# CELL-MATRIX ADHESION-DEPENDENT DIFFERENTIAL REGULATION OF CIS-MEDIAL VS TRANS-GOLGI ORGANISATION

A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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**20173512**

INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH

PUNE

2023

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# Acknowledgements

I express my heartfelt gratitude towards my PhD mentor, Dr. Nagaraj Balasubramanian, for entrusting me with this opportunity to work with him. His unwavering support and guidance throughout my doctoral journey have been invaluable. He has always encouraged me to take up new and challenging tasks in the lab and has taught me how to tackle research problems with a systematic approach. Furthermore, he has been an excellent mentor by allowing me to make mistakes and learn from them while providing me with the freedom to explore new avenues. I have gained immense knowledge from him not only in terms of research but also in handling challenging situations in life.

I thank Prof. M S Madhusudan for his help with the modelling studies done in this project. I would like to thank my Research Advisory Committee members, Prof. Richa Rikhy (IISER, Pune), Prof. Thomas (IISER, Pune) and Dr. Jeet Kalia (IISER, Bhopal) for their valuable suggestions during my RAC meetings and the constant guidance and support throughout my PhD.

I thank the IISER management for all the research facilities on campus and Council of Scientific & Industrial Research (CSIR) for funding me. I thank Integrated Decisions and Systems (India) Private Limited (IDeaS) for the scholarship received in 2018. I thank Wellcome-DBT India Alliance and the Indian Council of Medical Research (ICMR) for funding the lab. I am grateful to the microscopy facility for the smooth access to the microscopes and thankful to Vijay and Dr. Santhosh for the training and all the technical support during my experiments. I thank all the academic and biology office members at IISER, particularly Tushar and Sayalee, for their support in completing the necessary paperwork and administrative procedures. Additionally, I extend my gratitude to all the faculty members of the biology department for their technical aid, provision of reagents, and valuable guidance and recommendations during the project.

I am grateful to all the members of the adhesion lab for their invaluable contributions towards my experiments and insightful discussions on the project. I extend my sincere thanks to Keerthi and Natasha for their time and valuable suggestions that have helped me immensely in the project. I thank Siddhi for training me in various techniques used in the lab and guiding me through the project discussions. Prachi and Mayuresh's help and suggestions in the lab have been a significant boost, making it easier to work. I thank Nikita Shah for her assistance with conducting the experiments and for the enjoyable time we spent outside the lab. I extend my gratitude to Vishakha Kasherwal, who has been a great help in the lab, from arranging reagents to conducting experiments and even providing company during long overnight experiments. I also appreciate ShaunakKanade for his constant support and the much-needed tea breaks during work. I thank Tarun for being an inspiring junior, and I have enjoyed working with him in the lab. I thank Ajay, Omkar, Kajal, Sonam, Debasmita, Radhika, Arnav, and Antara for keeping the lab environment light-hearted and fun to work in. Finally, I would like to thank Dinesh Bhaiya for all his help in the lab that made it easier for us to carry out our experiments.

I express my gratitude to all the individuals who work on the first floor A-wing of IISER-Biology department for the cooperative and pleasant work atmosphere they created. Special thanks to Soumen Khan for his assistance with reagents, experimental design, and occasional treats. I would also like to thank Pooja Vaid for the delicious lunches that we thoroughly enjoyed. I thank Sanket Nagarkar for his support and valuable suggestions.

My experience at IISER wouldn't have been complete without the support of my dear friends, who made the journey enjoyable and fulfilling. I thank Shruti Marathe, whose suggestions were never valuable but for the great company and the amazing tea and breakfast sessions. I thank Dilsha for putting up with my work banter and keeping my spirits high. Saikat Panja and Fido were instrumental in helping me strike a balance between work and social life, and I will always cherish the conversations, discussions, and incredible food they made. Sandra was an amazing roommate whose company was always entertaining, and I doubt I will ever have a better one. I am grateful to Teju for the tea breaks and the delightful yet non-philosophical, straightforward chats. Swapna was incredibly helpful, and her breakfast and coffee made our Saturday mornings brighter. Finally, I extend my gratitude to Shirsa, Arjun, Ravi, Namrata, Visakh, and Lokesh for their unwavering support throughout my stay. I must express my gratitude to all the parents of my friends at IISER, whose support has been invaluable to us. I am particularly thankful for their generosity in sending us an abundance of food to share every time one of us visited home, which has been a great source of comfort and sustenance throughout our journey.

Completing this thesis would not have been possible without the support of my family. I extend my sincere gratitude to my parents for their unwavering belief in me, which has been more profound than my own. Their constant love and support have been my anchor throughout this journey. I also thank my siblings for lifting my spirits and helping me overcome the occasional blues.

Rajeshwari B R

# Contents


| Sl. No. | Title | Page no. |
|---|---|---|
| | Essential abbreviations | 9 |
| | Abstract | 11 |
| | Chapter 1: Introduction | 13 |
| 1.1. | The Golgi organization and its function in cells | 14 |
| 1.1.1. | The Golgi apparatus | 14 |
| 1.1.1.1. | The Golgi compartments | 14 |
| 1.1.1.2. | The cis-face of Golgi | 14 |
| 1.1.1.3. | The trans-face of Golgi | 15 |
| 1.1.2. | Organisation of the Golgi | 17 |
| 1.1.2.1. | Microtubules in Golgi organization | 17 |
| 1.1.2.2. | Role of ER in maintaining the Golgi structure | 18 |
| 1.1.2.3. | Role of matrix proteins in cisternal stacking | 19 |
| 1.1.2.4. | Role of small GTPases in the regulation of Golgi orgnization | 20 |
| 1.1.3. | The Golgi function | 22 |
| 1.1.3.1. | Transport through the Golgi | 23 |
| 1.1.3.2. | Glycosylation in the Golgi | 23 |
| 1.1.4. | Golgi structure-function relationship | 24 |
| 1.1.5. | Changes in Golgi organization: Role and regulation | 25 |
| 1.1.5.1. | Physiological conditions | 26 |
| 1.1.5.1.1. | Cell cycle | 26 |
| 1.1.5.1.2. | Apoptosis | 28 |
| 1.1.5.2. | Diseased conditions | 28 |
| 1.1.5.2.1. | Neurodegenration | 29 |
| 1.1.5.2.2. | Cancer | 31 |
| 1.2. | Small GTPase Arf1 and its role in regulating Golgi organization and function | 33 |
| 1.2.1. | ADP-ribosylation factor (Arf1) | 33 |
| 1.2.2. | Arf1 in Golgi organization and maintenance | 35 |
| 1.2.2.1. | Arf1 in Golgi biogenesis | 35 |
| 1.2.2.2. | Arf1 in the inheritance of Golgi | 37 |
| 1.2.2.3. | Arf1 mediated actin assembly at the Golgi | 39 |
| 1.2.2.4. | Arf1 based identity of Golgi compartments | 40 |
| 1.2.2.5. | Role of Arf1 in regulating Golgi function | 40 |
| 1.3. | Role of cytoskeleton in regulation of Golgi organization and function | 45 |
| 1.3.1. | Role of microtubules in regulating Golgi organization and function | 45 |
| 1.3.2. | Role of actin in regulating Golgi organization and function | 49 |

| | | |
|---|---|---|
| 1.3.3. | Regulation of actin and microtubules by integrin mediated cell-matrix adhesion | 51 |
| 1.3.3.1. | Integrin-actin cross-talk | 51 |
| 1.3.3.2. | Integrin-microtubule cross-talk | 52 |
| 1.4. | Hypothesis and objective of the thesis | 54 |
| | Chapter 2: Materials and methodology | 55 |
| 2.1. | Materials | 56 |
| 2.1.1. | Reagenst | 56 |
| 2.1.2. | Antibodies used for western blotting | 56 |
| 2.1.3. | Antibodies used for immunofluorescence | 56 |
| 2.1.4. | Plasmids | 56 |
| 2.2. | Mthodology | 57 |
| 2.2.1. | Cell culture and transfection | 57 |
| 2.2.2. | Suspension assay | 57 |
| 2.2.3. | Arf1 activity assay | 58 |
| 2.2.4. | GFP-ABD mediated detction of active Arf1 | 58 |
| 2.2.5. | RNA isolation and RT-PCR | 59 |
| 2.2.6. | BFA or GCA treatment of suspended WT-MEFs for imaging | 59 |
| 2.2.7. | Determining the Golgi distribution profile | 59 |
| 2.2.8. | Immunostaining of GBF1 | 60 |
| 2.2.9. | Confocal microscopy and deconvolution of z-stacks | 60 |
| 2.2.10. | Colocalization analysis | 60 |
| 2.2.11. | Cell spreading quatitation | 60 |
| 2.2.12. | Lectin labelling | 61 |
| 2.2.13. | Multiple sequence alignment | 61 |
| 2.2.14. | Structure modelling of the mouse GEFs (BIG1, BIG2, GBF1) and mouse Arf1. | 61 |
| 2.2.15. | Acetylated tubulin and total tubulin immunostaining | 62 |
| 2.2.16. | Measurement of intensity of acetylated tubulin staining | 62 |
| 2.2.17. | Isolation of membrane-rich fractions | 62 |
| 2.2.18. | Golgi isolation | 63 |
| 2.2.19. | Statistical analysis | 63 |
| | Chapter 3: Role of Arf1 and kinetics of change in Arf1 activation in regulating Golgi organization and function in non-adherent cells | 64 |
| 3.1. | Rationale | 65 |
| 3.2. | Results | 67 |
| 3.2.1. | Effect of loss of cell-matrix adhesion on Golgi organization and Arf1 activation | 67 |
| 3.2.2. | Localization of active Arf1 in suspended vs re-adherent WT-MEFs | 69 |
| 3.2.3. | Relative expression of Arf1-GEFs and structural analysis of Arf1-GEFs bound to Arf1 and BFA | 71 |
| 3.2.4. | BFA-mediated decrease in Arf1 activation regulates Golgi fragmentation in non-adherent WTMEFs | 75 |

| | | |
|---|---|---|
| 3.2.5. | GCA-mediated decrease in Arf1 activation regulates Golgi fragmentation in non-adherent WTMEFs | 79 |
| 3.2.6. | Localization of GBF1 in non-adherent WT-MEFs | 85 |
| 3.2.7. | Constitutive active Arf1 (Q71L) rescues BFA/GCA mediated Golgi fragmentation | 86 |
| 3.2.8. | Kinetics of Arf1 inactivation regulates loss of adhesion-mediated Golgi disorganization | 89 |
| 3.2.9. | Effect change in the kinetics of Arf1 activation has on Golgi function | 93 |
| 3.2.10. | Effect of BFA/GCA treatment on trans-Golgi compartment | 94 |
| 3.3. | Summary | 97 |
| 3.4. | Conclusion | 98 |
| 3.5. | Future directions | 98 |
| | Chapter 4: Adhesion-dependent regulation of microtubule acetylation | 99 |
| 4.1. | Rationale | 100 |
| 4.2. | Results | 102 |
| 4.2.1. | Loss of cell-matrix adhesion results in a drop in microtubule acetylation | 102 |
| 4.2.2. | Effect of tubacin treatment on microtubule acetylation levels in WT-MEFs | 104 |
| 4.2.3. | Effect of microtubule acetylation on the trans-Golgi organisation in non-adherent cells | 107 |
| 4.2.4. | Effect of microtubule acetylation on the cis-medial-Golgi organisation in non-adherent cells | 109 |
| 4.2.5. | Effect of active Arf1 (Q71L) expression on microtubule acetylation in WT-MEFs | 111 |
| 4.3. | Summary | 113 |
| 4.4. | Conclusion | 114 |
| 4.5. | Future directions | 114 |
| | Chapter 5: Isolation of Golgi from adherent vs non-adherent cells for proteomic profiling | 115 |
| 5.1. | Rationale | 116 |
| 5.2. | Results | 118 |
| 5.2.1. | Isolation of membrane and cytosolic fractions from adherent and suspended WT-MEFs | 118 |
| 5.2.2. | Isolation of Golgi from adherent and suspended WT-MEFs | 120 |
| 5.3. | Summary | 122 |
| 5.4. | Conclusion | 122 |
| 5.5. | Future directions | 122 |
| | Chapter 6: Discussion | 123 |
| 6.1. | Role kinetics of change in Arf1 activation has in regulating Golgi organization | 124 |

6.2. Adhesion-dependent regulation of microtubule acetylation 129
6.3. Proteomic profile of Golgi isolated from adherent vs non-adherent cells 132

Conclusion 134

References 135

# Essential Abbreviations

| | |
|---|---|
| µl | microliter |
| µM | micromolar |
| ml | milliliter |
| ER | Endoplasmic reticulum |
| MTOC | Microtubule organising center |
| MT | Microtubule |
| VTC | Vesicular-tubular clusters |
| ERES | Endoplasmic reticulum exit sites |
| Arf1 | ADP-ribosylation factor 1 |
| GEF | Guanine nucleotide exchange factor |
| GAP | GTPase-activating factor |
| GBF1 | Golgi specific brefeldin A-resistance nucleotide exchange factor 1 |
| BIG1 | Brefeldin A-inhibited guanine nucleotide exchange factor 1 |
| BIG2 | Brefeldin A-inhibited guanine nucleotide exchange factor 2 |
| ABD | Arf binding domain |
| GFP | Green fluorescent protein |
| RFP | Red fluorescent protein |
| ManII | Mannosidase II |
| GalTase | Beta-1,4-galactosyltransferase |
| BFA | Brefeldin-A |
| GCA | Golgicide-A |
| GM130 | Golgi matrix protein 130 |
| GGA3 | Golgi localised γ-ear containing Arf-binding protein 3 |
| WT-MEFs | Wild-type mouse embryonic fibroblasts |
| FN | Fibronectin |
| GT | Glycosyltransferase |
| FAPP1 | Four-phosphate-adaptor protein 1 |

# ABSTRACT

The characteristic structure of the Golgi apparatus is made up of flattened cisternal stacks and conserved across eukaryotes. The Golgi plays a central role in protein processing and trafficking. It is a dynamic structure that undergoes a rapid reorganisation in response to different stimuli, including cell-matrix adhesion. In response to loss of adhesion, Golgi rapidly disorganises and is distributed throughout the cell while staying attached to the microtubule network. Adhesion-dependent Golgi disorganisation is seen to be controlled by Arf1 activation. In mouse fibroblasts, loss of adhesion triggers a drop (~50%) in Arf1 activation that causes the Golgi to disorganise but not fragment. The extent of this disorganisation is interestingly seen to be different for different Golgi compartments. While the trans-Golgi is seen to be more disorganised and distributed throughout the cell, the cis/medial Golgi disorganises much less. This difference seen in non-adherent cells we find is supported by the differential localisation of active Arf1 to these compartments. This could be mediated by the differential activation of Arf1 by GEFs. Non-adherent fibroblasts hence provide a unique setting to test and understand the role GEF-mediated Arf1 activation has in regulating Golgi organisation. Using Man II-GFP expressing non-adherent fibroblasts treated with increasing concentrations of Brefeldin-A (BFA) (inhibits BIG1/2 and GBF1) and Golgicide A (GCA) (inhibits GBF1 only), we test this regulation. This reveals comparable inhibition of Arf1 to cause a concentration-dependent increase in cis-Golgi fragmentation and ER fallback. A distinct difference in the kinetics of Arf1 inhibition at low vs high BFA/GCA concentrations we find could mediate this by regulating cis-Golgi localised GBF1. In agreement with this, on loss of adhesion, slower kinetics of Arf1 inhibition (~50% inactivation in 120min) causes the Golgi to disorganise. In contrast, a comparable but rapid inhibition of Arf1 (using BFA or GCA) causes the Golgi to instead fragment dramatically. This affects Golgi function reflected in altered cell surface glycosylation and delayed re-adherent cell spreading. Loss of adhesion also causes a distinct drop in microtubule acetylation which could be more an effect of Golgi disorganisation rather than a mediator of the same. These studies, using the distinct change in Golgi organisation and function on loss of adhesion, help provide a novel insight into the Arf1-mediated regulation of the Golgi.

# CHAPTER 1

# Introduction and review of literature

## 1.1. Golgi organisation and its function in cells.

### 1.1.1. The Golgi apparatus

The Golgi apparatus is a conserved, membrane-bound subcellular organelle in all eukaryotes. It lies central to the secretory pathway and plays a significant role in lipid and protein processing and trafficking. It comprises flattened disc-like structures called cisternae (Mark S Ladinsky et al., 1999; Rambourg & Clermont, 1997) stacked upon each other to form the entire Golgi complex. The number of Golgi stacks can vary from 2-3 in lower eukaryotes to up to 30 in higher eukaryotes. In mammals, these Golgi stacks are interlinked to form a continuous ribbon-like structure (Klumperman, 2011; Marsh et al., 2001).

#### 1.1.1.1.The Golgi compartments

The Golgi complex is a highly dynamic organelle maintained through continuous input and out-flows of the membrane. It is structurally and functionally subdivided into cis-, medial and trans-Golgi (Fig. 1.1) flanked by Endoplasmic reticulum-Golgi complex intermediate compartment (ERGIC) and Trans-Golgi-Network (TGN) (Klumperman, 2011; Papanikou & Glick, 2014). The cargo vesicles arising from the Endoplasmic reticulum (ER) exit sites form vesicular-tubular clusters (VTCs) that fuse to form the ERGIC. These VTCs then give rise to the cis-Golgi compartment. The cargo that arrives at the cis-Golgi then moves to medial and later trans-Golgi. As the cargo molecules traverse the Golgi, they undergo several modifications by the Golgi resident proteins. Later, the cargo that arrives at the trans-Golgi is segregated into vesicles that comprise the TGN, from where they are further distributed to their destined locations (M S Ladinsky et al., 1999; Nakamura et al., 2012).

#### 1.1.1.2.The cis-face of Golgi

The vesicles carrying cargo that needs to be exported from the endoplasmic reticulum (ER) bud off from the endoplasmic reticulum exit sites (ERES)(Fan et al., 2003; A. Mironov et al., 2003). Activation of Sar1GDP at the ERES recruits Sec23/Sec24p and Sec31/Sec13p leading to the assembly of COPII coat around the vesicles arising from the ERES. The COPII coat facilitates the budding of the vesicles from the ER membrane(Barlowe, 2003; Barlowe et al., 1994). Later the uncoating of COPII leads to the fusion of these cargo vesicles forming the vesicular-tubular clusters (VTCs) that comprise the ERGIC (Fig. 1.2)(Sergei I. Bannykh et al., 1996).

Some of the VTCs can be branched and sometimes even appear to consist of few flat disc-shaped membranes (Klumperman et al., 1998; Sesso et al., 1994). COPI coat is known to assemble on VTCs and help retrieving the ER resident enzymes that could have escaped into the VTCs (Letourneur et al., 1994; Lewis & Pelham, 1990). Several VTCs are found to be associated with the ERES and are referred as peripheral VTCs. Small tubulovesicular membranes detach from peripheral VTCs and fuse with the central VTCs forming the ERGIC in a microtubule dependent manner (Scales et al., 1997; Sciaky et al., 1997). The central VTC then undergoes a process in which it transforms into small-sized cisternal membranes that are parallel to the cis-most Golgi cisternae. These membranes can either fuse directly with pre-existing cis-cisternae or laterally to form new ones (Hauri & Schweizer, 1992). A computational model proposed that the ability of VTC membranes to fuse with the cis-Golgi cisterna is determined by the rate at which the maturing cisterna loses its ability to fuse(Kühnle et al., 2010). This loss of fusion ability could be due to the recycling of SNARE proteins back to the endoplasmic reticulum (Kühnle et al., 2010).

It is hard to discern the boundary between the ERGIC and cis-Golgi, however several proteins like ERGIC53 show strong demarcation in their localisation to ERGIC over the cis-Golgi and these markers can help discern the boundary between the ERGIC and cis-Golgi(Klumperman, 2011; Mellman & Simons, 1992). It still remains unclear whether the VTCs of ERGIC fuse to give rise to the cis-Golgi cisternae or vesicles that bud out from the VTCs later go on to fuse to form cis-face of the Golgi (Klumperman, 2011).

The cargo that arrives at the cis-Golgi then moves to medial and later trans-Golgi. The cis, medial and trans-Golgi cisternae are very compactly packed (Glick, 2000; Klumperman, 2011). The cisternae appear dilated at the rims and are quite narrow at the centre. As the cargo molecules traverse the Golgi, they undergo several modifications carried out by the Golgi resident proteins. Later, the cargo that arrives at the trans-Golgi is segregated into vesicles that comprise the TGN, from where they are further distributed to their destined locations (Fig. 1.2) (M S Ladinsky et al., 1999; Nakamura et al., 2012).

#### 1.1.1.3.The trans-face of Golgi

The trans-Golgi is the final compartment of the Golgi apparatus in a mammalian cell, located on the opposite end from the cis-Golgi. It is responsible for sorting and packaging proteins and lipids into their final destinations, such as lysosomes, secretory vesicles, or the plasma

membrane (Maria Antonietta De Matteis & Luini, 2008). The trans-Golgi is characterized by its distinctive morphology, which includes several interconnected cisternae or sacs. Within the trans-Golgi, there is a sub-compartment known as the trans-Golgi network (TGN), which is responsible for directing proteins and lipids to their final destinations (Fig. 1.2). The TGN is also involved in the recycling of molecules back to the Golgi apparatus or the endoplasmic reticulum. The penultimate trans-cisternae and the trans-cisternae can project tubules that contribute to the formation of TGN (Marsh & Howell, 2002; Mogelsvang et al., 2004). While the cis-Golgi is generally coated with COPI, the trans-Golgi and TGN is coated with clathrin. Morphologically the COP and clathrin coats are disticntly different and can be distinguishiung even without immunolabelling (Kirchhausen et al., 1986; Mark S. Ladinsky et al., 1994; Oprins et al., 1993). Based on the sorting signals on the cargos, the cargos are packaged into transport vesichles destined to specific locations in the cell. Different carriers can be employed based on the location of delivery(Klumperman, 2011; Linton M. Traub & Kornfeld, 1997).

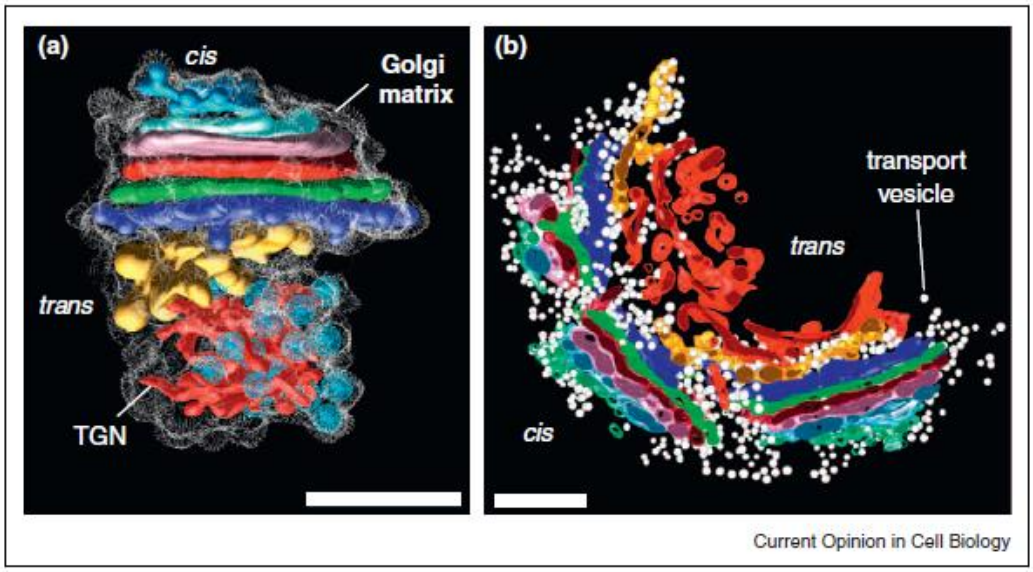



**Figure 1.1:** Golgi model generated by electron tomography (Adapted from Staehelin LA and Kang BH, Plant Physiol. 2009). A) A Golgi stack within a meristem cell of *Arabidopsis thaliana.* Different cisternae are represented with different colours, and the surrounding Golgi matrix is shown in white. Clathrin-coated vesicles emerging from the *trans*-Golgi are represented in blue. B) Golgi stacks from a pancreatic cell from hamster. Different cisternae are represented in different colours, and the transport vesicles are shown in white.

### 1.1.2. The Organisation of Golgi

The structure of the Golgi apparatus plays a vital role in regulating its functions. Export from the Endoplasmic Reticulum (ER) is essential for the biogenesis and maintaining the Golgi integrity (Marra et al., 2007; A. Mironov et al., 2003; A. A. Mironov et al., 2001; Ward et al., 2001). The structure and localisation of the mammalian Golgi are also governed by its interaction with the microtubules and the Golgi matrix proteins (Barr & Short, 2003; Marra et al., 2007; Short et al., 2005). Microtubules aid in the positioning of the Golgi, and the Golgi matrix proteins stack the cisternae, thus giving the Golgi a ribbon-like appearance (Lowe, 2011; Thyberg & Moskalewski, 1999). Trafficking across the Golgi also governs its structure. A dynamic equilibrium between the anterograde and retrograde transport across the Golgi is essential to maintain a stable Golgi structure (Nihal Altan-Bonnet et al., 2004; Ward et al., 2001). Small GTPases like Rab proteins and Arf1 aid vesicular transport across the Golgi. These small GTPases might be involved indirectly in maintaining the Golgi structure by maintaining trafficking and also by mediating the interaction of Golgi proteins with other regulatory molecules (Baschieri & Farhan, 2012; Short et al., 2005). The synchronized function of these regulatory molecules maintains the Golgi structure.

#### 1.1.2.1.Microtubules in Golgi organisation

In mammals, the Golgi apparatus has a peri-nuclear localisation, organised close to the Microtubule Organizing Center (MTOC). Given its localisation, it has been seen that microtubules play a significant role in organizing and maintaining the Golgi structure. Two different sets of microtubules are involved in organizing the Golgi structure. The microtubule arising from the MTOC facilitates the positioning of the Golgi near the centrosome (Burkhardt, 1998; Wang *et al.*, 2017). Also, the Golgi apparatus can nucleate and give rise to its own microtubules (Wu, de Heus, et al., 2016). The microtubules arising from the Golgi help bring the VTCs from ER-exit sites towards the MTOC by the “search and capture” method and facilitate their fusion with the Golgi cisternae. Disrupting microtubule organisation by nocodazole treatment resulted in the fragmentation of Golgi, supporting the role of microtubules in the organisation and maintenance of Golgi structure (Cole et al., 1996a).

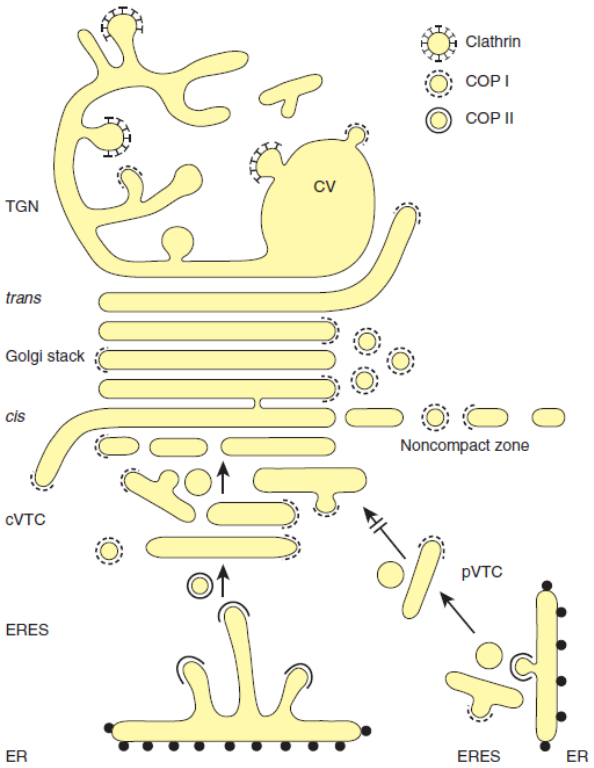



**Figure 1.2:** Schematic representation of the Golgi and associated membrane networks (Adapted from Klumperman, 2011). The above schematic gives an overview of the Golgi stack with respect to ERES, central (c) and peripheral (p) VTCs and TGN and the COPII, COPI, and clathrin coats. Membranes are drawn as if cross-sectioned in side view.

### 1.1.2.2. Role of ER in maintaining the Golgi structure

ER trafficking is a significant player in the maintenance of the Golgi structure. Initially, the Golgi apparatus was thought to be just an extension of the ER. It is now known that the Golgi apparatus carries out several functions distinct from the ER. Though different in function, ER trafficking has a significant role in Golgi maintenance. Blockage or disruption of anterograde transport from the ER results in Golgi fragmentation and redistribution of Golgi enzymes back to the ER (Klausner, 1992). As the anterograde transport from ER delivers cargo to the Golgi, retrograde transport from the Golgi returns escaped ER resident proteins back to the ER. Also, retrograde flow stabilizes the loss of membrane from ER due to anterograde flow and helps maintain ER (Ward et al., 2001).

Also, normal trafficking from the ER can facilitate the *de novo* biogenesis of the Golgi membrane (Nihal Altan-Bonnet et al., 2004). Golgi apparatus like ER is inherited during cell division. Hence, it was believed Golgi biogenesis occurs in a template-dependent manner only. However, experiments have shown that normal ER trafficking can lead to *de novo* synthesis of Golgi. Post Golgi ablation through laser nanosurgery, the VTCs arising from the ER-exit sites can fuse and form the Golgi apparatus.(Ronchi et al., 2014) However, this is an energy and time-consuming process. Hence, despite being capable of synthesizing the Golgi *de novo,* cells have adopted to inherit the Golgi apparatus to reduce the workload on newly formed daughter cells. Also, a pre-formed Golgi would facilitate faster processing and trafficking of proteins/lipids required for the newly formed cells (Yanzhuang Wang & Seemann, 2011).

#### 1.1.2.3.Role of matrix proteins in cisternal stacking

The Golgi cisternae are surrounded by the fibrous protein scaffold formed by the Golgi matrix proteins. The Golgi matrix is a ribosome-free zone around the Golgi apparatus, composed of several matrix proteins, including Golgi Reassembly and Stacking Proteins (GRASPs) (Fig. 1.3). Two GRASP proteins are important in mammals, GRASP65 and GRASP55 (Barr et al., 1997; Feinstein & Linstedt, 2008). GRASP65 is known to majorly localize to the cis-Golgi and GRASP55 to the medial and trans-Golgi (Xiang & Wang, 2010). These proteins help in the stacking of the cisternae and facilitate ribbon formation. GRASP65 and GRASP55 might have redundant roles, as a single knockdown of either protein does not drastically disrupt the Golgi structure. However, a double knockdown of these proteins results in ribbon unlinking and increases the inter-cisternal distance (Bekier et al., 2017).

The Golgi cisternae are also held together by the interconnecting tubular structures formed due to coordination between two opposing proteins, Phospholipase A2 (PLA2) and lysophospholipid acyltransferase (LPAT). PLA2 activity generates lysophospholipids that lead to negative membrane curvature locally by forming tubules, which is opposed by LPAT. Therefore, increased tubulation by blocking LPAT fragments the Golgi. Inhibition of tubulation by blocking PLA2 activity hinders the ribbon-like structure. This shows that tubulation is a highly controlled phenomenon, but the exact underlying mechanism is yet to be understood (Schmidt et al., 2010; Schmidt & Brown, 2009). The motor protein kinesin is also postulated to play a role in tubulation (Dorner et al., 1998; She et al., 2017). It was seen that an inactive mutant of kinesin KIFIC failed to induce tubule formation post-BFA treatment. However, a

knockout of the same protein had no significant effect on tubulation (Nakajima et al., 2002). This suggests that kinesin is involved in tubulation, but other proteins can take over this function in the absence of kinesin. On the other hand, binding of Golgi phosphoprotein (GLOLPH3) to the rare myosin, Myosin18A, can generate enough stretch and pull force necessary for tubule formation (Dippold et al., n.d.). Tubulation can also be brought about by binding the Golgi proteins to microtubules and actin filaments, generating a pulling force at the Golgi membrane surface (Copeland et al., 2016).

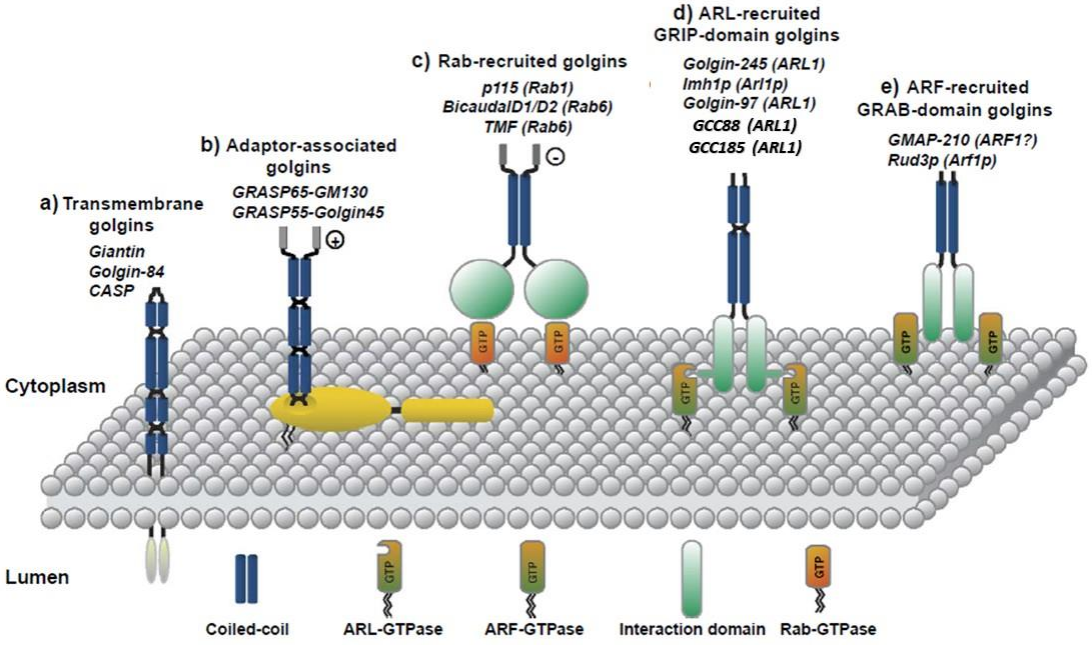



**Figure 1.3:** Schematic representation of Golgins that associate with Golgi membranes and the different ways in which they associate with the Golgi membranes (Adapted from Short, Haas and Barr, 2005).

### 1.1.2.4.Role of small GTPases in the regulation of Golgi organisation

Besides the above-mentioned mechanisms, several small-GTPases including Rab proteins and Arf1 have been implicated in maintaining the Golgi structure and function (Fig. 1.4) (Serguei I. Bannykh et al., 2005). Out of the 60 known mammalian Rab proteins, Rab 6, Rab 1, Rab 2, Rab 18, Rab 33b and Rab 43 have been shown to localize to the Golgi and play a role in the maintenance of Golgi organisation and trafficking across the Golgi (Goud & Gleeson, 2010; Short et al., 2005). However, it is still unclear whether Rab proteins' effect on the Golgi organisation is a direct effect or a side effect of disrupting their role in protein trafficking.

ADP-ribosylation factor 1(Arf1) localizes to the entire Golgi (Bottanelli et al., 2017). However, Arf1 regulators are differentially localised at the Golgi and regulate the interaction of Arf1 with its effectors. GBF1 and BIG1/2 are Arf1 GEFs that are localised to Golgi.(J.G. Donaldson & Honda, 2005) GBF1 predominantly localizes to the cis-Golgi, and BIG1/2 localizes to the trans-Golgi (Quilty et al., 2014; Richardson, McDonold, et al., 2012). These GEFs recruit Arf1 to the Golgi membrane and activate it by exchanging bound GDP to GTP on Arf1. Active Arf1 is involved in coatomer recruitment and vesicle formation. Loss of active Arf1 at the Golgi membrane post-BFA treatment leads to fragmentation. After, BFA washout recovery in active Arf1 facilitates Golgi re-organisation. Expressing a GDP-restricted dominant-negative form of Arf1 leads to Golgi fragmentation. This shows Arf1 activity is necessary for the maintenance of the Golgi structure. Other than Arf1, Arf3 is also known to localize Golgi. However, the role of Arf3 at the Golgi is yet to be identified (Helms et al., 1993; Quilty et al., 2014; Szul et al., 2007).

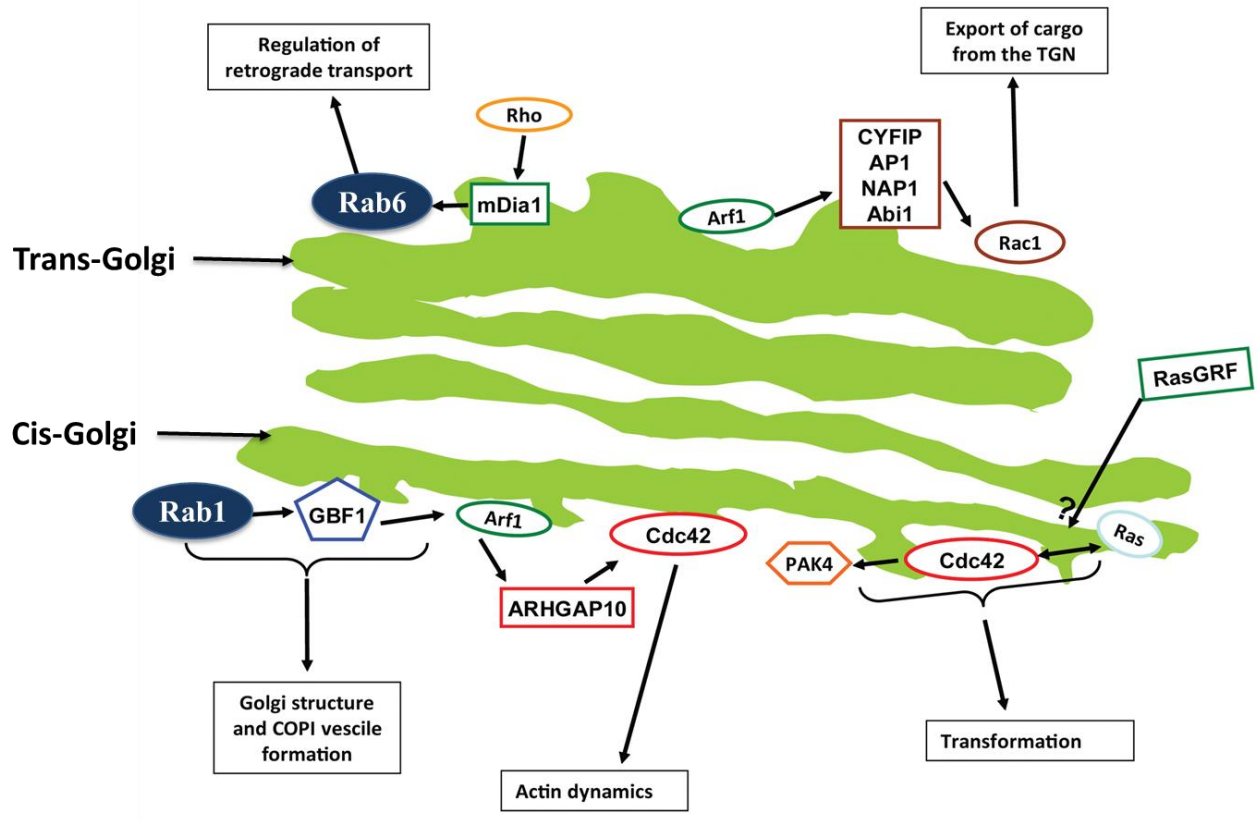



**Figure 1.4:** Schematic representation of small GTPases localised to the Golgi (Adapted from Baschieri and Farhan, 2012).

### 1.1.3. Golgi function

The Golgi apparatus is involved in protein and lipid modification, sorting and trafficking. Modifications of cargo molecules carried out in the Golgi govern the localisation and function of these molecules. The cargo molecules emerging from the ER enter the Golgi at the cis-face. As they transit across the Golgi, they are modified by the action of several enzymes localised to the Golgi compartments. Finally, the modified cargo is sorted at the trans-Golgi, packaged into carrier vesicles and delivered to the destined locations. Modifications of the cargo include glycosylation, sulfation, phosphorylation, and proteolysis (Yanzhuang Wang & Seemann, 2011).

#### 1.1.3.1. Transport through the Golgi

The dynamic nature of the Golgi structure also contributes to its function. Golgi is central to the secretory pathway. The proteins and lipids exiting the ER are processed and sorted in the Golgi. Cargo trafficking through the Golgi can be partitioned into (i) transport from ER to Golgi, (ii) transit through Golgi, and (iii) transport from the Golgi to the plasma membrane.

**(i) Transport from ER to Golgi**

Proteins newly synthesized in the ER are packaged into vesicles coated by COPII coatomer protein induced by the activation of Sar1 GTPases. These vesicles bud out of the ER and form the ERGIC (Memon, 2004).

**(ii) Transit through the Golgi**

Several models are proposed to describe cargo transit across the Golgi complex. However, most available evidence has pointed towards the “cisternal maturation” model. In this model, the cisternae are viewed as dynamic entities. They sequentially mature from cis to trans-cisternae, retaining the same cargo. These cisternae mature via retrograde transport, where enzymes from late cisternae are recycled back to early cisternae. This helps maintain a concentration gradient for different enzymes along the different compartments. This gradient of enzymes aids in the sequential processing of cargo (Nakano et al., 2010).

There has also been enough evidence to show the existence of a “vesicular shuttle” model of cargo transport through the Golgi. This model views the Golgi cisternae as static entities that house different enzymes in different stacks. The cargo is transported across the cisternae by COPI/COPII coated vesicles that bud out from each cisterna and fuse with the cisterna ahead.

However, this model fails to explain the exclusion of Golgi enzymes from the budding vesicles and the transport of cargo too large to fit into these vesicles. Hence currently, cargo transportation across Golgi is believed to be through a combination of both both "cisternal maturation" and "vesicular shuttling" (Jackson, 2009).

**(iii) Transport from Golgi**

The cargo vesicles between the *trans*-Golgi and PM comprise the TGN and lie central to exocytosis and endocytosis pathway. These vesicles are coated with API, clathrin and other coat proteins that transport them to different organelles within the cell or to be secreted into the external environment (Griffiths & Simons, 1986).

### 1.1.3.2. Glycosylation in the Golgi

Glycosylation is the most necessary modification out of the many other modifications in the Golgi. It is a process in which glycosyltransferases (GT) add carbohydrate moieties to the substrates. Each species carries several glycosyltransferase enzymes specific to the kind of conjugate they add, the type of substrate it acts on and the site of modification. As a result of this, each species can generate a wide range of glycol-conjugated cargos. These cargos can be glycoproteins, proteoglycans, glycophosphatidylinositol or glycolipids. Glycoconjugates added to the substrates regulate the biological role of these molecules. These biological functions include host-pathogen recognition and interaction and distinguishing self from non-self within and across species (P. Stanley, 2011).

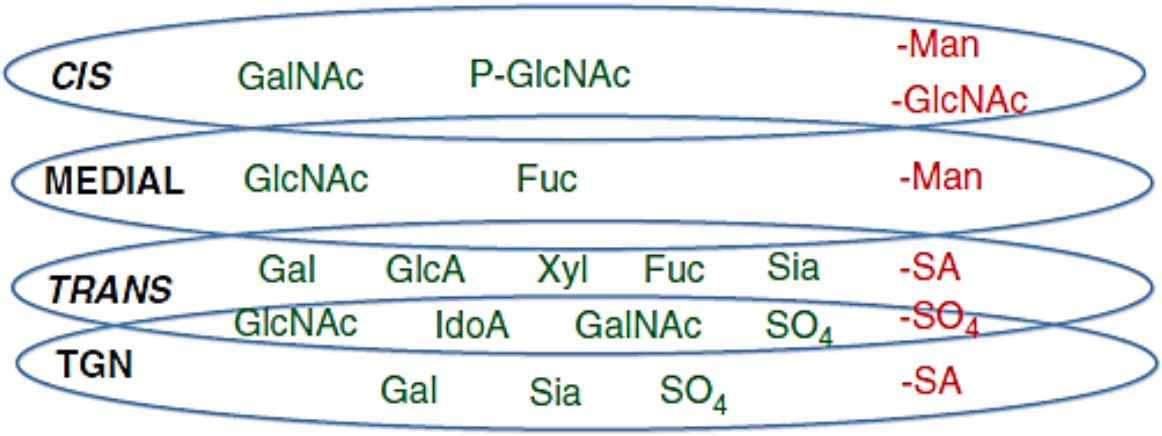



**Figure 1.5**: Schematic representation of specific glycosylation modifications that take place in different Golgi compartments (Adapted from Stanley, 2011).

Glycosylation might result in substrates carrying as little as a single glycan to a substrate with as many as 200 glycans. The sugar moiety transferred by the GT is donated by a high-energy nucleotide that is synthesized in the cytosol and transported into the Golgi apparatus in exchange for a low-energy nucleotide. Adding one sugar then allows the substrate to be acted on by the next GT. So, several GTs act upon the substrate sequentially to produce the final glycol-modified cargo that exits the Golgi (Fig. 1.5). The sugars added by the GTs can be removed by the action of glycosidases that reside within the Golgi. Also, these sugar moieties can be further modified by acetylases, sulphotransferases or epimerases (Opat et al., 2001).

The substrates are either N-glycosylated or O-glycosylated. The initial glycosylation in both cases is mainly initiated at the ER. The N-glycans that enter the Golgi can remain unprocessed throughout their transit within the Golgi. However, most N-glycans are processed at the cis-face, where α-mannosidases act upon the N-glycans, which removes several mannose molecules to generate an intermediate molecule, $Man_5GlcNAc_2An$ which is a substrate for enzymes residing at medial Golgi. In further processing, two of the five mannose residues can be replaced by GlcNac. This increases the complexity by including branching in the substrates. A single substrate can have as many as six branches (P. Stanley, 2011).

In the case of O-glycosylation, initiation in the ER includes the addition of a single sugar moiety to serine or threonine residue. Mucins and glycosaminoglycans are the most abundant class of O-glycosylated substrates. Around 15 different N-acetyl galactosamine transferases carry out O-glycosylation in mammals, whereas yeast lacks these transferases. Sulfotransferases of the Golgi further act upon glycosaminoglycans to add sulfate moieties at specific sites of these molecules.

Lipid glycosylation is also initiated at the ER by adding sugar to ceramide (Cer). The Glc-Cer can remain unmodified as it transits the Golgi or can be heavily modified by the sequential action of Golgi GTs (P. Stanley, 2011; Zhang & Wang, 2016a).

### 1.1.4. Golgi structure-function relationship

Ever since its discovery, the elaborate structure of mammalian Golgi has drawn the interest of many researchers. Though Golgi has been conserved in all eukaryotes, Golgi structure has evolved with the complexity of the cell. The role of the Golgi ribbon structure in mammalian

cells is yet to be understood entirely. Currently, the following roles can be attributed to the Golgi structure:

i. Cisternal stacking increases the efficiency of cargo trafficking between the cisternae. The vesicles from one cisterna can easily be docked to the adjacent cisternal membrane. This decreases diffusion and aids cargo's faster and efficient movement across the Golgi (Y Wang et al., 2008).
ii. During glycosylation, the glycosyltransferases and glycosidases work sequentially, where the product of one enzyme feeds in as a substrate for the following enzyme. These enzymes are localised across the Golgi in order of their action on the substrate. Stacking helps in the spatial separation of these enzymes, provides a suitable environment for enzymatic activity, and simultaneously aids in sequential localisation, ensuring precise modification of the cargo molecules (Lowe, 2011).
iii. Stacking also limits the kinetic rate of cargo movement as the total surface area available for vesicle budding is reduced. As a result, vesicles bud out and fuse only at the rims of the cisternae. This also ensures most of the cargo sorting occurs only at the *trans*-Golgi (J E Rothman, 1981; Y Wang et al., 2008).
iv. Stacking the Golgi and its positioning to the front of the cell aids in directional trafficking, thus facilitating cell polarization and migration. This helps deliver cargo molecules involved in cell migration to the plasma membrane faster (Xing et al., 2016; Yadav et al., 2009).
v. The Golgi apparatus undergoes fragmentation during the G2 phase. Failure to unstack the cisternae prevents the entry of cells into mitosis, thus establishing a "Golgi checkpoint" in cell division (Colanzi & Corda, n.d.; Persico et al., 2009).
vi. Recently, Golgi has been viewed as an organelle capable of sensing and transducing signals. Golgi senses and responds to signals like apoptosis and DNA damage by altering its phenotype (Hicks & Machamer, 2005). The change in Golgi organisation can aid in further signal transduction (like in apoptosis) or better cell survival (DNA damage response) brought about by altered function (Farber-Katz et al., 2014). Hence, the conserved Golgi structure might have a significant role to play in signalling events independent of its protein and lipid processing function.

### 1.1.5. Changes in Golgi organisation: Role and regulation

The stable structure of Golgi observed in the interphase stage of cells is known to change in several physiological and diseased conditions. The Golgi is fragmented in physiological

conditions like mitosis, meiosis, and apoptosis. Post mitosis and meiosis, the fragmented Golgi re-organises at the MTOC to form the ribbon-like structure(Tang & Wang, 2013). However, the fragmentation observed during apoptosis is irreversible(Mukherjee et al., 2007; Rabouille et al., 2015). Golgi is also reported to be fragmented in several cancers and neurodegenerative diseases. The fragmented phenotype of the Golgi is irreversible in these conditions (Ayala & Colanzi, 2017; Rabouille et al., 2015; Zappa et al., 2018). The role of Golgi fragmentation in these conditions is yet to be fully understood.

#### 1.1.5.1.Physiological conditions

Cell cycle and apoptosis are two physiological conditions where the Golgi is fragmented. The cell cycle is a process of duplication of cellular contents and cell division. The cell cycle is a highly regulated process and constitutes four different phases: G1 (Gap1), S (Synthesis phase), G2 (Gap2) and M (Mitosis) phase. G1 is the phase where the cell prepares for DNA duplication. It is followed by DNA duplication in S-phase. In the G2 phase, the cell grows and gets committed to entering mitosis at the end of the G2 phase. At the end of mitosis, the cell divides to give rise to daughter cells. It has been seen that Golgi fragmentation acts as a checkpoint to regulate the entry of a cell into mitosis(Mascanzoni et al., 2019; Persico et al., 2009).

Apoptosis is a process of programmed cell death. The cell undergoing apoptosis shrinks and condenses. The cellular components, like the nuclear envelope and the cytoskeleton, are disassembled, and the DNA is fragmented. The cellular components are then packaged into apoptotic bodies recognized and cleared by macrophages or the neighbouring cells. Golgi in these cells is fragmented during the early stages of apoptosis. This disrupts the processing and trafficking through the Golgi (Rabouille et al., 2015).

##### 1.1.5.1.1. Cell cycle

In mammalian cells, the Golgi apparatus starts fragmenting towards the end of the G2 phase. This is due to an increase in vesiculation followed by the failure of these Golgi vesicles to fuse with the adjacent membrane (Tang & Wang, 2013). In prophase, the Golgi is completely fragmented and distributed in two pools. One pool is randomly distributed throughout the cell, and the other is primarily localised to the spindle pole. The Golgi vesicles localised to the spindle pole are known to be rich in GRASP protein that aids in Golgi reassembly post-cell division (Yanzhuang Wang & Seemann, 2011).

