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Graham Warren

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Intracellular membrane morphology

GRAHAM WARREN

Imperial Cancer Research Fund, P.O. Box 123, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

SUMMARY

The membrane-bound organelles on the exocytic and endocytic pathways are linked by vesicles which bud from one membrane compartment, carrying selected cargo, and fuse with the next on the pathway. The principles underlying this vesicle-mediated traffic go a long way towards explaining the morphology of these intracellular organelles and their behaviour during mitosis.

1. INTRODUCTION

After synthesis and assembly in the endoplasmic reticulum (ER), secretory and membrane proteins are transported through each of the compartments that comprise the Golgi apparatus (see figure 1). The *cis* Golgi network (CGN) at the *cis*, or entry face of the Golgi stack appears to act as the last quality control step. Only correctly assembled proteins are allowed to proceed further and any escaped ER proteins are returned (Pelham 1989; Gething & Sambrook 1992).

The stack of closely apposed and flattened cisternae, the central feature of the Golgi apparatus, carries out the bulk of post-translational processing reactions, the best characterized being the modifications to the bound oligosaccharides (Roth 1987). When the modified proteins reach the *trans* Golgi network (TGN), at the exit face of the Golgi stack, they are sorted to their correct destinations which include the plasma membrane, lysosomes and secretory granules (Griffiths & Simons 1986). Plasma membrane receptors (such as the receptors for low density lipoprotein and transferrin) then move through the endocytic pathway as they carry out their function of bringing nutrients and other material into the cell (Brown *et al.* 1983).

Transport from organelle to organelle or from compartment to compartment within an organelle is mediated by vesicles that bud from one membrane and fuse with the next on the pathway (Rothman 1994). Though the precise molecules used depend on the pathway, the underlying principles are very similar and are illustrated in figure 2.

The first step involves budding of a patch of membrane containing selected cargo (both membrane and secretory proteins). Budding is initiated by a membrane-bound GTPase which recruits coat subunits from the cytoplasm. Stepwise assembly leads to deformation of the membrane which eventually brings the non-cytoplasmic (or periplasmic) membrane surfaces of the compartment together leading to membrane fusion. This releases a coated vesicle which then uncoats so that the coat subunits can be recycled for another round of coated vesicle budding.

The next step involves specific docking of the vesicle with the next compartment on the pathway. Docking is a complex process with several layers of checks and controls but at its heart is an interaction between cognate pairs of membrane proteins termed snares (Sollner *et al.* 1993). Each type of vesicle has a particular vesicle, or v-SNARE, which specifically interacts with the target, or t-SNARE in the acceptor membrane. There are different SNAp REceptor (SNARE) pairs for each of the vesicle-mediated steps on the exocytic and endocytic pathways (Rothman 1994).

The last step is the fusion of the docked vesicle with the target membrane. Soluble NSF Accessory Proteins

