FEBS Letters 583 (2009) 3827–3838

journal homepage: www.FEBSLetters.org

Review

The Golgi apparatus: Lessons from *Drosophila*

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ARTICLE INFO

Article history:
Received 9 July 2009
Revised 10 September 2009
Accepted 26 September 2009
Available online 1 October 2009

Edited by Antonella De Matteis

Keywords:
S2 cell
Golgi
Membrane traffic
RNAi
Development
tER site
Drosophila

ABSTRACT

Historically, *Drosophila* has been a model organism for studying molecular and developmental biology leading to many important discoveries in this field. More recently, the fruit fly has started to be used to address cell biology issues including studies of the secretory pathway, and more specifically on the functional integrity of the Golgi apparatus. A number of advances have been made that are reviewed below. Furthermore, with the development of RNAi technology, *Drosophila* tissue culture cells have been used to perform genome-wide screens addressing similar issues. Last, the Golgi function has been involved in specific developmental processes, thus shedding new light on the functions of a number of Golgi proteins.

1. Introduction: Drosophila, a suitable organism for studying the Golgi apparatus

The Golgi apparatus is situated at the heart of the secretory pathway, and its main functions are to modify and sort proteins and lipids that are transported through this organelle en route to their final destinations, such as the plasma membrane, the extracellular medium and the endosomal/lysosomal compartments. In mammalian tissue culture cells, it consists of flattened membrane-bound compartments, called cisternae, which form Golgi stacks, themselves interconnected by lateral tubules to form the Golgi ribbon, which displays a juxtanuclear localisation next to the microtubule organising center (MTOC). Both Golgi stacks and ribbon are polarised with an entry (cis) face, where cargo molecules synthesised in the endoplasmic reticulum (ER) reach the Golgi, and an exit (trans) face, where they leave for their downstream locations [1].

For decades, yeast has provided a tractable genetic system for studying the function of this organelle. Screens using *Saccharomyces cerevisiae* have resulted in the identification of many genes encoding proteins involved in secretion (*sec* genes [2,3]). However, the secretory pathway in *S. cerevisiae* lacks the structural complexity existing in higher eukaryotes and exhibits deviations in several features mentioned above. For instance, Golgi stacks are rarely observed under normal growth conditions [4,5].

Drosophila has recently been established as a good alternative model system to study the Golgi. In fly tissues and cell lines, it shares many morphological and functional similarities with the mammalian one.

(1) Golgi stacks are clearly visible, often displaying 2–3 cisternae per stack. In *Drosophila* tissue culture S2 cells, the stacks display an average cross sectional diameter of 370 nm [6,7], about half the size of a Golgi stack in HeLa cells despite that S2 cells are 4–8-fold smaller.
(2) *Drosophila* Golgi stacks also seem to be polarised with a cis and trans face, the cis one being defined by its close proximity to the ER exit sites (see lesson 1 below).
(3) Drugs known to inhibit anterograde protein transport in mammalian cells, such as Brefeldin A (BFA) or H89, have the same effect in S2 cells [6,8], although in BFA-treated S2 cells the Golgi stacks do not fuse back to the ER, at least after

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0014-5793/$36.00
doi:10.1016/j.febslet.2009.09.048

2 h treatment with the drug. Instead, they remain largely intact, enlarge, and sometimes cluster in one part of the cell [8].[1]

(4) Golgi stacks also undergo a disassembly-reassembly cycle during mitosis [7,9] as they do in mammalian cells [10].

(5) Most proteins shown to play an essential role in Golgi function and organization have homologues encoded in the *Drosophila* genome (Rab small GTPases, SNAREs, the so-called Golgi Matrix proteins GM130, p115 and GRASP65/55, other long coiled coil proteins of the Golgin family [11,12], COPI and COPII coat subunits, many glycosylation enzymes, cargo receptors, etc), suggesting that protein and lipid transport, glycosylation and sorting takes place in a similar way in *Drosophila* and mammalian cells (see Table 1). One exception is giantin, a mammalian golgin that has no sequence homologue in *Drosophila*, but whose structure resembles the coiled-coil protein Lava Lamp [13].

(6) In addition to striking similarities between *Drosophila* and mammalian Golgi, *Drosophila* provides several additional advantages for studying the early secretory pathway. One of these is the more limited gene redundancy compared to mammalian cells. For instance, only one Sec23 and Sar1 isoforms are encoded in *Drosophila* genome versus two in humans [14,15]. This is not to be overlooked in the era of gene knockdown by RNAi, a widely-used method in studies of membrane traffic (see below).

(7) As its development is studied in great detail, *Drosophila* offers a very good opportunity to address the role of Golgi proteins in development, using classical and reverse genetics including use of RNAi stocks that have been generated and available for the research community (http://stockcenter.vdrc.at/control/main; http://www.dgrc.kit.ac.jp/en/index.html).

(8) Exogenous (tagged) proteins can be expressed at moderate level, in S2 cells using the metallothionein promoter [16] that is activated by addition of low doses of copper sulphate, and in vivo using the UAS/GAL4 system [17]. The control of GAL4 by endogenous promoters as well as the temperature at which the flies are crossed allows protein expression in a dose-, time- and tissue-specific manner. In this way, a massive overexpression that creates artefacts of both localisation and function can be avoided.

In this review, we will highlight a number of lessons that *Drosophila* has taught us on the functional and structural organization of the Golgi complex leading to the development of novel concepts in the field. When possible, this information will be compared and extrapolated to mammalian systems.

[1] BFA inhibits ER to Golgi transport and therefore secretion in most cells where it has been tested (for review see [128]). BFA confers its effect by trapping the Arf1-GDP/GEF complex on membrane, preventing GDP/GTP exchange and activation of Arf1. As a result, COPI coat cannot be recruited onto Golgi membrane and COPI vesicles cannot be formed. This leads to an inhibition of retrograde transport of essential components for anterograde transport from Golgi back to ER and ultimately to a cessation of membrane exit from the ER. This rapid release of COPI coat subunits from the Golgi membrane into cytosol in response to BFA is a universal feature of all eukaryotic cells (including plants and yeast), and suggests that the BFA molecular target and mode of action are highly conserved [129,130].
In most mammalian cells (except MDCK cells), BFA treatment has a strong effect on the integrity of the Golgi apparatus as Golgi-resident membrane proteins are redistributed to the ER [128]. This is probably an indirect effect mediated by a COPI independent mechanism.
Upon BFA treatment of pancreatic acinar cells [131], plant cells [132] and yeast [133], COPI is released in the cytoplasm and secretion is blocked. Initially, the Golgi stacks in yeast and plant cells enlarge and these fragments in pancreatic cells, but Golgi-resident membrane proteins do not relocate to the ER although it can happen in later stages of treatment. Therefore, S2 cells do conform to the universal mode of BFA action.

