Histol Histopathol (1997) 12: 735-738
DOI: 10.14670/HH-12.735
http://www.hh.um.es

Histology and Histopathology
*From Cell Biology to Tissue Engineering*

# On the mechanism of the transport through Golgi apparatus

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**Summary.** The application of an ultrastructural cytochemical method for $K^+$-dependent paranitrophenylphosphatase ($Na^+$,$K^+$-ATPase) has revealed patterns of plasticity of the Golgi apparatus of neurons in the cerebral cortex of 15-day-old rats. The peripheral part of the *cis*-most cisterna, being usually reactive, deviates from its regular arrangement in the Golgi stack and contacts with adjacent profile of the granular endoplasmic reticulum or with the next cisterna. The findings prompt the hypothesis that active movements of the *cis*-cisternae may facilitate and accelerate the transport of nascent proteins to and through the Golgi apparatus. The question about the nature of the contacts between the pointed membrane-bound compartments remains open.

**Key words:** Golgi apparatus, $Na^+$, $K^+$-ATPase, Transport, Plasticity

## Introduction

A previous hypothesis, which has explained the transport of material through the Golgi apparatus by movement of the cisternae themselves from the *cis*-Golgi to the *trans*-Golgi as intact units (the so called cisternal progression), is considered to be not plausible (Rothman et al., 1984; Rothman, 1985). Today it is generally accepted that the Golgi transport is mediated by vesicles, the coat of which apparently provides the mechanism on what each vesicle chooses its target for fusion (Rothman et al., 1984; Rothman, 1985, 1994; Rothman and Orci, 1992; Pelham, 1994; Alberts et al., 1995; Lippincott-Schwartz et al., 1995; Lodish et al., 1995).

During our investigations on the $K^+$-dependent paranitrophenylphosphatase ($K^+$-pNPPase) distribution in the developing and mature nervous system (Dolapchieva, 1995, 1996) (as well as unpublished data), our attention was drawn by the peculiar positions of some *cis*-most cisternae in the stacks of Golgi complexes in the neurons of 15-day-old rat cerebral cortex. We found that parts of these cisternae deviate and establish close interrelationships with the neighbouring cisternae of the Golgi stack or with adjacent profiles of the granular endoplasmic reticulum (ER) of the neuron. The view of these interrelationships opens a question about a possible mechanism for a direct conveyance of the enzyme between the above mentioned membrane-bound compartments.

The integral membrane enzyme $Na^+$,$K^+$-ATPase is composed of two polypeptides, the catalytic α-subunit, which carries its functional properties, and the glycoprotein ß-subunit (Jogersen, 1982). The $Na^+$,$K^+$-ATPase passes to and through Golgi apparatus as a complex, because the two subunits (α and ß) of the enzyme assemble soon after their translation into the rough ER (Renaud et al., 1991). The enzyme takes part in the biogenesis of the neuronal plasma membrane, as well as in the fast axonal transport (Hammerschlag et al., 1982; Dolapchieva, 1996).

## Materials and methods

Fifteen-day-old Wistar rats were perfused with 2% paraformaldehyde and 0.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.2, 1 ml/g weight, for 20 min. Small pieces of the cerebral cortex were post immersed in the same fixative for 30 min at 4 ºC and stored in the same buffer, additionally containing 10% v/v dimethyl-sulfoxide (DMSO) and 0.25M sucrose, overnight. The material was sectioned on a Reichert-Jung cryostat (Germany) at a thickness of 40 μm. The incubation medium was prepared according to Mayahara et al. (1980). The paranitrophenylphosphate (pNPP) was obtained from Boehringer/Mannheim (Germany). In controls, sections were incubated in: a) medium without pNPP; b) in a full medium supplemented with 10mM ouabain. Following incubation, the sections were fixed in 1% $OsO_4$ for 1 h at 4 ºC, dehydrated and embedded in Durcupan ACM (Fluka, Switzerland). The ultrathin sections were made on an LKB ultratome (Sweden) and were observed unstained or slightly stained with lead citrate by a Hitachi H500 electron microscope.

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## Results

The right edge of the *cis*-most cisterna of the Golgi apparatus bent and contacted with a profile of the granular ER, disposed in proximity (Fig. 1). Both compartments were moderately reactive. The next cisterna of the *cis*-Golgi compartment was strongly stained.

The left edge of the reactive *cis*-most cisterna bent sharply and contacted with the next, also reactive cisterna of the Golgi stack (Fig. 2). The *trans*-most cisterna of the Golgi stack was strongly stained. Nearby, the perinuclear space as well as the profiles of the granular ER were also found reactive.