Towards the end of telophase, Golgi vesicles reorganise to form cisternae. A larger Golgi stack is formed opposite to the division furrow. A smaller Golgi stack is also formed facing the furrow. This smaller stack is believed to deliver membrane components to the cleavage furrow and thus aid membrane sealing (Fig. 1.6) (Corda et al., 2012).

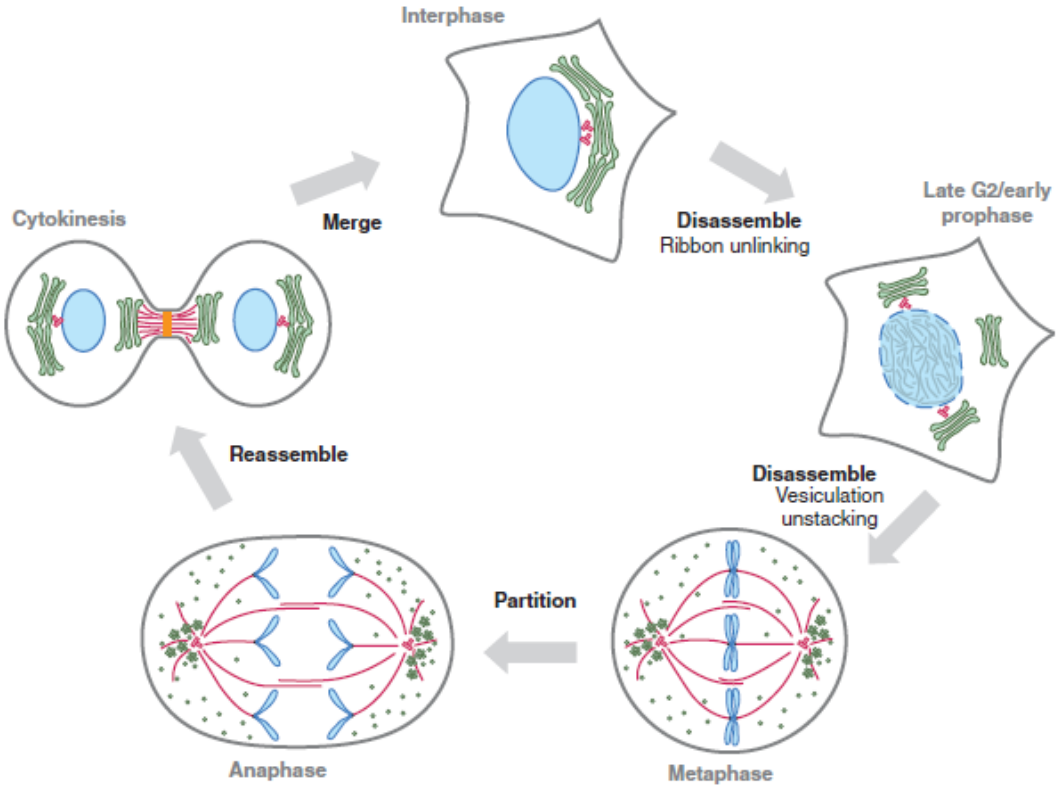



**Figure 1.6:** Inheritance of Golgi during cell division (Adapted from Wang and Seemann, 2011). The Golgi ribbon undergoes fragmentation towards the end of the G2 phase. Golgi fragments are then distributed throughout the cell and are partitioned into the daughter cells stochastically. Also, a fraction of these Golgi fragments are associated with the spindle poles and are inherited in a spindle pole-dependent manner. During the telophase, the Golgi stacks reform. A major Golgi stack is reformed opposite to the division furrow, and a minor Golgi stack is formed towards the cleavage furrow. Post cytokinesis, the minor stack moves and fuses with the major Golgi stack, thus creating a single ribbon structure of the Golgi.

The fragmented Golgi during cell division doesn't carry out any cargo processing and trafficking activity. In cells with the cell wall, where there is a need for continuous synthesis of cell wall components, the Golgi stacks do not fragment during mitosis as a functional Golgi is required to maintain the cell wall (e.g. Plants) (H. Stanley et al., 1997; Yanzhuang Wang & Seemann, 2011).

#### 1.1.5.1.2. Apoptosis

Apoptosis is programmed cell death, during which the cell components are disassembled and packaged into apoptotic bodies. These apoptotic bodies are later phagocytized and cleared off by the neighbouring cells (Atkin-Smith & Poon, 2017). The initiator and effector caspases mediate apoptosis. Caspase-2 is an initiator caspase localised to the Golgi apparatus. Stress-sensing by the Golgi apparatus can lead to activation of Golgi localised caspase-2 that might drive the cells to apoptosis. Activated Caspase 2 initially cleaves GM160, followed by caspase-3 activity at the Golgi. Activated caspase-3 cleaves several Golgi-associated coiled-coiled proteins like GM130, GRASP65, GRASP55, p115, synatxin5, and giantin. GM130 is rapidly lost from the Golgi membrane, leading to fragmentation. Expressing caspase-resistant forms of these Golgi proteins inhibits Golgi fragmentation and delays apoptosis (Chiu et al., 2002; Lane et al., 2002).

The caspase-2 cleaved product of GM160, known to bind Golgi-associated protein GCP60, is translocated to the nucleus post-cleavage. Within the nucleus, this fragment might be able to sense and respond to signals by altering gene expression. Prevention of nuclear localisation of this fragment increases the sensitivity of cells to apoptosis. Hence, this might protect against the initial stages of stress response and help alleviate stress. The caspase-3 cleavage fragment of p115 translocates and accumulates in the nucleus. This fragment can induce apoptosis on its own. The cleavage of p115 could be a later event in apoptosis as it is cleaved by an effector caspase (Chiu et al., 2002). It would be generated when the cell has already committed towards apoptosis and would further help in the transduction of death signals. This might suggest that Golgi proteins may be actively involved in stress sensing and signalling (Hicks & Machamer, 2005; Rabouille et al., 2015).

Golgi fragmentation is one of the early events during apoptosis. Golgi fragmentation occurs before the disassembly of the cytoskeletal components, suggesting it to be independent of cytoskeletal damage (Mukherjee et al., 2007). Trafficking from the Golgi is completely blocked following fragmentation, and the Golgi is now no longer localised at the MTOC.

#### 1.1.5.2.Disease conditions

Golgi is known to be fragmented in several cancers and neurodegenerative diseases. DNA damage in certain cancers can induce Golgi fragmentation. This fragmentation is known to induce resistance to apoptosis and increase survival in these cells. In the case of

neurodegeneration, it is still debatable whether Golgi fragmentation is a cause or an effect. Like cancer, the Golgi fragmentation in neurodegeneration is irreversible. However, inhibition of Golgi fragmentation has been shown to delay apoptosis in the cells undergoing degeneration. Hence, inhibitors of Golgi fragmentation can make attractive drug targets for treating neurodegeneration. Deciphering the role of Golgi structure in the initiation/progression of neurodegeneration would help us understand the significance of Golgi in this condition and thus design appropriate drug candidates (Jiang et al., 2011; Zappa et al., 2018).

#### 1.1.5.2.1. Neurodegeneration

Golgi fragmentation has been reported in several neurodegenerative disorders like Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's (Haase & Rabouille, 2015; G. Joshi et al., 2015). Alzheimer's is characterized by the extracellular deposition of insoluble β-amyloid peptides (Aβ), which is a by-product of Amyloid Precursor Protein (APP) cleavage by β-secretase. Under normal conditions, the APP protein is targeted to the plasma membrane, cleaved by α-secretase to generate a soluble ectodomain with a neuroprotective role. The sAPPα can further be exocytosed and acted upon by γ-secretase, further fragmenting it or directly degraded via the endosomal pathway.

In Alzheimer's, APP is cleaved by β-secretase generating insoluble Aβ-peptides, which tend to form aggregates. BACE-1 is the primary β-secretase implicated in this process, known to be activated by the NF-κB pathway. BACE-1 cycles between the Golgi and plasma membrane. At the plasma membrane, BACE-1 is internalized and trafficked back to the TGN or endosomes. At steady state conditions, both APP and BACE-1 show different localisation. Hence, these two would necessarily interact at the TGN or the endosomes (G. Joshi et al., 2015).

The initial deposition of Aβ-peptides triggers the influx of calcium, which leads to the activation of the cyclin-dependent kinase 5 (CDK-5). CDK-5 further phosphorylates two Golgi proteins, GM130 and p115, leading to Golgi's fragmentation. CDK-5 is also known to phosphorylate GRASP65, resulting in Golgi fragmentation (Miao et al., 2018). Fragmentation of the Golgi apparatus results in altered processing and trafficking of several cargo molecules. Additionally, fragmentation leads to increased APP trafficking across the Golgi. This leads to a decrease in the amount of APP retained within the Golgi cisternae at any given point in time as compared to normal conditions (Fig. 1.7). Unstacking the cisternae increases the total surface area available for vesiculation which might lead to increased trafficking. This also

disrupts the sequential transport of cargo across the Golgi cisternae leading to an altered pattern of cargo processing (G. Joshi et al., 2014).

CDK-5 also phosphorylates APP, which might alter its trafficking, processing, and sorting at the Golgi. Hence, it is still unclear whether the formation of Aβ plaques is a result of altered Golgi structure and function or is a result of defective APP processing due to APP phosphorylation. However, in mouse models, Golgi fragmentation has been observed much before the appearance of Aβ plaques, and reversal of Golgi structure has been shown to reduce plaque deposition (G. Joshi et al., 2015; G. Joshi & Wang, 2015).

CDK-5 further phosphorylates Tau protein leading to Tau polymerization and deposition. Tau protein is known to be associated with the cis and trans side of the Golgi. Hence this might further contribute to alteration in Golgi structure and function. Studies have also shown that the deletion of Golgin-84 can activate CDK-5 and ERK. Therefore, the altered Golgi phenotype through concurrent mechanism might further feedback to increase the Aβ deposits and thus create a deleterious loop of Golgi disruption and plaque deposition. Furthermore, it has been shown that inhibition of Golgi fragmentation in neuronal mice cells delays apoptotic cell death in these cells. Hence, even though Golgi has not been implicated in initiating the disease, it might still play a significant role in the progression of Alzheimer's (Antón-Fernández et al., 2017; Miao et al., 2018).

Alternatively, Golgi fragmentation in the adult neurons could be a result of the adherent entry of matured neuronal cells into mitosis or disruption of the cytoskeleton in these cells. In either case, inhibition of Golgi fragmentation has been viewed as a possible therapy to treat or delay the progression of Alzheimer's (Zappa et al., 2018).

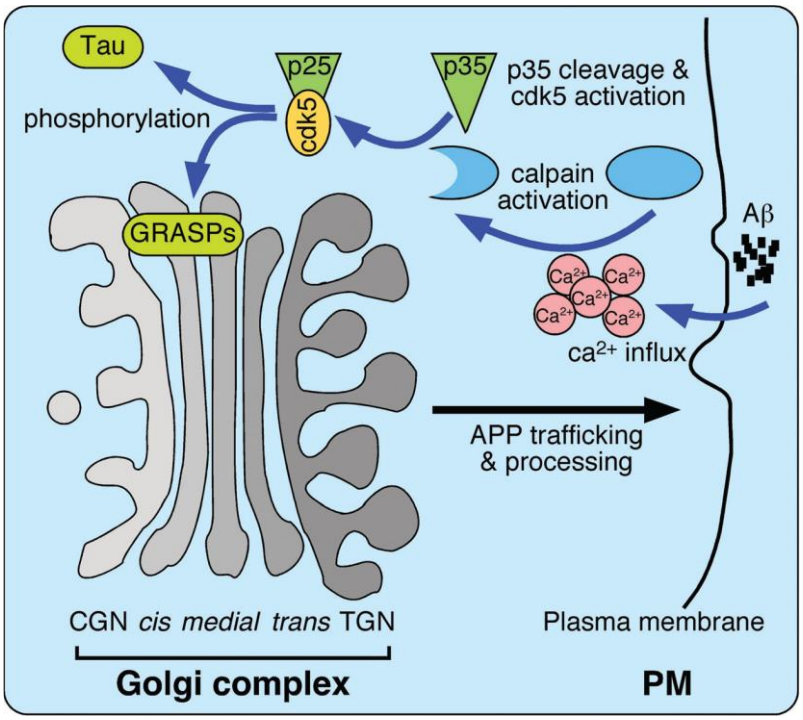



**Figure 1.7:** Golgi fragmentation in Alzheimer's disease (Adapted from Joshi and Wang, 2015). Accumulation of Aβ-peptides causes an influx of $Ca^{2+}$ ions, thus activating calpain. Calpain cleaves p35 to p25, which then activates Cdk5. Active Cdk5 phosphorylates GRASP65 leading to Golgi fragmentation. Golgi fragmentation results in accelerated trafficking of APP leading to increased production and accumulation of Aβ peptides. Calpain cleaves p35 to p25, which then activates Cdk5. Active Cdk5 phosphorylates GRASP65 leading to Golgi fragmentation. Golgi fragmentation results in accelerated trafficking of APP leading to increased production and accumulation of Aβ peptides.

#### 1.1.5.2.2. Cancer

Changes in Golgi morphology in several cancer types was reported about 50-60 years ago. However, it is only recently that the significance of this change is being understood. The Golgi apparatus is an essential organelle responsible for the post-translational modification, sorting, and packaging of proteins and lipids for transport to various cellular destinations. In cancer, the organisation and function of the Golgi are often altered, leading to changes in cell signalling, proliferation, and migration (Fig. 1.8). These changes can promote cancer progression, making the Golgi a potential target for cancer therapy(Makhoul et al., 2019; Zappa et al., 2018).

One of the critical changes observed in the Golgi of cancer cells is its fragmentation. The Golgi typically exists as a single, continuous ribbon-like structure with perinuclear localisation, but

in cancer cells, it breaks up into smaller, scattered fragments throughout the cytoplasm. This fragmentation is thought to be caused by dysregulation of the signalling pathways that control the integrity of the Golgi (Zhang, 2021). For example, the oncogene RAB1B, which is often upregulated in cancer, has been shown to cause Golgi fragmentation by activating the Golgi-disrupting protein GOLPH3 (Sechi et al., 2020).

Golgi fragmentation has been implicated in several aspects of cancer progression. One of the most well-studied effects is on protein glycosylation. The Golgi is responsible for adding sugar molecules to proteins, which can affect their function and stability. Over 250 glycosyltransferases are localised to the Golgi in an order dependent on the biosynthetic step of glycosylation they are involved. Changes in the localisation of these glycosyltransferases could lead to aberrant changes in the glycosylation of proteins and lipids (D'souza et al., 2021; Moremen et al., 2012; Zhang & Wang, 2016b). In addition, in cancer cells with fragmented Golgi, there is often an increase in the branching and sialylation of glycans, which can promote cancer cell migration and invasion (Dobie & Skropeta, 2021). This is thought to be because the altered glycosylation patterns on cell surface proteins can affect their interactions with the extracellular matrix and other cells, making cancer cells more mobile and able to invade surrounding tissues (Pinho and Reis, 2015; Zhang and Wang, 2016).

Golgi fragmentation has also been linked to the altered trafficking of proteins and lipids in cancer cells. For example, the Golgi normally sorts and packages proteins for secretion or transport to other organelles, but this process is disrupted in cancer cells with fragmented Golgi. This can result in aberrant localisation of proteins typically found in specific subcellular compartments, leading to dysregulation of signalling pathways and cell function (Zhang, 2021; Zhang & Wang, 2016a). Additionally, the Golgi is responsible for generating the lipid bilayer of the plasma membrane, and disruption of this process can affect cell membrane integrity and cell signalling (Kakugawa et al., 2002).

Another effect of Golgi fragmentation in cancer cells is the formation of secretory lysosomes. These are organelles that contain both digestive enzymes and membrane proteins that are typically found in the Golgi. In cancer cells, Golgi fragmentation can lead to the mis-localisation of these proteins, resulting in the formation of secretory lysosomes that have altered protease activity. This can promote cancer cell invasion by degrading the extracellular matrix and creating channels for cell migration (Bajaj et al., 2022; Tan et al., 2020).

Overall, the changes in Golgi organisation and function observed in cancer cells significantly impact cancer progression. The altered glycosylation patterns, disrupted protein and lipid trafficking, and formation of secretory lysosomes can all promote cancer cell migration, invasion, and survival. Targeting the Golgi and its associated pathways may be a promising cancer therapy approach (Zappa et al., 2018). For example, inhibitors of the Golgi-disrupting protein GOLPH3 have been shown to reduce cancer cell invasion and metastasis in preclinical models, and targeting specific glycosylation pathways has been proposed as a strategy for reducing cancer cell migration and invasion (Sechi et al., 2020).

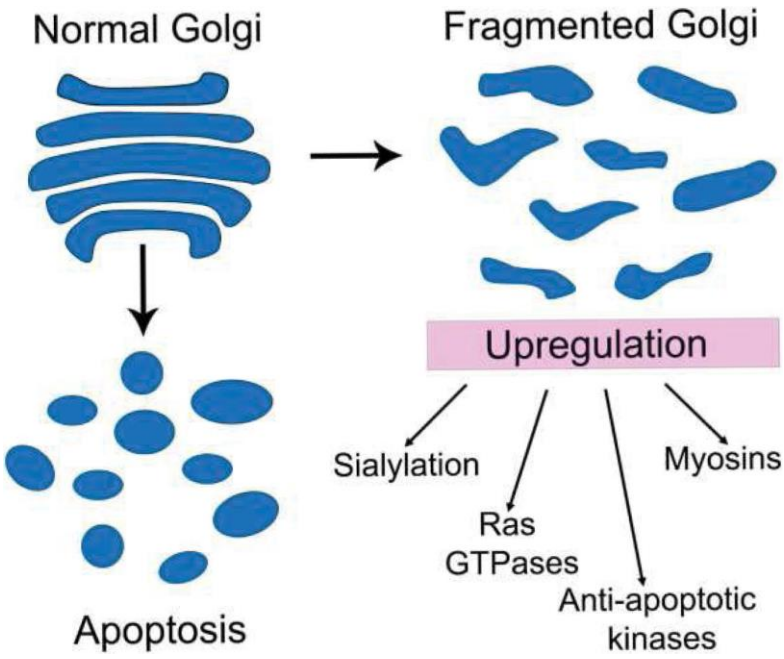



**Figure 1.8:** Fragmentation of the Golgi is the hallmark of both apoptosis and cancer (Adapted from Armen Petrosyan, Biochem Mol Biol J. 2015). The Golgi localised caspase-2 and caspase-3 mediate cleavage of several golgins and GRASPs, thus resulting in irreversible disorganisation of the Golgi (left panel). Cancer-specific Golgi fragmentation (right panel) is accompanied by activation of different pathways, including (a) O-and N-glycans sialylation, (b) overriding of Ras GTPases, (c) upregulation of anti-apoptotic protein kinases, and (d) myosin motor proteins association with Golgi.

## 1.2. Small GTPase Arf1 and its role in regulating Golgi organisation and function

### 1.2.1. ADP-ribosylation factor 1 (Arf1)

Arf1 is a protein of 181 amino acids of 20,697kda molecular weight. It belongs to the ARF multigene family of small GTPases, which were initially discovered as cofactors for the ADP-ribosylation G protein carried out by the cholera toxin and hence the name. Arf proteins can be subdivided into three groups based on the sequence and structural analysis. Arf1, Arf2 and Arf3

belong to class I of Arf proteins, Arf4 and Arf5 belong to the second class, and Arf6 is the sole protein in the third class. Class I and ClassII Arf proteins have been known to associate with the Golgi membrane. Arf1 and Arf3 localize to Golgi and differ by only seven amino acids. Arf1 localises to all the compartments of Golgi and Arf3 localises to the TGN and supports vesicular trafficking at the TGN. Arf4 and Arf5 are known to associate with the ERGIC and along with Arf1 support COPI dependent vesicular transport. Arf6 is not involved with the Golgi; it localizes and functions at the plasma membrane. Arf proteins share structural similarities with Arf-like proteins (Arls), which, unlike Arfs, do not act as cholera toxin activators (Alvarez et al., 2003; J.G. Donaldson & Honda, 2005; Pennauer et al., 2021; Wong-Dilworth et al., 2023).

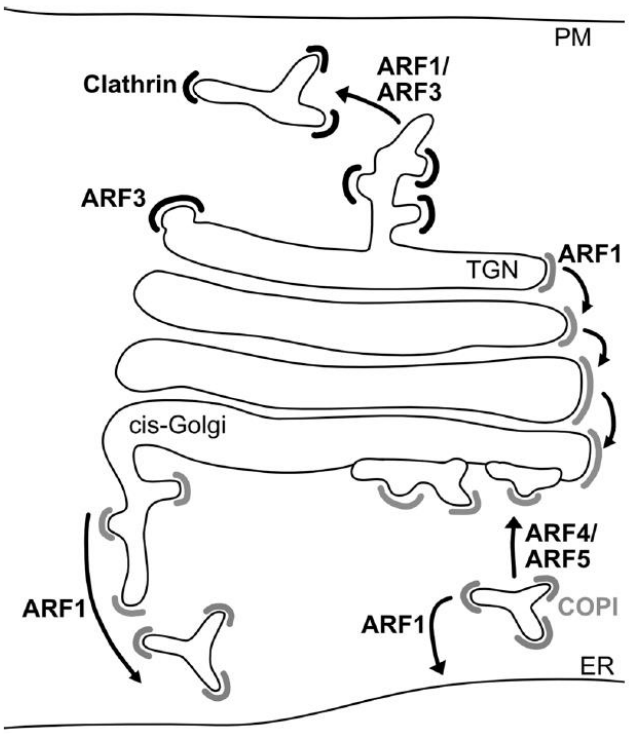



**Figure 1.9:** Different Class I and ClassII Arfs associated with the Golgi. (Adapted from Wong-Dilworth et al., 2023)

Class I and class II Arfs share redundant functions at the Golgi. Studies in cultured Hela cells show that a single knockdown of any of these Arfs doesn't affect the viability of cells. However, cells with a double knockdown of Arf1 and Arf4 or class II Arfs (4+5) are not viable. Arf4 was shown to be sufficient to sustain all the functions of class I and II Arfs when other three were absent. In these studies, Arf1 knockdown alone leads to an increase in the Golgi volume and decrease in the coat recruitment and retrograde and anterograde trafficking from the Golgi.

This indicates that Arf1 could be a major player in regulating the vesicular traffic across the Golgi apparatus (Pennauer et al., 2021).

The amino acid sequence required for GTP binding and its hydrolysis to GDP is conserved in all Arf proteins. The glycine in the N-terminal α-helix of all Arf proteins is myristoylated and is vital for the biological functions of these Arf proteins. GTP binding of Arf proteins occludes the hydrophobic region of the amphipathic helix and thus facilitates the insertion of the myristoylated N-terminal into the membranes. In the GDP-bound form, α-helix is inserted into the hydrophobic pocket, thus inhibiting the binding of effectors that interact only with the active form of Arf (GTP-bound) (Liu et al., 2009).

Arf1 binds to the entire Golgi membrane, from the cis to trans phase and also is associated with the TGN (Bottanelli et al., 2017). The biological function of Arf1 is regulated by its interaction with the effector proteins, Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). Arf GEFs can be classified into four families, SEC7/BIG1/BIG2, Gea/GBF1, EFA6 and Cytohesin/ARNO. In mammals, GBF1 and BIG1/2 associate with the Golgi and regulate Arf1 function at the Golgi. GEFs release the GDP bound to Arf1, allowing the GTP to bind to Arf1. The Sec7 domain, conserved in all Arf GEFs, is responsible for this nucleotide exchange activity. Arf1 is active in GTP-bound form and binds to membranes through its myristoylated N-terminal (Bui et al., 2009).

GAPs, on the other hand, have a conserved zinc finger domain through which they catalyze the hydrolysis of GTP-bound Arf. Arf GAP1 was the first to be identified of the 24 genes identified for coding for Arf GAPs. These GAPs are subdivided into six families. Arf1 interaction with the Arf GAP converts the Arf bound GTP to GDP, thus inactivating the Arf1 protein. The inactive form of Arf1 localizes to the cytosol. It has been shown that GTP hydrolysis of Arf1 by Arf GAPs is necessary to bring out its biological function (East & Kahn, 2011).

### 1.2.2. Arf1 in Golgi organisation and maintenance

#### 1.2.2.1.Arf1 in Golgi biogenesis

Unlike organelles composed of stable entities, Golgi is a dynamic organelle. It is not a conventional autonomous entity; instead, it's viewed as a steady-state entity emerging from the ER. Block in ER transport results in the disappearance of Golgi. Golgi can form *de nova* by fusion of the transport vesicles emerging from the ER. The activity of Sar1 GTPase is required

to differentiate ER membrane into transport vesicles and load them with cargo protein. Sar1 GTP binding and hydrolysis initiate the recruitment of COPII coatomers to the ER membrane and differential localisation of several Golgi membrane and matrix proteins into the emerging COPII-coated vesicles. p115 is recruited to the site of emerging vesicles by Sar1 GTPase activity. p115 can interact with several other proteins, ranging from Golgi membrane proteins, SNAREs, and Rab1 GTPases to Arf1 GEFs. Through its interaction with a wide range of proteins, it orchestras the biogenesis of the Golgi membrane (Nihal Altan-Bonnet et al., 2004).

By binding Arf1 GEF, GBF1 p115 recruits Arf1 to the site of Golgi biogenesis (Kawamoto et al., 2002). Arf1 binds to the ERGIC in its active form and recruits several effector proteins to the site. It recruits proteins like COPI coats, spectrin, ankyrin, lipid-modifying enzymes, and cytoskeletal components like tubulin, myosin, dynein and dynactin. Block in Arf activity via BFA treatment results in the segregation of Golgi components on the ER membrane by the activity of Sar1GTPases and p115, but these membrane sections fail to bud out from the ER, thus indicating the requirement of Arf1-GTP binding to initiate vesiculation at the ER exit site (Dascher & Balch, 1994; Kaczmarek et al., 2017a).

Arf1, through its interaction with lipid-modifying enzymes, helps in altering the lipid composition of the Golgi membrane, thus differentiating it from the ER membrane. It interacts and activates phospholipase D (PLD) and phosphoinositol 5-phosphate kinase (PIP5 kinase) at the Golgi membrane. The activity of PLD results in the production of phosphatidic acid (PA) from phosphatidylcholine, which initiates membrane fusion. PLD is involved in the signalling pathway through its activity of producing PA, as PA is eventually converted to Diacylglycerol (DAG), which activates protein kinase C (PKC). Also, DAGs are known to stimulate the activity of ArfGAP1, which leads to GTP hydrolysis on active Arf1 leading to its inactivation. PIP5 kinase can be activated directly by active Arf1 protein or PA. PIP5 Kinase phosphorylated phosphatidylinositol 4-phosphate (PI4P) to form phosphatidylinositol 4,5-phosphate ($PIP_2$). While PI4P is Golgi localised, $PIP_2$ is primarily localised to the plasma membrane. Arf1, through its stimulation of PIP5 Kinase, can thus regulate the interaction of PI4P with its downstream effectors on the Golgi (Julie G. Donaldson et al., 2005; Kaczmarek et al., 2017a).

Also, Arf1 has been shown to directly bind to and recruit the enzyme PI4-Kinase IIIβ to Golgi. The enzyme PI4- Kinase IIIβ is responsible for the synthesis of PI4P. Arf1-GTP and PI4P recruit FAPPI to the trans end of the Golgi. FAPPI functions in the formation of tubular and

vesicular structures important in the secretory pathway at the TGN. These interactions provide evidence for the ability of Arf1 to alter the lipid composition of the Golgi membrane and make it more distinct than the ER or PM lipid composition (Kaczmarek et al., 2017a; H.-W. Shin & Nakayama, 2004).

#### 1.2.2.2.Arf1 in the inheritance of Golgi

In vertebrates, the Golgi complex is fragmented during the start of mitosis, and it reorganises at the MTOC during the telophase in the daughter cells. The fragmented Golgi is uniformly distributed throughout the cell, which ensures equal distribution of Golgi fragments to the daughter cells upon division. In daughter cells, the Golgi complex is not formed *de nova; instead*, it is generated by the fusion of these fragmented Golgi elements (Persico et al., 2009).

Previously it was seen that treatment of interphase cells with BFA, an Arf-GEF inhibitor resulted in the inactivation of Arf1, which led to Golgi fragmentation, and the fragmented Golgi elements fell back into the ER (Lippincott-Schwartz et al., 1989a). This observation led researchers to hypothesize that Arf1 might be involved in mitotic Golgi disassembly. However, when cells undergoing mitosis were treated with H98, a protein kinase inhibitor that blocks the GTP hydrolysis on Arf1, it was seen that the mitotic Golgi failed to fragment and remained intact and localised perinuclearly. Also, the expression of a constitutively active form of Arf1, Q71L-Arf1, resulted in the same effect where the Golgi failed to fragment. These experiments also resulted in improper chromosome segregation and failure in furrow ingression during division, showing that Golgi fragmentation is necessary for the cell cycle to progress smoothly. (N Altan-Bonnet et al., 2003)

Early, during mitosis, GBF1 is phosphorylated by AMPK at Threonine 1773 (Mao et al., 2013). This inhibits the nucleotide exchange on Arf1, thus inactivating Arf1. Inactive Arf1 dissociates from the Golgi membrane, and is found in the cytoplasm. This release of Arf1 also leads to the release of effector proteins recruited by Arf1 onto the Golgi membrane. Dynein is one of the major proteins essential for Golgi organisation recruited by Arf1. Arf1 recruits GM160 to the Golgi membranes, which directly binds to dynein. Dynein then clips the Golgi membrane to the minus end of the microtubule and brings the Golgi vesicles together to fuse and form Golgi stacks. Also, dynein brings these stacks to the MTOC along the microtubules emerging from the MTOC. Loss of Arf1 leads to dissociation of GM160 and thus dynein from the Golgi

membrane, which leads to Golgi fragmentation and re-localisation throughout the cytoplasm (Yadav et al., 2012).

There has been conflicting evidence on the inactivation of Arf1 leading to mitotic Golgi disassembly. There have been studies that show the requirement of active Arf1 for the mitotic disassembly of Golgi. Golgi failed to disperse in the mitotic cytosol depleted of active Arf1. Supplementation with active Arf1 induced Golgi dispersal. However, these studies were carried out in cell-free systems. No complementary studies *in vivo* show the requirement of Arf1-GTP for mitotic Golgi dispersal. All *in vivo* studies have shown that the phosphorylation of GBF1 inhibits nucleotide exchange on Arf1, resulting in Golgi fragmentation during mitosis (Xiang et al., 2007).

Inheritance of Golgi during meiosis is different than that during mitosis. During meiosis, Golgi fragments fuse with the ER and are reformed post-fertilization (Payne & Schatten, 2003). Golgi is also known to fragment during apoptosis. This fragmentation is Arf1-independent and mediated by cleaved caspases (Mukherjee et al., 2007). Fragmented Golgi has been observed in several pathological conditions like cancer and neurodegenerative disorders (Zappa et al., 2018). Arf1 could be involved in this fragmentation. A study has shown that the crosstalk between Arf1 and Golgi localised tubulin binding Cofactor E (TBCE) leads to Golgi dispersal in progressive neuropathy (Bellouze et al., 2014). However, more studies are required to establish a definitive role of Arf1 in Golgi dispersal during pathological conditions. Also, the extent of Arf1 involvement in this phenomenon could vary with different cells leading to the development of the disease (Mukherjee et al., 2007).

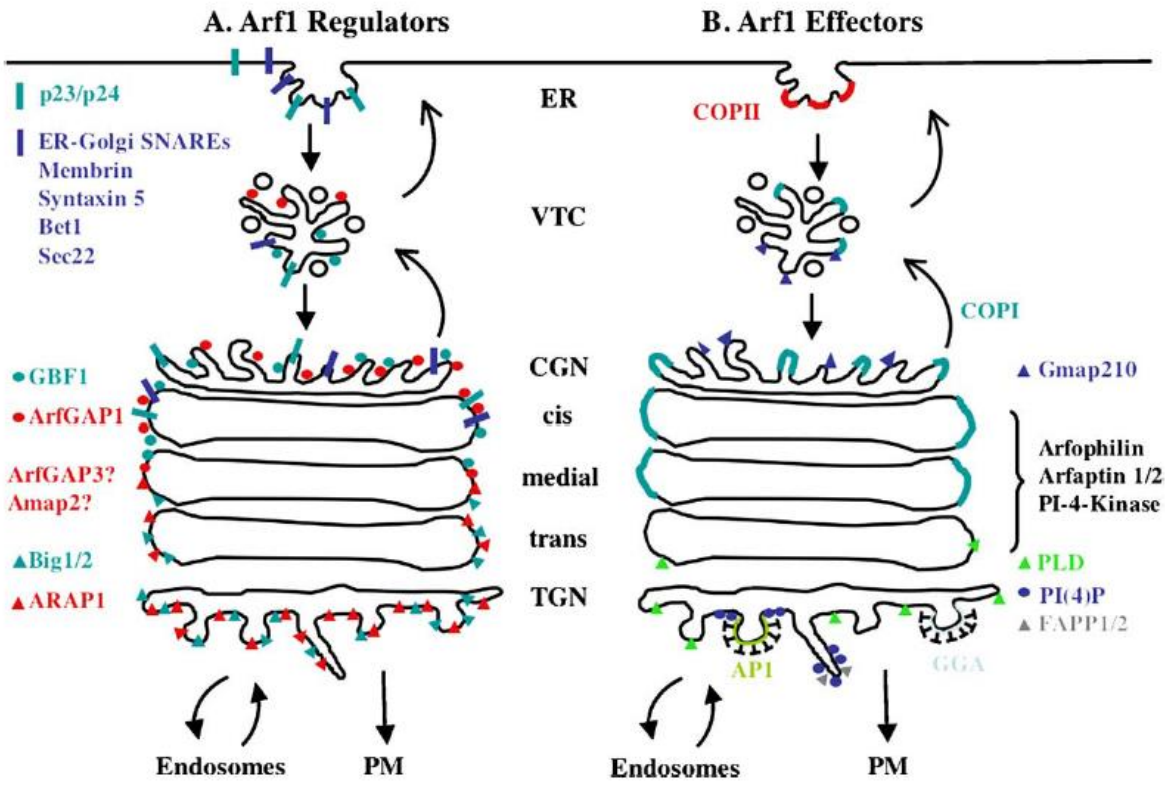



**Figure 1.10:** Differential localisation of Arf1 regulators and effectors on the Golgi membrane (Adapted from Donaldson and Weigert, 2005).

### 1.2.2.3.Arf1 mediated Actin assembly at the Golgi

Active Arf1 induces the assembly of actin and spectrin molecules around the Golgi. In addition to this, it also recruits Cdc42 to the Golgi membrane. Cdc42 interacts with the γ-COP coat protein at the Golgi membrane, and active Arf1 mediates this interaction. Cdc42 then activates Arp2/3 at the Golgi surface, initiates actin assembly, and recruits mAbp1. Arf1 also mediates the polymerization of drebrin-enriched actin scaffold across the Golgi. These actin filaments are formed by interaction with a coat protein other than γ-COP mediated by recruitment of Cdc42/Rac proteins or Rho family GTPases (Cao et al., 2005).

This actin assembly is postulated to play a role in transporting secretory vesicles. The actin comet tail formed during actin polymerization might generate enough force to carry the vesicles to the membrane. Or the actin filaments can provide a scaffold to transport vesicles across them by linking them to myosin motors. The two distinct pools of actin polymers formed may be involved in transporting vesicles of different coat compositions (Cherfils, 2014; D'Souza-Schorey & Chavrier, 2006).

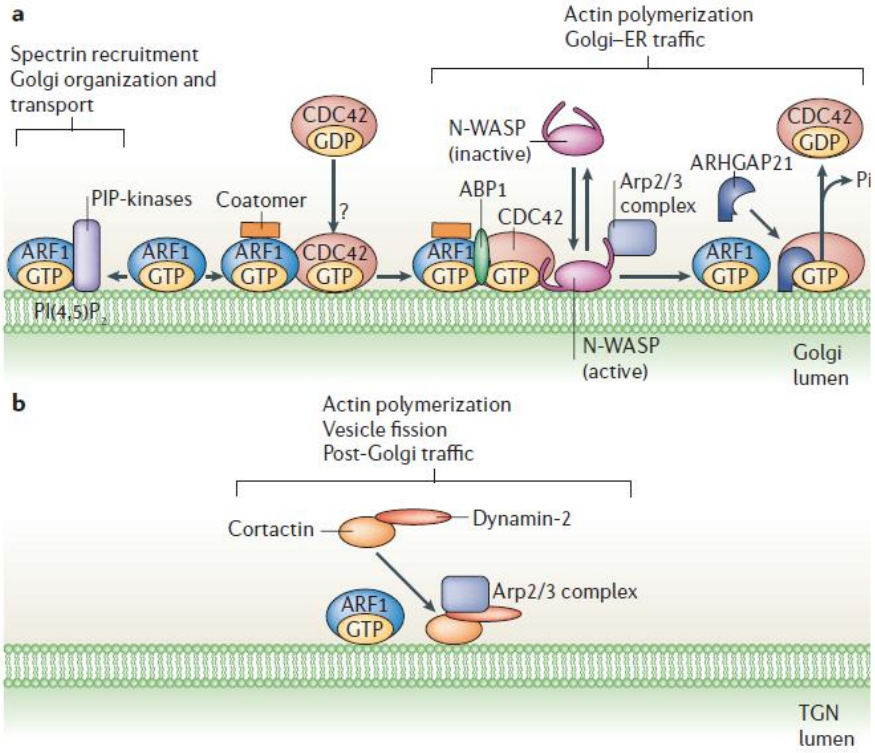



**Figure 1.11:** Arf1-induced actin assembly at the (a) Golgi membrane and (b) TGN (Adapted from D'Souza-Schorey and Chavrier, 2006).

### 1.2.2.4.Arf1-based identity of Golgi compartment.

Arf1, along with its effector molecules, provides a mechanism of Golgi compartment identification. Differential interaction of Arf1 with different Golgi cisternae is brought about by the Arf1 regulators that differentially localize to the Golgi membrane. Interaction of Arf1 with different Arf-GEFs and ArfGAPs along with proteins like p115 and ER-Golgi SNAREs and its association with membrane lipids and lipid-modifying enzymes is compartment-specific and provides a way of identifying Golgi compartments based on the localisation of Arf1 regulators and effectors to these compartments. Along with Arf1, other Arf proteins could localize to Golgi and work synergistically to mediate trafficking. Arf3 is one such molecule that localizes to the medial Golgi and is thought to function in ways similar to Arf1 (Fig 1.9) (D'Souza-Schorey & Chavrier, 2006).

### 1.2.2.5. Role of Arf1 in regulating Golgi function

#### COPI vesicle formation

The role of Arf1 and Arf1 GEF, GBF1 in the formation of COPI vesicles is well studied. GBF1 is recruited to the ERGIC vesicles by its interaction with Rab1 and p115(Alvarez et al., 2003;

García-Mata & Sztul, 2003). GBF1 then activates Arf1 at the cis-Golgi membrane(Helms & Rothman, 1992; Klausner et al., 1992). Active Arf1 binds to the Golgi membrane through its myristoylated α-helical end. Active Arf1, in turn, recruits effector proteins like coat protein complex I (COPI)(James E. Rothman, 1994). COPI induces differential sorting of cargo into vesicles. Coat proteins then polymerize on the membrane and induce membrane curvature. Eventually, COPI-coated vesicles are released from the membrane, fusing with the adjacent cisternae upon coat protein release. COPI release and binding are mediated by the Arf1 GTP-GDP cycle. Active Arf1 recruits COPI and initiates coat assembly. It is also known to interact with the p23 membrane protein. On activation, Arf1 dissociates from p23; this allows the coat proteins to access p23 and assemble into vesicles(Sohn et al., 1996). GTP hydrolysis of Arf1 by the action of ArfGAPs would later lead to the release of both Arf1 and COPI into the cytoplasm, thus uncoating the vesicles emerging from the Golgi, which would enable their fusion and release of contents to the adjacent Golgi/ER membrane(Nickel et al., 2002). There exist two molecules of Arf1 per coat protein on the vesicles(Yu et al., 2012). Dimerization of Arf1 proteins induces the membrane curvature required for budding out vesicles from the Golgi surface. Hydrolysis of Arf1-GTP is necessary for efficient sorting of cargo into transport vesicles as inhibition of GTP hydrolysis by expressing a non-hydrolysable form of Arf1 led to the formation of COPI-coated vesicles deficient of cargos (Faini et al., 2013; Nickel et al., 2002; Presley et al., 2002).

COPI-coated vesicles mediate retrograde transport across the Golgi, carrying back ER-resident proteins that have escaped into vesicles emerging at the ER exit site. Adaptor proteins recognize the KDEL, ER localisation signal on the cargo proteins. They bind to cytosolic regions of COPI and thus enable the partitioning of ER-resident proteins into COPI-coated vesicles(Pellett et al., 2013; James E. Rothman, 1994). COPI vesicles may also play a role in maintaining the gradient of Golgi resident proteins in the cisternal maturation model. COPI vesicles also play an essential role in transporting biosynthetic cargo to the Golgi complex. COPII vesicles mediate the initial formation of transport vesicles at the ER exit site. The switch from COPII to COPI-coated vesicle is brought about by the binding of GBF1 to these vesicles. GBF1 recruitment of Arf1 and its activation results in COPI assembly on these vesicles (Pevzner et al., 2012).

Arf1 also interacts with SNARE protein membrin, GS15 and YKT6. All these SNAREs have been shown to have a role in retrograde transport across Golgi cisternae. However, in the case

of the stationary cisternae model, COPI vesicles have been implicated in the anterograde transportation of cargo (Spang, 2002).

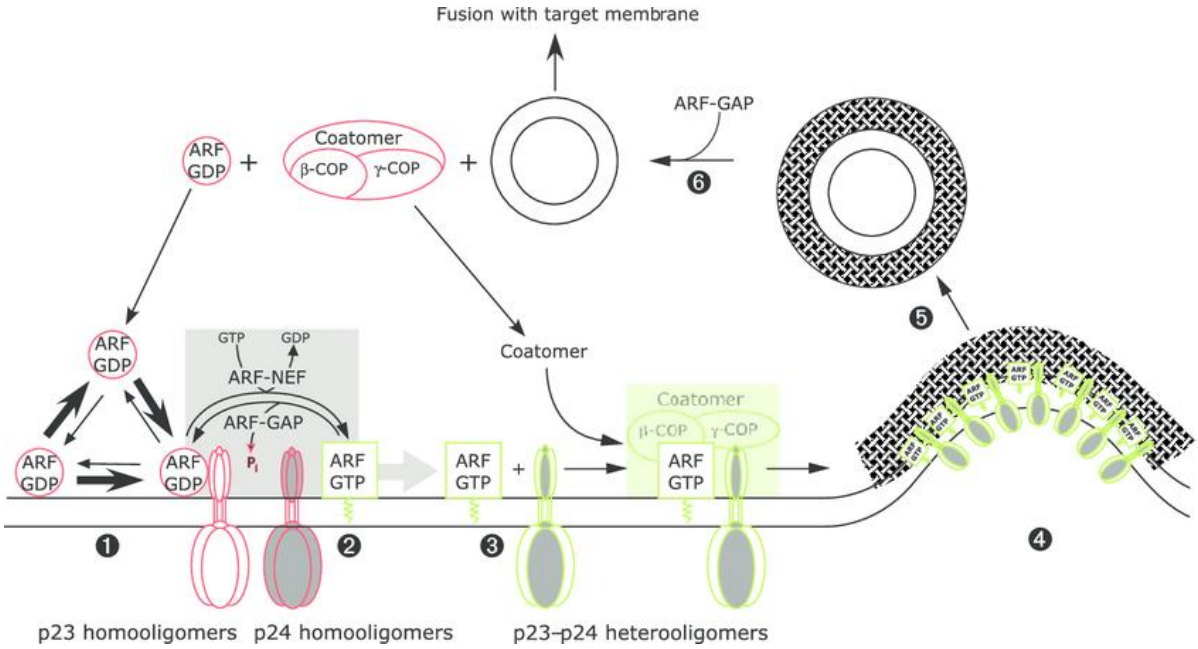



**Figure 1.12:** The schematic shows the core machinery involved in COP1 recruitment to Golgi membranes, COPI coat polymerization, vesicle budding and uncoating of COP1 coat, adopted from Nickel, Brügger and Wieland, JCS, 2002. (1) Binding of Arf-GDP to p23 initiates the recruitment of COP1 coat to membranes. (2) Upon activation, Arf1-GTP dissociates from p23 and stably associates with membranes. (3) p23/p24 oligomers are rearranged following multiple cycles of GTP hydrolysis and GDP to GTP exchange. (4) Arf1-GTP and p23/p24 heterooligomers might facilitate coatomer binding and polymerization. (5) Coat polymerization initiates vesicle budding. (6) Arf-GAP triggers uncoating of the vesicle. Components in green are in active state and those in red are in inactive state.

**Arf1 in the formation of secretory vesicles**

Arf1 GEF BIG 1/2 localizes to the trans-Golgi and regulates Arf1 function. BIG1 and BIG2 GEFs are known to dimerize, as a result of which their association with the Golgi membrane could be more robust than that of GBF1. Furthermore, serum starvation dissociates BIG1/2 from the Golgi membrane and localises to the nucleus. This might be a way to reduce the activity of Arf1 at the Golgi membrane.

Arf1 plays a role in vesicle formation at the trans-Golgi and TGN by recruiting adaptor proteins like AP1, AP3, AP4 and Golgi Associated, Gamm Adaptin Ear Containing, Arf binding Protein 3 (GGA3). These different coat proteins interact with different types of cargo molecules and thus enable the packaging of different cargos into other vesicles, which helps regulate a wide

range of cargo transport within and across the cell. In addition, AP1 and GGA3 interact with the mannose-6-phosphate receptor through its cytoplasmic tail and enable its movement from trans-Golgi to lysosomes (Memon, 2004; X. Ren et al., 2013).

The synergetic action of PI4P recruits AP1 localised to the Golgi membrane by interacting with Arf1 and Arf1 itself. AP1 assembly also require certain cytosolic factors that facilitate AP1 binding to cargo tails. GGA3 has a GAT domain (named based on its homology with GGA and TOM1) through which it binds to Arf1-GTP. The N-terminal helix-loop-helix subdomain of GGA3 interacts with the Arf1-GTP (Shinotsuka et al., 2002).

AP1 and GGA3 recruitment lead to Clathrin-Coated Vesicles (CCV) formation. These CCVs carry very low levels of Arf1, indicating that hydrolysis of Arf1-GTP on coated vesicles is of little significance for coat disassembly. This is unlike the requirement of Arf1-GTP hydrolysis for COPI coat disassembly. Arf1 at the TGN interacts with AGAP1 and AGAP2, Arf-GAPs that are different from the ArfGAP1 it interacts with during the formation and dissociation of COPI vesicles. The interaction of Arf1 with different GEFs and GAPs might facilitate the differential sorting of cargo into the respective transport vesicles at different sites of the Golgi membrane(Bottanelli et al., 2017; Julie G. Donaldson et al., 2005).

**Additional Arf1 interactions at the Golgi membrane**

**Arf-like proteins (Arls)**

Arls are structural homologues to Arf proteins. Initially, they were thought to be involved in functions different from Arf proteins. Later, it was discovered that these proteins localize to the mammalian Golgi membrane and are myristoylated at the N-terminal, facilitating their Golgi association. Arls are not known to be activated or inactivated by any known Arf-GEFs. However, they must associate GM245 (Van Valkenburgh et al., 2001) and GM97 (Lu & Hong, 2003) at the Golgi. Expression of the dominant negative form of Arl disperses the Golgi in a fashion similar to that caused due to expression of dominant negative Arf1. Expressing a constitutively active state of Arl protects the vesicular coats and can inhibit the BFA-induced disassembly of coated vesicles.(Julie G. Donaldson et al., 2005; Lu et al., 2001; Lu & Hong, 2003).

**Table 1:** Downstream effectors of Arf1 at the Golgi.

| Class | Name | Localisation | Function |
|---|---|---|---|
| Coat/Adaptor proteins | COPI | ERGIC and cis-Golgi membranes | Involved in the retrograde transport of cargo from Golgi to ER (Julie G. Donaldson & Klausner, 1994; Majoul et al., 2001; L. Zhao et al., 1999) |
| | AP/Clathrin | Trans-Golgi and TGN | Sorting of cargo proteins exiting the Golgi (Robinson, 2004) |
| | GGA/Clathrin | Trans-Golgi and TGN | Sorting of cargo proteins exiting the Golgi (Shiba et al., 2003; Suer et al., 2003) |
| | FAPP1 | Trans-Golgi and TGN | Formation of tubular, secretory membrane transport from the TGN (Godi et al., 2004) |
| | FAPP2 | Golgi | Transport of glucosylceramide lipids from the *cis*-Golgi to the trans-Golgi (M. A. De Matteis & Godi, 2004; Godi et al., 2004) |
| | MINT3 | TGN | Export of Amyloid Precursor Protein (APP) from Golgi (Shrivastava-Ranjan et al., 2008) |
| | | | |
| Lipid modifying enzymes | PIP5 Kinase | Trans-Golgi and TGN | Phosphorylates phosphatidylinositol 4-phosphate (PI4P) to form phosphatidylinositol 4,5-phosphate ($PIP_2$) (D'Souza-Schorey & Chavrier, 2006) |
| | PI4-kinase Type IIIβ | Cis, medial. trans-Golgi and TGN | Responsible for the synthesis of PI4P (Haynes et al., 2005) |
| | Phospholipase D (PLD) | Trans-Golgi and TGN | Production of phosphatidic acid (PA) from phosphatidylcholine, PA influences membrane fission (Brown et al., 1993; Cockcroft et al., 1994) |
| | Oxysterol binding protein (OSBP) | Endoplasmic reticulum (ER)-Golgi contact sites | Transports cholesterol and phosphatidylinositol 4-phosphate (PI-4P), and activates lipid transport and biosynthetic activities |
| | Ceramide Transfer (CERT) | Golgi | Transport of ceramide from ER to the cis-Golgi (M. A. De Matteis & Godi, 2004) |
| | | | |
| Coiled-coiled tethers | Golgin 160 | Cis-Golgi | Serves as a receptor to cytoplasmic Dynein-1 (Yadav et al., 2012) |
| | GMAP-210 | Cis-Golgi | Tethers small vesicles from ER to the Golgi cisternae and is involved in Golgi centrosome linking (Gillingham et al., 2004) |
| | | | |
| Others | Arfaptin 1 and 2 | TGN | Arfaptins have been shown to inhibit Arf1-GTP functions, such as activation of PLD (Kanoh et al., 1997; Williger et al., 1999) |

**Arfaptins and Arfophillins**

Yeast two-hybrid studies led to the identification of Arfaptins and Arfophillins as interactors of active Arf1. Arfaptins do not interact with the inactive GDP-bound form of Arf1. Upon BFA treatment, Arfaptin1 is released from the Golgi complex. This indicates that Arf1-GTP mediates Arfaptin1 association with the Golgi membrane. Arfaptin1 also interacts with Rho family GTPases in their GDP-bound form. Through this interaction, Arfaptin1 might be capable of negotiating a cross-talk between Arf and Rho family GTPases. Arfaptins have an inhibitory effect on Arf1-GTP activity. They inhibit PLD activation by competing with the PLD binding site on Arf1-GTP (Williger et al., 1999).

