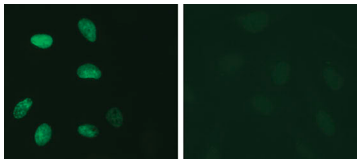
In This Issue

Text by Ben Short
bshort@rockefeller.edu

Cdks put Treslin on TopBP1



DNA synthesis (indicated by green nuclei) occurs in cells expressing wild-type Treslin (left) but not in cells expressing a nonphosphorylatable version of the protein (right).

Kumagai et al. describe how cyclin-dependent kinases (Cdks) initiate DNA replication by promoting the interaction of two proteins conserved from yeast to mammals.

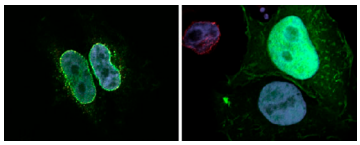
In budding yeast, Cdks phosphorylate two replication origin proteins called Sld2 and Sld3, allowing them to bind a third protein called Dpb11. Together, these proteins recruit Cdc45, which then promotes the activation of the MCM helicase complex to separate the two strands of DNA and initiate replication. Whether the same system operates in higher organisms is unclear because the vertebrate homologues of Sld2 and Sld3 have remained obscure.

Kumagai et al. previously identified a protein called Treslin as a candidate homologue of Sld3. Treslin is required for DNA replication in *Xenopus* egg extracts and human cells, and it binds to TopBP1—the vertebrate homologue of Dpb11—in the presence of Cdks. The researchers now show that Cdks phosphorylate Treslin on a specific serine residue in a region of the protein that associates with TopBP1 and that is relatively similar in sequence to yeast Sld3. When this serine was mutated to alanine, Treslin could no longer bind to TopBP1, and cells expressing this mutant instead of wild-type Treslin were unable to replicate their DNA. In addition, Treslin bound *Xenopus* Cdc45, suggesting that it really is the vertebrate orthologue of Sld3.

The authors now want to examine how Treslin and TopBP1 integrate Cdc45 into the replication initiation complex. They also want to investigate why Cdks apparently phosphorylate additional sites in the Treslin sequence.

Kumagai, A., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201102003.

Epac1 cAMPs out at nuclear pores



RanBP2 (red) anchors Epac1 (green) to the nuclear envelope in control cells (left), but RanBP2 knockdown leads to Epac1's enrichment in the nucleus and cytoplasm (right).

The nucleoporin RanBP2 tethers a GTPase regulatory protein to nuclear pores, thereby limiting its ability to promote cell adhesion, Gloerich et al. reveal.

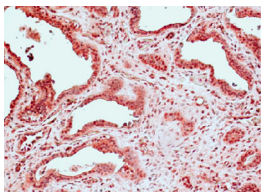
Epac1 is a guanine nucleotide exchange factor that activates the Rap1 GTPase to promote a variety of cellular functions, including intercellular and cell–matrix adhesion. Epac1 itself is activated by several cell signaling pathways through the second messenger cAMP. In addition, the exchange factor is anchored by various proteins to specific locations in the cell to control exactly where Rap1 is switched on.

Gloerich et al. discovered that Epac1 is anchored to the nuclear envelope by the nuclear pore protein RanBP2. However, the team found that Epac1 is kept inactive at this location because RanBP2's zinc fingers bind directly to the exchange factor's catalytic domain. Knocking down RanBP2 by RNAi released Epac1 into the cytoplasm and boosted its ability to activate Rap1 and promote cell adhesion.

cAMP was unable to free Epac1 from RanBP2's clutches, but the exchange factor was liberated at the onset of mitosis, when RanBP2's zinc fingers are phosphorylated by mitotic kinases. Senior author Johannes Bos doesn't yet know the identity of these kinases, but he speculates that Epac1's release in mitosis might help cells rapidly reestablish their contacts with neighboring cells and the extracellular matrix following cell division.

Gloerich, M., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201011126.

Aurora A limits calcium release



Red staining indicates the presence of activated Aurora A in the epithelial cells lining cysts in PKD patient kidneys.

The Aurora A kinase may contribute to polycystic kidney disease (PKD) by inactivating a key calcium channel in kidney cells, Plotnikova and Golemis say.

Aurora A is an oncogene best known as a regulator of mitotic progression. But the kinase has important functions during interphase as well, when it can promote cilia disassembly and can be activated by elevated calcium levels. Because both calcium signaling and cilia are defective in PKD, Plotnikova and Golemis wondered whether Aurora might contribute to the pathology of this common genetic disease.

The researchers found that Aurora A was up-regulated and activated in epithelial cells lining the cysts in PKD patient kidneys. In addition, Aurora A bound to and phosphorylated a calcium channel called polycystin-2, whose gene, *PKD2*, is often mutated in autosomal dominant forms of PKD. This interaction was enhanced by Aurora A's regulatory partner NEDD9.

Polycystin-2 mediates calcium influx into cilia and the release of calcium from storage in the endoplasmic reticulum. Inhibition or knockdown of Aurora A boosted intracellular calcium levels, but this effect was less pronounced in kidney cells lacking polycystin-2, indicating that the kinase normally lowers calcium levels by inactivating this channel. Only small doses of inhibitor were required to increase calcium levels, suggesting that Aurora A may be a viable therapeutic target for boosting polycystin-2 activity in certain PKD patients. Senior author Erica Golemis now wants to investigate how Aurora A becomes up-regulated in PKD and whether inhibitors of the kinase can slow cystogenesis in mouse models of the disease.

Plotnikova, O.V., and E.A. Golemis. 2011. *J. Cell Biol.* doi:10.1083/jcb.201012061.