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The Golgi Apparatus

State of the art 110 years after Camillo Golgi's discovery

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Preface

In 2008, we celebrate the 110th anniversary of the first description of the complex *apparato reticolare interno* by Camillo Golgi in the Bollettino della Società Medico-Chirurgica di Pavia. The biography of Camillo Golgi, and the Golgi apparatus history have impressively been described by Paolo Mazzarello in his book "The Hidden Structure. A Scientific Biography of Camillo Golgi" (Oxford University Press, 1999). During the 20th century, Camillo Golgi's discovery had a changing up and down and up history, timely being assessed as an artefact, and then again coming into the centre stage of cell biologic research. Today, it is well established that the Golgi apparatus constitutes a main crossroads in the intracellular transport routes of the biosynthetic, endocytic, and recycling systems. During the past decades, multiple new discoveries contributed to the understanding of the organization and the functions of the complex organelle (see Chapter 1.1). In 1997, the excellent book about the Golgi apparatus edited by J. Roth and E. Berger provided a comprehensive summarizing presentation of the state of research 100 years after the first description of the organelle. Now, after further 10 years, it is necessary to summarize again what it is known about the complex organization of Camillo Golgi's apparatus.

Our book is an attempt to bring together multiple new results obtained by different techniques, and addressing different aspects of the Golgi apparatus and intracellular transport. We are hopeful that the presentation of the state of the art 110 years after Camillo Golgi's discovery of the complex apparato reticolare interno will lead to an improved understanding, novel insights, and new perspectives for future research.

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Introduction

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All cells secrete a diversity of macromolecules to modify their environment or to protect themselves. On the other hand, there is the necessity to replace membrane proteins and lipids that are being constantly degraded in compartments of the secretory and endocytic pathways. Therefore, eukaryotic cells synthesize proteins for either export (secretion) or delivery to the secretory and endocytic compartments and to the plasma membrane (PM) for replacement of degraded proteins and lipids. The synthesis is carried out by ribosomes attached to the cytosolic surface of the endoplasmic reticulum (ER). The synthesis of most of cellular lipids and all fatty acids also occurs in the smooth ER. During or after the synthesis, the polypeptide chains containing transmembrane domains composed of hydrophobic amino acids are inserted into the ER membrane, whereas soluble proteins are transferred into the ER lumen. After the cleavage of their leading hydrophobic signal peptides and after protein folding both the groups of proteins are transported along the secretory pathway.

The Golgi apparatus (GA) is the central station along this pathway. While passing through the GA, proteins and lipids undergo posttranslational modifications (mainly glycosylation by Golgi glycosidases and transferases) and sorting. The transport of proteins and lipids from the ER to their destinations may be carried out in several ways: via the dissociation mechanism, the progression mechanism, and/or the lateral diffusion mechanism (see Chapter 1.2).

Over the past 30 years, the field of intracellular traffic has seen tremendous advances towards the identification of the relevant molecular machineries. Substantial progress in the isolation, cloning, and characterization of proteins involved in intracellular transport and its regulation, as well as in deciphering corresponding molecular events was achieved. Proteins involved in budding, fission, fusion, and sorting have been discovered, and, in some cases, a picture of how such proteins are assembled and work has been glimpsed. In contrast, perhaps surprisingly, a satisfactory understanding of how transport occurs *in vivo* at the organelle level has not been achieved, and the general picture of this process remains obscure. As a consequence, our present view of the overall mode of intracellular traffic (the “physiology” of traffic) in living cells is rather poorly developed. Although *in vitro* reconstitution experiments have been crucial in establishing the minimum number of components required for carrier budding using biochemical techniques, a more critical evaluation of intracellular transport could be complemented by *in vivo* experiments.

The reasons for this lag are both technological and conceptual. Technological, because intracellular traffic is an essentially dynamic event in time and

space. Yet, methods to study its dynamic features have been lacking until very recently. The recent advent of green fluorescent protein (GFP) technology has now partially filled this gap. Conceptual, because the field has been dominated until very recently by the model of intracellular traffic by anterograde vesicles. The vesicular scheme has been very successful in providing a framework to integrate an enormous amount of biochemical and genetic evidence collected over the past two decades into a coherent picture.

In the past years, the progress in development of new scientific methods accelerated. New microscopic and biochemical methods, new scientific instruments together with the development of molecular tools has given the possibility to study biochemical reactions and molecules in single cells with a resolution impossible to achieve before. Significant progress was made in the development of new synchronization protocols suitable for study of transport of several cargoes.

On the other hand, the complete deciphering of the full genetic code of yeast, humans, plants and several other species together with the development of proteomics provided a significant number of new proteins and details on protein–protein and protein–lipid interactions involved into the traffic. In the literature, there are thousands of details regarding protein interactions, their sorting signals, and the effects of their inhibition and deletion. As such, it seems that the field of intracellular transport appears to be facing a serious crisis. There are now so many proteins and inter-protein interactions involved that it has become almost impossible to follow and understand the meaning of all of these details.

The main idea of this book is thus to collect the full range of expertise and to examine the problems from different points of view and with different approaches.

This book is devoted to the molecular mechanisms of morpho-functional organization of the GA and summarizes most of new data related to the GA. The book is a collection of chapters written by different groups and therefore expresses different views especially on the mechanisms of traffic.

The possibility to follow the evaluation of the intracellular transport from different points of view and on the basis of different expertises could help to resolve this contradiction in the field.

There are several levels in the book. The main is the description of cell physiology with emphasis on the physiology of intracellular transport. The second level is the presentation of the morphology in wide term including light microscopy, analysis of live cells and so on. Finally, there are several chapters devoted to the molecular mechanisms involved in different physiological processes related to intracellular transport. In this book, we could not exclude completely some degree of overlapping. First, because the authors have different opinions about models of transport, and the second, it was necessary to illustrate some ideas from different points of view. We apologize to colleagues, whose relevant work has not been mentioned because of space limitations and focusing on work published most recently.

In the first part of the book there are two chapters. One is an overview of the history of this field of cell biology. The second one is a morpho-functional overview of the main steps of intracellular transport including functional organization and architecture of the GA.

In the second part of the book, main machineries operating along the secretory pathway are presented. These include SNAREs, Rabs, COPII, COPI, ARFs, ARFGEFs and ARLs, COG, TRAPP, dynamin and cortactin, Golgi enzymes and sugar transporters, ERGIC-53, and other luminal lectins, Golgins and Golgi matrix proteins. Lipids and lipid signalling, as well as common signalling mechanisms will be assessed. Additionally, the role of calcium and other ions in the regulation of intracellular transport, and the role of the cytoskeleton in Golgi function will be described.

