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ER Export: Call 14-3-3

Dispatch

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Forward transport of proteins from the ER to the plasma membrane requires escape from the ER's retention machinery. Recent studies suggest that 14-3-3 proteins may mediate ER export of potassium channels destined for the plasma membrane by interfering with dibasic-motif-mediated retention.

The endoplasmic reticulum (ER) is a major site of protein synthesis in the cell. After translocation of nascent chains into the ER, elaborate quality control mechanisms sort incorrectly from correctly folded proteins [1]. Incorrectly folded proteins are retained by chaperones and, if folding is unsuccessful, degraded by the proteasome after retrotranslocation to the cytosol. Correctly folded proteins destined either for different subcellular compartments or for secretion from the cell are packaged into COPII-coated vesicles [2] and transported to the Golgi via the ER–Golgi intermediate compartment [3]. Most membrane-bound and soluble proteins assemble into oligomers. As the function of these proteins depends on their oligomeric state, additional quality control mechanisms must ensure the acquistion of correct quarternary structure.

How is it ensured that only correctly assembled protein complexes leave the ER? Initial studies on T cell and immunoglobulin E receptors [4,5] suggested a mechanism that appears to apply to many other cell surface receptors: the association of subunits into heterooligomeric complexes masks retention signals that keep unassembled subunits in the ER. Studies on multimeric channels and receptors destined for the plasma membrane of neuronal cells confirmed this notion [6]. In these cases a distinct subunit is retained in the ER by a di-arginine motif — RR or RXR, where X is any amino acid — in its cytoplasmic tail. By subunit assembly the di-arginine motif is masked and the multimeric protein complex can leave the ER.

Two recent papers [7,8] suggest a novel mechanism for release from dibasic signal-mediated retention involving 14-3-3 proteins. 14-3-3 proteins constitute a family of at least 100 conserved proteins present in all eukaryotes. These proteins have attracted interest because they are involved in important cellular processes including signal transduction, cell-cycle control, apoptosis, stress response and malignant transformation [9,10]. Although the exact function of 14-3-3 proteins is still unknown, they can act as adapters in protein–protein interactions, regulate the subcellular localization of proteins and activate or inhibit enzymes.

In a yeast two-hybrid screen, O'Kelly *et al.* [7] identified 14-3-3β as a binding partner for the carboxy-terminal tail of the homodimeric KCNK3 potassium channel. This finding was unexpected, as the tail does not contain a consensus motif for 14-3-3 binding (RSXS^{P}XP or RXXXS^{P}XP, where S^{P} denotes a phosphorylated serine) [10]. The interaction of 14-3-3β with KCNK3 was confirmed by peptide pull-down, and the binding site narrowed to the most carboxy-terminal RRSS^{P}V motif. 14-3-3 binding was shown to require phosphorylation of the motif both *in vitro* and *in vivo*.

Mutagenesis revealed that the most carboxy-terminal valine residue was essential for 14-3-3β binding and surface localization and function of the channel. Moreover, appending a classical 14-3-3 binding motif to the KCNK3 tail rendered the channel fully functional in 14-3-3β binding and transport. ER retention of the valine-impaired mutant channel was due to a dibasic 'KR' motif in its cytoplasmic amino-terminal tail. Surprisingly, this motif bound β-COP, a component of the coat protein complex I (COPI) [11], and the channel bound to either β-COP or 14-3-3β in a mutually exclusive way.

These results led O'Kelly *et al.* [7] to propose that forward transport of proteins retained in the ER by COPI can be initiated by phosphorylation of the 14-3-3 binding motif and subsequent binding of 14-3-3β. Such a mechanism is consistent with previous studies on the major histocompatibility antigen class II-associated invariant chain, Iip35, which carries a di-arginine signal [12] and an overlapping 14-3-3 binding motif in its amino-terminal tail [13]. Anterograde transport of Iip35 in association with class II molecules is dependent on phosphorylation [14]. O'Kelly *et al.* [7] have now found that phosphorylation-dependent binding of 14-3-3β or β-COP to Iip35 is mutually exclusive. Similarly, the neuronal α4 acetylcholine receptor subunit carries a ER retention/retrieval motif overlapping with a 14-3-3 binding motif in a cytoplasmic loop domain [7].

In complementary work published very recently in *Current Biology*, Yuan *et al.* [8] studied trafficking of the K_{ATP} channel α subunit, Kir6.2. This protein is retained in the ER by a RKR motif in its cytoplasmic tail until correct assembly of the K_{ATP} multimer occurs [15]. By screening for RKR motif-interacting partners, Yuan *et al.* [8] identified 14-3-3 ε and ζ. The interaction of 14-3-3 with the RKR motif could be reconstituted with purified proteins. Binding to Kir6.2 was independent of phosphorylation and could be competed by a high-affinity-binding, non-phosphorylated 14-3-3 ligand. Moreover, there was a correlation between the oligomeric state of the Kir6.2 tail and the binding of 14-3-3 *in vitro* and *in vivo*: 14-3-3 proteins bound to a dimeric or tetrameric RKR motif, but not to a monomeric one, and only reporters with oligomeric-presented RKR motifs were able to reach the cell surface. They also showed that COPI components can specifically interact with the RKR motif and that there is competition for binding with 14-3-3 *in vitro* [8]. Like O'Kelly *et al.* [7], Yuan *et al.* [8] propose a mechanism for COPI-mediated retention of the Kir6.2 subunit that can be competed for by 14-3-3 binding provided that the K_{ATP} channel is correctly