The left edge of the *cis*-most cisterna abruptly curved to the left edge of the next cisterna (Fig. 3). Several little membrane-bound vesicles were seen to be interposed between the rims of both cisternae. The *cis*-most cisterna was well reactive whereas its bent edge, the neighbouring cisterna and vesicles were almost not reactive.

The *cis*-most cisterna and the next cisterna were bound by a vesicle (Fig. 4). The contacting structures appeared to fuse. The *cis*-most cisterna was moderately reactive, whereas the neighbouring cisterna was strongly stained. Reaction product was observed into the interposed vesicle, too.

In the control sections, the lumen of the Golgi and

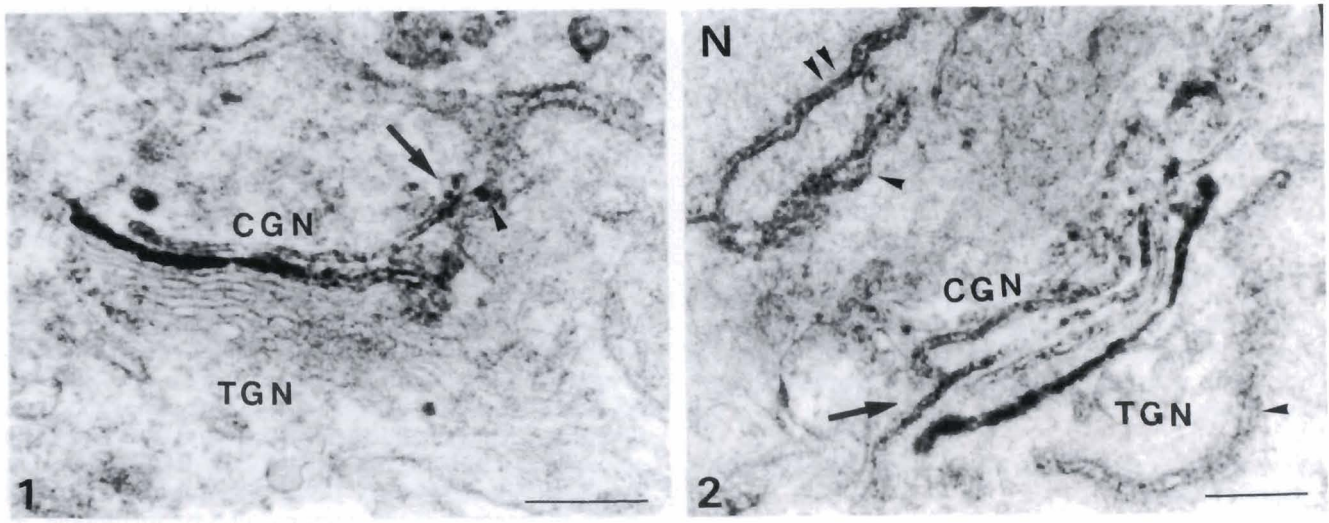


**Fig. 1.** The bent right edge of the *cis*-most cisterna contacts (arrow) with a profile of the ER (arrowhead). CGN: *cis*-Golgi network; TGN: *trans*-Golgi-network. x 40,000; Bar: 0.4 $\mu$m.

**Fig. 2.** The bent left edge of the *cis*-most cisterna contacts (arrow) with the next cisterna in the stack. The perinuclear space (double arrowhead) and profiles of ER (arrowheads) are also reactive. CGN: *cis*-Golgi-network; TGN: *trans*-Golgi-network; N: nucleus of the neuron. x 34,000; Bar: 0.4 $\mu$m.

