In This Issue

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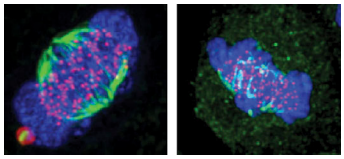
Hsp72 gives spindles a fiber supplement



Compared with a control cell (left), the number of cold-resistant K-fibers (green) is reduced in a cell lacking Hsp72 (right). Chromosomes (blue) and kinetochores (red) are also labeled.

O'Regan et al. reveal that the mitotic kinase Nek6 targets the chaperone Hsp72 to the mitotic spindle, where it promotes the formation of stable K-fibers.

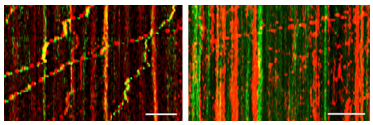
Nek6 is required for robust spindle assembly and mitotic progression, but its substrates are largely unknown. O'Regan et al. found that the kinase bound to Hsp72 and, during mitosis, phosphorylated a conserved threonine residue in the chaperone's nucleotide-binding domain. This promoted Hsp72 recruitment to the mitotic spindle. Knocking down the chaperone caused mitotic HeLa cells to form fewer K-fibers, stable microtubule bundles that connect chromosomes to the spindle poles. As a result, chromosomes often failed to congress at the cell equator during metaphase or failed to stay there once they had arrived, thus delaying anaphase onset.

Hsp72 promoted the interaction between ch-TOG and TACC3, which crosslink K-fiber microtubules. Both proteins were lost from mitotic spindles in the absence of either Hsp72 or Nek6, but a phosphomimetic version of Hsp72 rescued ch-TOG/TACC3 localization, K-fiber formation, and mitotic progression in Nek6-deficient cells.

Hsp72 and related chaperones are often overexpressed in human tumors, and cancer cells that frequently contain aneuploid genomes and amplified centrosomes are particularly sensitive to mitotic spindle defects. Hsp72 and/or Nek6 could therefore be attractive therapeutic targets to inhibit spindle assembly and cancer cell division. Senior author Andrew Fry now wants to investigate how Nek6 phosphorylation targets Hsp72 to the spindle. Elsewhere in this issue, Fry, together with Suzanna Prosser and colleagues, demonstrate that the related kinase Nek5 regulates the timing of centrosome separation, another process that orchestrates spindle assembly in mitosis.

O'Regan, L., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201409151.

Axonal autophagosomes use a rideshare service



Kymographs show that, in a control axon (left), markers of autophagosomes (green) and late endosomes (red) move together on amphisomes (yellow). When the organelles' fusion is inhibited (right), autophagosomes are selectively immobilized.

Autophagosomes gain the dynein motor complexes they need to move away from axonal terminals by fusing with late endosomes, Cheng et al. reveal.

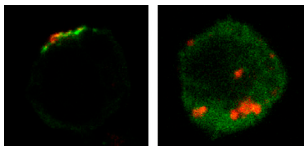
During autophagy, autophagosomes engulf cytoplasmic components and deliver them to lysosomes for degradation. In neurons, mature acidic lysosomes are enriched in the cell body, so autophagosomes formed at the distal ends of axons must be transported back to the soma by the motor protein dynein. How dynein is recruited to autophagosomes is unknown, however.

Cheng et al. found that the majority of axonal autophagosomes fuse with late endosomes to form intermediate organelles known as amphisomes. Unlike autophagosomes, but similar to late endosomes, amphisomes were associated with dynein and moved along axons toward the cell body. This suggested that autophagosomes might become mobile by fusing with late endosomes and sharing their complement of dynein motors. Accordingly, blocking dynein's recruitment to late endosomes by inhibiting the adaptor protein snapin impaired the movement of amphisomes toward the cell body, and reducing the ability of autophagosomes to fuse with late endosomes caused immobile autophagic compartments to accumulate in axon terminals.

Live imaging revealed that autophagosomes can fuse with late endosomes and move retrogradely along axons within one minute of their formation. Senior author Zu-Hang Sheng says that similar events occur much more slowly in nonneuronal cells. He therefore wants to investigate whether local calcium levels speed up autophagosome fusion in synaptic terminals.

Cheng, X.-T., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201412046.

Rab27a builds a platform for HIV-1



Compared with a control T cell (left), knocking down Rab27a blocks the delivery of late endosomes (red) to the plasma membrane, inhibiting the recruitment of $Pr55^{Gag}$ (green) to HIV-1 assembly sites.

The endosomal trafficking protein Rab27a supports HIV-1 replication by promoting $PI(4,5)P_2$ production at the plasma membrane (PM), Pereyra Gerber et al. reveal.

New HIV-1 particles assemble at specialized PM domains that are enriched in the phospholipid $PI(4,5)P_2$ and recruit the viral polyprotein $Pr55^{Gag}$. Because endosomal trafficking has been implicated in viral assembly and release, Pereyra Gerber et al. investigated whether HIV-1 replication was controlled by Rab27a, a small GTPase that promotes the delivery of late endosomes and multivesicular bodies to the PM.

Viral replication was impaired in T cells lacking Rab27a, the researchers found. These cells showed reduced levels of $PI(4,5)P_2$ at the PM and thus failed to recruit $Pr55^{Gag}$ to form viral assembly sites. Knocking down Rab27a also suppressed $PI(4,5)P_2$ production and viral replication in macrophages, which normally recruit $Pr55^{Gag}$ to PM invaginations called virus-containing compartments.

Rab27a boosted $PI(4,5)P_2$ production at the PM by delivering the late endosome-associated lipid kinase PI4KIIα, which generates the $PI(4,5)P_2$ precursor PI(4)P. Several Rab27a effectors were also required for HIV-1 replication. T cells lacking the endosomal docking protein Slp2a, for example, also failed to deliver PI4KIIα to the PM to promote $PI(4,5)P_2$ production and $Pr55^{Gag}$ recruitment.

Senior author Matías Ostrowski says that these results open a path to investigate whether manipulating endosomal traffic could be a new target for anti–HIV-1 therapies. He now wants to investigate how endosomes carrying PI4KIIα fuse with the PM once they have been docked there by Rab27a and its effectors.

Pereyra Gerber, P., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201409082.