PERSPECTIVES: DRUG DELIVERY

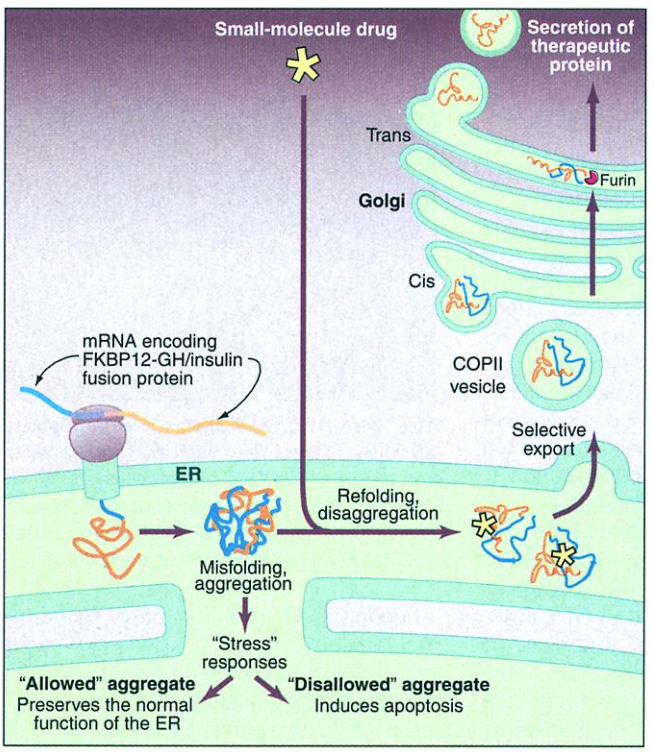
Regulating Export of ER Cargo

Meir Aridor and William E. Balch

Mammalian cells control the release of a variety of biologically active peptides and proteins into the circulation by regulating their secretion through the exocytic pathway. These secreted proteins serve as key chemical messengers, directing communication between cells. They are released with fast kinetics to activate signaling pathways, allowing the body to respond quickly and efficiently to physiological changes—a necessity if homeostasis is to be maintained. Large quantities of these chemical messengers are synthesized in advance by specialized secretory (endocrine and exocrine) cells and are stored in granules in the cytoplasm. They are released in response to signaling events initiated at the cell surface by the binding of a ligand to its receptor.

Remarkably, the research article by Rivera *et al.* (*1*) on page 826 of this issue now demonstrates that the endoplasmic reticulum (ER), the first compartment in the secretory pathway, can also serve as a practical storage container for genetically engineered proteins such as insulin or growth hormone. The investigators demonstrate that the secretion of these therapeutic proteins can be regulated by administering exogenously a synthetic small-molecule drug to cultured cells or mice. By manipulating the therapeutic protein of interest within the ER, they bypassed the need for highly specialized endocrine or exocrine cells. With this approach, storage and secretion of biologically active peptides and other proteins should be possible in any type of cell. Furthermore, the authors showed that controlled secretion from the ER was specific for an engineered therapeutic fusion protein (as opposed to bulk release of total secretory granule content induced by regulated exocytosis). The ability to artificially regulate the secretory response in cells may enable us to adjust the delivery of hormones and enzymes introduced by gene therapy to correct a range of pathophysiological conditions (*2*).

The ER is the initial cellular compartment for proteins destined for the cell surface (see the figure). It houses crucial machinery that directs protein folding and oligomerization. After protein folding, sorting motifs are exposed on the proteins (*3, 4*) that direct their selective incorporation into coat complex II (COPII) vesicles (*5–11*), which bud off from the ER (*12*). Although it has traditionally been assumed that protein secretion from the ER is a constitutive process (that is, one that occurs continuously), Rivera *et al.* capitalized on the recent realization that the ER can be induced to export a target protein (*2, 7, 9*). Endogenous ER proteins, such as receptor-associated protein and Nina A, are specific ligands for cargo proteins destined for secretion. They bind their cargo protein targets inducing conformational changes that then direct incorporation of the target proteins into COPII vesicles (see the figure) (*2, 13*). Examples of this type of regulatory secretion include: the binding of receptor-associated protein to low density lipoprotein–like receptors, the binding of Nina A to rhodopsin, and the binding of the invariant chain to major histocompatibility complex (MHC) class II proteins.



