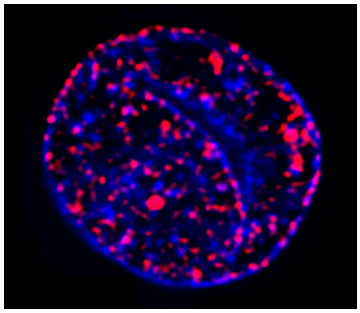
In This Issue

Text by Mitch Leslie
mitchleslie@comcast.net

Cdks set the replication schedule



DNA replication sites (red) light up in a nucleus stimulated with cyclin A.

The cyclin-dependent kinases (Cdks) have an even bigger role in DNA replication than researchers thought, Thomson et al. reveal. The enzymes not only activate individual replication sites, they also control the large-scale pattern of DNA duplication by turning on clusters of replication forks known as replication factories.

DNA replication sticks to a script. Large sections of the genome duplicate in a particular sequence during S phase. What drives the replication timing program is a mystery. Possible candidates are the Cdks, which spur individual replication origins to start copying. The researchers tested how Cdks contribute using hamster cell nuclei in *Xenopus* egg extracts, a setup that accelerates DNA duplication while preserving the copying pattern.

Thomson et al. found that inhibiting Cdks slowed or even stopped progression through the timing program. Stimulating Cdk activity had the opposite effect. Those results indicate that Cdks ensure that cells move from stage to stage in the replication program. The researchers then determined how this is achieved. Boosting Cdk activity increased the number of working replication factories, in many cases without changing the number of replication forks within each factory. Overall, the findings suggest that Cdks can advance the replication timing program by enlisting additional replication factories. How Cdks switch the factories on remains to be determined.

Thomson, A.M., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200911037.

Misfolded proteins go their separate ways

Bernasconi et al. clarify how mammalian cells remove misfolded ER proteins for disposal—how a particular protein exits depends on its ER location.

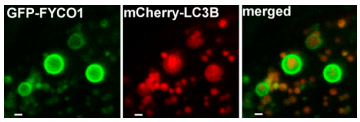
Newly made proteins idle in the ER while they fold into shape. Proteins that make a mistake during the process are usually expelled from the ER and destroyed by the proteasome. Several protein complexes embedded in the ER membrane can remove these rejects, and evidence from yeast suggests that the location of the error within the protein determines which complex handles its elimination. For example, proteins with flaws in their cytoplasmic section are shunted through the complex that contains the enzyme DOA10. But if the damage falls in the section of the protein protruding into the ER lumen, the HRD1-containing complex clears the damaged molecule. Bernasconi et al. wanted to determine what happens in mammals, where the situation is hazy.

The researchers tested how mammalian cells dispose of the enzyme β-secretase (BACE) and CD3-δ, which is part of the T cell receptor. Both proteins had folding errors in the lumenal region, which in yeast would mean the same pathway would expel them from the ER. But Bernasconi et al. found that another factor was important in mammalian cells: whether the protein was soluble or connected to the ER membrane. CD3-δ normally carries a membrane-tethering segment. Removing this anchor made the protein soluble in the ER and changed which pathway was used for disposal. Deleting the tether from BACE also altered its exit route.

Bernasconi et al. also showed that two mysterious proteins serve as shuttles, delivering faulty soluble proteins to the ER membrane for disposal. The shuttles didn't transport tethered proteins, however—membrane-anchored proteins are already near the disposal complexes and don't need to be fetched. A key question that needs to be answered, the researchers say, is which protein channel ushers the misfolded proteins through the ER membrane.

Bernasconi, R., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200910042.

FYCO1 gets autophagy on track



FYCO1 (green) and LC3 (red) occur together on the rim of autophagosomes (right).

A newly discovered protein helps clean up cellular trash, Pankiv et al. show. The protein attaches cytoplasmic refuse collectors to cargo-hauling motors.

Autophagy allows cells to dispose of worn-out or damaged proteins and organelles. During the process, a membrane pouch called an autophagosome surrounds a portion of the cell's contents and ferries the material to the lysosome for digestion. Researchers suspect that autophagosomes travel to their destinations along microtubules, but how they move is uncertain. Pankiv et al. report that the previously undescribed protein FYCO1 connects to three key autophagy molecules and appears to couple autophagosomes to molecular motors.

The researchers identified the molecule because it latches onto LC3, a protein embedded in the surface of autophagosomes. But FYCO1 isn't faithful. It also sticks to the phospholipid PI3P, a component of the autophagosome membrane, and to the GTPase Rab7, which helps autophagosomes merge with lysosomes. The researchers surmise that FYCO1 and its partners form a trailer hitch that connects an autophagosome to a molecular motor.

When Pankiv et al. cranked up FYCO1 production in cells, they found that vesicles carrying Rab7 cruised toward the plus ends of microtubules and accumulated at the cell's edges. The direction of movement suggests that FYCO1 links to kinesin molecular motors. A separate connection would attach the autophagosome to dynein motors that tug in the opposite direction, allowing travel toward the other end of a microtubule.

Pankiv, S., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200907015.