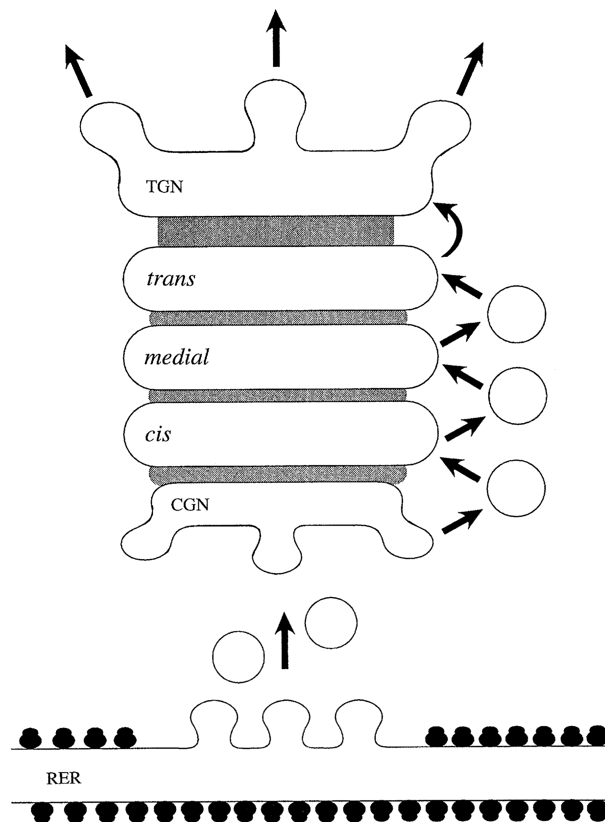

Figure 1. Schematic view of the exocytic pathway comprising the rough endoplasmic reticulum (RER), the *cis* Golgi network (CGN), the *cis*, *medial* and *trans* cisternae of the Golgi stack, and the *trans* Golgi network (TGN).

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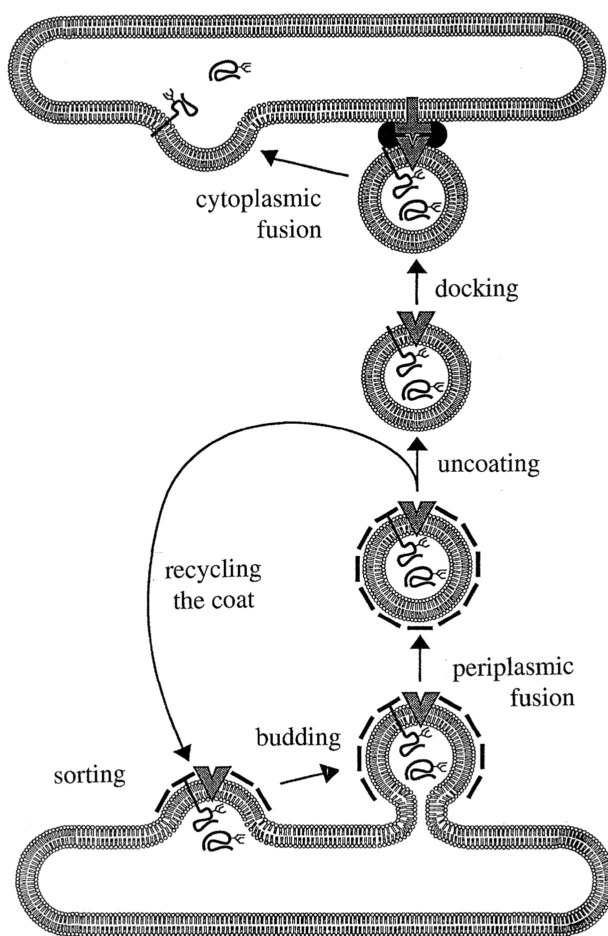

Figure 2. Schematic view of vesicle-mediated transport between two membrane compartments. The release of a coated vesicle is dependent upon periplasmic fusion of the non-cytoplasmic surfaces of the membrane compartment whereas delivery of the cargo depends upon the heterotypic type of cytoplasmic fusion. (Adapted with permission from Warren 1991).

(SNAPs) and *N*- ethylmaleimide sensitive factor (NSF) bind to the SNARE pairs and separate them by the hydrolysis of ATP (Sollner *et al.* 1993; Rothman 1994). In a manner yet to be elucidated, this separation is coupled to fusion between the cytoplasmic surfaces of the docked membranes.

There are two types of cytoplasmic fusion. Heterotypic fusion is the best characterized and involves fusion between dissimilar membranes. An example is the fusion between a transport vesicle and its target membrane (Rothman 1994). Homotypic fusion involves similar or equivalent membranes. The best examples are seen at the end of mitosis when fragmented membranes reassemble (Warren 1993).

2. MITOTIC FRAGMENTATION

When animal cells enter mitosis, membrane traffic ceases and membranes on the exocytic pathway break down (Warren 1993). The nuclear envelope breaks down completely whereas the rest of the ER breaks down to a lesser extent. The single copy of the Golgi apparatus is almost completely vesiculated yielding up to 10000 vesicles which become randomly dispersed throughout the mitotic cell cytoplasm. Fragmentation is thought to aid the partitioning of membranes between the two daughter cells by a stochastic process (Birky 1983; Warren 1993).