In the third part of the book, different transport steps of intracellular transport will be evaluated, namely, ER-to-Golgi transport, intra-Golgi transport, Golgi-to-PM transport, Golgi-to-endosome transport, transport of lysosomal enzymes, retrograde endosome-to-TGN transport, and retrograde PM-to-Golgi transport. Mechanisms of regulated secretion will be explained in a separate chapter and in particular, mechanisms involved in formation of mucin granules are described. Interactions between endocytosis and secretory transport, the relationship between the GA and cell polarity, as well as structure and domain organization of the *trans*-Golgi network, and the questions of GA formation and inheritance will be discussed in separate chapters.

Finally, in the last part of the book the reader will find some aspects about the peculiarities of intracellular transport in different organisms: plants, yeast, and protists. A separate chapter is devoted to the endomembrane ultrastructure and dynamics in yeasts. Finally, the models of Golgi evolution will be discussed in the final chapter of this part.

This book is intended for cell biologists and histologists, who work with students, and also for scientists working in other fields of biology as well as for students per se. The most important item for teaching is the understanding of not a single or several mechanisms but the comprehensive view upon the full drama of development of scientific models. The readers should understand the logic of model replacements. Therefore, the aim of the book is to make the field of intracellular transport more understandable by keeping it as simple as possible, but also as full as possible, and at minimal cost.

Finally, we would like to mention the question of terminology. Since the original term was “Golgi apparatus”, this term is used in most of the chapters. However, both terms, Golgi apparatus and Golgi complex, are customary today.

General considerations

The Golgi apparatus and main discoveries in the field of intracellular transport

Alexander A. Mironov and Margit Pavelka

In this chapter, we summarize important findings in the field of intracellular transport, which have considerably contributed to the understanding of the function and organization of the Golgi apparatus (GA). It is not possible to mention all authors in this huge field. We apologize for gaps and incompleteness, and are thankful for suggestions and corrections.

The GA is named after its discoverer Camillo Golgi, who first described the complex *apparato reticolare interno* in 1898 (Golgi 1898a,b; reviewed by Berger 1997; Dröscher 1998). Although Camillo Golgi had presented his discovery convincingly, for a long time his data have been considered as an artifact of cell staining (Farquhar and Palade 1981). Only after the electron microscopic confirmation of the existence of the GA in cells by Dalton in 1951, scientists started to believe in its reality. Therefore, we will not list the discoveries within the area of intracellular transport made in the time, before the existence of the GA was confirmed electron microscopically. However, the names of A. Negri, H. Fuch, A. Perroncito, S. Ramon y Cajal, D.N. Nassonov, R.H. Bowen, G.S. Carter, H.W. Beams and R.L. King, V.M. Emmel, H.W. Deane and E.M. Dempsey, W.C. Schneider et al. should be mentioned, because they have considerably contributed to the understanding of the Golgi function (reviewed in Berger 1997). Here, we want to address most important discoveries within the area of intracellular transport after 1951 (Table 1).

Additionally, we would like to mention further important contributions to this field. The hypothesis of lipid rafts was proposed and developed by van Meer and Simons. The Lodish group made the invention of the synchronization of the transport of cargoes. The role of lectins in ER-to-Golgi transport was discovered by H.-P. Hauri. The most important contribution to the characterization of Rab machinery (although in the endocytic pathway) was made by M. Zerial. W. Hong, R. Sheller and R. Jahn made important contributions to the understanding of the function of the SNARE machinery. R. Schekman and W. Balch deciphered the functions of the COPII coat. A. Rambourg, Y. Clermont, G. Griffiths, A. Staehelin and K. Howell made significant contributions to the 3D-analysis of the GA in different cell types. J. Slot and H. Geuze provided new insight into the morphology of the endocytic system and its interaction with exocytosis. The important contribution into the analysis of the kinetics of the plant GA was made by C. Hawes. The characterization of the 3D-structure of several proteins important for intracellular transport, and protein coat complexes in their crystal state is linked with W. Balch and J. Goldberg's names. We apologise again for possible

Table 1. The Golgi apparatus and main discoveries in the field of physiology of intracellular transport

1898	Discovery of the GA
1951	Confirmation of the presence of the GA (Dalton 1951)
1961	The regional distribution of the thiamine-pyrophosphatase activity within the GA (Novikoff and Goldfischer 1961)
1964	The *trans* ER (Novikoff 1964; Novikoff et al. 1964)
1964	GERL concept (Novikoff 1964)
1964	Isolation of Golgi membranes from cells (Morré and Mollenhauer 1964)
1964	The process of sulphation in the GA (Godman and Lane 1964)
1966	The sugar–nucleotide transport from the cytosol to the Golgi lumen across the Golgi membranes, the role of the GA in glycosylation (Neutra and Leblond 1966)
1966	The origin of lysosomes and the function of clathrin-coated vesicles during protein absorption (Bainton and Farquhar 1966; Friend and Farquhar 1967)
1967	The intracellular transport (Jamieson and Palade 1967a,b)
1969	Galactosyltransferase as a Golgi marker (Whur et al. 1969; Morré et al. 1969)
1976	Isolation of clathrin-coated vesicles (Pearse 1976)
1977	The PM-to-Golgi transport of the endogenously added marker (Herzog and Farquhar 1977)
1980	M6P-mediated sorting of Golgi enzymes at the GA (Tabas and Kornfeld 1980)
1981	Clathrin-coated buds in the *trans* side of the GA (Griffiths et al. 1981)
1982	Immunocytochemical localization of galactosyltransferase (Roth and Berger 1982)
1983	Topology of N-glycosylation (Dunphy and Rothman 1983)
1984	Reconstitution of intra-Golgi transport *in vitro* (Balch et al. 1984)
1984	The 15°C temperature block (Saraste and Kuismanen 1984)
1985	Clathrin-independent endocytosis (Moya et al. 1985; Sandvig et al. 1985)
1985–1987	The mitotic form of the GA and mechanisms of mitotic Golgi transformation in animal cells (Featherstone et al. 1985; Lucocq et al. 1987)
1986	The COPI-coated vesicles and characterization of molecular mechanisms involved into the function of COPI coat (Orci et al. 1986; Serafini et al. 1991)
1986	The structure and function of the TGN and the 20°C temperature block (Griffiths and Simons 1986)
1987	KDEL-signal for the retention of luminally located proteins (Munro and Pelham 1987)
1989	BFA was applied for the study of intra-Golgi transport (Doms et al. 1989; Lippincott-Schwartz et al. 1989)
1990	SNAREs (Newman et al. 1990)
1990	The main genes involved in intracellular transport, the genetic evidence in favour of the vesicular model of the transport in yeast (Kaiser and Schekman 1990)
1991	A Golgi retention signal in the membrane-spanning domain (Swift and Machamer 1991)
1993	The role of oligomerization for the retention of Golgi enzymes (Weisz et al. 1993)
1993	The role of PM-derived signalling for intra-Golgi transport (De Matteis et al. 1993)
1994	Golgi matrix (Slusarewicz et al. 1994) and *cis*-Golgin, GM130 (Nakamura et al. 1995)
1994	COPI-dependent retrieval sorting signals (Cosson and Letourneur 1994)