2. Lesson 1: the tER-Golgi units are the basic secretory units

One of the main differences in the Golgi organization between the mammalian and *Drosophila* cells is that fly Golgi stacks are not interconnected to form a single-copy organelle, the Golgi ribbon, as in mammals. Instead, they remain dispersed throughout the cytoplasm [18,19] and are almost always found in close association to tER sites (also referred to as ER exit sites or ERES), thus forming what we and others call "tER-Golgi units" ([6]; Fig. 1A and D).

The same basic organization of the early secretory pathway in discrete tER-Golgi units is similar to that described in lower eukaryotes, such as yeast *Pichia pastoris* [5,20], protozoa *Trypanosoma brucei* [21] and *Toxoplasma gondii* [22], and plants [23]. In *Drosophila* S2 cells, as in *P. pastoris*, the number of tER-Golgi units is fairly constant (about 20 in interphase S2 cells versus 2–5 in *Pichia*). However, as a multicellular and genetically manipulable organism, *Drosophila* is a more attractive model system when compared to lower eukaryotes, since the significance of the cellular function of Golgi-related proteins can be directly tested in the developing fly (see introduction and lesson 10).

At first sight, the scattered tER-Golgi units in *Drosophila* differ from the organization of the early secretory pathway in mammalian cells, whose tER sites are dispersed throughout the cytoplasm and Golgi ribbon is located at the MTOC (Fig. 1A). This difference reflects the microtubule-dependence of membrane transport from peripheral tER sites to the perinuclear area in mammalian cells. However, an intimate spatial relationship between mammalian tER sites and Golgi stacks exists and is reminiscent of tER-Golgi units. First, although tER sites are found all over the cytoplasm, a significant proportion of them concentrates next to the Golgi ribbon [24], as if they were forming a giant tER-Golgi unit (Fig. 1A and B). Second, upon microtubule depolymerisation, the Golgi ribbon is reorganised into Golgi stacks that are found very close to tER sites, thus forming tER-Golgi units ([24,25]; Fig. 1C). After an initial lagging period, these units support anterograde transport in the absence of microtubules [25], as do *Drosophila S2* cells [7]. We propose that the presence scattered tER-Golgi units represent the archetypal organization of the early secretory pathway. Late in evolution this organization became dependent on the microtubule network and microtubule minus end-directed movement of membrane from tER sites to the Golgi complex [26], presumably leading to Golgi stack gathering and Golgi ribbon formation around the MTOC. However, the biological implications of the Golgi ribbon pericentriolar localisation are not yet completely understood.

3. Lesson 2: the paired Golgi stack: a Golgi ribbon in *Drosophila*?

Despite the presence in the *Drosophila* genome of genes encoding proteins involved in the building/maintenance of the mammalian Golgi ribbon, such as GRASP65, GRASP55, GM130 [27,28] and golgin 84 [29], most *Drosophila* cells/tissues conspicuously lack a Golgi ribbon, although it can sometimes be observed, for instance in the onion stage spermatids, where a juxtanuclear Golgi ribbon, called acroblast, is clearly visible (Fig. 2A). This indicates that the molecular machinery to build a ribbon is present in *Drosophila* but is not used for reasons that remain to be determined.

What is clear, however, is that each scattered tER-Golgi unit comprises a pair of Golgi stacks (Fig. 2B), defined by a distance of less than 70 nm between the cisternal rims of the two adjacent stacks [7]. The presence of tubules interconnecting the neighbouring stacks has been suggested from 3D electron tomography [7], but their existence and nature (transient or stable) still need to be confirmed. If these tubules were to exist, this paired Golgi stack would represent the smallest conceivable ribbon. Strikingly, the

Table 1
List of Golgi-associated proteins whose localisation, cellular function and role in development have been studied.

Drosophila protein	Mammalian homologue	Features	Subcellular localisation in *Drosophila*	Cellular/molecular function in *Drosophila*	Tissue-specific role in *Drosophila* development	Refs.
GRASPs						
dGRASP	GRASP65 GRASP55	Per N-Myr	tER-Golgi PM (lesson 7)	Subtle role in Golgi stacking; Unconventional secretion	Transport of integrins during epithelial remodeling (lesson 7)	[8,44]
Golgins						
p115	p115	Per	tER-Golgi	Golgi integrity and tER site organization	ND	[6]
GM130	GM130	Per	tER sites cis-Golgi	Golgi integrity only when co-depleted with dGRASP; Rab1/30 binding	ND	[6,8,118]
Lava lamp	Not found	Per	Golgi	MT/Actin binding and Golgi positioning	Embryo cellularization (lesson 8.2)	[13]
dGMAP	GMAP-210	Per	cis-Golgi	Golgi organization and anterograde transport; Rab2 binding		[118,119]
GRIP domain proteins (golgin-97, golgin-245, GCC88, GCC185)	GRIP domain proteins (golgin-97, golgin-245, GCC88, GCC185)	Per	trans-Golgi	No Golgi disorganisation upon single RNAi depletions; Binding to Rab2, 6, 19, 30 and Arl1	Not found	[11,118]
Tethering complex						
COG5	COG5	Per	cis-Golgi	Cytokinesis	Spermatogenesis	[35,120]
GTPases						
Rab6	Rab6	Per	Golgi	Golgi to PM transport; cytokinesis	Oocyte development	[109,121,122]
Rab1	Rab1	Per		dGM130 binding	Dendrite outgrowth	[37,118]
COP coat						
COPI	COPI	Per	Golgi	Intracellular transport; lipid biogenesis; virus replication (lessons 8 and 9)	Embryonic dorsal closure; Tracheal dorsal branching, lumen expansion and tube formation (lesson 8.1)	[76,77,99,100,102,123]
COPII	COPII	Per	tER	Formation of COPII-coated vesicles	Dendrite outgrowth; Tracheal dorsal branching, lumen expansion (lesson 8.1)	[37,76,78,124]
SNAREs						
Syntaxin 5 (dSed5)	Syntaxin 5	TM	Golgi	Transport to and through the Golgi; cytokinesis	Spermatogenesis	[19,125]
Golgi enzymes						
Fringe	Rfng, Lfng, Mfng	TM	Golgi	O-glycosylation (lesson 8.3)	Wing/leg/eye development (lesson 8.3)	[88,89,91,126]
dGMII		TM	Golgi	N-glycosylation	ND	[19]
Others						
Cornichon	Erv14	TM		Gurken cargo receptor	Oocyte ventralisation	[40,127]
TANGO1	TANGO1	TM	tER-Golgi	ER-Golgi transport block	ND	[94,96]

Per, peripheral membrane protein; TM, Transmembrane protein; PM, Plasma membrane; ND, not determined.
Interacting proteins of most of the mammalian homologues are reviewed in [12].

same type of pair has been observed in other *Drosophila* tissues and organisms, such as Toxoplasma [22].

