ER transport signals and trafficking of potassium channels and receptors
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Introduction

Channels and receptors on the plasma membrane allow a cell to respond to signals and changes in its environment. The functions of channels and receptors depend critically on their numbers on the cell surface, which are determined jointly by secretory and endocytic pathways. Compared to extensive studies on the endocytic pathway [1–4], relatively little is known about regulation of receptors and channels in the secretory pathway, particularly the transport between the endoplasmic reticulum (ER) and the Golgi apparatus. As the first station of the secretory pathway, the ER is where membrane proteins are synthesized, folded and assembled.

These functions require a variety of ER resident proteins; the ER also exerts quality control of proteins synthesized therein [5]. How do ER resident proteins remain in the ER? Both retention and retrieval mechanisms have been implicated [5]. Whereas the ER retention mechanism remains poorly defined, two types of retrieval motifs have been extensively characterized. For example, the peptide motifs KDEL and KKXX (X is any amino acid) are found at the carboxy (C)-termini of many luminal and transmembrane ER resident proteins, respectively. Both motifs act to facilitate the retrieval of escaped ER resident proteins from the ER–Golgi intermediate compartment (ERGIC) or the Golgi compartment [5]. How does the ER ensure the export of only fully folded and assembled proteins after their synthesis? The ER retains proteins that are incompletely or incorrectly folded via their interaction with the quality control machinery, leading to their degradation [5]. In addition to ER retention, post-ER retrieval may also contribute to the quality control mechanism [6,7]. It is generally thought that, once properly folded and assembled, proteins targeted to later compartments in the secretory pathway leave the ER by default, with no need for specific traffic signals [8].

A different picture has emerged from recent studies that have yielded a number of surprises on the trafficking of channels and receptors targeted to the plasma membrane. First, a novel form of ER retention/retrieval signal functions as a quality control checkpoint for the assembly of properly folded channels and receptors [9,10,11•,12•,13•]. Second, contrary to received wisdom, exit of fully folded and assembled channel proteins from the ER can be under the control of anterograde ER export signals [14•,15•,16]. Third, the interaction of channels and receptors with scaffold proteins such as PDZ (PSD95/DLG/ZO-1) domain-containing proteins and Homers can affect their ER to Golgi transport [11•,12•,13•,17–19]. Fourth, the ER to Golgi transport of certain receptors may be regulated by phosphorylation [12•,13•]. In this review, we discuss these issues individually.

ER retention/retrieval motifs

Functional ATP-sensitive potassium channels (K\textsubscript{ATP}) consist of four inwardly rectifying potassium channel (Kir) α subunits (Kir6.1/6.2) and four regulatory sulphonylurea (SUR)-binding β subunits (SUR1/2A/2B). In a manner typical of multimeric protein complexes, assembly of K\textsubscript{ATP} channels occurs in the ER, but only octameric channels can exit the ER. Zerangue et al. [9] found that an ER retention/retrieval motif, RKR, causes ER retention of Kir6 tetramers, SUR monomers, and other partial complexes with fewer than eight subunits. The RKR motifs are found in the cytoplasmic domains of Kir6 and SUR, and they are
shielded only after the complete assembly of octameric $K_{ATP}$ channels [9]. When fused to the plasma membrane protein CD4, this motif is sufficient to cause the accumulation of CD4 fusion protein in the ER [9]. The RKR motif differs from the previously known KDEL and KXXX motifs; it does not need to be located at the C-terminus of a protein [9], and retrieval of these different motifs from the Golgi likely involves different machinery (see below).

Similar arginine-based motifs also function as ER retention/retrieval signals during the assembly of the $\gamma$-amino butyric acid B (GABA$_B$) receptor [10] and N-methyl-D-aspartate (NMDA) receptors [11*,12*,13*]. Removal of four arginine-based motifs has also been reported to cause surface expression of a misfolded $\Delta F508$ cystic fibrosis transmembrane conductance regulator (CFTR) mutant channel, which is otherwise retained in the ER by the quality control machinery [20]. Thus, the arginine-based motif may be used to monitor both the folding (CFTR) and the assembly ($K_{ATP}$, GABA$_B$ and NMDA receptors) of membrane proteins.