Table 1. *(Continued)*

1994	COPII coat. Isolation of COPII-dependent small vesicles in cell-free system (Barlowe et al. 1994)
1996	Application of GFP-technology for the study of the GA in living cells (Cole et al. 1996)
1996	Characterization of the ER exit sites (Bannykh et al. 1996)
1997	The AP3 and AP4 coats (Dell'Angelica et al. 1997, 1999)
1997	Characterization of ER-to-Golgi transport carriers in living cells (Presley et al. 1997; Scales et al. 1997; Mironov et al. 2003)
1997	Characterization of post-Golgi transport carriers in living cells (Wacker et al. 1997; Hirschberg et al. 1998; Polishchuk et al. 2000)
1998	Intra-Golgi transport of large cargo aggregates (Bonfanti et al. 1998)
1998	The role of endocytic TGN in the formation of the most-*trans* Golgi cisterna (Pavelka et al. 1998)
1998	Discovery of R- and Q-SNAREs (Fasshauer et al. 1998)
1999	Tomographic reconstruction of the GA (Ladinsky et al. 1999)
2001	The concentration of regulatory secretory proteins within the Golgi cisternae (Oprins et al. 2001)
2003	The understanding of the evolution of small GTPases had changed the model of the Golgi evolution (Jékely 2003)
2003	Characterization of Golgi-to-apical PM transport carriers in living cells (Kreitzer et al. 2003)
2004	Intercisternal connections in transporting GA (Marsh et al. 2004; Trucco et al. 2004)
2006	Characterization of the Golgi-to-endosome carriers in living cells (Polishchuk et al. 2006)
2006	The role of GM130 in the maintenance of the Golgi ribbon (Puthenveedu et al. 2006)
2007	The role of ER-to-Golgi transport in the maintenance of the Golgi ribbon (Marra et al. 2007)

gaps (all authors quoted in the consecutive chapters deserve to be listed here). The list is open for suggestions.

The development of the research in the field of intracellular transport has been comprehensively discussed in 1998 at the conference in Pavia devoted to the 100th anniversary of the Golgi discovery.

History of models of intracellular transport

Historically, the first mechanism that had been proposed for intracellular transport was the progression. The origin of the progression model (or the concept of *cis*-to-*trans* flow) links to Grasse's name (1957) who proposed that the continuous formation of *cis* Golgi cisternae balances the conversion of *trans* one into secretory granules. However, the first experimental data in favour of the progression concept were obtained in 1971 (Franke et al. 1971).

In 1967, it has been demonstrated that proteins newly synthesized in the ER appeared, after a few minutes, not only over Golgi stacks but also over

round profiles surrounding the GA and the conclusion that secretory proteins bypass the GA was made (Jamieson and Palade 1967a,b, 1968a,b). Then, in 1981, the vesicular model replaced the progression model because the main support for the progression model, the *cis–trans* movement of scales in algae has been considered to be a rare formula connected with unusual geometry and size of the product (Farquhar and Palade 1981). Ironically, the major supporting data for the vesicular model at that time was based on the isolation of Golgi-derived clathrin-coated vesicles (Rothman et al. 1980). However, after the discovery of coat protein I (COPI) (Orci et al. 1986), the vesicular model was changed, and instead of clathrin-dependent vesicles, COPI-dependent vesicles were proposed to serve as anterograde carriers. The strongest support for the vesicular model appeared from the experiments in yeast with the temperature sensitive *Sec* genes (Kaiser and Schekman 1990). The *in vitro* isolation of functional (containing VSVG and able to fuse with acceptor Golgi membranes) COPI-coated vesicles (Osterman et al. 1993) was interpreted as the second proof for the role of COPI-coated vesicles in the anterograde intra-Golgi transport. Importantly, however, that the first author of this paper later stressed, that actually, these data support the cisterna maturation model (Ostermann 2001).

On the other hand, it has also been demonstrated that 20 min after fusion of two (or more) cells (one cell is VSV-infected, another is a non-infected cell) and formation of a heterokaryon, VSVG seems to move from the GA derived from the infected cell to the GA derived from non-infected cells (Rothman et al. 1984). These results were interpreted as confirmation of the ability of vesicular carriers to diffuse through the cytosol of the heterokaryon from one GA to another. However, later, the Rothman group (Orci et al. 1998) laid less emphasis on the heterokaryon experiments, suggesting that those observations appeared as a result of the treatment of cells with an acidic medium. Instead, the “string theory” was proposed, according to which a proteinaceous-like string links vesicles to cisternal elements and prevents budded vesicles from diffusing away, while still allowing them to diffuse laterally.

With time, due to accumulation of contradictions, the current vesicular paradigm became less and less effective in the explanation of growing body of observations (Mironov et al. 1997). As a result, the original version of the vesicular paradigm began to be modified not only by the opponents of the vesicular model but also by its authors and proponents (Orci et al. 1998).

In order to resolve accumulated contradictions within the field, almost simultaneously several groups (Bannykh and Balch 1997; Mironov et al. 1997; Glick et al. 1997; Schekman and Mellman 1997) have published the cisterna maturation-progression model based on the COPI vesicles-mediated Golgi enzyme recycling.

The first experimental confirmation that large aggregated cargo, such as procollagen I, can be transported through the GA by maturation mechanism came in 1998 (Bonfanti et al. 1998). Previous stereological observations in

P. scheffelii suggesting that their scales being much too large to be packaged into vesicles are transported by the progression of Golgi cisternae towards the plasmalemma were published not in an original paper but in a review (Becker et al. 1995) and were not confirmed later because glycoprotein and polysaccharide synthesis are uncoupled during flagella regeneration (Perasso et al. 2000).