Arfophillins were initially identified based on their interaction with Arf5, a class II Arf protein. Arfophillins have a juxtanuclear positioning in the Golgi, which leads to the possibility that they might be associated with the Golgi membrane. However, additional studies are required to prove their Golgi association and identify their function at the Golgi membrane (O. H. Shin et al., 2001). GMAP210 has also been identified to be associated with the Golgi membrane based on its interaction with Arf1-GTP. GMAP210 is a Golgi scaffolding protein that localizes to the cis-face of the Golgi. In mammalian cells, GMAP210 overexpression induces Golgi fragmentation and disrupts anterograde and retrograde transport across the Golgi (Gillingham et al., 2004; Pernet-Gallay et al., 2002).

## 1.3. Role of cytoskeleton in the regulation of Golgi organisation and function.

Cytoskeleton plays a significant role in regulating the size and shape of the cell. Additionally, it plays a vital role in regulating the positioning of subcellular organelles, trafficking between the organelles, cellular endocytosis and exocytosis (Alberts et al., 2017). Both microtubules and actin are known to play an essential role in regulating the morphology and the function of the Golgi apparatus (Egea et al., 2013; Kreis, 1990).

### 1.3.1. Role of microtubules in regulating the Golgi organisation and function.

Microtubules are hollow tube-like structures made up of the protofilaments formed by polymerizing the microtubule subunits, α-tubulin and β-tubulin. Several proteins, referred to as Microtubule-associated Proteins (MAPs) bind to the microtubules and are essential in regulating the dynamics and functions of microtubules (Goodson & Jonasson, 2018). In

mammals, centrioles are the major sites of microtubule nucleation and are referred to as microtubule organizing center (MTOC). γ-tubulin associates with other initiator proteins to form the γ-tubulin ring complex at the centrioles that acts as the nucleation site for the polymerization of α-tubulin and β-tubulin (Muroyama & Lechler, 2017). Microtubules are polarized, with β-tubulin being exposed at the plus end, generally pointing towards the MTOC. α-tubulin is exposed at the minus-end (growing end) that points away from the MTOC. Microtubules are dynamic; in the presence of GTP, they are known to polymerize and depolymerize rapidly (Wade & Hyman, 1997). Cells utilise the dynamic nature of microtubules in regulating the trafficking of proteins and the morphology of the cell in response to stimuli. The motor proteins associated with the microtubules are vital in guiding vesicular movement. Dynein acts as a minus-end motor protein, and several kinesins act as plus-end motor proteins (Goodson & Jonasson, 2018; Muroyama & Lechler, 2017).

The earliest studies that revealed the role of microtubules in regulating Golgi organisations were based on the effects seen upon treatment with anti-microtubular drugs (A. M. Tartakoff, 1989; Cole et al., 1996b; Yang & Storrie, 1998). Recent advances have now led to a better understanding of the role of microtubules and the associated motor proteins in regulating the organisation of the Golgi. In interface cells, the Golgi is organised into a ribbon-like structure consisting of disc-like stacks at the MTOC. The depolymerization of microtubules with anti-microtubular drugs led to the separation of the Golgi stacks and their dispersal through the cytoplasm (Cole et al., 1996b). The disintegration of Golgi stacks upon anti-microtubular drug treatments is less dramatic than the treatment with other drugs like Brefeldin A (BFA). While treatments with drugs like BFA results in the complete break up of Golgi and its fusion back to ER, treatment with anti-microtubular drugs only results in the dispersal of Golgi stacks throughout the cytoplasm. These dispersed Golgi stacks remain functional, and the trafficking of proteins is only seen to be marginally affected (Lippincott-Schwartz et al., 1989a; Yang & Storrie, 1998). The Golgi elements dispersed upon the anti-microtubular drug treatment were seen to localize to the ER exit sites. These studies reveal that though microtubules are essential in regulating the Golgi organisation, they do not have a vital role in the genesis of Golgi elements from the ER (Cole et al., 1996b). Nocodazole is an anti-microtubular drug that binds β-tubulin and disrupts the microtubule polymerization/depolymerization dynamics. Golgi unstacking and dispersal are seen in cells treated with nocodazole. On nocodazole washout,

microtubule network reforms and dispersed Golgi elements move along microtubules to reorganise into a stacked structure near the Golgi (Storrie & Yang, 1998).

Given that the Golgi is organised near the MTOC, it was speculated that dynein, the minus-end motor protein, might play an essential role in the movement of the Golgi towards the MTOC. Studies revealed that dynein interacts with the Golgi matrix protein Golgin160 and is responsible for the organisation of the Golgi at MTOC. Golgin160 is recruited to the Golgi membranes by the small GTPase Arf1 (Yadav et al., 2012). Dynein association with the Golgi membranes facilitates the movement of Golgi vesicles from the ER exit sites towards the MTOC. Dynein could also play a role in the movement and recycling of membranes from the plasma membrane to the trans and from the trans to the cis face of the Golgi (Yadav et al., 2012). Several kinesins are also known to associate with the Golgi membrane and facilitate the trafficking of vesicles from the Golgi to the plasma membrane and other cell locations (Allan et al., 2002).

The microtubules that arise from the MTOC have distinct radial symmetry. The microtubule network in interphase is asymmetric, and their radial symmetry could be disadvantageous in these cells for the targeted delivery of vesicles. Non-centrosomal scaffolds of microtubules give rise to asymmetric microtubule networks. In many cells, Golgi is seen to act as a scaffold for the nucleation of microtubules. This has so far been reported in neurons, hepatocytes, epithelial cells, pancreatic beta cells and skeletal muscle (Chabin-Brion, Marceiller, et al., 2001; Efimov et al., n.d.; Miller et al., 2009; Rivero et al., 2009). Factors responsible for the nucleation of microtubules at the centrosome are also found to be localised to the Golgi membranes, suggesting that the process of microtubule nucleation at the Golgi and centrosome are similar. γ-tubulin and γ-tubulin ring complex proteins AKAP450, myomegalin, CAMSAP2 and pericentrin are seen to localize to the Golgi membranes and depleting any one of these components can reduce the nucleation of microtubules at the Golgi (Sanders & Kaverina, 2015).

A high concentration of γ-tubulin is required to facilitate microtubule nucleation at the centrosome. The concentration of γ-tubulin found at the Golgi is not higher than that of γ-tubulin located near the γ-tubulin ring complexes formed in the cytoplasm. Yet, the nucleation of microtubules at the Golgi might result from the stabilization of newly formed microtubules at the Golgi. CLASPs (CLASP1 and CLASP2) are known to bind GCC185 and accumulate at

the trans-Golgi. CLASPs play a vital role in the nucleation of microtubules and are also known to stabilize the microtubules. Though there is no clear understanding of the mechanism by which CLASPs aid nucleation, they are thought to stabilize the newly formed microtubules across the Golgi stacks (Sanders & Kaverina, 2015; Wu, de Heus, et al., 2016).

Additionally, Golgi-localised myomegalin binds to microtubule end-binding proteins (EB1 and EB3). EBs, upon recruitment, could assist in the nucleation of microtubules. Increasing the concentration of α and β-tubulin subunits could also facilitate the nucleation and polymerization of microtubules (Roubin et al., 2013; Sanders & Kaverina, 2015; Z. Wang et al., 2014). Tubulin-specific chaperones play a significant role in folding the α and β-tubulin to aid their incorporation into the microtubule lattice. There are five tubulin-specific chaperones; TBCE, a chaperone that assists the folding of β-tubulin, is recruited to the Golgi in an Arf1-dependent manner. Chaperone localisation at the Golgi could increase the concentration of properly folded α and β-tubulin subunits around the Golgi membranes and aid the polymerization of microtubules (Baillat et al., 2014). Additionally, the Golgi environment could contribute to the nucleation and stabilization of microtubules at the Golgi. A recent study has shown that GM130 can bind to TPX2 and initiate microtubule nucleation (Wei et al., 2015). This suggests that there could be additional mechanisms of microtubule nucleation at the Golgi.

Golgi-associated microtubules are more stable and have shown to be post-translationally modified. Golgi-associated microtubules are hyperacetylated and detyrosinated; these modifications increase the stability of microtubules and reduce their depolymerization rates. These post-translational modifications could be made on microtubules nucleated at the Golgi or microtubules originating from the MTOC associated with the Golgi. Still, the association with Golgi is necessary for the modifications (Thyberg & Moskalewski, 1993a, 1999). The increased stability of Golgi-associated microtubules could facilitate rapid rates of trafficking across the Golgi as microtubule acetylation has been shown to increase the binding of Kinesin-1 (Balabanian et al., 2017; Mizuno & Singer, 1994).

The microtubules originating from the MTOC and the Golgi together could contribute to the organisation of the Golgi at the MTOC. The COPII vesicles exiting the ER exit site fuse to form the ERGIC (Endoplasmic reticulum Golgi intermediate compartment), and the ERGIC fuse to form the cis-Golgi which matures to form the medial and trans-Golgi compartments. As the Golgi stacks form, the nucleation of microtubules is initiated. The microtubules arising

from the Golgi help bring together the Golgi stacks formed throughout the cell by a search and capture method. Later, the association of these stacks with the microtubules from the MTOC facilities the movement of these stacks towards the MTOC. Further, microtubules associated with the Golgi extend towards the leading edge of the cells and facilitate the cargo transport to the leading edge. Hence, coordination between the microtubules arising from the MTOC and the Golgi is essential for organisation of the Golgi into a ribbon-like stacked structure at the MTOC (Lowe, 2011).

### 1.3.2. Role of actin in regulating the Golgi organisation and function.

The role of microtubules in the Golgi organisation and function has been studied extensively. In comparison, the role of the actin cytoskeleton in regulating Golgi has not been explored enough. Comitin, an actin-binding protein and actin-related proteins centractin, spectrin, ankyrin and filamin have been known to localize to Golgi. Disruption of the actin cytoskeleton results in the collapse of the Golgi cisternae near the MTOC. EM studies show that the collapsed cisternae appear swollen, similar to the swelling of cisternae observed during the disruption of ion flux at the Golgi. Disruption of actin could affect the Golgi's ionic balance and result in the cisternae's swelling (Egea et al., 2006, 2013).

Further disruption of microtubules in these cells leads to unlinking of the Golgi stacks and their dispersal throughout the cytoplasm. Disruption of actin does not affect the anterograde and retrograde transport across the Golgi. In the absence of actin, retrograde transport disruption leads to Golgi enzymes' fallback to ER. The reassembly of Golgi from ER is not affected by actin disruption. The microtubule-dependent reassembly of Golgi stacks at MTOC does not require actin fibres. This suggests that actin and microtubules have independent roles in regulating Golgi morphology. While microtubules contribute to linking Golgi stacks and their assembly at the MTOC, actin could contribute to the flattening of the cisternae (Copeland et al., 2016; Egea et al., 2006; Kreis, 1990).

The clathrin coat at the trans-Golgi recruits the WAVE complex to the Golgi membranes in an Arf1-dependent manner. Rac1 activates the WAVE complex at the Golgi and leads to the recruitment of the Arp2/3 complex. Arp2/3 complex initiates the polymerization of actin filaments at the TGN, which generates the force necessary for vesicle budding. Myosin II is also seen to localize to the TGN and is required for the scission of vesicles from the Golgi membrane (Chakrabarti et al., 2021). Additionally, phosphatidylinositol-4-phosphate (PI4) at

the trans-Golgi directly binds to GOLPH3, which binds to the unconventional myosin, Myosin18A. Mysoin18A links the filamentous actin (F-actin) to the Golgi membranes. F-actin has been demonstrated to play an essential role in the trafficking between the Golgi and plasma membrane. Disruption of F-actin assembly at the trans-Golgi impairs the trafficking between the Golgi and plasma membrane. It alters the Golgi morphology, leading the Golgi to condense and form a compact structure around the centrosome. Over-expression of GOLPH3 has been shown to result in over extension of Golgi membranes leading to Golgi fragmentation in an F-actin-dependent manner. F-actin hence would generate a tensile force at the Golgi that contributes to the stretching and flattening of the Golgi cisternae (Dippold et al., n.d.; Egea et al., 2013; Farber-Katz et al., 2014).

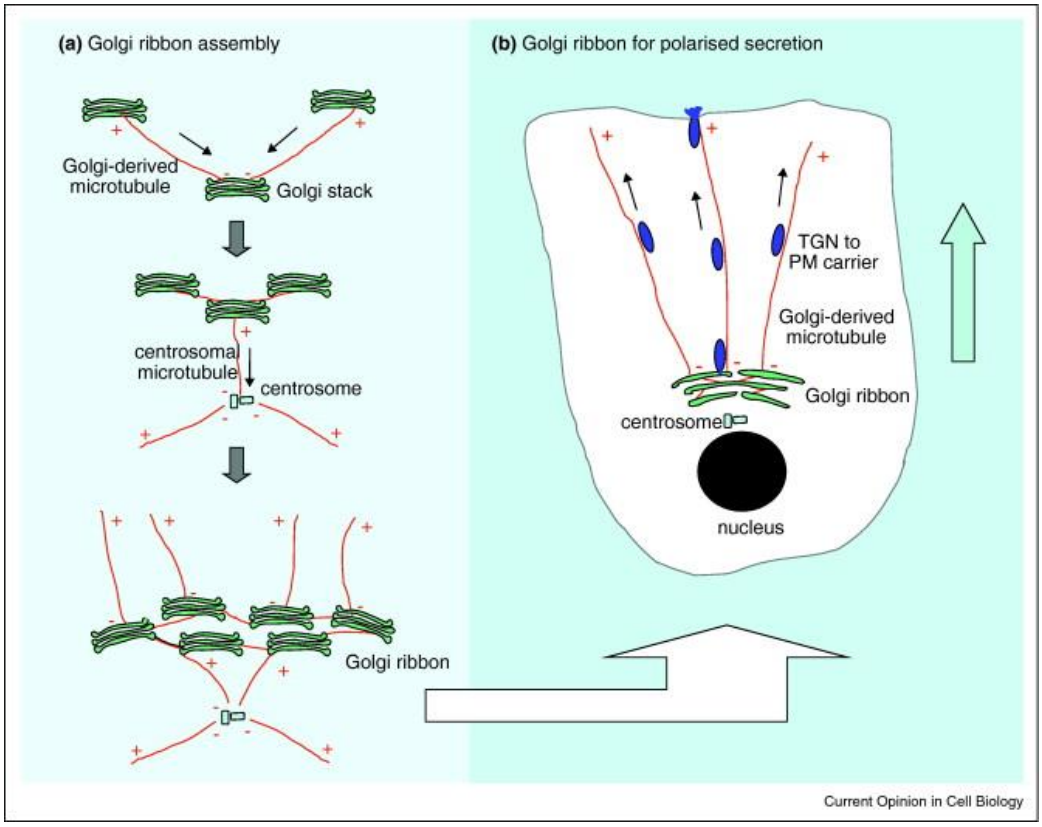



**Figure 1.13:** Role of microtubule in organisation of the Golgi apparatus. (Adapted from Lowe, 2011). (a) The Golgi derived microtubules bring the Golgi stacks together by a search and capture method. (b) The association of Golgi membranes with microtubules originating from the centrosome facilitates the organisation of the Golgi near the centrosome.

Actin has also been speculated to play a role in COPI-mediated retrograde transport. Cdc42 and N-WASP are known to associate with Golgi membrane through Arf1-COPI interaction and activate Arp2/3 complex to polymerase actin (Gomez & Billadeau, 2009). Mutants of

Cdc42 disrupt the retrograde transport of cargo from Golgi to ER. Cdc42 competes with p23 to bind γCOPI subunit, p23 mediated cargo loading to COPI vesicles leads to dissociation of Cdc42 and association of dynein to COPI vesicles leading to dynein-dependent retrograde transport. Hence, Cdc42 could help coordinate microtubule and actin-dependent transport of COPI vesicles at the Golgi. Like its role in endocytosis at the plasma membrane, actin could provide the structural support necessary to form the Golgi's vesicles (Copeland et al., 2016; Egea et al., 2013; Fucini et al., 2000).

### 1.3.3. Regulation of actin and microtubules by integrin-mediated cell-matrix adhesion.

Cells bind to the extracellular matrix through several interactions, out of which integrin-mediated interactions are well established and are known to play an essential role in regulating several cellular processes, including cell division, cell spreading, gene expression, protein synthesis, cell migration and cytoskeleton arrangement. Integrins are heterodimers made of α and β subunits. About eighteen α and eight β subunits in mammals are known to form around 24 canonical combinations (Hynes, 2002). Integrins are transmembrane proteins that bind to fibronectin, Lamins, collagen and other proteins in the extracellular matrix. The binding of integrins to extracellular matrix proteins leads to integrin clustering and brings about a conformational change in the integrins that exposes their intracellular tail. The intracellular tail interacts with 180 proteins like talin, vinculin, paxillin, tensin, ERK, and FAK, forming the adhesome. These interactions trigger a cascade of signalling events that ultimately lead to the formation and stabilization of focal adhesions (FAs), which are large macromolecular complexes that connect the actin cytoskeleton to the ECM and transmit mechanical forces from the outside to the inside of the cell(Berrier & Yamada, 2007; De Arcangelis & Georges-Labouesse, 2000).

#### 1.3.3.1.Integrin-actin cross-talk

One of the critical signalling pathways that integrins use to modulate the actin cytoskeleton is the Rho family of GTPases, which includes RhoA, Rac1, and Cdc42. Activation of integrins leads to the recruitment and activation of the Rho guanine nucleotide exchange factors (GEFs), which promote the conversion of RhoA, Rac1, and Cdc42 from their inactive GDP-bound form to their active GTP-bound form (Arthur et al., 2002). Active RhoA promotes the formation of actin stress fibers, contractility, and cell spreading by activating downstream effectors such as ROCK and myosin light chain kinase (MLCK). In contrast, active Rac1 and Cdc42 promote the formation of lamellipodia and filopodia, respectively, which are thin protrusions that drive

cell migration and adhesion by activating effectors such as the WAVE complex and N-WASP (Arthur et al., 2002; Etienne-Manneville & Hall, 2002; Schaller, 2001).

Integrin signalling also affects the actin cytoskeleton through the regulation of actin-binding proteins (ABPs), which include actin nucleators, bundlers, cross-linkers, and severing proteins. ABPs play a crucial role in controlling the organisation and dynamics of the actin cytoskeleton by regulating actin polymerization, bundling, and turnover (Calderwood et al., 2000; Romero et al., 2020). For example, integrin signalling promotes the recruitment and activation of the actin nucleator Arp2/3 complex, which catalyzes the branching of actin filaments and the formation of lamellipodia (Pollard et al., 2000). Integrin signalling also promotes the recruitment and activation of the actin-bundling protein α-actinin, which cross-links actin filaments and stabilizes FAs. Conversely, integrin signalling inhibits the activity of the actin-severing protein cofilin, which promotes the depolymerization of actin filaments and the turnover of FAs (Adams et al., 1998; Jamora & Fuchs, 2002; Pollard et al., 2000).

#### 1.3.3.2.Integrin-microtubule cross-talk.

Integrin-mediated cell-matrix adhesion is also known to play a vital role in regulating microtubule polymerization (LaFlamme et al., 2018). Defects in integrin signalling impairs the repolymerization of microtubules post-nocodazole treatment. It was discovered that the defect in the regrowth of microtubules was due to the suppression of microtubule nucleation owing to reduction in γ-tubulin recruitment to the centrosome (Colello et al., 2012; Reverte et al., 2006). Downstream of cell adhesion, integrins activate Src, FAK and ERK signalling pathways. Inhibition of Src and ERK pathways using specific inhibitors leads to defects in the recruitment of γ-tubulin. Defects in integrin signalling suppress the activation of Src and ERK pathways that could affect the recruitment of γ-tubulin to the centrosome (Colello et al., 2010, 2012). It has been shown that activation of Src and ERK in cells defective in integrin signalling restores the recruitment of γ-tubulin to the centrosome and the microtubule nucleation rates. γ-tubulin is assembled into γ-tubulin ring complex (γTRC) in the cytoplasm and then recruited to the centrosome. The molecule that affects γ-tubulin recruitment downstream of integrins is not known yet. It is speculated that integrin signalling could affect the assembly of γTRC and thus modulate the γ-tubulin recruitment to the centrosomes(Colello et al., 2012).

Integrin signalling also contributes to the stability of microtubules through the activation of FAK (Palazzo et al., 2004). Loss or defects in integrin signalling result in the loss of stable

microtubules. The Rho family of GTPases, RhoA, Rac, and Cdc42, known to regulate actin polymerization, also play a vital role in regulating the growth and stability of microtubules (Del Pozo et al., 2000; Etienne-Manneville & Hall, 2003; X. D. Ren et al., 1999). Activation of Rho-dependent signalling leads to the activation of mDia and formin that initiate the formation of a microtubule-stabilizing complex consisting of APC, EB1 and IQGAP proteins (Wickström et al., 2010).

Integrin signalling also contributes to microtubule stabilization by inhibiting stathmin, a known microtubule destabilizing protein (Cassimeris, 2002; LaFlamme et al., 2008). Integrin-mediated adhesion to ECM is also known to inhibit the activity of glycogen synthase kinase 3β (GSK3β**).** GSK3β phosphorylates MAPs that inhibit their ability to bind to and stabilise growing microtubules (Akhmanova & Steinmetz, 2008; Kumar et al., 2009). Proteomic analysis shows that protein complexes formed with activated integrins include several microtubule-stabilizing proteins like mDia, CLASPs, EB1 and IQGAP and thus suggest that integrin signalling could play a vital role in stabilizing microtubules at the cell cortex (Byron et al., 2015; LaFlamme et al., 2018).

Loss of adhesion does not disrupt the microtubule network (Singh et al., 2018). Given the role of integrin signalling in regulating microtubule stability (Cassimeris, 2002; LaFlamme et al., 2018; Palazzo et al., 2004), loss of adhesion could still affect the stability of microtubules by altering the PTMs on the microtubule filaments. PTMs, such as acetylation, phosphorylation, and polyglutamylation, have been shown to modulate the stability and function of microtubules (Janke & Montagnac, 2017; Macrae, 1997; Thyberg & Moskalewski, 1999). Alterations in these PTMs upon loss of adhesion could significantly affect the association of motor proteins with microtubules which could in turn impact cellular trafficking pathways.

However, it remains to be tested whether loss of adhesion indeed alters microtubule PTMs microtubules and whether this has any significant impact on the organization and function of the Golgi apparatus. How loss of adhesion affects microtubule stability and cellular trafficking pathways is also an open question that remains to be addressed.

## 1.4. Hypothesis and objectives of the thesis.

**Hypothesis: The differential effect of loss of cell-matrix adhesion on cis-medial and trans-Golgi can be mediated by the regulation of Arf1 activity at these compartments.**

Loss of cell-matrix adhesion leads to Golgi disorganisation in mouse fibroblasts. Interestingly, the trans-Golgi disorganises to a greater extent and fills up the entire cell volume. The cis-medial Golgi disorganises to a much lesser extent and retains its perinuclear localisation. Upon re-adhesion the Golgi compartments reorganise along the microtubule network to form a compact Golgi. Disruption of microtubules in non-adherent cells leads to further disorganisation of the cis-medial Golgi compartment, which now like the trans-Golgi compartment fills up the entire cell volume (Singh et al., 2018).

Loss of adhesion results in a drop in Arf1 activation. As a consequence of this drop, there is a drop in the amount of Dynein associated with the Golgi membranes which drives Golgi disorganisation (Singh et al., 2018). Arf1 is known to localize to all Golgi compartments in adherent cells and plays a vital role in regulating their organisation and function (Bottanelli et al., 2017). It remains to be tested if differential regulation of Arf1 at the cis-medial vs. trans-Golgi drives differences in the extent of disorganisation of these compartments in non-adherent cells. The role microtubules could have in the differential regulation of the Golgi compartments in non-adherent could further contribute to this regulation.

**Specific objectives of the thesis:**

I. Role of arf1 and kinetics of change in arf1 activation in regulating Golgi organisation and function in non-adherent cells.
II. Adhesion-dependent regulation of MT acetylation. Its role in Golgi organisation.
III. Isolation of Golgi from adherent vs. non-adherent cells for proteomic profiling

# CHAPTER 2

# Materials and Methodology

## 2.1. Materials.

### 2.1.1. Reagents.

Fibronectin (FN) (cat. no. F2006), Brefeldin A (cat. no. B7651), Golgicide A (cat. no. G0923) and Tubacin (cat. no. SML0065) were purchased from Sigma. Fluoromount-G (cat. no. 0100-01) was purchased from Southern Biotech. Concanavalin A Alexa-488 (ConA Alexa-488, cat. No. C11252) was purchased from Thermo Fisher Scientific.

### 2.1.2. Antibodies used for western blotting.

anti-Arf1 (clone 1D9, Abcam, #AB2806)(Kulasekaran et al., 2021; Sadakata et al., 2010; Singh et al., 2018) was used at dilution of 1:500, anti-acetylated tubulin (Sigma Aldrich, # T7451) at dilution 1:2000, 12G10 anti-alpha tubulin (Developmental Studies Hybridoma Bank, # AB_1157911) at dilution 1:5000, anti-GM130 (clone 35, BD transduction, #BD-610822) at dilution 1:250, anti-dynein ((clone 74.1, Millipore, #MAB1618) at dilution 1:2000, anti-p115(BD transduction, BD-612260), anti-Syntaxin 6 (BD transduction, #BD-610635) , anti-Vti1a (BD transduction, #BD-611404), anti-Vti1b (BD transduction, #BD-611220) at dilution 1:1000 and anti-GAPDH (Abcam, cat. No G9545)(Lobato-Márquez et al., 2021; Singh et al., 2018) at a dilution of 1:5000. Secondary antibodies conjugated to horseradish peroxidase (HRP) were purchased from Jackson Immuno Research and used at a dilution of 1:5000.

### 2.1.3. Antibodies used for immunofluorescence.

anti-GBF1 antibody (Abcam cat no. ab86071) was used at dilution of 1:100, anti-acetylated tubulin antibody (Sigma Aldrich, cat. no. T7451) at dilution 1:250, 12G10 anti-alpha tubulin (Developmental Studies Hybridoma Bank, cat. no. AB_1157911) at dilution 1:250, Phalloidin Alexa Fluor 488 (Invitrogen, cat. No. A12379) was used at dilution 1:500.(Buwa et al., 2021; B. Joshi et al., 2012) Secondary antibodies with Alexa conjugate (488 or 568) were purchased from Invitrogen molecular probes and were used at dilution 1:1000.

### 2.1.4. Plasmids.

GalTase-RFP, Mannosidase II-GFP and ss-RFP-KDEL, constructs were obtained from Dr. Jennifer Lippincott-Schwartz (HHMI). GFP-tagged (WT-Arf1 and T31N-Arf1)
constructs were obtained from Dr. Satyajit Mayor (National Centre for Biological Sciences,

Bangalore, India). GFP-tagged Arf1-Q71L construct was made by site-directed mutagenesis using GFP-Arf1-WT as a template and following primers:
(forward) 5'-GACGTGGGTGGCCTGGACAAGATCCGG-3' and
(reverse) 5'-CCGGATCTTGTCCAGGCCACCCACGTC-3'.
mCherry-tagged Arf1-WT and Arf1-Q71L constructs were made by releasing the Arf1 gene from GFP constructs (using BglII and BamH1 sites) and cloning the same into an empty mCherry-N1 vector. GFP-ABD construct was provided by Prof. Satyajit Mayor (NCBS).

## 2.2 Methodology.

### 2.2.1. Cell culture and transfection.

Mouse embryonic fibroblasts (WT-MEFs) from Dr. Richard Anderson (University of Texas Health Science Center, Dallas, TX) were cultured in complete Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) with 5% fetal bovine serum (FBS) and penicillin-streptomycin (Pen-Strep; Invitrogen) at 37°C in a 5% $CO_2$ incubator. Cell lines used were routinely tested for Mycoplasma contamination and used only when they were clean. Cells were transfected using Lipofectamine 2000 (Invitrogen) in 6-cm dishes with complete medium using 4µg DNA for 12h (for all constructs). 48h after transfection, cells were serum-deprived for 14h in DMEM with 0.2% FBS and then used for experiments.

### 2.2.2. Suspension assay.

Pathways regulated by cell-matrix adhesion are also known to be regulated by several growth factors present in serum. Hence, cells were serum-deprived to clearly understand the effects arising from loss of cell-matrix adhesion. Serum-deprived cells were detached using trypsin-EDTA (Invitrogen) at 37°C and washed with low-serum DMEM. Cells suspended in 15ml of low-serum DMEM were gently mixed with an equal volume of 2% methylcellulose in low-serum DMEM and incubated at 37°C for 120min (120′ SUSP cells). Following this incubation, cells were collected at the required time, carefully washed twice with low-serum DMEM, and centrifuged at 1000 rpm for 5min at 4°C. They were then reconstituted in low-serum DMEM. One aliquot of the cells was centrifuged at 1000rpm and the pellet was processed for 120' SUS. Reconstituted cells were then re-plated on coverslips coated with 2µg/ml FN for 10 or 15min (referred to as 10′ FN or 15' FN cells). Cells re-plated on FN were allowed to stay adherent for 4h and defined as being stable adherent. For western blotting studies, suspended and re-

adherent cells were lysed in 1× Laemmli's buffer, heated at 95°C for 10min, and stored at −80°C. For confocal microscopy, cells suspended or re-adherent on coverslips were washed twice with 1XPBS and fixed with 3.5% paraformaldehyde (PFA) for 15 min at RT. These cells were eventually washed with 1XPBS and mounted using Fluoromount-G and imaged using a Zeiss laser scanning microscope.

### 2.2.3. Arf1 activity assay.

To determine active Arf1 levels, $6*10^5$ cells (suspended or adherent) following their respective incubation or drug treatment were washed, frozen, and lysed in 400µl activity assay buffer, and lysates were incubated with 60µg of glutathione S-transferase (GST)-tagged Golgi localised γ-ear containing Arf-binding protein 3 (GGA3) fusion protein (GST-GGA3) (Pawar et al., 2016) to pull down active Arf1. 30µl of the whole-cell lysate (6% of the total WCL) and all of the GGA3 pulldown sample (100% of total) was resolved by SDS-PAGE, western blotted using the anti-Arf1 antibody (Clone 1D9, Abcam). Blots were developed and imaged using the ImageQuant LAS 4000 (Fujifilm-GE); densitometric band analysis was done using ImageJ software (NIH). The ratio of band intensities of Arf1 from GGA3 pull-down was normalized to their respective whole cell lysate. WTMEFs treated with varying BFA and GCA concentrations for varying times were similarly processed and their active Arf1 levels were calculated and normalized to their respective control samples.

### 2.2.4. GFP-ABD mediated detection of active Arf1.

Arf binding domain (ABD) encompasses a pleckstrin homology domain of ARHGAP10 that specially binds to active Arf1(Kumari & Mayor, 2008). WTMEFs were transfected with GFP-ABD (4 µg). 48hours post-transfection cells were serum-deprived for 14hrs, detached and held in suspension for 120min and replated on FN, and allowed to re-adhere for 10 mins (considered as early re-adhesion time point) and for 4hrs (considered as stable adherent time point). Cells were fixed with 3.5% PFA and immunostained with anti-GM130 antibody (1:100 dilution), mounted, and imaged using the confocal microscope. WTMEFs were similarly transfected with GFP-ABD-GFP (4µg) and GalTase-RFP (4µg). Cells were suspended for 120mins and replated on FN for 10min (considered as an early re-adhesion time point) and for 4hrs (considered as stable adherent time point). Fixed cells were then mounted and imaged using a Zeiss Confocal Microscope.

### 2.2.5. RNA isolation and RT-PCR.

RNA was isolated from WT-MEFs using Trizol reagent (Invitrogen) and cDNA was prepared using Oligo-dT primers and Reverse Transcriptase (Promega). Quantitative PCR was done using SYBR SAFE qPCR master mix in BioRad CFX96 Real-Time PCR System. The primers used are as follows:

GBF1 Forward: CGCACTCATAGATCCAACTC,
GBF1 Reverse: TCATCAGGACAACTTCATCAC,
BIG1 Forward: GCACATTGTCACTCTTGTATTT,
BIG1 Reverse: GTCGGATTGCTTCCATACTT,
BIG2 Forward: CTGCTAGGTTCTCTCACATTC,
BIG2 Reverse: TCGTGGGACTTTGGATCT

### 2.2.6. BFA or GCA treatment of suspended WTMEFs for imaging.

$4\times10^5$ WTMEFs transiently transfected with ManII-GFP were serum-deprived for 14 h in low-serum DMEM (0.2% FBS), detached, and held in suspension for 60 min in DMEM with 1% methylcellulose. Cells were then treated with the required concentration of BFA or GCA and incubated for the required interval of time (10, 20, or 30 min respectively) at 37°C. Control cells were treated with an equivalent volume of solvent (DMSO/methanol). Cells were processed as described above and samples were collected at the required times.

### 2.2.7. Determining the Golgi distribution profile.

Cells, expressing the cis-Golgi marker ManII-GFP or trans-Golgi marker GalTase-RFP, were imaged using a confocal microscope; the organisation of the Golgi was then observed and classified as organised, disorganised, partially fragmented, and fragmented based on the distribution of Golgi elements in the cells imaged by Confocal microscopy. To characterise the organisation of the Golgi on inhibitor treatment cis-medial ManII-GFP labelled cells were used. In cells with an **intact/organised Golgi phenotype**, the cis-medial ManII-GFP labelled Golgi has a distinct compact organisation. Cells with a **disorganised Golgi** are characterised by this compact organisation being marginally disrupted to cause the cis-medial ManII-GFP labelled Golgi to be more dispersed but keep their perinuclear localisation in suspended cells. **Partially fragmented** cis-medial ManII-GFP labelled Golgi has a dispersed perinuclear Golgi pool but also shows some diffused distribution throughout the cell. Occasionally GFP labelled tubular extensions are seen to form from the perinuclear pool in these cells. Man II-GFP labelled Golgi shows some overlap with the ssRFP-KDEL labelled ER in these cells. The **fragmented** cis-medial ManII-GFP labelled Golgi lacks a perinuclear localisation and is distributed throughout

the cell, appearing completely diffused in most cells. ManII-GFP labelled tubular extensions are also seen through the cell. The fragmented Golgi is seen to overlap extensively with the ss-RFP-KDEL labelled ER. Randomly selected cells (200 or more) in each population were evaluated for their Golgi organisation, classified as listed above and the percentage distribution of cells with each phenotype was calculated and plotted.

### 2.2.8. Immunostaining for GBF1.

WT-MEFs fixed with 3.5% PFA for 15 mins at room temperature (RT) were permeabilized with 0.1% TritonX in 5% BSA for 30 mins at RT. They were blocked with 5% BSA in 1XPBS containing 10% horse serum, overnight at 4ºC. Coverslips were incubated with anti-GBF1 antibody (1:100 dilution) overnight at 4ºC. Washed with 1XPBS and incubated with anti-mouse Alexa-568 (1:1000 dilution), at RT for 1hr. Cells were then mounted with fluoromount and imaged using a confocal microscope.

### 2.2.9. Confocal microscopy and deconvolution of z-stacks.

All imaging of cells was done using a Zeiss 710 or 780 laser scanning confocal microscope with a 63× oil objective (NA 1.4). Acquisition settings were, laser power=2%, Pinhole=1 AU, gain=650–900, and these settings were kept constant. Images were acquired at a resolution of 1024×1024, at a scan speed of 5. Z-stacks were acquired at 0.2µm intervals and scan speed 7, de-convoluted using the Huygens Professional version 16.10 (Scientific Volume Imaging, The Netherlands, http://svi.nl). De-convoluted images were rendered as a maximum-intensity projection (MIP; by using the MIP renderer plug-in).

### 2.2.10. Colocalisation analysis.

Colocalisation analysis was done by using the Colocalisation Analyzer plug-in in the SVI Huygens Professional software (version 16.10). Pearson coefficients calculated were compared between treatments.

### 2.2.11. Cell spreading quantitation.

WT-MEFs re-adherent on FN for 15 minutes were stained with Phalloidin (1:500 dilution) and imaged using the EVOS FL Auto Imaging System (Thermo Fisher, Waltham, MA, USA, AMAFD1000) at 40X magnification and analysed using the ImageJ software (NIH, Bethesda,

MA, USA). Images of phalloidin-stained cells were processed and a threshold was set such that it covers the entire stained cell. This was used to define the boundary of each cell using the tracing tool. The total area within this mapped boundary for each cell was calculated using the "measure" option under the "analyse" tool in Image J. The area determined for 100 or more cells for each treatment or time point was used to calculate the mean cell spread area, which was then compared across treatments.

### 2.2.12. Lectin labelling.

WT-MEFs serum-deprived for 14hrs were detached using Accutase, washed, and held in suspension for 10 mins without or with BFA (3.6µM) or GCA (0.5µM). Post suspension, cells were harvested and incubated with ConA-Alexa 488 (0.05µg/ml) for 15mins, on ice in dark. Post incubation, cells were washed twice with cold 1XPBS and fixed with 3.5% paraformaldehyde for 15mins, washed and resuspended in 1X PBS, and analyzed using the instrument BD Celesta (BD Bioscience) and BD FACS Diva software. For each experiment, unlabelled WTMEFs were used as a control to set voltage gates using FSC-A and SSC-A parameters. The gated population (P1) was selected to generate histograms for the FITC channel detecting the labelling of ConA Alexa488 lectin. The median fluorescent intensity values obtained were used for comparing lectin labelling between samples.

### 2.2.13. Multiple sequence alignment.

The sequences of the mouse GEFs: BIG1(G3X9K3), BIG2 (A2A5R2), and GBF1(Q6DFZ1) were extracted from Uniprot. The Sec7 domains of these sequences were aligned along with the human ARNO sequence (Q99418) using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [DOI: 10.1038/msb.2011.75]. Note that the Sec7 domains of the mouse GEFs were manually curated keeping the human ARNO Sec7 domain from the structure of the ARNO-Arf1 complex (PDB ID:1R8Q) as a reference. The entire lengths of the 4 proteins were also aligned, not just their sec7 domains as mentioned above. These results are not shown here and were not pursued further.

### 2.2.14. Structure modelling of the mouse GEFs (BIG1, BIG2, GBF1) and mouse ARF1.

The alphafold2 generated structures of the mouse BIG1, BIG2, GBF1, and Arf1 were taken from the EBI repository (see supplement for the coordinate files). These structures encompass

the whole-length structures of all proteins. From these structures, the sec7 domains of the GEFs, as defined for the alignment above, were extracted. To build complexes of mouse Arf1GEF-Brefeldin-Arf1 complexes, the MODELLER suite of programs (Šali & Blundell, 1993) was used for comparative modelling with the PDB structure of the human ARNO-BFA-Arf1 complex (PDB ID:1R8Q) serving as a template. The BrefeldinA bound Arf1 undergoes a conformational change (well documented in DOI: 10.1038/nature02197). The alphafold2 models did not incorporate these conformational changes and hence necessitated the use of MODELLER to construct models of the complex.

### 2.2.15. Acetylated tubulin and total tubulin immunostaining.

WT-MEFs (suspended or adherent) were fixed with BRB80 buffer for 5 mins at RT, followed by chilled methanol at -20°C for 5mins. They were then washed twice with 1XPBS. Cells were then permeabilization with 0.05% Triton X (in 5% PBS) for 10 mins at RT, followed by overnight blocking with 10% horse serum (in 5% BSA) at 4°C. Cells were then incubated with anti-acetylated tubulin (1:250) or total alpha-tubulin (1:250) antibody overnight at 4°C. Secondary antibody (anti-mouse Alexa 488 or anti-mouse Alexa 568, Dilution-1:1000) staining was done at RT for 1 hr. Cells were then washed twice with 1XPBS and mounted with Fluoromount-G and imaged using confocal microscopy.

### 2.2.16. Measurement of intensity of acetylated tubulin staining.

Images taken on Zeiss 710 or 780 were opened using Image J. The total cell area or the region of WT/Q71L-Arf1 localisation was selected using the tracing tool. The threshold of these images was set to 27 and the intensity within the mapped area was measured using the 'measure' option under the 'analyze' tool in ImageJ. Intensity was measured for at least 30 cells per sample and the integrated density values for each cell was plotted using GraphPad.

### 2.2.17. Isolation of membrane-rich fractions.

WT-MEFs (adherent/suspended) (~100µl pellet) were resuspended in 500µl of Homogenizing buffer (250mM sucrose, 10mM HEPES pH 7.2, 1mM DTT, 1mM EDTA and protease inhibitors (1X), Cells were homogenized by passing them through 26-gauge needle (~200 strokes) and centrifuged at 3000g, for 10mins at 4°C. The pellet (Nuclear fraction) was lysed in 500µl of RIPA. The supernatant (Post Nuclear Supernatant (PNS)) was then centrifuged at

100,000g for 1hr at 4°. The pellet (Membrane fraction) was lysed in 200µl of RIPA. The supernatant was TCA precipitated and the pellet obtained (Cytosolic fraction) was lysed in 200µl of RIPA. Protein estimation was done with Peirce BCA estimation kit.

### 2.2.18. Golgi isolation.

WT-MEFs (~1-3 $X10^8$) (adherent/suspended) were washed twice in homogenizing medium (0.25M sucrose, 1mM Tris, pH 7.4). Cells were homogenized in 3ml of homogenizing medium by giving 15-20 strokes in a tight-fitting pestle Dounce homogenizer. Then 2 volumes of 2M sucrose gradient solution was added to bring the cell homogenate to 1.4M with respect to sucrose. 4.5ml of the homogenate was transferred to a 13.2 ml tube and overlaid with 4.5ml of 1.2M sucrose gradient and 2.5 ml of 0.8M sucrose gradient solution. Further, 1.5 ml of 1.6M sucrose solution is laid under the sample and centrifuged for 2 hours in an SW41 ultracentrifuge at 110,000g at 4°C. Golgi membranes being less dense than other membrane particles partition at the interface of 1.2M/0.8M gradient. 500ul fractions were collected from the top of the tube and lysed in laemmli's buffer. Fractions 4-7 lie at the interface of 1.2M/0.8M sucrose. These fractions were analyzed through western blotting to check for relative enrichment of Golgi proteins.

### 2.2.19. Statistical analysis.

All the analysis was done using Prism GraphPad analysis software. Statistical analysis for Arf1 activity was done using a two-tailed unpaired Mann–Whitney test. Normalized data were analyzed, using the one-sample t-test.

# CHAPTER 3

# Role of Arf1 and kinetics of change in Arf1 activation in regulating Golgi organisation and function in non-adherent cells.

## 3.1. Rationale.

The extracellular matrix (ECM) is a complex network of carbohydrates and proteins surrounding the cell. Cell matrix adhesion refers to the interaction between the cells and their extracellular matrix. This interaction is crucial in regulating several cellular processes like cell division, proliferation, polarity, migration and survival. Integrins are trans-membrane proteins made up of alpha and beta subunits. Integrins are vital in binding to the ECM proteins and mediating several downstream cellular signalling pathways (Berrier and Yamada, 2007; Caswell et al., 2009; Danen and Yamada,2001). Loss of cell-matrix adhesion affects endocytosis and trafficking within the cells, which leads to the downregulation of growth signalling pathways (M.A. Arnaout et al., 2005, Annu. Rev. Cell Dev. Biol., Berrier and Yamada, 2007, Journal of Cellular Physiology)

Previous studies from the lab have shown that cell-matrix adhesion can regulate Golgi organisation by regulating Arf1 activation in cells (Singh et al., 2018). It was observed that loss of adhesion causes a ~50-60% drop in active Arf1 levels in WT-MEFs, which drives the disorganisation of the Golgi. This disorganisation of the Golgi was distinctly different from the known Golgi fragmentation (Lippincott-Schwartz et al., 1989a; Singh et al., 2018). Upon re-adhesion, the cis-medial and trans-Golgi reorganised rapidly along the microtubule network to form a compact Golgi. Interestingly, the extent of disorganisation observed in the non-adherent WT-MEFs was distinctly different for the different Golgi compartments. The trans-Golgi is prominently disorganised to fill up the entire cell, while the cis-medial Golgi is less disorganised and stayed perinuclear. In non-adherent WTMEFs this raises the possibility that the changing activation status of Arf1 across these Golgi compartments could regulate their organisation. A further reduction in active Arf1 levels in these non-adherent WT-MEFs, through BFA/GCA treatment, is seen to drive Golgi fragmentation. This further highlights the role changing active Arf1 levels could have in regulating Golgi organisation in response to adhesion (Singh et al., 2018).

Hence, non-adherent mouse fibroblasts provide a unique scenario where we can evaluate and understand the role Arf1 activation has in regulating the organisation of Golgi compartments. In our current study, we have used Arf1 inhibitors BFA/GCA to titrate active Arf1 levels in non-adherent WT-MEFs to understand the effect Arf1 activation has on Golgi organisation and function. Our studies have been conducted under serum-starved conditions. This was necessary

as several pathways regulated by cell-matrix adhesion are also regulated by growth factors found in serum. Serum starvation would significantly reduce the impact of serum growth factors and help us better understand the role of the loss of cell-matrix adhesion. These studies were conducted in WT-MEFs with the help of markers that would aid in identifying the effect on cis, cis-medial and trans-Golgi compartments.

## 3.2 Results.

### 3.2.1. Effect of loss of cell-matrix adhesion on Golgi organisation and Arf1 activation.

Earlier studies have shown cell-matrix adhesion regulates Golgi organisation in mouse embryonic fibroblasts. Loss of adhesion leads to disorganisation of Golgi compartments, and re-adhesion was seen to rapidly reorganise the Golgi compartments(Singh et al., 2018). This depends on the Arf1 activation status regulated by cell-matrix adhesion. To confirm this, we co-expressed the cis-medial (ManII-GFP) and trans-Golgi (GalTase-RFP) in WT-MEFs. In serum-deprived stable adherent WTMEFs, the cis-medial Golgi marker Mannosidase II–GFP and trans-Golgi marker GalTase-RFP show an almost complete overlap in the organised Golgi (Fig 3.1.A). When cells were detached and held in suspension for 120 mins (120' SUS), the cis-medial and trans-Golgi were seen to disorganise (Fig. 3.1.A). As reported earlier, the extent of disorganisation was different for different compartments of the Golgi. The trans-Golgi is extensively dispersed and occupies most of the cell volume, while the cis-Golgi is less dispersed and stays largely perinuclear (Fig. 3.1.A). This is the predominant phenotype observed and confirmed by their distribution profile in suspended cell populations (Fig 3.1.C). Upon re-adhesion on fibronectin for 15 mins (15' FN), both Golgi compartments rapidly reorganise (Fig. 3.1.A) around the MTOC (Singh et al., 2018), confirmed by their distribution profile (Fig. 3.1.B). The differential extent of disorganization of Golgi compartments is further confirmed by the drop in the extent of colocalization of cis-medial Golgi and trans-Golgi in cells suspended for 120 mins as compared to stable adherent and early re-adherent cells (15' FN)(Fig 3.1.B).We further tested and confirmed the adhesion-dependent regulation of Arf1 by GST-conjugated GGA3 pull-down assay (Pawar et al., 2016; Singh et al., 2018). Loss of adhesion caused a significant decrease in Arf1 activation that is restored on re-adhesion (Fig. 3.1.D)(B. R. et al., 2023).

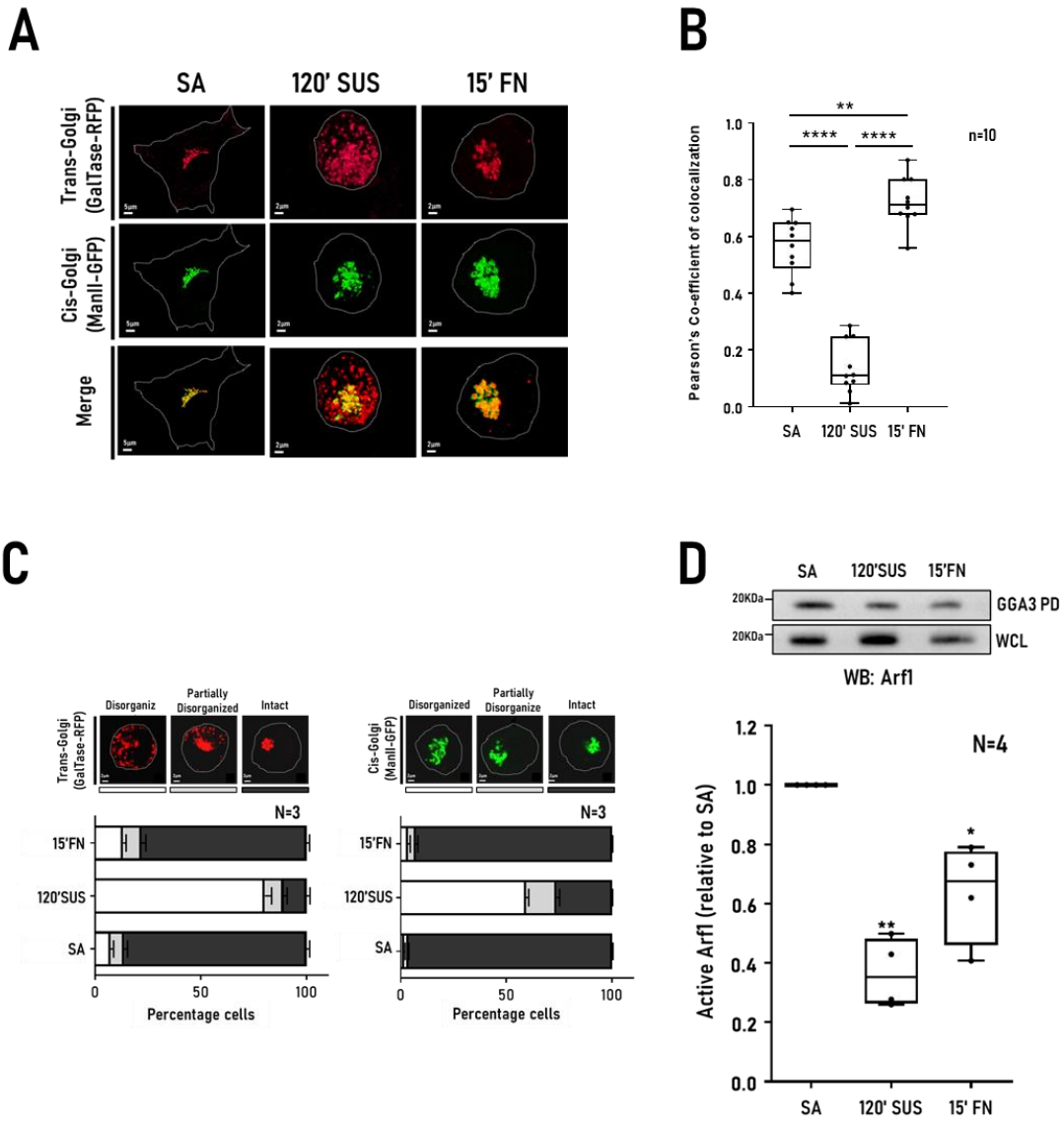



**Figure 3.1: Adhesion-dependent Arf1 activation differentially regulates cis/medial- vs trans-Golgi organisation in WT-MEFs. (A)** Representative de-convoluted maximum intensity projection (MIP) images of the Golgi in WT-MEFs transfected with GalTase-RFP (trans-Golgi marker) and ManII-GFP (cis-Golgi marker) when stable adherent (SA), held in suspension for 120min (120' SUS) and re-adherent on FN for 15min (15' FN). **(B)** The box and whisker plot represents Pearson's coefficient of co-localization for cis-medial (ManII-GFP) and trans-Golgi (Galtase-RFP) for WT-MEFs SA, 120' SUS and 15' re-adherent on FN. **(C)** The percentage distribution profile of WT-MEFs with disorganised (clear), partially disorganised (grey), and intact (black) cis-medial Golgi (ManII-GFP) and trans-Golgi (GalTase-RFP) was calculated. The graph represents their mean ± standard error (SE) of percentage distribution in SA, 120' SUS, and 15'FN from 3 independent experiments. **(D)** Western blot detection of active Arf1 (WB: Arf1) pulled down using GST-GGA3 (GGA3 PD) and total Arf1 in the whole-cell lysate (WCL) from stable adherent (SA), suspended (120′ SUS) and re-adherent (15′ FN) WT-MEFs. The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 from 4 independent experiments, normalised to stable adherent cells.