A similar checkpoint exists during the assembly of the high voltage-activated calcium channel [21*]. An ER retention/retrieval signal present in the cytoplasmic loop linking the first two repeats of the pore forming $\alpha$ subunit, when transplanted to another ion channel, retards its surface expression. Coassembly with the auxiliary $\beta$ subunit shields this signal in the $\alpha 1$ subunit, allowing the channel complex to reach the cell membrane [21*].

**ER export signals**

It is generally assumed that proteins, once folded and assembled, exit the ER by a default pathway [8]. However, certain membrane proteins become concentrated during the process of ER export [22,23], raising the possibility that export signals for selective enrichment of export cargos might mediate the ER export of membrane proteins. A motif containing DXE [24] and neighboring residues [25] accelerates the ER export of a viral membrane protein, vesicular stomatitis virus glycoprotein (VSVG). Until recently, it was unclear whether ER export signals exist among endogenous plasma membrane proteins such as receptors and channels, and, if so, whether they affect the steady-state surface density of these plasma membrane proteins.

Studies of the trafficking of the Kir family revealed an ER export signal, FCYENE, in the Kir2.1 channel [14*,15*]. Mutations of FCYENE greatly reduced the steady-state surface density of Kir2.1 channels without affecting their folding and assembly; those mutant channels that reached the cell membrane exhibited normal single channel properties and their surface expression may have been enhanced by ER export signals grafted to ectopic positions [14*]. Significantly, when fused to several unrelated proteins, FCYENE promoted the ER export and thus the steady-state surface density of these membrane proteins [14*].

Similar but distinct ER export signals are present in other Kir family members, including Kir1.1 [14*], Kir3.2A, and Kir3.4 [16]. Kir3.2A or Kir3.4 coassemble with Kir3.1 to form heterotetrameric channels. When expressed alone, Kir3.1 resides in the ER due to the lack of an ER export signal [16]. When coexpressed with Kir3.2A or Kir3.4, the heterotetrameric channels exit the ER efficiently, due to the ER export signals present in Kir3.2A and Kir3.4 [16]. Thus, in addition to the arginine-based ER retention/retrieval signals, ER export signals can also be utilized to specify the subunit composition of multimeric membrane protein complexes.

A cytosolic amphipathic helix containing evenly spaced hydrophobic residues, FXXXFXXXF, is important for the ER export of the dopamine D1 receptor [26*]. This segment with highly conserved hydrophobic residues is adjacent to the last transmembrane domain of many G-protein-coupled receptors [26*]. An ER resident protein, DRIP78, is able to interact with these residues and mediate the ER export of the D1 receptor [26*]. These results suggest that FXXXFXXXF may encode an ER export signal.

Two other motifs, the C-terminal VXVSL of Kv1.4 [27] and the last 25 residues of SUR1 [28], may also play a role in the ER to Golgi transport of Kv1.4 and $K_{ATP}$ channels, respectively. Mutating these motifs decreases the number of channels with mature glycosylation, a modification occurring in the Golgi apparatus [27,28]. Because glycosylation status reflects the steady-state subcellular distribution of channels, these results suggest that either the mutated channels have a defect in their ER to Golgi transport, which could result from defective folding or removal of a traffic signal, or they are degraded soon after acquiring mature glycosylation in the Golgi. A resolution of this issue will be obtained by pulse chase analysis, and by testing the effectiveness of potential traffic signals fused to unrelated membrane proteins.