Next, it has been demonstrated (Mironov et al. 2001) that both diffusible and non-diffusible cargoes are transported in the same carriers through the Golgi stacks. It has been proved that vesicles are not transport carriers for cargo in the intra-Golgi transport not only *in situ*, but also *in vitro*, in cell-free assay (Happe and Weidman 1998). After these publications, there was a short period when the cisterna maturation model became dominant.

With time new contradictions not compatible with the cisterna maturation-progression model have accumulated (Mironov et al. 2005). The attempts to use transport models based on combination of basic principles were not successful (see Chapter 3.2). Therefore now, there is no consensus on the models of intra-Golgi transport. The existence of the maturation mechanism is almost finally established for the secretion of large polymeric structures incompatible in size with COPI-dependent vesicles in many types of cells and under the infection of some viruses.

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The Golgi apparatus as a crossroads in intracellular traffic

Alexander A. Mironov and Margit Pavelka

In this chapter, we will briefly describe the structure and list main functions of different compartments along the secretory pathway.

ER–Golgi interface

After its synthesis, folding and quality control a cargo exits from the endoplasmic reticulum (ER) and moves to the Golgi apparatus (GA) through the ER–Golgi interface. Here, the most peculiar structures are vesicular–tubular clusters (VTC) defined as clusters of a few small vesicles and tubular–saccular elements associated with the region of the rough ER and even with the nuclear envelope containing COPII-coated buds (Bannykh et al. 1996; Mironov et al. 2003). On routine EM sections, the size of VTCs varies from 200 to 1,000 nm. They are positive for glucose-6-phosphatase (Thorne-Tjomsland et al. 1991) and at immunofluorescence level, appear as COPII-positive distinct sites (0.5–1 μm in size) associated with the ER (Stephens et al. 1997). In interphase cells at steady state, the average number of VTC remains constant varying from several dozens to several hundreds of VTC per one cell (Bannykh et al. 1996; Aridor et al. 1999; Hammond and Glick 2000).

Typically, COPII-coated buds are described as elevations on the surface of the ER with a width of 65–85 nm, extruded from the membrane by at least 50% of their diameter. Buds are covered with an 8–10 nm thick electron-dense COPII coat. On grazing sections, the buds possess a lattice-like appearance due to semi-regular array of 4–5 nm elongated particles arranged in a semi-regular pattern with mostly tetrahedron organization (Bannykh et al. 1996). Separated COPII-coated vesicles do exist (Zeuschner et al. 2006) although they are few and mostly are devoid of secretory proteins (Mironov et al. 2003). Some elongated profiles (which might represent cross-sections of saccules or tubules) within VTCs have a dense COPI-like coat at their tips and on their central parts (Martinez-Menarguez et al. 1999).

VTCs are not carriers, which undergo centralization and deliver cargo to the GA (Stephens et al. 2000; Mironov et al. 2003; see details in Chapter 3.1). For this purpose, ER-to-Golgi carriers (EGCs) are used. These appear mostly as saccular containers filled with either the large supramolecular cargo (i.e. procollagen) or the small diffusible cargo proteins. They arise through cargo concentration and direct en bloc protrusion of specialized ER domains in the vicinity of VTC (Mironov et al. 2003).

ER–Golgi connections

Direct membrane continuity between the ER and the GA has been described in many papers over the years in variety of tissues and cell types under different functional conditions (Flickinger 1969, 1973; Maul and Brinkley 1970; Claude 1970; Bracker et al. 1971; Holzman 1971; Morre et al. 1974; Franke and Kartenbeck 1976; Novikoff and Yam 1978; Uchiyama 1982; Broadwell and Cataldo 1983; Sasaki et al. 1984; Williams and Lafontane 1985; Lindsey and Ellisman 1985a,b; Tanaka et al. 1986; Krijnse-Locker et al. 1994; Sesso et al. 1994; Stinchcombe et al. 1995; Trucco et al. 2004) and using different methods of analysis, including three-dimensional (3D) observation in high voltage electron microscope (Lindsey and Ellisman 1985a,b), scanning electron microscopy (Tanaka et al. 1986), reconstruction of serial sections (Sesso et al. 1994) and even functional analysis of transport (Krijnse-Locker et al. 1994). Connections were described between the ER and EGC (Stinchcombe et al. 1995; Mironov et al. 2003).

After 3D tomographic reconstruction (Ladinsky et al. 1999), a connection between the ER and the membrane disk integrated between Golgi cisternae has been found. However, the nature of this disk is not established. The author interpreted this disk as the specialized domain of the ER.

Recently, two reports about ER-to-Golgi connections have been published. In one (Koga and Ushiki 2006) the existence of connections between the ER and the GA was not confirmed. However, the method used is not completely free from artefacts. ER-to-Golgi connections cannot be a result of fixation, because fixative usually disrupt pre-existing tubules rather than induce their formation (McIntosh 2001). In the report by Vivero-Salmeron et al. (2008), the existence of ER-to-Golgi connections was confirmed. The relatively low frequency of these observations might simply be due to the fact that thin sections are technically unsuitable for revealing a convoluted and transient (Vivero-Salmeron et al. 2008) structure extending through a large three-dimensional space or due to temporality of the connections (Fig. 1, 2c–e).

The function of compartments within the ER-to-Golgi interface

Within the ER-to-Golgi interface, several important posttranslational functions are performed: COPII-mediated concentration of defined membrane and soluble cargoes (to improve the efficiency of transport), delivery of cargo to the GA (see Chapter 3.1), O-glycosylation (Tooze et al. 1988), acylation (Rizzolo et al. 1985), generation of mannose-6-phosphate signal for lysosomal protein targeting (Pelham et al. 1988), protein palmitoylation (Bonatti et al. 1989), retrieval of misfolded proteins (Hammond and Helenius 1994), and segregation of secretory cargoes, namely, regulatory secretory