### 3.2.2. Localisation of active Arf1 in suspended vs re-adherent WT-MEFs.

The drop and recovery of active Arf1 detected through the GGA3-pull-down assay represents cellular Arf1 activation status. Given that the extent of cis-medial vs trans-Golgi disorganisation is different, we speculated that this difference could result from the difference in Arf1 activation at these compartments. To evaluate this, we used the GFP-ABD construct. This construct contains the Arf binding domain (ABD) that encompasses a pleckstrin homology domain of ARHGAP10. ABD is known to bind to active Arf1 specifically, and hence localisation of GFP-ABD could allow us to visualise active Arf1 localisation in cells.

To test the activation status of Arf1 at the Golgi compartments, we expressed the GFP-ABD construct with cis- (GM130) and trans-Golgi (GalTase) compartment markers in WTMEFs. Colocalisation of GFP-ABD with GalTase-RFP (Trans-Golgi) is high in stable adherent cells. However, it drops significantly in suspended cells where the trans-Golgi is extensively dispersed but recovers on re-adhesion (Fig. 3.2A). In contrast, the observed colocalisation of GFP-ABD with GM130 (cis-Golgi) in stable adherent cells drops only marginally in suspended cells and recovers in re-adherent cells (Fig. 3.2B). This suggests on the loss of adhesion, most of the decrease in net Arf1 activation seen (Fig. 3.1.C) is likely at the trans-Golgi and not at the cis-Golgi (B. R. et al., 2023). Arf1 GEF, GBF1 is known to localize to cis-Golgi while BIG1/2 localizes to the trans-Golgi in cells (Kawamoto et al., 2002; Lowery et al., 2013; Manolea et al., 2008). The differential localisation and regulation of Arf GEFs in the trans- vs cis-Golgi, could be responsible for differences in Arf1 activation between these compartments.

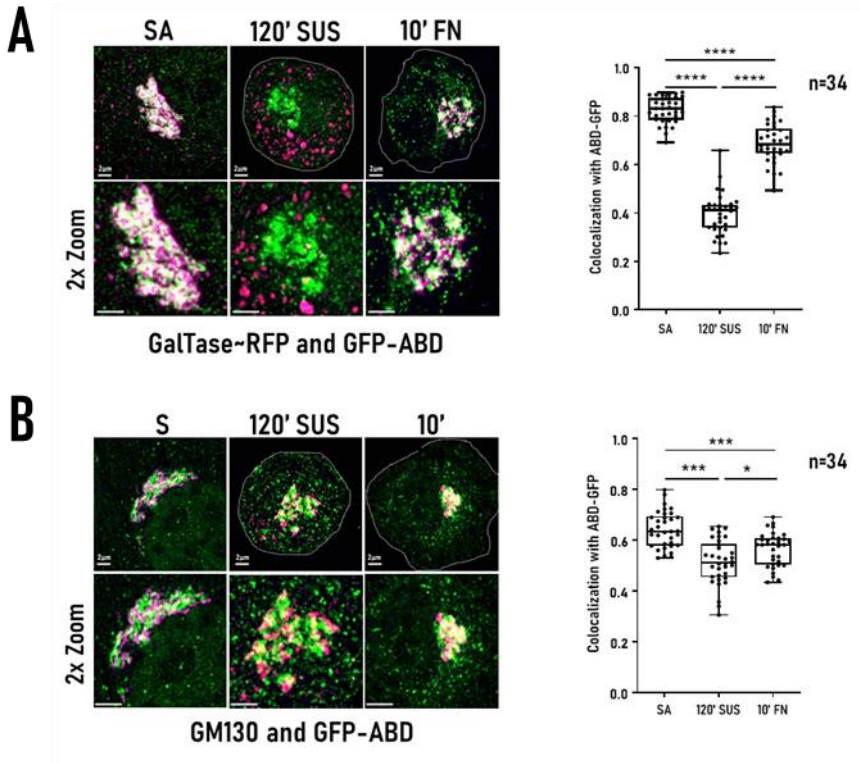



**Figure 3.2: Localisation of GFP-ABD relative to Golgi compartments.** Representative de-convoluted MIP images of stable adherent (SA), suspended (120' SUS), and re-adherent (15' FN) WT-MEFs expressing GFP-ABD (shown in green) with **(A)** GalTase-RFP (shown in magenta) (top panel) or **(B)** immunostained with GM130 (shown in magenta) (lower panel). The box and whisker plot represents Pearson's co-localisation coefficient for GFP-ABD with **(A)** GalTase-RFP or **(B)** GM130 in 34 cells from 3 independent experiments. Statistical analysis was using the Mann-Whitney test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001) or with normalised data using single sample T-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001)

### 3.2.3. Relative expression of Arf1-GEFs and structural analysis of Arf1-GEFs bound to Arf1 and BFA.

The localisation and activation of Arf1 at the different Golgi compartments are regulated by the spatially localised Arf GEFs. GBF1 governs the activation of Arf1 at the ERGIC and cis-Golgi compartments (Kawamoto et al., 2002; Lowery et al., 2013; Manolea et al., 2008), and BIG1/2 regulates Arf1 activation at the trans-Golgi . Upon loss of adhesion, BIG1/2 have been shown to play a predominant role in regulating Golgi organisation (Manolea et al., 2008; Stephens, 2010; Yamaji et al., 2000). Loss of BIG1/2 activity at the trans-Golgi membranes could result in low activation of Arf1 at the trans-Golgi leading to its extensive dispersal in non-adherent cells. In non-adherent cells, retention of active Arf1 at the cis-medial Golgi possibly suggests the retention of GBF1 at the cis-Golgi (Fig. 3.2). This means that downstream of cell-matrix adhesion, Arf-GEFs could be regulated differentially and contribute to the differences observed in the extent of disorganisation of the cis-medial and trans-Golgi compartments.

To determine the relative role of these Arf GEFs in adherent vs non-adherent WT-MEFs, we first tested the relative expression of GBF1, and BIG1/BIG2 by qRT-PCR. We identified the expression of BIG1 was the highest, followed by GBF1 and BIG2 in serum-deprived stable adherent cells (Fig. 3.3A) (BIG1 > GBF1 > BIG2). In suspended (120' SUS) WT-MEFs, all 3 GEFs showed a ~15% drop in their mRNA levels (Fig. 3.3B), which could, in turn, affect their protein levels. However, their relative expression in non-adherent cells stayed comparable to stable adherent cells (BIG1 > GBF1 > BIG2)(B. R. et al., 2023).

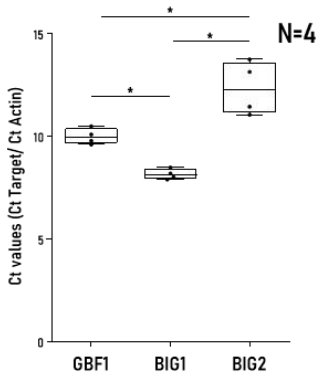
The relative roles of these Arf GEFs in regulating the activation of Arf1 can be studied using the inhibitors BFA and GCA. BFA is an uncompetitive inhibitor of a sub-family of Arf GEFs, including GBF1 and BIG1 / BIG2 (BFA-inhibited GEFs) (Casanova, 2007). GCA, however, inhibits, specifically GBF1, without affecting BIG1/BIG2 (Sáenz et al., 2009). There are no available inhibitors that would target only BIG1/BIG2. A comparative study using both inhibitors, BFA and GCA, could help decipher the relative roles of these Arf GEFs in regulating Arf1.

As BFA is known to inhibit the activity of all three GEFs, we wanted to test if BFA affects the activity of all three GEFs comparably. Therefore, we used molecular modelling to evaluate the

binding of BFA with these Arf1 GEFs. BFA is known to bind a highly conserved Sec7 domain in Arf GEFs to inhibit Arf1 GDP-GTP exchange (Niu et al., 2005, Peyroche et al., 1999). Using the available human ARNO Sec7-BFA-Arf1 structure (PDB ID:1R8Q) crystal structure, 3D models were generated for the Sec7 domain of mouse BIG1, BIG2, and GBF1 in a complex with BFA and mouse Arf1 (representative image for BIG1-BFA-Arf1 is shown in Fig 3.3C). A comparison between the ARNO-BFA-Arf1 and the predicted mouse Arf1 GEF-BFA-Arf1 showed that the residues vital for the interaction of BFA with these GEFs are conserved across human (ARNO) and mouse Arf1 GEFs (BIG1, BIG2, GBF1) (Fig. 3.3D marked by blue arrows). This suggests that the inhibitory effect of BFA should be comparable across these GEFs (B. R. et al., 2023). GCA also binds the same Sec7 pocket, but its interaction extends beyond the Sec7 domain to contact the tripeptide loop made by the QNA (Glutamine-Asparagine-Alanine) residues in GBF1(Fig. 3.3D, highlighted with a red box). The interaction with the tripeptide loop is essential for the inhibitory effect of GCA, and contributes to its specificity for GBF1(Saenz et al., 2009).

Figure 3.2

A

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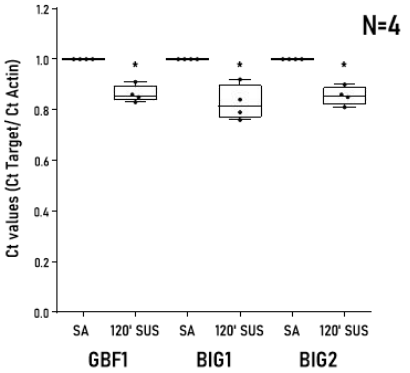


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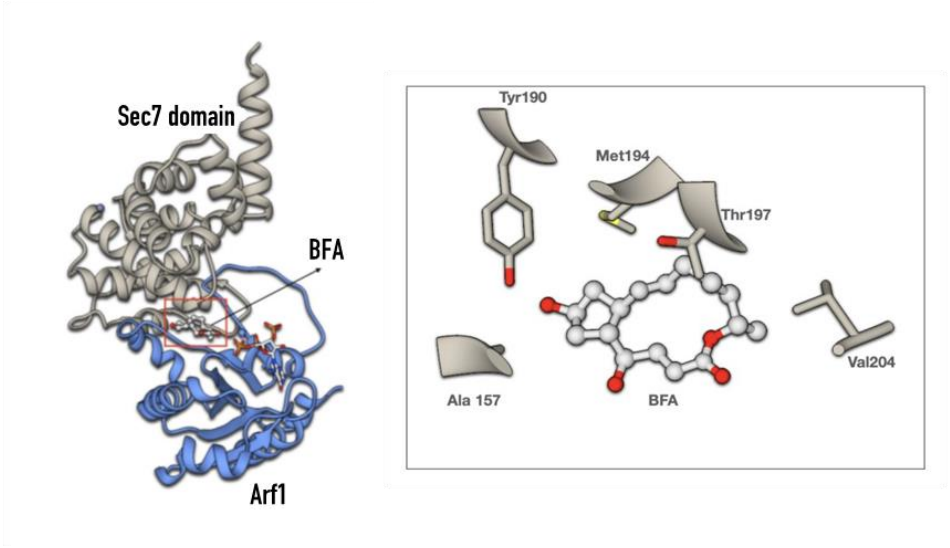



D

```
hARNO - sp|Q99418|56-251   ---SKTLQRNRKMAMGRKKFNMDPKKGIQFLVENELLQNT--PEEIARFLYKGEGLNKTA
mGBF1 - tr|Q6DFZ1|692-892  -ELIEIKNKKKLLITGTEQFNQKPKKGIQFLQEKGLLTIPMDNTEVAQWLRENPRLDKKM
mBIG1 - sp|G3X9K3|688-887  -QFEVLKQQKEIIEQGIDLFNKKPKRGIQYLQEQGMLGTT--PEDIAQFLHQEERLDSTQ
mBIG2 - sp|A2A5R2|642-842  EQFEVIKQQKEIIEHGIELFNKKPKRGIQFLQEQGMLGAA--VEDIAQFLHQEERLDSTQ
                                  :::. :  * . ** .**:***:* *: :*       ::*::* :   *:..

hARNO - sp|Q99418|56-251   IGDYLGEREELNLAVLHAFVDLHEFTDLNLVQALRQFLWSFRLPGEAQKIDRMMEAFAQR
mGBF1 - tr|Q6DFZ1|692-892  IGEFVSDRK--NMDLLESFVSTFSFQGLRLDEALRLYLEAFRLPGEAPVIHRLLEVFTEH
mBIG1 - sp|G3X9K3|688-887  VGEFLGDNDKFNKEVMYAYVDQHDFSGKDFVSALRLFLEGFRLPGEAQKIDRLMEKFAAR
mBIG2 - sp|A2A5R2|642-842  VGEFLGDSTRFNKEVMYAYVDQLDFCEKEFVSALRTFLEGFRLPGEAQKIDRLMEKFAAR
                           :*::::.:    *  :: ::*.  .*    : .*** :* .*******  *.*::* *: :

hARNO - sp|Q99418|56-251   YCLCNPG--VFQSTDTCYVLSYSVIMLNTDLHNPNVRD---KMGLERFVAMNRGINEGGD
mGBF1 - tr|Q6DFZ1|692-892  WRSCNGS--PFANSDACFALAYAVIMLNTDQHNHNVRKQNAPMTLEEFRKNLKGVNGGKD
mBIG1 - sp|G3X9K3|688-887  YLECNQGQTLFASADTAYVLAYSIIMLTTDLHSPQVKN---KMTKEQYIKMNRGINDSKD
mBIG2 - sp|A2A5R2|642-842  YIECNQGQTLFASADTAYVLAYSIIMLTTDLHSPQVKN---KMTKEQYIKMNRGINDSKD
                           :  ** .   * .:*:.:.*::::***.*. *. :*:.       *.:    :*:* . *

hARNO - sp|Q99418|56-251   LPEELLRNLYDSIRNEPFKIPEDDGN
mGBF1 - tr|Q6DFZ1|692-892  FEQDILEDMYHAIKNEEIVMPEEQTG
mBIG1 - sp|G3X9K3|688-887  LPEEYLSAIYNEIAGKKISMKETKEL
mBIG2 - sp|A2A5R2|642-842  LPEEYLSSIYDEIEGKKIAMKETKEH
                           : :: *  :*. * .: : : * .
```

**Figure 3.3: Arf1-GEFs expression in WT-MEFs. (A)** Arf GEF expression profile in stable adherent WTMEFs detected by RT-PCR shows BIG1 to be better expressed (lower Δ Ct values) than GBF1 and BIG2. The box and whisker plot represents the Δ Ct values relative to actin from 4 independent reactions. Statistical analysis of normalised data was done using the single sample T-test (*P<0.05) **(B)** Arf GEF expression profile in WTMEFs detected by RT-PCR compares the expression of GBF1, BIG1, BIG2 in stable adherent (SA) and suspended (120' SUS) cells. The box and whisker plot represent the Δ Ct values for each GEF in 120' SUS cells relative to SA cells relative to respective Δ Ct values of actin from 4 independent reactions. All data were analysed using the single sample t-Test, and P values are as indicated (* p<0.05). **(C)** Ribbon representation of the Arf-GEF Sec7 domain in complex with BFA. Only the BIG1 Sec7-BFA-Arf1 complex is represented here. The sec7 domain of BIG1 is in blue ribbons, and Arf1 is represented in grey ribbons. The BFA sandwiched between the Arf1 and GEF is in a ball-and-stick representation. The inset shows the residues marked by blue arrows in Fig. 2D represented as sticks interacting with BFA (in a ball and stick representation). The inset was rendered using UCSF Chimera (Pettersen et al., 2004). **(D)** Multiple sequence alignment of the sec7 domains of the mouse BIG1, BIG2, GBF1, and human ARNO as constructed by Clustal Omega. The amino acids highlighted in green interact with Arf1 (within 4.0Å) in the homology models. The residue positions marked with blue arrows are the residues making contact (within 4.0 Å) with BFA in these models. The red box marks the QNA residues unique to GBF1 and responsible for its specific interactions with GCA(Sáenz et al., 2009).

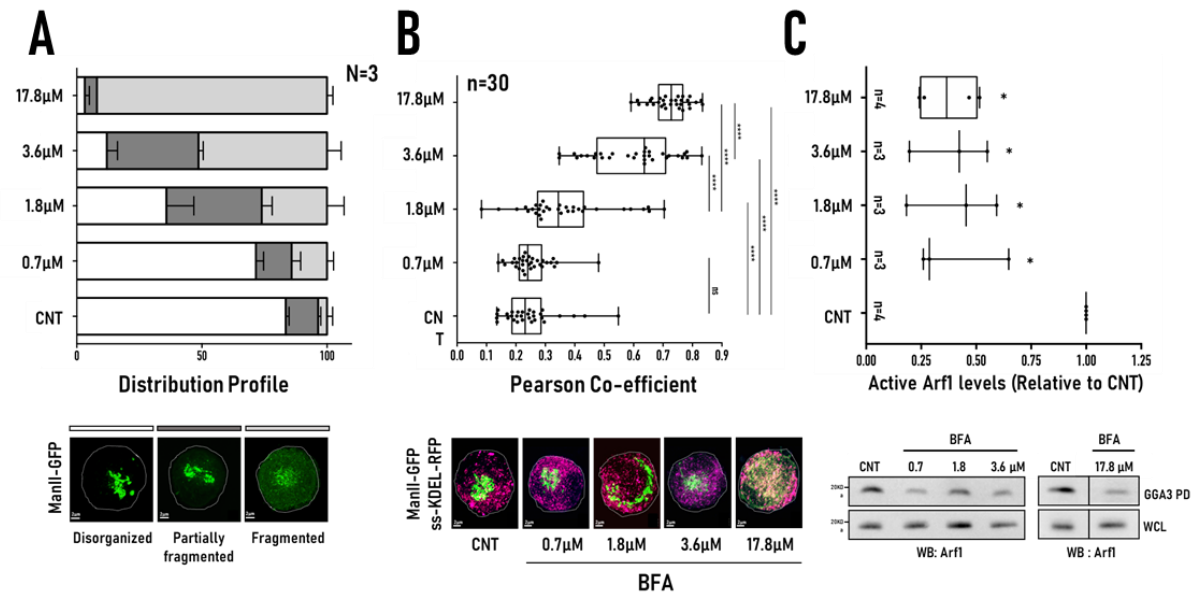
### 3.2.4. BFA-mediated decrease in Arf1 activation regulates Golgi fragmentation in non-adherent WTMEFs.

Non-adherent WTMEFs provide a unique cellular context to evaluate the role of Arf GEFs in regulating Arf1 activation at the Golgi. BFA, as an uncompetitive inhibitor of GBF1 and BIG1/2 (Casanova, 2007) inhibits Arf1 activation at the Golgi that causes the cis/cis-medial Golgi to fragment and fall back into the ER in stable adherent (Klausner et al., 1992) and non-adherent cells (Singh et al., 2018). In non-adherent WT-MEFs, active Arf1 is retained at the cis-medial Golgi compartment, and hence we choose to evaluate the effect GEF inhibitor mediated inhibition of Arf1 activation on the cis-medial compartment. WT-MEFs transfected with a cis-medial Golgi marker ManII-GFP were suspended for 60 mins and then treated with increasing BFA concentrations (0.7µM, 1.8µM, 3.6µM, 17.8µM) for 30mins. The disorganised cis-medial Golgi (ManII-GFP) in suspended cells shows a concentration-dependent increase in its fragmentation (Fig. 3.4A).

BFA-mediated Golgi fragmentation is known to cause the ER fall back of Golgi-localised enzymes (Lippincott-Schwartz et al., 1989b). To analyse this, we used ss-RFP-KDEL construct (Alton-bonnet et al., 2006), which localizes exclusively to ER lumen and could help determine fragmented Golgi fallback into the ER. A BFA concentration-dependent increase in the extent of cis-medial Golgi fragmentation was reflected by the rise in the cis-medial Golgi (ManII-GFP) overlap with the ER (ss-RFP-KDEL). This is further confirmed by measuring their Pearson's coefficient of colocalisation (3.4B). The inhibitor concentration-dependent fragmentation of the Golgi could be the result of BFA-mediated differential inhibition of Arf1 activation. Therefore, we checked the Arf1 activation status in these cells. We found that Arf1 activation in these cells dropped by ~50-60% relative to control across all BFA concentrations (Fig. 3.4C). No significant change in the total Arf1 levels was observed across all treatments (Fig. 3.4F). Taken together, this suggests the net active Arf1 levels in non-adherent fibroblasts alone may not regulate Golgi fragmentation.

The kinetics of change in Arf1 inactivation on treatment with increasing BFA concentration could drive Golgi fragmentation. Treatment with 0.7µM and 17.8µM BFA results in a comparable decrease in net active Arf1 levels with distinct differences observed in Golgi fragmentation (Fig. 3.4A, 3.4C). We chose these concentrations to test their effect on the kinetics of Arf1 inactivation in 30min of treatments. With 0.7µM BFA treatment, Arf1 activity

drops gradually by ~30% (10min), ~39% (20min), and ~53% (30min) over time (Fig. 3.4D). In comparison, 17.8µM BFA causes a ~58% drop in Arf1 activation within the first 10mins that is retained at 20min (~59% decrease) and increases at 30 mins (~70% decrease) (Fig. 3.4E). However, no significant change in total Arf1 levels were observed in cells treated with 0.7µM or 17.8uM BFA for 10min, 20min or 30min (Fig. 3.4G & 3.4H). Furthermore, non-adherent WTMEFs expressing the cis-medial Golgi marker ManII-GFP when treated with 0.7µM BFA (causing a gradual drop in Arf1 activity) did not cause Golgi fragmentation, even when the decrease in Arf1 activation was ~53% after 30min (Fig. 3.4D & 3.4I). In contrast, cells treated with 17.8µM BFA, showing a steep drop in Arf1 activation by a comparable ~58% in 10min, caused the Golgi to rapidly fragment (Fig. 3.4E & 3.4J). This suggests that BFA-mediated inhibition of Arf GEFs and the resulting kinetics of change in Arf1 activation could differentially affect Golgi organisation in non-adherent WTMEFs (B. R. et al., 2023).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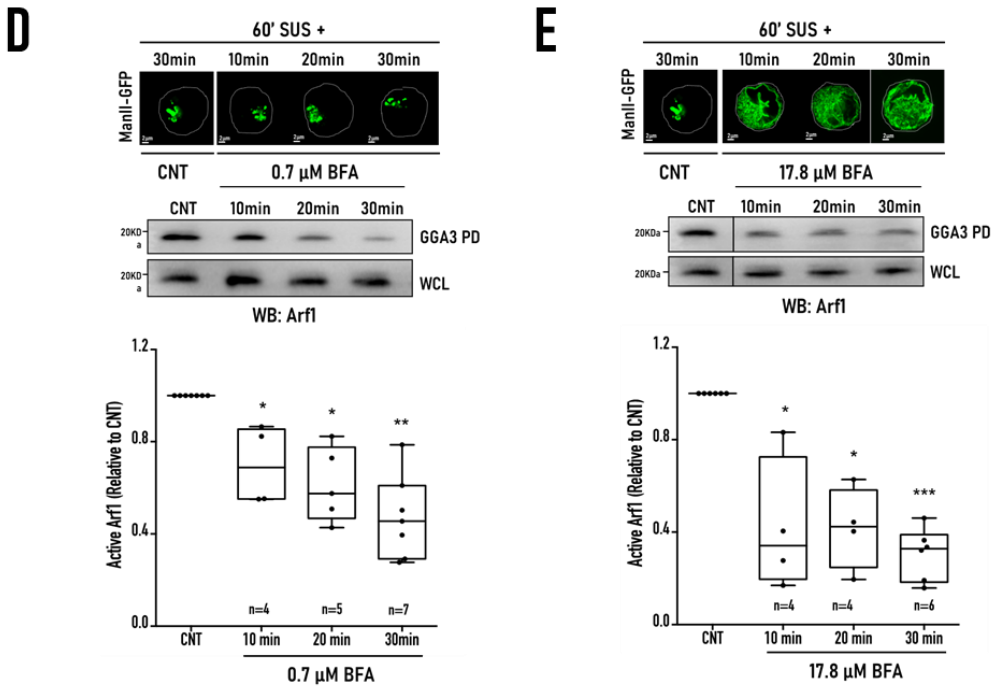
3.6µM
1.8µM
0.7µM
CNT
0
50
100
Distribution Profile
ManII-GFP
Disorganized
Partially fragmented
Fragmented
B
n=30
17.8µM
3.6µM
1.8µM
0.7µM
CN T
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
Pearson Co-efficient
ManII-GFP
ss-KDEL-RFP
CNT
0.7µM
1.8µM
3.6µM
17.8µM
BFA
C
n=4
n=3
n=3
n=3
n=4
17.8µM
3.6µM
1.8µM
0.7µM
CNT
0.00 0.25 0.50 0.75 1.00 1.25
Active Arf1 levels (Relative to CNT)
BFA
CNT 0.7 1.8 3.6 µM
BFA
CNT 17.8 µM
GGA3 PD
WCL
WB: Arf1
WB : Arf1


D
60' SUS +
30min 10min 20min 30min
ManII-GFP
CNT
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20KD
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WCL
WB: Arf1
Active Arf1 (Relative to CNT)
1.2
0.8
0.4
0.0
n=4 n=5 n=7
CNT 10 min 20 min 30min
0.7 µM BFA
E
60' SUS +
30min 10min 20min 30min
ManII-GFP
CNT
17.8 µM BFA
CNT 10min 20min 30min
20KDa
GGA3 PD
WCL
WB: Arf1
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1.2
0.8
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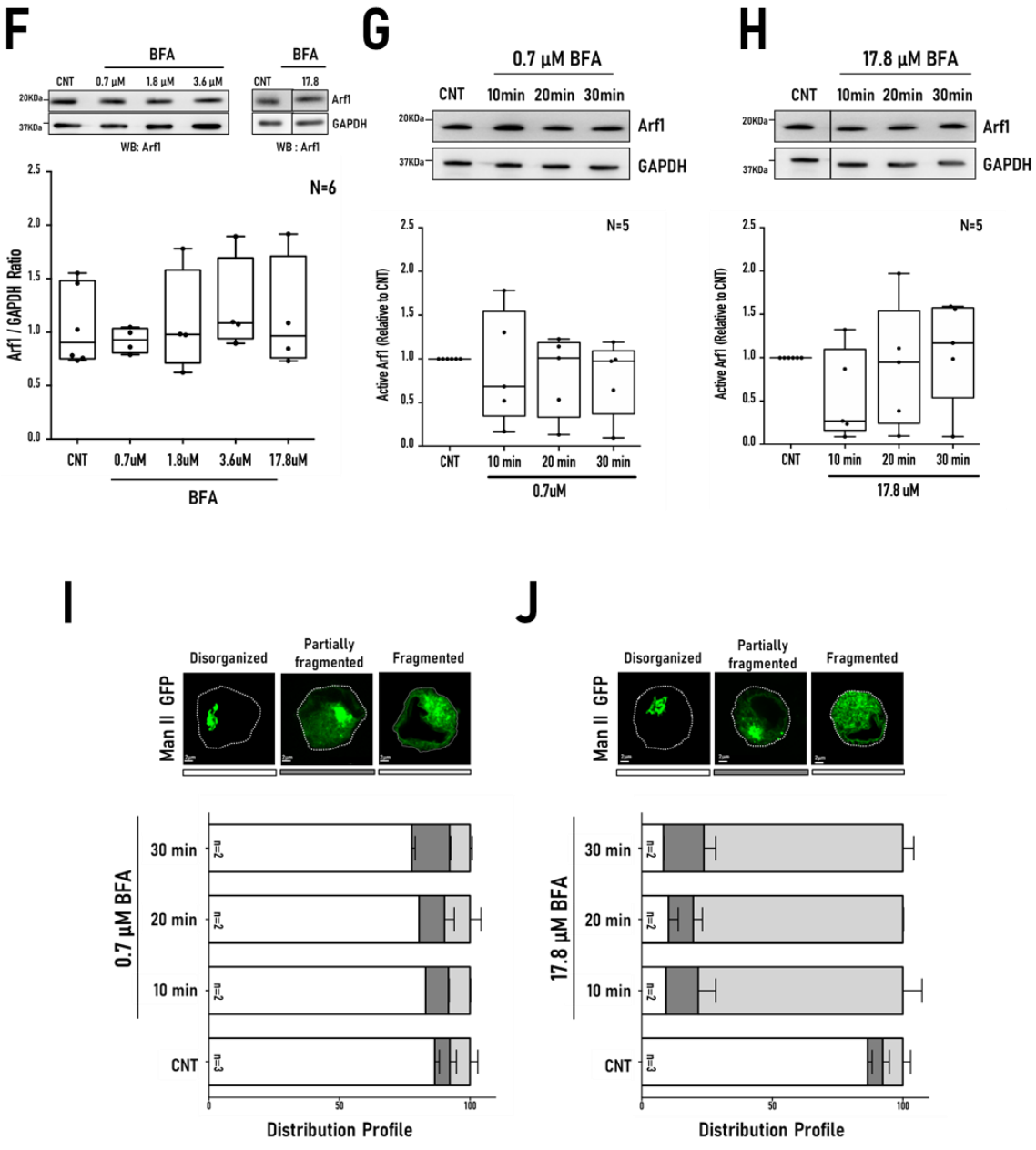
F
BFA
BFA
CNT
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CNT
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GAPDH
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WB : Arf1
N=6
Arf1 / GAPDH Ratio
CNT
0.7uM
1.8uM
3.6uM
17.8uM
BFA
G
0.7 µM BFA
CNT
10min
20min
30min
Arf1
GAPDH
N=5
Active Arf1 (Relative to CNT)
CNT
10 min
20 min
30 min
0.7uM
H
17.8 µM BFA
CNT
10min
20min
30min
Arf1
GAPDH
N=5
Active Arf1 (Relative to CNT)
CNT
10 min
20 min
30 min
17.8 uM
I
Disorganized
Partially fragmented
Fragmented
Man II GFP
0.7 µM BFA
30 min
20 min
10 min
CNT
n=2
n=2
n=2
n=3
Distribution Profile
J
Disorganized
Partially fragmented
Fragmented
Man II GFP
17.8 µM BFA
30 min
20 min
10 min
CNT
n=2
n=2
n=2
n=3
Distribution Profile

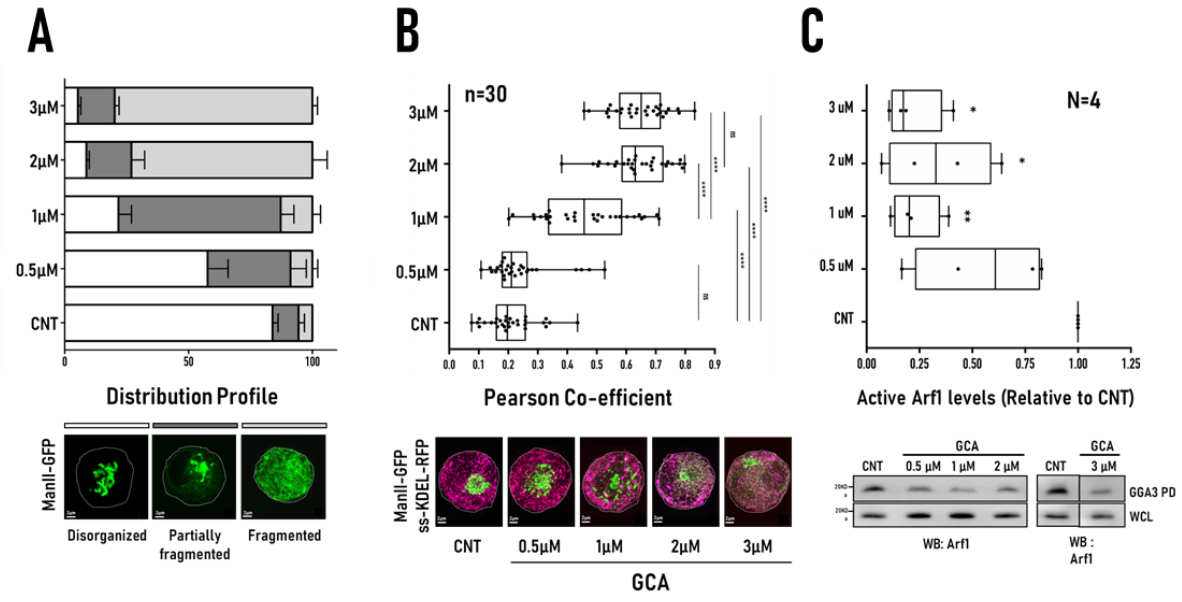
**Figure 3.4: Concentration-dependent BFA treatment of suspended WT-MEFs. (A)** WTMEFS expressing cis-medial Golgi (ManII-GFP) marker were held in suspension for 60 min and treated for 30min without (CNT) or with increasing concentrations of BFA (0.7µM, 1.8µM, 3.6µM, 17.8µM). The distribution of cells with disorganised (clear), partially fragmented (dark grey), and fragmented (grey) Golgi were manually counted, and the graph represents their mean±SE of percentage distribution from 3 independent. Selected representative images for each phenotype are presented below the graph. **(B)** The box and whisker plot represents the Pearson's coefficient of co-localisation for cis-Golgi (ManII-GFP) and ER (ss-KDEL-RFP) in WT-MEFs suspended for 60 min and treated for 30min without (CNT) or with BFA (0.7µM, 1.8µM, 3.6µM, 17.8µM), 30 cells from 3 independent experiments. Selected representative images for the prominent phenotype of each BFA concentration are presented below the graph, ManII-GFP is shown in green, and ss-KDEL-RFP is shown in magenta**. (C)** Western blot detection of active Arf1 pulled down using GST-GGA3 and equated to total Arf1 in whole-cell lysate from WT-MEFs in suspension for 60 min and treated for 30min without CNT or with BFA (0.7µM, 1.8µM, 3.6µM, 17.8µM). The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 normalised to respective control (CNT) from 3 independent experiments. Representative blots are shown below the graph. 17.8µM BFA treated samples were run on a separate gel with the respective control sample. The gel image break represents a gap in the lanes where these samples were run. **(D, E)** Representative images of cis-medial Golgi (ManII-GFP) phenotype in cells held in suspension for 60min (60' SUS+), treated without (CNT) or with **(D)** low BFA **(**0.7µM) or **(E)** high BFA (17.8µM) for 10min, 20min and 30min, respectively. The middle panel shows the respective representative blots. The break in gel images shown represents a gap in the lanes where these samples were run. The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 normalised to respective control (CNT) from 3 to 6 independent experiments. Statistical analysis was done using the Mann-Whitney test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001) or with normalised data using single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001). **(F)** Western blot detection of total Arf1 and GAPDH in WT-MEFs held in suspension for 60min and treated for 30min without or with increasing concentration of BFA (0.7µM, 1.8µM, 3.6µM and 17.8µM). The box and whisker plot represents the densitometric band intensity ratio of total Arf1 to GAPDH levels from 6 independent experiments. Representative blots are shown above the graph.17.8µM BFA treated samples were run on a separate gel with the respective control sample. The gel image break represents a gap in the lanes where these samples were run. **(G, H)** Western blot detection of total Arf1 and GAPDH in WT-MEFs untreated (CNT) or treated with BFA 0.7µM **(G)** or 17.8µM **(H)**for 10min, 20min, and 30min, respectively. The gel image break represents a gap in the lanes where these samples were run. The box and whisker plots represent the western blot intensity ratio of Arf1 to GAPDH levels from 5 independent experiments. (**I, J)** Distribution profile depicting the percentage of cells with disorganised (white), partially fragmented (deep grey), and fragmented (grey) Golgi phenotype in non-adherent WT-MEFs, held in suspension for 60 min and treated without (CNT) or with 0.7µM **(I)** or 17.8µM BFA **(J)** for 10min, 20min and 30min, respectively. Representative images are shown for each Golgi phenotype described.

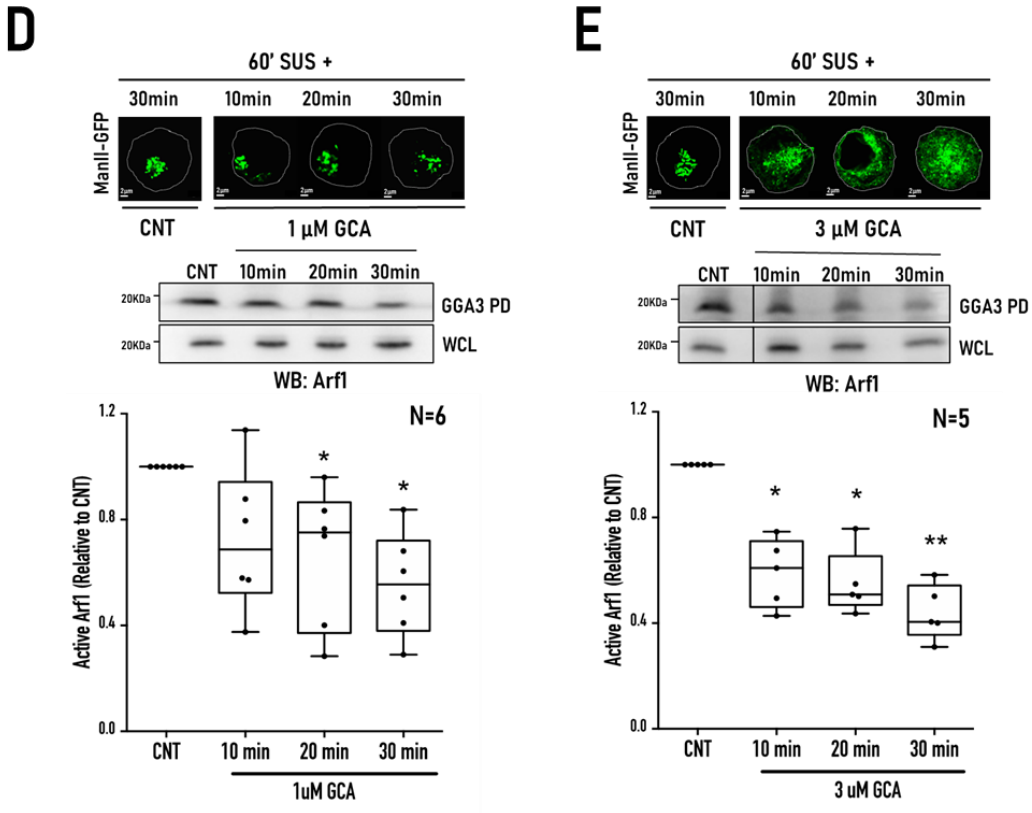
### 3.2.5. GCA-mediated decrease in Arf1 activation regulates Golgi fragmentation in non-adherent WTMEFs.

Arf1 activation at the cis-medial Golgi is regulated by the Arf GEF GBF1 (Kawamoto et al., 2002; Manolea et al., 2008; X. Zhao et al., 2002, 2006b). The fragmentation of cis-medial Golgi observed upon BFA treatment could result from the inhibition of GBF1 activity. To further confirm this, we used GCA, which specifically inhibits the activity of GBF1 (Sáenz et al., 2009). WT-MEFs transfected with ManII-GFP suspended for 60min were treated with increasing concentrations of GCA (0.5µM, 1µM, 2µM, 3µM) for 30min. The disorganised Golgi (ManII-GFP) in suspended cells showed a concentration-dependent increase in its fragmentation (Fig. 3.5A). GCA concentration-dependent increase in the extent of cis-medial Golgi fragmentation was reflected by the rise in the cis-medial Golgi overlap (ManII-GFP) with the ER (ss-RFP-KDEL) represented by the Pearson's coefficient of colocalisation (3.5B). The higher 2µM and 3µM GCA concentrations had comparable effects on the Golgi distribution profile and ER fallback. We further checked the effect of GCA on active Arf1 levels. We found that active Arf1 levels reduced comparably by ~65-75% relative to control across GCA concentrations (Fig. 3.5C), except for 0.5µM GCA (~45% decrease) (Fig. 4C). There was no significant change in the total Arf1 levels across all treatments (Fig. 3.4F). This is comparable to how increasing BFA concentrations affect Golgi organisation and Arf1 activation (Fig. 3.4B, 3.4C). This further suggests that the drop in net active Arf1 levels might not be sufficient to regulate Golgi organisation in these suspended cells.

Treatment with 1µM and 3uM GCA results in a comparable decrease in net active Arf1 levels but shows a distinct difference in their Golgi fragmentation profile (Fig. 3.5B, 3.5C). To test the effect, they have on the kinetics of Arf1 inactivation, WTMEFs suspended for 60min were incubated with GCA (1µM and 3µM) for 10min, 20min, and 30mins, respectively, and their Arf1 activation status was compared. 1uM GCA treatment caused Arf1 activity to drop by ~28% (10min), ~34% (20min) and ~45% (30min) (Fig. 3.5D). In comparison, 3µM GCA treatment causes a ~41% drop in Arf1 activation within the first 10min that decreases by ~45% at 20min and ~56% at 30min (Fig. 3.5E). No significant change in total Arf1 levels was observed across these treatments (Fig. 3.5G & 3.4H). Non-adherent WTMEFs expressing the cis-medial Golgi marker ManII-GFP when treated with 1µM GCA (causing a gradual drop in Arf1 activity) did not cause complete Golgi fragmentation, even when the drop in Arf1 activation is by ~45% after 30min (Fig. 3.5D & 3.5I). In contrast, cells treated with 3µM GCA

where the active Arf1 levels dropped rapidly (~41% in 10min) caused the Golgi to fragment (Fig. 3.4E & 3.4J). This and the results obtained with BFA suggest that the kinetics of Arf1 drop and the net Arf1 levels are vital for regulating Golgi organisation in suspended cells and suggest that the Arf GEF, GBF1, could predominantly regulate these effects (B. R. et al., 2023).


A
3μM
2μM
1μM
0.5μM
CNT
0
50
100
Distribution Profile
ManII-GFP
Disorganized
Partially fragmented
Fragmented
B
n=30
3μM
2μM
1μM
0.5μM
CNT
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
Pearson Co-efficient
ManII-GFP
ss-KDEL-RFP
CNT
0.5μM
1μM
2μM
3μM
GCA
C
N=4
3 uM
2 uM
1 uM
0.5 uM
CNT
0.00 0.25 0.50 0.75 1.00 1.25
Active Arf1 levels (Relative to CNT)
GCA
CNT
0.5 μM
1 μM
2 μM
GCA
CNT
3 μM
GGA3 PD
WCL
WB: Arf1
WB : Arf1


D
60' SUS +
30min
10min
20min
30min
ManII-GFP
CNT
1 μM GCA
CNT
10min
20min
30min
20KDa
GGA3 PD
20KDa
WCL
WB: Arf1
N=6
Active Arf1 (Relative to CNT)
1.2
0.8
0.4
0.0
CNT
10 min
20 min
30 min
1uM GCA
E
60' SUS +
30min
10min
20min
30min
ManII-GFP
CNT
3 μM GCA
CNT
10min
20min
30min
20KDa
GGA3 PD
20KDa
WCL
WB: Arf1
N=5
Active Arf1 (Relative to CNT)
1.2
0.8
0.4
0.0
CNT
10 min
20 min
30 min
3 uM GCA

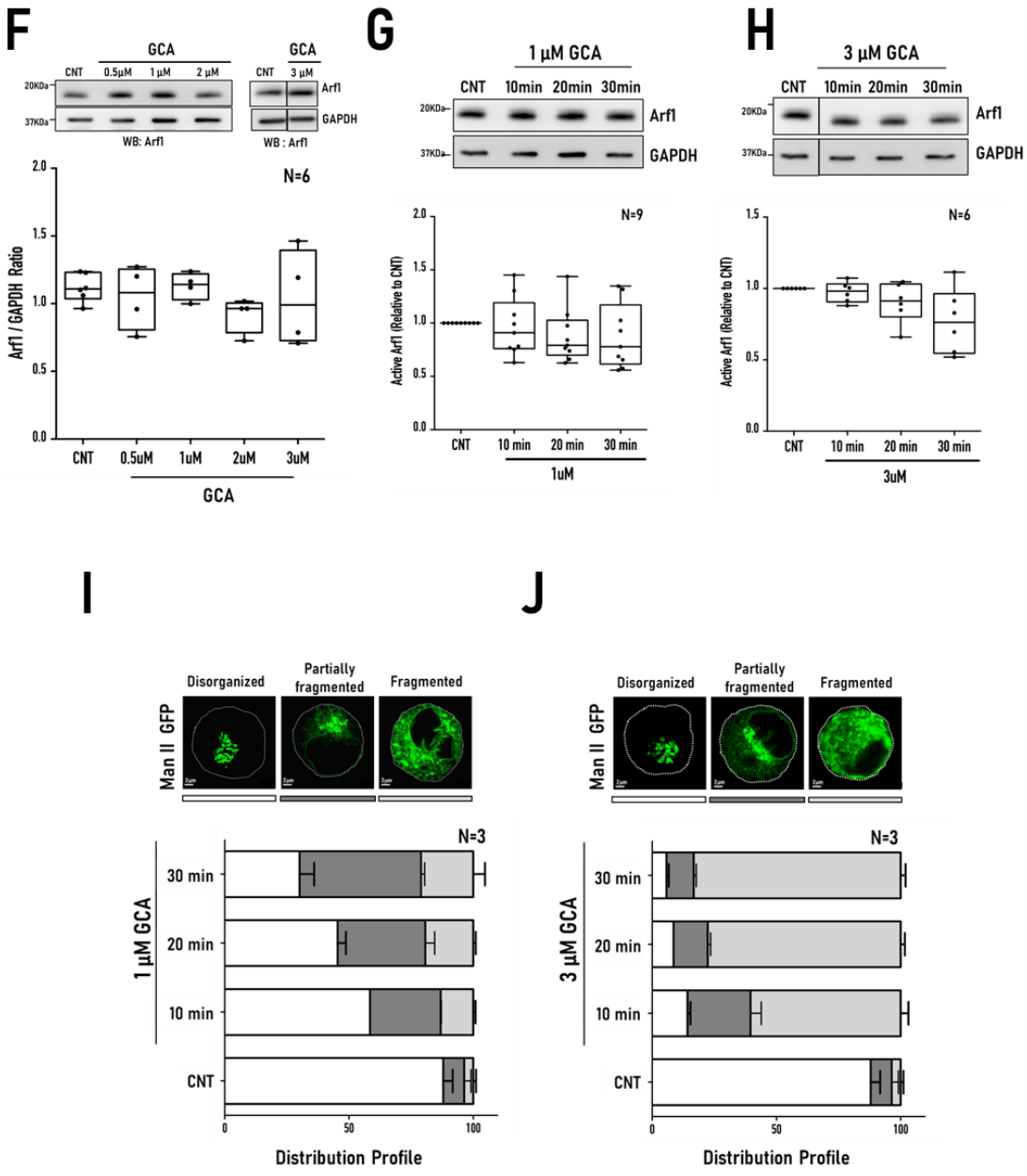

F
GCA
CNT
0.5µM
1 µM
2 µM
GCA
CNT
3 µM
20KDa
37KDa
Arf1
GAPDH
WB: Arf1
WB : Arf1
N=6
Arf1 / GAPDH Ratio
CNT
0.5uM
1uM
2uM
3uM
GCA
G
1 µM GCA
CNT
10min
20min
30min
Arf1
GAPDH
N=9
Active Arf1 (Relative to CNT)
CNT
10 min
20 min
30 min
1uM
H
3 µM GCA
CNT
10min
20min
30min
Arf1
GAPDH
N=6
Active Arf1 (Relative to CNT)
CNT
10 min
20 min
30 min
3uM
I
Disorganized
Partially fragmented
Fragmented
Man II GFP
N=3
1 µM GCA
30 min
20 min
10 min
CNT
Distribution Profile
J
Disorganized
Partially fragmented
Fragmented
Man II GFP
N=3
3 µM GCA
30 min
20 min
10 min
CNT
Distribution Profile

**Figure 3.5: Concentration-dependent GCA treatment of suspended WT-MEFs. (A)** WTMEFS expressing cis-medial Golgi (ManII-GFP) marker were held in suspension for 60min treated for 30min without (CNT) or with increasing concentrations of GCA (0.5µM, 1µM, 2µM, 3µM). The distribution of cells with disorganised (clear), partially fragmented (dark grey), and fragmented (grey) Golgi were manually counted, and the graph represents their mean±SE of percentage distribution from 3 independent experiments. Selected representative images are presented below the graph. **(B)** The box and whisker plot represents the Pearson's coefficient of co-localisation for cis-medial Golgi (ManII-GFP), nd ER (ss-KDEL-RFP) in WT-MEFs suspended for 60 min and treated for 30min without (CNT) or with GCA (0.5µM, 1µM, 2µM, 3µM), 30 cells from 3 independent experiments. Selected representative images for the prominent phenotype of each GCA concentration are presented below the graph, ManII-GFP is shown in green, and ss-KDEL-RFP is shown in magenta. **(C)** Western blot detection of active Arf1 pulled down using GST-GGA3 and equated to total Arf1 in whole-cell lysate from WT-MEFs suspended for 60 min and treated for 30min without (CNT) or with GCA (0.5µM, 1µM, 2µM, 3µM). The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 normalised to respective control (CNT) from 3 independent experiments. Representative blots are shown below the graph. 3µM GCA treated samples were run on a separate gel with the respective control sample. The gel image break represents a gap in the lanes where these samples were run. **(D, E)** Representative images of cis-medial Golgi (ManII-GFP) phenotype in cells held in suspension for 60min (60' SUS+), treated without (CNT) or with **(D)** low GCA (1µM) or **(E)** high GCA (3µM) for 10min, 20 min and 30 min. The middle panel shows the respective representative blots. The gel image break represents a gap in the lanes where these samples were run. The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 from 5 or 6 independent experiments. Statistical analysis was using the Mann-Whitney test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001) or normalised data using single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001). **(F)** Western blot detection of total Arf1 and GAPDH in WT-MEFs held in suspension for 60min and treated for 30min without or with increasing concentration of GFA (0.5µM, 1µM, 2µM and 3µM). The box and whisker plot represents the densitometric band intensity ratio of total Arf1 to GAPDH levels from 6 independent experiments. Representative blots are shown above the graph.3µM BFA treated samples were run on a separate gel with the respective control sample. The gel image break represents a gap in the lanes where these samples were run. **(G, H)** Western blot detection of total Arf1 and GAPDH in WT-MEFs untreated (CNT) or treated with GCA 1µM **(G)** or 3µM **(H)**for 10min, 20min, and 30min, respectively. The gel image break represents a gap in the lanes where these samples were run. The box and whisker plots represent the western blot intensity ratio of Arf1 to GAPDH levels from 9 and 6 independent experiments, respectively. **(I, J)** Distribution profile depicting the percentage of cells with disorganised (white), partially fragmented (deep grey), and fragmented (grey) Golgi phenotype in non-adherent WT-MEFs, held in suspension for 60 min and treated without (CNT) or with 1µM **(I)** or 3µM GCA **(J)** for 10min, 20min and 30min, respectively. Representative images are shown for each Golgi phenotype described.

