In This Issue

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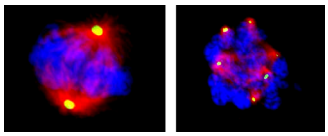
MLL5 limits PLK1 aggregation



In the absence of MLL5 (right), PLK1 aggregates in the cytoplasm and forms additional spindle poles containing pericentrin (green). Microtubules are labeled red. DNA is blue.

Zhao et al. reveal that the prospective tumor suppressor MLL5 helps mitotic cells maintain a bipolar spindle by preventing the kinase PLK1 from aggregating in the cytosol.

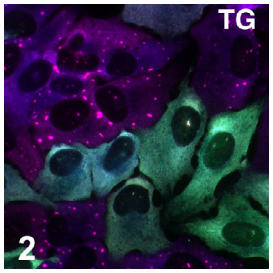
MLL5, whose gene is often deleted in patients with acute myeloid leukemia, localizes to nuclear speckles and regulates chromatin organization and the cell cycle. During mitosis, cells lacking MLL5 form multipolar spindles with misaligned chromosomes, resulting in genomic instability. MLL5 promotes chromosome alignment by stabilizing the Aurora B kinase–containing chromosomal passenger complex, but how MLL5 limits the formation of multipolar spindles remains unknown.

Zhao et al. found that, in addition to nuclear speckles, MLL5 localizes to centrosomes throughout the cell cycle. During mitosis, MLL5 delocalized from chromatin and bound to PLK1, a key regulator of centrosome maturation that recruits and phosphorylates several microtubule-organizing proteins. In the absence of MLL5, PLK1's localization to centrosomes was partially reduced, and the kinase also formed numerous aggregates dotted throughout the cytoplasm. Many of these aggregates recruited additional proteins, such as pericentrin and γ-tubulin, to form acentrosomal microtubule-organizing centers (aMTOCs) that contributed to the assembly of multipolar spindles.

The researchers discovered that a motif in MLL5's central region binds to the polo-box domain of PLK1. Mutating this motif inhibited MLL5's ability to prevent PLK1 aggregation and multipolar spindle formation. Senior author Lih-Wen Deng thinks that, by limiting PLK1 aggregation, cytosolic MLL5 enhances the kinase's incorporation into centrosomes. She now wants to investigate how, in the absence of MLL5, PLK1 aggregates develop into aMTOCs.

Zhao, W., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201501021

A switch for stress granule assembly



Cells overexpressing USP10 (green) fail to form stress granules (magenta) when translation initiation is inhibited.

Kedersha et al. describe how phosphorylation and the competition between mutually exclusive binding partners regulate G3BP's ability to mediate stress granule assembly.

When translation initiation is inhibited, mammalian polysomes disassemble into ribonucleoprotein particles that form microscopically visible stress granules (SGs). The RNA-binding protein G3BP can nucleate SG assembly—its overexpression induces SG formation even in translation competent cells—but how the protein does this, and how its activity is regulated, remains unknown.

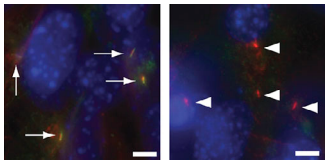
Kedersha et al. found that cells lacking both isoforms of G3BP were unable to form SGs in response to stresses that inhibit translation initiation via the phosphorylation of eIF2α, though they were still capable of assembling SGs in response to other insults, such as osmotic stress. G3BP can be phosphorylated on a serine residue in a disordered region adjacent to its N-terminal domain. Unlike a non-phosphorylatable version of G3BP1, a phosphomimetic mutant failed to rescue SG assembly in G3BP-deficient cells, indicating that the protein's activity is regulated by phosphorylation.

Two proteins—Caprin1 and USP10—bind to a region of G3BP neighboring this critical phosphorylation site. Kedersha et al. found that the proteins compete with each other for G3BP binding and that, whereas Caprin1 promotes SG formation, USP10's interaction with G3BP inhibits SG assembly. G3BP phosphorylation may regulate this competition, because the phosphomimetic version of G3BP preferentially bound to USP10.

G3BP is a highly dynamic protein that shuttles in and out of SGs. Lead author Nancy Kedersha thinks that phosphorylation and Caprin1/USP10 binding may regulate this shuttling process by altering the protein's conformation.

Kedersha, N., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201508028

How Gpr161 exits from cilia



Gpr161 (green) is removed from primary cilia (red) upon Smoothened activation (right).

Pal et al. describe how a G protein–coupled receptor (GPCR) that inhibits sonic hedgehog (Shh) signaling is removed from the primary cilium in response to activation of the pathway.

Shh signaling is associated with trafficking of proteins into and out of the primary cilium. For example, upon activation of the pathway, the signal transducer Smoothened accumulates in the cilium, while the constitutively active orphan GPCR Gpr161, which suppresses Shh signaling, is quickly expelled. How Gpr161 is removed from the cilium is unknown; GPCRs are generally removed from the plasma membrane by clathrin-mediated endocytosis, but clathrin only localizes to the base of the cilium—at the ciliary pocket—rather than in the cilium itself.

Pal et al. found that Gpr161's removal from the cilium is triggered by the accumulation of active Smoothened and is dependent on the kinase Grk2 and the β-arrestin family of proteins that link GPCRs to the clathrin machinery. Active Smoothened promoted Gpr161's interaction with β-arrestins inside the cilium. Deleting *β-arrestin1/2*, or mutating the β-arrestin binding site in Gpr161, prevented the receptor's disappearance from cilia and suppressed Shh signaling. Knocking down clathrin also inhibited Gpr161's removal from the cilium, suggesting that, after associating with β-arrestins inside the cilium, Gpr161 is linked to the endocytic machinery at the ciliary pocket.

Senior author Saikat Mukhopadhyay now wants to investigate the physiological consequences of blocking Gpr161's removal from the cilium, particularly in the hippocampus and cerebellum where Shh signaling is crucial for neurodevelopment.

Pal, K., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506132

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