front-fanged coral snakes have exceptionally short tails and elongate bodies.

75. H. M. Smith and D. A. Langebartel, *J. Wash. Acad. Sci.* **39**, 409 (1950).
76. F. R. Gehlbach (*23*, p. 319) concluded that "color resemblance alone does not confer special protection on colubrid snakes, hence may simply represent concealing (disruptive) coloration." However, javelinas (*Tayassu*) and coatis (*Nasua*) responded significantly more often to his coral snake model with "fright" and to the normal snake model with "predation" [Fisher Exact Test, $P < .05$, for data in table 1 of (*23*)].
77. Gehlbach (*23*) also found similar frequencies of a banded morph in populations of a colubrid snake, *Sonora episcopa*, where they occur allopatrically or sympatrically to *Micrurus fulvius*. He concluded (*23*, p. 318) that "no mimetic advantage is apparent." We agree, but suggest that no such advantage is to be expected because even the banded morph of *S. episcopa* bears no special resemblance to a local coral snake; the banded *S. episcopa* morph is bicolored, brown or reddish brown with narrow dark crossbars—see color illustrations of it and *M. fulvius* in R. Conant, *A Field Guide to Reptiles and Amphibians of Eastern and Central North America* (Houghton Mifflin, Boston, 1975).
78. In the Gómez Farías region of Tamaulipas, Mexico, *Pliocercus* occurs only in cloud forest, and *M. fulvius* is found in tropical deciduous and semievergreen forests [P. S. Martin, *Misc. Publ. Mus. Zool. Univ. Mich.* **101**, 1 (1958)]. Note that mimicry requires only that model and mimic exist within the foraging range of relevant predators, not actual syntopy (*5*).
79. P. M. Sheppard and J. R. G. Turner, *Evolution* **31**, 452 (1977).
80. R. I. Vane-Wright, *Biol. J. Linn. Soc.* **8**, 25 (1976).
81. Grobman (*27*) concluded that little or no predation occurs on coral snakes because no records exist in the U.S. Fish and Wildlife Service files, and, therefore, that the banded color patterns of coral snakes are not adaptive. We suspect that few records of predation exist for most snakes, and that this does not necessarily reflect actual predation rates. Grobman also cited brightly patterned bellies in other burrowing snakes as evidence that this feature is not adaptive, but did not mention that some such species (for example, *Farancia abacura*, *Diadophis punctatus*) have defensive displays that suddenly expose the bright colors [H. W. Greene, *J. Herpetol.* **7**, 143 (1973)]. Grobman [table 1 in (*27*)] examined the distributions of 28 taxa said to resemble coral snakes, and concluded (p. 7) that "the frequency of races of nonvenomous snakes 'mimicking' the coral snake is independent of the presence of a model." However, we can reach exactly the opposite conclusion on the basis of figures, maps, and color pattern descriptions in Conant (*77*) and R. C. Stebbins [*A Field Guide to Western Reptiles and Amphibians* (Houghton Mifflin, Boston, 1966)]. *Chionactis palarostris* and eastern populations of *C. occipitalis annulatus* closely resemble the sympatric *Micruroides euryxanthus*. *Chilomeniscus cinctus*, three subspecies of *Chionactis occipitalis*, *Lampropeltis triangulum multistrata*, and *L. t. syspila* are largely or entirely allopatric to and do not particularly resemble front-fanged coral snakes. We must point out that *Ficimia quandrangularis* is sympatric with *Micruroides*, but in the northern part of its range it does not especially resemble a coral snake. However, the intensity of red in the color pattern increases to the south where it is sympatric with *Micrurus distans* and *Micruroides euryxanthus* (R. W. McDiarmid, unpublished). We agree that four subspecies of *L. triangulum*, one subspecies of *L. pyromelana*, five subspecies of *L. zonata*, and two subspecies of *Rhinocheilus lecontei* are largely or completely allopatric to venomous coral snake models and that they do bear some resemblance to the latter. However, none of them resembles *Micrurus* as much as do the subspecies of *L. triangulum* (*amaura*, *annulata*, *elapsoides*) that are sympatric with *M. fulvius* (*54*). *Rhinocheilus lecontei antoni* has much brighter and more contrasting red, yellow, and black markings in northwestern Mexico where it occurs sympatrically with *M. distans* and *Micruroides euryxanthus neglectus* (R. W. McDiarmid, unpublished).
82. Color patterns in the figures are based on preserved museum specimens. Museum numbers and specific localities are on file with the authors.
83. We thank the many herpetologists who permitted us to study specimens, loaned color slides, or assisted in other ways. Versions of the manuscript were criticized by W. Altimari, G. M. Burghardt, J. E. Cadle, J. A. Campbell, A. C. Echternacht, M. S. Foster, J. D. Groves, F. H. Pough, W. F. Pyburn, J. A. Roze, A. H. Savitzky, R. L. Seib, W. W. Tolbert, and D. B. Wake. F. J. Irish prepared the excellent illustrations. Greene was supported by the University of Texas at Arlington, Foundation for Environmental Education, Field Museum of Natural History (Karl P. Schmidt Fund), Center for Latin American Studies (UCB), Smithsonian Tropical Research Institute (Noble Fund), and Museum of Vertebrate Zoology (Alexander Fund); McDiarmid was supported by the University of South Florida, Organization for Tropical Studies, U.S. Fish and Wildlife Service, and National Science Foundation (grant to O. B. Berlin, UCB).

The Golgi Apparatus: Two Organelles in Tandem

James E. Rothman

Summary. The Golgi apparatus consists of distinct *cis* and *trans* compartments that may act sequentially to refine the protein export of the endoplasmic reticulum by removing escaped endoplasmic reticulum proteins. Refinement may be a multistage process akin to fractional distillation; the stack of cisternae comprising the *cis* Golgi may be the plates in this distillation tower. The *trans* Golgi, consisting of the last one or two cisternae, may be the receiver that collects from the *cis* Golgi only its most refined fraction for later distribution to specific locations throughout the cell.

Present in all eukaryotic cells, the Golgi apparatus is generally agreed to be of fundamental importance in the processing and sorting of newly synthesized proteins. However, the underlying principles that must somehow relate the Golgi's striking stacklike structure to its exact functions have been elusive. The possibility that the Golgi may carry out a previously unsuspected form of sorting, the sorting of endoplasmic reticulum proteins in multiple stages, is explored in this article, and may provide the needed connection between structure and function.