Although, as mentioned above, the microtubule network is critical for the maintenance of the mammalian Golgi ribbon, it does not affect the *Drosophila* Golgi pairing when depolymerised. Conversely, the integrity of actin cytoskeleton plays a crucial role in *Drosophila* Golgi stack pairs since drug induced F-actin depolymerisation induces their splitting [7]. Using a combination of RNAi depletions and expression of dominant negative mutants, Abi and Scar/WAVE have been shown to regulate the polymerisation of a subset of F-actin around the tER-Golgi units that keeps the two stacks in a pair. This regulatory process, which is likely downstream of Rac signalling pathway, is also suggested by the partial localisation of Abi and Scar/WAVE to the early secretory pathway in *Drosophila* S2 cells [7].

The effect of F-actin depolymerisation in *Drosophila* cells is seemingly very different from what has been reported in mammalian cells, at least at light microscopy level. F-actin depolymerisation drugs lead to a more compact appearance of the Golgi ribbon, which still remains around the MTOC ([30,31] and references therein). At ultrastructural level, though, and despite the fact that there is no significant disruption of cisternal stacking, the Golgi ribbon was shown to undergo fragmentation with swelling of Golgi cisternae [32]. Interestingly, when mammalian cells are treated first with nocodazole, thus generating Golgi stacks (see lesson 1), and then with F-actin depolymerising drugs, splitting of paired Golgi can also be observed [7,33], suggesting that F-actin may have an equivalent structural role in mammalian Golgi, and the paired Golgi stack organization could be conserved.

These observations highlight another advantage in working with cells exhibiting scattered distribution of their tER-Golgi units, such as *Drosophila* cells. More specifically, it allows visualisation of subtle changes in their organization that can be missed when Golgi stacks are interconnected into a Golgi ribbon that is confined

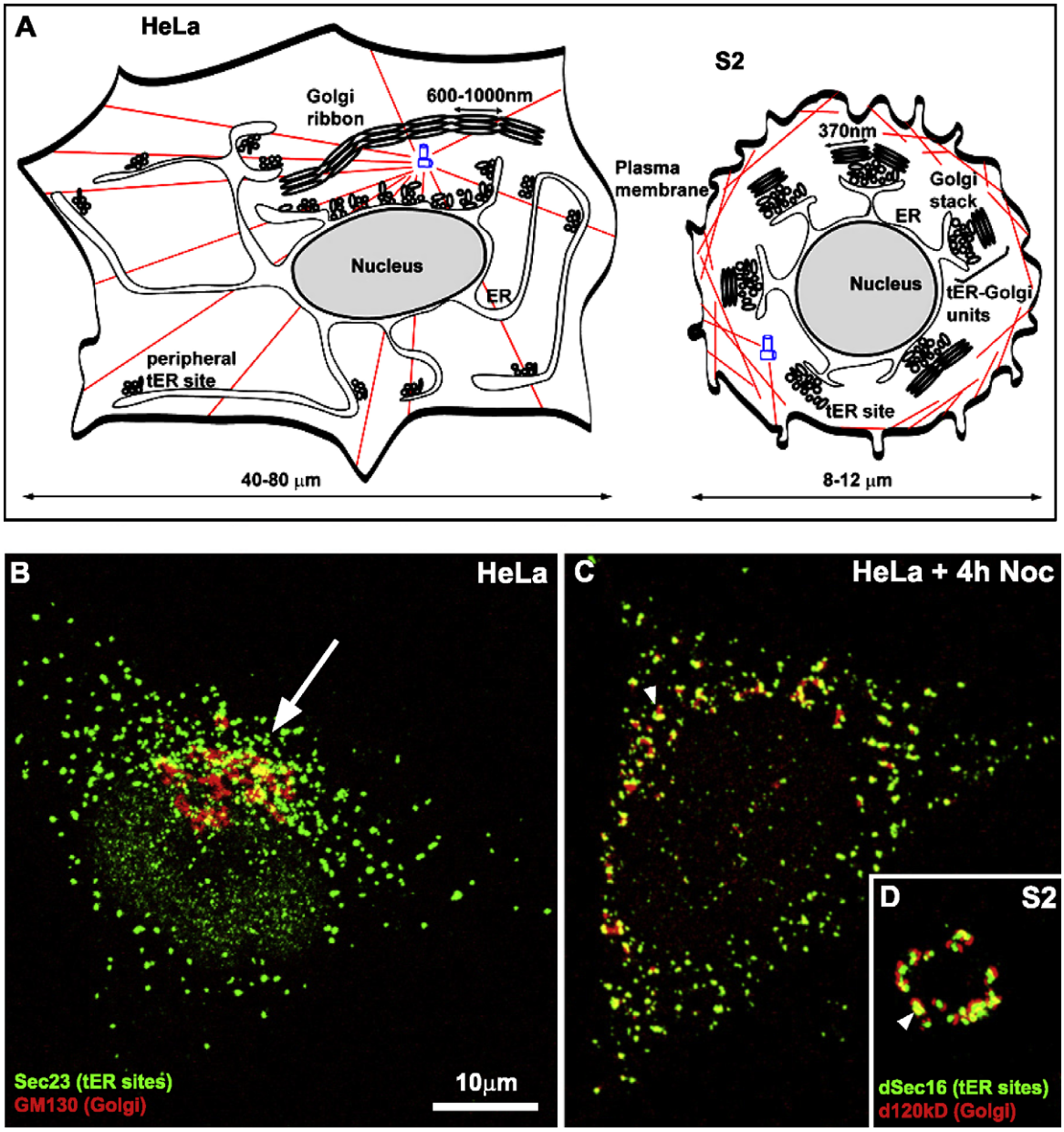

Fig. 1. The tER-Golgi units in mammalian and *Drosophila* S2 cells. (A) Schematic representation of the early secretory pathway organization in HeLa and *Drosophila* S2 cells. The centrosome is depicted in blue and the microtubules in red. (B) Visualisation by immunofluorescence of the early secretory pathway of HeLa cells highlighting the concentration of a large portion of tER sites (green) in close proximity to the Golgi apparatus (red) that resembles a giant tER-Golgi unit (arrow). (C,D) Mammalian tER-Golgi units are best exemplified when HeLa cells are treated with nocodazole to depolymerise microtubules. There, each scattered Golgi stack is found in close proximity to one (or two) ER exit sites (C), as in *Drosophila* S2 cells (D). All pictures represent confocal sections. Note that S2 cells are 4–8 times smaller than HeLa cells, but tER-Golgi units are about the same size as those generated in HeLa cells upon nocodazole treatment. Arrowheads in C andf D indicate one tER-Golgi unit. Bar: 10 μM (B–D).

around MTOC, a cellular location cramped with many other organelles, such as tER sites, centrosome and recycling endosomes.

4. Lesson 3: the tER-Golgi units can function differentially

The presence of tER-Golgi units scattered in different areas of the cell cytoplasm suggests that they could sustain different functions independently from one another. This has indeed been shown for at least two aspects. First, the subset of glycosylation enzymes seems to differ between Golgi stacks of the same cell, suggesting that different tER-Golgi units perform different glycosylation functions and process different substrates [34]. This can be beneficial to differentially modulate the biological activity of crucial plasma membrane proteins. Additionally, it may contribute to the establishment of apico-basal polarity, if subsets of tER-Golgi units sorting apical or basal determinants are located close to their respective plasma membrane portion. Second, studies in the *Drosophila* oocyte have shown that tER-Golgi units are able to differentially transport different proteins. This could be the result of mRNA localisation and local translation combined with efficient export from tER sites. For example, units situated at the dorso-anterior corner of a stage 9 oocyte transport Gurken protein that is locally synthesised from its localised mRNA, whereas all units distributed throughout the oocyte are able to transport Yolkless [35], a plasma membrane receptor that is synthesised from a pool of non targeted mRNA and delivered all over the entire oocyte cortex [36].

