Polycystins: inhibiting the inhibitors

Robert Benezra

Polycystin-1 and -2 — two integral membrane proteins that are mutated in polycystic kidney disease — regulate the cell cycle by preventing nuclear localization of the pro-proliferative helix-loop-helix (HLH) protein Id2. This novel mechanism for restraining Id proteins has important implications for our understanding of the nature of polycystic kidney disease and perhaps other proliferative disorders.

Autosomal dominant polycystic kidney disease (ADPKD) affects 1 in 1,000 people worldwide and is a hyperproliferative disorder characterized by the appearance of fluid-filled cysts in the tubules and collecting ducts of the affected kidney[1]. Although key players in the proliferative response have been identified, a detailed molecular process has yet to be described. On page 1202 of this issue, Jing Zhou and colleagues uncover an important mechanistic insight into the nature of the proliferative response in affected patients: nuclear translocation and activation of the growth stimulatory protein Id2 (ref. 2).

ADPKD arises when a loss-of-function mutation occurs in one copy of either of the genes (*PKD1* or *PKD2*) encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively, and the remaining normal copy is subsequently inactivated[3]. PC1 and PC2 are membrane glycoproteins with complex structures. PC1 has 11 predicted transmembrane-spanning segments that follow an extracellular amino-terminal portion containing several adhesive domains implicated in cell–cell and cell–matrix interactions. PC1 also contains a short intracellular carboxyl terminus that has a G-protein activation site and a coiled-coiled protein interaction domain. The latter domain mediates interaction with PC2 (ref. 4), an ion-channel protein with six predicted membrane-spanning segments and a short intracellular C-terminal region containing a calcium-binding domain, an endoplasmic reticulum retention signal and a coiled-coiled PC1-binding domain. PC1 recruits PC2 to the plasma membrane[5], and the PC1–PC2 complex is thought to function as a receptor–ion-channel complex[5] in which PC1 regulates both the activity of PC2 and intrinsic G-protein signalling[6,7].

Robert Benezra is in the Memorial Sloan-Kettering Cancer Center, Box 241, 1275 York Avenue, New York City, NY 10021, USA.
e-mail: r-benezra@ski.mskcc.org