Many different proteins that must ultimately reside in such diverse cellular compartments as the surface membrane, secretion granules, and lysosomes are synthesized or initially found in the same compartment, the endoplasmic reticulum (ER). Herein lies a sorting problem of considerable proportions (*1*). The newly synthesized membrane proteins destined for export from the ER are present in only trace amounts at any given moment. Yet, within minutes of synthesis, these proteins are removed from the ER and delivered to their separate destinations, virtually free of any contamination by the vast excess of characteristic ER membrane proteins that are left behind. The question then arises as to how this vital task of purification can be accomplished.

The Golgi apparatus is strategically located in the midst of this sorting process, interposed between the ER and the final destinations. To help clarify the relation between Golgi structure and function, we must understand (i) why the mixture of proteins exported from ER needs to pass through the Golgi before sorting can be completed, and (ii) why all of the necessary purification cannot be completed at the level of the ER, without the involvement of a Golgi apparatus.

Clues to these central problems have now emerged from several independent lines of investigation. After a brief review of the biochemistry and morphology of the Golgi apparatus, I point out how these recent developments suggest a novel but speculative view of this organelle. Specifically, I propose that the

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Golgi actually consists of two attached but different organelles (the *cis* and the *trans* Golgi) having related but distinct purposes, and operating sequentially to purify exported proteins from those of the ER on the basis of an underlying principle akin to that of fractional distillation. The Golgi stack would be needed to permit purification in sequential stages, mainly in its *cis* portion. The closely applied *trans* Golgi, consisting in many instances of only the last one or two cisternae of the stack, would be needed to collect the purest fraction of this export for further sorting and delivery to multiple cellular locations.

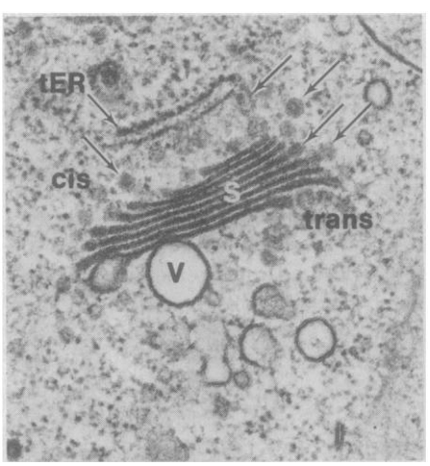
Review of Golgi Structure and Function

The structure of the Golgi apparatus appears to be simple in its essentials and closely conserved in all eukaryotes (*2–9*): a stack of about a half-dozen or more flattened, membrane-bound cisternae (about 1 micrometer in diameter) from which many small vesicles (in the range of 5 to 10 nanometers in diameter) bud or fuse (or both), especially at the rims (Figs. 1 and 2). These platelike cisternae appear to be discrete because several membrane properties (*2–9*), including the density of intramembrane particles (*10*), change from one to the next (Fig. 2).

The stack is an asymmetric structure whose two ends differ (*2–9*). The *cis* face is frequently nearer the nucleus and adjacent to a specialized portion of rough ER that lacks bound ribosomes and is called "transitional ER" (Fig. 1). The *trans* face is at the opposite end of the stack. Newly formed secretion granules are associated with the *trans* but not the *cis* face; certain enzymes (such as thiamine pyrophosphatase) and concentrated secretions can be demonstrated only within cisternae at the *trans* end (Fig. 3). Osmium deposits selectively into cisternae at the *cis* end of the stack.

Proteins destined for multiple intracellular compartments (*2–9*), including the plasma membrane, secretion granules, and probably lysosomes (*11, 12*), appear to pass through the Golgi after an initial association with the ER. Covalent modifications of these transported proteins (Table 1), including glycosylation (*13*), selective proteolysis (*14*), sulfation (*15*), phosphorylation (*16*), and the addition of fatty acids (*17*), can be the biochemical hallmarks of this passage. Of these, the most thoroughly studied is glycosylation. Asparagine-linked oligosaccharides of glycoproteins found in all three of the aforementioned compartments are derived by processing of a common precursor oligosaccharide, acquired by the polypeptides in the ER (*13*). The degree and nature of this processing depends on the attached protein and its destination, but almost every step takes place in the Golgi, as shown by electron microscopic autoradiography (*4*) and by subcellular fractionation to locate the site of sugar incorporation and the enzymes responsible (*3, 8, 12, 13*). Immunocytochemical electron microscopy reveals that examples of proteins destined for both secretion granules (*18, 19*) and for the plasma membrane (*19, 20*) are found in every cisterna of the stack. Comparable experiments with lysosomal enzymes have not yet been reported.

Various observations suggest that proteins exported from the ER enter the stack at its *cis* face and exit from its *trans* face (*2–9*). The *cis* face is frequently proximal to the "transitional" region of ER (Fig. 1). A viral-encoded plasma membrane protein enters the stack at one end, generally that facing the nucleus (*20*). This probably corresponds to the *cis* face. Newly formed secretory granules and concentrated secretions are at the *trans* face (Fig. 3C). Extracellular tracers (believed to follow the pathway by which membrane transport vesicles are returned to the Golgi after the delivery of their cargo to the plasma membrane) appear almost exclusively in the *trans*-most cisterna or two of the Golgi stack (Fig. 3B), marking these as the points of exit (*21*). Rat liver Golgi can be fractionated on sucrose density gradients to yield two principal fractions (termed GF_3 and GF_{1+2}) that are respectively enriched in *cis* and *trans* portions of the



