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Potassium Channels: Some Assembly Required

Minireview

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Many types of channels and receptors are expressed in the nervous system, contributing to the complex and diverse functional repertoires of neurons. Regulation of transcription and translation of the relevant genes exerts significant control over the phenotype of individual neurons. Also important, however, are posttranslational events that regulate the subunit composition of channels and receptors, their localization to specialized regions of the plasma membrane, and their density on the cell surface. Each of these factors has significant consequences for the functional capabilities and behavioral output of a neuron.

This minireview focuses on one such posttranslational event, the regulation and quality control of subunit assembly during potassium channel biogenesis. Although there are a few notable exceptions among channel proteins, most membrane proteins, including potassium channels, fold and assemble in the endoplasmic reticulum (ER) (Hegde and Lingappa, 1997, and references therein). Before transfer to the medial-Golgi compartment and beyond, these newly minted molecules are required to pass muster. The ER contains a stringent quality control system that recognizes and retains misfolded or incompletely assembled proteins, preventing their transport to locations where aberrant functional properties could disrupt cellular physiology (Kopito, 1997, and references therein). The biogenesis of several types of potassium channels is known to be strictly monitored in the ER (Schulteis et al., 1998; Zerangue et al., 1999).

After a brief review of the subunit structure and biogenesis of potassium channels, this article will describe some recent results concerning two key steps in subunit assembly and quality control: the formation of tetramers during the biogenesis of voltage-gated potassium channels and the formation of octamers during the biogenesis of ATP-sensitive potassium (K_{ATP}) channels.

Potassium channels contain four subunits, which surround a water-filled, K^+-selective pore (Doyle et al., 1998). A reentrant loop (the P loop) from each subunit contributes to the selectivity filter. The most familiar potassium channel subunits additionally contain either six (Shaker-like) or two (inward-rectifier type) transmembrane segments (Figure 1). A variety of "two P domain" subunits has also been described (Goldstein et al., 1998). Presumably, two such subunits would form a channel to retain a tetrameric arrangement of P loops around the pore.

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In addition to the pore-forming subunits, many potassium channels have additional subunits that regulate function and/or trafficking of the channel. A family of cytoplasmic β subunits of the Kvβ family associate with voltage-gated potassium channels. Some of these β subunits regulate inactivation, whereas others promote the maturation and cell surface localization of pore-forming subunits in heterologous expression systems (Shi et al., 1996, and references therein). In the heart and the ear, a single-spanning transmembrane protein, minK, coassembles with KvLQT1 tetramers to form a delayed rectifier that plays an essential role in repolarization of the cardiac action potential and in potassium secretion in the inner ear. Assembly with minK alters both the gating and permeation properties of KvLQT1 channels (Splawski et al., 1997). In pancreatic β cells and other tissues, including neurons, the sulfonylurea receptor (SUR), a multispanning membrane protein, associates with Kir6.1 or 6.2 pore-forming subunits of the inward rectifier family, to form K_{ATP} channels (Figure 1B) (Babenko et al., 1998). SUR, a member of the ATP-binding cassette family of proteins that includes the P glycoprotein and cystic fibrosis transmembrane conductance regulator (CFTR), is important for regulating both the function and trafficking of the K_{ATP} channel (Zerangue et al., 1999).

Sequence of Events and Quality Control during K^+ Channel Biogenesis

Biogenesis of a potassium channel involves a complex series of events. Individual pore-forming subunits are targeted to the ER membrane and insert, adopting a multispanning topology. Compatible subunits assemble with each other and with the appropriate auxiliary subunits. Along the way, important hydrophilic functional domains are formed that span the membrane, including the pore for potassium conduction and, in voltage-gated channels, a charged voltage sensor. The pore contains water (Doyle et al., 1998), and there is probably significant intrusion of water into the voltage sensor (Papazian and Bezanilla, 1997, and references therein). These hydrophilic structures are presumably shielded from the hydrophobic membrane bilayer in the final, assembled structure, but there is little information about how these structures are generated. Neither is it known whether these structures form in some protected environment such as the protein translocation channel (translocon) used for protein import into the ER or in the hydrophobic bilayer itself.