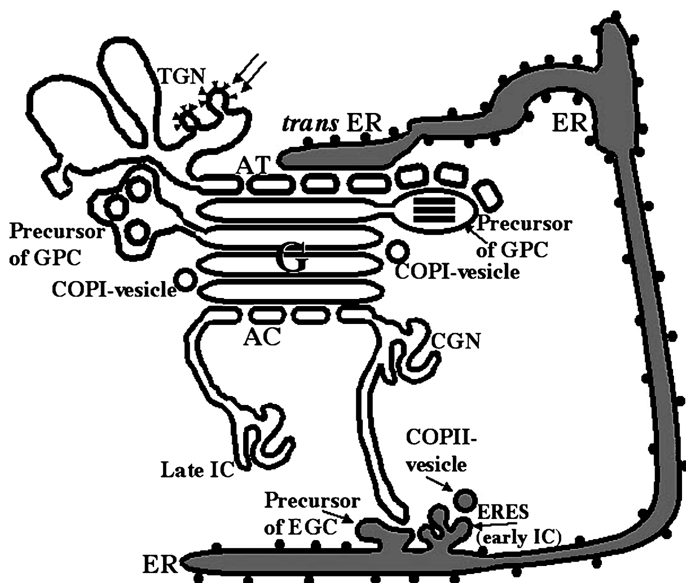


Figure 1. Scheme of structures within the ER–Golgi interface. The ER is shown as the structure filled with a grey content and covered with ribosomes (dark dots). The ER has a specialized domain that is attached to the Golgi stack (*trans*-ER). At the *cis*-side of the stack, the ER contains the ER exit site (ERES, or early intermediate compartment [IC] or vesicular tubular cluster; arrow). The ERES can include COPII-dependent vesicles (arrow). Near the ERES, there is the forming precursor of an ER-to-Golgi carrier (EGC, arrow). The *cis* Golgi network is composed of several domains. One represents the highly perforated disk similar in shape with Golgi cisternae. It is attached to the Golgi stacks (G) and is named as attached CGN (AC). There is a small part of the total CGN that appears as the three-dimensional tubular network or a cage (indicated as CGN) near the Golgi stacks. This part produces tubules moving towards the ERES (in the center of the image). Another part of the CGN is connected with the AC by tubules and has similar shape with the CGN. It localizes out of the Golgi stack and appears as the late IC that could be rather stable compartment. At the *trans*-side of the Golgi stack (G), there is the *trans*-Golgi network (TGN), parts of which reside apart of the stack, and are connected with the other part, the attached TGN (AT). The TGN contains clathrin-coated buds (double arrow). Precursors of Golgi-to-PM carriers (GPC) could form from the last two COPI-positive (with COPI-buds) cisternae, or from the entire TGN. COPI-vesicles are present near the rims of Golgi cisternae.

proteins, constitutive secretory proteins, proteins destined for the apical plasma membrane (PM) and basolateral PM, endosomal and lysosomal proteins by elimination of the mechanisms responsible to their retention within the ER.

The role of microtubules in centralization of EGCs is described in Chapter 2.14. However, this function is absent in plant and yeast cells (Nebenfuhr and Staehelin 2001) and even in several cell types in mammals (such as oocytes, Motta et al. 1995) and, thus, might be not directly related to ER–Golgi transport per se.

Transient ER–Golgi connections could serve for the diffusion of cargo proteins and recycling of resident proteins.

Morphology of the Golgi apparatus

The structural organization of the GA varies among species (see Chapters 4.1–4.4). In yeast (Chapter 4.3), and some protists (Chapter 4.4), the GA is composed of tubular networks and isolated disks. In animal and plant cells, the GA appears as a series of closely associated flattened membrane 'sacs' aligned in parallel to form a stack (Polishchuk and Mironov 2004; Chapter 4.1). In plants, *S. cerevisiae*, protists and some insect cells, stacks remain separated from each other whereas in mammalian cells stacks form the single ribbon (Chapter 2.12). Here, we describe mainly the mammalian GA.

The GA is embedded into a (so-called) "zone of exclusion", a polymer-based derivative of the cytosol that is especially evident in plant. This zone does not contain ribosomes but cytoskeleton elements can pass through it (Mollenhauer and Morre 1978). In epithelial cells of epididymis, goblet cells in the jejunum, gonadotrophs in pituitary glands and dorsal root ganglion cells, an "empty" zone of exclusion also surrounds the GA with the thickness of about 200 nm (Koga and Ushiki 2006).

The GA represents 2% of hepatocellular membrane (Blouin 1983) or 20% of that of the ER system (Griffiths et al. 1989). The GA is capable to undergo rapid and reversible reorganization in response to a variety of experimental manipulations (Polishchuk and Mironov 2004).

According to Griffiths et al. (1995), the GA begins from the place where mannosidase I is localized. Man I exhibits a highly polarized staining at the *cis*-pole of Golgi stacks usually composed of the first, sometimes of the first two cisternae (Marra et al. 2001).

The canonical GA consists of a series flattened cisternal membranes closely associated, aligned in parallel and forming a stacked structure, abundant tubular–reticular networks and vesicles. In the perinuclear area, dozens or hundreds of Golgi stacks are linked together to form an interconnected, ribbon-like structure as a single organelle with alternating compact (stacked cisternae) and non-compact (tubular–reticular) zones (Mogelsvang et al. 2004; see also Chapter 2.12).

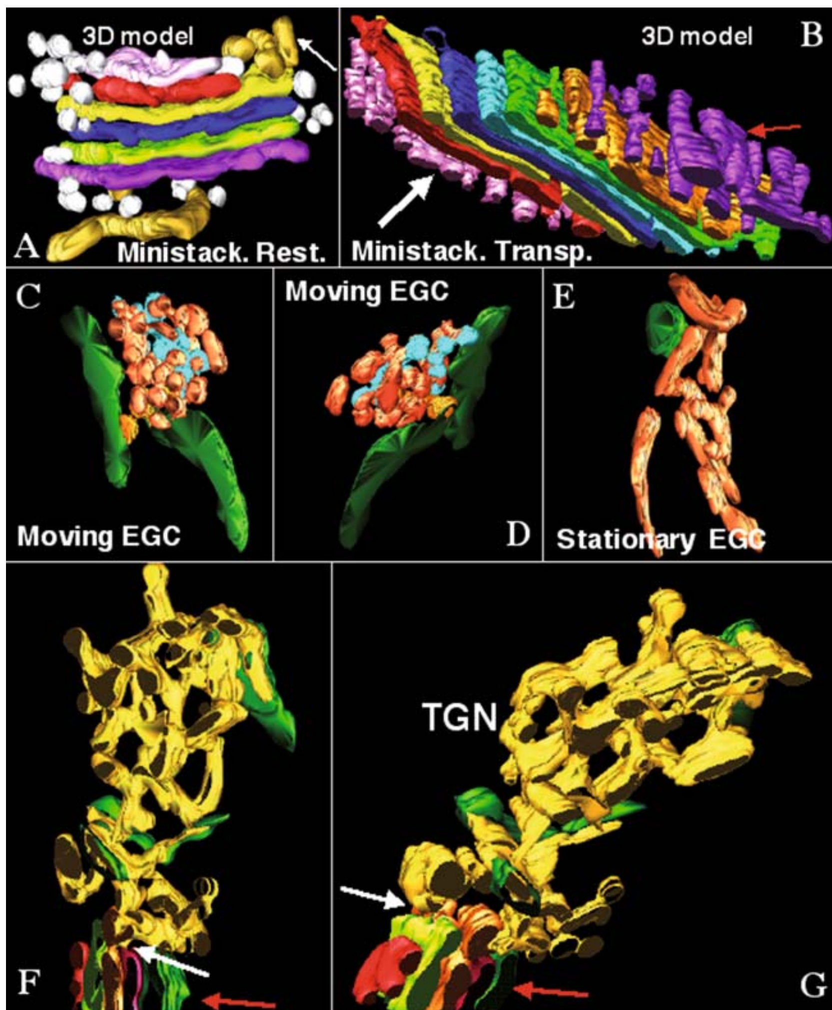
The GA can be viewed schematically as being composed of three main compartments: the *cis*-, *medial*- and *trans*-Golgi. Both the *cis*- and the *trans*-most Golgi elements are largely tubular. The *medial*-Golgi stacked cisternal compartment resides between these two networks (Rambourg and Clermont 1997; Polishchuk and Mironov 2004).