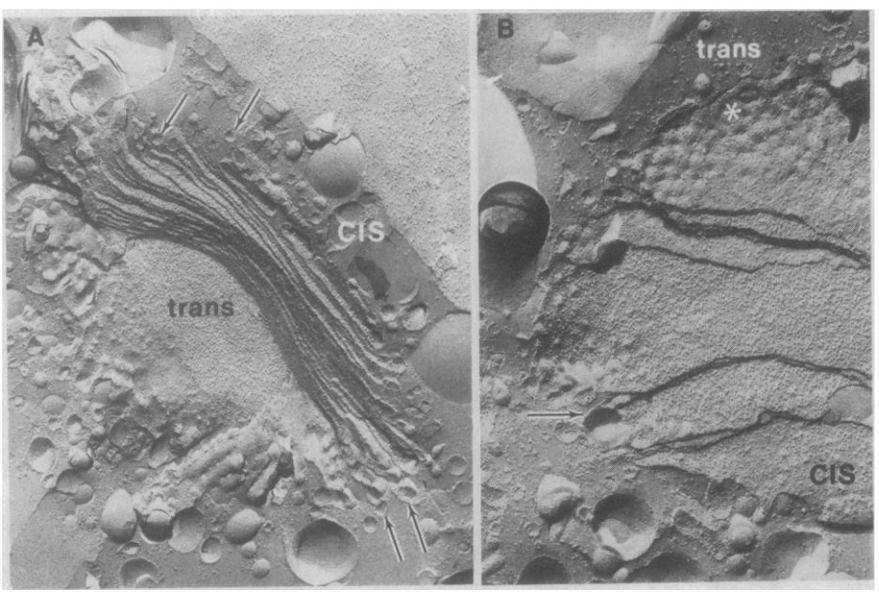


Fig. 1 (left). Electron micrograph of Golgi complex of *Chlamydomonas reinhardii*, showing the stack of cisternae (*S*) with small vesicles (indicated by arrows) budding or fusing with the edges of each. The *cis* face is proximal to the smooth-surfaced face of an element of transitional ER (*tER*). Ribosomes are present on the side of this element distal from the Golgi. The *trans* face is associated with a variety of larger vesicles (*V*) (×30,000). [Courtesy of Andrew Staehlin, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder] Fig. 2 (right). Freeze-fracture electron micrographs of a Golgi complex of *Micrasterias denticulata*, revealing the interiors of the cisternal membranes (*10*). The fracture plane proceeded either along the long axis of the stack (A) or in the planes of the cisternae (B). Of the cisternae thus revealed, the *cis*-most and *trans*-most are labeled. In (A), vesicular profiles (arrows) are in continuity with the rims of the cisternae (×10,000). In (B), a gradual increase in the density of intramembrane particles (presumed to be membrane proteins) appears in successive cisternae in the *cis* → *trans* direction (×20,000). Numerous invaginations are visible at the rims of the *trans*-most cisterna (*). Each indentation represents a vesicle that is budding (or fusing) from the rim of this cisterna, as seen from inside the membrane looking out. [Courtesy of the *Journal of Cell Science*]

stack, as judged by electron microscopy with the enclosed lipoproteins and thiamine pyrophosphatase histochemistry as markers for cisterna and vesicles from the *trans* side (*22*). Secretory proteins move from the *cis*-rich to the *trans*-rich subfractions (*23*). The ionophore monensin appears to interrupt this movement in some manner (*24*).

Within the stack, protein appears to reach the *trans* end from the *cis* end primarily via movement between adjacent cisternae. Thus, the density of freeze-fracture particles (*10*), the average membrane thickness (*25*), the content of complex carbohydrate (*4*), and probably the concentration of cholesterol (*26*) change progressively from one cisterna to the next. However, the detailed pattern of protein flow cannot yet be discerned (*3*). At one extreme, proteins could be transported between neighboring but static cisternae by vesicles budding from their rims or flat faces, or even by diffusion through membrane continuities. At the other extreme is the concept that the cisternae themselves move as intact units from the *cis* to the *trans* face. But, it would appear to be difficult to reconcile such a cisternal progression with the existence of distinct compartments. The properties of the Golgi apparatus reviewed above can be understood on the basis of the biochemical studies described below and the hypothesis that follows.

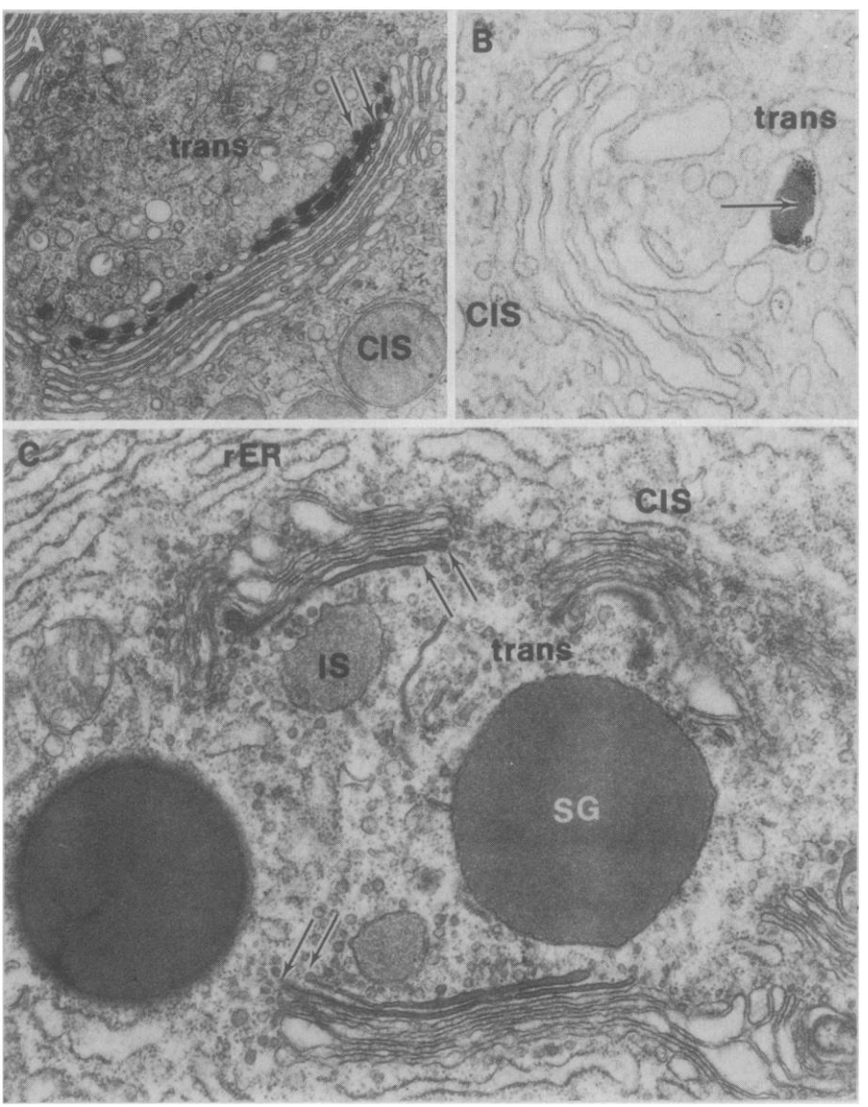


Fig. 3. (A) Selective staining of the last two cisternae (arrows) on the *trans* side of the Golgi apparatus of rat epididymal epithelial cells when the enzyme thiamine pyrophosphatase is localized (*39, 54*) (×25,000). [Courtesy of *Journal of Cell Biology*] (B) Golgi complex of a prolactin-secreting cell (mammotroph) of the pituitary gland after the endocytosis of cationized ferritin (*21*). Ferritin is seen in the *trans*-most cisterna, in this case attached to a granule of concentrated secretory product (×70,000). [Courtesy of *Journal of Cell Biology*] (C) Golgi complex from gastrodermal cell of a coelenterate medusa (*2*). The last two cisternae of each Golgi stack (arrows) are filled with product not evident in earlier cisternae. Large secretion granules (*SG*) and immature secretion granules (*IS*) are present on the *trans* side of every stack, with rough ER (*rER*) on the *cis* side (×23,600). [Courtesy of *International Review of Cytology*]