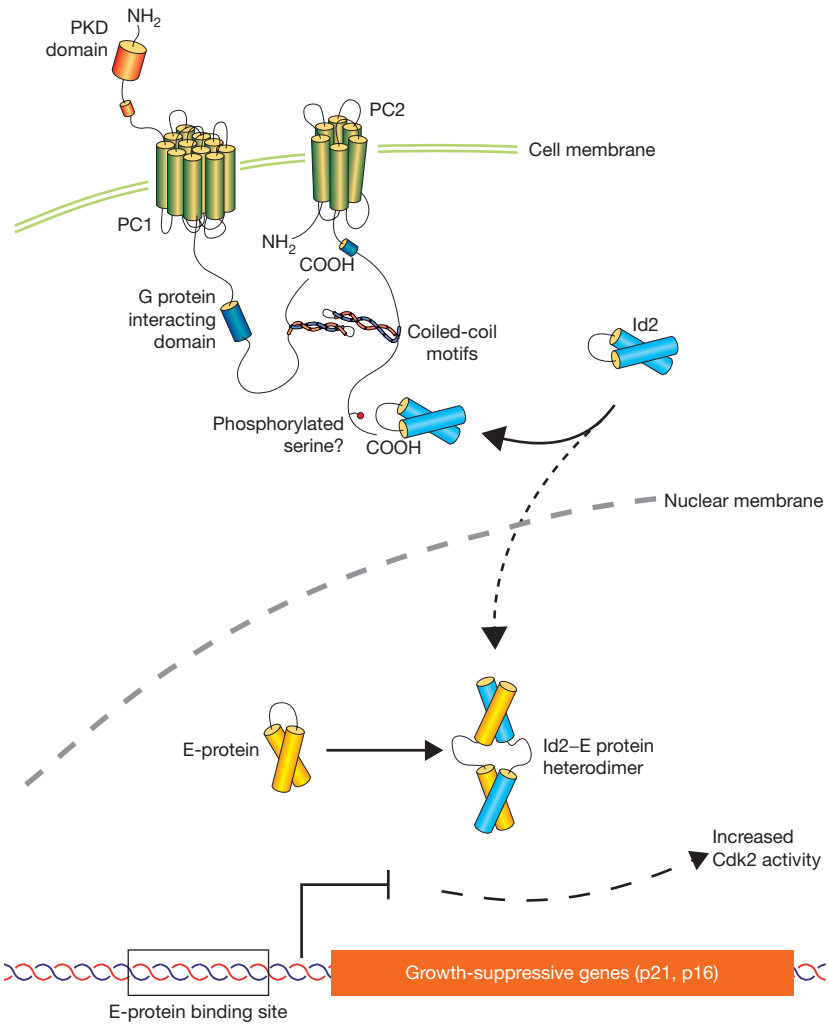

Figure 1 PC2 sequesters Id2 in the cytoplasm. Id2 is normally prevented from entering the nucleus through its association with the phosphorylated C-terminal domain of PC2. When PC1 or PC2 are inactivated, Id2 translocates to the nucleus (dashed arrow) where it blocks the ability of E proteins to turn on growth-suppressive genes, either alone or in combination with other basic-HLH proteins. This results in increased Cdk2 activity. PKD, polycystic kidney disease repeat motifs.

So how does this complex affect proliferation? Activation of the transcription factor AP-1 (ref. 8) and Jak–STAT signalling[9] by the PC1–PC2 complex has been postulated, and the interaction of this complex with E-cadherin at adherens junctions suggests a connection with the Wnt–β-catenin cell-cycle signalling machinery. Indeed, the C-terminal region of PC1 has been shown to stabilize β-catenin[10]. Zhou and colleagues now show that when PC2 is overexpressed, it can physically associate with the HLH protein Id2 and block its translocation to the nucleus[2]. This is significant because Id

proteins have been identified as key regulators of differentiation and proliferation in a variety of cellular contexts.

There are four members of the Id family (called Id1–4) and all are expressed at high levels in various regions throughout the early embryo[11]. Id proteins lack a DNA-binding domain and exert their effects by binding to positively acting HLH proteins and preventing them from associating with DNA. The best-characterized binding partners for Id proteins are the ubiquitously expressed E protein family members — obligate heterodimerizing partners of tissue-restricted HLH transcription factors, such as MyoD and NeuroD, that regulate cell differentiation. By sequestering E proteins away from these partners, the Id proteins can alter cell fate and cell-cycle progression in multiple tissue types. Id2 is particularly interesting as it is the only member of the Id family that not only associates with E proteins and blocks their ability to activate transcription of growth-suppressive genes such as p21 (ref. 12), but it is also restrained by the tumour suppressor Rb at the G1/S cell-cycle boundary[13]. Zhou and colleagues show that PC1 is required for the PC2–Id2 interaction by demonstrating PC1-dependent phosphorylation of a C-terminal serine residue in PC2 that promotes its association with Id2[2]. These data predict that when PC2 or PC1 is lost in ADPKD, Id2 can enter the nucleus and overwhelm the growth-restraining effects of E proteins and Rb.

This prediction is confirmed by two lines of experimental evidence. First, the authors show that mutation of *PKD1* and *PKD2* in patients and gene targeting of *Pkd1* in mice leads to the accumulation of Id2 in the nucleus of kidney epithelial cells[2]. This translocation, at least in mouse kidney tissue, is associated with a sharp decrease in p21 expression. Moreover, in mouse embryonic kidney cells derived from wild-type and *Pkd1*-mutant mice, the nuclear E protein–Id2 complex is observed only when PC1 is absent and the PC2–Id2 interaction is disrupted. This competition between E proteins and PC2 for Id2 was confirmed in overexpression experiments in which PC2 was shown to override the ability of E proteins to escort Id2 into the nucleus. Given the nanomolar affinity of E proteins for Id2, these results indicate a strong Id2–PC2 interaction and make a compelling case for its physiological relevance. Second, the cell-cycle profile of PC1 mutant cells was normalized when Id2 levels were reduced with RNA interference, consistent with the model proposed.

