The ins and outs of polycystin-2 as a calcium release channel

Michael D. Cahalan

Mutations in either of two polycystin genes can cause kidney failure, but controversy remains regarding the cellular localization and function of the protein products. Polycystin-2 may be a calcium release channel located within the endoplasmic reticulum (ER), and yet may be physically linked to polycystin-1 in the surface membrane.

Genetic diseases of ion channels (channelopathies) have been identified rapidly over the past ten years. Most are rare disorders, but others are among the most common genetic disorders known. Frances Ashcroft summarizes mutations of various ion channel genes that result in disorders of the nervous system, skeletal muscle, heart, lungs, kidney and other organs[1]. Affecting 1 in 400–1000 live births, autosomal dominant polycystic kidney disease (ADPKD) constitutes the most common genetic cause of kidney failure in man. ADPKD results in the formation of numerous fluid-filled cysts within the kidney and liver, enlargement of these organs and renal failure in 50% of affected individuals. ADPKD is caused by mutations in either of two genes named for the disorder, *PKD1* and *PKD2,* which encode polycystin-1 and -2, respectively. These transmembrane proteins share some sequence similarity and physically interact at their carboxy termini, suggesting that they may form a heteromultimeric complex that functions as a receptor or an ion channel[2]. Polycystin-2 has sequence similarity to *TRP* genes (named for the transient receptor potential gene of Drosophila), within a putative pore-forming region. A new report on page 191 of this issue of *Nature Cell Biology*[3] addresses how polycystin-2 functions biophysically as a channel, where within the cell is it localized and how it functions within the context of the whole cell.

Recent studies indicate that polycystin-2 can indeed form an ion channel when expressed exogenously in *Xenopus* oocytes or in mammalian cell lines, or when reconstituted into lipid bilayer membranes from native placental membranes or affinity-purified protein[4–6]. Koulen *et al.*[3] show that polycystin-2 exhibits channel behaviour reminiscent of ryanodine receptors and IP_3 receptors. ER microsomes from kidney cells that were transfected to overexpress normal or truncated polycystin-2 were fused to lipid bilayers. The wild-type protein yielded a high-conductance channel that is permeable to calcium, with open probability enhanced by calcium elevation on the cytosolic side of the membrane, setting up the possibility of positive feedback between calcium flux and channel activity. An unusual twist to the story is that the channel is also voltage-dependent, being closed whenever the cytosolic side of the membrane is made positive, relative to the opposite side. The pathogenic C-terminal truncation mutant found in some affected families exhibited altered voltage dependence and its open probability was unaffected by calcium. These functional differences tended to close the mutant channel and further suggest that polycystin-2 itself is the channel.

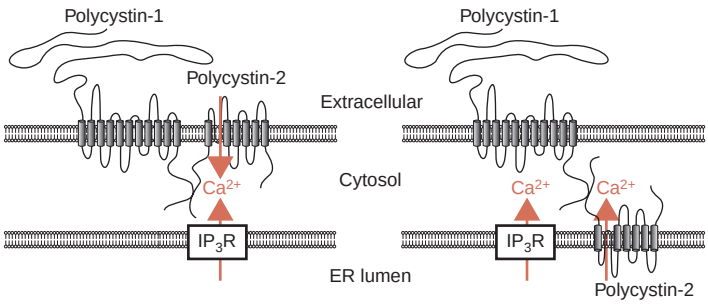

Figure 1 **Proposed cellular localization of polycystin-2. Left, the model of Hanakoa *et al.*[5], with polycystin-2 dragged to the surface membrane by polycystin-1 functioning as a chaperone. Right, the model of Koulen *et al.*[3], with polycystin-2 linked to polycystin-1, but functioning as a calcium release channel to augment calcium transients initiated by the IP_3 receptor (IP_3R).**

The subcellular localization of polycystin-2 has been a matter of some debate. Polycystin-2 has ER retention signals within a C-terminal domain, which prevents trafficking to the surface membrane when expressed on its own[7]. However, polycystin-2 has been shown to localize to the plasma membrane when abundantly expressed by a baculovirus vector in Sf9 cells. Moreover, polycystin-1 and polycystin-2 are physically associated proteins, as demonstrated by co-immunoprecipitation[8]. Co-expression of both polycystins resulted in the localization of polycystin-2 at or near the surface membrane by confocal microscopy and the detection of novel channel activity by whole-cell recording[5]. Thus, it was natural to believe that polycystin-2 might function as a surface membrane calcium-permeable channel, leading to the model shown in Fig. 1, left. Here, the new study by Koulen *et al.*[3] tips the pendulum back to the view that polycystin-2 functions as a calcium release channel located in the endoplasmic reticulum, but tethered close to the surface membrane by its physical association with polycystin-1 (Fig. 1, right). In cell lines and in native kidney, polycystin-1 was shown to be expressed in the endoplasmic reticulum, as judged by its localization to intracellular compartments by immunofluorescence confocal imaging, co-fractionation with ER markers, such as calnexin, and by sensitivity to endoglycosidase H digestion.