Scattering of tER-Golgi units is also observed in *Drosophila* [37] and mammalian neurons [38], whose dendrites are populated by

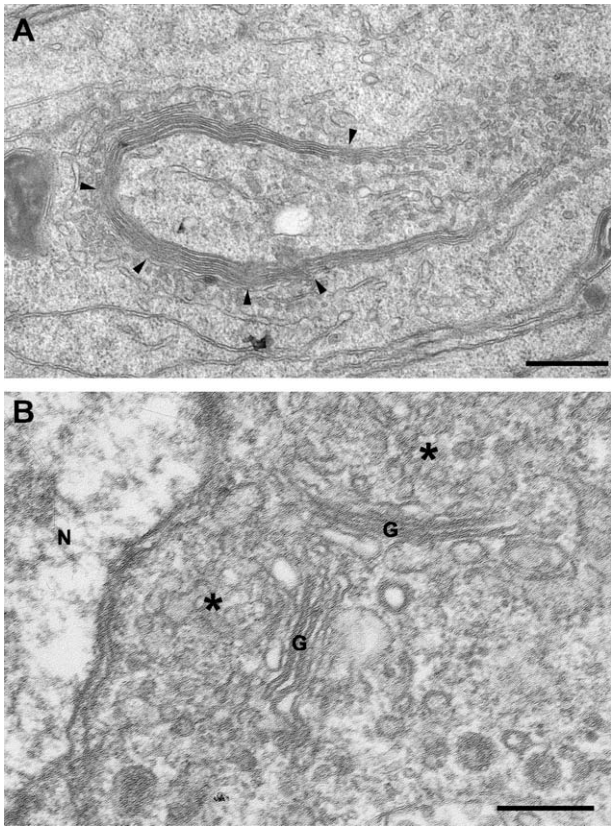

Fig. 2. Golgi ribbons in *Drosophila*. (A) EM (epon) visualisation of the Golgi apparatus in *Drosophila* spermatids (acroblast) that resembles the Golgi ribbon in mammalian cells including the non compact zones (arrows) likely to correspond to lateral tubules connecting adjacent and equivalent cisternae in two stacks. This shows that the machinery to build a ribbon is present in *Drosophila* but not used in most tissues and developmental stages. (B) EM (epon) visualisation of the paired Golgi stacks in S2 cells. Although the tubules connecting the two stacks have not been formally demonstrated, this paired stack can be considered as the smallest ribbon possible. Asterisks (*) indicate tER sites. Bars: 200 nm.

the so-called Golgi outposts that have characteristic features of tER-Golgi units [39,40]. These outposts have been proposed to sustain membrane delivery necessary for dendrite outgrowth as well as local transport and deposition of transmembrane proteins that are locally synthesised, such as AMPA receptors, independently from the Golgi ribbon situated in the soma. The presence of peripherally-distributed tER-Golgi units in very large cells, like neurons, is biologically relevant when their proper function depends on their fast response to extracellular stimuli leading to extensive membrane remodelling.

One remaining question is whether all stacks in a mammalian Golgi ribbon are functionally equivalent. Work by Linstedt and colleagues has shown that upon ribbon unlinking (after depleting GM130), the glycosylation pattern at the plasma membrane was affected when compared to control cells, suggesting that the ribbon allows lateral diffusion of glycosylation enzymes between cisternae of adjacent stacks leading to their even distribution and facilitating optimal processing of proteins transisting through the Golgi apparatus [27]. If this uniformity is lost upon Golgi ribbon unlinking, this suggests that the Golgi stacks are not equivalent to start with. Furthermore, given the size of the ribbon in mammalian cells, it is possible that one part of the ribbon is in contact to several restricted organelles or moieties, such as localised mRNAs, and that only a given number of stacks within the ribbon mediate transport of the encoded proteins.

5. Lesson 4: efficient anterograde transport does not depend on the Golgi stack structural integrity

Although the stacking of Golgi cisternae is a feature that makes this organelle unique, the presence of Golgi stacks is not essential for efficient anterograde transport. Until a few years ago, this concept was not clear, at least in higher eukaryotes. This was probably due to the fact that the disorganisation of the Golgi apparatus (ribbon and stacks) was often coupled to a cessation in anterograde transport, e.g. upon BFA treatment or during mitosis. However, several studies in *Drosophila* have shifted this paradigm.

Strikingly, at numerous *Drosophila* developmental stages, cells do not exhibit any Golgi stacks. Instead, the Golgi complex comprises clusters of vesicles and tubules, for instance during early embryogenesis [41,42], in early/mid third instar larval imaginal discs [43] and in follicle cells surrounding the growing oocyte (Fig. 3A [44]). In all these situations, it is clear that Golgi lacking stacks do support robust secretion. For example, follicle cells produce a very large amount of secreted yolk proteins that is then endocytosed by the oocyte for storage into yolk granules (unpublished data, Fig. 3B). Efficient secretion without Golgi stacks is, of course, also observed in budding yeast and many lower eukaryotes, such as *E. cuniculi* that only exhibit isolated Golgi cisternae or branching tubular networks with Golgi identity [4,45,46].