**Involvement of scaffold proteins in ER to Golgi transport**

Scaffold proteins, such as PDZ domain-containing proteins and Homers, are important for protein complex assembly and signaling at the synapse [29–32]. In addition to their organizational role, scaffold proteins may associate with channels and receptors early in the secretory pathway; these associations can facilitate or inhibit the ER to Golgi transport of channels and receptors [11*,12*,13*,17–19]. For example, coexpression of the PDZ protein SAP97 with the Kv1.4 channel results in the ER retention of both proteins, whereas Kv1.4 can reach the surface efficiently when expressed alone or coexpressed with another PDZ protein, PSD95 (postsynaptic density protein 95kDa) [17]. Coexpression of the metabotropic glutamate receptor mGlur5 with Homer 1b, a different type of scaffold protein, also causes accumulation of both proteins in the ER [18]. Conversely, Syntenin, also a PDZ protein, interacts with the C-terminus of pro-transforming growth factor $\alpha$ (proTGF$\alpha$) and increases its surface density, probably by
facilitating its ER to Golgi transport [19]. Studies of the NR1 subunit of the NMDA receptor provide another example of a PDZ protein stimulating ER export. Among the alternative splice variants of NR1, NR1-1a and NR1-1b contain only an arginine-based ER retention/retrieval motif, whereas NR1-3a and NR1-3b contain both the arginine-based motif and a consensus PDZ domain-binding site [11•,12•,13•]. Whereas NR1-1a/1b reside in the ER, due to the arginine motif-mediated retention/retrieval, binding of PDZ proteins to the C-terminus of NR1-3a/3b NMDA receptor subunits antagonizes the action of the arginine-based motif and allows the surface expression of NR1-3a/3b [11•,12•,13•]. The ability of similar PDZ proteins to cause either inhibition or facilitation of ER to Golgi transport may be due to their different expression levels, different subcellular localizations, different interactions with other cellular constituents, and/or the different cell types used in these studies. The mechanism by which the PDZ proteins affect ER to Golgi transport remains unknown. Presumably, binding of PDZ proteins to channels and receptors changes the relative effectiveness of retention/retrieval signals and ER export signals. How they do it is an intriguing open question.

**Regulation of ER to Golgi transport**

There is precedence for regulated ER to Golgi transport. For example, cholesterol homeostasis is maintained by a feedback mechanism that controls the sterol-dependent cleavage of a membrane-bound transcription factor, sterol regulatory element binding protein (SREBP) [33•]. The sterol-dependent cleavage of SREBPs is regulated at the level of ER export of these proteins along with their cleavage-activating protein [33•]. This elegant example illustrates how a cell can regulate the ER export of specific proteins according to relevant physiological conditions. The presence of retention/retrieval signals and ER export signals in channels and receptors raises the possibility that the ER to Golgi transport of these proteins destined for the cell membrane might also be regulated. Although definitive evidence is still lacking, several observations suggest this is likely to be the case.

Firstly, the ER retention mediated by arginine-based motifs in the NR1-1 or NR1-3 subunits of NMDA receptors can be potentially suppressed by phosphorylating two neighboring serine residues [12•,13•]. These serine residues are known protein kinase C (PKC) and protein kinase A (PKA) sites. When fused to the C-termini of two heterologous reporter proteins Tac or CD8, the arginine-based motif of NR1 is sufficient to cause their ER retention. However, mutating the neighboring serine residues to aspartate abolishes ER retention of these fusion proteins. Moreover, activation of PKC by phorbol 12-myristate 13-acetate (PMA) results in the surface expression of the Tac–NR1a fusion protein [12•]. It remains to be determined whether the additional NR1 on the cell surface

![Diagram](image-url)
Conclusions and future directions

ER to Golgi transport of channels and receptors appears to be more complicated than previously thought. Both ER retention/retention signals and ER export signals have been identified in various channels and receptors recently, and these signals govern their surface density. The functions of these ER transport signals may be subjected to regulation by phosphorylation or the binding of scaffold proteins.

In the future, it will be important to identify the cellular machineries that recognize the arginine-based ER retention/retention motifs and the various ER export signals. Coat protein I (COPI) and II (COPII) complexes are known to mediate the retrograde and anterograde transport between ER and Golgi compartments, respectively. Because the A508 CFTR mutant channel is localized to the ERGIC compartment in cells from cystic fibrosis patients [34] and in stably transfected tissue culture cells [35], it seems likely that the arginine-based motif functions as a retrieval signal. Whether it interacts directly with COPI component(s) as in the case of KXXX [36–38], or indirectly via an adaptor molecule, as in the case of KDEL [39,40], remains open to investigation. An alternative possibility is that the arginine-based motifs facilitate the retrieval of proteins through a COPI-independent pathway [38,41]. Similarly, channel ER export signals may be recognized directly by coat protein II (COPII) component(s) [42,43], indirectly by an adaptor molecule linked to COPII coat [44,45], or by an as yet unknown mechanism. In this regard, it is noteworthy that efficient ER export of a yeast plasma membrane protein requires a special homolog of Sec24, in addition to the general COPII coat components [46•]. Alternatively, the recruitment of peptide-loaded major histocompatibility complex (MHC) class I molecules to the ER export site may be facilitated by their association with transport receptors such as BAP31 [47•].