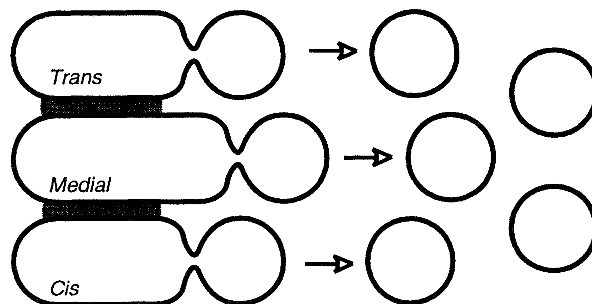

Figure 3. Vesiculation of the Golgi stack during mitosis can be explained by continued budding of transport vesicles in the absence of docking and fusion.

We proposed a simple model to explain the vesiculation of the Golgi apparatus by suggesting that the observed inhibition of membrane traffic is caused by an inhibition of cytoplasmic membrane fusions (Warren 1985). Vesicles would continue to bud from the cisternal rims of the Golgi stack but they could no longer fuse (see figure 3). Because the membrane lost by the budding of vesicles is normally compensated by the fusion of an incoming vesicle, the inevitable consequence would be the conversion of cisternae into vesicles.

At the level of the Golgi apparatus, COat Protomer (COP) coats are the device used to bud transport vesicles (Rothman 1994). COP-coated vesicles have been shown to bud at similar rates under interphase and mitotic conditions and to be required for the observed accumulation of vesicles (Misteli & Warren 1994, 1995). These data provide strong support for the hypothesis.

COP-coated vesicles cannot, however, consume all of the Golgi membrane and there is another pathway that converts cisternae into tubular networks and then into smaller tubules and vesicles (Misteli & Warren 1995). The mechanism is unknown but one simple proposal (Rothman & Warren 1994) utilizes the fact that, unlike cytoplasmic fusion, periplasmic fusion continues during mitosis. Otherwise, COP-coated vesicles could not bud from the rims of Golgi cisternae.

4. PERIPLASMIC FUSION

Periplasmic fusion differs from cytoplasmic fusion in that it is a non-specific fusion event. The layers of checks and controls needed for cytoplasmic fusion are unnecessary for periplasmic fusion because the only two membranes that can fuse are part of the same compartment.

Another possible difference and one which permits the following speculation is that periplasmic fusion might be triggered simply by close apposition of the lumenal membrane surfaces (Rothman & Warren 1994). Coats would not locate the putative periplasmic fusion proteins at the neck of the budding vesicle; they would simply deform the membrane, bringing the

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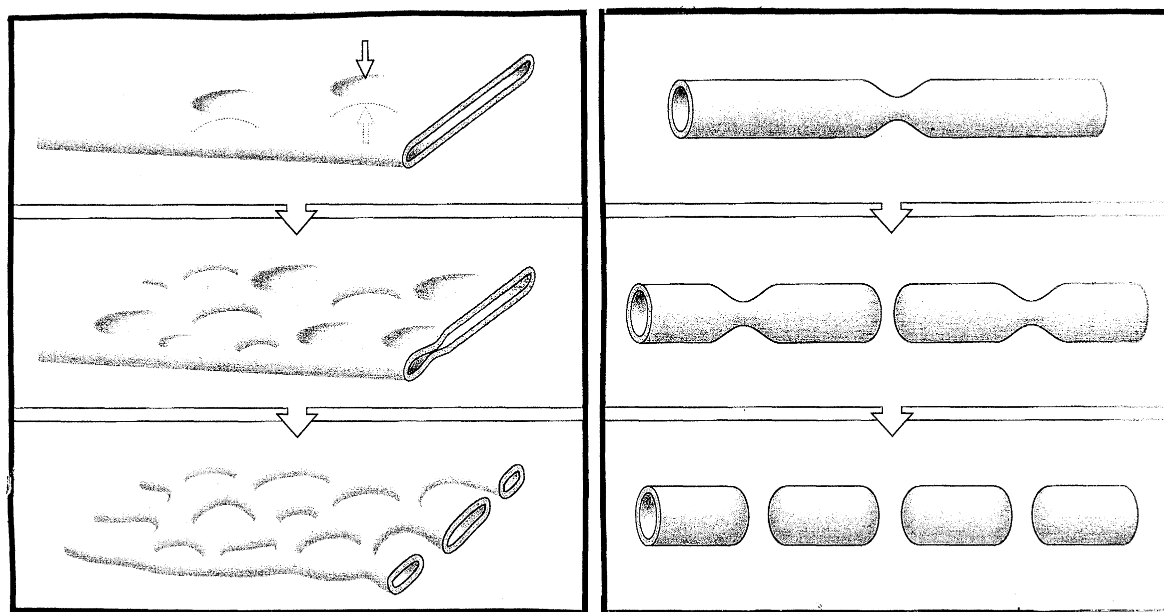