### 3.2.6. Localisation of GBF1 in non-adherent WT-MEFs.

In adherent cells, GBF1 is known to localize and regulate Arf1activation at the ERGIC and cis-medial Golgi (Kawamoto et al., 2002; Manolea et al., 2008; X. Zhao et al., 2002, 2006b). In non-adherent WT-MEFs, we have seen that the activation status of Arf1 is sustained at cis-medial Golgi, as a result of which the cis-medial Golgi is disorganised to a lesser extent. Like adherent cells, the activity of Arf1 at the cis-medial Golgi in non-adherent cells could be regulated by GBF1. To confirm this, we looked at the localisation of GBF1 in non-adherent cells relative to the cis-medial Golgi. In non-adherent WT-MEFs, GBF1 overlap with the cis-medial Golgi is comparable to that seen in adherent and early re-adherent (15' FN) WT-MEFs (Fig. 3. 6A).

To check if the kinetics of drop in Arf1 activation is mediated through GBF1 activity at the cis-medial Golgi, we looked at GBF1 localisation in WT-MEFs treated with low vs high inhibitor concentration. WT-MEFs transfected with ManII-GFP were held in suspension for 60min and then treated with BFA (0.7µM or 17.8µM) or GCA (1µM or 3µM) for 30mins. These cells were then immunostained with anti-GBF1 antibody to look at GBF1 localisation. Treatment with 0.7µM BFA or 1µM GCA, which did not cause Golgi fragmentation, did not displace GBF1 from the cis-medial Golgi (Fig. 3.6B). Treatment with 17.8µM BFA or 3µM GCA, which fragmented the Golgi displaced GBF1 from the Golgi membranes (Fig. 3.6C). This suggests that BFA and GCA-mediated inhibition of GBF1 drives the differential kinetics of change in Arf1 activation to affect cis-medial Golgi fragmentation (B. R. et al., 2023).

### 3.2.7. Constitutive active Arf1 (Q71L) rescues BFA/GCA mediated Golgi fragmentation.

BFA and GCA inhibit Arf1 activation at the Golgi by inhibiting the Arf GEFs responsible for the GDP to GTP exchange on Arf1. BFA also affects the ADB-ribosylation of cytosolic proteins like BARS50 and GAPDH (Di Girolamo et al., 1995; Weigert et al., 1997). BARS50 has been shown to protect against BFA-mediated Golgi fragmentation. ADP-ribosylation of BARS50 inhibits the activity of BARS50 at the Golgi membranes and aids Golgi fragmentation (A. Mironov et al., 1997; Weigert et al., 1997). To confirm that Golgi fragmentation mediated by BFA/GCA is driven by the loss of active Arf1 at the Golgi, we expressed constitutively active Q71L-Arf1-mCherry (or WT-Arf1-mCherry) with ManII-GFP in WT-MEFs. These cells were held in suspension for 60min and then treated with 17.8uM BFA or 3uM GCA for 30min, the highest concentration of BFA or GCA seen to completely fragment the Golgi in earlier experiments (Fig. 3.4A, 3.5A). The effect constitutively active Arf1 (Q71L) and WT Arf1 have on Golgi organisation in these inhibitor-treated cells was compared. In WT Arf1 expressing cells treated with BFA and GCA, ~87% and ~85% of cells had a fragmented Golgi (Fig. 3.6D). Constitutively active Arf1 (Q71L) causes this percentage of fragmented Golgi-containing cells to drop to ~19% and ~17%, respectively (Fig. 3.6E). This is also reflected in a distinct increase in the percentage of cells with disorganised Golgi in Q71L Arf1 expressing cells treated with BFA (~35%) and GCA (~46%), relative to WT Arf1 expressing cells (BFA – 3.6%, GCA – 3.9%) (Fig. 3.6D, 3.6E). Together these studies reveal constitutively active Arf1 can rescue Arf GEF inhibitor (BFA / GCA) mediated fragmentation of Golgi in suspended WTMEFs. It hence confirms that the fragmentation of the Golgi upon Arf GEF inhibitor treatment is indeed mediated by loss in Arf1 activity (B. R. et al., 2023).

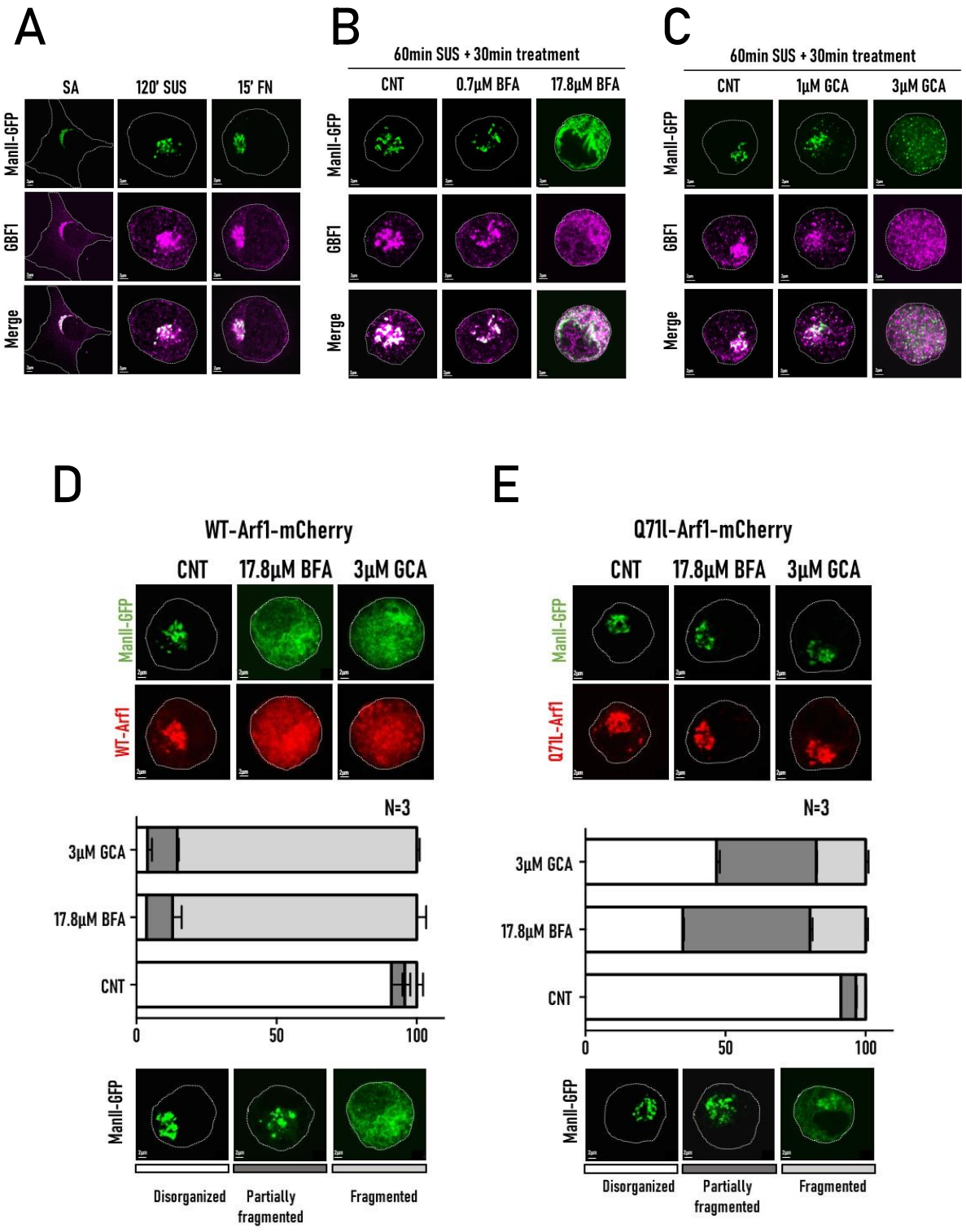


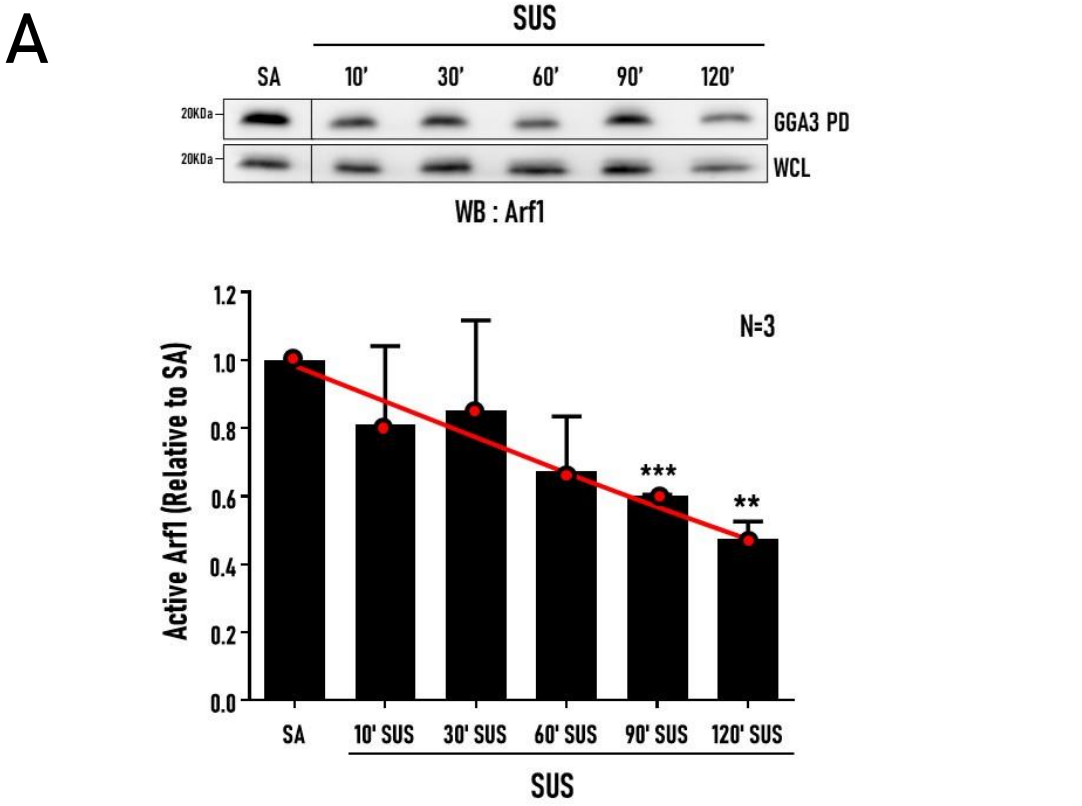

**Figure 3.6 (A)** Representative immunostained images of GBF1 in ManII-GFP expressing WTMEFs, stable adherent (SA), suspended (120' SUS), and re-adherent on fibronectin (15' FN). **(B, C)** ManII-GFP expressing WT-MEFs (CNT) suspended for 60min and treated with **(B)** 0.7μM BFA or 17.8μM BFA for 30min (60' SUS+30min treatment) or **(C)** 1μM GCA or 3μM GCA for 30min (60' SUS+30min treatment) and immunostained with GBF1. Representative ManII-GFP, GBF1, and merged images are shown. **(D, E)** WT-MEFs expressing **(D)** WT-Arf1-mCherry or **(E)** Q71L-Arf1-mCherry held in suspension for 60min

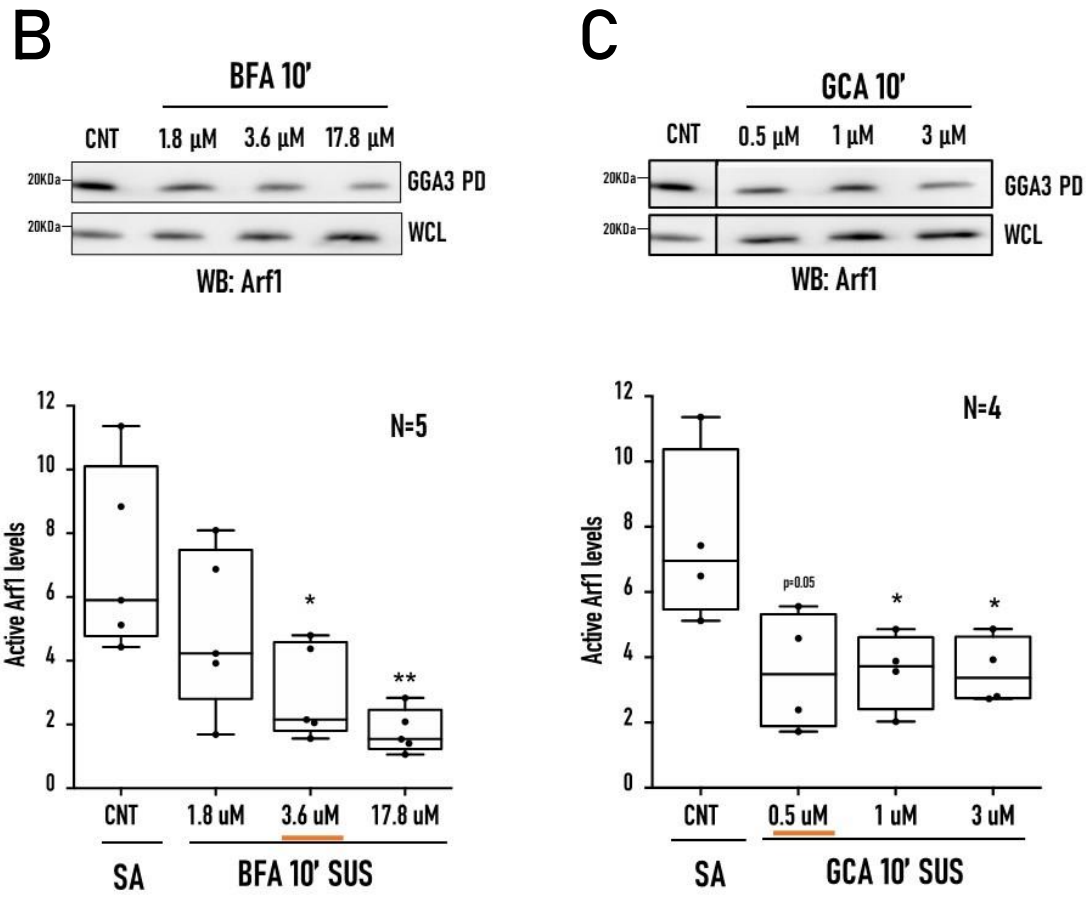
and treated with 17.8μM BFA or 3μM GCA for30min (60' SUS+30min treatment). The upper panel shows representative images of the predominant Golgi phenotype (ManII-GFP) in **(D)** WT-Arf1-mCherry or **(E)** Q71L-Arf1-mCherry expressing cells. The graph shows the percentage distribution of cells with disorganised (clear), partially fragmented (dark grey), and fragmented (grey) Golgi, represented as their mean±SE from 3 independent experiments. Selected representative images for each Golgi phenotype (ManII-GFP) are presented below the graph.

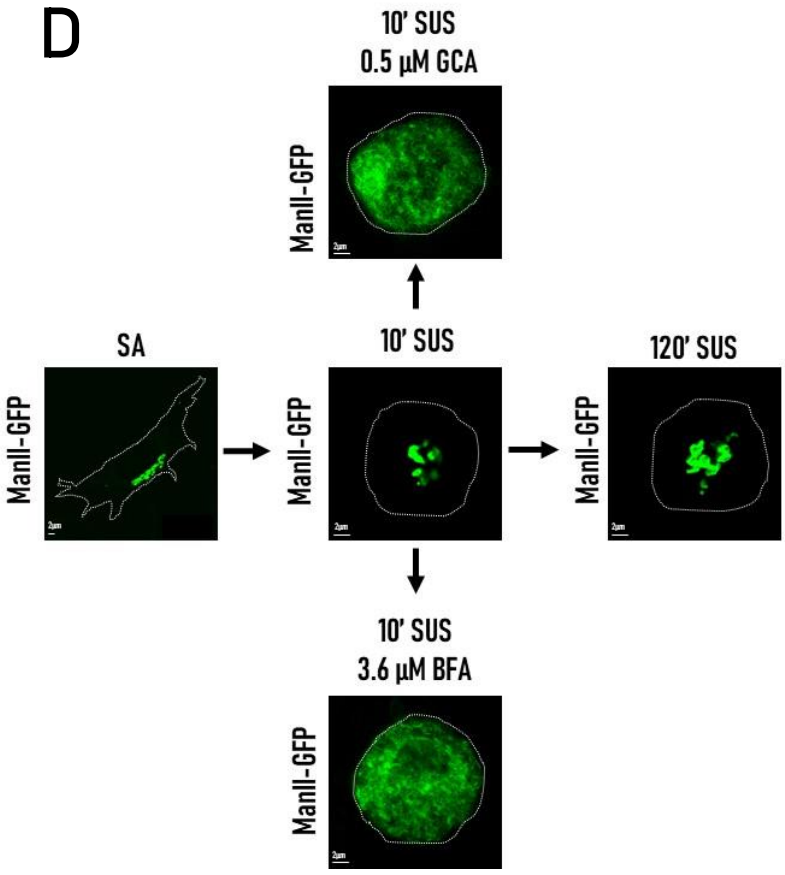
### 3.2.8. Kinetics of Arf1 inactivation regulates loss of adhesion-mediated Golgi disorganisation.

The inhibitor studies in non-adherent WT-MEFs done so far suggest that the kinetics of change in active Arf1 level and total active Arf1 level are both vital in regulating Golgi organisation in these cells. The drop in active Arf1 levels seen upon loss of cell-matrix adhesion and the change in Golgi organisation could similarly be affected by the kinetics of the change in active Arf1 levels. (Fig. 1C, (Singh et al., 2018)). To evaluate this, WTMEFs were suspended for 10, 30, 60, 90, and 120 min, and their respective active Arf1 levels determined. We observed a steady decrease in active Arf1 levels over time. A 15-20% drop in Arf1 activation in 10 and 30min further drops to ~33% in 60min, ~40% in 90min, and eventually, ~53% in 120min relative to stable adherent cells (Fig. 3.7A).

We wanted to test if the kinetics of change in active Arf1 level were vital to the Golgi phenotype in non-adherent cells. To test this, we asked if disrupting the kinetics without significantly affecting the net active Arf1 levels can affect the Golgi disorganisation observed upon loss-of-adhesion. Arf1 activity drops by ~50% in WT-MEFs suspended for 120 min. Hence, we used a range of BFA and GCA concentrations to determine the lowest concentration of each inhibitor that causes a similar ~50% drop in Arf1 activity, but in 10min of treatment of detached cells. We found 3.6µM BFA and 0.5µM GCA treatment led to ~54% and ~45% drop in active Arf1 levels, respectively, in WT-MEFs detached and held in suspension for 10 min (Fig. 3.7B & 3.7C, respectively). 0.5uM GCA failed to fragment the Golgi when cells were detached and held in suspension for 60 min and then treated for 30 min. However, when cells were detached and immediately treated with 0.5uM GCA for 10 min the Golgi fragments. This difference could be due to a distinct difference in the kinetics of change in Arf1 activation observed in the above two conditions. Golgi organisation in detached cells (with Golgi that is beginning to disorganize) treated with 0.5uM GCA (Fig. 3.7C) is fragmented vs it being disorganized in cells already suspended for 60mins (with disorganised Golgi) and then treated with GCA (Fig. 3.5A). Both 3.6µM BFA and 0.5µM GCA treatments and the resulting decrease in Arf1 activation in detached cells cause the cis-medial Golgi (Man II GFP) to fragment, unlike on loss of adhesion for 120 min where the Golgi is disorganised (Fig. 3.7D). This is despite both having comparable net active Arf1 levels (Fig. 3.7A, 3.7B, 3.7C), suggesting the difference in kinetics of decrease in active Arf1 levels could mediate the differences seen in Golgi organisation (B. R. et al., 2023).


A
SUS
SA
10'
30'
60'
90'
120'
20KDa
GGA3 PD
WCL
WB : Arf1
Active Arf1 (Relative to SA)
N=3
SA
10' SUS
30' SUS
60' SUS
90' SUS
120' SUS
SUS


B
BFA 10'
CNT
1.8 µM
3.6 µM
17.8 µM
GGA3 PD
WCL
WB: Arf1
Active Arf1 levels
N=5
CNT
1.8 uM
3.6 uM
17.8 uM
SA
BFA 10' SUS
C
GCA 10'
CNT
0.5 µM
1 µM
3 µM
GGA3 PD
WCL
WB: Arf1
Active Arf1 levels
N=4
p=0.05
CNT
0.5 uM
1 uM
3 uM
SA
GCA 10' SUS


D
10' SUS
0.5 µM GCA
ManII-GFP
SA
ManII-GFP
10' SUS
ManII-GFP
120' SUS
ManII-GFP
10' SUS
3.6 µM BFA
ManII-GFP

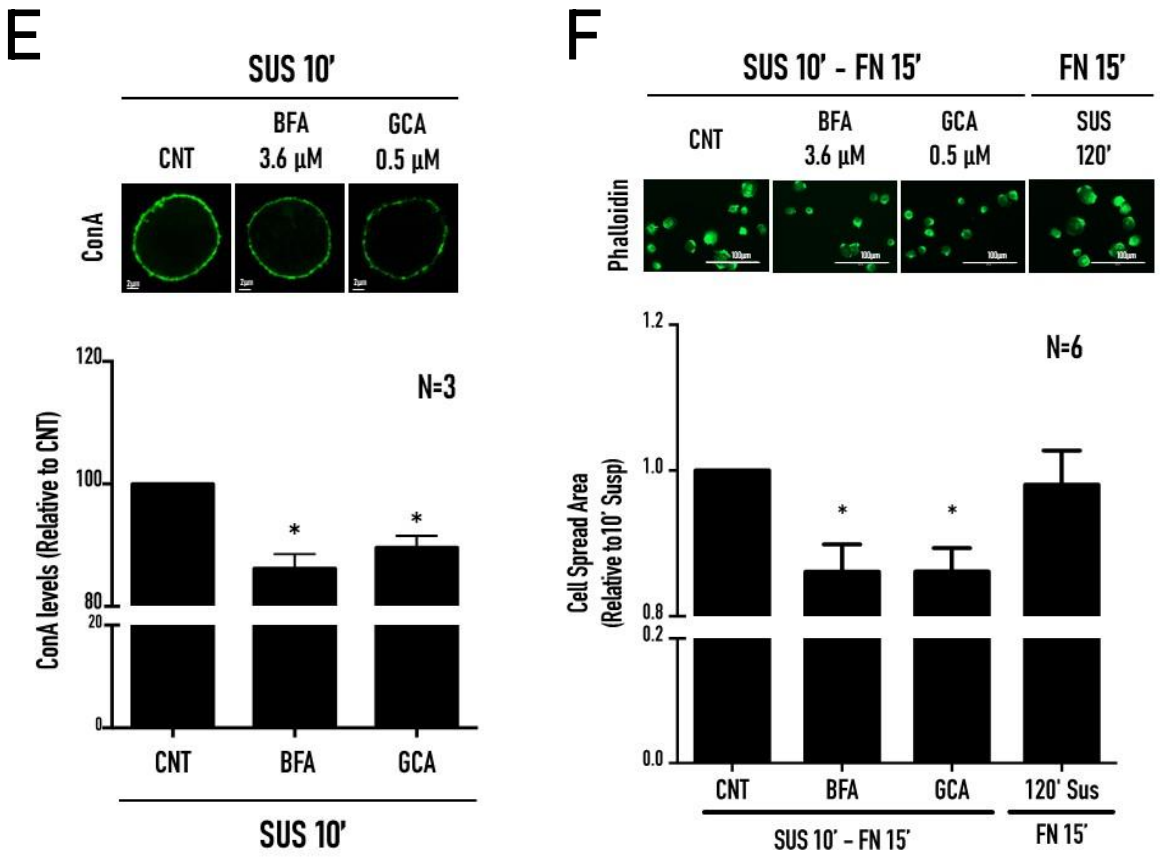

E
SUS 10'
CNT
BFA
3.6 µM
GCA
0.5 µM
ConA
N=3
ConA levels (Relative to CNT)
120
100
80
20
0
*
*
CNT
BFA
GCA
SUS 10'
F
SUS 10' - FN 15'
FN 15'
CNT
BFA
3.6 µM
GCA
0.5 µM
SUS
120'
Phalloidin
N=6
Cell Spread Area
(Relative to10' Susp)
1.2
1.0
0.8
0.2
0.0
*
*
CNT
BFA
GCA
120' Sus
SUS 10' - FN 15'
FN 15'

**Figure 3.7: Kinetics of Arf1 inactivation on the loss of adhesion in WT-MEFs.** Western blot detection of active Arf1 (WB: Arf1) pulled down using GST-GGA3 (GGA3 PD) and total Arf1 in the whole-cell lysate (WCL) from WT-MEFs, **(A)** detached and held in suspension for increasing time (10', 30', 60', 90', 120' SUS) relative to stable adherent (SA) cells or **(B, C)** suspended and treated for 10min in suspension, with **(B)** BFA 1.8µM, 3.6µM and 17.8µM or **(C)** GCA 0.5µM, 1µM and 3µM relative to respective stable adherent control cells (CNT, untreated). The box and whisker plot represents the densitometric band intensity ratio of active to total Arf1 normalised to respective control (CNT) from 3 independent experiments. **(D)** Representative cross-section images of cis-Golgi (ManII-GFP) phenotype in WT-MEFs, stable adherent (SA), suspended for 10min (10' SUS), 120min (120' SUS), or suspended for 10min (10' SUSP) with 0.5µM GCA and 3.6µM BFA. **(E)** WT-MEFs held in suspension for 10min (SUS 10'), without (CNT), or with 3.6uM BFA or 0.5uM GCA were surface labelled with ConA-Alexa-488. The graph represents the median surface-bound ConA fluorescence normalised to CNT from 3 independent experiments. Representative images are shown above the graph. **(F)** WT-MEFs held in suspension for 10min (SUS 10'), without (CNT), or with 3.6uM BFA or 0.5uM GCA or in suspension for 120min, were replated on fibronectin for 15min (FN15') and cell spreading was evaluated by phalloidin staining. The box and whisker plot represents six independent experiments' mean cell spread area. Representative images are shown above their respective graph. Statistical analysis was using the Mann-Whitney test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001) or for normalised data using single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001).

### 3.2.9. Effect change in the kinetics of Arf1 activation has on Golgi function.

The primary function of the Golgi apparatus is to process and modify the newly synthesized proteins and lipids. The proteins and lipids processed in the Golgi are then packaged and delivered to their respective destinations. Golgi also plays a very crucial role in regulating cellular signalling pathways and homeostasis. Glycosylation of proteins is one of the major functions of the Golgi. Glycosylation plays a significant role in assisting protein folding and determining the stability and functions of the proteins (Pokrovskaya et al., 2011; P. Stanley, 2011).

Golgi organisation is known to affect the trafficking and processing of proteins (Pokrovskaya et al., 2011). Furthermore, loss of cell-matrix adhesion-mediated disorganisation of the Golgi affects cell surface glycosylation (Singh et al., 2018). Hence, we wanted to determine if the kinetics of change in activation of Arf1 and resulting effect on Golgi organisation impact Golgi function. To check this, we determined the cell surface ConA levels of WT-MEFs detached and held in suspension for 10mins, untreated or treated with 3.6µM BFA or 0.5µM GCA. Treatment with 3.6µM BFA or 0.5µM GCA showed a significant reduction in cell surface ConA binding relative to control cells (Fig. 3.7E).

Also, for non-adherent cells, re-adhesion to the cell matrix is one of the crucial phenomena to help them re-establish the normal signalling pathways. Re-adhesion can be affected by changes in cell surface glycosylation. Hence, we looked at the early adhesion-dependent spreading of WT-MEFs detached and held in suspension for 10min treated without or with 3.6µM BFA or 0.5µM GCA. Compared to control WT-MEFs and WT-MEFs suspended for 120min, the BFA/GCA treated WT-MEFs showed a significant reduction in the cell spread area upon replating on fibronectin for 15min. Together these results reveal that the kinetics of Arf1 activation in cells controls Golgi organisation and function.

### 3.2.10 Effect of BFA/GCA treatment on trans-Golgi compartment.

Loss of cell-matrix leads to a drop in cellular levels of active Arf1, which leads to Golgi disorganisation. We see that most of the active Arf1 is lost from the trans-Golgi membrane, which leads to its greater disorganisation when compared to the cis-medial Golgi compartment. The loss in active Arf1 levels upon loss of adhesion is predominately mediated by BIG1/2(Singh et al., 2018). We find that GBF1 is retained at the cis-medial Golgi facilitating Arf1 activation. Further, BFA/GCA treatment of non-adherent WT-MEFs leads to the displacement of GBF1 from the Golgi causing Golgi fragmentation.

Though we saw that most of the active Arf1 is lost from the trans-Golgi membranes in non-adherent cells, we were interested in checking if inhibitor mediated inactivation of Arf1 in these cells could affect the trans-Golgi organisation. Therefore, we treated WT-MEFs expressing GalTase-RFP (trans-Golgi marker) with increasing concentrations of BFA or GCA. We observed that the treatment with BFA or GCA led to a further drop in cellular active Arf1 levels (Fig. 3.4C, 3.5C) but had no effect on the trans-Golgi compartment organisation. Even in the cells treated with the highest concentration of BFA and GCA, the trans-Golgi remained disorganised, unlike the fragmented cis-medial Golgi (Fig. 3.8A, 3.8B). This is further confirmed by the distribution profile (Fig. 3.8C, 3.8D). Loss of cell-matrix adhesion is known to predominantly affect the activity of BIG1/2 at the Golgi (Singh et al., 2018). The loss of BIG1/2 activity at the Golgi could be due to the loss or decrease in the association of BIG1/2 with the Golgi membranes in non-adherent cells. This could cause a reduction in Arf1 activation at the trans-Golgi (Fig 3.2A) as BIG1/2 is known to predominantly localise to the trans-Golgi and regulates the activation of Arf1 at the trans-Golgi (García-Mata et al., 2003; Manolea et al., 2008; Yamaji et al., 2000). As a result of this, the disorganised trans-Golgi in non-adherent cells may no longer be regulated by active Arf1. Hence, further changes in total active Arf1 levels through inhibitor treatments may not affect the disorganised trans-Golgi (Fig. 3.8A, 3.8B)(B. R. et al., 2023).

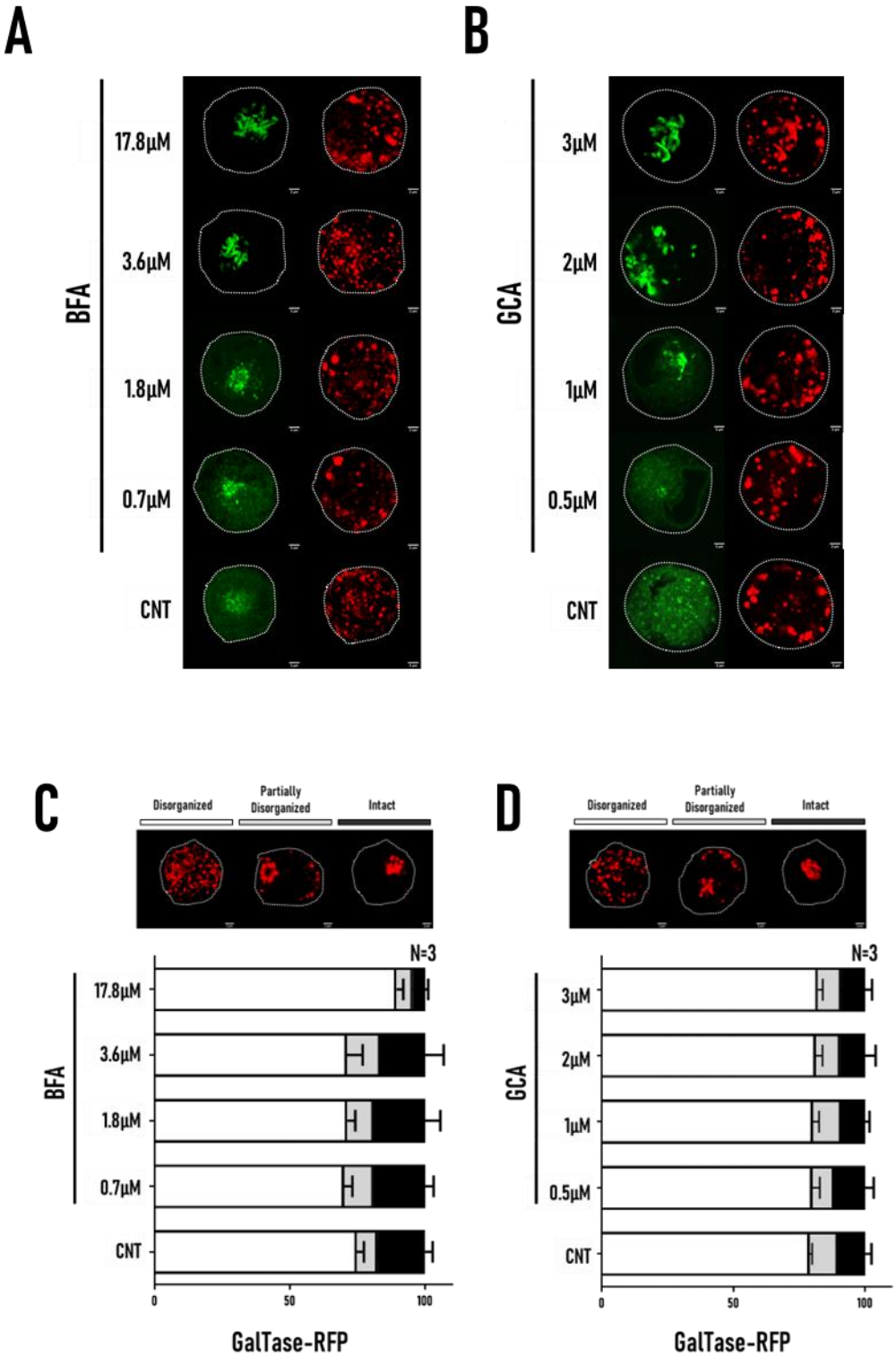

A
BFA
17.8μM
3.6μM
1.8μM
0.7μM
CNT
B
GCA
3μM
2μM
1μM
0.5μM
CNT
C
Disorganized
Partially Disorganized
Intact
N=3
BFA
17.8μM
3.6μM
1.8μM
0.7μM
CNT
0
50
100
GalTase-RFP
D
Disorganized
Partially Disorganized
Intact
N=3
GCA
3μM
2μM
1μM
0.5μM
CNT
0
50
100
GalTase-RFP

**Figure 3.8: Concentration-dependent BFA and GCA treatment of suspended WT-MEFs.** WTMEFS expressing cis-medial Golgi (ManII-GFP) marker and trans-Golgi marker (GalTase-RFP) were held in suspension for 60 min and treated for 30min without (CNT) or with increasing concentrations of **(A)** BFA (0.7µM, 1.8µM, 3.6µM, 17.8µM) or **(B)** GCA (0.5µM, 1µM, 2µM, 3µM). **(C, D)** The distribution of cells with disorganised (clear), partially disorganised (grey), and intact (black) trans-Golgi was manually counted in cells treated with BFA **(C)** or GCA **(D)**, and the graph represents their mean±SE of percentage distribution from 3 independent. Selected representative images for each phenotype are presented below the graph.

## 3.3. Summary.

Arf1 acts as a vital regulator of Golgi organisation and functions downstream of multiple stimuli. In mouse fibroblasts, loss of adhesion leads to a ~50% drop in active Arf1 levels which causes the Golgi to disorganise (Fig. 3.1A, 3.1B, 3.1C, (Singh et al., 2018)). This disorganisation of Golgi observed upon loss of adhesion is distinctly different from the Golgi fragmentation, where the Golgi falls back to ER. (Nihal Altan-Bonnet et al., 2006a; Lippincott-Schwartz et al., 1989b; Singh et al., 2018). The differential disorganisation of the trans-Golgi further illustrates the distinct regulation of the Golgi on loss-of-adhesion (Fig. 1B). This difference could be mediated by the difference in the activation status of Arf1 at the cis/cis-medial vs trans Golgi compartments. We find that upon loss of adhesion, the cis-Golgi retains active Arf1, whereas the trans-Golgi does not (detected using GFP-ABD, Fig. 3.2A & 3.2B). The active Arf1 at the cis-medial Golgi allows us to further test the role of Arf1 in regulating Golgi organisation and function in non-adherent fibroblasts.

The activity of Arf1 at the Golgi is regulated by the Arf GEFs, GBF1, BIG1 and BIG2 ((Manolea et al., 2008). Their relative expression in WTMEFs (BIG1> GBF1> BIG2) is unaffected by loss of adhesion (Fig. 3.3A, 3.3B). The preferential localisation of GBF1 to the ER-Golgi intermediate compartment (ERGIC) and cis-Golgi, with BIG1/2 localising to the trans-Golgi (García-Mata et al., 2003; Manolea et al., 2008; Yamaji et al., 2000) could support their differential regulation of Golgi compartments in non-adherent cells. Like in stable adherent cells, in non-adherent cells, GBF1 remains localised to the cis-medial Golgi (Fig 3.6A), and could contribute to the activation of Arf1 at the cis-medial Golgi.

Further inhibition of Arf1 in non-adherent cells leads to the fragmentation of the disorganised Golgi (Singh et al., 2018). This provides a unique cellular context to study the role of Arf1 GEFs mediated regulation of Arf1 activity at the Golgi and the role it has in regulating the Golgi organisation and function in non-adherent cells. This study treating suspended WT-MEFs with increasing concentrations of BFA and GCA reveals Golgi fragmentation to be dependent on the kinetics of the drop in Arf1 activity and net active Arf1 levels (Fig. 3.4 & 3.5). Over-expression of active-Arf1 is seen to prevent the Golgi fragmentation by BFA/GCA treatment (Fig. 3.6D & Fig. 3.6E). In non-adherent cells, BFA and GCA have a comparable effect on the organisation of Golgi, and this suggests that the inhibition of the activity of GBF1 at the cis-medial Golgi could mediate the effect seen upon BFA/GCA treatment. This is confirmed by the localisation of GBF1 at the cis-medial Golgi, which is dispersed on Golgi fragmentation during BFA/GCA treatment (Fig. 3.6A, 3.6B & 3.6C). The trans-Golgi disorganised upon loss

of addition remains disorganised on BFA/GCA treatment (Fig. 3.8). This could be supported by the observation that, in non-adherent cells, most of the active Arf1 is retained at the cis-medial Golgi compared to the trans-Golgi (Fig. 3.2). There is almost no active Arf1 at the trans-Golgi. Hence a further drop in Arf1 activation upon BFA/GCA treatment might not affect the organisation of trans-Golgi.

On loss of adhesion, the gradual drop (over 120min) in active Arf1 levels to ~50% of levels seen in SA (Fig. 3.7A) supports Golgi disorganisation (but not fragmentation), which in turn regulates Golgi function (Singh et al., 2018). On the other hand, BFA and GCA treatment of non-adherent fibroblasts, by rapidly decreasing active Arf1 levels to ~50% of levels seen in SA cells in 10min causes the Golgi to fragment, disrupting its function (Fig. 3.7B-F). This suggests the kinetics Arf1 inactivation by regulating Arf GEFs and also possibly Arf GAPS (which remains to be tested) has vital implications for Golgi organisation in cells.

## 3.4. Conclusion.

Along with the net active Arf1 levels, the kinetics of Arf1 activation also significantly contributes to the regulation of cis-medial Golgi organisation in non-adherent cells. Such a regulation could have implications for how the Golgi organisation is controlled in cells.

## 3.5. Future directions

This study shows that, the loss of adhesion does not displace GBF1 from the Golgi membranes. However, more research is required to determine if loss of adhesion affects the localisation of BIG1/BIG2 to the trans-Golgi. Furthermore, the specific molecule(s) responsible for the signal transduction between integrin and Arf GEFS is yet to be identified.

# CHAPTER 4

# Adhesion-dependent regulation of microtubule acetylation. Its role in Golgi organisation.

# 4.1. Rationale.

In mammals, the Golgi apparatus has a peri-nuclear localisation, being organised close to the Microtubule Organizing Center (MTOC) (Nakamura et al., 2012). Given its localisation, it has been seen that microtubules play a significant role in organising and maintaining the Golgi structure. Two different sets of microtubules are involved in organising the Golgi structure. The microtubule arising from the MTOC facilitates the positioning of the Golgi near the MTOC. Also, the Golgi apparatus can nucleate and give rise to microtubules (Chabin-Brion, Me Marceiller, et al., 2001; Wu, De Heus, et al., 2016)(Chabin-Brion, Me Marceiller, et al., 2001). Furthermore, the microtubules arising from the Golgi help bring the Vesicular-tubular clusters (VTCs) from ER-exit sites towards the MTOC by the "search and capture" method and facilitate their fusion with the Golgi cisternae. Disruption in microtubule organisation by nocodazole treatment has resulted in Golgi fragmentation, proving the role of microtubules in the organisation and maintenance of Golgi structure (Sandoval et al., 1984).

Active Arf1 is known to recruit Golgin-160 to the Golgi membranes. Golgin-160 binds to dynein, a minus end-motor protein (Yadav et al., 2012). Golgi membrane association with dynein results in its organising at the MTOC. Upon loss of adhesion, active Arf1 levels drop and recover post-re-adhesion. Similarly, it has been seen that dynein levels associated with active Arf1 drop during suspension and, recover post-re-adhesion (Singh et al., 2018). Inhibition of dynein with ciliobrevin inhibits re-organisation of the Golgi post-re-plating on FN for 15 mins. This shows that Golgi disorganisation upon loss of adhesion could be due to the loss of dynein motors from the Golgi membrane, and dynein is required to reorganise Golgi downstream of adhesion (Singh et al., 2018).

Loss of cell-matrix adhesion does not affect the integrity and function of microtubules (Singh et al., 2018). However, disruption of microtubules leads to further disorganisation of the Golgi and inhibits Golgi re-organisation on re-adhesion. Disruption of microtubules upon loss of adhesion does not have a significant effect on the trans-Golgi compartment. However, it disorganises the cis-medial compartment comparable to the trans compartment in these cells (Singh et al., 2018). This suggests that Golgi disorganisation is an active process with Golgi compartments actively held on functional microtubules.

Given that the extent of disorganisation is different for cis/medial *vs* trans-Golgi compartments, the loss of dynein between these compartments could be variable. More dynein lost from the

trans-Golgi compared to the cis-Golgi, could result in its more significant disorganisation? Also, if dynein is essential in keeping the Golgi organised at the MTOC, could a kinesin also be involved in the disorganisation of the Golgi in suspension?

The Golgi apparatus also acts as an MTOC-independent microtubule nucleating centre. The Golgi-derived microtubules are known to bring Golgi vesicles originating from the ER towards the Golgi apparatus and assist their fusion with the *cis*-Golgi. Furthermore, the Golgi-associated microtubules are reported to carry several post-translation modifications like hyperacetylation that increase their stability and help trafficking from the Golgi apparatus (Thyberg & Moskalewski, 1993a, 1999). The post-translational modifications on the microtubules also affect the association of motor proteins to the microtubules (Reed et al., 2006a). Given this, we wanted to evaluate if the loss of cell-matrix adhesion could affect PTMs on microtubules which could contribute to the disorganisation of Golgi.

## 4.2. Results.

### 4.2.1. Loss of cell-matrix adhesion results in a drop in microtubule acetylation.

Most known microtubule PTMs occur on the outer surface of the microtubule strands. Microtubule acetylation is unique in that it appears on the inner lumen of the microtubule (Hernault & Rosenbaum, 1985). Lysine 40 (K40) residue of α-tubulin is acetylated by the enzyme acetyltransferase αTAT1 (Ledizet & Piperno, 1987), and the activity of αTAT1 is 100 times higher towards the α-tubulin subunits in the polymerised state than those in unpolymerised state (Maruta et al., 1986). Hence, the free cytosolic monomers of α-tubulin are not found to be acetylated. Microtubule acetylation influences the properties and functions of the microtubule network in the cells. It has been shown to affect the binding of motor proteins to the microtubules (Balabanian et al., 2017; Reed et al., 2006b).

Given the role of acetylation in regulating the binding and motility of motor proteins, we evaluated the effect loss of adhesion had on microtubule acetylation. Serum-deprived WT-MEFs were detached and held in suspension for 120min and replated on fibronectin for 15min (15' FN) and 4 hours (Stable adherent). The microtubule acetylation levels were detected in these cells through western blots. We found that compared to the stable adherent cells, there was a significant drop in the acetylated alpha-tubulin levels in suspension and levels stayed low even after replating on fibronectin for 15min (Fig. 4.1).

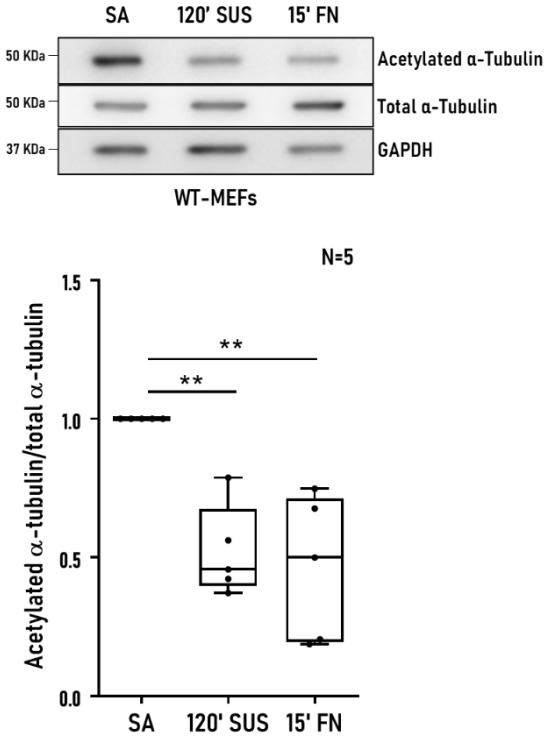



**Figure 4.1: Adhesion-dependent regulation of microtubule acetylation in WT-MEFs.** Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysate (WCL) from stable adherent (SA), suspended (120′ SUS) and re-adherent (15′ FN) WT-MEFs. The box and whisker plot represents the densitometric band intensity ratio of acetylated alpha-tubulin to total alpha-tubulin, normalised to SA from 5 independent experiments and normalised to stable adherent cells. Statistical analysis for normalised data was done using a single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001).

### 4.2.2. Effect of tubacin treatment on microtubule acetylation levels in WT-MEFs.

Golgi-associated microtubules are known to be hyperacetylated and can contribute to the Golgi organisation and trafficking across the Golgi. Therefore, a drop in acetylated alpha-tubulin levels in suspension might influence the observed Golgi disorganisation in suspended cells. HDAC6 (Hubbert et al., 2002) and sirtuin type 2 (SIRT2) (North et al., 2003) have been implicated in the deacetylation of α-tubulin, and HDAC6 is seen to play a more prominent role (Zilberman et al., 2009). To check if the drop in acetylated microtubules led to the disorganisation of Golgi in suspended cells, we used tubacin (an HDAC6 inhibitor) to prevent the decrease in alpha-tubulin acetylation and look at its effect on the Golgi organisation.

We first used increasing concentrations of tubacin to check if the treatment caused an increase in the acetylation of alpha-tubulin in serum-deprived cells without causing any cytotoxicity Stable adherent WT-MEFs, when treated with increasing concentrations (0.5μM, 1μM, 2μM, and 3μM) of tubacin under serum-deprived conditions for 14h, showed a steady increase in acetylated alpha-tubulin levels (Fig. 4.2A). The immunostaining in serum-deprived WT-MEFs treated with tubacin for 14h confirmed the increase in acetylated microtubules in stable adherent cells (Fig. 4.2B). Tubacin treatment at 2μM and 3μM for 14h increased acetylation levels but caused significant cell death in serum-deprived cells. However, cells treated with 0.5μM and 1μM Tubacin stayed healthy for up to 24h. We hence chose to use 1μM tubacin treatment for further experiments.

Further, we wanted to check if tubacin treatment prevented the drop in microtubule acetylation in suspended cells. To test this, we pre-treated WT-MEFs with 1μM tubacin for 14h, detached them, held them in suspension for 120min and replated on fibronectin for 15min and 4hrs. 1μM tubacin was maintained through suspension and re-adherent conditions. Compared to untreated WT-MEFs, a significant increase in the acetylated alpha-tubulin levels were detected in tubacin-treated stable adherent WT-MEFs (Fig. 4.3). These levels stayed high when treated cells were held in suspension and were replated on FN for 15min (Fig. 4.3).

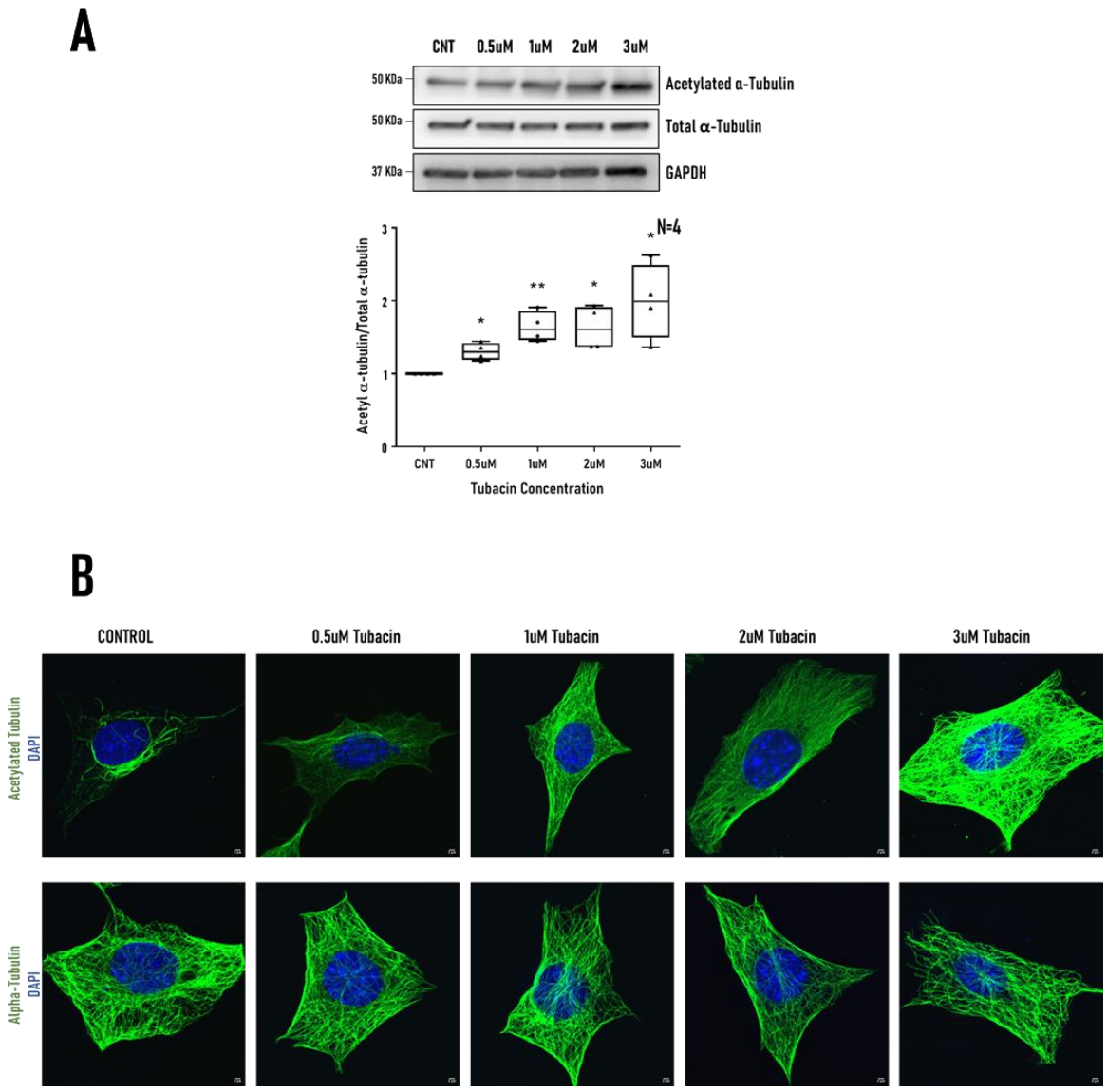



**Figure 4.2: Effect of tubacin treatment on microtubule acetylation in serum-deprived, stable-adherent WT-MEFs. (A)** Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysate (WCL) from WT-MEFs untreated or treated with increasing concentrations of tubacin (0.5μM, 1μM, 2μM or 3μM). The box and whisker plot represents the densitometric band intensity ratio of acetylated alpha-tubulin to total alpha-tubulin, normalised to untreated CNT from 5 independent experiments and normalised to stable adherent cells. Statistical analysis for normalised data was done using a single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001) **(B)** Representative images of WT-MEFs untreated or treated with increasing concentration of tubacin (0.5μM, 1μM, 2μM or 3μM) and immunostained for acetyl-α-tubulin (upper panel) or total-α-tubulin (lower panel). Statistical analysis for normalised data was done using a single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001).