Evidence for Distinct *cis* and *trans* Compartments

The marked polarity of the Golgi stack could signify a fundamental division of the stack into functionally distinct compartments, or could instead be a reflection of gradients of composition within a single, multicisternal compartment. These possibilities cannot readily be distinguished by morphological studies that are essentially static in nature. Rather, a functional test that would be expected to be sensitive to the movement of exported proteins is needed. The reconstitution in cell-free systems (*27*) of what appears to be a segment of the pathway of intracellular transport involving the Golgi has provided the starting point for such an assay, and the results imply that *cis* and *trans* portions of Golgi are functionally distinct subcellular organelles (*28, 29*).

These reconstitution experiments have been performed with animal cells infected with the enveloped vesicular stomatitis virus (VSV), whose limited genetic capacity forces it to follow pathways provided by its host. In particular, the VSV-encoded membrane glycoprotein (G protein) follows a route to the plasma membrane (*20*) of infected animal cells, which is indistinguishable from that believed to be taken by most of the cell's own surface membrane proteins prior to infection (*30*). The G protein is synthesized by ribosomes bound to the ER membrane, where the precursor oligosaccharide (*13*) is added. Within 10 minutes, the G protein is transported into the Golgi stack (*20*), where its oligosaccharides are processed (*13*). During the next hour or so, G is gradually delivered to the plasma membrane, from which it enters progeny viral particles as they bud from the cell surface (*30*). Processing of the oligosaccharides in the Golgi proceeds in two major stages (*13*). First, certain sugars, especially mannose, are removed (*31*). Second, other sugars, such as galactose and sialic acid, are added in the terminal stage of glycosylation (*13, 32*). Enzymes catalyzing both stages of processing (*31, 32*) are typically concentrated 100-fold or more in Golgi fractions of liver that are nearly homogeneous as judged by electron microscopy (*33*), and thus can serve as enzymatic markers for portions of this organelle.

To attempt reconstitution (*27, 28*), a crude membrane fraction (containing ER and Golgi membranes) was prepared from the homogenate of VSV-infected cells of a mutant type (*34*) and incubated with a comparable fraction from uninfected, normal cells. The mutant cells can remove mannose, but cannot initiate

the second and terminal stage of oligosaccharide processing because their Golgi lack a key glycosyltransferase (*34*). Therefore, G protein provided by mutant cell membranes should be terminally glycosylated only after movement to Golgi membranes from the normal cells that contain the glycosyltransferase missing in the mutant. Terminal glycosylation dependent on the addition of membranes from the normal cells is in fact observed, provided that both adenosine triphosphate (ATP) and a soluble fraction are also supplied (*27*). Highly purified Golgi membranes from rat liver will substitute for the crude membrane fraction of normal cells, and the G protein donated by mutant cell membranes can be recovered in the exogenously supplied Golgi membranes after appropriate incubation (*27*). Fractionation and short-term labeling experiments (*28*) revealed that the G protein whose oligosaccharide processing could be completed in vitro was provided by the Golgi membranes of the mutant cell and not by those of the ER.

In particular, it was a pool of G protein whose oligosaccharides had just passed through the first stage of processing in vivo that could be transferred in vitro (*28*). Remarkably, the capacity of the Golgi membranes to provide G protein in vitro declined precipitously (the half time was about 5 minutes) as transport proceeded further in vivo (*28*). Therefore, the in vitro system provides a functional assay that distinguishes two pools of G protein present in Golgi membranes: a transferable pool that can reach the portion of another Golgi that houses terminal glycosyltransferases; and another, nontransferable pool that cannot do so. The G protein moves irreversibly from the transferable pool to the nontransferable pool in about 5 minutes. The simplest interpretation (*28*) would be that the two pools signify two distinct compartments through which G protein passes successively. In a cell, G protein would generally be transported from the first to the second compartment of the same Golgi apparatus. The transport observed in vitro might represent transfer from the first compartment of one Golgi to the second compartment of another. Because the appearance of G protein in the first compartment (housing the transferable pool) coincides with the removal of mannose from its oligosaccharides (*28*), and because transport in vitro to a normal Golgi results in terminal glycosylation (*27*), it would then be expected that the enzymes responsible for the first stage of oligosaccharide processing would be concentrated in the first Golgi compartment, and that the terminal glycosyltransferases (*32*) responsible for the second stage would be present in the second Golgi compartment. These respective enzymes would then serve as markers for the two compartments. In that the direction of protein transport in the Golgi is *cis* to *trans*, the first and second compartments will be termed the "cis" Golgi and the "trans" Golgi, respectively.

Table 1. Posttranslational modifications of proteins executed sequentially during transit through the major compartments of the endoplasmic reticulum–Golgi system.