Figure 4. The effect of spontaneous periplasmic fusion. A flattened cisterna (top left) would become increasingly fenestrated leading, eventually, to a tubular network (bottom left). The tubules (top right) in these networks would also be susceptible, breaking down into small tubular remnants (bottom right). (Adapted, with permission, from Rothman & Warren 1994, Copyright Current Biology Publications).

lumenal surfaces close together. Freely diffusing fusion proteins might then sense this close apposition perhaps because they could now interact across the narrow gap. This would then trigger periplasmic fusion.

The clear advantage of such a mechanism is that it can be used by any device that needs to sever membranes (for references see Rothman & Warren 1994). There are many different types of coat protein, all of which generate vesicles from membranes. Yeast undergo closed mitosis which means that nuclear and ER membranes must be severed when the cell divides. Cytokinesis could provide the necessary device that brings the periplasmic surfaces of the nuclear envelope and ER together, leading to membrane fusion. A final example is the insertion of nuclear pores into the nuclear envelope during S phase. If the nuclear pore complex binds to the outer nuclear envelope and partially 'buds' into the intermembrane space, this would bring the periplasmic surface of the outer nuclear envelope into contact with the periplasmic surface of the inner nuclear envelope. Triggering fusion in this manner would ensure insertion of the nuclear pore complex without leakage of nuclear contents.

Despite this clear advantage there is a disadvantage in using a common fusion mechanism for different severing devices. The cytoplasm is a turbulent environment and membranes are constantly deformed and stretched. This could bring together the lumenal surfaces and trigger unwanted, or spontaneous, periplasmic fusion. The consequence of such spontaneous periplasmic fusion is illustrated in figure 4, starting with a flattened cisterna. If opposite sides touch and fuse, a hole – or fenestration – will form. Further touching will eventually lead to a tubular network. The tubules in this network will also be susceptible to further periplasmic fusion events leading, eventually, to small tubular fragments. This sequence of events is observed during mitotic fragmentation of Golgi stacks in the presence or absence of COP coats (Misteli & Warren 1994, 1995). This provides strong evidence that continued periplasmic fusion (both spontaneous and triggered by COP coats) in the absence of heterotypic fusion (the fusion of transport vesicles with their target membrane), is responsible for the observed mitotic fragmentation of Golgi stacks.

5. SCAFFOLDS AND HOMOTYPIC FUSION

The consequences of spontaneous periplasmic fusion are not observed under interphase conditions even when the mitotic inhibition of vesicle fusion is mimicked using inhibitors either of uncoating or fusion (Orci *et al.* 1989; Misteli & Warren 1994). Transport vesicles continue to bud showing that triggered periplasmic fusion is still occurring. The vesicles also accumulate showing that heterotypic fusion is inhibited. However, the Golgi stacks remain intact; they are not converted into tubular networks.