Modulation of Id protein localization during differentiation is not unprecedented. Id1 and Id2 proteins have been reported to redistribute to the cytoplasm during muscle and oligodendrocyte differentiation before they degrade[14,15]. This may be partly caused by the loss of association with E proteins, which can chaperone Id proteins into the nucleus[16]. The Zhou study expands on these observations by identifying a molecular anchor that is required to sequester Id2 in the cytoplasm and by making a case for the involvement of this mechanism in the pathophysiology of ADPKD.

Although overexpression of Id proteins has been shown to affect the growth properties of normal and transformed cells in culture, the timing and location of *in vivo* overexpression is a controversial subject[11]. This study opens up the possibility that re-localization of the Id proteins may represent another cellular strategy to enhance or to restrain their activity.

As with all significant advances, a number of important follow-up experiments are suggested. As *Id2* knockout mice are viable, it will be of great interest to determine whether Id2 loss ameliorates the phenotype of the *Pkd*-mutant mice in a genetic cross of these strains. Conditional loss of Id2 in the kidney would be the most informative experimental approach. Is loss of p21 (through translocation of Id2 to the nucleus) the major determinant of the proliferative response or are targets other than p21 contributing to the disease phenotype? Is Rb part of a larger molecular complex involving the PC proteins and Id2? Are other Id proteins (such as Id1 or 3) regulated in similar ways? The answers to these questions should provide a more thorough understanding of the molecular basis of ADPKD and perhaps guide us towards new strategies for targeted therapeutic intervention. □

1. Delmas, P. *et al. Biochem. Biophys. Res. ommun.* **322**,1374–1383 (2004).
2. Li, X. *et al. Nature Cell Biol.* **7,** 1202–1212 (2005).
3. Wu, G. *et al. Cell,* **93,** 177–188 (1998).
4. Newby, L.J. *et al. J. Biol. Chem.* **277,** 20763–20773 (2002).
5. Hanaoka, K. *et al. Nature* **408,** 990–994 (2000).
6. Delmas, P. *et al. FASEB J.* **18,** 740–742 (2004).
7. Delmas, P. *et al. J. Biol. Chem.* **277,** 11276–11283 (2002).
8. Parnell, S.C. *et al. J. Biol. Chem.* **277,** 19566–19572 (2002).
9. Bhunia, A.K. *et al. Cell* **109,** 157–168 (2002).
10. Kim, E. *et al. J. Biol. Chem.* **274,** 4947–4953 (1999).
11. Perk, J., Iavarone, A. & Benezra, R. *Nature Rev. Cancer* **5,** 603–614 (2005).
12. Prabhu, S., Ignatova, A., Park, S.T. & Sun, X.H. *Mol. Cell Biol.* **17,** 5888–5896 (1997).
13. Lasorella, A., Noseda, M., Beyna, M. & Iavarone, A. *Nature* **407,** 592–598 (2000).
14. Sun, L., Trausch-Azar, J.S., Ciechanover, A. & Schwartz, A.L. *J. Biol. Chem.* **280,** 26448–26456 (2005).
15. Wang, S. *et al. Neuron* **29,** 603–614 (2001).
16. Deed, R.W., Armitage, S. & Norton, J.D. *J. Biol. Chem.* **271,** 23603–23606 (1996).

nature cell biology

ERRATUM

Owing to a technical error, the pages of this manuscript were originally mis-numbered by a 100 pages. This has now been corrected online. The corrected online manuscript is numbered 100 pages higher than the mis-numbered version.