The Shaker potassium channel has been used as a model for investigating the folding and assembly events that occur during biogenesis (Schulteis et al., 1998, and references therein). It presents several significant advantages for such studies. First, the maturation, folding, and assembly of the wild-type protein occur rapidly and efficiently in diverse expression systems (Schulteis et al., 1998, and references therein). Second, a biochemical hallmark of the native structure has been identified, providing a way to assess the structural integrity of mutant proteins that do not express on the cell surface (Schulteis et al., 1996). Third, the ER quality control system

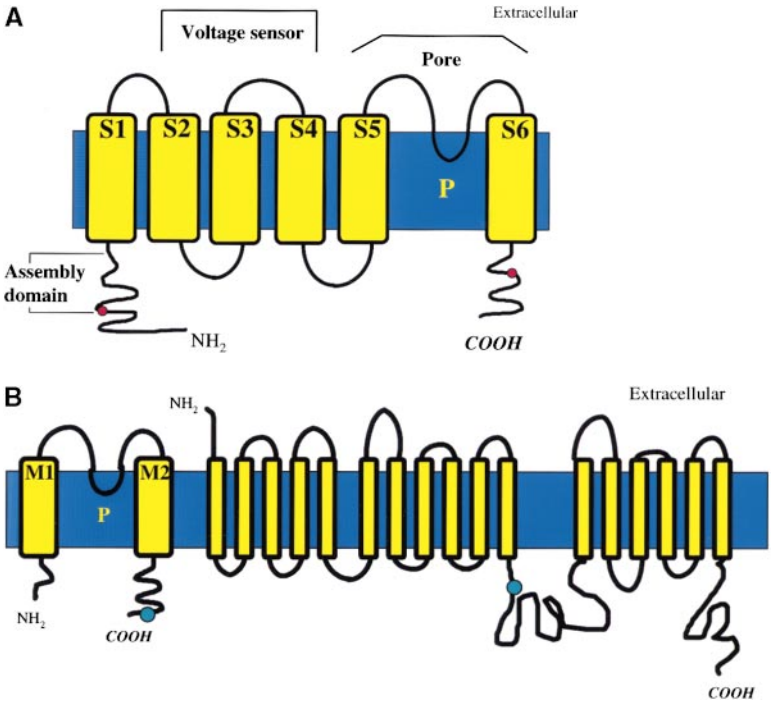

Figure 1. Putative Membrane Topologies of K^+ Channel Subunits

(A) Membrane topology of a voltage-gated potassium channel α subunit. Shaker-like potassium channel subunits have cytoplasmic amino and carboxyl termini, six transmembrane segments, and a reentrant P loop. Approximate locations of the amino-terminal assembly domain, the voltage sensor, and the pore are shown. Red dots indicate the approximate locations of two cysteines, C96 and C505, in Shaker that can be oxidized to form an intersubunit disulfide bond (Schulteis et al., 1996).

(B) Membrane topology of components of the K_{ATP} channel.

(Left) Topology of Kir6.1 or 6.2, the pore-forming subunits of K_{ATP}.

(Right) Putative topology of SUR (Zerangue et al., 1999, and references therein).

Blue dots indicate the approximate locations of the RKR retention/recycling signals in the Kir6.2 and SUR proteins.

discriminates extremely well between native and nonnative forms of the protein (Schulteis et al., 1998, and references therein). Finally, mutations that disrupt discrete steps in biogenesis have been identified and investigated, providing insights into the pathway of folding and assembly events that occur in the ER (Schulteis et al., 1998).

The full-length Shaker protein is detected first in the ER as a core-glycosylated protein. Transfer of the protein to the Golgi apparatus results in modification of the glycan chains (Nagaya and Papazian, 1997). The immature and mature forms of the protein can be readily distinguished because they have different electrophoretic mobilities (Schulteis et al., 1996). Unlike some mammalian Kv subunits (Shi et al., 1996), β subunits are not needed for Shaker to progress beyond the ER (Nagaya and Papazian, 1997). A fortuitous structural hallmark of the channel is provided by two cysteine residues, one in the amino terminus (C96) and one in the carboxyl terminus (C505) of adjacent subunits, that come into relatively close proximity in the properly folded and assembled conformation. Although these cytoplasmic residues do not form an intersubunit disulfide bond under normal cellular conditions, such a bond can be formed by exposing intact cells to mild oxidants (Schulteis et al., 1996). Using this marker, it has been shown that an excellent correlation exists between protein maturation and the generation of a native structure (Schulteis et al., 1996, 1998). Mutant proteins that lack this structural feature of the native state are efficiently retained in the ER.