This restricted expression pattern is consistent with the physiology of calcium signalling. The most intriguing finding in the new paper by Koulen *et al.*[3] is that polycystin-2 increases calcium release from the ER when IP_3 is generated by surface receptor stimulation. A kidney cell line transfected with polycystin-2 exhibited greatly augmented calcium release transients in response to vasopressin, compared to wild-type cells or cells expressing either the C-terminal truncation mutant or

another pathogenic point mutant. Putting everything together — the calcium-dependent calcium channel activity, the localization to the ER and the enhanced calcium release activity — it seems that polycystin-2 may be a new type of calcium release channel, with properties that allow it to mediate calcium-induced calcium release (CICR). Moreover, channel function and the ability to augment calcium release were inhibited in the pathogenic mutants.

The paper will be significant for channel physiologists interested in intracellular calcium signalling, because it proposes that polycystin-2 is a new type of calcium release channel, in addition to ryanodine receptors and IP_3 receptors. This paper also resonates with the TRP field. There are about 20 *TRP*-related genes that can often function as nonselective cation channels, which often exhibit calcium permeability and can be activated in numerous ways. In addition to having a pore region similar to TRP, polycystin-2 can physically associate with TRPC1 (ref. 9). Physical linkage between proteins in the surface membrane and the endoplasmic reticulum may mediate capacitative calcium entry and most certainly underlies excitation–contraction coupling in skeletal muscle. Among the mysteries that remain are how polycystin-2 coupled to polycystin-1 can result in channel activity[5] and how polycystin-2 channel dysfunction and possible calcium dysregulation can cause the disease phenotype found in ADPKD. □

Michael D. Cahalan is in the Department of Physiology and Biophysics, University of California, Irvine, CA 92697-4561, USA
e-mail: mcahalan@uci.edu

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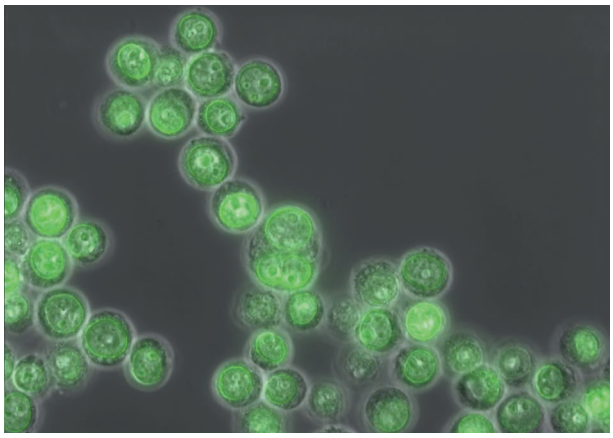
Getting hit by SUMO

Proteins can be targeted to different fates through post-translational modifications. For instance, the addition of ubiquitin (or some other ubiquitin-like proteins) to lysine residues or to the amino terminus of proteins can induce degradation by the proteasome, intracellular trafficking or activation. Targeting by one particular ubiquitin-like protein, the small ubiquitin-related modifier 1 (SUMO1) can affect protein interactions, subcellular localization or stability. Now Frauke Melchior and her colleagues at the Max-Planck Institute in Martinsried, Germany, describe a new link between protein SUMOylation and nuclear transport.

Modification of proteins by ubiquitin is a three-step process that involves three classes of enzymes, E1–E3. The E1 activating enzyme and the E2 conjugating enzyme prime ubiquitin for ligation to a substrate by an E3 ubiquitin ligase. There are several E3 ligases that are responsible for transferring activated ubiquitin to their substrates. These confer substrate specificity. An E1 (Aos1/Uba2) and an E2 (Ubc9) enzyme have been previously described for SUMO1. More recently, RING-domain-containing proteins of the protein inhibitors of activated STATs (PIAS) family have been identified as E3 ligase enzymes for SUMO1. Now, Melchior and colleagues show that the nucleoporin RanBP2, which has been previously characterized for its function in nuclear transport, is also an E3 ligase for SUMO1. They find that purified RanBP2 exerts E3 ligase activity on some SUMO1 targets *in vitro* (such as Sp100) but not on all of them (indeed p53 could be SUMOylated by PIAS1, but not by RanBP2), indicating substrate specificity. Furthermore, depletion of RanBP2 and its binding partner RanGAP1 from cytosolic extracts results in the loss of SUMOylation activity, which can be rescued by adding back immunoprecipitates containing RanBP2.

Interestingly, the domain of RanBP2 that contains the E3 ligase activity bears no structural, and apparently no functional, homology to either RING finger motifs or HECT domains, two domains that have been previously shown to bear ubiquitin E3 ligase activity in other proteins. This suggests that the mechanism of RanBP2 activity is different to ubiquitin E3 ligases.



Melchior and coworkers also describe the localization of a fluorescently tagged SUMO1 protein (shown in green in the figure) to the nucleus and to the cytoplasmic side of the nuclear pore complex (NPC). As RanBP2 is part of the machinery that mediates nucleocytoplasmic transport of proteins with a nuclear localization signal (NLS), Melchior's findings provide a rationale for the observation that most SUMO1 target-proteins require an NLS for *in vivo* modification. Active import seems to contribute to the accumulation of tagged SUMO1 in the nucleus. Furthermore, tagged SUMO1 at the cytoplasmic side of the NPC seems to be conjugated to unknown proteins, consistent with the idea that conjugation of SUMO1 to targets by RanBP2 precedes import. Altogether, these findings raise some interesting questions about the relationship between nuclear transport and SUMOylation. These two events seem to be coordinated, and one can even speculate that SUMOylation could be involved in the translocation process itself.

VALERIE FERRIER