Several gradients exist within a Golgi stack: (i) a gradient in the cisterna fenestration; (ii) a gradient in the cisterna thickness; (iii) a gradient in the localization of the Golgi enzymes; (iv) a gradient in the lipid bilayer thickness; (v) a gradient in the pH; (vi) a gradient in concentration of cholesterol.

The concentration of cholesterol is higher at the *trans*-side of a Golgi stack. Especially high concentration of cholesterol is found in endosomes (Orci et al. 1981; Cluett et al. 1997; Möbius et al. 2003).

The *cis*-to-*trans* changes in cisterna fenestration and thickness mean that the size of the fenestrae and the wells initially become smaller towards the medial cisternae, while the thickness of the cisternae decreases from the *cis* to the *trans*-pole. Then, still in a *trans*-wise direction, the cisternae become more perforated again (Ladinsky et al. 1999).

The enzymes involved in the early stages of glycosylation are located mostly at the *cis*-side, whereas the late and terminal glycosylation enzymes are situated at the *trans*-side of the stacks. The *trans*-compartments are enriched in terminal processing enzymes involved in sialylation. However, a significant difference in the distribution of the Golgi enzymes can be detected only between the *cis*-most and *trans*-most cisternae of the central



Golgi domain, and not between adjacent cisternae of the same stack (Rabouille et al. 1995). Thus, a single cisterna does not necessarily represent a separated Golgi compartment from either the biochemical or the structural point of view, potentially representing instead a part of a larger compartment.

Cis-Golgi network (CGN)

The *cis*-Golgi network is composed of several domains. One represents the highly perforated disk similar in shape with Golgi cisternae. It is attached to the Golgi stacks and is named as the attached CGN or the *Cis*-perforated cisternae of the intermediate compartment or CGN (CISCIC). CISCIC appears as a disk with 30 nm perforations. This is especially evident in epithelial cells of epididymis, goblet cells in the jejunum, gonadotrophs in pituitary glands and dorsal root ganglion cells (Koga and Ushiki 2006). It is highly labelled for COPI (Oprins et al. 1993; Kweon et al. 2004). The second part of the CGN is a small tubular part of the total CGN that appears as the three-dimensional tubular network or a cage near the Golgi stacks. Let's name it as the free CGN. This part produces tubules moving towards the ER-export sites (ERES; Marra et al. 2001; Mironov et al. 2003). Another part of the CGN is connected with the attached CGN by tubules and has similar shape with the CGN. It localizes out of the GA and appears as the late intermediate compartment (Marra et al. 2001) that could be rather a stable compartment (Ben-Tekaya et al. 2005).

In spermatides, the *cis*-most cisterna appears as a regular network of anastomotic membranous tubules, and the medial saccules usually have fewer but larger irregular fenestrations in them (Ho et al. 1999). There, the *cis*-elements of Golgi stacks were slightly reactive for G6Pase. Labelling can be seen in some *cis*-Golgi cisternae. The CGN contains also early processing enzymes such as alpha-mannosidases that trim high mannose N-linked

Figure 2. Tomographic 3D reconstruction of different compartments of the secretory pathway. **A**,**B** Comparison of the resting (**A**) and transporting ministacks (**B**) in fibroblast-like cells. Ministacks are formed after the complete depolymerization of MTs (3 h of treatment with nocodazole). The resting stack (the stack before the release of the block of intra-Golgi transport) contains more vesicles (white spheres) and less cisternae. In resting stack (**A**), the *cis*-most and the *trans*-most cisternae are almost absent whereas in the transporting stack (**B**) both the *trans*-cisterna (white arrow) and the *cis*-cisterna (red arrow) are visible. In the resting stack (**A**), the *cis*-most cisterna is replaced by the tubular network (white arrow). **C**–**E** Structure of moving (**C**,**D**) and stationary (**E**) ER-to-Golgi carriers. The ER is pictured in green. The uncoated domains of the EGCs are pictured into brownish. The domains coated with COPII-like coat are pictured into yellow whereas the buds coated with COPI-like coat are indicated by light-blue colour. Samples were prepared according to the correlative light-electron microscopy (Mironov et al. 2003) 20 min after the restoration of ER-to-Golgi transport of tsVSVG. **F**,**G** Structure of the Golgi exit site at the moment of the formation of Golgi-to-PM carriers filled with tsVSVG (12 min after the release of the ER exit block according to the small pulse-chaise protocol (Mironov et al. 2001). Connections (arrow) between the COPI-positive Golgi cisterna and the TGN (yellow network) are shown from different points of view. Red arrows show the Golgi stack. The ER is pictured in green. Models **A** and **B** were made by A. Trucco, Models **C**–**G** were made by G. V. Beznoussenko. Bar: 120 nm.

oligosaccharides added to the nascent chain in the ER (Thorne-Tjomsland et al. 1991).

The CGN receives newly synthesized or recycled polypeptides from the endoplasmic reticulum, which are then posttranslationally modified by glycosylation, sulphation, phosphorylation, palmitoylation, myristoylation or methylation (De Graffenried and Bertozzi 2004).