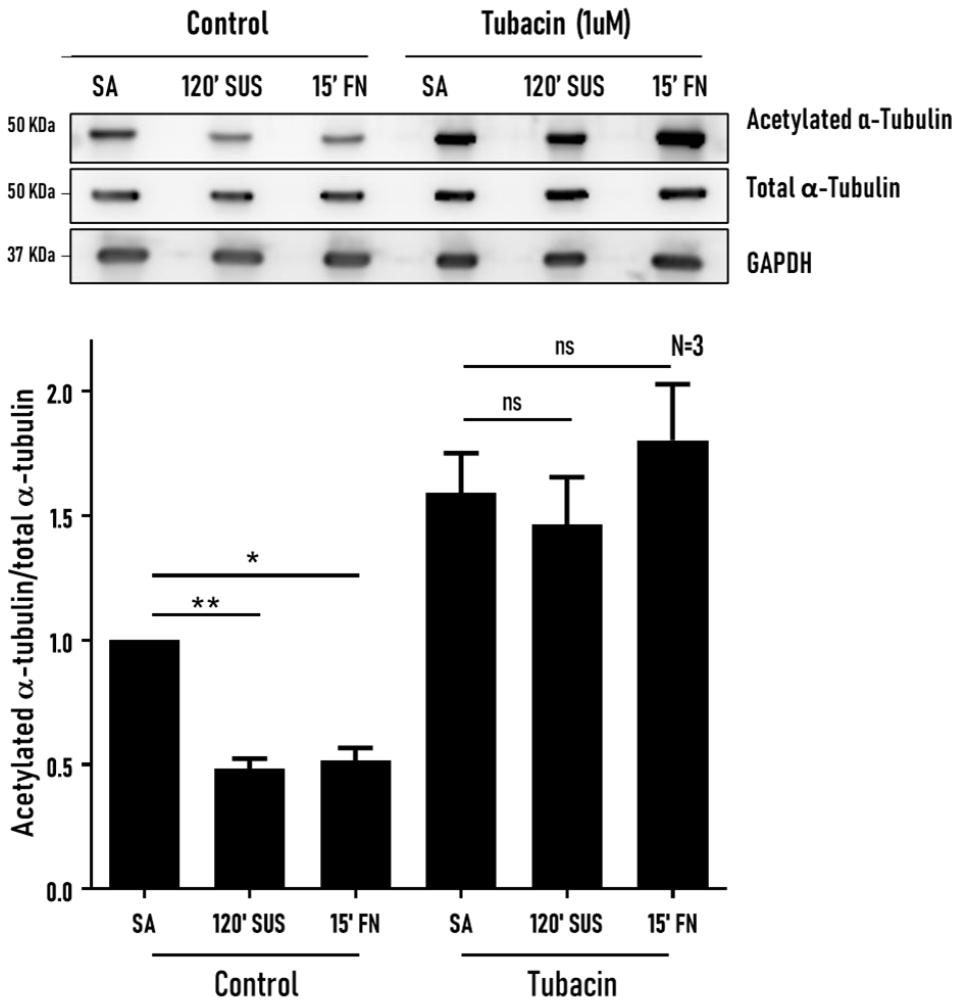



**Figure 4.3: Effect of tubacin treatment on microtubule acetylation in WT-MEFs held in suspension.** Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysate (WCL) from stable adherent (SA), suspended (120′ SUS) and re-adherent (15′ FN) WT-MEFs untreated or treated with 1μM tubacin. The bar graph represents the densitometric band intensity ratio of acetylated alpha-tubulin to total alpha-tubulin, normalised to untreated CNT from 3 independent experiments and normalised to untreated stable adherent cells. Statistical analysis for normalised data was done using a single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001).

### 4.2.3. Effect of microtubule acetylation on the trans-Golgi organisation in non-adherent cells.

We wanted to evaluate if the drop in microtubule acetylation reversed by tubacin treatment could affect the loss of adhesion-mediated disorganisation of Golgi. In non-adherent cells, the trans-Golgi is more disorganised; hence, first, we checked the effect of tubacin treatment on GalTase-RFP labelled trans-Golgi in WT-MEFs. Pre-treated with 1uM tubacin for 14h, cells were detached, held in suspension for 120min and replated on FN for 15min and 4hr. The trans-Golgi phenotype in these cells were compared to untreated cells. A fraction of untreated and treated cells, held in suspension, were also lysed and run on a gel to check for the microtubule acetylation levels. These western blots confirmed that the acetylated tubulin levels in tubacin-treated cells were as expected higher than in untreated cells (Fig. 4.4A).

However, when we looked at the trans-Golgi phenotype, we saw that, like the control cells, the tubacin-treated suspended WT-MEFs also showed a distinctly disorganised Golgi phenotype. The trans-Golgi in tubacin-treated cells stayed predominantly organised in stable adherent and re-adherent cells (Fig. 4.4B and Fig. 4.4C)). This showed that the microtubule acetylation levels in suspended cells did not affect trans-Golgi organisation.

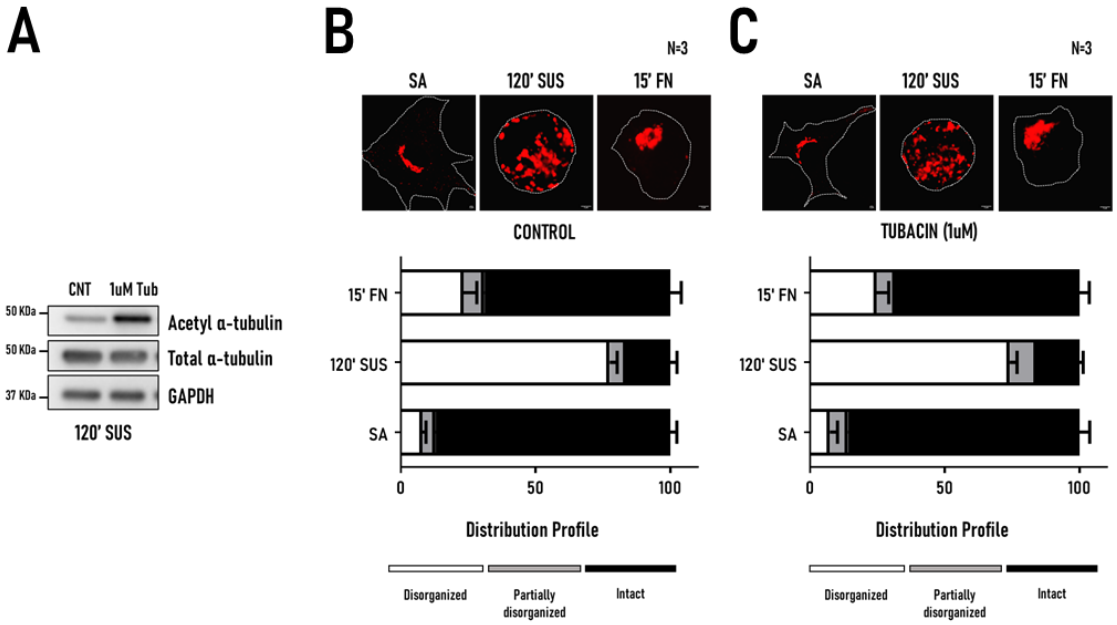



**Figure 4.4: Effect of tubacin treatment on trans-Golgi organisation in WT-MEFs. (A)**Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysates of cells suspended (120′ SUS) untreated or treated with 1μM tubacin. **(B, C)** The trans-Golgi organisation in cells untreated **(B)** or treated with 1μM tubacin **(C)**. The upper panel represents the predominant trans-Golgi phenotype observed in stable adherent (SA), suspended (120' SUS) and re-adherent on FN (FN' 15). The graph represents the percentage distribution profile of WT-MEFs with disorganised (clear), partially disorganised (grey), and intact (black) trans-Golgi (GalTase-RFP).

### 4.2.4. Effect of microtubule acetylation on the cis-medial-Golgi organisation in non-adherent cells.

The increased microtubule acetylation did not affect the organisation of trans-Golgi in non-adherent WT-MEFs. Dynein is required for the organisation of the Golgi at the MTOC. Earlier work from the lab has shown loss of adhesion causes a drop in active Arf1 at the Golgi that affects dynein recruitment. The dispersal of the Golgi in these cells is likely supported by the presence of Golgi-associated Kinesins (microtubule plus end motor proteins). Knowing that microtubule acetylation assists binding and recruitment of kinesins (Reed et al., 2006a), we wanted to test if increased acetylation in non-adherent cells leads to increased recruitment of kinesins to the Golgi, which could mediate further dispersal of the Golgi.

In non-adherent WT-MEFs, unlike the trans-Golgi, the cis-medial Golgi is disorganised to a lesser extent. If the increased microtubule acetylation could lead to increased recruitment of kinesins to the Golgi and assist further dispersal of the Golgi, this could be reflected in a greater extent of disorganisation of the cis-medial Golgi. ManII-GFP (cis-medial Golgi marker) expressing WT-MEF were pre-treated with 1μM tubacin for 14h, detached and held in suspension for 120min and replated on FN for 15min and 4hr. The cis-medial Golgi phenotype in these cells was compared to that in untreated cells. In addition, a small fraction of untreated and treated cells, held in suspension, were lysed and run on a gel to check if the microtubule acetylation levels were maintained high in tubacin-treated cells. The western blots confirmed that the acetylated tubulin levels in tubacin-treated cells were higher than in untreated cells (Fig. 4.5A).

However, no significant difference was observed in the extent of disorganisation of the cis-medial Golgi in untreated and treated cells. Like the untreated control cells, the tubacin-treated cells showed an organised Golgi when stably adherent, disorganised in suspension and reorganised upon replating on FN for 15min (Fig. 4.5B and Fig. 4.5C). This showed that even if the increased microtubule acetylation had any effect on recruiting the motor proteins, this did not affect the organisation of the cis-medial or the trans-Golgi.

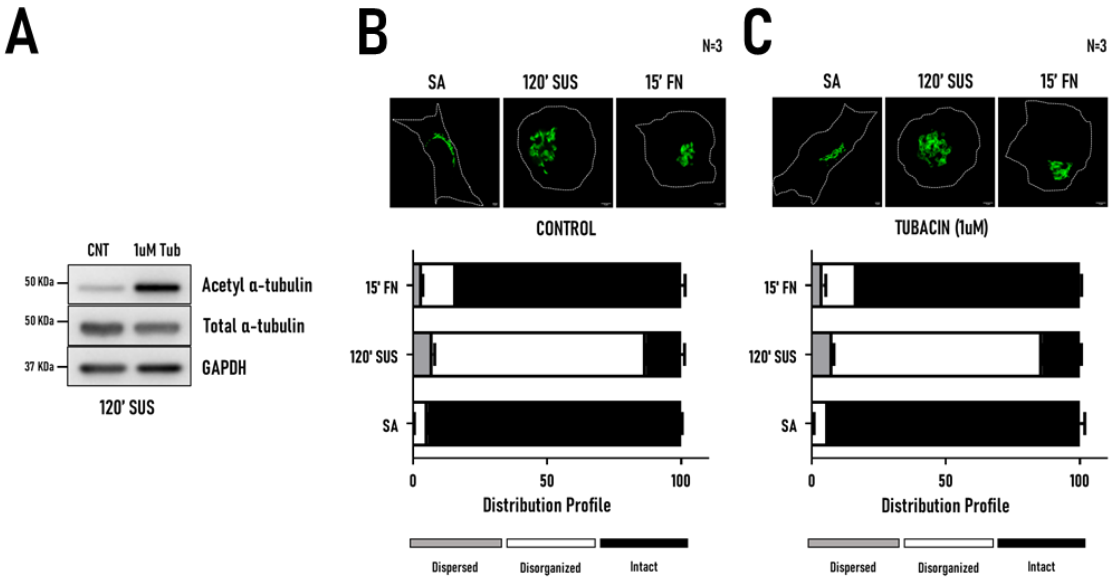


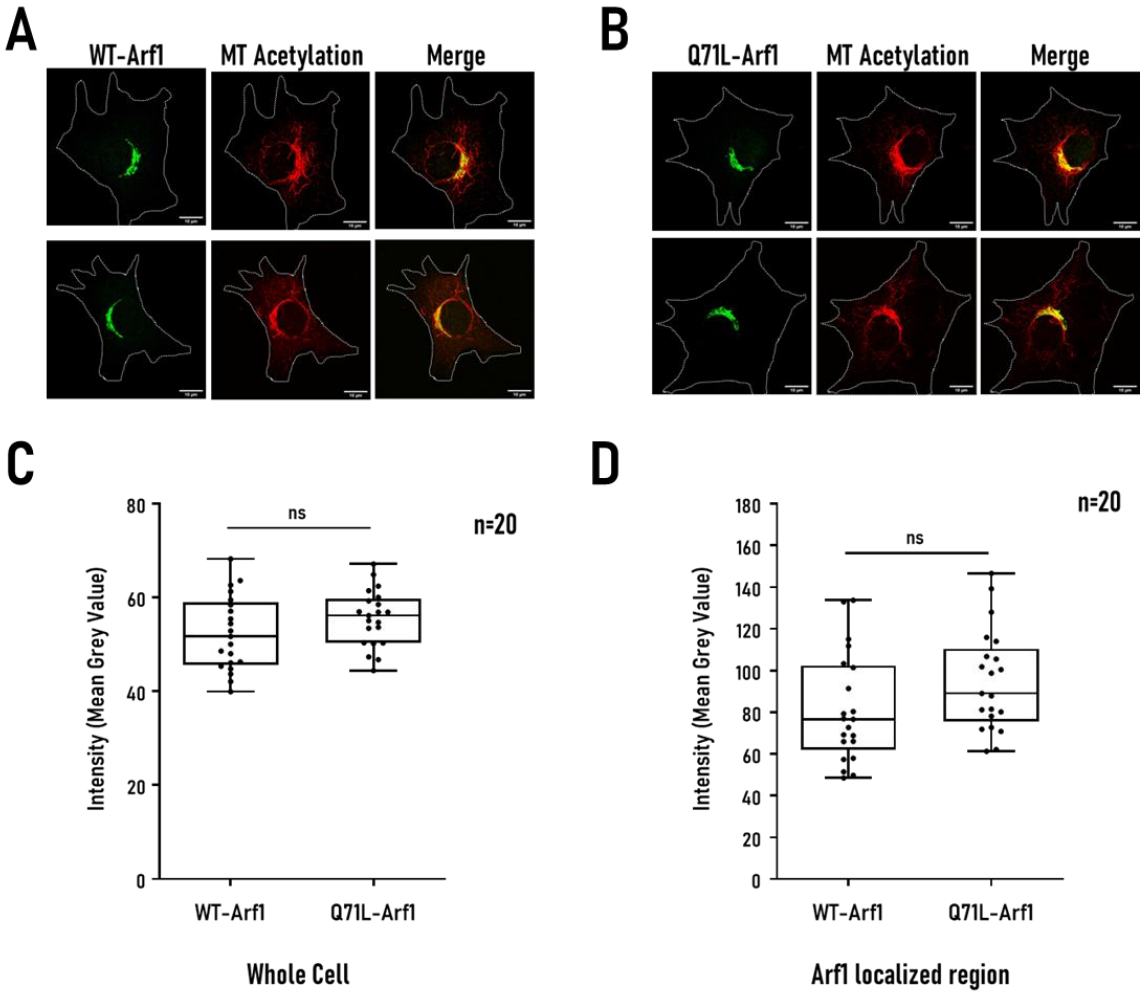

**Figure 4.5: Effect of tubacin treatment on cis-medial-Golgi organisation in WT-MEFs.** **(A)**Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysates of cells suspended (120′ SUS) untreated or treated with 1μM tubacin. **(B, C)** The cis-medial Golgi organisation in cells untreated **(B)** or treated with 1μM tubacin **(C)**. The upper panel represents the predominant cis-medial Golgi phenotype observed in stable adherent (SA), suspended (120' SUS) and re-adherent on FN (FN' 15). The graph represents the percentage distribution profile of WT-MEFs with dispersed (grey), disorganised (clear) and intact (black) cis-medial Golgi (ManII-GFP).

### 4.2.5. Effect of active Arf1 (Q71L) expression on microtubule acetylation in WT-MEFs.

Arf1 plays vital for the regulation of Golgi organisation. Studies show that, *Chlamydia* an intercellular pathogen hijacks the host cytoskeleton to induce its uptake (Clausen et al., 1997; Jiwani et al., 2012). *Chlamydia* recruits and activates the small GTPases, Arf1 and Arf4, to reposition the Golgi complex. The activation of Arf1 was vital in mediating Golgi's re-organisation around the *Chlamydia* inclusion. Additionally, Arf1 activation was also crucial in regulating the Microtubule PTMs (acetylation and detyrosination), which increased the microtubule stability (Wesolowski et al., 2017).

Loss of cell-matrix adhesion leads to a drop in Arf1 activation (Fig. 3.2.1C, (Singh et al., 2018)) and a decrease in microtubule acetylation (Fig. 4.1). This made us ask if the activation status of Arf1 directly regulates the microtubule acetylation downstream of cell-matrix adhesion. To address this, we expressed WT-Arf1-GFP and constitutively active Q71L-Arf1-GFP and analysed the effect Arf1 activation could have on microtubule acetylation in stable adherent WT-MEFs (Fig. 4.6A and 4.6B). The immunofluorescence intensity for the acetylated tubulin staining showed the overexpression of active Arf1 (Q71L) to have no significant effect on the acetylation levels in cells when compared to WT-Arf1 expressing cells (Fig. 4.6). This was confirmed by quantitation of the same (Fig. 4.6C). We further looked at the microtubule intensity around the Arf1 (Q71L/WT) localised region by marking the region of interest based on the Arf1 (Q71L/WT) fluorescence and measuring the intensity of microtubule acetylation within the selected region. There was no significant difference in the extent of microtubule acetylation around this region between the WT-Arf1 and Q71L-Arf1 expressing cells (Fig. 4.6D).

This suggests that activation of Arf1 might not have a direct role in regulating microtubule acetylation downstream of cell-matrix adhesion. However, Arf1 could still regulate microtubule acetylation by regulating the Golgi organisation. Hence, the drop in microtubule acetylation we see in suspension could be the result of (and not a cause) of Arf1-dependent Golgi disorganisation.




**<u>Figure 4.6:</u> Effect of tubacin treatment on cis-medial-Golgi organisation in WT-MEFs.** **(A)**Western blot detection of acetylated α-tubulin, total α -tubulin and GAPDH levels in the whole-cell lysates of cells suspended (120′ SUS) untreated or treated with 1μM tubacin. **(B, C)** The cis-medial Golgi organisation in cells untreated **(B)** or treated with 1μM tubacin **(C)**. The upper panel represents the predominant cis-medial Golgi phenotype observed in stable adherent (SA), suspended (120' SUS) and re-adherent on FN (FN' 15). The graph represents the percentage distribution profile of WT-MEFs with dispersed (grey), disorganised (clear) and intact (black) cis-medial Golgi (ManII-GFP).

## 4.3. Summary.

In this chapter, we have looked at how loss of adhesion mediated Golgi disorganisation and microtubule acetylation could regulate each other. Golgi-associated microtubules are known to be hyperacetylated. Hyperacetylation and detyrosination are known to increase the stability of microtubules, which assist in regulating Golgi organisation and function (Thyberg & Moskalewski, 1999). Given this, we were interested in testing if changes in MT-acetylation could prevent the observed Golgi disorganisation in suspended cells.

We find loss of adhesion to cause a significant drop in MT-acetylation levels, that does not recover on re-adhesion to fibronectin for 15' (Fig. 4.1). It is worth noting that the Golgi is rapidly re-organised on re-adhesion for 15min (Fig. 3.1A). We further used the HDAC6 inhibitor tubacin to inhibit MT-deacetylation in non-adherent cells, and were able to successfully increase their basal levels (Fig. 3.2, 3.3). Increased MT-acetylation however, did not have any effect on the disorganised trans-Golgi in suspended cells (Fig. 3.4). Tubacin mediated increased MT acetylation could recruit Kinesins, which could influence Golgi disorganisation on loss of adhesion by causing further dispersal of the Golgi elements. In such a case, the cis-medial Golgi which is disorganised to a lesser extent in suspended (Fig 3.1A) could further be dispersed throughout the cell. However, the cis-medial Golgi organisation in tubacin treated cells showed no significant difference when compared to the cis-medial Golgi in untreated control cells (Fig. 3.5). Considering tubacin treatment did not lead to further dispersal of the cis-medial Golgi elements in suspended cells, or its reorganisation on re-adhesion suggests the stabilisation of microtubules and possible recruitment of dependent kinesis does not affect the same. Hence, increase in microtubule acetylation was not sufficient to influence the Golgi organisation in non-adherent mouse fibroblasts.

Our earlier studies had shown active Arf1-dependent loss of dynein from Golgi membranes mediates Golgi disorganisation (Singh et al., 2018). The differential activation of Arf1 in the cis-medial vs trans-Golgi could affect their dynein recruitment (Fig. 3.2). Loss of cell-matrix adhesion leads to a drop in Arf1 activity and MT-acetylation. Earlier studies suggest that Arf1 could regulate MT-acetylation by regulating the Golgi organisation (Wesolowski et al., 2017). In our study, constitutively active Arf1 had no significant effect on MT-acetylation in stable adherent suggesting Arf1 might not have a direct role in altering the MT-acetylation levels.

This also suggests, Arf1, through its effect on Golgi organisation, might contribute to changes in MT acetylation observed.

## 4.4 Conclusion.

Change in microtubule acetylation is not a cause but the result of loss of adhesion-mediated Golgi disorganisation.

## 4.5. Future directions

Further research is required to determine if the drop in microtubule acetylation upon loss of adhesion effects trafficking through the Golgi. It needs to also be tested if loss-of adhesion affects microtubule PTMs other than acetylation, and if this change in microtubule PTMs affect the association of motor proteins to Golgi membranes and contributes to the adhesion-dependent regulation of Golgi organization and function.

# CHAPTER 5

# Isolation of Golgi from adherent vs non-adherent cells for proteomic profiling.

# 5.1. Rationale.

The Golgi apparatus plays a central role in regulating the secretory pathway. The organisation of Golgi, from aligning the stacks to localizing the enzymes in respective cisternae, is tightly regulated by several signalling pathways. The organisation of the Golgi is tightly linked to its function. The processing of proteins and lipids in the Golgi influences their localisation and function, and thus Golgi plays a central role in maintaining organellar and cellular homeostasis. Changes in the organisation of the Golgi lead to alterations in the protein and lipid modifications that can severely affect cellular homeostasis. In addition to its role in cargo processing and trafficking, the Golgi, has also emerged as a hub for several signalling pathways (Colanzi et al., 2007; Litvak et al., 2004; Royle et al., 2005). Signalling molecules implicated in cell cycle progression and apoptosis have been known to localize to the Golgi membranes(Hicks & Machamer, 2005; Litvak et al., 2004; Mancini et al., 2000). Changes in the organisation of the Golgi can lead to the release of these molecules, which could lead to their recruitment to other cellular locations where they are involved in activating different downstream pathways that regulate cell survival (Gervais et al., 2002; Hicks & Machamer, 2005; Litvak et al., 2004; Rabouille et al., 2015). Clathrin, Nir2, Sak1, and cullin-2 are known to associate with Golgi membranes in interphase cells. During prophase, accompanied by Golgi fragmentation, these molecules are released from the Golgi membranes and have been shown to involved in functions like mitotic spindle, separation of chromosomes, and cytokinesis (Nihal Altan-Bonnet et al., 2006b; Litvak et al., 2004; Royle et al., 2005). Hence, change in the Golgi organisation can also act as an initiator of changes in signalling cascade upon loss of adhesion, which remains to be explored.

Cell-matrix dependent Golgi disorganisation can lead to the association or dissociation of several regulator and effector molecules which could affect Golgi and cellular functions. Though we now know that the activation status of Arf1 plays a predominant role in the adhesion-dependent organisation of Golgi, there are still several unanswered queries about how the change in Arf1 activation is brought about, the impact this has on other Golgi regulators and/or Golgi structural proteins. In addition, it remains unclear how changes in integrin activation are communicated to the Golgi.

Obtaining a proteomic profile of the Golgi membranes of stable adherent and suspended WT-MEFs will be a direct way to get an insight into regulators and effectors that could be differentially localised and or regulated on the Golgi membranes in adherent vs suspended

cells. It could also help us identify kinesin motor proteins differentially associated with the Golgi when organised vs disorganised that could work with dynein to regulate the Golgi.

Hence, establishing a protocol for the isolation of Golgi membranes from stable adherent and suspended WT-MEFs would be an important first step towards generating their proteomic profiles. Additionally, in suspended cells, the differential disorganisation of the cis-medial vs trans-Golgi could impact their isolation. Careful evaluation of cis-medial vs trans-Golgi markers could help determine if they can be separated in such an isolation protocol owing to the differences in their volume and object numbers.

## 5.2. Results.

### 5.2.1. Isolation of membrane and cytosolic fractions from adherent and suspended WT-MEFs.

As a preliminary analysis of how the loss of adhesion can affect Golgi-associated proteins, we isolated membrane-enriched fraction from post-nuclear supernatant obtained from 100µl cell pellet of stable adherent and suspended WT-MEFs each. Membrane-enriched fraction (MF) would be enriched in proteins from the Golgi, plasma membrane, mitochondria and ER membranes.

Equal volumes of these samples were then analyzed by western blotting to check the relative levels of Rho-GDI (a cytosolic protein) and Calnexin (ER marker). As expected Calnexin was detected only in the Membrane Fraction (MF), and Rho-GDI was predominantly detected in Cytosolic Fraction (CF). GAPDH was found in similar amounts in both fractions indicating that the total protein levels of MF and CF might not be drastically different and the differences in levels of Rho-GDI and Calnexin seen are a result of their differential association with membranes (Fig. 5.1A). We further probed the MF for relative levels of proteins we knew associated differentially with Golgi membranes in adherent vs suspended cells. This includes Arf1 and dynein. We observed a drop in Arf1 and dynein levels in the membrane fractions from suspended cells (120' SUS) compared to stable adherent cells. A significant drop in GM130 levels, a known Golgi matrix protein that is important in regulating the Golgi organisation was also observed in membrane fractions obtained from suspended cells (Fig. 5.1B).

Our earlier study shows that, there is a drop in active Arf1 levels and dynein associated with active Arf1 in WCL of suspended cells as compared to adherent cells (Singh et al., 2018). Arf1 associates very weekly with membranes in its GDP bound form. The association of Arf1 with membranes is stabilized after its activation by the exchange of GDP to GTP, mediated by Arf GEFs. The drop in activation of Arf1 in suspended cells might lead to the loss of Arf1 from membranes is reflected in the detection of lower Arf1 levels in MFs isolated from suspended cells as compared to stable adherent cells.

Silver staining of equal (2ug) protein from MFs of SA and 120' SUS cells was used to evaluated changes in the protein bands detected in the fractions. There were a few protein bands whose intensity (reflective of protein levels) varied in membrane fractions from SA vs 120' SUS.

These could be protein candidates of interest whose identity once established could help us explore their possible role in adhesion-dependent Golgi organisation (Fig. 5.1C).

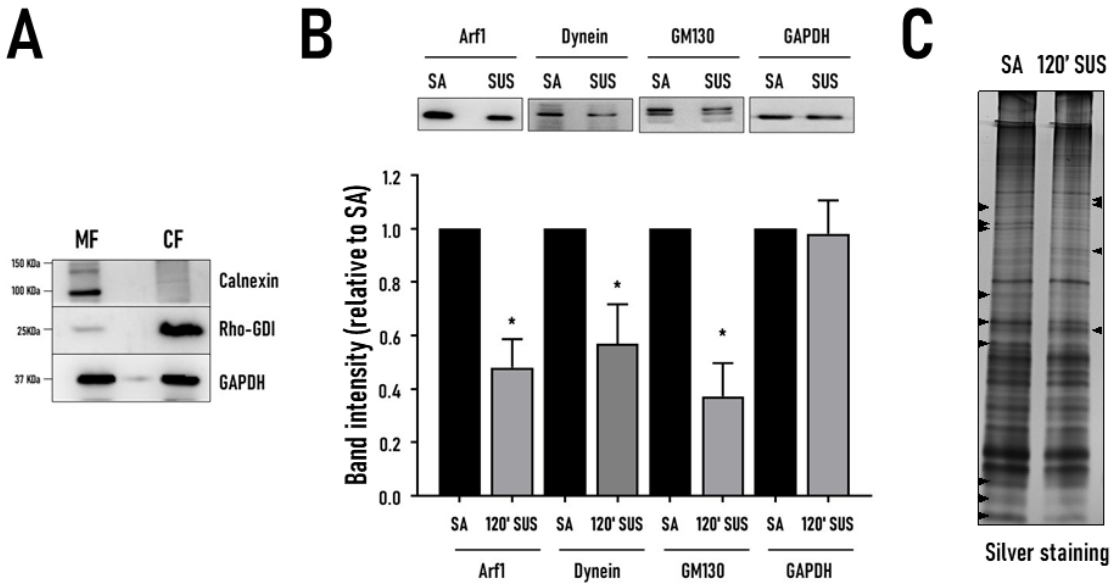



**Figure 5.1: Membrane-enriched and cytosol-enriched fractions isolated from WTMEFS.** **(A)** Western blot detection of Calnexin, Rho-GDI and GAPDH in membrane-enriched (MF) and cytosol-enriched fractions (CF) isolated from stable adherent cells. **(B)** Western blot detection of Arf1, Dynein, GM130 and GAPDH in membrane fractions isolated from WT-MEFs, stable adherent (SA) and suspended for 120min (120' SUS/SUS). The bar graph represents the band intensity of each protein normalized to SA band intensity. Statistical analysis for normalized data was done using a single sample t-test (*P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001). **(C)** Silver staining to detect protein bands in 2ug each of membrane fractions isolated from WT-MEFs, stable adherent (SA) and suspended for 120 min (120' SUS).

### 5.2.2. Isolation of Golgi from adherent and suspended WT-MEFs.

Isolation of enriched or pure Golgi fractions should allow us to obtain a proteomic profile of these fractions, leading to valuable insight into the molecules that play a role in adhesion-dependent regulating Golgi organisation and function. Changes in signalling pathways involved in Golgi organisation and function could affect presence and levels of proteins associated with Golgi membranes. The proteomic profile of Golgi membranes from stable adherent and suspended cells could hence help reveal cellular signalling pathways that are affected by changes in Golgi organisation.

Using the discontinuous sucrose method, we aimed to isolate pure Golgi fractions from stable adherent and 120' suspended cells. After ultracentrifugation, based on their density Golgi membranes are known to concentrate at the interface between 0.8M and 1.2M sucrose fractions. 500ul fractions collected were used to analyse the discontinuous gradient and interfaces therein (Figure 5.2A). These fractions were analyzed by western blotting to detect cis-Golgi (p115 and GM130), trans-Golgi (vti1a and vti1b) and TGN (Syntaxin 6) marker proteins. Golgi fractions isolated from suspended cells showed reduced levels of cis-Golgi markers than fractions from stable adherent cells. There was however no significant change in levels of trans-Golgi markers between these fractions. Calnexin and Rho-GDI were used as non-Golgi markers to check for contamination with cytosolic and ER fractions. Relatively low levels of calnexin and Rho-GDI were detected in these fractions. The samples were also probed for Arf1 and Dynein (known regulators of adhesion-dependent Golgi organisation (Singh et al., 2018)). Lower amounts of Arf1 and Dynein detected in Golgi fractions isolated from suspended cells as compared to Golgi fractions from stable adherent cells (Figure 5.2B). This agrees with our understanding (Singh et al., 2018) of how Arf1 activation and resultant recruitment of dynein could be affected in Golgi from suspended cells.

However, when we analysed all the fractions collected after ultracentrifugation by western blotting most of the Golgi associated proteins were still retained at the 1.4M sucrose fraction (Figure 5.2C). This was the case for both fractions obtained from stable adherent and suspended WT-MEFs in these preliminary experiments. This said, we haven't yet been able to optimise the protocol to efficiently isolate Golgi membrane. This work while initiated by me is ongoing in the lab. The approach I am hopeful will help reveal possible candidates as originally envisaged, be it in the near future.

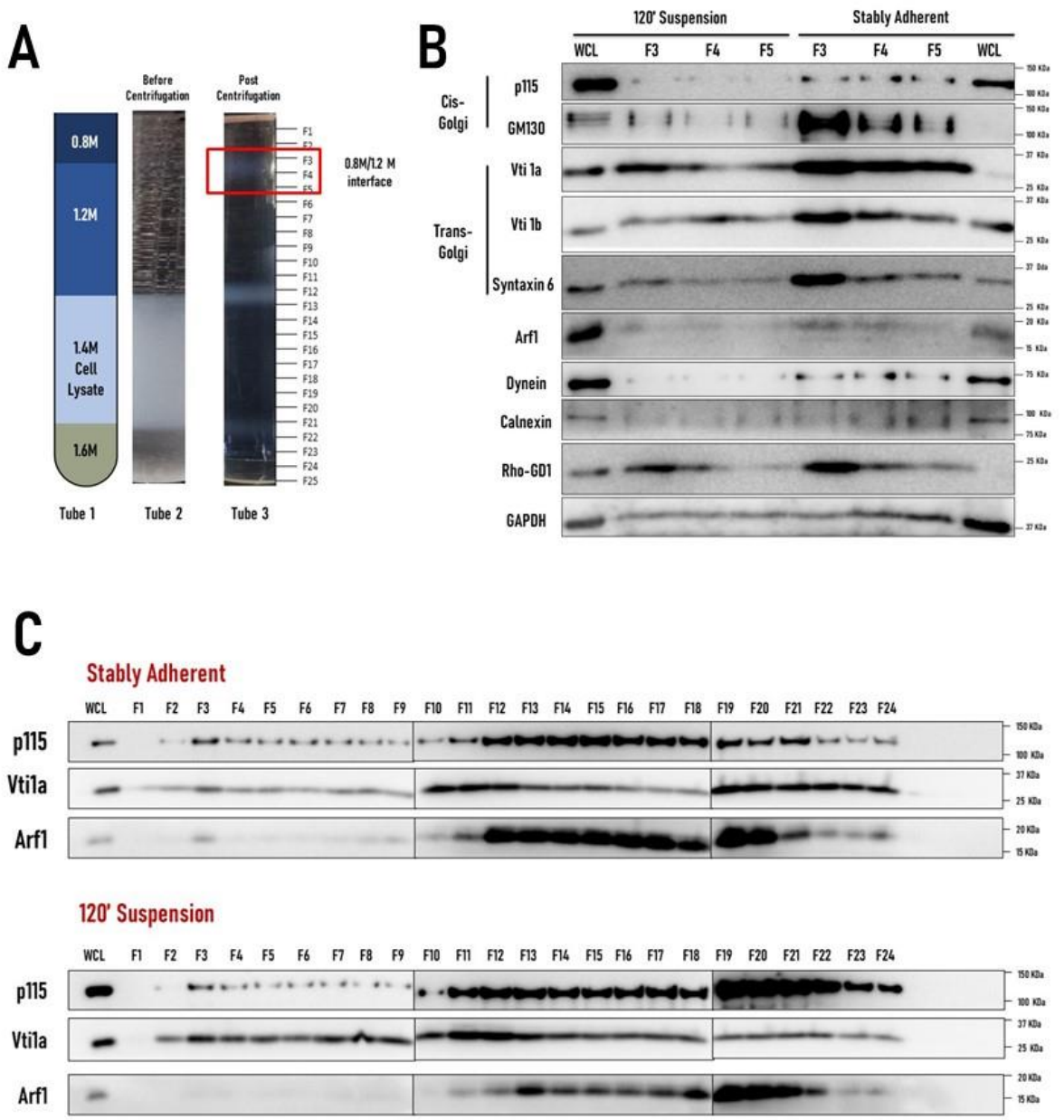



**Figure 5.2: Isolation of Golgi from WTMEFS. (A)** Tube 1 is a picture representing the layering of sucrose gradients before ultracentrifugation. Tube 2 is an actual image of the tube in which the cell lysate has been layered with other sucrose gradients. Tube 3 is the image of tube two post ultracentrifugation. The F1-F25 markings represent the 500ul fractions collected post ultracentrifugation. F3-F5 fractions lie at the interface of 0.8M and 1.2M. **(B)** Western blot detection of cis-Golgi markers (GM130, p115), trans-Golgi markers (Vti1a, Vti1b. Syntaxin 6) and other proteins of interest (Arf1, Dynein, Calnexin, Rho-GDI and GAPDH) in fractions F3-F5 isolated from WT-MEFs suspended for 120min and stable adherent WT-MEFs. **(C)** Western blot detection of p115, Vti1a and Arf1 in the fractions F1-F25 collected post ultracentrifugation.

## 5.3 Summary.

In this chapter, we have aimed to enrich and isolate Golgi-rich membrane fractions from adherent and suspended cells and evaluate them for differences in proteins that associate differentially. We found the association of Arf1, Dynein and GM130 to membranes in suspended cells was lower than in adherent cells. Silver staining of these fractions also helped detect differences in the intensity of protein bands detected in adherent membrane fractions vs suspended membrane fractions (Fig. 5.1). This led us to try isolating pure Golgi membranes from stable adherent and 120' suspended WT-MEFs, which could eventually allow us to do proteomic profile and identify molecules that could differentially regulate the Golgi in these cells. Preliminary studies with a discontinuous sucrose gradient did detect a few cis and trans-Golgi proteins at the interface of 0.8M/1.2M sucrose, but failed to show sufficient enrichment of Golgi proteins (Fig. 5.2). Further optimisation of this protocol leading to the isolation of pure Golgi membrane fractions would help us obtain a proteomic profile that could help us detect proteins that could differentially associate with Golgi membranes regulating their organisation and function downstream of adhesion.

## 5.4 Conclusion.

We have initiated studies to isolate pure Golgi membranes from stable adherent WT-MEFs and WT-MEFs suspended for 120 mins and test and compare their proteomic profile.

## 5.5. Future directions

The Golgi isolation protocol needs to be standardized to consistently yield fractions enriched with Golgi from adherent and non-adherent cells. Alternatively, Golgi immunoprecipitation protocols can be used to isolate pure Golgi fractions. Recent advancements in the field have shown that GolgiTAG (TMEM15) protein can be expressed in cells and used to immunoprecipitate intact Golg that can be coupled with advanced omics studies to reveal the contents of the Golgi (Fasimoye et al., 2023). Further, the proteomic differences in the Golgi membranes isolated from adherent and non-adherent cells could help identify proteins that are differentially recruited and could affect adhesion-dependent Golgi organisation and function.

# CHAPTER 6

# Discussion

# 6. Discussion.

This thesis uses adhesion-dependent regulation of the Golgi to evaluate the role of Arf1 in regulating the organization and function of Golgi in non-adherent mouse fibroblasts. In doing so this study reveals how Arf1 inactivation kinetics could impact Golgi organisation and function. Additionally, this study also evaluates the role cell-matrix adhesion could have in regulating microtubule acetylation and its possible effect on Golgi organization.

## 6.1. Role kinetics of change in Arf1 activation has in regulating Golgi organization.

The Golgi apparatus is crucial in the secretory pathway and facilitates the processing and transportation of proteins and lipids within cells (Mark S Ladinsky et al., 1999; Rambourg & Clermont, 1997). Golgi organization is essential for its functions, as it controls the sequential localization of enzymes that modify proteins and lipids processed through the Golgi. Moreover, the arrangement of the Golgi cisternae determines the pace of cargo movement through the Golgi compartments and affects the type of modifications the cargo undergoes (Lowe, 2011; Y Wang et al., 2008).

The Golgi complex comprises of disc-like cisternae stacked upon each other and is organised near the MTOC in adherent mammalian cells (Klumperman, 2011; Rambourg & Clermont, 1997). In mouse fibroblasts, loss of cell-matrix adhesion leads to the disorganization of the Golgi (Fig. 3.1, (Singh et al., 2018)). Interestingly, the trans-Golgi disorganises more significantly and fills up the entire cells. In contrast, the cis-medial Golgi disorganises distinctly less and stays largely perinuclear. In adherent cells, the Golgi compartments are organised into a tightly stacked and compact structure (Fig. 3.1A, 3.1B, (Singh et al., 2018)). However, in non-adherent cells, the organisation of Golgi compartments is disrupted, leading to a spatial separation of these compartments. This presents a unique opportunity to investigate the distinct regulation and unique roles of individual compartments in the Golgi.

In adherent cells, the small GTPase, Arf1, is known to localise to all the compartments of the Golgi and plays a crucial role in regulating the organization and function of the Golgi (Bottanelli et al., 2017; J.G. Donaldson & Honda, 2005; Presley et al., 2002). Arf1 has a myristoylated amphipathic helix at its N-terminus, which allows it to anchor to the Golgi membrane (Goldberg, 1998; Haun et al., 1993). When Arf1 is loaded with GTP and activated, the myristoylated N-terminal amphipathic helix is released, increasing its membrane affinity. Golgi-localised Arf-GEFs do not have a PH domain, but they do have multiple regulatory

domains (Bui et al., 2009; Mouratou et al., 2005) that enable them to bind to the Golgi membrane and regulate Arf1 activation (Bouvet et al., 2013; Christis & Munro, 2012; Gustafson & Fromme, 2017; Meissner et al., 2018; Pocognoni et al., 2018; Richardson, Mcdonold, et al., 2012). Arf1 is responsible for the recruitment of Golgin 160 to the Golgi membranes, which in turn binds to Dynein and facilitates the association of Golgi membranes with the microtubules arising from the centrosome. Dynein association helps the organization of the Golgi at the MTOC (Yadav et al., 2012). Arf1 is also involved in vesicular trafficking at the Golgi. At the cis-medial Golgi, active Arf1 recruits COPI coat to the Golgi membranes and regulates the retrograde transport of cargo from the Golgi to ER (Dascher & Balch, 1994; Presley et al., 2002). At the trans-Golgi, Arf1 recruits adaptor proteins like AP1, AP3, AP4 and GGA3 involved in the packaging and trafficking of processed cargo (Memon, 2004; Ooi et al., 1998; X. Ren et al., 2013; L. M. Traub et al., 1993).

Our studies have shown a ~50% drop in activation of Arf1 in non-adherent cells to drive the disorganization of Golgi (Fig 3.1A, 3.1B, (Singh et al., 2018)). Over-expression of constitutively active Arf1 (Q71L) prevents this disorganisation of the Golgi (Singh et al., 2018). In non-adherent cells, a drop in Arf1 activation reduces the association of Dynein with the Golgi membranes affecting Golgi organisation on the microtubule network (Singh et al., 2018). The differential activation status of Arf1 at the Golgi compartments could drive the distinctly different disorganisation of the compartments in non-adherent cells. On loss of adhesion, the trans-Golgi loses active Arf1 (detected using GFP-ABD), whereas the cis-medial Golgi retains it (Fig. 3.2).

Arf GEFs, GBF1, BIG1, and BIG2 regulate the recruitment and activation of Arf1 at the Golgi membranes. GBF1 preferentially localises to the ER-Golgi intermediate compartment (ERGIC) and cis-Golgi, BIG1/2 localise to the trans-Golgi (García-Mata et al., 2003; Manolea et al., 2008; Yamaji et al., 2000). In WT-MEFs, the relative expression levels of the Arf GEFs are unaffected by loss of adhesion (BIG1>GBF1>BIG2) (Fig. 3.3A, 3.3B). Like in adherent WT-MEFs, GBF1 localises to the cis-medial Golgi compartment in suspended and re-adherent cells (Fig. 3.6A). This suggests that GBF1 could sustain the activation of Arf1 at the cis-medial Golgi compartment in non-adherent cells. Active Arf1 retained at the cis-medial Golgi allows us to test further its role and regulation in the Golgi organisation in suspended cells. Suspended cells by eliminating the impact of adhesion-dependent signalling pathways also provide a cleaner system to study the regulation of Golgi organisation by Arf GEF-dependent Arf1 activation.

The interaction of GBF1 with Golgi membranes is vital for Arf1 activation at the cis-medial Golgi (Niu et al., 2005) and regulated by Rab1-mediated activation of PI4KIIIα and the resulting production of PtdIns(4)P on the Golgi membrane (Alvarez et al., 2003; Dumaresq-doiron et al., 2010; Monetta et al., 2007). Recent studies with Gea2, the yeast paralog of the human Arf-GEF, GBF1, confirm the amphipathic helix to be specifically required for activating Arf1 on the membrane (Muccini et al., 2022). Active Arf1 at the Golgi is involved in the recruitment of COPI (Dascher & Balch, 1994), the Golgi-localised γ-ear-containing, Arf-binding proteins (GGAs), and adaptor protein (AP)-1 (Ooi et al., 1998; L. M. Traub et al., 1993). GBF1-mediated Arf1 activation supports the recruitment of COPI (Alvarez et al., 2003; Deng et al., 2009) and GGAs to Golgi membranes (Lefrançois & McCormick, 2007). Inhibition of GBF1 and the resulting loss of Arf1 activation leads to the dissociation of COPI driving the fragmentation of the Golgi and its fallback into the ER, inhibiting secretory traffic (Serafini et al., 1991; Tanigawa et al., 1993).

BFA inhibits the exchange of GDP to GTP on Arf1 by binding to the Sec7 domain of Arf GEFs. While BFA can inhibit all three Arf GEFs (Peyroche et al., 2001), GCA specifically targets GBF1(Sáenz et al., 2009). GCA binds to the Sec7 domain of GBF1, but it also interacts with a tripeptide loop beyond the Sec7 domain. This interaction with the QNA amino acids in the tripeptide loop is necessary for GCA to inhibit GBF1. The tripeptide loop is not present in BIG1/2, which makes GCA specific to GBF1 (Sáenz et al., 2009). The Sec7 domains of the mouse Arf GEFs, GBF1, BIG1, and BIG2 are highly conserved. The residues in the Sec7 domain vital for the interaction with BFA identified through 3D models constructed using MODELLER are conserved across GBF1, BIG1, and BIG2, confirming that BFA should comparably inhibit these GEFs (Fig. 3.3C-D).

In non-adherent fibroblasts, inhibiting Arf1 GEFs causes the disorganised cis-medial Golgi to fragment. This distinct change in Golgi organisation provides a distinct cellular context to study how GBF1-Arf1 regulates Golgi organisation. A concentration gradient of BFA and GCA in steadily increasing the inhibition of Arf1 GEFs leads to differences in the rate of decrease in active Arf1 levels causing varied fragmentation of the cis-medial Golgi (Fig 3.4, 3.5). Interestingly, this treatment with increasing concentration of BFA/GCA does not significantly alter the total Arf1 levels (Fig. 3.3F-H, 3.4F-H). Over-expression of constitutively active Arf1 (Q71L) prevents the BFA/GCA mediated Golgi fragmentation (Fig. 3.6D-E), confirming that the changes in active Arf1 levels is indeed what mediates this. BFA/GCA have a similar effect on the organisation of Golgi and the reduction in active Arf1 levels. BFA and GCA both can

inhibit GBF1, suggesting that inhibiting GBF1 activity at the cis-medial Golgi could drive the fragmentation of the cis-medial Golgi in non-adherent cells. Supporting this, GBF1 is seen to localise to the cis-medial Golgi in non-adherent cells and is dispersed upon treatment with a higher concentration of BFA (17.8μM) or GCA (3μM) (Fig. 3.6B-C). Treatment with a lower BFA/GCA concentration fails to disperse GBF1 completely from the cis-medial Golgi membranes, which might prevent the complete fragmentation of cis-medial Golgi membranes (Fig. 3.6B-C).

Interestingly, BFA/GCA treatment have no effect on the organisation of trans-Golgi in non-adherent cells (Fig. 3.8). BIG1/2 are localised to the trans-Golgi and are responsible for the activation of Arf1 at the trans-Golgi (Manolea et al., 2008; X. Zhao et al., 2002). Loss of adhesion predominately affects the activity of BIG1/2 which could result in a more significant disorganisation of the trans-Golgi (Singh et al., 2018). Active Arf1 (detected by GFP-ABD) localises with trans-Golgi in adherent and 15min re-adherent (on FN) cells (Fig. 3.2B). However, the extent of active Arf1 and trans-Golgi localisation significantly drops in non-adherent cells (Fig. 3.2B). This suggests that in non-adherent cells, there could be substantially low activation levels of Arf1 at the trans-Golgi, and hence further inhibition of Arf1 activation (via BFA/GCA treatment) might not have a significant effect on the organisation of trans-Golgi.

GBF1-mediated regulation of the rate of Arf1 activation will affect the levels of active Arf1 associated with the Golgi. GBF1 and Arf1 are soluble proteins that rapidly cycle between their cytosolic and membrane-associated pools (Godi et al., 2004; X. Zhao et al., 2006a). GBF1-mediated Arf1 activation and coatomer binding, combined with the recruitment of accessory proteins (McMahon & Mills, 2004; Orci et al., 1986; Reinhard et al., 2003), ultimately results in COPI vesicle formation. GBF1 and Arf1 membrane dynamics could significantly affect COPI assembly and disassembly (Serguei I. Bannykh et al., 2005; Kaczmarek et al., 2017b; McMahon & Mills, 2004; Yu et al., 2012). Interestingly two GTP-bound Arf1 molecules bind sequentially to recruit the COPI heptameric coatomer (Dodonova et al., 2017; Yu et al., 2012). This suggests the complete disassembly of COPI coatomer would require the inhibition of both associated Arf1 molecules, which its kinetics of activation could influence. A rapid decrease in active Arf1 levels could hence allow COPI disassembly to be significantly enhanced, driving ER fallback. A more gradual reduction in active Arf1 levels could allow the known cycling of GBF1 and active Arf1 to rapidly restore their Golgi membrane levels to prevent complete COPI disassembly and Golgi fragmentation.