Using insights gained from the extensive structure–function analysis that has been carried out on voltage-gated channels, it has been possible to disrupt specifically the biogenesis of several key functional domains in the channel, including the voltage sensor and the pore. The capability of these mutant proteins to undergo various steps of biogenesis has been analyzed (Schulteis et al., 1998, and references therein). In Shaker channels, tetramerization mediated by a conserved domain located in the amino terminus is required for subsequent pore formation and either precedes or is independent of folding of the voltage sensor. Late during biogenesis, the amino and carboxyl termini of adjacent subunits come into proximity. Subunits with folding defects oligomerize into tetramers in a step mediated by the amino-terminal assembly domain. These results indicate that proper folding of subunits is not a prerequisite for assembly. Furthermore, association with native subunits can weakly promote the proper folding of some mutant subunits, suggesting that steps of folding and assembly alternate during channel biogenesis (Schulteis et al., 1998).

In contrast to the wild-type Shaker protein, some mammalian Kv subunits do not efficiently leave the ER in expression systems (Shi et al., 1996). Currently, it is not clear whether this reflects inefficient folding and assembly and/or a deficiency of specialized molecules (including β subunits) needed for transport beyond the ER.

Formation of Tetramers

Among voltage-gated (Kv) potassium channels, only compatible α subunits, chosen from a subfamily of closely related proteins, are able to coassemble (Li et al., 1992). The role of the amino terminus in the oligomerization of Shaker subunits was first recognized by the laboratory of Lily and Yuh Nung Jan (Li et al., 1992). They identified a cytoplasmic sequence preceding the first transmembrane segment that was conserved among Kv1 subfamily members (Figure 1). In Shaker channels, the conserved region is found between residues 97 and 196. This domain, sometimes called T1 for "tetramerization domain 1," self-associates to form tetramers (Shen

et al., 1993). In the Shaker (Kv1) subfamily, the amino-terminal domain mediates oligomerization and prevents heteromultimer formation with members of other Kv subfamilies (Li et al., 1992). The structure of the domain has been solved for representative members of several Kv subfamilies, providing information about the protein interfaces that are important for subfamily-specific assembly (Bixby et al., 1999). These studies reveal that only a few characteristic residues are crucial.

In Shaker potassium channels, this amino-terminal domain appears to be required for efficient assembly and trafficking beyond the ER (Schulteis et al., 1998). Deletion of the most highly conserved region, between residues 96 and 197, abolishes tetramerization. Detergent-stable oligomers can not be detected, and there is no evidence for assembly in the membrane from either dominant-negative experiments or from cross-linking in situ. This finding is not universal among Kv1 subfamily members, however. For instance, the Kv1.3 protein can form functional channels after deletion of the amino terminus (Tu et al., 1996). Therefore, it is possible that the primary role of the amino-terminal assembly domain in some Kv1 proteins is to prevent assembly with incompatible subunits which are members of other subfamilies.

The laboratory of Carol Deutsch has investigated the pathway of oligomerization for potassium channel subunits. A recent paper focuses on whether tetramers form by successive addition of monomers or by dimerization of dimers (Tu and Deutsch, 1999). Do both pathways occur? Does either predominate? Using a strategy of coexpression and covalent linkage of subunits, their analysis of subunit assembly suggests that potassium channels form as the result of dimerization of dimers. Interestingly, the results suggest that two different faces of the amino-terminal domain mediate association of monomers to form dimers and dimerization of dimers to form tetramers. This is satisfying because one interaction surface would not suffice to explain the formation of radially symmetric tetramers.

Oligomerization mediated by the amino-terminal region is likely to occur early during the folding and assembly of voltage-gated potassium channels (Schulteis et al., 1998). Some investigators have even suggested that it is a cotranslational event (Deal et al., 1994). Given its amino-terminal location, the domain emerges from the ribosome prior to the transmembrane segments, which presumably serve to target the protein to the ER membrane. Several lines of evidence indicate that the amino-terminal domain can fold and assemble independently of the rest of the protein (Li et al., 1992; Schulteis et al., 1998; Bixby et al., 1999). However, it is difficult to reconcile the idea of cotranslational assembly with the current picture of the insertion of proteins into the ER membrane (Hegde and Lingappa, 1997). Strong evidence supports the idea that one ribosome associates with one translocon during the insertion of membrane proteins (reviewed by Hegde and Lingappa, 1997). Given the large size of ribosomes, it is doubtful that they can approach one another closely enough to allow coordinated insertion of two nascent polypeptide chains into the same translocon. One RNA molecule can be simultaneously translated by multiple ribosomes, raising the possibility that subunits translated on such a polysome might preferentially assemble and insert into the membrane coordinately. This, however, seems unlikely for potassium channel tetramers. When assembly of heteromultimers has been assessed by tagging individual subunits with toxin sensitivity or an inactivation ball and chain, results consistent with a random association of subunits encoded by different mRNA molecules have been obtained (MacKinnon, 1991).

