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Golgi Export of the Kir2.1 Channel Is Driven by a Trafficking Signal Located within Its Tertiary Structure

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SUMMARY

Mechanisms that are responsible for sorting newly synthesized proteins for traffic to the cell surface from the Golgi are poorly understood. Here, we show that the potassium channel Kir2.1, mutations in which are associated with Andersen-Tawil syndrome, is selected as cargo into Golgi export carriers in an unusual signal-dependent manner. Unlike conventional trafficking signals, which are typically comprised of short linear peptide sequences, Golgi exit of Kir2.1 is dictated by residues that are embedded within the confluence of two separate domains. This signal patch forms a recognition site for interaction with the AP1 adaptor complex, thereby marking Kir2.1 for incorporation into clathrin-coated vesicles at the *trans*-Golgi. The identification of a trafficking signal in the tertiary structure of Kir2.1 reveals a quality control step that couples protein conformation to Golgi export and provides molecular insight into how mutations in Kir2.1 arrest the channels at the Golgi.

INTRODUCTION

The Golgi apparatus is well appreciated to act as the central biosynthetic sorting station, responsible for properly targeting newly synthesized membrane proteins to their appropriate subcellular destinations (De Matteis and Luini, 2008). Endosomal sorting processes provide a framework for understanding the underlying molecular mechanisms. It is well established that endosome- and lysosome-destined proteins are recognized at the Golgi as cargo for inclusion into clathrin-coated vesicles by short, linear tyrosine-containing or dihydrophobic signals that serve as recognition sites for interaction with clathrin adaptor complexes and GGA proteins (Bonifacino and Traub, 2003). Although much less is known about how newly synthesized plasma membrane proteins are sorted to the cell surface, an emerging body of evidence indicates that select plasmalemma proteins may also leave the Golgi by signal-dependent processes, rather than by default trafficking pathways as once believed (Rodriguez-Boulan and Müsch, 2005). Subsets of membrane proteins in polarized epithelial cells, for example, are sorted at the Golgi for traffic to the basolateral membrane in a clathrin-dependent manner (Deborde et al., 2008) by short linear peptide sequences (Campo et al., 2005). Although these signals share remarkable similarity with endosomal sorting signals, the clathrin-associated sorting proteins that interact with them at the Golgi remain to be discovered. Other cell surface proteins (see below) rely on completely unrelated structures for Golgi export, and the sorting machineries that decode these putative signals also remain unknown. Different coat proteins (Wang et al., 2006), Golgi tethers (Lock et al., 2005), and scaffolding molecules (Godi et al., 2004) have been implicated in Golgi-to-cell-surface traffic, but these are considered to play important roles in carrier vesicle formation rather than in cargo recognition.

Signal-dependent Golgi export processes have been implicated in controlling the surface density of inwardly rectifying K^+(Kir) channels (Nichols and Lopatin, 1997). In recent years, it has become evident that different trafficking processes regulate Kir channels to control neuronal excitability, action potential cessation, hormone secretion, heart rate, and salt balance. Several Kir channels have been postulated to leave the Golgi in a signal-dependent manner (Stockklausner and Klocker, 2003; Yoo et al., 2005). In the Kir2.1 channel (Kubo et al., 1993), a short cluster of highly conserved basic amino acids in the cytoplasmic N terminus is required for Golgi exit (Stockklausner and Klocker, 2003). A nearly identical structure in the kidney potassium channel Kir1.1 (ROMK) is necessary for forward trafficking in the secretory pathway (Yoo et al., 2005), consistent with a shared signal-dependent Golgi export process. Nevertheless, the sequences exhibit no resemblance to known trafficking signals, and it is completely unknown how they might control Golgi exit.

In the present study, a human Kir2.1 channel disease, Andersen-Tawil syndrome (ATS1) (Plaster et al., 2001), provided a new insight into the Golgi mechanism. The Kir2.1 channel (Kubo et al., 1993) is responsible for controlling membrane excitability in many cell types. Because it is especially important in ventricular cardiomyocytes (Zaritsky et al., 2001) and skeletal muscle (Fischer-Lougheed et al., 2001), loss of