There are two likely reasons for this. The first is that, during interphase, the Golgi cisternae are stacked one on top of the other and are likely held together by both intercisternal and lumenal scaffolds (see Slusarewicz *et al.* 1994, for references). Such scaffolds would keep the periplasmic surfaces of the cisternae apart, thereby preventing periplasmic fusion. The second reason is that any damage done by periplasmic fusion is likely repaired by homotypic fusion (Rothman & Warren 1994).

Homotypic fusion, like heterotypic fusion, is inhibited during mitosis (Warren 1993). It resumes during telophase (Souter *et al.* 1993) when large numbers of vesicles fuse with the rims of cisternal remnants, permitting cisternal regrowth (Rabouille *et al.* 1995). Compound fusion at the rims generates extensive

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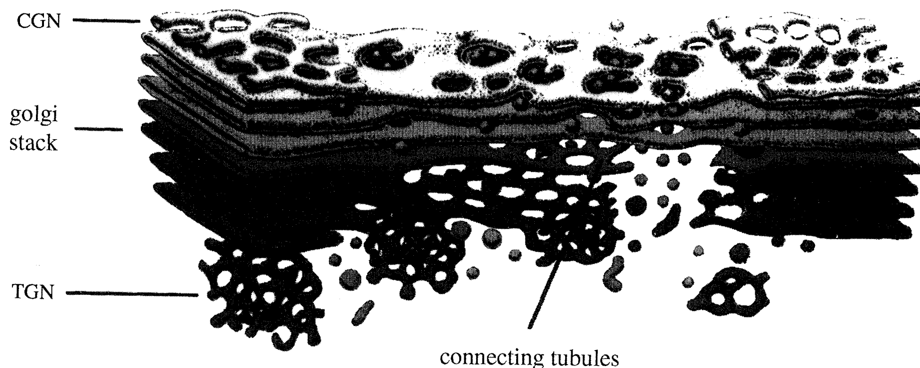

Figure 5. Schematic view of the Golgi apparatus derived from high voltage electron microscopy. (Adapted with permission from Rambourg *et al.* 1987).

tubular networks that gradually flatten and become less fenestrated (Rabouille *et al.* 1995). The loss of fenestrations is likely the consequence of homotypic fusion since closing a hole involves cytoplasmic fusion of the membranes surrounding it.

Because homotypic fusion can close fenestrations it could repair the damage done by spontaneous periplasmic fusion during interphase. If, for example, the Golgi cisternal faces did touch, despite the presence of membrane scaffolds, the fenestration formed could be closed by homotypic fusion. During mitosis, the scaffolds would break down and homotypic fusion would be inhibited. Spontaneous periplasmic fusion would generate fenestrations that could not be repaired leading to the formation of tubular networks and tubular remnants. COP-coated vesicles, utilising the same periplasmic fusion mechanism, would ensure both rapid and efficient fragmentation of the Golgi apparatus and consequent equal partitioning of membrane between daughter cells.

6. IMPLICATIONS FOR MORPHOLOGY

The morphology of many intracellular membranes can now be seen as the balance between periplasmic fusion on the one hand and both membrane scaffolds and homotypic fusion on the other.

A schematic view of the Golgi apparatus is shown in figure 5. The membrane scaffolds would ensure the relative absence of fenestrations in the central, stacked regions. The CGN and TGN probably lack these scaffolds (at least on one surface) so they would be more susceptible to periplasmic fusion. This would explain their reticular morphology (cf. figures 4 and 5). The cisternal rims also lack these scaffolds because they are the site from which COP-coated vesicles bud. This would explain their more fenestrated appearance.

The Golgi apparatus in animal cells is a ribbon-like structure in which discrete stacks are linked by tubules that connect equivalent cisternae in adjacent stacks (Rambourg *et al.* 1987). Such connecting tubules (see figure 5) would be constantly severed by periplasmic fusion and repaired by homotypic fusion. Repair would be enhanced by the action of microtubule motors that concentrate Golgi membranes in the pericentriolar region. In the absence of microtubules, severing the connecting tubules would lead to separation of discrete stacks that would then preclude repair by homotypic fusion. This would explain the action of anti-microtubule agents such as nocodazole (Thyberg & Moskalewski 1985).