Cisternal shape

Cisternae are oriented along microtubules. Although the number of cisternae in the Golgi stacks varies from one cell type to another (three to eight cisternae, in the majority of cases), however within the same cell line, the number of cisternae could be constant representing a specific characteristic of cell type (Ladinsky et al. 1999) or varies depending on the functional state. At least, this number is reproduced in cells washed out after brefeldin A and nocodazole treatment. The number of not perforated COPI-positive cisternae is almost not changed after the arrival of cargo (Trucco et al. 2004; see Chapter 2.16).

All Golgi cisternae have roughly the same surface area, although they can differ in volume by as much as 50% (Ladinsky et al. 1999). The length of all of the cisternae within the stack is equal. When the GA becomes fragmented, this feature becomes particularly evident even when cargo is being transported through the GA. Even after arrival of cargo to the *cis*-side of the GA, the length of all Golgi cisternae rapidly became equal (Trucco et al. 2004).

Both the *cis*-most perforated cisterna and all *medial* cisternae contain COPI-coated buds, whereas the *trans*-most perforated cisterna(e) contains only clathrin-coated buds and usually have no COPI-coated buds (Ladinsky et al. 1999).

All Golgi cisternae are fenestrated; the existence of Golgi cisternae without these fenestrae has yet to be demonstrated, at least in mammalian cells. The large openings in cisternae can often form "wells" (Ladinsky et al. 1999). These fenestrae are necessary for movement of secretory granules (SGs; Rambourg and Clermont 1997).

The lumen of a Golgi cisterna is usually quite narrow (10–20 nm). There is a systematic decrease in luminal diameter in the *trans*-direction in quick-frozen NRK cells (Ladinsky et al. 1999). Mechanisms responsible for Golgi cisterna stacking and maintenance of the cisterna shape remain mostly unknown. Attempts to explain stacking by the presence of so-called Golgi matrix proteins or Golgins (see Chapter 2.12) were not successful (Seemann et al. 2000) because these proteins are not present between all Golgi cisternae.

Low affinity antiparallel dimerization of cytosolic domains of sugar transporters might be responsible for attachment of Golgi cisternae to each other. At least overexpression of GDP-mannose transporter in the yeast *Saccharomyces cerevisiae* induces formation of the stacked GA (Hashimoto et al. 2002). Stacks are also formed in *S. cerevisiae* after deletion of function of Sec7

(Rambourg et al. 1993). In any case, additional analysis is necessary to resolve this problem.

Golgi vesicles

There are several known coats along the secretory pathway participating in the formation of coat-dependent vesicles. The most important of them are the following: COPII (see above), COPI, clathrin. Each of them contains several variants of the coat. COPII-based coat has two main forms (see Chapter 2.3), COPI-based coat also has two forms (see Chapter 2.4), and clathrin coat has two or four variants interacting with different adaptors, namely, AP1, AP2, AP3 and AP4 (reviewed by Traub 2005). Membrane budding with the help of these coats could induce generation of corresponding coat-dependent vesicles.

So far, only four types of coat-dependent small vesicles have been found in cells, namely, clathrin-coated vesicles that could be formed from clathrin/AP1-coated buds present on the TGN attached to COPI-positive stacked Golgi cisternae, secretory granules, endosomes and clathrin/AP2-coated buds found on the PM. COPII-dependent vesicles are formed near the ER exit sites. COPI-dependent vesicles are formed at the level of the ER-to-Golgi carriers, the intermediate compartment, the *cis*-Golgi and medial Golgi (reviewed by McMahon and Mills 2004).

One of the most important features of the GA is the presence of small 52 nm COPI-dependent vesicles surrounding each Golgi cisterna (Ladinsky et al. 1999; Marsh et al. 2001). COPI vesicles do not appear to be really "free" because most of them are unmistakably tethered to neighbouring vesicles and/or to the Golgi membranes (Orci et al. 1998). This explains why vesicles do not diffuse towards the cell periphery. In contrast, most of the clathrin-dependent or irregularly shaped "vesicles" are clustered away from the GA (Ladinsky et al. 1999).

A dense COPI coat observed on Golgi buds/vesicles on thin sections appears as a lace-like cytoplasmic structure closely attached to the lipid bilayer and composed of a series obtuse spikes separated by an average of 20 nm center-to-center. These spikes do not have the "bristle" or "spiny" appearance of clathrin subunits in basketworks (Orci et al. 1986). The thickness of COPI coat is about 10 nm whereas the thickness of clathrin coat is 18 nm (Oprins et al. 1993).

The number of vesicles is the result of the equilibrium between two processes – the activity of COPI machinery and the activity of SNARE/Ca machinery. Inhibition of COPI activity causes reduction of the number of COPI-dependent vesicles. Similar effects are observed, when the COPI/ARF machinery is inhibited with brefeldin A In contrast, when the SNARE machinery is inhibited, the number of vesicles increases (Kweon et al. 2004).

Most of data suggest that these vesicles are formed from COPI-coated buds abundant along the cisternal rims. COPI-dependent vesicles could derive

from varicosities within tangential tubules along the rims of Golgi cisternae (Weidman et al. 1993).

There could be following functions for COPI vesicles.

1. Formation of COPI vesicles could be specific mechanisms controlling the geometry of the Golgi elements (see Chapter 2.16).
2. COPI vesicles could control the fusion between adjacent Golgi cisternae extracting Qb SNAREs from there. COPI-dependent vesicles are two-fold enriched in Qb-SNAREs of the same type: membrin and GOS28 (Trucco et al. 2004; our unpublished observations) and depleted of syntaxin 5 (Orci et al. 2000a,b). Extracting GOS28 and membrin from the Golgi membrane, COPI vesicles prevent fusion between the cisternae (Trucco et al. 2004).
3. Formation of coat-dependent vesicles could be the way for fast uncoating.

The Role of COPI vesicles as anterograde or retrograde carriers will be analyzed in Chapter 3.2.

Intercisternal connections

One of the important questions of Golgi morphology is the issue of intercisternal heterogeneous connections (Tanaka et al. 1986; Rambourg and Clermont 1990, 1997; Sesso et al. 1994). In some cells, the Golgi forms even a single continuous membranous system (Tanaka et al. 1986; Inoue 1992; Rambourg and Clermont 1990, 1997). At steady state and in unstimulated cells, the connections are rare (Marsh et al. 2004). Intercisternal connections are augmented after arrival of cargo to the GA (Trucco et al. 2004) or when the islet beta cells have been stimulated for 1 h with 11 mM glucose (Marsh et al. 2004). Recently, the existence of such connections has been confirmed at steady state (Beznoussenko et al. 2006; Vivero-Salmeron et al. 2008).