Drug-induced ER export. A fusion protein (FKBP12 linked to a therapeutic hormone) forms aggregates that are retained in the ER, possibly because sorting motifs essential for ER export are masked. These protein aggregates either induce apoptosis of the cell through activation of stress signaling pathways ("disallowed" aggregates) or become normal intermediates in the export pathway that are well tolerated by the ER ("allowed" aggregates). Addition of a small-molecule drug (yellow) that promotes disaggregation triggers incorporation of the properly folded fusion protein into COPII vesicles for export. Processing in the Golgi by the protease furin releases the hormone from FKBP12.

Small, diffusible molecules can also serve as ER ligands for cargo protein targets. Retinol is required to mobilize the retinol binding protein from the ER, peptides are required to initiate MHC class I export, and heme is necessary for folding and export of myeloperoxidase. In fact, the ability to modify protein conformation in the ER by small ligands is currently being tested as a therapeutic approach to promote the export of ABC transporters such as the cystic fibrosis transmembrane regulator (CFTR). In cystic fibrosis patients, this protein fails to assume a transport-competent conformation and is consequently degraded in the ER, resulting in the pathology of this disease.

Building on these examples, Rivera *et al.* (*1*) engineered a mutant form of the monomeric soluble human protein FKBP12 that, when translocated into the ER, forms protein aggregates that cannot be exported (see the figure). Aggregates of FKBP12 dissolve upon binding a small, membrane-permeable drug (that can be added exogenously to cells), and export of the protein begins. To harness this system to the regulated export of therapeutic hormones, the authors linked FKBP12 to either growth hormone or insulin with a cleavage motif recognized by furin (a protease associated with the final compartments of the Golgi apparatus). When these FKBP12-hormone fusion proteins were expressed either in cultured cell lines in vitro or in cells implanted into the muscles of mice, they aggregated and accumulated in the ER (see the figure). Upon addition of the small, membrane-permeable drug, the FKBP12-hormone aggregates assumed a transport-competent conformation and were efficiently delivered to the Golgi, where they were cleaved by furin; the mature hormone was then rapidly secreted. In the case of insulin, this led to a striking temporal control of serum glucose levels that mimicked the natural response to elevated blood sugar in a mouse model of hyperglyce-

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mia. The Rivera study clearly demonstrates that the compartments of the secretory pathway and their regulated sorting sites can be adapted to achieve rapid and efficient secretion of engineered therapeutic proteins.

This new technology may provide a simple platform from which to regulate secretion of therapeutic proteins in any type of cell. However, there remain intriguing scientific challenges that will need to be overcome if the systematic success of this approach is to be assured. Protein folding and accumulation are constantly monitored by the ER (*14*). Proteins that fail to assume a transport-competent conformation are targeted for removal by an ER-specific degradative pathway (*15*). Although both peptide signals and sugar modifications play a role in regulating protein stability (*14*), the exact mechanisms by which proteins are selected for degradation are unknown. Moreover, accumulation of unfolded proteins in the ER requires that both the size of the ER and its accessory components undergo continuous adjustment. This is achieved through signaling pathways from the ER to the nucleus whose components include the ER-associated transmembrane proteins Ire1, PERK (PKR-like ER kinase), and potentially other signaling receptors (*16, 17*). These pathways trigger the synthesis of a variety of resident ER and cytosolic proteins, which are required for proper protein folding, regulation of translation, membrane lipid synthesis, and transport. In a number of cases, signaling pathways from the ER can induce apoptosis of cells and tissue degeneration associated with diseases such as liver cirrhosis, peripheral neuropathies, and perhaps Alzheimer's disease (*2, 17*). Intriguingly, mobilization of the ER—although clearly observed by Rivera and colleagues as an expansion of the ER in response to accumulation of engineered proteins—did not cause programmed cell death, the activation of PERK, or degradation of the FKBP12-fusion protein.