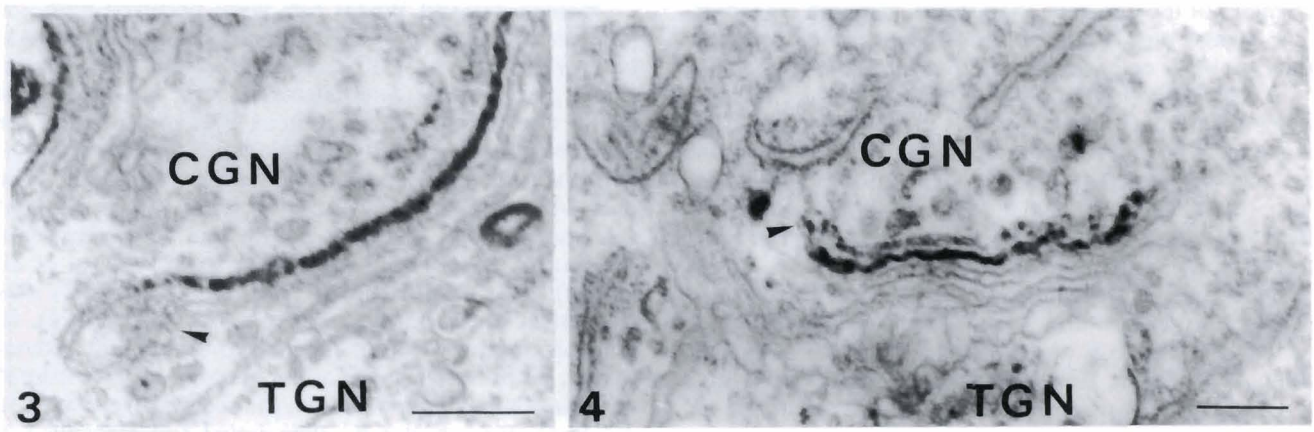


**Fig. 3.** Several transport vesicles (arrowhead) are seen between the bent edge of the *cis*-most cisterna and the edge of the next cisterna. CGN: *cis*-Golgi-network; TGN: trans-Golgi-network. x 40,000; Bar: 0.4 $\mu$m.

**Fig. 4.** The *cis*-most cisterna and the next cisterna are bound by a transport vesicle (arrowhead). CGN: *cis*-Golgi-network; TGN: *trans*-Golgi-network. x 30,000; Bar: 0.4 $\mu$m.

ER cisternae, as well as the perinuclear space were not stained.

## Discussion

It is generally accepted that the traffic of nascent proteins proceeds from the ER to and through Golgi apparatus by transporting vesicles at the cisternal rims (Rothman et al., 1984; Rothman, 1985, 1994; Pelham, 1994). The demonstrated deviations of the *cis*-most cisternae appear to reveal the plasticity of the neuronal Golgi apparatus. The ability of the CNS to make adaptive changes related to its structure and function is well known (Zilles, 1992). We admit that the movements of the *cis*-most cisterna may facilitate the transfer of the $Na^+,K^+$-ATPase from the granular ER to *cis*-Golgi as well as through the Golgi apparatus. Such supplementary mechanism might help the acceleration of the intracellular protein transport in periods of intensive production of membrane proteins. In our case, the necessity for $Na^+,K^+$-ATPase in the developing cerebral neurons of 15-day-old rats increases more along with the initiation (10th-12th postnatal days) and progression of the myelinization, as well as along with the enzyme equipment of the new-built up nodes of Ranvier (Dolapchieva, 1996).

The peripheral parts of the reactive «plastic» *cis*-most cisternae are probably able to move in two directions: to the «donor» of the enzyme (ER) and to the «acceptor» of the enzyme (the next cisterna in the stack). Such interpretation of the findings correlates with the concept of the forward (ER-to-Golgi) transport of $Na^+,K^+$-ATPase. The movement to the elements of the ER could be interpreted, however, in one another aspect. The *cis*-most cisterna might convey material from Golgi apparatus to the ER in the sense of the concept of the reverse (Golgi-to-ER) pathway (Lippincott-Schwartz et al., 1989, 1995), such as the retrograde transport to ER for recycling of specific proteins, necessary for the fusion (Pavelka and Ellinger, 1993; Pelham, 1994).

Our findings do not furnish assays about the possible mechanism of conveyance of the material in the area of contact. Rothman (1994) emphasizes that the «donor» and «acceptor» compartments are prevented from fusing directly by their membrane coats and that the fusion always follows budding. In this sense, the most probable way of conveyance of the protein between the adjoining compartments has to be the classical vesicle transport pathway. The finding in figure 3 might be interpreted as an assay for the benefit of such reason. The demonstrated deviation of the *cis*-most cisterna obviously helps the vesicular intra-Golgi transfer.

A mode of transport, quite similar to the classical one, could be seen in figure 4. A large vesicle serves the transfer between the two cisternae, which preserve their position in the stack. Besides, the transporting vesicle simultaneously presents a dual state of budding from the rim of the one cisterna and fusion to the rim of the other cisterna, escaping the classical full dissociation from the Golgi stack.

Further ultrastructural cytochemical and specialised immunocytochemical studies are necessary to clarify the nature of the phenomena arising between the contacting ER and Golgi compartments.

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Accepted January 28, 1997