The connections between cisternae at different levels of the GA are of three types. The first type is observed at points, where the Golgi ribbon branches (Rambourg and Clermont 1997). Cisternae could be connected at both equivalent and non-equivalent levels. This "Y" configuration of cisternae at the branch in the Golgi ribbon also means that there is direct continuity between cisternae at different levels around the periphery of the upper stack of Golgi membranes. The second type of connection occurs when one cisterna projects through an opening (fenestration) in an adjacent cisterna to form a continuous lumen with its next-nearest neighbour. In the third type of connection, membrane tubules connecting non-equivalent cisternae bypass interceding cisternae at the periphery of the stack in Golgi regions where the ribbon is unbranched (Marsh et al. 2004; Trucco et al. 2004). Intercisternal connections were detected rarely because on conventional EM sections luminal continuity between consecutive cisternae is almost undetectable (Marsh 2005).

The tubules connecting different stacks have been proposed to participate in intra-Golgi traffic (Mellman and Simons 1992; Weidman 1995). These connections could be used for the transport of soluble cargo, like albumin, or serve for the movement of proton from the *trans*- to the *cis*-compartments, or for Golgi enzyme diffusion (Trucco et al. 2004). The speed diffusion of ions, lipids and even transmembrane proteins along Golgi lumen and membranes is high. For instance, the speed of cholesterol diffusion in clear lipid bilayer is about 2 μm/s. The speed of ceramide diffusion is about 0.4–0.5 μm/s (Cooper et al. 1990). If connections are constant, the ionic, lipid and protein gradients that are known to exist between the Golgi poles (reviewed in Mironov et al. 1998, 2005) have to be expected to dissipate through these continuities. However, since this is not the case the connections have to be transient and highly regulated as within the framework of kiss-and-run models of transport (see Chapter 3.2).

Function of the Golgi apparatus

In mammalian cells, occupying a central position, the GA plays a central role in the classical secretory pathway, as well as in endocytic pathways, and in multiple recycling routes.

1. The GA exchanges membrane components with several other subcellular organelles, including endosomes, caveosomes, autophagosomes, and lipid droplets participating in sorting (Mironov et al. 2005).
2. The GA is the main station of cellular glycosylation. During movement along the Golgi membranes, cargoes undergo glycosylation. The Golgi stack is composed of a series of compartments containing oligosaccharide processing and other enzymes that are generally arranged in a *cis*-to-*trans* orientation (Rabouille et al. 1995).
3. Assembly of triglycerides with apoB and other apolipoproteins occurs in the GA. During this process, apoB undergoes conformational changes, and the expanding lipoproteins recruit more apoE (Gusarova et al. 2007).
4. The GA is involved in various other cellular processes such as transcription, apoptosis, and mitosis via signalling pathways mediated by Ras proteins, protein kinases, and G proteins (Helms et al. 1998; DeBose-Boyd et al. 1999; Lane et al. 2002; Sutterlin et al. 2002; Bivona et al. 2003; Nardini et al. 2003; Preisinger et al. 2004).
5. The GA provides a connection between exocytosis and endocytosis.

Structure of the Golgi exit site

After its passage through the GA cargo exits from it, and moves to the sites of its destinations. In the literature, this Golgi exit site (GES) is usually called as the *trans*-Golgi network (TGN, Griffiths and Simons 1986; Ladinsky et al. 1994; Clermont et al. 1995) suggesting that the TGN is composed of distinct tubules

with little indication of anastomosis. However, this name is misleading because the term TGN reflects mostly the structural organization of only one compartment, the whole TGN, within GES (Fig. 2f, g).

The identity of the GES could be defined by membrane components delivered to the TGN from both anterograde and retrograde transport, together with the recruitment from the cytosol of coat proteins, regulatory GTPases, fusion and Golgi matrix and motor proteins. Among the most important proteins localized within the TGN, one could observe clathrin, M6PR, TGN38, AP1, furin, GGA, EEA1, clathrin, Golgin-97, and other *trans*-Golgins (reviewed in Robinson and Bonifacino 2001). Two to four Golgi cisternae are stained with ceramide (Pagano et al. 1989) and TPPase (Novikoff et al. 1971).

The GESs are composed of the two last (*trans*) COPI-positive cisternae of the Golgi stack (Ladinsky et al. 2002), the *trans*-most perforated cisterna(e) with clathrin-coated buds, and the network of tubules surrounding the stack near its *trans*-pole and sometimes being continuous with either multiple *trans*-cisternae (Rambourg et al. 1979) or with only the *trans*-most cisterna in the stack (Griffiths and Simons 1985; Griffiths et al. 1989).

"Peeling off" configurations of the last Golgi cisterna with clathrin-coated buds are frequently described in the past (Ladinsky et al. 1994; Clermont et al. 1995). This *trans*-most cisterna contains exclusively clathrin-coated buds, whereas the other cisternae have COPI-coated buds only (Ladinsky et al. 1999, 2002).

Only 12% of the total TGN surface area is attributable to the flattened cisternal part of the TGN which is labelled by the presence of TPPase and which is morphologically indistinguishable from the other cisternae of the Golgi stack. In many cases most of the tubules located within the TGN area are devoid of the reaction for TPPase (Griffiths et al. 1989).

The *trans*-most cisterna containing clathrin-coated buds actually represents a highly perforated disk containing clathrin-coated buds along its rims (Ladinsky et al. 1999). This perforated cisterna is accessible for WGA-HRP added from outside (Pavelka et al. 1998). Thus, it represents a part of the endocytic TGN connected with endosomes. As such, this *trans* cisterna of the endocytic *trans*-Golgi network could be named as the TRANSCET or the attached TGN. In epithelial cells of the epididymis, goblet cells in the jejunum, gonadotrophs in pituitary glands and dorsal root ganglion cells, the TRANSCET appears as perforated sheet, varicose tubules or small plates connected with each other two-dimensionally by tubules (Koga and Ushiki 2006).

Structures within GES are highly dynamic and continuously undergo renewal (Clermont et al. 1995). Tubules continuously emanate from the Golgi cisternae going towards the cell periphery (Cooper et al. 1990).

The structure of the GES depends on the cell type. In cells, where secretory granules (SGs) are not seen being associated with the GA, the non-attached TGN appears as a tubular network connected with last two Golgi cisternae. However, the TGN does not form the continuous ribbon along the Golgi