Indeed, it is becoming increasingly apparent that transient protein aggregation is a normal intermediate step in the folding and assembly of a variety of proteins, enabling the ER machinery to cope with a heavy or unbalanced protein synthesis load. Such aggregation allows for the temporary sorting and storage of specific proteins without the induction of protein degradation or the programmed death of cells. An example would be the accumulation and release of immunoglobulin from the distended ER of mature B cells. Cells can compensate for inefficient protein transport by accumulating proteins and expanding the ER (this is seen in some forms of congenital hyperthyroid goiter) (*2*). Furthermore, normal tissue homeostasis can be controlled by endogenous circulating molecules that trigger ER export of accumulated protein. For example, C reactive proteins stored in the ER are rapidly released during tissue injury. Therefore, under certain conditions the ER can avoid activation of unwanted stress signaling pathways, which are usually mobilized in response to protein aggregation and accumulation.

A better understanding of the events that direct ER export and its integration with degradative and stress signaling pathways will no doubt improve our ability to generate "designer" cargo target proteins and to control their secretion. The Rivera *et al.* study represents a promising step in this direction. Furthermore, it confirms the importance of selective protein export from the ER as a key step in regulating cellular, tissue, and body homeostasis (*5, 7*).

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PERSPECTIVES: BEHAVIOR

Measuring Beelines to Food

Thomas Collett

Many decades ago, von Frisch (*1*) showed that honeybees are able to measure the distance that they fly from their hive to a foraging site. Knowing this distance helps bees to return to the site and also enables them to communicate the location of the site to other bees. A forager bee imparts this information on its return to the hive by performing a waggle dance (see the figure). A missing piece to this story is an understanding of the way in which bees measure this distance. Led by the bees' errors when flying with tail winds or against head winds, von Frisch supposed that the bees' estimate of distance was derived from the energy they expended during a flight—a curiously unreliable tape measure for bees to use.

The issue was reexamined by two groups in the mid-1990s, one in Notre Dame headed by Esch and another at the Australian National University led by Srinivasan, who reports his latest findings on page 851 of this issue (*2*). Both groups rejected the energy consumption hypothesis. They showed instead that bees monitor distance visually. Bees integrate over time the motion of images crossing the retina as they fly through a landscape. By this means they can both measure and control the distance that they travel. The experimental proofs of both groups depended on a fundamental limitation of the insect neural system that measures self-generated image motion (optic flow). This system is ignorant of the distance of features in the world that generate image motion on the retina. Consequently, flying a short distance close to the ground will generate the same integrated motion signal as flying a longer distance high above the ground.

Esch and Burns (*3*) trained bees to forage from the top of a 50-m-high building to a feeder placed on the roof of another tall building 230 m away. The waggle dances of these bees indicated a distance that was about half the length signaled by bees traveling the same distance from a hive to a feeder on the ground. Srinivasan *et al.* (*4*) took a different tack. They analyzed the search behavior of bees that were trained to fly several meters down a narrow, well-lit tunnel and to forage at a feeder partway along its length. When the feeder was absent, bees searched persistently at the expected location. Evidence for the use of optic flow came from testing these trained bees in tunnels of different widths. Making the tunnel narrower than the standard 22 cm caused bees to search closer to the entrance. When the tunnel was widened, bees searched further from the entrance. To maximize the perceived image motion, the tunnel was decorated with black and white vertical stripes on the walls (see the cover of this issue). But if the stripes were horizontal, so that there were no contrast changes to activate the motion detection system, bees did not know where to search when the feeder was missing and flew from one end of the tunnel to the other without stopping in the middle. Srinivasan and his colleagues have now woven these two strands of research together, and in this issue of *Science* they pre-

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