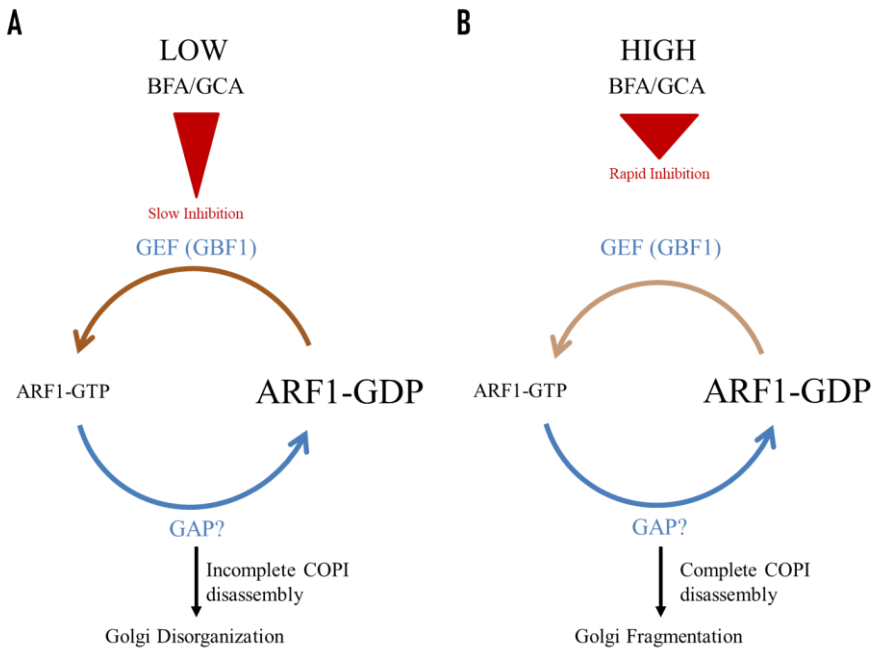



**Figure 6.1:** Schematics of the proposed model for the role of Arf1 kinetics in regulation of Golgi organization in non-adherent cells. **(A)** Lower concentration of the inhibitor (BFA/GCA) may result in slower inhibition of GBF and thus Arf1 activation. A more gradual reduction in active Arf1 levels could allow the known cycling of GBF1 and active Arf1 to rapidly restore their Golgi membrane levels to prevent complete COPI disassembly and help prevent Golgi fragmentation. **(B)** Higher concentration of the inhibitor (BFA/GCA) may lead to rapid inhibition of GBF1 and thus a rapid loss in active Arf1. A rapid decrease in active Arf1 levels could hence result in enhanced COPI disassembly which drives Golgi fragmentation. The role of GAPs in this scenario remains to be explored.

On loss of adhesion, the gradual drop (over 120min) in active Arf1 levels to ~50% of levels seen in SA (Fig. 3.7A) results in Golgi disorganisation (but not fragmentation), which in turn regulates Golgi function (Singh et al., 2018). BFA and GCA treatment of non-adherent fibroblasts, rapidly decreasing active Arf1 levels to a similar ~50% (of levels seen in SA cells) but in 10min causes the Golgi to now fragment, disrupting its function (Fig.3.76B-F). This suggests that the kinetics Arf1 inactivation (by regulating GEF) has vital implications for Golgi organisation in cells. While on the loss of adhesion, this regulation causes a drop in Arf1 activation, a similar role for the kinetics of increase in Arf1 activation to restore Golgi organisation could also exist. Along with Arf GEFs, Arf GAPs could also contribute to the observed kinetics of change in active Arf1 levels to affect Golgi organisation and function. There could be a role for BFA-mediated regulation of ADP-ribosylation of cytosolic proteins like BARS in driving Golgi fragmentation (Colanzi et al., 2013; Corda et al., 2006; A. Mironov et al., 1997). However, this role is likely limited because GCA, which is not known to regulate

ADP-ribosylation, similarly affects Arf1 inactivation kinetics and Golgi fragmentation in this study.

Along with loss-of-adhesion and possibly re-adhesion, such regulation of Arf1 and the Golgi could have implications for processes like cell division. A rapid change in cell adhesion accompanies it, and mitotic cell rounding (Dao et al., 2009; Dix et al., 2018) was accompanied by Golgi fragmentation and vesiculation, leading to its fallback into the mitotic ER (Colanzi & Corda, n.d.; Corda et al., 2012; Shorter & Warren, 2002). This is mediated by AMPKα, Cdk1 and Casein kinase-2 mediated phosphorylation of GBF1, which regulates Arf1 activation (Magliozzi et al., 2018; Morohashi et al., 2010; Shaul & Seger, 2006) and possibly its kinetics. The dynamic nature of Golgi organization and its impact on cellular function makes its regulation very important to cells. The kinetics of Arf1 inactivation or activation in controlling Golgi organization will provide cells with an additional means to fine-tune Golgi and cell function.

Additionally, Arf1 is also know to play an important role in regulation of the organization and function of mitochondria. GBF1 is known to mediate the association of active Arf1 with Miro which blocks the Miro-mediated transport of mitochondria towards the MTOC, thus regulating the positioning of mitochondria in cells (Walch et al., 2018). Arf1 regulates mitochondrial fusion and fission in yeast (Ackema et al., 2014; Nagashima et al., 2020). Arf1 regulates the lipid transfer to mitochondria and thus is involved in energy metabolism (Enkler et al., n.d.). Arf1 localises to the plasma membrane and regulates the CLIC/GEEC endocytic pathway (Kumari & Mayor, 2008; Thottacherry et al., n.d.). Arf1 is also involved in the maintenance of the integrity of the endosomal compartment where its activity is regulated by BIG2 (Shen et al., 2006; H. W. Shin et al., 2004). Given the myriad roles of Arf1 in the cell, it would be interesting to see if the loss of adhesion-mediated drop in Arf1 activity affects the roles of Arf1 other than the regulation and function of the Golgi apparatus.

### 6.2. Adhesion-dependent regulation of microtubule acetylation.

The structure and organization of the Golgi are primarily dependent on microtubules, which originate from both the microtubule-organizing centre (MTOC) and the Golgi itself. Together, these microtubules help shape the Golgi into its characteristic stacked disc-like structure around the MTOC. In addition, Golgi-derived microtubules play a crucial role in bringing together vesicles from the endoplasmic reticulum and facilitating their fusion with the cis-Golgi

cisternae. Furthermore, the association between Golgi membranes and centrosome-derived microtubules leads to the positioning of the Golgi near the centrosomes and helps to maintain its organisation (Chabin-Brion, Me Marceiller, et al., 2001; Wu, De Heus, et al., 2016).

Loss of cell-matrix adhesion does not significantly disrupt the microtubule network or the centrosomes(Singh et al., 2018). However, adhesion-mediated drop in active Arf1 levels reduces the association of Dynein to Golgi membranes resulting in the disorganisation of Golgi in non-adherent mouse fibroblasts (Singh et al., 2018). It remains to be tested if the loss of Dynein is sufficient to drive the disorganisation of Golgi or if there is a role for kinesins in this phenomenon. The differential disorganisation of cis- vs trans-Golgi in non-adherent cells could be the outcome of differential loss and retention of dynein and kinesins respectively.

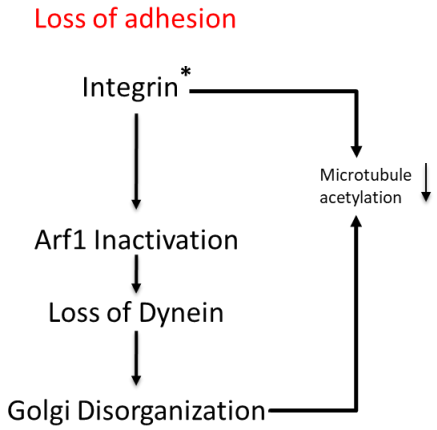



**Figure 6.2:** Schematic representation of proposed model for the loss of adhesion mediated drop in microtubule acetylation. Loss of adhesion mediated drop in Arf1 activity leads to the dissociation of Dynein from the Golgi membranes leading to Golgi disorganization. The drop in microtubule acetylation observed upon loss of adhesion could be a result of Golgi disorganization.

The Golgi-associated microtubules are known to be hyperacetylated and detyrosinated, which contributes to the increased stability of these microtubules. The increased stability could facilitate rapid rates of trafficking from the Golgi (Thyberg & Moskalewski, 1993b, 1999). BFA treatment to disrupt the Golgi complex does not destabilize the network of hyperacetylated and detyrosinated microtubules, nor does this prevent the reformation of the network post-nocodazole treatment (Burgess et al., 1991). Furthermore, the association of motor proteins to the microtubules could be modulated by modifying the PTMs on microtubules (Reed et al., 2006b). Changes in PTMs of microtubules could affect microtubule dynamics in the cell and alter the association of vesicles and membranes to microtubules by altering the binding efficiency of motor proteins.

Loss of adhesion leads to a ~50% drop in microtubule acetylation levels compared to stable adherent cells, which does not recover at an early re-adhesion time-point (15' FN). When suspended cells are replated on FN, the reorganisation of Golgi happens at a very rapid rate. The Golgi compartments are reorganised to form a compact structure within 15min of replating on FN (Fig. 3.1A-B, (Singh et al., 2018)). As microtubule acetylation levels do not recover in 15min of replating on FN, this suggests that microtubule acetylation might not have a role to play in the reorganisation of the Golgi apparatus upon re-adhesion. It's also possible that despite the drop in acetylation the levels present in suspended cells can in effect support the disorganised Golgi in non-adherent cells and the re-organisation of the Golgi in re-adherent cells.

Increasing microtubule acetylation levels (through tubacin treatment) did not affect the organization of cis-medial or the trans-Golgi membrane in stable adherent, suspended and re-adherent cells (Fig. 4.4, 4.5). Increased microtubule acetylation may affect the binding of motor proteins to microtubules in these cells. However, if basal acetylation levels and recruitment of motor proteins is sufficient for the adhesion-dependent regulation of the Golgi this increase in acetylation may not impact the organisation of Golgi. Loss of adhesion-mediated mediated disorganisation of the Golgi does appear to be independent of the drop in microtubule acetylation.

Arf1 is known to recruit tubulin-binding Cofactor E (TBCE) to the Golgi membranes. TBCE is a chaperone involved in the proper folding of β-tubulin. Localisation of tubulin chaperones to the Golgi is speculated to increase the concentration of correctly folded tubulin subunits around the Golgi and facilitate microtubule nucleation at the Golgi. Disruption of the cross-talk between Arf1-TBCE is known to impair the polymerization of Golgi-derived microtubules and drive Golgi fragmentation (Bellouze et al., 2014). Loss of adhesion-mediated drop in Arf1 activity could disrupt this cross-talk and impair the polymerization of Golgi-derived microtubules in non-adherent cells.

As Golgi-associated microtubules are hyperacetylated, it was suspected that Arf1 could influence microtubule acetylation. However, overexpression of active Arf1 (Q71L) had no significant effect on microtubule acetylation levels in stable adherent cells (relative to overexpression of WT-Arf1). This challenges the notion that active Arf1 could regulate microtubule acetylation and function, particularly at the Golgi. In suspended cells , active Arf1 is known to regulate Golgi organisation, preventing its dispersal (Singh et al., 2018). Loss of

adhesion is also seen to distinctly affect MT acetylation. Active Arf1 by regulating Golgi organisation could hence affect microtubule acetylation levels in these cells. This however remains to be tested.

While our understanding the cause-effect relationship between MT acetylation and Golgi organisation remains incomplete, these studies do suggest adhesion-dependent regulation of MT acetylation could be more of an effect rather and cause of Golgi disorganisation in non-adherent cells.

### 6.3. Proteomic profile of Golgi isolated from adherent vs non-adherent cells.

The structure of the Golgi apparatus plays a vital role in the regulation of its function. The structure influences the localization of the enzymes involved in processing proteins and lipids and the rate of trafficking across the Golgi compartments. Both these factors together influence the modifications of the proteins and lipids as they pass through the Golgi (Lowe, 2011; Y Wang et al., 2008). Several signalling pathways contribute to maintaining the Golgi structure, and changes in Golgi structure could severely affect cellular homeostasis (Nakamura et al., 2012). The Golgi apparatus has also been viewed as a sequester of several signalling molecules involved in cell cycle progression and apoptosis. Changes in Golgi organization could release these molecules from the Golgi and help them localise to other cellular locations where they activate other downstream pathways (Gervais et al., 2002; Hicks & Machamer, 2005; Litvak et al., 2004; Rabouille et al., 2015). Arf1 localises at the Golgi and is prominent in regulating the Golgi organization and function. Arf1 is also involved in the CLIC/GEEC (CG) endocytic pathway. The CG pathway in cells is limited by the availability of active Arf1 at the plasma membrane. Fragmentation of the Golgi with BFA has been shown to free Arf1, which now localises to the plasma membrane and increases the rate of CG pathway in CHO cells (Kumari & Mayor, 2008; Thottacherry et al., n.d.).

Similarly, changes in Golgi organization could significantly affect the association and dissociation of several molecules from the Golgi membranes. Golgi membranes isolated from adherent and suspended cells could be processed through mass spectrometry to obtain a proteomic profile of these membranes. Such a proteomic profile could help identify molecules that differentially associate with the Golgi membranes in adherent vs suspended cells. This could help us identify other regulators and regulatory pathways that are involved in the

regulation of Golgi organisation downstream of adhesion and also identify pathways that could be affected by changes in Golgi organisation.

Loss of adhesion-mediated Golgi disorganisation is distinctly different for cis-medial and trans-Golgi compartments (Fig. 3.1, (Singh et al., 2018)). As a result, there is a significant difference in the volume and surface area of the cis-medial and trans-Golgi compartments (Singh et al., 2018). It would be interesting to try if these differences could help isolate separate cis-medial and trans-Golgi compartments from non-adherent cells. If such isolation is possible, the proteomic profile of cis-medial and trans-Golgi compartments could provide great insight into the regulation and functioning of these compartments.

As a preliminary step, membrane-rich fractions were isolated from cells to check if differences in protein levels could be detected. Membrane-rich fractions isolated from the post-nuclear supernatant (PNS) of stable adherent and suspended WT-MEFs showed significant differences in the amount of Arf1, Dynein, and GM130 detected in these fractions. There was a drop in the levels of Arf1, Dynein and GM130 seen in membrane fractions from suspended cells compared to stable adherent cells (Fig. 5.1). This suggested differences in protein levels could be detected upon isolation of large membrane fractions.

The discontinuous sucrose gradient method was employed to obtain Golgi membrane-rich fractions. The discontinuous sucrose gradient method is a density-based separation method in which the Golgi membranes are expected to partition at the interface of 0.8M/1.2M sucrose. In both adherent and suspended WT-MEFs, cis-Golgi (p115 & GM130) and trans-Gogi (Vti1a, Vti1b & Syntaxin 6) markers were detected in fractions collected from the interface of 0.8M/1.2M sucrose gradient (Fig. 5.2B). However, the extent of protein separation into these fractions was very low. Most of the protein loaded did not fractionate to the 0.8M/1.2M interface and was instead detected with 1.4M fractions. This suggested that the fractionation efficiency was extremely low and the level of proteins obtained was insufficient and non-ideal for protein estimation via mass spectrometry.

Further standardization of the Golgi isolation method would be needed and if optimised to get pure Golgi membrane fractions from adherent vs suspended cells could contribute immensely to our understanding of adhesion-dependent Golgi organisation.

# CONCLUSION

The work presented in this thesis in using suspended mouse fibroblasts has added to our understanding of how the small GTPase Arf1 can regulate Golgi organisation and function. It has helped reveal that Arf GEF, GBF1, plays a significant role in regulating Arf1 activity at the cis-medial Golgi. Arf GEF inhibitors, BFA, and GCA reveal that along with total active Arf1 levels, the kinetics of drop in active Arf1 levels, influences the organization and function of the Golgi in non-adherent cells. This hence suggests that cells could effectively alter the kinetics of Arf1 activation/inactivation to achieve distinct change in Golgi organization and hence function. Such a regulation could have implications for how Golgi fragmentation is regulated in processes like cell division. Loss of adhesion-mediated regulation of microtubule acetylation, while not regulating Golgi organisation, could have implications for other MT stability dependent events at the Golgi. It also reveals how adhesion-dependent regulation of the Golgi can be relevant to not just test how the Golgi is regulated, but also extend our understanding of existing Golgi regulatory pathways in cells.

# REFERENCES


A. M. Tartakoff, J. R. T. (1989). The Response of the Golgi Complex to Microtubule Alterations: The Roles of Metabolic Energy and Membrane Traffic in Golgi Complex Organization. *Journal of Cell Biology*, *109*(November), 2081–2088.

Ackema, K. B., Hench, J., Böckler, S., Wang, S. C., Sauder, U., Mergentaler, H., Westermann, B., Bard, F., Frank, S., & Spang, A. (2014). The small GTP ase Arf1 modulates mitochondrial morphology and function . *The EMBO Journal*, *33*(22). https://doi.org/10.15252/embj.201489039

Adams, C. L., Chen, Y.-T., Smith, S. J., & Nelson, W. J. (1998). Mechanisms of Epithelial Cell-Cell Adhesion and Cell Compaction Revealed by High-resolution Tracking of E-Cadherin-Green Fluorescent Protein. *The Journal of Cell Biology*, *142*(4), 1105–1119. http://www.jcb.org

Akhmanova, A., & Steinmetz, M. O. (2008). Tracking the ends: A dynamic protein network controls the fate of microtubule tips. In *Nature Reviews Molecular Cell Biology* (Vol. 9, Issue 4). https://doi.org/10.1038/nrm2369

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2017). Molecular Biology of the Cell. In *Molecular Biology of the Cell*. https://doi.org/10.1201/9781315735368

Allan, V. J., Thompson, H. M., & McNiven, M. A. (2002). Motoring around the Golgi. *Nature Cell Biology*, *4*(10), E236–E242. https://doi.org/10.1038/ncb1002-e236

Altan-Bonnet, N, Phair, R. D., Polishchuk, R. S., Weigert, R., & Lippincott-Schwartz, J. (2003). A role for Arf1 in mitotic Golgi disassembly, chromosome segregation, and cytokinesis. *Proc Natl Acad Sci U S A*, *100*(23), 13314–13319. https://doi.org/10.1073/pnas.2234055100

Altan-Bonnet, Nihal, Sougrat, R., & Lippincott-Schwartz, J. (2004). Molecular basis for Golgi maintenance and biogenesis. *Current Opinion in Cell Biology*, *16*(4), 364–372. https://doi.org/10.1016/j.ceb.2004.06.011

Altan-Bonnet, Nihal, Sougrat, R., Liu, W., Snapp, E. L., Ward, T., & Lippincott-Schwartz, J. (2006a). Golgi Inheritance in Mammalian Cells Is Mediated through Endoplasmic Reticulum Export Activities. *Molecular Biology of the Cell*, *17*(2), 990–1005. https://doi.org/10.1091/mbc.e05-02-0155

Altan-Bonnet, Nihal, Sougrat, R., Liu, W., Snapp, E. L., Ward, T., & Lippincott-Schwartz, J. (2006b). Golgi inheritance in mammalian cells is mediated through endoplasmic reticulum export activities. *Molecular Biology of the Cell*, *17*(2), 990–1005. https://doi.org/10.1091/MBC.E05-02-0155/ASSET/IMAGES/LARGE/ZMK0020675010009.JPEG

Alvarez, C., Garcia-Mata, R., Brandon, E., & Sztul, E. (2003). COPI recruitment is modulated by a Rab1b-dependent mechanism. *Molecular Biology of the Cell*, *14*(5), 2116–2127. https://doi.org/10.1091/MBC.E02-09-0625/ASSET/IMAGES/LARGE/MK0532208009.JPEG

Antón-Fernández, A., Aparicio-Torres, G., Tapia, S., DeFelipe, J., & Muñoz, A. (2017). Morphometric alterations of Golgi apparatus in Alzheimer's disease are related to tau hyperphosphorylation. *Neurobiology of Disease*, *97*, 11–23. https://doi.org/10.1016/j.nbd.2016.10.005

Arthur, W. T., Noren, N. K., & Burridge, K. (2002). Regulation of Rho family GTPases by cell-cell and cell-matrix adhesion. *Biological Research*, *35*(2). https://doi.org/10.4067/S0716-97602002000200016

Atkin-Smith, G. K., & Poon, I. K. H. (2017). Disassembly of the Dying: Mechanisms and

Functions. *Trends in Cell Biology*, *27*(2), 151–162. https://doi.org/10.1016/j.tcb.2016.08.011

Ayala, I., & Colanzi, A. (2017). Alterations of Golgi organization in Alzheimer's disease: A cause or a consequence? *Tissue and Cell*, *49*(2), 133–140. https://doi.org/10.1016/j.tice.2016.11.007

B. R., R., Shah, N., Joshi, P., Madhusudan, M. S., & Balasubramanian, N. (2023). Kinetics of Arf1 inactivation regulates Golgi organisation and function in non-adherent fibroblasts. *Biology Open*. https://doi.org/10.1242/BIO.059669

Baillat, G., Bellouze, S., Scha, M. K., Rabouille, C., & Haase, G. (2014). *Golgi fragmentation in pmn mice is due to a defective ARF1 / TBCE cross-talk that coordinates COPI vesicle formation and tubulin polymerization*. *23*(22), 5961–5975. https://doi.org/10.1093/hmg/ddu320

Bajaj, R., Warner, A. N., Fradette, J. F., & Gibbons, D. L. (2022). Dance of The Golgi: Understanding Golgi Dynamics in Cancer Metastasis. *Cells*, *11*(9). https://doi.org/10.3390/cells11091484

Balabanian, L., Berger, C. L., & Hendricks, A. G. (2017). Acetylated Microtubules Are Preferentially Bundled Leading to Enhanced Kinesin-1 Motility. *Biophysical Journal*, *113*(7). https://doi.org/10.1016/j.bpj.2017.08.009

Bannykh, Sergei I., Rowe, T., & Balch, W. E. (1996). The organization of endoplasmic reticulum export complexes. *Journal of Cell Biology*, *135*(1). https://doi.org/10.1083/jcb.135.1.19

Bannykh, Serguei I., Plutner, H., Matteson, J., & Balch, W. E. (2005). The role of ARF1 and Rab GTPases in polarization of the Golgi stack. *Traffic*, *6*(9), 803–819. https://doi.org/10.1111/j.1600-0854.2005.00319.x

Barlowe, C. (2003). Signals for COPII-dependent export from the ER: What's the ticket out? In *Trends in Cell Biology* (Vol. 13, Issue 6). https://doi.org/10.1016/S0962-8924(03)00082-5

Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M. F., Ravazzola, M., Amherdt, M., & Schekman, R. (1994). COPII: A membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell*, *77*(6). https://doi.org/10.1016/0092-8674(94)90138-4

Barr, F. A., Puype, M., Vandekerckhove, J., & Warren, G. (1997). GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell*, *91*(2), 253–262. https://doi.org/10.1016/S0092-8674(00)80407-9

Barr, F. A., & Short, B. (2003). Golgins in the structure and dynamics of the Golgi apparatus. In *Current Opinion in Cell Biology* (Vol. 15, Issue 4, pp. 405–413). https://doi.org/10.1016/S0955-0674(03)00054-1

Baschieri, F., & Farhan, H. (2012). *Crosstalk of small GTPases at the Golgi apparatus Do not distribute . © 2012 Landes Bioscience . Do not distribute . 3*(2), 80–90.

Bekier, M. E., Wang, L., Li, J., Huang, H., Tang, D., Zhang, X., & Wang, Y. (2017). Knockout of the Golgi stacking proteins GRASP55 and GRASP65 impairs Golgi structure and function. *Molecular Biology of the Cell*, *28*(21), 2833–2842. https://doi.org/10.1091/mbc.E17-02-0112

Bellouze, S., Schäfer, M. K., Buttigieg, D., Baillat, G., Rabouille, C., & Haase, G. (2014). Golgi fragmentation in pmn mice is due to a defective ARF1/TBCE cross-talk that coordinates COPI vesicle formation and tubulin polymerization. *Human Molecular Genetics*, *23*(22), 5961–5975. https://doi.org/10.1093/hmg/ddu320

Berrier, A. L., & Yamada, K. M. (2007). Cell–matrix adhesion. *Journal of Cellular Physiology*, *213*(3), 565–573. https://doi.org/10.1002/JCP.21237

Bottanelli, F., Kilian, N., Ernst, A. M., Rivera-Molina, F., Schroeder, L. K., Kromann, E. B.,

Lessard, M. D., Erdmann, R. S., Schepartz, A., Baddeley, D., Bewersdorf, J., Toomre, D., & Rothman, J. E. (2017). A novel physiological role for ARF1 in the formation of bidirectional tubules from the Golgi. *Molecular Biology of the Cell*, *28*(12), 1676–1687. https://doi.org/10.1091/mbc.E16-12-0863

Bouvet, S., Golinelli-Cohen, M.-P., Contremoulins, V., & Jackson, C. L. (2013). Targeting of the Arf-GEF GBF1 to lipid droplets and Golgi membranes. *Journal of Cell Science*, *126*(20), 4794–4805. https://doi.org/10.1242/jcs.134254

Brown, H. A., Gutowski, S., Moomaw, C. R., Slaughter, C., & Sternwels, P. C. (1993). ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. *Cell*, *75*(6). https://doi.org/10.1016/0092-8674(93)90323-I

Bui, Q. T., Golinelli-Cohen, M. P., & Jackson, C. L. (2009). Large Arf1 guanine nucleotide exchange factors: Evolution, domain structure, and roles in membrane trafficking and human disease. *Molecular Genetics and Genomics*, *282*(4), 329–350. https://doi.org/10.1007/s00438-009-0473-3

Burgess, T. L., Skoufias, D. A., & Wilson, L. (1991). Disruption of the Golgi apparatus with brefeldin A does not destabilize the associated detyrosinated microtubule network. *Cell Motility and the Cytoskeleton*, *20*(4). https://doi.org/10.1002/cm.970200405

Burkhardt, J. K. (1998). The role of microtubule-based motor proteins in maintaining the structure and function of the Golgi complex. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1404*(1–2), 113–126. https://doi.org/10.1016/S0167-4889(98)00052-4

Buwa, N., Kannan, N., Kanade, S., & Balasubramanian, N. (2021). Adhesion-dependent Caveolin-1 Tyrosine-14 phosphorylation is regulated by FAK in response to changing matrix stiffness. *FEBS Letters*, *595*(4), 532–547. https://doi.org/10.1002/1873-3468.14025

Byron, A., Askari, J. A., Humphries, J. D., Jacquemet, G., Koper, E. J., Warwood, S., Choi, C. K., Stroud, M. J., Chen, C. S., Knight, D., & Humphries, M. J. (2015). A proteomic approach reveals integrin activation state-dependent control of microtubule cortical targeting. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms7135

Calderwood, D. A., Shattil, S. J., & Ginsberg, M. H. (2000). Integrins and actin filaments: Reciprocal regulation of cell adhesion and signaling. *Journal of Biological Chemistry*, *275*(30), 22607–22610. https://doi.org/10.1074/jbc.R900037199

Cao, H., Weller, S., Orth, J. D., Chen, J., Huang, B., Chen, J. L., Stamnes, M., & McNiven, M. A. (2005). Actin and Arf1-dependent recruitment of a cortactin-dynamin complex to the Golgi regulates post-Golgi transport. *Nature Cell Biology*, *7*(5), 483–492. https://doi.org/10.1038/ncb1246

Casanova, J. E. (2007). Regulation of Arf activation: The Sec7 family of guanine nucleotide exchange factors. In *Traffic* (Vol. 8, Issue 11, pp. 1476–1485). John Wiley & Sons, Ltd. https://doi.org/10.1111/j.1600-0854.2007.00634.x

Cassimeris, L. (2002). The oncoprotein 18/stathmin family of microtubule destabilizers. In *Current Opinion in Cell Biology* (Vol. 14, Issue 1). https://doi.org/10.1016/S0955-0674(01)00289-7

Chabin-Brion, K., Marceiller, J., Perez, F., Settegrana, C., Drechou, A., Durand, G., & Poüs, C. (2001). The Golgi Complex Is a Microtubule-organizing Organelle. *Molecular Biology of the Cell*, *12*(7), 2047–2060. https://doi.org/10.1091/mbc.12.7.2047

Chabin-Brion, K., Me Marceiller, J., Perez, F., Settegrana, C., Drechou, A., Ve Durand, G., & Poü, C. (2001). The Golgi Complex Is a Microtubule-organizing Organelle. In *Molecular Biology of the Cell* (Vol. 12). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC55652/pdf/mk002047.pdf

Chakrabarti, R., Lee, M., & Higgs, H. N. (2021). Multiple roles for actin in secretory and endocytic pathways. In *Current Biology* (Vol. 31, Issue 10).

https://doi.org/10.1016/j.cub.2021.03.038
Cherfils, J. (2014). Arf GTPases and their effectors: Assembling multivalent membrane-binding platforms. *Current Opinion in Structural Biology*, *29*, 67–76. https://doi.org/10.1016/j.sbi.2014.09.007
Chiu, R., Novikov, L., Mukherjee, S., & Shields, D. (2002). A caspase cleavage fragment of p115 induces fragmentation of the Golgi apparatus and apoptosis. *Journal of Cell Biology*, *159*(4), 637–648. https://doi.org/10.1083/jcb.200208013
Christis, C., & Munro, S. (2012). The small G protein Arl1 directs the trans-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2. *Journal of Cell Biology*, *196*(3), 327–335. https://doi.org/10.1083/jcb.201107115
Clausen, J. D., Christiansen, G., Holst, H. U., & Birkelund, S. (1997). Chlamydia trachomatis utilizes the host cell microtubule network during early events of infection. *Molecular Microbiology*, *25*(3), 441–449. https://doi.org/10.1046/J.1365-2958.1997.4591832.X
Cockcroft, S., Thomas, G. M. H., Fensome, A., Geny, B., Cunningham, E., Gout, I., Hiles, I., Totty, N. F., Truong, O., & Hsuan, J. J. (1994). Phospholipase D: A downstream effector of ARF in granulocytes. *Science*, *263*(5146). https://doi.org/10.1126/science.8290961
Colanzi, A., Carcedo, C. H., Persico, A., Cericola, C., Turacchio, G., Bonazzi, M., Luini, A., & Corda, D. (2007). The Golgi mitotic checkpoint is controlled by BARS-dependent fission of the Golgi ribbon into separate stacks in G2. *The EMBO Journal*, *26*, 2465–2476. https://doi.org/10.1038/
Colanzi, A., & Corda, D. (n.d.). *Mitosis controls the Golgi and the Golgi controls mitosis*. https://doi.org/10.1016/j.ceb.2007.06.002
Colanzi, A., Grimaldi, G., Catara, G., Valente, C., Cericola, C., Liberali, P., Ronci, M., Lalioti, V. S., Bruno, A., Beccari, A. R., Urbani, A., De Florah, A., Nardini, M., Bolognesi, M., Luini, A., & Corda, D. (2013). Molecular mechanism and functional role of brefeldin A-mediated ADP-ribosylation of CtBP1/BARS. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(24), 9794–9799. https://doi.org/10.1073/PNAS.1222413110
Cole, N. B., Sciakyt, N., Marotta, A., Song, J., & Lippincott-Schwartz, J. (1996a). Golgi Dispersal during Microtubule Disruption: Regeneration of Golgi Stacks at Peripheral Endoplasmic Reticulum Exit Sites. In *Molecular Biology of the Cell* (Vol. 7). https://www.molbiolcell.org/doi/pdf/10.1091/mbc.7.4.631
Cole, N. B., Sciakyt, N., Marotta, A., Song, J., & Lippincott-Schwartz, J. (1996b). Golgi Dispersal during Microtubule Disruption: Regeneration of Golgi Stacks at Peripheral Endoplasmic Reticulum Exit Sites. In *Molecular Biology of the Cell* (Vol. 7). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC275914/pdf/mbc00011-0138.pdf
Colello, D., Mathew, S., Ward, R., Pumiglia, K., & LaFlamme, S. E. (2012). Integrins regulate microtubule nucleating activity of centrosome through mitogen-activated protein kinase/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signaling. *Journal of Biological Chemistry*, *287*(4). https://doi.org/10.1074/jbc.M111.254128
Colello, D., Reverte, C. G., Ward, R., Jones, C. W., Magidson, V., Khodjakov, A., & LaFlamme, S. E. (2010). Androgen and Src signaling regulate centrosome activity. *Journal of Cell Science*, *123*(12). https://doi.org/10.1242/jcs.057505
Copeland, S. J., Thurston, S. F., & Copeland, J. W. (2016). Actin- and microtubule-dependent regulation of Golgi morphology by FHDC1. *Molecular Biology of the Cell*, *27*(2), 260–276. https://doi.org/10.1091/mbc.e15-02-0070
Corda, D., Barretta, M. L., Cervigni, R. I., & Colanzi, A. (2012). Golgi complex fragmentation in G2/M transition: An organelle-based cell-cycle checkpoint. *IUBMB Life*, *64*(8), 661–670. https://doi.org/10.1002/iub.1054

Corda, D., Colanzi, A., & Luini, A. (2006). The multiple activities of CtBP/BARS proteins: The Golgi view. In *Trends in Cell Biology* (Vol. 16, Issue 3). https://doi.org/10.1016/j.tcb.2006.01.007

D'Souza-Schorey, C., & Chavrier, P. (2006). ARF proteins: Roles in membrane traffic and beyond. *Nature Reviews Molecular Cell Biology*, *7*(5), 347–358. https://doi.org/10.1038/nrm1910

D'souza, Z., Sumya, F. T., Khakurel, A., & Lupashin, V. (2021). Getting sugar coating right! the role of the golgi trafficking machinery in glycosylation. In *Cells* (Vol. 10, Issue 12). https://doi.org/10.3390/cells10123275

Dao, V. T., Dupuy, A. G., Gavet, O., Caron, E., & de Gunzburg, J. (2009). Dynamic changes in Rap1 activity are required for cell retraction and spreading during mitosis. *Journal of Cell Science*, *122*(16). https://doi.org/10.1242/jcs.041301

Dascher, C., & Balch, W. E. (1994). Dominant inhibitory mutants of ARF1 block endoplasmic reticulum to Golgi transport and trigger disassembly of the Golgi apparatus. *Journal of Biological Chemistry*, *269*(2), 1437–1448.

De Arcangelis, A., & Georges-Labouesse, E. (2000). Integrin and ECM functions: Roles in vertebrate development. In *Trends in Genetics* (Vol. 16, Issue 9). https://doi.org/10.1016/S0168-9525(00)02074-6

De Matteis, M. A., & Godi, A. (2004). Protein-lipid interactions in membrane trafficking at the Golgi complex. In *Biochimica et Biophysica Acta - Biomembranes* (Vol. 1666, Issues 1–2). https://doi.org/10.1016/j.bbamem.2004.07.002

De Matteis, Maria Antonietta, & Luini, A. (2008). Exiting the Golgi complex. In *Nature Reviews Molecular Cell Biology* (Vol. 9, Issue 4). https://doi.org/10.1038/nrm2378

Del Pozo, M. A., Price, L. S., Alderson, N. B., Ren, X. D., & Schwartz, M. A. (2000). Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *EMBO Journal*, *19*(9). https://doi.org/10.1093/emboj/19.9.2008

Di Girolamo, M., Silletta, M. G., De Matteis, M. A., Braca, A., Colanzi, A., Pawlak, D., Rasenick, M. M., Luini, A., & Corda, D. (1995). Evidence that the 50-kDa substrate of brefeldin A-dependent ADP-ribosylation binds GTP and is modulated by the G-protein βγ subunit complex. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(15). https://doi.org/10.1073/pnas.92.15.7065

Dippold, H. C., Ng, M. M., Farber-Katz, S. E., Lee, S.-K., Kerr, M. L., Peterman, M. C., Sim, R., Wiharto, P. A., Galbraith, K. A., Madhavarapu, S., Fuchs, G. J., Meerloo, T., Farquhar, M. G., Zhou, H., & Field, S. J. (n.d.). GOLPH3 Bridges Phosphatidylinositol-4- Phosphate and Actomyosin to Stretch and Shape the Golgi to Promote Budding. *Cell*, *139*, 337–351. https://doi.org/10.1016/j.cell.2009.07.052

Dix, C. L., Matthews, H. K., Uroz, M., McLaren, S., Wolf, L., Heatley, N., Win, Z., Almada, P., Henriques, R., Boutros, M., Trepat, X., & Baum, B. (2018). The Role of Mitotic Cell-Substrate Adhesion Re-modeling in Animal Cell Division. *Developmental Cell*, *45*(1). https://doi.org/10.1016/j.devcel.2018.03.009

Dobie, C., & Skropeta, D. (2021). Insights into the role of sialylation in cancer progression and metastasis. In *British Journal of Cancer* (Vol. 124, Issue 1). https://doi.org/10.1038/s41416-020-01126-7

Dodonova, S. O., Aderhold, P., Kopp, J., Ganeva, I., Röhling, S., Hagen, W. J. H., Sinning, I., Wieland, F., & Briggs, J. A. G. (2017). 9Å structure of the COPI coat reveals that the Arf1 GTPase occupies two contrasting molecular environments. *ELife*, *6*. https://doi.org/10.7554/eLife.26691

Donaldson, J.G., & Honda, A. (2005). Localization and function of Arf family GTPases. *Biochemical Society Transactions*, *33*(4), 639–642. https://doi.org/10.1042/BST0330639

Donaldson, Julie G., Honda, A., & Weigert, R. (2005). Multiple activities for Arf1 at the Golgi

complex. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1744*(3 SPEC. ISS.), 364–373. https://doi.org/10.1016/j.bbamcr.2005.03.001

Donaldson, Julie G., & Klausner, R. D. (1994). ARF: a key regulatory switch in membrane traffic and organelle structure. *Current Opinion in Cell Biology*, *6*(4). https://doi.org/10.1016/0955-0674(94)90072-8

Dorner, C., Ciossek, T., Müller, S., Møller, N. P. H., Ullrich, A., & Lammers, R. (1998). Characterization of KIF1C, a New Kinesin-like Protein Involved in Vesicle Transport from the Golgi Apparatus to the Endoplasmic Reticulum. *Journal of Biological Chemistry*, *273*(32), 20267–20275. https://doi.org/10.1074/jbc.273.32.20267

Dumaresq-doiron, K., Savard, M., Akam, S., Costantino, S., & Lefrancois, S. (2010). *The phosphatidylinositol 4-kinase PI4KIII  is required for the recruitment of GBF1 to Golgi membranes*. *4*. https://doi.org/10.1242/jcs.055798

East, M. P., & Kahn, R. A. (2011). Models for the functions of Arf GAPs. *Seminars in Cell and Developmental Biology*, *22*(1), 3–9. https://doi.org/10.1016/j.semcdb.2010.07.002

Efimov, A., Kharitonov, A., Efimova, N., Loncarek, J., Miller, P. M., Andreyeva, N., Gleeson, P., Galjart, N., Maia, A. R. R., Mcleod, I. X., Yates, J. R., Maiato, H., Khodjakov, A., Akhmanova, A., & Kaverina, I. (n.d.). *Developmental Cell Asymmetric CLASP-Dependent Nucleation of Noncentrosomal Microtubules at the trans-Golgi Network*. https://doi.org/10.1016/j.devcel.2007.04.002

Egea, G., Lázaro-Diéguez, F., & Vilella, M. (2006). Actin dynamics at the Golgi complex in mammalian cells. *Current Opinion in Cell Biology*, *18*(2), 168–178. https://doi.org/10.1016/j.ceb.2006.02.007

Egea, G., Serra-Peinado, C., Salcedo-Sicilia, L., & Gutiérrez-Martínez, E. (2013). Actin acting at the golgi. *Histochemistry and Cell Biology*, *140*(3), 347–360. https://doi.org/10.1007/s00418-013-1115-8

Enkler, L., Szentgyörgyi, V., Pennauer, M., Prescianotto-Baschong, C., Riezman, I., Wiesyk, A., Avraham, R. E., Spiess, M., Zalckvar, E., Kucharczyk, R., Riezman, H., & Spang, A. (n.d.). *Nature Cell Biology nature cell biology Arf1 coordinates fatty acid metabolism and mitochondrial homeostasis*. https://doi.org/10.1038/s41556-023-01180-2

Etienne-Manneville, S., & Hall, A. (2002). Rho GTPases in cell biology. In *Nature* (Vol. 420, Issue 6916). https://doi.org/10.1038/nature01148

Etienne-Manneville, S., & Hall, A. (2003). Cdc42 regulates GSK-3β and adenomatous polyposis coli to control cell polarity. *Nature 2003 421:6924*, *421*(6924), 753–756. https://doi.org/10.1038/nature01423

Faini, M., Beck, R., Wieland, F. T., & Briggs, J. A. G. (2013). Vesicle coats: Structure, function, and general principles of assembly. *Trends in Cell Biology*, *23*(6), 279–288. https://doi.org/10.1016/j.tcb.2013.01.005

Fan, J. Y., Roth, J., & Zuber, C. (2003). Ultrastructural analysis of transitional endoplasmic reticulum and pre-Golgi intermediates: A highway for cars and trucks. *Histochemistry and Cell Biology*, *120*(6). https://doi.org/10.1007/s00418-003-0597-1

Farber-Katz, S. E., Dippold, H. C., Buschman, M. D., Peterman, M. C., Xing, M., Noakes, C. J., Tat, J., Ng, M. M., Rahajeng, J., Cowan, D. M., Fuchs, G. J., Zhou, H., & Field, S. J. (2014). DNA damage triggers golgi dispersal via DNA-PK and GOLPH3. *Cell*, *156*(3), 413–427. https://doi.org/10.1016/j.cell.2013.12.023

Feinstein, T. N., & Linstedt, A. D. (2008). GRASP55 Regulates Golgi Ribbon Formation. *Molecular Biology of the Cell*, *19*(7), 2696–2707. https://doi.org/10.1091/mbc.e07-11-1200

Fucini, R. V., Navarrete, A., Vadakkan, C., Lacomis, L., Erdjument-Bromage, H., Tempst, P., & Stamnes, M. (2000). Activated ADP-ribosylation factor assembles distinct pools of actin on Golgi membranes. *Journal of Biological Chemistry*, *275*(25), 18824–18829.

https://doi.org/10.1074/jbc.M000024200
García-Mata, R., & Sztul, E. (2003). The membrane-tethering protein p115 interacts with GBF1, an ARF guanine-nucleotide-exchange factor. *EMBO Reports*, *4*(3). https://doi.org/10.1038/sj.embor.embor762
García-Mata, R., Szul, T., Alvarez, C., & Sztul, E. (2003). ADP-ribosylation factor/COPI-dependent events at the endoplasmic reticulum-Golgi interface are regulated by the guanine nucleotide exchange factor GBF1. *Molecular Biology of the Cell*, *14*(6). https://doi.org/10.1091/mbc.E02-11-0730
Gervais, F. G., Singaraja, R., Xanthoudakis, S., Gutekunst, C. A., Leavitt, B. R., Metzler, M., Hackam, A. S., Tam, J., Vaillancourt, J. P., Houtzager, V., Rasper, D. M., Roy, S., Hayden, M. R., & Nicholson, D. W. (2002). Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. *Nature Cell Biology 2002 4:2*, *4*(2), 95–105. https://doi.org/10.1038/ncb735
Gillingham, A. K., Tong, A. H. Y., Boone, C., & Munro, S. (2004). The GTPase Arf1p and the ER to Golgi cargo receptor Erv14p cooperate to recruit the golgin Rud3p to the cis-Golgi. *Journal of Cell Biology*, *167*(2). https://doi.org/10.1083/jcb.200407088
Glick, B. S. (2000). Organization of the Golgi apparatus. *Current Opinion in Cell Biology*, *12*(4), 450–456. https://doi.org/10.1016/S0955-0674(00)00116-2
Godi, A., Di Campli, A., Konstantakopoulos, A., Di Tullio, G., Alessi, D. R., Kular, G. S., Daniele, T., Marra, P., Lucocq, J. M., & De Matteis, M. A. (2004). FAPPS control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. *Nature Cell Biology*, *6*(5), 393–404. https://doi.org/10.1038/ncb1119
Goldberg, J. (1998). Structural basis for activation of ARF GTPase: Mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell*, *95*(2). https://doi.org/10.1016/S0092-8674(00)81754-7
Gomez, T. S., & Billadeau, D. D. (2009). A FAM21-Containing WASH Complex Regulates Retromer-Dependent Sorting. *Developmental Cell*, *17*(5). https://doi.org/10.1016/j.devcel.2009.09.009
Goodson, H. V, & Jonasson, E. M. (2018). *Microtubules and Microtubule-Associated Proteins*.
Goud, B., & Gleeson, P. A. (2010). TGN golgins, Rabs and cytoskeleton: Regulating the Golgi trafficking highways. *Trends in Cell Biology*, *20*(6), 329–336. https://doi.org/10.1016/j.tcb.2010.02.006
Griffiths, G., & Simons, K. (1986). The trans Golgi network: sorting at the exit site of the Golgi complex. *Science*, *234*(4775), 438–443. https://doi.org/10.1126/science.2945253
Gustafson, M. A., & Fromme, J. C. (2017). Regulation of Arf activation occurs via distinct mechanisms at early and late Golgi compartments. *Molecular Biology of the Cell*, *28*(25). https://doi.org/10.1091/mbc.E17-06-0370
Haase, G., & Rabouille, C. (2015). Golgi fragmentation in ALS motor neurons. New mechanisms targeting microtubules, tethers, and transport vesicles. *Frontiers in Neuroscience*, *9*(DEC). https://doi.org/10.3389/fnins.2015.00448
Haun, R. S., Tsai, S. C., Adamik, R., Moss, J., & Vaughan, M. (1993). Effect of myristoylation on GTP-dependent binding of ADP-ribosylation factor to Golgi. *Journal of Biological Chemistry*, *268*(10). https://doi.org/10.1016/s0021-9258(18)53145-8
Hauri, H. P., & Schweizer, A. (1992). The endoplasmic reticulum-Golgi intermediate compartment. *Current Opinion in Cell Biology*, *4*(4). https://doi.org/10.1016/0955-0674(92)90078-Q
Haynes, L. P., Thomas, G. M. H., & Burgoyne, R. D. (2005). Interaction of Neuronal Calcium Sensor-1 and ADP-ribosylation Factor 1 Allows Bidirectional Control of Phosphatidylinositol 4-Kinase ␣ and trans -Golgi Network-Plasma Membrane Traffic *. *Journal of Biological Chemistry*, *280*(7), 6047–6054.

https://doi.org/10.1074/jbc.M413090200
Helms, J. B., Palmer, D. J., & Rothman, J. E. (1993). Two distinct populations of ARF bound to Golgi membranes. *Journal of Cell Biology*, *121*(4), 751–760.
Helms, J. B., & Rothman, J. E. (1992). Inhibition by brefeldin a of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. *Nature*, *360*(6402). https://doi.org/10.1038/360352a0
Hernault, S. W., & Rosenbaum, J. L. (1985). Chlamydomonas a-Tubulin Is Posttranslationally Modified by Acetylation on the e-Amino Group of a Lysine^. *J. Cell Biol*, *24*, 258–263. https://pubs.acs.org/sharingguidelines
Hicks, S. W., & Machamer, C. E. (2005). Golgi structure in stress sensing and apoptosis. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1744*(3 SPEC. ISS.), 406–414. https://doi.org/10.1016/j.bbamcr.2005.03.002
Hubbert, C., Guardiola, A., Shao, R., Kawaguchi, Y., Ito, A., Nixon, A., Yoshida, M., Wang, X. F., & Yao, T. P. (2002). HDAC6 is a microtubule-associated deacetylase. *Nature*, *417*(6887). https://doi.org/10.1038/417455a
Hynes, R. O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell*, *110*(6). https://doi.org/10.1016/S0092-8674(02)00971-6
Jackson, C. L. (2009). Mechanisms of transport through the Golgi complex. *Journal of Cell Science*, *122*(4), 443–452. https://doi.org/10.1242/jcs.032581
Jamora, C., & Fuchs, E. (2002). Intercellular adhesion, signalling and the cytoskeleton. *Nature Cell Biology*, *4*(4), 101–108. https://doi.org/10.1038/ncb0402-e101
Janke, C., & Montagnac, G. (2017). Causes and Consequences of Microtubule Acetylation. *Current Biology*, *27*(23), R1287–R1292. https://doi.org/10.1016/J.CUB.2017.10.044
Jiang, Z., Hu, Z., Zeng, L., Lu, W., Zhang, H., Li, T., & Xiao, H. (2011). *The role of the Golgi apparatus in oxidative stress: is this organelle less significant than mitochondria?* https://doi.org/10.1016/j.freeradbiomed.2011.01.011
Jiwani, S., Ohr, R. J., Fischer, E. R., Hackstadt, T., Alvarado, S., Romero, A., & Jewett, T. J. (2012). Chlamydia trachomatis Tarp cooperates with the Arp2/3 complex to increase the rate of actin polymerization. *Biochemical and Biophysical Research Communications*, *420*(4), 816. https://doi.org/10.1016/J.BBRC.2012.03.080
Joshi, B., Bastiani, M., Strugnell, S. S., Boscher, C., Parton, R. G., & Nabi, I. R. (2012). Phosphocaveolin-1 is a mechanotransducer that induces caveola biogenesis via Egr1 transcriptional regulation. *Journal of Cell Biology*, *199*(3). https://doi.org/10.1083/jcb.201207089
Joshi, G., Bekier, M. E., & Wang, Y. (2015). Golgi fragmentation in Alzheimer's disease. *Frontiers in Neuroscience*, *9*, 340. https://doi.org/10.3389/fnins.2015.00340
Joshi, G., Chi, Y., Huang, Z., & Wang, Y. (2014). Aβ-induced Golgi fragmentation in Alzheimer's disease enhances Aβ production. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(13), E1230-9. https://doi.org/10.1073/pnas.1320192111
Joshi, G., & Wang, Y. (2015). Golgi defects enhance APP amyloidogenic processing in Alzheimer's disease. *BioEssays*, *37*(3), 240–247. https://doi.org/10.1002/bies.201400116
Kaczmarek, B., Verbavatz, J. M., & Jackson, C. L. (2017a). GBF1 and Arf1 function in vesicular trafficking, lipid homoeostasis and organelle dynamics. *Biology of the Cell*, *109*(12), 391–399. https://doi.org/10.1111/boc.201700042
Kaczmarek, B., Verbavatz, J. M., & Jackson, C. L. (2017b). GBF1 and Arf1 function in vesicular trafficking, lipid homoeostasis and organelle dynamics. *Biology of the Cell*, *109*(12), 391–399. https://doi.org/10.1111/boc.201700042
Kakugawa, Y., Wada, T., Yamaguchi, K., Yamanami, H., Ouchi, K., Sato, I., & Miyagi, T. (2002). Up-regulation of plasma membrane-associated ganglioside sialidase (Neu3) in

human colon cancer and its involvement in apoptosis suppression. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(16). https://doi.org/10.1073/pnas.152597199

Kanoh, H., Williger, B. T., & Exton, J. H. (1997). Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to golgi membranes. *Journal of Biological Chemistry*, *272*(9). https://doi.org/10.1074/jbc.272.9.5421

Kawamoto, K., Yoshida, Y., Tamaki, H., Torii, S., Shinotsuka, C., Yamashina, S., & Nakayama, K. (2002). GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the cis-Golgi and involved in membrane association of the COPI coat. *Traffic*, *3*(7), 483–495. https://doi.org/10.1034/j.1600-0854.2002.30705.x

Kirchhausen, T., Harrison, S. C., & Heuser, J. (1986). Configuration of clathrin trimers: Evidence from electron microscopy. *Journal of Ultrastructure Research and Molecular Structure Research*, *94*(3). https://doi.org/10.1016/0889-1605(86)90067-4

Klausner, R. D. (1992). Brefeldin A: insights into the control of membrane traffic and organelle structure. *The Journal of Cell Biology*, *116*(5), 1071–1080. https://doi.org/10.1083/jcb.116.5.1071

Klausner, R. D., Donaldson, J. G., & Lippincott-Schwartz, J. (1992). Brefeldin A: Insights into the control of membrane traffic and organelle structure. *Journal of Cell Biology*, *116*(5), 1071–1080. https://doi.org/10.1083/jcb.116.5.1071

Klumperman, J. (2011). Architecture of the mammalian Golgi. *Cold Spring Harbor Perspectives in Biology*, *3*(7), 1–19. https://doi.org/10.1101/CSHPERSPECT.A005181

Klumperman, J., Schweizer, A., Clausen, H., Tang, B. L., Hong, W., Oorschot, V., & Hauri, H. P. (1998). The recycling pathway of protein ERGIC-53 and dynamics of the ER-Golgi intermediate compartment. *Journal of Cell Science*, *111*(22). https://doi.org/10.1242/jcs.111.22.3411

Kreis, T. E. (1990). Role of microtubules in the organisation of the Golgi apparatus. In *Cell Motility and the Cytoskeleton* (Vol. 15, Issue 2). https://doi.org/10.1002/cm.970150202

Kühnle, J., Shillcock, J., Mouritsen, O. G., & Weiss, M. (2010). A modeling approach to the self-assembly of the Golgi apparatus. *Biophysical Journal*, *98*(12). https://doi.org/10.1016/j.bpj.2010.03.035

Kulasekaran, G., Chaineau, M., Crescenzo Piscopo, V. E., Verginelli, F., Fotouhi, M., Girard, M., Tang, Y., Dali, R., Lo, R., Stifani, S., & McPherson, P. S. (2021). An Arf/Rab cascade controls the growth and invasiveness of glioblastoma. *Journal of Cell Biology*, *220*(2). https://doi.org/10.1083/JCB.202004229

Kumar, P., Lyle, K. S., Gierke, S., Matov, A., Danuser, G., & Wittmann, T. (2009). GSK3beta phosphorylation modulates CLASP-microtubule association and lamella microtubule attachment. *The Journal of Cell Biology*, *184*(6), 895–908. https://doi.org/10.1083/JCB.200901042

Kumari, S., & Mayor, S. (2008). ARF1 is directly involved in dynamin-independent endocytosis. *Nature Cell Biology*, *10*(1), 30–41. https://doi.org/10.1038/ncb1666

Ladinsky, M S, Mastronarde, D. N., McIntosh, J. R., Howell, K. E., & Staehlin, L. a. (1999). Golgi structure in three dimensions: functional insights from the {NRK} cell. *J. of Cell Biology*, *144*(6), 1135–1149. https://doi.org/10.1083/jcb.144.6.1135

Ladinsky, Mark S., Kremer, J. R., Furcinitti, P. S., McIntosh, J. R., & Howell, K. E. (1994). HVEM tomography of the trans-Golgi network: structural insights and identification of a lace-like vesicle coat. *The Journal of Cell Biology*, *127*(1), 29. https://doi.org/10.1083/JCB.127.1.29

Ladinsky, Mark S, Mastronarde, D. N., Mcintosh, J. R., Howell, K. E., & Staehelin, L. A. (1999). Golgi Structure in Three Dimensions: Functional Insights from the Normal Rat Kidney Cell. In *The Journal of Cell Biology* (Vol. 144, Issue 6). http://www.jcb.org

LaFlamme, S. E., Mathew-Steiner, S., Singh, N., Colello-Borges, D., & Nieves, B. (2018). Integrin and microtubule crosstalk in the regulation of cellular processes. *Cellular and Molecular Life Sciences*, *75*(22), 4177–4185. https://doi.org/10.1007/s00018-018-2913-x

LaFlamme, S. E., Nieves, B., Colello, D., & Reverte, C. G. (2008). Integrins as regulators of the mitotic machinery. In *Current Opinion in Cell Biology* (Vol. 20, Issue 5). https://doi.org/10.1016/j.ceb.2008.06.006

Lane, J. D., Lucocq, J., Pryde, J., Barr, F. A., Woodman, P. G., Allan, V. J., & Lowe, M. (2002). Caspase-mediated cleavage of the stacking protein GRASP65 is required for Golgi fragmentation during apoptosis. *Journal of Cell Biology*, *156*(3), 495–509. https://doi.org/10.1083/jcb.200110007

Ledizet, M., & Piperno, G. (1987). *Identification of an acetylation site of Chlamydomonas a-tubulin (posttranslational acetylation/epitope mapping/a-tubulin isoforms/microtubule stability)*. *84*, 5720–5724. https://www.pnas.org

Lefrançois, S., & McCormick, P. J. (2007). The Arf GEF GBF1 is required for GGA recruitment to Golgi membranes. *Traffic*, *8*(10). https://doi.org/10.1111/j.1600-0854.2007.00623.x

Letourneur, F., Gaynor, E. C., Hennecke, S., Démollière, C., Duden, R., Emr, S. D., Riezman, H., & Cosson, P. (1994). Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. *Cell*, *79*(7). https://doi.org/10.1016/0092-8674(94)90011-6

Lewis, M. J., & Pelham, H. R. B. (1990). A human homologue of the yeast HDEL receptor. *Nature*, *348*(6297). https://doi.org/10.1038/348162a0

Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S., & Klausner, R. D. (1989a). Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: Evidence for membrane cycling from Golgi to ER. *Cell*, *56*(5). https://doi.org/10.1016/0092-8674(89)90685-5

Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S., & Klausner, R. D. (1989b). Rapid Redistribution of Golgi Proteins into the ER in Cells Treated with Btefeldin A: Evidence for Membrane Cycling from Golgi to ER. In *Cell* (Vol. 56).