The morphology of the ER could also be explained using these arguments. The nuclear envelope has a number of membrane scaffolds (the lamina, nuclear pores and ribosomes)(Gerace & Burke 1988) which would inhibit the appearance of fenestrations. The rough ER (RER) is coated with only one of these scaffolds (ribosomes) which would explain why it is often fenestrated. Smooth ER lacks even this scaffold which explains its tubular-reticular appearance.

The balance between periplasmic and homotypic fusion in animal cells appears to result in a tubular network. The presence of membrane scaffolds shifts this balance towards flattened cisternal structures.

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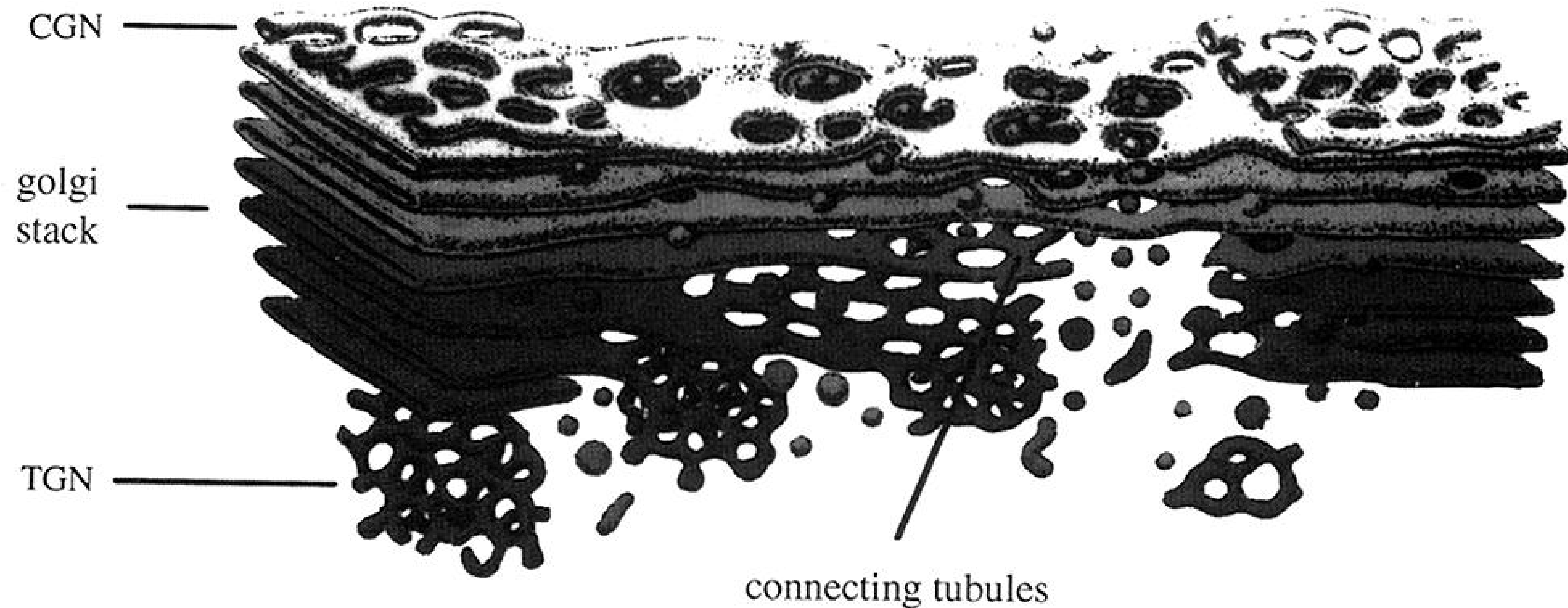

gure 5. Schematic view of the Golgi apparatus derived from high voltage electron microscopy. (Adapted with rmission from Rambourg *et al*. 1987).

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