Editorial

Unlocking the Mysteries of Na^+-K^+-ATPase Endocytosis

Phosphorylation Is the Key

Any understanding of endocytosis of receptors and transporters begins with the classic studies on the LDL receptor and patients with familial hypercholesterolemia by Brown and Goldstein (reviewed in Ref. 1). They demonstrated that the LDL receptor rapidly internalized via clathrin-coated pits because it contained a tyrosine-based signal within the receptor's 50–amino acid cytoplasmic domain (2). Soon related signals in other receptors such as the transferrin receptor were identified, and thus it appeared that a common mechanism could explain how all cell surface proteins were endocytosed. For transport proteins like the LDL receptor and the transferrin receptor where cargo (i.e., cholesterol and iron) are taken in by the cell, the process appeared to be constitutive except for the interruption that occurs during cell division. Interestingly, later studies demonstrated that the clathrin-mediated endocytic pathway is inhibited by mitotic phosphorylation (3), illustrating that the classic phosphorylation–dephosphorylation regulation paradigm is an integral regulatory component of the endocytic cycle.

After establishing that signals within the cytoplasmic tails were recognized by components of the clathrin-coated pit, a key player in this process was identified as an assembly protein referred to as adaptor protein-2, or AP-2 (reviewed in Ref. 4). This protein served as a bridge between the receptors and clathrin and through its recognition of the tyrosine-based signal, promoted two things: clustering of the relevant receptors and clathrin assembly. Thus the model established was that AP-2 was a common adaptor that recognized and promoted the internalization of all cell surface proteins. This simple view, however, was soon dispelled when it became clear that a large number of other "adaptor" proteins existed for other receptors, including the β-arrestins that mediate internalization of some G protein–coupled receptors (GPCR) (5). AP-2, however, either directly or indirectly, still remains a central component of clathrin-mediated endocytosis of cell surface proteins.

The mechanism for recognition of the tyrosine-based signal, YXXΦ (where X is any amino acid and Φ is a bulky hydrophobic residue [6]) by AP-2 involves a direct interaction between one of the subunits of the AP-2 complex, μ2, and the 4-residue motif in the cytoplasmic tail of the cell surface protein (7). The AP-2 complex is a cytosolic heterotetramer consisting of two 100-kD chains (α and β2), one 50-kD chain (μ2), and a 17-kD chain (σ2). The crystal structure for part of the AP-2 heterotetramer revealed that the C-terminal domain of the μ2 subunit could accommodate the tyrosine-based signal into a hydrophobic pocket in the μ2 subunit (8). Interestingly, however, this hydrophobic binding pocket for the tyrosine-based motif is normally buried, suggesting that a conformation change would be required for interaction with the tyrosine-based signal (9). Subsequent studies provided evidence that phosphorylation of threonine 156 of the μ2 subunit is required for high-affinity binding and receptor internalization (10, 11), supporting the original model. This phosphorylation switch provided a key regulatory mechanism for controlling AP-2 function and subsequent receptor internalization.

Two kinases, AAK1 and GAK, have been proposed to phosphorylate this site *in vivo* and thus facilitate the AP-2 interaction with cargo (12, 13). The work by Chen and colleagues published in this issue of *AJRCMB* (pp. 127–132) provides evidence that a third kinase, PKC-ζ, may be involved as well (14). In an elegant series of studies, Chen and coworkers have developed a fascinating model for the complex regulation of the Na^+-K^+-ATPase pump, a membrane protein that is essential for vectorial movement of sodium across epithelia and for maintaining very different cytosolic and extracellular sodium concentrations to establish a sodium gradient for influx into the cell.

The Na^+-K^+-ATPase is a pump that uses ATP to transport Na^+ out of and K^+ into the cell and is composed of two major subunits: the α-subunit that uses ATP hydrolysis to exchange intracellular Na^+ for extracellular K^+, and the glycosylated β-subunit that controls heterodimer assembly (15). In renal tubule epithelial cells, hormones regulate Na^+-K^+-ATPase activity by controlling the surface expression of this molecule, thus providing a mechanism for controlling transepithelial sodium transport. Chen and coworkers demonstrate that this regulation is extremely complicated and that the distribution of the pumps in response to different agonists is tissue specific. In rodent renal epithelia, dopamine promotes Na^+-K^+-ATPase endocytosis, and this internalization is dependent upon phosphorylation of the Na^+-K^+-ATPase α_1-subunit at residue Ser-18 in the amino-terminal tail (16). This phosphorylation event provides the initiation event and a scaffold for organizing the assembly of molecules necessary for Na^+-K^+-ATPase α_1-subunit endocytosis in response to G protein–coupled receptor signals. The model proposed by Efendiev and coworkers (17) is that dopamine binding facilitates Ser-18 phosphorylation, and this allows for 14-3-3 binding to the amino-terminus of the α_1-subunit. 14-3-3 binding then facilitates PI-3 kinase recruitment to a nearby region in the amino-terminus of the α_1-subunit (a proline-rich region). Activated PI-3 kinase produces phosphatidylinositol 3-phosphate that is required for increasing the affinity of the μ2 subunit of AP-2 for the tyrosine-based motif in the C-terminal tail of the α_1-subunit, Y^{537}LEL (17). Phosphatidylinositol 3-phosphate is also responsible for recruiting dynamin to the coated pit, which is necessary in one of the last steps in Na^+-K^+-ATPase endocytosis, namely fission of the clathrin-coated pit.

What is the kinase that starts this process? In renal proximal tubules and lung alveolar epithelia, PKC-ζ is the kinase (14), based on the work from Chen and coworkers. They have shown that PKC-ζ phosphorylation of the α_1-subunit occurs in response to two different stimuli, dopamine and hypoxia (16, 18). This kinase begins the process, but interestingly, also appears to be involved in enhancing the interaction between the tyrosine-based motif in the α_1-subunit and the central player in this process, AP-2 through phosphorylation of the μ2 subunit (14). Although the role of AAK1 cannot be excluded from the present studies (14), the fact that a dominant-negative form of PKC-ζ blocked dopamine-dependent phosphorylation of μ2 indicates that PKC-ζ is somehow involved in this process. Inhibitor studies, however, suggest that AAK1 is involved as well, suggesting a potential kinase cascade. Are both kinases involved somehow?

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The lack of specific AAK1 kinase inhibitors makes this question difficult to address.

The studies by Chen and coworkers provide a framework for understanding how stimulation by a G protein–coupled receptor in renal epithelial cells and or by hypoxia (generated by mitochondrial reactive oxygen species) in lung alveolar epithelia result in similar cellular consequences, that is, removal of Na^+-K^+-ATPase pumps from the cell surface. What is the functional significance of this type of response? In the renal epithelia, the answer is straightforward: regulation of sodium and phosphate excretion. In airway epithelia, the answer is less clear, since a decrease in fluid clearance during hypoxia leads to airway complications that include edema and impaired gas exchange. Future studies investigating the relationship between PKC-ζ and AAK1 would help clarify the final steps in this process. Understanding the molecular details of Na^+-K^+-ATPase endocytosis in both renal and lung epithelia has important implications in treating hypertension as well as severe hypoxia or pulmonary edema. The fact that similar cellular mechanisms in different cell types regulate endocytosis of this essential transporter after a physiologic stimulus (dopamine) and after a pathologic stimulus (hypoxia) will continue to make this an intriguing area of investigation.

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