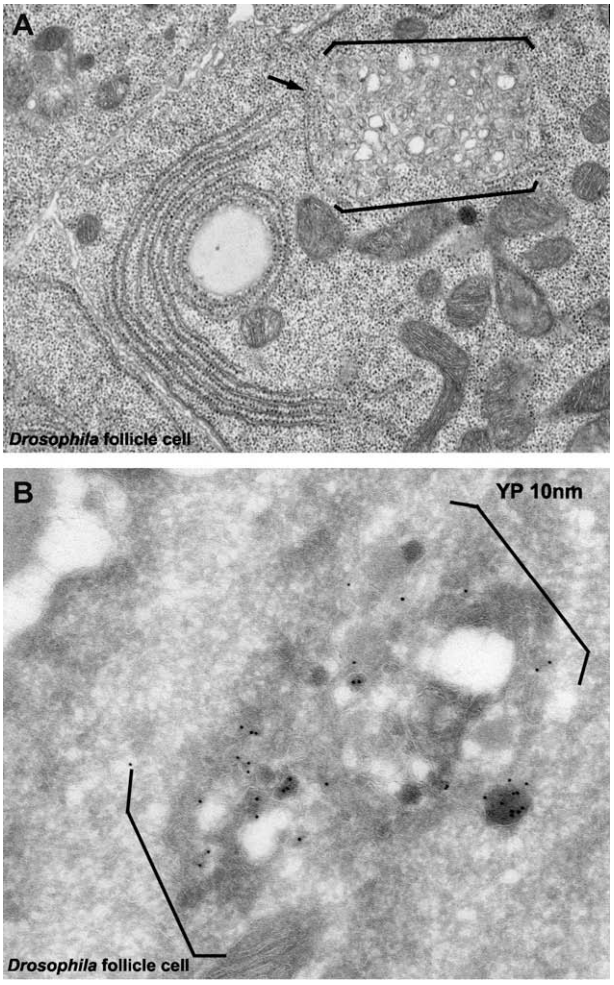

Fig. 3. A tER-Golgi unit without cisternae. (A) EM (epon) visualisation of a tER-Golgi unit (between brackets) in a *Drosophila* follicle cell as a cluster of tubules and vesicles of various sizes in close proximity to a cup shaped ER cisterna (arrow). (B) Immuno-EM visualisation of the abundant secreted yolk protein (10 nm gold particles) in a tER-Golgi unit (between brackets) of a *Drosophila* follicle cell showing that this unit is active in transport.

In agreement with this observation, experimental conditions can lead to fragmentation of the Golgi stacks without a significant inhibition of anterograde transport. For instance, S2 cells depleted of the *Drosophila* orthologue of p115 (dp115; [6]), or the single *Drosophila* orthologue of GRASP65 and 55 (dGRASP) combined with GM130 homologue (dGM130; [8]) result in a quantitative conversion of Golgi stacks into vesicles and tubules, but anterograde transport of the plasma membrane transmembrane protein Delta is only marginally affected. Similarly, depletion of the *Drosophila* and human sphingomyelin synthase-related protein, SMSr, leads to a structural collapse of Golgi stacks, yet keeping anterograde transport unaffected [47]. Supporting these results, cell proliferation is not affected in any of these conditions, suggesting that endogenous proteins are likely to be transported as efficiently in depleted and non-depleted cells [6,8]. In contrast, when exocytosis is blocked upon depletion of SNARE dSyntaxin5 (dSed5), cell proliferation is significantly inhibited [6].

Evidence supporting this notion has also accumulated in mammalian cells. First, the disruption of the Golgi ribbon is not detrimental to anterograde transport [27,29,48,49]. Second, BHK cells infected by the Uukuniemi virus have dilated and vacuolised Golgi stacks [50], but transport of Semliki virus glycoproteins to the plasma membrane occurs normally [51]. Moreover, depletion of p125 that affects the organization of the tER sites, and ultimately the Golgi structure (at least at the cis side) does not inhibit forward transport of VSV-G [52]. Taken together, these results show that the integrity of the Golgi stacks and cisternae is not needed for competently transporting the bulk of proteins to the plasma membrane.

A remaining issue is the role of the stacked cisternal architecture, if not to sustain anterograde transport. One possibility is that the Golgi stacks may increase the secretion efficiency compared to the Golgi clusters. This could be important in certain tissues and developmental stages in which elevated transport/secretion is needed. Additionally, Golgi stacks, but not Golgi clusters, may have a role in retrograde transport through the early secretory pathway (although this should eventually affect anterograde transport) and/or a role in recycling from endosomes. Furthermore, the proper and complete maturation of protein-borne O- and N-linked oligosaccharide moieties and the addition of sorting signals might require a Golgi stack. Last, it is in theory possible that in the absence of Golgi stacks (or under experimental conditions that disrupt their structure), secreted proteins reach the plasma membrane by an unconventional route bypassing the Golgi (see lesson 7). However, experimental evidence argues against this possibility. For instance, in S2 cells with disrupted Golgi stacks upon dSMSr depletion [47], the plasma membrane reporter Delta colocalises with Golgi markers at the early stages of its transport (our unpublished data), suggesting that it still follows its usual route through the Golgi. In addition, yolk proteins that are produced in *Drosophila* follicle cells, which exhibit vesicular-tubular Golgi membrane (Fig. 3), have been localized to the Golgi (Fig. 3B) and become properly glycosylated, phosphorylated and secreted [53].

6. Lesson 5: Golgi biogenesis is different from Golgi morphogenesis

That anterograde transport is able to efficiently proceed in fragmented Golgi stacks reveals a conceptual difference between the biogenesis of a functional organelle and its structural integrity. This might explain in part the debate that has been taking place about the status of the Golgi apparatus with two opposing models, the Golgi Matrix and the de novo Golgi formation.

In the Golgi Matrix model, the Golgi apparatus is considered to be an autonomous organelle built on a pre-existing template, as suggested by data from a variety of organisms. In mammalian cells, the template is proposed to be a Golgi matrix [10,54] containing Golgi matrix proteins, such as p115 and GM130, which localize to Golgi membranes, as well as F-actin/spectrin [30]. These proteins have been proposed to take part in building and/or maintaining Golgi stack architecture and ribbon formation.

In the de novo Golgi formation model, the Golgi apparatus is considered as an ER outgrowth. The membrane exiting the ER at tER sites are proposed to carry all the necessary molecular information to trigger the building of a functional Golgi apparatus by a mechanism of self-organization [55–57]. In this model, it is the structural integrity of tER sites and anterograde transport that are crucial for Golgi stack formation.

The first prediction of the de novo formation model is that proteins involved in Golgi structure and organization should be located both on the Golgi but also at tER sites. In mammalian cells, so far, only p115 has been shown to colocalise with ERGIC53 in addition to Golgi [58]. The localisation of other Golgi matrix proteins at tER sites has only been observed under conditions blocking anterograde transport. For instance, GM130 and GRASP65 colocalise extensively with COPII coat markers in cells expressing Sar1 dominant negative mutants or treated with BFA and H89 [56,59]. In contrast, the *Drosophila* Golgi matrix proteins that were studied (dp115, dGM130 and dGRASP) all localise to a significant extent to tER sites in addition to the Golgi area [6,8]. A clear role in tER site organization has even been unravelled for dp115. In dp115-depleted S2 cells, tER sites lose their typical focused organization and reorganise into more numerous smaller, yet functional sites, suggesting a role for p115 in their structural homeostasis. This is not reported in mammalian cells, perhaps because of functional redundancy, or due to the very large number of mammalian tER sites that may hide an increase in their number.

The second prediction is the existence of a causal link between the structural integrity of tER sites and the efficiency of anterograde transport, and Golgi stack formation. However, as mentioned above, depletion of dGRASP/dGM130 quantitatively disorganises the Golgi stacks, yet tER site organization remains intact and anterograde transport is as efficient as in non-depleted cells [8]. This suggests that, as predicted, these two parameters are sufficient for the biogenesis of a functional Golgi, but not for the morphogenesis of the Golgi apparatus comprising stacked cisternae. This shows that additional factors, such as a Golgi matrix, are needed for building a stack and these are independent of tER organization and exit.

The fine difference between Golgi biogenesis and morphogenesis should be pointed out as it is not easily detected by light microscopy methods, and highlights the importance of using ultrastructural EM analyses in deciphering subtle phenotypes.