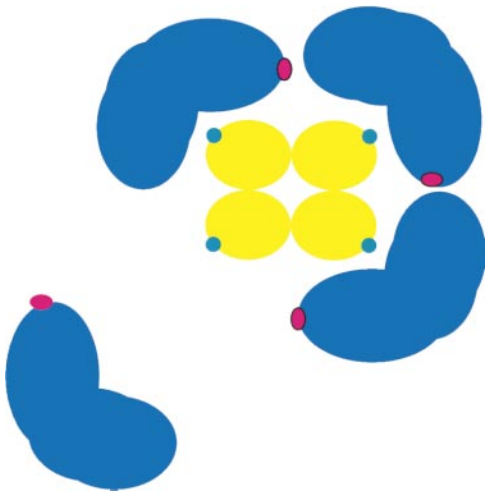

Figure 2. Model for RKR Signaling during K_{ATP} Biogenesis

The model shows a partially assembled complex of four Kir6.2 subunits (yellow) and three SUR subunits (dark blue), plus a monomeric SUR subunit that has not yet joined the complex. RKR sequences in Kir6.2 and SUR are indicated by light blue and red dots, respectively. These RKR sequences are exposed in Kir6.2 tetramers, monomeric SUR, and K_{ATP} complexes with fewer than eight subunits (Zerangue et al., 1999). Presumably, RKR interacts with components of the ER quality control system to prevent trafficking of partially assembled complexes beyond the *cis*-Golgi compartment.

Formation of Octamers

The Jan laboratory has once again led the way in identifying the role of retention/recycling signals in the quality control of unassembled or partially assembled potassium channel subunits (Zerangue et al., 1999). K_{ATP} channels are gated by the metabolic conditions in a cell, specifically the ADP/ATP ratio. These channels couple the excitability of a cell to its metabolic state. Molecularly, K_{ATP} channels are octamers, containing four pore-forming Kir6.1 or 6.2 subunits plus four SUR molecules (Babenko et al., 1998).

Studying the assembly and cell surface expression of K_{ATP} channels, the Jan laboratory has identified a simple retention/recycling signal, RKR, which is present in both the Kir and SUR components of K_{ATP}. The existence of such an inherent signal was suggested by the finding that carboxy-terminal deletions of Kir6.2 allow the cell surface expression of potassium channels in the absence of SUR (Tucker et al., 1997). Mutating or deleting the RKR sequence allows cell surface expression of monomeric SUR and incompletely assembled complexes, including Kir6.2 tetramers (Zerangue et al., 1999). Illustrating the potential dangers of quality control failure, these channels exhibit aberrant functional activity, with a high degree of basal activity unregulated by the metabolic state of the cell. In pancreatic β cells,

uncoupling channel activity from metabolic control would be expected to reduce insulin secretion.

The results reported by Zerangue et al. (1999) suggest a straightforward model for how the RKR sequence might function: exposure of RKR on incompletely assembled complexes would lead to retention via interaction with some ER resident protein (Figure 2). The RKR at the cytoplasmic end of Kir6.2 would be exposed in 6.2 tetramers and become inaccessible only upon addition of SUR to the complex. In turn, RKR on the large intracellular loop of SUR would be exposed when fewer than four SUR molecules had joined the complex. It would become inaccessible by interactions between adjacent SUR molecules only in fully assembled octamers.

The RKR signal may be part of a more general system for ER retention/recycling with roles in addition to the regulation and quality control of potasssium channel assembly (Zerangue et al., 1999). Proteins normally found on the cell surface are retained in the ER if RKR is inserted into them. In addition, the RKR sequence leads to ER retention in a variety of eukaryotic cell types including yeast, *Xenopus* oocytes, and mammalian cells.

One fascinating question for the future is the identity of ER components that recognize the RKR retention/recycling signal. Given the effectiveness of the RKR signal in divergent expression systems, it is likely that this component has been conserved throughout eukaryotic evolution. In addition, it will be interesting to determine whether additional retention/recycling signals serve to identify channel molecules with other structural defects, such as a misfolded voltage sensor or pore.

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