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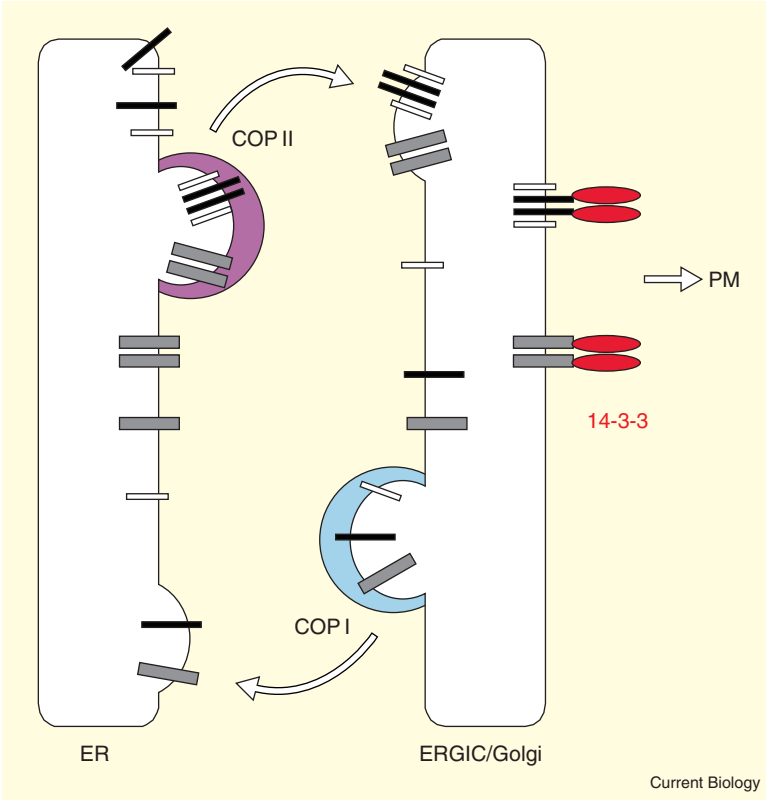

Figure 1. A model for 14-3-3 regulated trafficking of multimeric channels and receptors.

Unassembled subunits — white, black and grey rectangles — are retained in the ER via an arginine motif by a still unknown mechanism. Subunit assembly then leads to masking of the arginine motif and efficient ER export via COPII vesicles. Some unassembled subunits can also escape from ER by default. In the ER–Golgi intermediate compartment (ERGIC) and the Golgi, the assembly state is probed by 14-3-3 proteins. Correctly oligomerized proteins are transported forward to the plasma membrane. Unassembled subunits bind to COPI via their arginine motif and recycle to the ER in COPI vesicles for a next round of quality control. PM, plasma membrane.

assembled. 14-3-3 would thus probe the assembly state of the channel.

Integrating the new findings with previous data on trafficking of channels and receptors and knowledge on vesicular transport, a model emerges in which unassembled subunits are retained in the ER via an arginine motif by a mechanism that is still not clear (Figure 1). It is important to note that COPI binds to the ER–Golgi intermediate compartment and the Golgi complex, but not to the ER. Subunit assembly then leads to masking of the arginine motif and efficient ER export via COPII vesicles. Some unassembled subunits would also escape from ER by default. In the ER–Golgi intermediate compartment and the Golgi, the assembly state would be probed by 14-3-3 proteins. Correctly oligomerized proteins would be transported forward to the plasma membrane; unassembled subunits would bind to COPI via their arginine motif and recycle to the ER in COPI vesicles for a next round of quality control.

This model is speculative and some important questions remain to be answered. Are arginine-based ER localization signals indeed recognized by COPI *in vivo*? Up until now this interaction has only been documented *in vitro* [7,8] and di-lysine ER retention/retrieval signals — KKXX or KXKXX — recognized by COPI are nonfunctional if the lysines are replaced by arginines [16]. Functional inactivation of COPI may provide an answer. Does the arginine signal mediate both retention in the true sense and retrieval from post-ER compartments, as known for di-lysine signals [17]? That 14-3-3 can bind to the tail of a subunit of the K_{ATP} channel has been documented convincingly [8], but does it indeed recognize assembled K_{ATP} channel *in vivo*? Where in the cell does the binding of 14-3-3 to the channels occur? Although mutually exclusive binding of COPI or 14-3-3 [7,8] is consistent with an interaction early in the secretory pathway, the possibility remains that 14-3-3 binds the channels only at the cell surface.

Numerous studies have demonstrated a role for regulatory proteins, such as 14-3-3 [9,10] or PDZ-domain proteins [18], in post-Golgi sorting of receptors and channels and their clustering into membrane subdomains. The new work [7,8] suggests an additional role of 14-3-3 in facilitating transport of correctly assembled oligomeric proteins early in the secretory pathway and a similar role was proposed for PDZ-binding proteins [6]. How exactly these regulators mediate protein traffic is a challenging question for the future.

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