7. Lesson 6: the Golgi ribbon is involved in cell cycle control

As mentioned above, although the role of intact Golgi stacks is not clear (lesson 4), a functional role for the Golgi ribbon is emerging. Data reported by a number of groups clearly indicate that unlinking of mammalian Golgi ribbon into stacks is part of a putative G2/M checkpoint. Indeed, blocking this unlinking inhibits/delays cell entry into mitosis. More specifically, phosphorylation of GRASP65 [60–62] or GRASP55 [28,63] and activation of BARS [64] promotes Golgi ribbon unlinking, and preventing any of these events leads to cell cycle arrest in G2 [65,66].

In *Drosophila*, Golgi stack separation occurs physiologically at G2, at least in S2 cells, and this is likely to correspond to a local depolymerisation of F-actin around tER-Golgi units, perhaps by the specific inactivation of Abi and/or Scar [7]. Remarkably, when Golgi unpairing is inhibited by overexpression of Abi, the mitotic

index drops dramatically suggesting that this unpairing could also be part of a G2/M checkpoint [65].

How does Golgi ribbon/stack unlinking promote cell entry into mitosis is not yet clear. One scenario is that Golgi stack separation could allow an equal partitioning of the Golgi during cell division. Alternatively, severing inter-cisternal membrane connections may release signaling molecules, such as kinases or other enzymes, from the Golgi leading to their activation and function at another cytoplasmic location. It could also facilitate the recruitment of such molecules that are required to modify important cell cycle regulators localized to Golgi membrane, such as Myt1 (see [65] for a detailed review).

Considering that *Drosophila* paired Golgi stacks could represent the smallest possible ribbons (see above), Golgi stack separation in S2 cells and Golgi ribbon unlinking in mammalian cells at G2 appear to be equivalent events. In this view, this would be a case of convergent evolution, where a similar Golgi stack unlinking has evolved as a G2/M checkpoint, while the underlying molecular mechanism is different.

8. Lesson 7: the new roles of the Golgi protein dGRASP

An interesting case of Golgi-localised proteins that appear to have novel emerging functions are GRASPs. Several functions have been already assigned to GRASP65 and 55 (summarised in [12]). In mammalian cells, these two proteins were initially identified as factors required for stacking of Golgi stacks in vitro, and partly in vivo [67,68]. Recently, a role in the Golgi ribbon formation has been shown by Linstedt and colleagues using RNAi depletion of these two proteins in vivo [27,28], although other groups using similar approaches have not reported this result [67,69]. Furthermore, as mentioned above, the G2 specific phosphorylation of both GRASP65 and 55 leads to Golgi ribbon unlinking and interfering with this leads to a cell cycle arrest or delay. Last, GRASP65 [67] and GM130 [70] have also been show to have a role in the spindle formation.

In *Drosophila*, depletion of dGRASP (the single *Drosophila* orthologue of GRASP65 and 55) leads to a marginal effect on the Golgi stack structure but, as mentioned above, this can be significantly strengthened by simultaneously depleting dGM130, suggesting that it is involved in the maintenance of Golgi stack architecture. However, dGRASP is abundant on Golgi membranes in tissues, where no Golgi stacks are present (such as in follicle cells [44]) and depletion of dGRASP does not lead to Golgi stack unpairing.

These observations implied that dGRASP may have additional cellular functions. Indeed, we have recently shown that dGRASP is necessary for the unconventional secretion of alpha PS1 subunit of integrins at very specific developmental stages in which epithelium are remodelled. We find that this integrin subunit is transported to the basal plasma membrane of epithelial cells in a dGRASP dependent manner but without passing through the Golgi, as it is insensitive to BFA treatment and to loss of Syntaxin 5 [44]. Consequently, in *Drosophila* mutants for dGRASP, integrins are not properly deposited, and some epithelia are strongly disorganised, such as the wing and the oocytes-surrounding follicular epithelium. However, classical anterograde transport as a whole is not affected [44,71].

Interestingly, removing the single gene encoding a GRASP homologue in *Dictyostelium*, GrhA, also shows that this protein is required for another type of unconventional secretion of a cellular non-membrane associated factor AcbA. AcbA is produced in the cytoplasm of spore cells and released in the extracellular medium, where it binds to a specific spore receptor and elicits signaling leading to spore development [71–73].

Although the nature of its substrate and the type of secretion is different, it is remarkable that GRASP, a bonafide Golgi protein, exhibits additional and new functions both in *Dictyostelium* and *Drosophila*. Whether mammalian GRASPs have also similar functions in unconventional secretion of specific proteins is under investigation.

9. Lesson 8: the role of the Golgi in development

A rapidly emerging field of study related to the Golgi (and more generally to the early secretory pathway) concerns the biological significance of its functions and structural organization during development. Progress in this field has been recently reviewed elsewhere [40,74], therefore we will focus, here, on few examples illustrating how Golgi-related genes affect specific aspects of *Drosophila* development.

9.1. Tissue- and time-specific requirement of Golgi-related proteins

Despite the broad view that most proteins functioning along the early secretory pathway are essential, not all of them are expressed or required in every tissue and developmental stage to the same level. This is the case, for instance, for COPI[2] and COPII,[3] two protein coat complexes crucial for secretion. First, in *Drosophila* wing imaginal discs at different stages of elongation, *sec23* mRNA among others are upregulated in an ecdysone (the fly steroid hormone) dependent manner, leading to increased expression of the encoded proteins, whereas β′COP mRNA is downregulated [75].

Furthermore, mutations in COPI and COPII subunits do not affect the development of all *Drosophila* tissues similarly. COPI mutants exhibit defects in embryonic dorsal closure and trachea development [76–78]. Interestingly, although dorsal closure is severely inhibited in γCOP mutants, it still occurs normally in Sar1 mutants, indicating a specific COPI requirement for this process (although COPII might also be needed, albeit at lower amount). Regarding the trachea development, dorsal branching and lumen expansion depends on both COPII and COPI, but tube fusion exclusively depends on γCOP. The γCOP mutation could affect COPI coat formation as a whole, as it is suggested by the fact that δCOP mutants recapitulate γCOP mutant phenotype [78]. Alternatively, it acts in a COPI-independent fashion through specific interactions with other proteins in the fusion cells, such as ARL3. Differential requirement for COPI and COPII has also been observed in zebrafish, although the affected tissues are different. COPI mutant fish show defects primarily in notochord formation, while COPII mutations affect skeleton development, in particular chondrogenesis (reviewed in [40].

Last, COPII have also been shown to be specifically involved in dendrite, but not axon, outgrowth in *Drosophila* larval neurons [37]. Mutations in *Drosophila* Sar1, Sec23 (and Rab1, a small GTPase involved in ER-Golgi trafficking) all prevent neuronal dendritic growth, whereas axon growth remains largely unaffected. A similar result has been obtained in culture rat hippocampal neurons [38–40]). This is likely to be due to the presence of Golgi outposts in dendrites (see lesson 3).