Compartment	Covalent modification
Endoplasmic reticulum	Addition of common precursor oligosaccharide to asparagine (*13*) Removal of glucose from precursor oligosaccharide (*13*)
cis Golgi	Removal of outer mannose from precursor oligosaccharide (*29, 31*) (?) Addition of fatty acid to serine (*17, 29*) (?) Addition of *N*-acetylglucosaminyl-phosphoryl units to precursor oligosaccharides of proteins destined for lysosomes (*12, 13*)
trans Golgi	Addition of terminal sugars (such as *N*-acetylglucosamine, galactose, sialic acids, fucose) to oligosaccharides (*29, 32*) (?) Removal of terminal *N*-acetylglucosamine, unmasking the phosphoryl groups of the oligosaccharides of proteins destined for lysosomes (*12*) (?) Sulfation of glycoproteins (*15*) (?) Selective proteolysis, removing the propeptide from proteins destined for secretion (*14*)

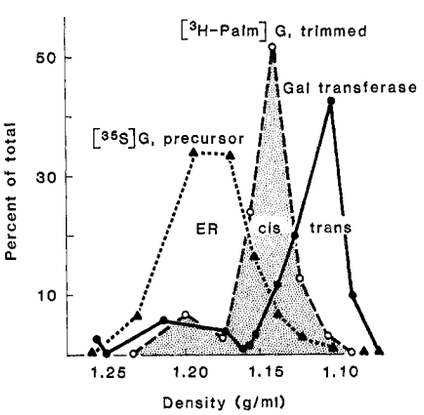


Fig. 4. Resolution of cis and trans Golgi units. Membranes from the postnuclear supernatant of VSV-infected Chinese hamster ovary clone 15B cells were fractionated by centrifugation to equilibrium in sucrose density gradients (*27, 28*). The distribution of radioactivity in G protein or of total enzyme activity (as appropriate) is plotted as a function of the density of the gradient fraction. (▲) The distribution of [^{35}S]methionine-labeled G protein when infected cells were incubated with isotope for 3 minutes at 37°C, determined from sodium dodecyl sulfate–polyacrylamide gels of fractions. All of this labeled G protein carried a high-mannose precursor oligosaccharide, as judged by electrophoretic mobility and sensitivity to endoglycosidase H. (○) The distribution of the ^{3}H incorporated into a "trimmed" form of G protein (from which some mannose units have been removed) when infected cells were incubated with ^{3}H-labeled palmitate for 5 minutes. This ^{3}H-labeled G protein moves faster on electrophoresis than the ^{35}S-labeled G protein. (●) The distribution of galactosyltransferase in infected cell membranes with ovalbumin as acceptor. [Data are redrawn from Dunphy *et al.* (*29*)]

Two portions of Golgi membranes with the properties expected of the putative cis and trans compartments can be resolved when crude membrane fractions are analyzed by centrifugation through sucrose density gradients (*29*). These fractionation experiments have depended on the discovery that fatty acyl groups are added covalently to G protein well after the addition of the precursor oligosaccharide in the ER but only a few minutes prior to the terminal stage of processing in the Golgi (*17*). This suggested that fatty acid might be added to G protein near or within the cis Golgi compartment. In fact, membranes containing freshly acylated G protein (whose oligosaccharides had already undergone the first stage of processing) were separated in the sucrose gradient from those containing enzymes (such as galactosyltransferase) that catalyze steps in the process of terminal glycosylation (Fig. 4). Assay of a mannosidase (*31*) capable of selectively removing mannose from precursor oligosaccharides revealed (*29*) that this activity was distributed in the gradient in the same manner as freshly acylated and processed G protein (Fig. 4). The distinct denser and lighter peaks of Golgi markers both differ from the distribution of ER membranes (Fig. 4). The sequence of oligosaccharide processing dictates that G is transported successively from ER fractions to the denser (cis) Golgi peak and then to the lighter (trans) Golgi peak. That the two peaks of Golgi makers can indeed be equated with the two functionally defined compartments follows because most of the freshly acylated G protein in the denser (cis) peak is transferable in vitro (*29*). Also, G passes into the nontransferable pool at the same rate as it encounters terminal glycosyltransferases (in the lighter, trans peak) after acylation in normal cells (*29*).

What portions of the Golgi apparatus

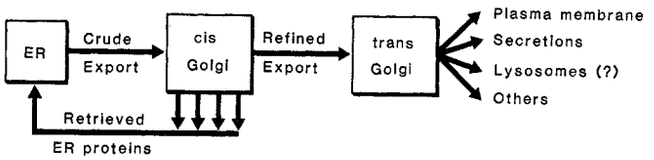


Fig. 5. The proposed division of labor in the Golgi apparatus. During passage through the cis Golgi, characteristic ER membrane proteins would be removed from the export. The now refined mixture of exported proteins would then be passed on to the trans Golgi unit for further sorting to yield separate fractions for delivery to multiple cellular compartments.

constitute the biochemically identified cis and trans compartments? Because G protein seems to enter the Golgi stack at its *cis* face (*20*), it is natural to attempt to equate the cis compartment with cisternae at the *cis* end of the stack and the trans compartment with cisternae at the *trans* end. The evidence that enzymatic markers of the trans Golgi compartment are concentrated within cisternae at the *trans* end of the stack helps to establish this link. Galactose and sialic acid are incorporated almost exclusively into an apparently *trans*-rich portion of rat liver Golgi (*35*). Galactosyltransferase activity is concentrated in cholesterol rich portions of Golgi (*36*); cholesterol is concentrated at the *trans* end of the stack (*26*). Thiamine pyrophosphatase [apparently the same enzyme as uridine diphosphatase (UDPase) (*8, 37*)] can also serve as a marker of the trans compartment because this enzyme fractionates with galactosyltransferase in subfractions of rat liver Golgi (*38*). [UDPase activity is needed at the site of terminal glycosylation to prevent inhibition of galactosyl transferase by its product, UDP (*8*)]. The histochemical stain to localize this phosphatase activity results in deposits in the last one or two cisternae at the *trans* end of the stack (Fig. 3A) in a wide variety of cells (*6, 39*).

If the implication of these correlations is correct, then (as the simplest possibility) the *cis* Golgi would consist of the entire remainder of the stack beginning from the *cis* end. Certainly, it is possible that the Golgi stack is composed of more than two distinct compartments, but there is as yet no reason to adopt this more complex point of view. Because these two portions of Golgi have distinct properties in a functional assay (*28*), contain different concentrations of certain enzymes (*29*), and can be physically resolved (*29*), it may be useful to consider them to be different organelles that happen to be attached.

The existence of distinct *cis* and *trans* compartments can explain a variety of observations. For example, the selective deposition of certain extracellular tracers (*21*) in the *trans*-most cisterna or two (Fig. 3B) would occur because the *trans* compartment would be engaged in transport to the cell surface; the *cis* compartment would not. Concentrated secretions (Fig. 3C) within these same *trans*-most cisternae (*2, 40*) could result from the vectorial transport of secreted protein across the boundary that separates *cis* and *trans* compartments. This capacity of the *trans* compartment to trap exported proteins as they arrive also offers a simple explanation of why net transport proceeds in the *cis* to *trans* direction. The presence of such a "sink" would create a flux of the trapped proteins across the stack into the *trans* compartment. The mechanisms that underlie vectorial transport in this and other instances (*7*) represent an important unsolved problem. It is possible that the covalent modifications that are carried out sequentially during transport (Table 1) are related to the mechanism that makes transport irreversible (*41*).

