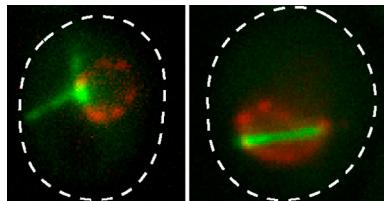


Microtubules support nuclear nonproliferation arrangement



Microtubules (green) form in the cytoplasm of a proliferating yeast (left) but span the nucleus (red) of a quiescent cell (right).

Laporte et al. describe how a microtubule array reorganizes the nucleus to aid the survival of quiescent budding yeast.

Eukaryotic cells undergo a dramatic rearrangement when they temporarily exit the cell cycle and become quiescent. Budding yeast, for example, reorganize their actin cytoskeleton into immobile structures called actin bodies when they stop proliferating, but what happens to microtubules in these cells is unknown.

Laporte et al. found that, when yeast ran out of nutrients and entered quiescence, they formed an array of stable microtubules emanating across the nucleus from the spindle pole body (the yeast

equivalent of the centrosome). These microtubules caused a drastic reorganization of the cell nucleus. The nucleolus was pushed to the side instead of lying opposite the spindle pole body as it does in proliferating yeast. And the centromeres of each chromosome—attached to microtubule plus-ends—were spread out along the nuclear microtubule array, instead of clustering near the spindle pole body as they do in G1.

Quiescent yeast required the kinesin Kar3 and the dynein/dynactin motor complex to form the nuclear microtubule array. In the absence of these proteins, yeast no longer rearranged their nuclei and were less able to survive quiescence and reenter the cell cycle when nutrients became available again. Senior author Isabelle Sagot speculates that microtubule-induced changes in nuclear organization might alter the expression of genes that help establish and maintain quiescence. She now wants to investigate how quiescent yeast assemble the nuclear microtubule array and to examine its effects on gene expression.

Laporte, D., et al. 2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201306075>.

AKT shuts down the TOR network

Hálová et al. identify a new mechanism by which yeast and mammalian cells regulate the activity of TOR protein kinases.

In response to environmental stimuli, TOR kinases regulate a range of cellular processes, from growth and proliferation to differentiation and cytoskeletal organization. The kinases assemble into two distinct complexes, TORC1 and TORC2, whose activities are tightly regulated by multiple signaling pathways. Hálová et al. wondered whether any of these pathways might regulate TOR by controlling the kinase's phosphorylation.

The researchers initially focused on fission yeast Tor1. This kinase, which assembles into the TORC2 complex, was phosphorylated on a conserved residue, threonine 1972, in the enzyme's ATP-binding domain. A nonphosphorylatable version of Tor1 showed increased kinase activity, and yeast expressing this mutant showed increased resistance to oxidative and osmotic stress. Compared with

wild-type cells, however, yeast expressing nonphosphorylatable Tor1 were less able to arrest and differentiate in response to nitrogen starvation, a process that requires Tor1 activity to be suppressed.

Hálová et al. found that the kinase Gad8 inhibited Tor1 by phosphorylating threonine 1972 under low nitrogen conditions. How the inhibition of Tor1 promotes arrest and differentiation is unclear; Gad8 itself is a target of Tor1, but other, yet-to-be identified substrates may be more significant.

In mammalian cells, the Gad8 homologue AKT also phosphorylated and inhibited mTOR. As in fission yeast, this phosphorylation was remarkably stable, which could help environmentally induced changes in TOR activity to persist. Senior author Janni Petersen now wants to investigate whether protein turnover, rather than phosphatase activity, is required for cells to reactivate Tor1 signaling when conditions change once more.

Hálová, L., et al. 2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201305103>.

A call for oxygen in the ER

Translated proteins require oxygen to form disulfide bonds, Koritzinsky et al. reveal, which may explain why low oxygen levels activate the unfolded protein response (UPR).

The UPR alleviates stress in the endoplasmic reticulum (ER) by suppressing translation and up-regulating chaperones and other factors that promote protein folding in the ER lumen. The fact that hypoxia induces the UPR suggests that oxygen is somehow required for the ER to process and export secretory cargo. But which of the ER's many activities depends on oxygen is unknown.

Koritzinsky et al. followed the maturation and transport of several secretory proteins in hypoxic cells and found that the absence of oxygen didn't inhibit protein glycosylation or vesicle transport. But low oxygen levels did impair the ability of newly synthesized proteins to form disulfide bonds, a critical step in the folding and maturation of many secretory proteins.

Disulfide bonds are introduced by a redox relay involving ER-localized protein disulfide isomerases (PDIs) and oxidases. The process can start while an ER cargo protein is still being translated but continues post-translationally, with multiple cysteine residues breaking and re-forming disulfide bonds until the correct conformation is achieved. Oxygen has been shown to accept the electrons that pass through PDIs and oxidases when disulfide bonds are formed in vitro. But Koritzinsky et al. found that, *in vivo*, oxygen was only required for the formation of post-translational disulfide bonds, suggesting that disulfide bonds formed during translation are created by distinct PDIs and oxidases that can use an alternative electron acceptor. The researchers now want to identify these enzymes and to determine what electron acceptor they use instead of oxygen.

Koritzinsky, M., et al. 2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201307185>.