9.2. Lava lamp and Drosophila cellularization

Cellularization is a process by which ~6000 cells are formed in a synchronous fashion during early *Drosophila* embryogenesis. Two

[2] COPI protein coat comprises 7 subunits (α, β, β′, γ, δ, ε, ξ), which are recruited to membranes by small GTPase Arf1. COPI-coated vesicles mediate retrograde transport of resident enzymes between the Golgi cisternae and from the Golgi back to the ER, but they may also be involved in forward cargo transport through the Golgi [123].

[3] COPII coat comprises 5 core proteins (Sar1, Sec23, Sec24, Sec13, Sec31). COPII vesicle formation is regulated by Sar1, a small GTPase triggering the membrane association of the other coat components. This takes place at tER sites whose biogenesis is regulated by Sec16. COPII vesicles mediate ER-Golgi transport [124,134].

hours after egg fertilization, the developing embryo undergoes 13 nuclear divisions within a single cytoplasm, yielding approximately 6000 nuclei that are positioned very close to the plasma membrane of the so-called syncytial embryo. Cellularization starts by forming shallow plasma membrane invaginations, called furrows, between the adjacent nuclei. As the pre-existing plasma membrane is pulled inwards by an actin–myosin based mechanism, deposition of a large amount of new membrane is needed [79]. At least part of the additional membrane needed for furrow canal progression was shown to derive from the secretory pathway (Golgi membrane or post-Golgi vesicles) [80], and consequently is inhibited upon BFA treatment [13,81].

Interestingly, the early secretory pathway is compartmentalised already before cellularization in such a way that one nucleus is closely associated to a functionally independent secretory units [82]. The mechanism underlying this compartmentalisation is shown to be microtubule-dependent. In particular, astral microtubules (organised by the centrosome that is itself closely associated to each nuclei) create a cage/frame that leads to sequestration of these secretory units around individual nuclei. This ensures the equivalent partition of these compartments before cellularization. Additionally, this early secretory pathway reorganisation could mediate the establishment of localised protein expression patterns and support the membrane transport required for cellularization [82].

One particular protein essential for cellularization is the Golgi peripheral protein, Lava Lamp, which was originally identified in a biochemical screen for proteins associated with both microtubule and actin filaments [13]. In the absence of *lava lamp* available mutants, injection of inactivating antibodies led to an inhibition furrow progression and an apparent Golgi membrane dispersal [13]. More recently, Lava lamp was also shown to interact with dynein/dynactin microtubule motor complex used to mediate transport of Golgi units or Golgi-derived membrane apically, where delivery of new membrane is required [83].

9.3. Fringe and wing development

A large proportion of the enzymes catalysing the maturation of N- and O-linked oligosaccharide chains are localised to the Golgi. Although protein glycosylation is instrumental for many aspects mammalian development, including Congenital Glycosylation Disorders [84–86], a role for N-linked glycosylation in *Drosophila* still remains to be demonstrated. O-linked glycosylation, on the other hand, has been shown to play a crucial role in wing development. This process requires the restricted activation of Notch signalling at the dorsoventral margin of the wing imaginal disc. Notch is a transmembrane protein being transported to the plasma membrane of all cells across the dorsoventral margin where it acts as a receptor for proteins expressed on the surface of neighbouring cells. Delta and Serrate, the two ligands that can activate Notch signalling, are produced by the cells on the ventral and dorsal side of the margin, respectively. However, genetic evidence suggests that Delta activates Notch only in dorsal cells (where it is not itself expressed), whereas Serrate only in the ventral cells of the margin [87].

The mechanism underlying this spatial Notch-ligand specificity depends on Fringe, which is expressed only in dorsal cells. Fringe is a Golgi resident N-acetylglusosamine transferase to O-linked fucose residues, and Notch is one of its substrates in *Drosophila* [88,89]. Once modified through this single sugar addition, Notch displays higher binding affinity for Delta and reduced affinity for Serrate, a critical property for the formation and maintenance of the dorsoventral wing boundary [88,90]. In loss-of-function *fringe* mutants, this boundary is disturbed and wings fail to develop properly [91]. Interestingly, O-fucosylated Notch has been shown to be itself a substrate of all three mammalian Fringe homologues [92].

Taken together, these examples demonstrate the complexity in defining roles for Golgi proteins and Golgi function in developing tissues and illustrate that the use of model organisms goes far beyond what tissue culture cells have allowed.

10. Lesson 9: assessing the Golgi functional organization by genome-wide RNAi screens in *Drosophila* cell lines

Drosophila derived cell lines have been widely used to perform genome-wide RNAi screens. Although the high number of off-target effects was a major shortcoming faced by many initial RNAi screens [93], developing new libraries of double-stranded RNAs (dsRNAs) using appropriate software combined with hit genes validation using 2 or more independent dsRNAs has resulted in minimizing these effects (http://flyrnai.org/; http://www.dkfz.de/signaling2/rnai/index.php).

Information on the Golgi apparatus has been gathered through the analysis of two kinds of genome-wide RNAi screens in *Drosophila* cell lines: the first directly aimed at identifying novel factors involved in anterograde transport through the early secretory pathway (including the Golgi). The second identified (sometimes unexpectedly) Golgi/transport-related proteins as regulators of a large variety of cellular processes.

At least two independent screens have been performed to identify new regulators of anterograde transport ([94]; Sean Munro personal communication). The read-out in both screens was the secretion of signal sequence containing chimeric proteins coupled to horseradish peroxidase [94] or firefly luciferase (Sean Munro personal communication). The first screen identified about 100 novel genes affecting constitutive protein secretion (the so-called TANGO genes). Overexpression of 20 of them has been performed showing that several localize in compartments of the early secretory pathway and therefore could directly regulate secretion [94,95]. The second screen has been performed more recently and made use of a new dsRNA library predicted to have minimal off-target effects. Furthermore, the amount of secreted reporter protein was normalized to the total protein level produced, a crucial step to eliminate hits genes that affect secretion indirectly by reducing cell growth and viability. This is presumably one of the reasons why this screen led to a very different list of novel hits than the first one (Sean Munro personal communication). Nevertheless, the transmembrane protein TANGO-1 is a hit common to both screens and has been characterized further [96]. Its mammalian homologue also localizes to tER sites and its depletion blocks collagen secretion. At this location, it interacts both with COPII components Sec23/24 and soluble cargos guiding them into COPII-coated vesicles. Furthermore, TANGO-5 and TANGO-13 have also been identified as rat liver Golgi proteins in a proteomics analysis [97], although surprisingly, knockout mice for both mammalian TANGO-13 homologues have no obvious defects in secretion [98]. These examples justify the use of genome-wide RNAi screens in *Drosophila* cell lines as a powerful method for the discovery of new "Golgi" genes (some of them even essential for secretion), but also demonstrate that such results should not be readily generalized to whole organisms.