Sorting of Endoplasmic Reticulum Proteins in the Golgi

It is widely accepted that the sorting of proteins exported from the ER for separate delivery to multiple destinations is a major process carried out in the Golgi. Yet, the concentration in the *trans* Golgi of the terminal glycosyltransferases that act on proteins destined for plasma membranes, secretion granules, and lysosomes (*11*) would suggest that all three types of exported proteins must pass into the *trans* compartment before they are separated. In other words, the kind of sorting generally attributed to the Golgi would appear not to have taken place within earlier cisternae. If this implication is correct, then it would seem that another kind of sorting process within the *cis* compartment needs to be invoked in order to explain the progressive changes in cisternal composition [membrane differentiation (*5*)] evident throughout most of the Golgi stack.

An important clue to the nature of this process may have come from the recent findings (*33, 36, 38, 42, 43*) suggesting that enzymes concentrated in ER membranes are also found in large amounts in *cis* but not *trans* Golgi fractions. Therefore, it may be worth considering the possible need for a different type of sorting, the sorting of "ER membrane proteins" (*44*) in the Golgi. It seems to me that these findings raise the possibility that there is for some reason a high rate of export of ER membrane proteins from ER, and that cells need a Golgi mainly to refine this export by removing those ER membrane proteins that had escaped (*45*).

Why might the ER be unable to avoid exporting its own membrane proteins? Of course, it is possible that export from the ER is nonselective by design. But a more likely possibility is suggested by the realization that those membrane proteins intended for export from the ER and subsequent distribution throughout the cell represent only a trace of the total membrane protein present at any given time in the ER. This situation probably pertains only to membrane proteins of the ER. Most if not all of the soluble protein in the lumen of the ER appears to be destined for export (*46*).

It may be necessary to keep this diverse array of precursors at the trace level to prevent the ER from assuming the characteristics of other compartments. For example, it appears that as little as one part in ten thousand of the total membrane proteins present in ER are precursors of the plasma membrane (Table 2); these precursors would then need to be purified as much as 10,000-fold, with only traces of ER membrane proteins remaining! This degree of purification may be impossible to achieve in a single step at the level of export from the ER. If so, multiple stages of purification (to remove contaminating ER membrane proteins) would be needed, presumably in a Golgi. Unfortunately, there is as yet no quantitative information concerning the selectivity of export from ER. But, the qualitative aspects of this analysis make it plausible to consider that essentially every protein that can diffuse freely in the ER membrane will be unavoidably exported to Golgi membranes in significant amounts.

Several lines of evidence support the view that the ER membrane proteins in Golgi fractions (*33, 36, 38, 42, 43*) are in Golgi membranes and not an artefact of contamination by ER membranes. The specific activity of such enzymes (including cytochrome P-450, cytochrome P-450 reductase, glucose-6-phosphatase, cytochrome b_5, cytochrome b_5 reductase, and several phospholipid biosynthetic enzymes) in Golgi fractions is typically ~ 0.3 to ~ 0.5 of their specific activity in ER fractions, and even higher in some instances (*36*). This appears to be too high a level to be attributed to contaminating ER vesicles; in those Gol-

gi fractions analyzed, identifiable Golgi elements comprised 88 percent of total membrane profiles and ER elements only 5 percent (*33*).

Second, individual ER membrane enzymes are contained in Golgi membranes that can be readily identified by electron microscopy. Thus, membranes that contain cytochrome P-450 reductase in a Golgi fraction were specifically adsorbed to beads via antibody to reductase (*43*). The adsorbed membranes consisted primarily of morphologically distinct Golgi vesicles and cisternae.

Third, the same ER enzymes are housed in differing bulk membrane environments in ER and Golgi fractions. The ER membrane enzymes of the Golgi fraction distributed differently (by a factor of ~ 10) in aqueous two-phase polymer systems from the same set of enzymes found in the ER fraction, and behaved as a single unit (*38*) in that individual ER membrane enzymes were never resolved. Digitonin (a detergent that forms a complex with cholesterol) shifts the set of ER membrane enzymes in the Golgi fraction to slightly higher densities on a sucrose gradient, but not the same enzyme activities found in ER membranes (*36*). The Golgi contains more cholesterol than the ER (*5, 26*).

Several lines of evidence, taken together, suggest that ER membrane enzymes are asymmetrically distributed within the Golgi stack, with large amounts in the *cis* Golgi and much less in the *trans* Golgi.

1) When liver Golgi is subfractionated in two-phase aqueous polymer systems, the specific activity of ER membrane enzymes in *trans*-rich fractions is ~ 0.05 of that in *cis*-rich fractions (*38*). Other subfractionation methods may be subject to a higher degree of cross-contamination between *cis*- and *trans*-rich fractions, yielding *trans*/*cis* ratios of specific activity in the range of ~ 0.1 to ~ 0.3 (*36*) or ~ 0.5 (*43*).

2) The ER membrane proteins in Golgi membranes can be resolved from enzymatic markers of *trans* Golgi. The set of ER membrane enzymes found in Golgi membranes partitions quite distinctly from *trans* Golgi "markers" galactosyltransferase and thiamine pyrophosphatase in two-phase polymer systems (*38*). Antibody to cytochrome P-450 reductase binds most of reductase and the other ER membrane enzymes of Golgi fractions to beads, but results in little binding of galactosyltransferase (*43*).

3) The digitonin density shift is greater for galactosyltransferase than for the ER membrane enzymes contained in Golgi membranes, suggesting that most of the ER membrane enzymes are housed in Golgi membranes that contain less cholesterol than the membranes housing terminal glycosyltransferases (*36*). Considering that cholesterol concentration increases from the *cis* to the *trans* side of the stack (*26*), the digitonin experiment would also place most of the ER membrane enzymes on the *cis* side.

Table 2. Estimate of the instantaneous content of precursors to the plasma membrane in the endoplasmic reticulum of a hepatocyte based on transit and turnover times and relative surface area.

