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Visualizing Protein Trafficking: Membrane Proteins Follow Multiple Trafficking Pathways to the Basolateral Cell Surface in Polarized Epithelial Cells

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Our current view of membrane protein trafficking and sorting began to take shape roughly 40 years ago, when Jamieson and Palade combined a pulse-chase labeling protocol with ultrastructural analysis to visualize the post-synthetic route pursued by newly synthesized proteins in pancreatic acinar cells [1-4]. The unique properties of the acinar cell, in which ~95% percent of protein synthesis is dedicated to producing zymogens that will be packaged into secretory granules, justified this study's simple and elegant design, since it was safe to assume that when metabolic radiolabeling was combined with EM autoradiography, the pattern of silver grains observed in the electron microscope would correspond to the subcellular distribution of a temporally defined cohort of secretory proteins. Until recently, and with very few exceptions, it has not been possible to generalize this sort of analysis to other proteins and tissues of interest. The development of the SNAP tag and related labeling technologies provides the tools required to not only elucidate the post-synthetic itinerary of any protein in any cell type, but also to identify that protein's set of interaction partners as a function of time.

The Na,K-ATPase, or sodium pump, generates the ion gradients responsible for most fluid and electrolyte transport processes in the kidney. To follow the post synthetic fate of the Na,K-ATPase, we have adapted a technique that permits direct observation of temporally defined cohorts of proteins via the combination of fluorescence microscopy with pulse-chase labeling protocols [5]. The 20-kDa SNAP-Tag is a modified version of the DNA repair protein 0^6-alkylguanine-DNA alkyltransferase, which cleaves para-substituted benzyl guanines (BGs) by covalently transferring the substituted benzyl group to its active thiol [6]. The resulting thioether bond irreversibly prevents the reacted SNAP-Tag from participating in any further labeling reactions. Fluorescent BG derivatives allow for the labeling and detection of SNAP-Tagged fusion proteins in either live or fixed cells [6,7,8] (Fig. 1). Through the combination of a "pre-pulse" blocking step with non-fluorescent BG, followed by selective labeling of newly synthesized protein with fluorescent BG, we can follow a cohort of protein as it is synthesized and trafficked. Similarly, BG-sepharose can be used to purify a temporally defined cohort of proteins for biochemical analysis. We have prepared a construct encoding a SNAP-tagged Na,K-ATPase α-subunit. We have shown that the resultant protein assembles with β-subunits to form functionally active sodium pumps that travel appropriately to the basolateral plasma membrane in stably transfected renal epithelial cells (Fig. 2). We have demonstrated that we can follow the trafficking of a temporally defined cohort of pumps and analyze the partner proteins with which this cohort specifically interacts.

The newly synthesized apical and basolateral membrane proteins of polarized epithelial cells are sorted from one another en route to the plasma membrane. While the trans-Golgi Network has long been thought to play a critical role in this sorting process, recent reports have shown that basolateral proteins whose sorting is dependent upon the AP-1B complex travel from the Golgi to recycling endosomes prior to their delivery to the membrane. We have found that the basolateral delivery of newly synthesized Na,K-ATPase, an AP-1B independent protein, occurs via a pathway distinct from that pursued by AP-1B dependent cargo. Sodium pump trafficking occurs faster than that of the AP-1B dependent vesicular stomatitis virus glycoprotein G protein. In addition, sodium pump trafficking is not regulated by the same small GTPase proteins as AP-1B dependent cargo, does not require passage through recycling endosomes for delivery to the membrane, and can be detected in separate post-Golgi transport intermediates from those utilized by VSV-G. Our results suggest that multiple routes carry proteins from the Golgi to the basolateral membrane in polarized epithelial cells [9].

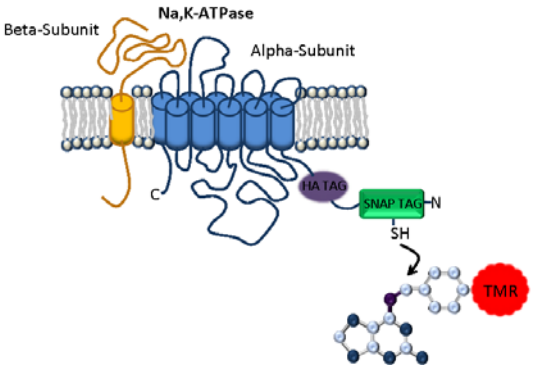

Fig.1 Labeling SNAP-tagged Na,K-ATPase with fluorescent benzyl guanine

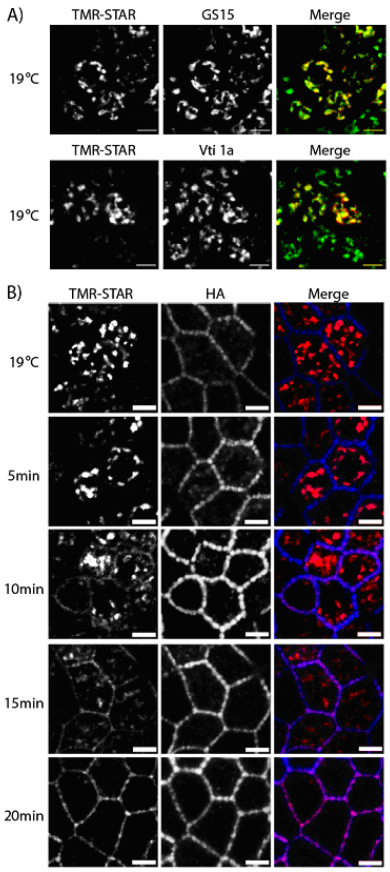

Fig. 2 Newly synthesized pumps accumulate in the Golgi at 19oC and move to the surface after release of the Golgi block

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