Litvak, V., Argov, R., Dahan, N., Ramachandran, S., Amarilio, R., Shainskaya, A., & Lev, S. (2004). Mitotic Phosphorylation of the Peripheral Golgi Protein Nir2 by Cdk1 Provides a Docking Mechanism for Plk1 and Affects Cytokinesis Completion. *Molecular Cell*, *14*(3), 319–330. https://doi.org/10.1016/S1097-2765(04)00214-X

Liu, Y., Kahn, R. A., & Prestegard, J. H. (2009). Structure and Membrane Interaction of Myristoylated ARF1. *Structure*, *17*(1), 79–87. https://doi.org/10.1016/j.str.2008.10.020

Lobato-Márquez, D., Xu, J., Güler, G. Ö., Ojiakor, A., Pilhofer, M., & Mostowy, S. (2021). Mechanistic insight into bacterial entrapment by septin cage reconstitution. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-24721-5

Lowe, M. (2011). Structural organization of the Golgi apparatus. In *Current Opinion in Cell Biology* (Vol. 23, Issue 1, pp. 85–93). Elsevier Ltd. https://doi.org/10.1016/j.ceb.2010.10.004

Lowery, J., Szul, T., Styers, M., Holloway, Z., Oorschot, V., Klumperman, J., & Sztul, E. (2013). *The Sec7 Guanine Nucleotide Exchange Factor GBF1 Regulates Membrane Recruitment of BIG1 and BIG2 Guanine Nucleotide Exchange Factors to the Trans -Golgi Network ( TGN ) *. *288*(16), 11532–11545. https://doi.org/10.1074/jbc.M112.438481

Lu, L., & Hong, W. (2003). Interaction of Arl1-GTP with GRIP domains recruits autoantigens Golgin-97 and Golgin-245/p230 onto the Golgi. *Molecular Biology of the Cell*, *14*(9). https://doi.org/10.1091/mbc.E03-01-0864

Lu, L., Horstmann, H., Ng, C., & Hong, W. (2001). Regulation of Golgi structure and function by ARF-like protein 1 (Arl1). *Journal of Cell Science*, *114*(Pt 24), 4543–4555. http://www.ncbi.nlm.nih.gov/pubmed/11792819

Macrae, T. H. (1997). *nbulin post-translational modifications Enzymes and their mechanisms of action*. *278*, 265–278.

Magliozzi, R., Carrero, Z. I., Low, T. Y., Yuniati, L., Valdes-Quezada, C., Kruiswijk, F., van Wijk, K., Heck, A. J. R., Jackson, C. L., & Guardavaccaro, D. (2018). Inheritance of the Golgi Apparatus and Cytokinesis Are Controlled by Degradation of GBF1. *Cell Reports*, *23*(11). https://doi.org/10.1016/j.celrep.2018.05.031

Majoul, I., Straub, M., Hell, S. W., Duden, R., & Söling, H. D. (2001). KDEL-Cargo Regulates Interactions between Proteins Involved in COPI Vesicle Traffic: Measurements in Living Cells Using FRET. *Developmental Cell*, *1*(1), 139–153. https://doi.org/10.1016/S1534-5807(01)00004-1

Makhoul, C., Gosavi, P., & Gleeson, P. A. (2019). Golgi Dynamics: The Morphology of the Mammalian Golgi Apparatus in Health and Disease. *Frontiers in Cell and Developmental Biology*, *7*(July), 1–7. https://doi.org/10.3389/fcell.2019.00112

Mancini, M., Machamer, C. E., Roy, S., Nicholson, D. W., Thornberry, N. A., Casciola-Rosen, L. A., & Rosen, A. (2000). Caspase-2 Is Localized at the Golgi Complex and Cleaves Golgin-160 during Apoptosis. *The Journal of Cell Biology*, *149*(3), 603. https://doi.org/10.1083/JCB.149.3.603

Manolea, F., Claude, A., Chun, J., Rosas, J., & Melançon, P. (2008). Distinct functions for Arf guanine nucleotide exchange factors at the Golgi complex: GBF1 and BIGs are required for assembly and maintenance of the Golgi stack and trans-Golgi network, respectively. *Molecular Biology of the Cell*, *19*(2), 523–535. https://doi.org/10.1091/mbc.E07-04-0394

Mao, L., Li, N., Guo, Y., Xu, X., Gao, L., Xu, Y., Zhou, L., & Liu, W. (2013). AMPK phosphorylates GBF1 for mitotic Golgi disassembly. *Journal of Cell Science*, *126*(6), 1498–1505. https://doi.org/10.1242/jcs.121954

Marra, P., Salvatore, L., Mironov, A., Campli, A. Di, Tullio, G. Di, Trucco, A., Beznoussenko, G., & De Matteis, M. A. (2007). The Biogenesis of the Golgi Ribbon: The Roles of Membrane Input from the ER and of GM130 □ D □ V. *Molecular Biology of the Cell*, *18*, 1595–1608. https://doi.org/10.1091/mbc.E06

Marsh, B. J., & Howell, K. E. (2002). The mammalian Golgi - Complex debates. *Nature Reviews Molecular Cell Biology*, *3*(10), 789–795. https://doi.org/10.1038/nrm933

Marsh, B. J., Mastronarde, D. N., Buttle, K. F., Howell, K. E., & McIntosh, J. R. (2001). Organellar relationships in the Golgi region of the pancreatic beta cell line, HIT-T15, visualized by high resolution electron tomography. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(5), 2399–2406. https://doi.org/10.1073/PNAS.051631998/SUPPL_FILE/MOVIE7_15LEGEND.HTML

Maruta, H., Greer, K., & Rosenbaum, J. L. (1986). The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *Journal of Cell Biology*, *103*(2), 571–579. https://doi.org/10.1083/JCB.103.2.571

Mascanzoni, F., Ayala, I., & Colanzi, A. (2019). Organelle Inheritance Control of Mitotic Entry and Progression: Implications for Tissue Homeostasis and Disease. In *Frontiers in Cell and Developmental Biology* (Vol. 7). https://doi.org/10.3389/fcell.2019.00133

McMahon, H. T., & Mills, I. G. (2004). COP and clathrin-coated vesicle budding: Different pathways, common approaches. In *Current Opinion in Cell Biology* (Vol. 16, Issue 4). https://doi.org/10.1016/j.ceb.2004.06.009

Meissner, J. M., Bhatt, J. M., Lee, E., Styers, M. L., Ivanova, A. A., Kahn, R. A., & Sztul, E. (2018). The ARF guanine nucleotide exchange factor GBF1 is targeted to Golgi membranes through a PIP-binding domain. *Journal of Cell Science*, *131*(3). https://doi.org/10.1242/jcs.210245

Mellman, I., & Simons, K. (1992). The Golgi complex: In vitro veritas? In *Cell* (Vol. 68, Issue 5). https://doi.org/10.1016/0092-8674(92)90027-A

Memon, A. R. (2004). The role of ADP-ribosylation factor and SAR1 in vesicular trafficking in plants. *Biochimica et Biophysica Acta - Biomembranes*, *1664*(1), 9–30. https://doi.org/10.1016/j.bbamem.2004.04.005

Miao, F. F., Kong, C. C., Wu, Y., Fan, L., & Wang, T. L. (2018). Golgi fragmentation induced by overactivated cyclin-dependent kinase 5 is associated with isoflurane-induced neurotoxicity. *NeuroReport*, *29*(4), 241–246. https://doi.org/10.1097/WNR.0000000000000931

Miller, P. M., Folkmann, A. W., Maia, A. R. R., Efimova, N., Efimov, A., & Kaverina, I. (2009). *Golgi-derived CLASP-dependent microtubules control Golgi organization and polarized trafficking in motile cells*. https://doi.org/10.1038/ncb1920

Mironov, A. A., Beznoussenko, G. V., Nicoziani, P., Martella, O., Trucco, A., Kweon, H. S., Giandomenico, D. Di, Polishchuk, R. S., Fusella, A., Lupetti, P., Berger, E. G., Geerts, W. J. C., Koster, A. J., Burger, K. N. J., & Luini, A. (2001). Small cargo proteins and large aggregates can traverse the Golgi by a common mechanism without leaving the lumen of cisternae. *Journal of Cell Biology*, *155*(7). https://doi.org/10.1083/jcb.200108073

Mironov, A., Beznoussenko, G. V., Trucco, A., Lupetti, P., Smith, J. D., Geerts, W. J. C., Koster, A. J., Burger, K. N. J., Martone, M. E., Deerinck, T. J., Ellisman, M. H., & Luini, A. (2003). ER-to-Golgi carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains. *Developmental Cell*, *5*(4). https://doi.org/10.1016/S1534-5807(03)00294-6

Mironov, A., Colanzi, A., Silletta, M. G., Fiucci, G., Flati, S., Fusella, A., Polishchuk, R., Mironov, A., Di Tullio, G., Weigert, R., Malhotra, V., Corda, D., De Matteis, M. A., & Luini, A. (1997). Role of NAD+ and ADP-ribosylation in the maintenance of the Golgi structure. *Journal of Cell Biology*, *139*(5), 1109–1118. https://doi.org/10.1083/jcb.139.5.1109

Mizuno, M., & Singer, S. J. (1994). A possible role for stable microtubules in intracellular transport from the endoplasmic reticulum to the Golgi apparatus. *Journal of Cell Science*, *107*(5), 1321–1331. https://doi.org/10.1242/jcs.107.5.1321

Mogelsvang, S., Marsh, B. J., Ladinsky, M. S., & Howell, K. E. (2004). Predicting function from structure: 3D structure studies of the mammalian golgi complex. In *Traffic* (Vol. 5, Issue 5). https://doi.org/10.1111/j.1398-9219.2004.00186.x

Monetta, P., Slavin, I., Romero, N., & Alvarez, C. (2007). *Rab1b Interacts with GBF1 and Modulates both ARF1 Dynamics and COPI Association □*. *18*(July), 2400–2410. https://doi.org/10.1091/mbc.E06

Moremen, K. W., Tiemeyer, M., & Nairn, A. V. (2012). Vertebrate protein glycosylation: Diversity, synthesis and function. In *Nature Reviews Molecular Cell Biology* (Vol. 13, Issue 7). https://doi.org/10.1038/nrm3383

Morohashi, Y., Balklava, Z., Ball, M., Hughes, H., & Lowe, M. (2010). Phosphorylation and membrane dissociation of the ARF exchange factor GBF1 in mitosis. *Biochemical Journal*, *427*(3). https://doi.org/10.1042/BJ20091681

Mouratou, B., Biou, V., Joubert, A., Cohen, J., Shields, D. J., Geldner, N., Jürgens, G., Melançon, P., & Cherfils, J. (2005). The domain architecture of large guanine nucleotide exchange factors for the small GTP-binding protein Arf. *BMC Genomics*, *6*. https://doi.org/10.1186/1471-2164-6-20

Muccini, A. J., Gustafson, M. A., & Fromme, J. C. (2022). Structural basis for activation of Arf1 at the Golgi complex. *Cell Reports*, *40*(9), 111282. https://doi.org/10.1016/J.CELREP.2022.111282

Mukherjee, S., Chiu, R., Leung, S. M., & Shields, D. (2007). Fragmentation of the Golgi apparatus: An early apoptotic event independent of the cytoskeleton. *Traffic*, *8*(4), 369–

378. https://doi.org/10.1111/j.1600-0854.2007.00542.x
Muroyama, A., & Lechler, T. (2017). *Microtubule organization , dynamics and functions in differentiated cells*. 3012–3021. https://doi.org/10.1242/dev.153171
Nagashima, S., Tábara, L. C., Tilokani, L., Paupe, V., Anand, H., Pogson, J. H., Zunino, R., McBride, H. M., & Prudent, J. (2020). Golgi-derived PI(4)P-containing vesicles drive late steps of mitochondrial division. *Science*, *367*(6484). https://doi.org/10.1126/science.aax6089
Nakajima, K., Takei, Y., Tanaka, Y., Nakagawa, T., Nakata, T., Noda, Y., Setou, M., & Hirokawa, N. (2002). Molecular motor KIF1C is not essential for mouse survival and motor-dependent retrograde Golgi apparatus-to-endoplasmic reticulum transport. *Molecular and Cellular Biology*, *22*(3), 866–873. https://doi.org/10.1128/MCB.22.3.866-873.2002
Nakamura, N., Wei, J. H., & Seemann, J. (2012). Modular organization of the mammalian Golgi apparatus. *Current Opinion in Cell Biology*, *24*(4), 467–474. https://doi.org/10.1016/j.ceb.2012.05.009
Nakano, A., Luini, A., Pfeffer, S., & Novick, P. (2010). Passage through the Golgi This review comes from a themed issue on Membranes and organelles Edited. *Current Opinion in Cell Biology*, *22*, 471–478. https://doi.org/10.1016/j.ceb.2010.05.003
Nickel, W., Brügger, B., & Wieland, F. T. (2002). Vesicular transport: The core machinery of COPI recruitment and budding. *Journal of Cell Science*, *115*(16). https://doi.org/10.1242/jcs.115.16.3235
Niu, T. K., Pfeifer, A. C., Lippincott-Schwartz, J., & Jackson, C. L. (2005). Dynamics of GBF1, a brefeldin A-sensitive Arf1 exchange factor at the Golgi. *Molecular Biology of the Cell*, *16*(3). https://doi.org/10.1091/mbc.E04-07-0599
North, B. J., Marshall, B. L., Borra, M. T., Denu, J. M., & Verdin, E. (2003). The Human Sir2 Ortholog, SIRT2, Is an NAD+-Dependent Tubulin Deacetylase. *Molecular Cell*, *11*(2), 437–444. https://doi.org/10.1016/S1097-2765(03)00038-8
Ooi, C. E., Dell'Angelica, E. C., & Bonifacino, J. S. (1998). ADP-ribosylation factor 1 (ARF1) regulates recruitment of the AP-3 adaptor complex to membranes. *Journal of Cell Biology*, *142*(2). https://doi.org/10.1083/jcb.142.2.391
Opat, A. S., Van Vliet, C., & Gleeson, P. A. (2001). Trafficking and localisation of resident Golgi glycosylation enzymes. *Biochimie*, *83*(8), 763–773. https://doi.org/10.1016/S0300-9084(01)01312-8
Oprins, A., Duden, R., Kreis, T. E., Geuze, H. J., & Slot, J. W. (1993). Beta-COP localizes mainly to the cis-Golgi side in exocrine pancreas. *The Journal of Cell Biology*, *121*(1), 49–60. https://doi.org/10.1083/JCB.121.1.49
Orci, L., Glick, B. S., & Rothman, J. E. (1986). A new type of coated vesicular carrier that appears not to contain clathrin: Its possible role in protein transport within the Golgi stack. *Cell*, *46*(2). https://doi.org/10.1016/0092-8674(86)90734-8
Palazzo, A. F., Eng, C. H., Schlaepfer, D. D., Marcantonio, E. E., & Gundersen, G. G. (2004). Localized Stabilization of Microtubules by Integrin- and FAK-Facilitated Rho Signaling. *Science*, *303*(5659). https://doi.org/10.1126/science.1091325
Papanikou, E., & Glick, B. S. (2014). Golgi compartmentation and identity. *Current Opinion in Cell Biology*, *29*(1), 74–81. https://doi.org/10.1016/j.ceb.2014.04.010
Pawar, A., Meier, J. A., Dasgupta, A., Diwanji, N., Deshpande, N., Saxena, K., Buwa, N., Inchanalkar, S., Schwartz, M. A., & Balasubramanian, N. (2016). Ral-Arf6 crosstalk regulates Ral dependent exocyst trafficking and anchorage independent growth signalling. *Cellular Signalling*, *28*(9). https://doi.org/10.1016/j.cellsig.2016.05.023
Payne, C., & Schatten, G. (2003). Golgi dynamics during meiosis are distinct from mitosis and are coupled to endoplasmic reticulum dynamics until fertilization. *Developmental*

*Biology*, *264*(1). https://doi.org/10.1016/j.ydbio.2003.08.004
Pellett, P. A., Dietrich, F., Bewersdorf, J., Rothman, J. E., & Lavieu, G. (2013). Inter-Golgi transport mediated by COPI-containing vesicles carrying small cargoes. *ELife*, *2013*(2), 1–20. https://doi.org/10.7554/eLife.01296.001
Pennauer, M., Buczak, K., Prescianotto-Baschong, C., & Spiess, M. (2021). Shared and specific functions of Arfs 1–5 at the Golgi revealed by systematic knockouts. *Journal of Cell Biology*, *221*(1). https://doi.org/10.1083/jcb.202106100
Pernet-Gallay, K., Antony, C., Johannes, L., Bornens, M., Goud, B., & Rios, R. M. (2002). The overexpression of GMAP-210 blocks anterograde and retrograde transport between the ER and the Golgi apparatus. *Traffic*, *3*(11). https://doi.org/10.1034/j.1600-0854.2002.31107.x
Persico, A., Cervigni, I., Luisa Barretta, M., & Colanzi, A. (2009). *Mitotic inheritance of the Golgi complex*. https://doi.org/10.1016/j.febslet.2009.10.077
Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13). https://doi.org/10.1002/jcc.20084
Pevzner, I., Strating, J., Lifshitz, L., Parnis, A., Glaser, F., Herrmann, A., Brügger, B., Wieland, F., & Cassel, D. (2012). Distinct Role of Subcomplexes of the COPI Coat in the Regulation of ArfGAP2 Activity. *Traffic*, *13*(6), 849–856. https://doi.org/10.1111/j.1600-0854.2012.01349.x
Peyroche, A., Courbeyrette, R., Rambourg, A., & Jackson, C. L. (2001). The ARF exchange factors Gea1p and Gea2p regulate Golgi structure and function in yeast. *J Cell Sci*, *114*(Pt 12), 2241–2253. http://www.ncbi.nlm.nih.gov/pubmed/11493664
Pinho, S. S., & Reis, C. A. (2015). Glycosylation in cancer: Mechanisms and clinical implications. In *Nature Reviews Cancer* (Vol. 15, Issue 9). https://doi.org/10.1038/nrc3982
Pocognoni, C. A., Viktorova, E. G., Wright, J., Meissner, J. M., Sager, G., Lee, E., Belov, G. A., & Sztul, E. (2018). Highly conserved motifs within the large Sec7 ARF guanine nucleotide exchange factor GBF1 target it to the Golgi and are critical for GBF1 activity. *American Journal of Physiology - Cell Physiology*, *314*(6). https://doi.org/10.1152/ajpcell.00221.2017
Pokrovskaya, I. D., Willett, R., Smith, R. D., Morelle, W., Kudlyk, T., & Lupashin, V. V. (2011). Conserved oligomeric Golgi complex specifically regulates the maintenance of Golgi glycosylation machinery. *Glycobiology*, *21*(12), 1554–1569. https://doi.org/10.1093/GLYCOB/CWR028
Pollard, T. D., Blanchoin, L., & Mullins, R. D. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. In *Annual Review of Biophysics and Biomolecular Structure* (Vol. 29). https://doi.org/10.1146/annurev.biophys.29.1.545
Presley, J. F., Ward, T. H., Pfeifer, A. C., Siggia, E. D., Phair, R. D., & Lippincott-Schwartz, J. (2002). Dissection of COPI and Arf1 dynamics in vivo and role in Golgi membrane transport. *Nature*, *417*(6885), 187–193. https://doi.org/10.1038/417187a
Quilty, D., Gray, F., Summerfeldt, N., Cassel, D., & Melancon, P. (2014). Arf activation at the Golgi is modulated by feed-forward stimulation of the exchange factor GBF1. *Journal of Cell Science*, *127*(2), 354–364. https://doi.org/10.1242/jcs.130591
Rabouille, C., Yoshida, H., Seemann, J., Woodman, P., & Machamer, C. E. (2015). *The Golgi complex in stress and death*. *9*, 421. https://doi.org/10.3389/fnins.2015.00421
Rambourg, A., & Clermont, Y. (1997). Three-dimensional structure of the Golgi apparatus in mammalian cells. *The Golgi Apparatus*, 37–61. https://doi.org/10.1007/978-3-0348-8876-9_2

Reed, N. A., Cai, D., Blasius, T. L., Jih, G. T., Meyhofer, E., Gaertig, J., & Verhey, K. J. (2006a). *Report Microtubule Acetylation Promotes Kinesin-1 Binding and Transport*. 2166–2172. https://doi.org/10.1016/j.cub.2006.09.014

Reed, N. A., Cai, D., Blasius, T. L., Jih, G. T., Meyhofer, E., Gaertig, J., & Verhey, K. J. (2006b). Microtubule Acetylation Promotes Kinesin-1 Binding and Transport. *Current Biology*, *16*(21), 2166–2172. https://doi.org/10.1016/J.CUB.2006.09.014

Reinhard, C., Schweikert, M., Wieland, F. T., & Nickel, W. (2003). Functional reconstitution of COPI coat assembly and disassembly using chemically defined components. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(14). https://doi.org/10.1073/pnas.1432391100

Ren, X. D., Kiosses, W. B., & Schwartz, M. A. (1999). Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO Journal*, *18*(3). https://doi.org/10.1093/emboj/18.3.578

Ren, X., Farías, G. G., Canagarajah, B. J., Bonifacino, J. S., & Hurley, J. H. (2013). Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1. *Cell*, *152*(4), 755–767. https://doi.org/10.1016/j.cell.2012.12.042

Reverte, C. G., Benware, A., Jones, C. W., & LaFlamme, S. E. (2006). Perturbing integrin function inhibits microtubule growth from centrosomes, spindle assembly, and cytokinesis. *Journal of Cell Biology*, *174*(4). https://doi.org/10.1083/jcb.200603069

Richardson, B. C., McDonold, C. M., & Fromme, C. J. (2012). The Sec7 Arf-GEF Is Recruited to the trans-Golgi Network by Positive Feedback. *Developmental Cell*, *22*(4), 799–810. https://doi.org/10.1016/j.devcel.2012.02.006

Richardson, B. C., Mcdonold, C. M., & Fromme, J. C. (2012). Article The Sec7 Arf-GEF Is Recruited to the trans -Golgi Network by Positive Feedback. *Developmental Cell*, *22*(4), 799–810. https://doi.org/10.1016/j.devcel.2012.02.006

Rivero, S., Cardenas, J., Bornens, M., & Rios, R. M. (2009). Microtubule nucleation at the cis-side of the Golgi apparatus requires AKAP450 and GM130. *The EMBO Journal*, *28*, 1016–1028. https://doi.org/10.1038/

Robinson, M. S. (2004). Adaptable adaptors for coated vesicles. In *Trends in Cell Biology* (Vol. 14, Issue 4). https://doi.org/10.1016/j.tcb.2004.02.002

Romero, S., Le Clainche, C., & Gautreau, A. M. (2020). Actin polymerization downstream of integrins: Signaling pathways and mechanotransduction. *Biochemical Journal*, *477*(1), 1–21. https://doi.org/10.1042/BCJ20170719

Ronchi, P., Tischer, C., Acehan, D., & Pepperkok, R. (2014). Positive feedback between Golgi membranes, microtubules and ER exit sites directs de novo biogenesis of the Golgi. *Journal of Cell Science*, *127*(21), 4620–4633. https://doi.org/10.1242/jcs.150474

Rothman, J E. (1981). The golgi apparatus: two organelles in tandem. *Science (New York, N.Y.)*, *213*(4513), 1212–1219. http://www.ncbi.nlm.nih.gov/pubmed/7268428

Rothman, James E. (1994). Mechanisms of intracellular protein transport. In *Nature* (Vol. 372, Issue 6501). https://doi.org/10.1038/372055a0

Roubin, R., Acquaviva, C., Chevrier, V., Sedjaï, F., Zyss, D., Birnbaum, D., & Rosnet, O. (2013). Myomegalin is necessary for the formation of centrosomal and Golgi-derived microtubules. *Biology Open*, *2*(2). https://doi.org/10.1242/bio.20123392

Royle, S. J., Bright, N. A., & Lagnado, L. (2005). Clathrin is required for the function of the mitotic spindle. *Nature*, *434*(7037). https://doi.org/10.1038/nature03502

Sadakata, T., Shinoda, Y., Sekine, Y., Saruta, C., Itakura, M., Takahashi, M., & Furuichi, T. (2010). Interaction of Calcium-dependent Activator Protein for Secretion 1 (CAPS1) with the class II ADP-ribosylation factor small GTPases is required for dense-core vesicle trafficking in the trans-Golgi network. *Journal of Biological Chemistry*, *285*(49). https://doi.org/10.1074/jbc.M110.137414

Sáenz, J. B., Sun, W. J., Chang, J. W., Li, J., Bursulaya, B., Gray, N. S., & Haslam, D. B. (2009). Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. *Nature Chemical Biology*, *5*(3), 157–165. https://doi.org/10.1038/nchembio.144

Šali, A., & Blundell, T. L. (1993). Comparative protein modelling by satisfaction of spatial restraints. *Journal of Molecular Biology*, *234*(3). https://doi.org/10.1006/jmbi.1993.1626

Sanders, A. A. W. M., & Kaverina, I. (2015). *Nucleation and Dynamics of Golgi-derived Microtubules*. *9*(November), 1–7. https://doi.org/10.3389/fnins.2015.00431

Sandoval, I. V, Bonifacino, J. S., Klausner, R. D., Henkart, M., & Wehland, J. (1984). *Role of Microtubules in the Organization and Localization of the Golgi Apparatus Manipulation of Microtubule Polymerization and Effect of Changes in Microtubule Polymerization and Organization on the Location and Organization of the Golgi Apparatus in*.

Scales, S. J., Pepperkok, R., & Kreis, T. E. (1997). Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell*, *90*(6). https://doi.org/10.1016/S0092-8674(00)80379-7

Schaller, M. D. (2001). Biochemical signals and biological responses elicited by the focal adhesion kinase. In *Biochimica et Biophysica Acta - Molecular Cell Research* (Vol. 1540, Issue 1). https://doi.org/10.1016/S0167-4889(01)00123-9

Schmidt, J. A., & Brown, W. J. (2009). Lysophosphatidic acid acyltransferase 3 regulates Golgi complex structure and function. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.200904147

Schmidt, J. A., Kalkofen, D. N., Donovan, K. W., & Brown, W. J. (2010). A Role for Phospholipase A 2 Activity in Membrane Tubule Formation and TGN Trafficking HHS Public Access. *Traffic*, *11*(12), 1530–1536. https://doi.org/10.1111/j.1600-0854.2010.01115.x

Sciaky, N., Presley, J., Smith, C., Zaal, K. J. M., Cole, N., Moreira, J. E., Terasaki, M., Siggia, E., & Lippincott-Schwartz, J. (1997). Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *Journal of Cell Biology*, *139*(5), 1137–1155. https://doi.org/10.1083/jcb.139.5.1137

Sechi, S., Frappaolo, A., Karimpour-Ghahnavieh, A., Piergentili, R., & Giansanti, M. G. (2020). Oncogenic roles of GOLPH3 in the physiopathology of cancer. In *International Journal of Molecular Sciences* (Vol. 21, Issue 3). https://doi.org/10.3390/ijms21030933

Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R. A., & Rothman, J. E. (1991). ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: A novel role for a GTP-binding protein. *Cell*, *67*(2), 239–253. https://doi.org/10.1016/0092-8674(91)90176-Y

Sesso, A., De Faria, F. P., Miazato Iwamura, E. S., & Corrêa, H. (1994). A three-dimensional reconstruction study of the rough ER-Golgi interface in serial thin sections of the pancreatic acinar cell of the rat. *Journal of Cell Science*, *107*(3), 517–528. https://doi.org/10.1242/JCS.107.3.517

Shaul, Y. D., & Seger, R. (2006). ERK1c regulates Golgi fragmentation during mitosis. *Journal of Cell Biology*, *172*(6), 885–897. https://doi.org/10.1083/jcb.200509063

She, Z.-Y., Pan, M.-Y., Tan, F.-Q., & Yang, W.-X. (2017). *Minus end-directed kinesin-14 KIFC1 regulates the positioning and architecture of the Golgi apparatus* (Vol. 8, Issue 22). www.impactjournals.com/oncotarget

Shen, X., Xu, K. F., Fan, Q., Pacheco-Rodriguez, G., Moss, J., & Vaughan, M. (2006). Association of brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2) with recycling endosomes during transferrin uptake. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(8), 2635–2640. https://doi.org/10.1073/pnas.0510599103

Shiba, T., Kawasaki, M., Takatsu, H., Nogi, T., Matsugaki, N., Igarashi, N., Suzuki, M., Kato,

R., Nakayama, K., & Wakatsuki, S. (2003). Molecular mechanism of membrane recruitment of GGA by ARF in lysosomal protein transport. *Nature Structural Biology*, *10*(5). https://doi.org/10.1038/nsb920

Shin, H.-W., & Nakayama, K. (2004). Dual control of membrane targeting by PtdIns(4)P and ARF. *Trends in Biochemical Sciences*, *29*(10), 513–515. https://doi.org/10.1016/j.tibs.2004.08.007

Shin, H. W., Morinaga, N., Noda, M., & Nakayama, K. (2004). BIG2, A guanine nucleotide exchange factor for ADP-ribosylation factors: Its localization to recycling endosomes and implication in the endosome integrity. *Molecular Biology of the Cell*, *15*(12). https://doi.org/10.1091/mbc.E04-05-0388

Shin, O. H., Couvillon, A. D., & Exton, J. H. joh. (2001). Arfophilin is a common target of both class II and class III ADP-ribosylation factors. *Biochemistry*, *40*(36). https://doi.org/10.1021/bi0107391

Shinotsuka, C., Waguri, S., Wakasugi, M., Uchiyama, Y., & Nakayama, K. (2002). Dominant-negative mutant of BIG2 an ARF-guanine nucleotide exchange factor specifically affects membrane trafficking from the trans-Golgi network through inhibiting membrane association of AP-1 and GGA coat proteins. *Biochemical and Biophysical Research Communications*, *294*(2), 254–260. https://doi.org/10.1016/S0006-291X(02)00456-4

Short, B., Haas, A., & Barr, F. A. (2005). Golgins and GTPases, giving identity and structure to the Golgi apparatus. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1744*(3 SPEC. ISS.), 383–395. https://doi.org/10.1016/j.bbamcr.2005.02.001

Shorter, J., & Warren, G. (2002). Golgi architecture and inheritance. In *Annual Review of Cell and Developmental Biology* (Vol. 18, pp. 379–420). Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA . https://doi.org/10.1146/annurev.cellbio.18.030602.133733

Shrivastava-Ranjan, P., Faundez, V., Fang, G., Rees, H., Lah, J. J., Levey, A. I., & Kahn, R. A. (2008). Mint3/X11γ is an ADP-ribosylation factor-dependent adaptor that regulates the traffic of the Alzheimer's precursor protein from the trans-Golgi network. *Molecular Biology of the Cell*, *19*(1). https://doi.org/10.1091/mbc.E07-05-0465

Singh, V., Erady, C., & Balasubramanian, N. (2018). *Cell-matrix adhesion controls Golgi organization and function through Arf1 activation in anchorage-dependent cells*. *2*. https://doi.org/10.1242/jcs.215855

Sohn, K., Orci, L., Ravazzola, M., Amherdt, M., Bremser, M., Lottspeich, F., Fiedler, K., Helms, J. B., & Wieland, F. T. (1996). A major transmembrane protein of Golgi-derived COPI-coated vesicles involved in coatomer binding. *Journal of Cell Biology*, *135*(5). https://doi.org/10.1083/jcb.135.5.1239

Spang, A. (2002). ARF1 regulatory factors and COPI vesicle formation. *Current Opinion in Cell Biology*, *14*(4), 423–427. https://doi.org/10.1016/S0955-0674(02)00346-0

Stanley, H., Botas, J., & Malhotra, V. (1997). The mechanism of Golgi segregation during mitosis is cell type-specific. *Proceedings of the National Academy of Sciences*, *94*(26), 14467–14470. https://doi.org/10.1073/pnas.94.26.14467

Stanley, P. (2011). Golgi glycosylation. *Cold Spring Harbor Perspectives in Biology*, *3*(4), 1–13. https://doi.org/10.1101/cshperspect.a005199

Stephens, D. J. (2010). *Specific Functions of BIG1 and BIG2 in Endomembrane Organization*. *5*(3). https://doi.org/10.1371/journal.pone.0009898

Storrie, B., & Yang, W. (1998). Dynamics of the interphase mammalian Golgi complex as revealed through drugs producing reversible Golgi disassembly. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1404*(1–2). https://doi.org/10.1016/S0167-4889(98)00053-6

Suer, S., Misra, S., Saidi, L. F., & Hurley, J. H. (2003). Structure of the GAT domain of human

GGA1: A syntaxin amino-terminal domain fold in an endosomal trafficking adaptor. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(8). https://doi.org/10.1073/pnas.0831133100

Szul, T., Grabski, R., Lyons, S., Morohashi, Y., Shestopal, S., Lowe, M., & Sztul, E. (2007). Dissecting the role of the ARF guanine nucleotide exchange factor GBF1 in Golgi biogenesis and protein trafficking. *Journal of Cell Science*, *120*(22), 3929–3940. https://doi.org/10.1242/jcs.010769

Tan, X., Banerjee, P., Pham, E. A., Rutaganira, F. U. N., Basu, K., Bota-Rabassedas, N., Guo, H. F., Grzeskowiak, C. L., Liu, X., Yu, J., Shi, L., Peng, D. H., Leticia Rodriguez, B., Zhang, J., Zheng, V., Duose, D. Y., Solis, L. M., Mino, B., Raso, M. G., … Kurie, J. M. (2020). PI4KIIIβ is a therapeutic target in chromosome 1q-amplified lung adenocarcinoma. *Science Translational Medicine*, *12*(527). https://doi.org/10.1126/scitranslmed.aax3772

Tang, D., & Wang, Y. (2013). Cell cycle regulation of Golgi membrane dynamics. *Trends in Cell Biology*, *23*(6), 296–304. https://doi.org/10.1016/j.tcb.2013.01.008

Tanigawa, G., Orci, L., Amherdt, M., Ravazzola, M., Helms, J. B., & Rothman, J. E. (1993). Hydrolysis of bound GTP by ARF protein triggers uncoating of golgi-derived COP-coated vesicles. *Journal of Cell Biology*, *123*(6 PART 1), 1365–1371. https://doi.org/10.1083/jcb.123.6.1365

Thottacherry, J. J., Kosmalska, A. J., Kumar, A., Singh Vishen, A., Elosegui-Artola, A., Pradhan, S., Sharma, S., Singh, P. P., Guadamillas, M. C., Chaudhary, N., Vishwakarma, R., Trepat, X., Del Pozo, M. A., Parton, R. G., Rao, M., Pullarkat, P., Roca-Cusachs, P., & Mayor, S. (n.d.). *Mechanochemical feedback control of dynamin independent endocytosis modulates membrane tension in adherent cells*. https://doi.org/10.1038/s41467-018-06738-5

Thyberg, J., & Moskalewski, S. (1993a). *Cell & Tissue Relationship between the Golgi complex and microtubules enriched in detyrosinated or acetylated -tubulin : studies on cells recovering from nocodazole and cells in the terminal phase of cytokinesis*. 457–466.

Thyberg, J., & Moskalewski, S. (1993b). Relationship between the Golgi complex and microtubules enriched in detyrosinated or acetylated α-tubulin: studies on cells recovering from nocodazole and cells in the terminal phase of cytokinesis. *Cell & Tissue Research*, *273*(3). https://doi.org/10.1007/BF00333700

Thyberg, J., & Moskalewski, S. (1999). *REVIEW Role of Microtubules in the Organization of the Golgi Complex*. http://www.idealibrary.com

Traub, L. M., Ostrom, J. A., & Kornfeld, S. (1993). Biochemical dissection of AP-1 recruitment onto Golgi membranes. *Journal of Cell Biology*, *123*(3). https://doi.org/10.1083/jcb.123.3.561

Traub, Linton M., & Kornfeld, S. (1997). The trans-Golgi network: A late secretory sorting station. *Current Opinion in Cell Biology*, *9*(4), 527–533. https://doi.org/10.1016/S0955-0674(97)80029-4

Van Valkenburgh, H., Shern, J. F., Sharer, J. D., Zhu, X., & Kahn, R. A. (2001). ADP-ribosylation factors (ARFs) and ARF-like 1 (ARL1) have both specific and shared effectors. Characterizing ARL1-binding proteins. *Journal of Biological Chemistry*, *276*(25). https://doi.org/10.1074/jbc.M102359200

Wade, R. H., & Hyman, A. A. (1997). Microtubule structure and dynamics. *Current Opinion in Cell Biology*, *9*(1). https://doi.org/10.1016/S0955-0674(97)80146-9

Walch, L., Pellier, E., Leng, W., Lakisic, G., Gautreau, A., Contremoulins, V., Verbavatz, J. M., & Jackson, C. L. (2018). GBF1 and Arf1 interact with Miro and regulate mitochondrial positioning within cells. *Scientific Reports 2018 8:1*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-35190-0

Wang, J., Xu, H., Jiang, Y., Takahashi, M., Takeichi, M., & Meng, W. (2017). CAMSAP3-dependent microtubule dynamics regulates Golgi assembly in epithelial cells. *Journal of Genetics and Genomics*, *44*(1), 39–49. https://doi.org/10.1016/j.jgg.2016.11.005

Wang, Y, Wei, J.-H., Bisel, B., Tang, D., & Seemann, J. (2008). Golgi Cisternal Unstacking Stimulates COPI Vesicle Budding and Protein Transport. *PLoS ONE*, *3*(2), 1647. https://doi.org/10.1371/journal.pone.0001647

Wang, Yanzhuang, & Seemann, J. (2011). Golgi biogenesis. *Cold Spring Harbor Perspectives in Biology*, *3*(10), 1–12. https://doi.org/10.1101/cshperspect.a005330

Wang, Z., Zhang, C., & Qi, R. Z. (2014). A newly identified myomegalin isoform functions in Golgi microtubule organization and ER-Golgi transport. *Journal of Cell Science*, *127*(22). https://doi.org/10.1242/jcs.155408

Ward, T. H., Polishchuk, R. S., Caplan, S., Hirschberg, K., & Lippincott-Schwartz, J. (2001). Maintenance of Golgi structure and function depends on the integrity of ER export. *Journal of Cell Biology*, *155*(4), 557–570. https://doi.org/10.1083/jcb.200107045

Wei, J. H., Zhang, Z. C., Wynn, R. M., & Seemann, J. (2015). GM130 Regulates Golgi-Derived Spindle Assembly by Activating TPX2 and Capturing Microtubules. *Cell*, *162*(2), 287–299. https://doi.org/10.1016/j.cell.2015.06.014

Weigert, R., Colanzi, A., Limina, C., Cericola, C., Di Tullio, G., Mironov, A., Santini, G., Sciulli, G., Corda, D., De Matteis, M. A., & Luini, A. (1997). Characterization of the endogenous mono-ADP-ribosylation stimulated by brefeldin A. *Advances in Experimental Medicine and Biology*, *419*. https://doi.org/10.1007/978-1-4419-8632-0_44

Wesolowski, J., Weber, M. M., Nawrotek, A., Dooley, C. A., Calderon, M., St. Croix, C. M., Hackstadt, T., Cherfils, J., & Paumet, F. (2017). Chlamydia Hijacks ARF GTPases To Coordinate Microtubule Posttranslational Modifications and Golgi Complex Positioning. *MBio*, *8*(3). https://doi.org/10.1128/MBIO.02280-16

Wickström, S. A., Lange, A., Hess, M. W., Polleux, J., Spatz, J. P., Krüger, M., Pfaller, K., Lambacher, A., Bloch, W., Mann, M., Huber, L. A., & Fässler, R. (2010). Integrin-linked kinase controls microtubule dynamics required for plasma membrane targeting of caveolae. *Developmental Cell*, *19*(4). https://doi.org/10.1016/j.devcel.2010.09.007

Williger, B. T., Provost, J. J., Ho, W. T., Milstine, J., & Exton, J. H. (1999). Arfaptin 1 forms a complex with ADP-ribosylation factor and inhibits phospholipase D. *FEBS Letters*, *454*(1–2). https://doi.org/10.1016/S0014-5793(99)00771-1

Wong-Dilworth, L., Rodilla-Ramirez, C., Fox, E., Restel, S. D., Stockhammer, A., Adarska, P., & Bottanelli, F. (2023). STED imaging of endogenously tagged ARF GTPases reveals their distinct nanoscale localizations. *The Journal of Cell Biology*, *222*(7). https://doi.org/10.1083/jcb.202205107

Wu, J., de Heus, C., Liu, Q., Bouchet, B. P., Noordstra, I., Jiang, K., Hua, S., Martin, M., Yang, C., Grigoriev, I., Katrukha, E. A., Altelaar, A. F. M., Hoogenraad, C. C., Qi, R. Z., Klumperman, J., & Akhmanova, A. (2016). Molecular Pathway of Microtubule Organization at the Golgi Apparatus. *Developmental Cell*, *39*(1), 44–60. https://doi.org/10.1016/j.devcel.2016.08.009

Wu, J., De Heus, C., Liu, Q., Qi, R. Z., Klumperman, J., & Akhmanova, A. (2016). Molecular Pathway of Microtubule Organization at the Golgi Apparatus Highlights d CAMSAP2 stabilizes microtubule minus ends at the Golgi d A complex of AKAP450 and myomegalin recruits CAMSAP2-bound microtubules to the Golgi d A single Golgi can be maintained in the absence of centrosome and Golgi microtubules d Golgi microtubules facilitate cell reorientation and migration in 3D matrix. *Developmental Cell*, *39*, 44–60. https://doi.org/10.1016/j.devcel.2016.08.009

Xiang, Y., Seemann, J., Bisel, B., Punthambaker, S., & Wang, Y. (2007). Active ADP-ribosylation factor-1 (ARF1) is required for mitotic Golgi fragmentation. *Journal of*

*Biological Chemistry*, *282*(30), 21829–21837. https://doi.org/10.1074/jbc.M611716200
Xiang, Y., & Wang, Y. (2010). GRASP55 and GRASP65 play complementary and essential roles in Golgi cisternal stacking. *Journal of Cell Biology*, *188*(2), 237–251. https://doi.org/10.1083/jcb.200907132
Xing, M., Peterman, M. C., Davis, R. L., Oegema, K., Shiau, A. K., & Field, S. J. (2016). GOLPH3 drives cell migration by promoting Golgi reorientation and directional trafficking to the leading edge. *Molecular Biology of the Cell*, *27*(24), 3828–3840. https://doi.org/10.1091/mbc.E16-01-0005
Yadav, S., Puri, S., & Linstedt, A. D. (2009). A Primary Role for Golgi Positioning in Directed Secretion, Cell Polarity, and Wound Healing. *Molecular Biology of the Cell*, *20*, 1728–1736. https://doi.org/10.1091/mbc.E08
Yadav, S., Puthenveedu, M. A., & Linstedt, A. D. (2012). Article Golgin160 Recruits the Dynein Motor to Position the Golgi Apparatus. *Developmental Cell*, *23*, 153–165. https://doi.org/10.1016/j.devcel.2012.05.023
Yamaji, R., Adamik, R., Takeda, K., Togawa, A., Pacheco-Rodriguez, G., Ferrans, V. J., Moss, J., & Vaughan, M. (2000). Identification and localization of two brefeldin A-inhibited guanine nucleotide-exchange proteins for ADP-ribosylation factors in a macromolecular complex. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(6), 2567–2572. https://doi.org/10.1073/pnas.97.6.2567
Yang, W., & Storrie, B. (1998). Scattered Golgi Elements during Microtubule Disruption Are Initially Enriched in *Trans* -Golgi Proteins. *Molecular Biology of the Cell*, *9*(1), 191–207. https://doi.org/10.1091/mbc.9.1.191
Yu, X., Breitman, M., & Goldberg, J. (2012). A Structure-based mechanism for Arf1-dependent recruitment of coatomer to membranes. *Cell*, *148*(3), 530–542. https://doi.org/10.1016/j.cell.2012.01.015
Zappa, F., Failli, M., & De Matteis, M. A. (2018). The Golgi complex in disease and therapy. *Current Opinion in Cell Biology*, *50*, 102–116. https://doi.org/10.1016/j.ceb.2018.03.005
Zhang, X. (2021). Alterations of Golgi Structural Proteins and Glycosylation Defects in Cancer. In *Frontiers in Cell and Developmental Biology* (Vol. 9). https://doi.org/10.3389/fcell.2021.665289
Zhang, X., & Wang, Y. (2016a). Glycosylation Quality Control by the Golgi Structure. *Journal of Molecular Biology*, *428*, 3183–3193. https://doi.org/10.1016/j.jmb.2016.02.030
Zhang, X., & Wang, Y. (2016b). Glycosylation Quality Control by the Golgi Structure. *Journal of Molecular Biology*, *428*(16), 3183–3193. https://doi.org/10.1016/j.jmb.2016.02.030
Zhao, L., Helms, J. B., Brunner, J., & Wieland, F. T. (1999). GTP-dependent binding of ADP-ribosylation factor to coatomer in close proximity to the binding site for dilysine retrieval motifs and p23. *Journal of Biological Chemistry*, *274*(20). https://doi.org/10.1074/jbc.274.20.14198
Zhao, X., Claude, A., Chun, J., Shields, D. J., Presley, J. F., & Melançon, P. (2006a). GBF1, a cis-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic. *Journal of Cell Science*, *119*(18). https://doi.org/10.1242/jcs.03173
Zhao, X., Claude, A., Chun, J., Shields, D. J., Presley, J. F., & Melançon, P. (2006b). *implicated in ER-to-Golgi protein traffic*. https://doi.org/10.1242/jcs.03173
Zhao, X., Lasell, T. K. R., & Melançon, P. (2002). Localization of large ADP-ribosylation factor-guanine nucleotide exchange factors to different golgi compartments: Evidence for distinct functions in protein traffic. *Molecular Biology of the Cell*, *13*(1). https://doi.org/10.1091/mbc.01-08-0420
Zilberman, Y., Ballestrem, C., Carramusa, L., Mazitschek, R., Khochbin, S., & Bershadsky, A. (2009). Regulation of microtubule dynamics by inhibition of the tubulin deacetylase HDAC6. *Journal of Cell Science*, *122*(19), 3531–3541.

https://doi.org/10.1242/JCS.046813