Compartment	Area*	Residence time†	Fraction in transit‡
ER (rough and smooth)	38	~ 10 minutes	~ .0001§
Plasma membrane	[1]	~ 2 days	[1]

*Relative surface area (*52*). The bracket indicates base of normalization. †Estimated transit or turnover time of a resident plasma membrane protein (*5, 53*). ‡Estimated fraction of total membrane protein destined for the plasma membrane. §Transit time through ER divided by turnover time in the plasma membrane, divided in turn by the relative surface area. This calculation considers that the cell surface as a whole needs to be replaced every 2 days, and that about 10 minutes worth of precursors are present in ER at any one time. A similar estimate could be made for a dividing cell with qualitatively similar results, in which case the doubling time of the cell replaces the turnover time of plasma membrane protein.

Most newly synthesized ER proteins are found in rough ER fractions several minutes before arrival in smooth membrane fractions (*47*). Therefore, it is likely that most of the ER membrane proteins in the Golgi arrive there after export from ER rather than by independent insertion into both ER and Golgi membranes (*48*). Given that the apparent flow of the export through the Golgi stack is in the *cis* to *trans* direction, the lower concentration of ER membrane proteins in *trans* as compared to *cis* portions makes it seem necessary to conclude that ER membrane proteins are continuously removed from the export as it passes through the Golgi stack in a filtration process that can be termed refinement.

One possible mechanism of refinement would be for ER membrane proteins to be removed from the Golgi and returned to the ER. The major alternative mechanism of refinement, that ER membrane proteins are selectively degraded as the export passes through the Golgi, seems less likely because of the great disparity between the time scales of turnover of ER proteins (days) (*5*) and of export (minutes) (Table 2). The subsequent discussion is based on the assumption of a retrieval mechanism for refinement, but the essence is quite independent of the actual mechanism of removal of ER membrane proteins from the Golgi.

The need to improve the fidelity of export from the ER, accomplished by refinement, could readily explain why all eukaryotes need a Golgi. Without one, the sorting of exported proteins into types (plasma membrane, secretion granule, and the like) could conceivably be carried out at the level of the ER, but the selective export to each of the final destinations would be heavily contaminated with ER membrane proteins. By imposing the Golgi apparatus as a filter between the ER and the final destinations (Fig. 5), the contaminating ER membrane proteins can be removed before further sorting takes place.

Altogether, the sorting of proteins in the Golgi may proceed in two sequential stages in attached *cis* and *trans* units of the Golgi. (Fig. 5). The crude export of ER may first be refined by removal of ER membrane proteins during transit through the *cis* Golgi and then passed on to *trans* Golgi for further sorting, probably during exit from the Golgi stack (*49*). Refinement could of course continue in the *trans* Golgi.

The Stacking of Cisternae of the Golgi

A stack could improve the overall efficiency of refinement by making it a multistage process. In the retrieval mechanism, a stack would allow repeated extraction of ER membrane proteins from the export at successive cisternae. This would be crudely analogous to the procedure of countercurrent distribution (*50*), in which proteins are efficiently separated when the same purification step is applied sequentially in a series of test tubes. Such a multistage process would naturally give rise to the kinds of progressive changes in cisternal properties so characteristic of the Golgi stack, and could also generate differences in lipid composition from ER to plasma membrane.

It is useful to consider what is required to ensure that the Golgi operates as a series of sequential stages under conditions of continuous flow. An analysis can help explain why a stack of flattened cisternae with associated vesicles and a closely applied but distinct *trans* unit may be necessary for proper Golgi function.

1) The ER membrane proteins would need to be removed from each of the cisternae (Fig. 6). The vesicles that stud

the rims of the cisternae (Figs. 1 and 2) could mediate this process.

2) The pattern of transfer of exported proteins would need to involve individual cisternae in a distinct sequence (Fig. 6); otherwise, the multistage effect would be lost. Certainly, there could be some randomness, so long as there is a strong bias for transfers between adjacent cisternae. There are two ways to achieve this. One is to have multiple cisternal compartments with a specific and distinct mechanism for each transfer to ensure sequential and vectorial delivery at each step. The simpler alternative would be to have a stack of copies of the same compartment with a single mechanism for transfer between them. In the absence of stacking, transfer among such identical compartments would be at random. The stacking of cisternae would be needed to constrain the pattern of transfer to favor adjacent cisternae. This geometric constraint would be most effective if the transfer of exported protein were to take place in the extensive, flattened zones of approximation of adjacent cisternae.

In the above scheme the stacking itself could not impart an overall sense of direction to these transfers; exported protein would pass with equal probability from a given cisternae to its indistinguishable nearest neighbors on either side. A detailed analysis (Fig. 7) based on chemical engineering principles (*50*) reveals that a consistent direction of transfer is, in fact, not necessary. Provided that a "crosscurrent" pattern of retrieval is used, all that is needed for multistage refinement is that the export encounter successive cisternae sequentially. The actual mechanism of transfer of protein between cisternae is a major unsolved problem in both the context of this model and in general. Refinement will be most efficient when both retrieval and intercisternal transfer processes are selective, but one or the other must be. Clathrin-coated vesicles (*49*) may be responsible for those transfers that are selective, as has been considered (*41*). Many possibilities, ranging from transport vesicles to diffusion through membrane continuities, would be consistent with a multistage operation (Fig. 7).

3) Only the purest, most fully refined fraction of the export should be utilized for further sorting and delivery to multiple destinations. Of course, the purest fraction in the stack (having had ER membrane proteins extracted the maximum number of times) would be found in its last, *trans*-most cisterna. The *trans* Golgi compartment, acting as a sink, would continuously receive and trap this optimally refined mixture of exported proteins. If there were no such receiver on the *trans* side, constantly siphoning off the cleanest fraction and providing a distinct site for exit from the stack, then the cell would be forced to take proteins from the cisternae at random (provided that, as before, the stack consists of multiple copies of the same cisternal

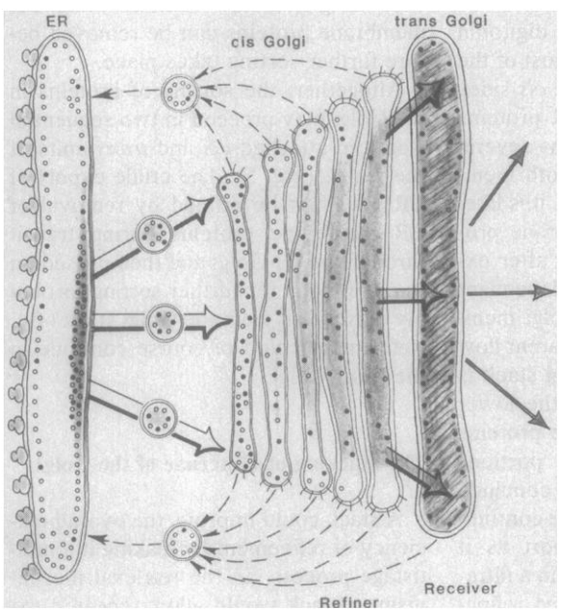


Fig. 6. An illustration of the process of multistage refinement that may take place in the Golgi stack. The density of shading portrays the progressive transport of exported proteins from ER through the *cis* to the *trans* Golgi. The closed circles represent some of these exported proteins. The open circles (whether drawn as membrane-bound or not) represent ER membrane proteins that would most likely be removed from the rims of Golgi cisternae by budding vesicles that then fuse with ER (dashed arrows). This corresponds to a crosscurrent (Fig. 7); other flow patterns are also possible. The thick arrows represent transport steps in which exported proteins are transported vectorially into the *cis* and into the *trans* Golgi compartments.