Of relevance to the mechanisms governing the traffic of channels and receptors from the ER is the question of how scaffold proteins affect their ER to Golgi transport. Do scaffold proteins act by masking or unmasking ER transport signals? Or do they serve as adaptor-like molecules coupling the channels and receptors to the ER export machinery? Another important area for future investigation is how the ER export of channels and receptors is regulated in vivo and how this type of regulation contributes to neuronal functions such as synaptic plasticity. Recent studies of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors have demonstrated the exciting link between neuronal plasticity and receptor trafficking [4,48–52]. Moreover, recent electron microscope morphological studies suggest the existence of satellite secretory pathways in the dendritic spines of neurons [53•], the primary site of synaptic plasticity. Traffic regulation and synaptic plasticity have thus emerged as two intimately linked events in neurons.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• Of special interest
**Of outstanding interest

11. Stanley SD, Roche KW, McCallum J, Sans N, Wenthold RJ: PDZ + domain suppression of an ER retention signal in NMDA receptor NR1 splice variants. Neuron 2000, 28:887-898. The NR1 subunit of the NMDA receptor exists in eight alternatively spliced isoforms, NR1-1a/1b, NR1-2a/2b, NR1-3a/3b, and NR1-4a/4b. When expressed alone, some splice variants are expressed efficiently on the cell surface and some are mainly localized to the ER. These different subcellular distributions are due to the combined action of two cytoplasmic motifs, an ER retention/retention motif (RRR) similar to the RKR motif found in KATP channels [9] and a consensus PDZ domain-binding motif (STTV). Whereas the RRR motif alone retains NR1b in the ER, the presence of the additional STTV motif in NR3b suppresses the action of RRR and allows for surface expression.

Signalling mechanisms
The authors fuse the C-terminal domains of NR1 splice variants to a plasma membrane reporter, CD8, and analyze the trafficking patterns of these fusion proteins. They reach the same conclusions as Standley et al. [11], also independently identified the Kir2.1 ER export signal.


46. Spilioti ET, Osorio M, Zuuniga MC, Edidin M: Selective export of MHC class I molecules from the ER after their dissociation from TAP. Immunity 2000, 13:441-451. MHC class I molecules are assembled in the ER, where they are associated with transporter for antigen process (TAP) and other ER resident proteins. It is generally thought that peptide-loaded MHC class I molecules dissociate from TAP and exit the ER by bulk flow. However, the authors find that MHC class I molecules concentrate on the ER export sites after their dissociation from TAP, indicating a selective mechanism for their ER export. Because this concentration does not depend on the cytoplasmic tails of MHC class I molecules, cargo receptors that recognize the transmembrane or lumenal domain of MHC class I molecules may be involved.

47. Shimoni Y, Kurihara T, Ravazzola M, Amherdt M, Orci L, Schekman R: Lst1p and Sec24p cooperate in sorting of plasma membrane ATPase into COPII vesicles in Saccharomyces cerevisiae. J Cell Biol 2001, 151:973-984. Sec24p is an essential component of the COPII coat. Lst1p is one of two nonessential Sec24p homologs in yeast. Although not required for the packaging of several soluble and membrane cargos into COPII vesicles in a cell-free budding assay, Lst1p is necessary for the efficient packaging of Pma1p, the plasma membrane ATPase. The authors demonstrate that both Lst1p and Sec24p are required to promote the efficient packaging of Pma1p into the COPII vesicles. They suggest that the different Sec24p homologs serve to expand the range of cargos that can be packaged to the COPII vesicles, possibly by changing the geometry of the COPII coat.