Conversely, many RNAi screens aiming to identify regulators of various cellular processes have picked up genes encoding proteins with known functions in the early secretory pathway. However, these are often among hundreds of other genes with different functions, and their biological significance needs to be precisely determined. A very interesting case, though, is a new role for the COPI machinery in lipid homeostasis. More specifically, two independent screens [99,100] using microscopy-based assays have shown that depletion of COPI coat subunits as well as Arf79F (Arf1 homologue, the GTPase that recruits the COPI components) and Garz

(GBF1 homologue acting as an Arf1 GEF) lead to an increase in lipid storage. COPI activity seems to exert its role in lipolysis by mediating the recruitment of ATGL, an enzyme crucial for lipid catabolism, on the lipid droplets [99,100]. Interestingly, this COPI-mediated recruitment of ATGL appears to be independent of its known role in protein transport, since depletions of COPII components and clathrin do not have an effect on lipid droplets [99,100]. However, this has been contradicted by a recent study reporting that ATGL delivery to lipid droplets is COPI and COPII dependent [101].

A novel role for COPI coat components has been shown in another genome-wide RNAi screen searching for proteins involved in picorna virus replication in *Drosophila* cells. This screen identified COPI subunits to be important for the formation of vesicular compartments, where virus replication takes place. Again, COPI activity is protein transport-independent, as COPII or Syntaxin 5 depletion did not affect virus replication [102].

Of note, εCOP is the only subunit that appears dispensable for the above mentioned COPI functions both in *Drosophila* and mammalian cells. Although εCOP mRNA can be efficiently lowered by RNAi [102], it has been missed systematically in almost all genome-wide screens that identify hits among the COPI subunits. Either εCOP is an extremely long-lived protein, or it can be substituted on the COPI coat by another yet unidentified protein, or it is not essential in higher eukaryotes.

Several components of the early secretory pathway have also been identified in screens looking for genes involved in S2 cell infection efficiency by a variety of pathogens, such as *Mycobacterium* [103,104], *Listeria* [104], *E. coli* [103,105], *Candida ablicans* [105], *Brucella* [106] and *Chlamydia* [107]. The general trend coming out from these studies is that vesicular traffic is required for the completion of pathogens' life cycle inside the host cells. However, the significance of specific vesicular transport genes that are required for the pathogenicity of specific pathogens is currently unclear.

Genes with Golgi-related functions have also come up in RNAi screens for genes involved cytokinesis [108,109]. Depletion of proteins that strongly inhibit anterograde transport, such as Rab1, Syntaxin5, α-SNAP or COPI subunits (except εCOP), leads to a significant increase of binucleated cells indicating that membrane delivery from the exocytic pathway is required along with that supplied from the endocytic pathway for the completion of cytokinesis (Table 1). In addition to cytokinesis, efficient anterograde transport may also be important for cell entry into mitosis, as for instance COPI or Syntaxin5 depletion increases the G2/M population ([110]; our unpublished observations).

Last, Sec23 and ζCOP have also been shown to promote cell death (DIAP1- and Doxorubicin-mediated caspase activation) as their depletion prevents cells from undergoing apoptosis [111], thus highlighting yet a possible additional role for both COP subunits.

In conclusion, RNAi screens have revealed a number of interesting new roles for proteins of the early secretory pathway, especially COPI. Whether the role for COPI subunits relates to its established function in protein transport remains to be established. The advantage of *Drosophila* RNAi screens is the generation of very large data sets available for comparison, thus allowing the identification of crosstalk between different cellular functions (http://flight.licr.org/). This could also help dissecting direct from indirect effects of gene depletions in different cellular processes, an issue that remains a major challenge in using this technology.

11. The missing lesson 10: what's next?

By no means have we exhausted the discoveries on the Golgi apparatus using *Drosophila* (and any other organisms, for that matter). In principle, it is difficult to foresee what will be discovered next, but we outline here areas where progress is likely to be made.

One area could be in the secretion of proteins that are crucial for development, such as morphogens Wingless and Hedgehog that are lipidated and cholesterol-bound (in the case of Hedgehog). This analysis would help unravel what adaptations the secretory pathway acquires for such a specialised secretion, and whether the morphogen-producing cells develop new strategies/compartments to perform it adequately. Genetic screens for mutants affecting *Drosophila* eye development and genome-wide RNAi in S2 cells have already identified a number of proteins that are specific for Wingless secretion. The seven transmembrane domain protein Evi/Wntless is suggested to bind Wingless in the TGN and chaperone it to the plasma membrane [112,113] before being endocytosed and recycled using the retromer complex (for review see [114]). A similar strategy could be used for Hedgehog as well as other receptors and ligands that are essential for development.

Second, we still do not have a clear picture for the role of the Golgi organization, and the proteins responsible for it, in development and disease. This may be a case we will never win, as Golgi seems to be plastic to such an extent that the absence of stacked structure at certain developmental stages does not seem to affect secretion. Nevertheless, systematic reverse and forward genetics approaches for Golgi structural proteins, by using inducible RNAi fly stocks or generating large collections of mutants, is likely to shed light on this issue leading to the discovery of unexpected links between Golgi structure and developmental processes. However, designing an appropriate readout is far from fruition. Alternatively, RNAi screens in S2 cells for factors involved in Golgi organization could be performed and the role of individual hits for the developing organism could be investigated using alleles created by targeted mutagenesis (such as imprecise P-element excision) and RNAi fly stocks. For instance, this strategy could be followed for the genes identified in the Malhotra's lab, such as TANGO-1, 5 and 13 (see lesson 9) which, from localisation studies [94], seem promising for having a direct effect on secretion. In this way, the knowledge of cellular protein functions can be directly transferred/tested in development, and this is likely to unravel requirements that have not been picked up in tissue culture cells.

Third, genome-wide screens for factors affecting a large variety of cellular functions (either in vivo in the whole animals, or by RNAi on S2 cells) have generated numerous dataset, which upon bioinformatics analysis could identify unexpected functions of Golgi proteins/genes. These could either be unrelated to their known function in the Golgi functional organization, or reveal so far unidentified crosstalk between the secretory pathway and other cellular processes.

Last, *Drosophila* could be useful in the identification of signaling molecules at the surface of the Golgi. We have already shown that F-actin regulators and Rac effectors, Abi and Scar/Wave, are partly confined on *Drosophila* tER-Golgi units [7], and the prediction is that the Golgi, as the endosomal system and the ER can act as platforms for regulated signalling. This notion is exemplified in mammalian cells, where certain Ras isoforms localise to the Golgi and trigger signalling cascades [115]. Conversely, the function of the Golgi can be regulated by signalling, and although this has been illustrated in few cases [116,117], much remains to be discovered. One could design RNAi screens aiming at the identification of kinases whose depletion leads to a disruption of the Golgi morpho-functional organization. Due to the lack of gene redundancy and the possibility of doing RNAi screens transferable to the whole animal, *Drosophila* studies are likely to shed light on this issue in a short time frame.

Acknowledgements

We thank our colleagues of the department of Cell Biology, UMC Utrecht as well as Cathy Jackson (Gif Sur Yvette, France) and Sean Munro (Cambridge, UK) for helpful discussions and for sharing unpublished data.

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