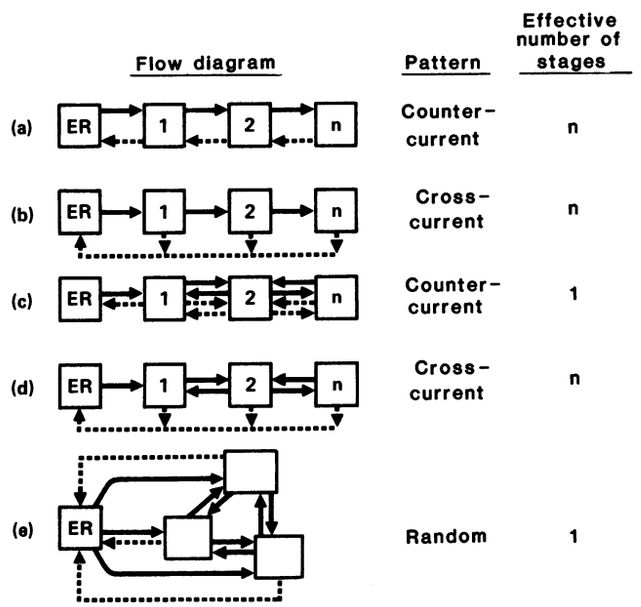


Fig. 7. An analysis of some of the possible patterns of protein transport in a Golgi stack from the viewpoint of chemical engineering (*50*). Each stage (a place where transported proteins can mix) corresponds to a single cisterna of the Golgi. Solid arrows denote transfers that either (i) are selective for proteins destined to locations such as plasma membranes, lysosomes, or secretion granules, or (ii) exclude ER membrane proteins; dashed arrows indicate transfers that enrich for ER membrane proteins. The different schemes (a to e) represent different flow patterns in which these transfers are executed continuously. The schemes can be evaluated on the basis of whether or not the steady state concentration of ER membrane proteins in the last stage (n) declines exponentially with n as n increases; that is, whether the stack refines in an efficient, stage-dependent fashion. Accordingly, schemes (a) and (b) will both refine efficiently, but both require that flow in the stack be vectorial throughout. This would not be possible in the simpler view (see text) in which the cisternae are multiple copies of the same compartment, indistinguishable to each other. Patterns (c) and (d) take this into account. The countercurrent version (c) will not refine any better than a single stage. In the crosscurrent version (d), where transfers selective for ER membrane proteins result in exit from the stack, neighboring cisternae on the left and right need not be distinguished, and refinement is stage-dependent. This analysis makes it seem likely that a crosscurrent rather than a countercurrent pattern of flow is used in the Golgi. In scheme (e) the cisternae are unstacked, transfers among them are random, and the Golgi becomes the equivalent of a single stage. These schemes were evaluated by applying the principle of mass balance to each stage, by means of standard methods (*50*) and solving for the steady-state distribution of ER membrane proteins in each case.

compartment). This mixed output of multiple cisternae would of course be much more contaminated by ER membrane proteins than the best product at the *trans* end of the stack. The process of multistage refinement would work best when the export from ER is received in the *cis*-most cisterna of the stack. Proximity of this cisterna to the transitional ER undoubtedly helps to ensure that the export is optimally delivered, but might not be absolutely necessary. Even if the stack were randomly disposed, the *trans* compartment, closing off one end of the stack, might suffice to restrict entry to the opposite, *cis* face.

The purpose and principle of the Golgi may therefore be essentially that of fractional distillation (*50*), and the apparatus used may be fundamentally the same. In the "distillation hypothesis," the Golgi stack operates under continuous flow to effect a binary separation of ER membrane proteins from other proteins intended for export. The *cis* Golgi stack is the distillation tower of this apparatus and its membrane-bound cisternae are the plates. The *trans* Golgi acts as the receiver, bleeding off the most refined product, often condensing it so that it can be more easily distributed in a concentrated form. The cisternae of the Golgi stack may in fact be no more complex than the plates in a distillation tower, simply a series of places where proteins can be delivered and removed, their purification effected by repetitive use of the same basic steps.

The Golgi Apparatus and the Sorting Problem

The views on the structure and function of the Golgi apparatus expressed in this article are meant to be taken as a working hypothesis. The new observations that have been emphasized are that

1) The Golgi stack is precisely divided into *cis* and *trans* compartments through which exported proteins are transported sequentially. Glycosyltransferases that seem to act on exported proteins destined for plasma membranes, secretion granules, and lysosomes are concentrated in the *trans* compartment, probably corresponding to the last one or two cisternae only.

2) Enzymes concentrated in ER membranes seem also to be abundant in *cis* portions of Golgi, but rare in *trans* portions.

These two points lead to the following specific proposals:

1) The sorting of exported proteins into multiple types is postponed until the *trans* compartment is reached.

2) A novel form of sorting, the removal of ER membrane proteins from the export, takes place mainly in the *cis* compartment. As a result, the *trans* compartment receives a refined mixture of exported proteins that can now be further sorted to yield fractions destined for several compartments, each freed of contamination by ER membrane proteins.

3) The stack of cisternae increases the overall efficiency of refinement by forcing the removal of ER membrane proteins to take place in a series of stages. The distinct *trans* Golgi compartment ensures that only the most fully refined fraction of the export is collected for further distribution in the cell. In analogy to distillation, the *cis* Golgi cisternae would be the plates of a distillation tower, and the *trans* Golgi the receiver of the distilled product. More than 80 years after its discovery (*51*) the Golgi apparatus may yet reveal secrets like those that have been known to chemical engineers all along.

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