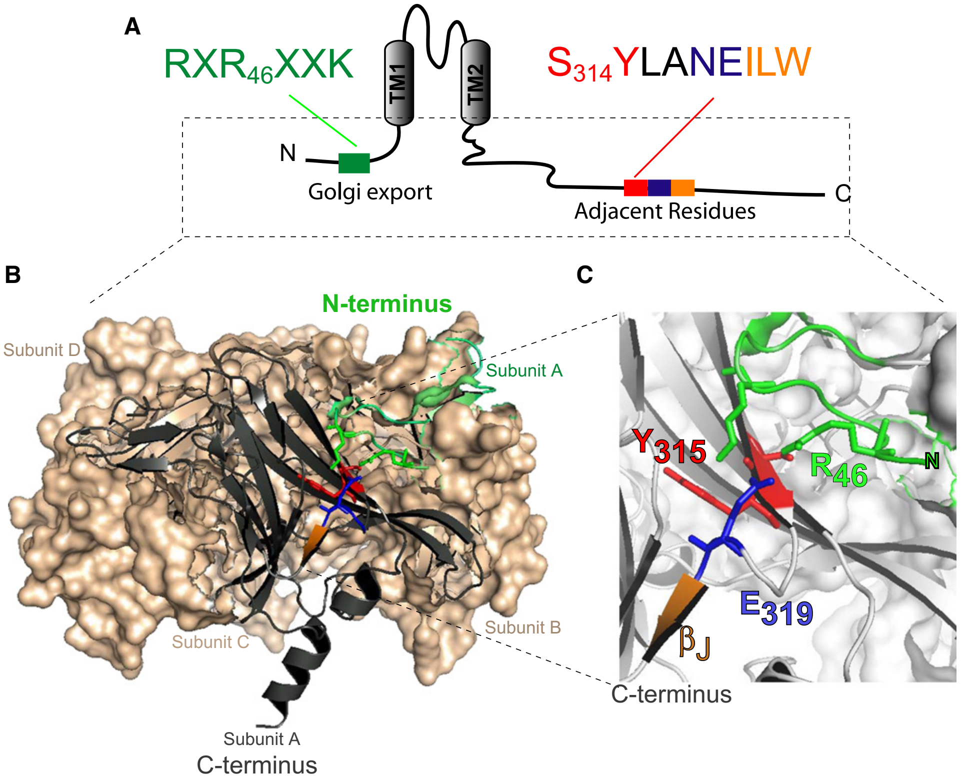

Figure 1. The Kir2.1 N-Terminal Golgi Export Structure Juxtaposes the Cytoplasmic C Terminus and an ATS1 Mutation

(A) A single Kir2.1 channel subunit with relevant residues color coded. N terminus, green; ATS1 mutation (Δ314-315), red; NE319, blue; hydrophobic residues in the β J stand, orange; transmembrane domains (TM1 and TM2), gray.

(B) Atomic structure Kir2.1 cytoplasmic domains channel tetramer, forming the cytoplasmic pore as shown (from Pegan et al., 2005, PDB1u4f). Standard ribbon display of one subunit (subunit A) is highlighted against a surface rendering of the other three subunits.

(C) Magnified view of residues at the cytoplasmic domain interface. Residues affected by the Δ314-15 ATS1 mutation, SY$_{314\text{-}15}$(red), lie under the N-terminal trafficking determinant (green).

Kir2.1 function in ATS1 is manifested as a disorder of ventricular arrhythmias, periodic paralysis, and skeletomuscular dysplasia (Andersen et al., 1971; Sansone et al., 1997). Of the many ATS1 mutations, we found that one of them in the cytoplasmic C terminus of Kir2.1 surprisingly blocks Golgi export. Our investigation into the underlying pathologic mechanism revealed that Golgi exit of Kir 2.1 is dictated by an unusual signal. Unlike conventional short, linear trafficking signals, the Golgi export signal in Kir2.1 is formed by a patch of residues that are located within the confluence of cytoplasmic N- and C-terminal domains. This signal patch creates an interaction site for the AP1 adaptin complex, allowing properly folded Kir2.1 channels to be incorporated into clathrin-coated vesicles at the *trans*-Golgi for export to the cell surface.

RESULTS

Kir 2.1 Channels, Bearing an ATS1 Mutation, Accumulate in the Golgi

Exploration of the Golgi export mechanism in Kir2.1 was guided by mapping the location of an ATS1 mutation, Δ314-15 (Plaster et al., 2001), in the atomic resolution structure (Pegan et al., 2005). Remarkably, the involved C-terminal residues, SY$_{315}$, juxtapose the N-terminal Golgi trafficking determinant at a domain interface (Figure 1). The mutation has been reported to block surface expression (Bendahhou et al., 2003), but the mechanism is not known. Because our studies revealed that the mutation does not impair subunit assembly or cause misfolding or ER retention (Figure S1 available online), we wondered whether it might instead disrupt Golgi trafficking and provide a model for understanding the export signal. In fact, Kir2.1Δ314-15 channels exhibit an unusual mistrafficking phenotype (Figure 2). As measured by HA-antibody binding of external HA-epitope-tagged channels (Figure 2A), the Δ314-15 mutation reduced cell surface expression to background levels, comparable to the effects of removing the “ER export” signal (ΔFCYE) (Ma et al., 2001). Confocal microscopy (Figure 2B) revealed that Kir2.1Δ314-15 channels colocalize with markers of the medial Golgi (GM130; Nakamura et al., 1995) and *trans*-Golgi network (TGN46 or TGN38; Prescott et al., 1997), unlike the robust surface localization of the wild-type channel and the ER retention phenotype of ΔFCYE mutant channels. As evaluated by Pearson’s colocalization analysis, the degree of Kir2.1Δ314-15 channel localization in the Golgi is significant.