

Clathrin helps maintain the centrosome's integrity

Inactivating clathrin in S phase reveals the protein's involvement in centrosome maturation.

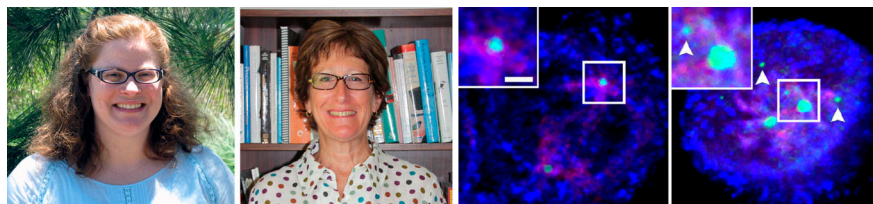
FOCAL POINT

Clathrin has long been known for its role in endocytosis and other membrane-trafficking events. But in recent years the protein has been linked to several other processes, including organization of the actin cytoskeleton and stabilization of the mitotic spindle. Foraker et al. now reveal a function for clathrin in maintaining the integrity of centrosomes during mitosis (1).

Clathrin appears to have several roles in mitosis, including directing the membrane transport required for cytokinesis and abscission (2) and stabilizing the mitotic spindle by forming a complex with the microtubule-binding proteins ch-TOG and TACC3 (3). In addition, cells lacking several different clathrin-associated proteins show defects in centrosome maturation during the G2/M phase of the cell cycle, raising the possibility that clathrin functions at this stage of mitosis as well.

Amy Foraker, Stéphane Camus, Frances Brodsky, and colleagues at the University of California, San Francisco found that clathrin localizes to centrosomes and that knocking down the clathrin heavy chain CHC17 (and/or its associated light chains) for periods of 48–72 hours resulted in multinucleate cells with multipolar spindles and increased numbers of centrosomes (1). The centrosomes in these cells appeared to be fragmented and showed reduced staining for key proteins such as γ -tubulin and pericentrin.

"We wanted to figure out where all these cell cycle phenotypes were coming from," explains Brodsky. Centrosomal defects, for example, could potentially be caused by errors in spindle stability or cytokinesis that accumulate over multiple rounds of division during the time course of clathrin knockdown. Brodsky and colleagues therefore wanted to find a way of inactivating clathrin much more rapidly. "Could we inactivate clathrin at a defined



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(Left to right) Amy Foraker, Frances Brodsky, and colleagues (not pictured) reveal that clathrin promotes the maturation and structural integrity of centrosomes during mitosis. To demonstrate that clathrin affects centrosomes directly, rather than indirectly through its functions in spindle stabilization and cytokinesis, the researchers acutely inactivated clathrin during S phase, just before centrosome maturation. Compared with control cells (left), cells with cross-linked clathrin (right) have fragmented centrosomes (γ -tubulin, green; arrowheads) in early mitosis. Clathrin (with a SNAP-tagged light chain) is blue, and α -tubulin is red. Clathrin appears to promote centrosome integrity by forming a complex with the microtubule-binding proteins ch-TOG and TACC3.

time in the cell cycle to figure out when and where it's operating?" Brodsky asks.

To achieve this, Timothy Evans recombinantly fused the clathrin light chain uLCA to SNAP tag, a protein that binds covalently to cell-permeable derivatives of the small molecule O⁶-benzylguanine (4). By adding a bivalent form of O⁶-benzylguanine to synchronized cell populations, the researchers could crosslink neighboring SNAP tag-uLCA molecules and acutely inactivate the clathrin complex at selected points in the cell cycle.

Specifically, Foraker et al. inactivated clathrin during S phase. "This is before there would be any spindle defects," explains Brodsky, "so we could look directly to see if there was a problem with centrosome maturation, which occurs just following this period." Fragmented centrosomes and multipolar spindles appeared

within hours of uLCA crosslinking during S phase, indicating that centrosome defects are an immediate, rather than long-term, consequence of clathrin inactivation.

Similar phenotypes arise when the Aurora A kinase is inhibited, so, because this kinase regulates the formation of the clathrin-ch-TOG-TACC3 complex on mitotic spindles, Foraker et al. wondered whether this complex might stabilize centrosomes as well as spindle microtubules. Indeed,

ch-TOG and TACC3 are known to maintain the integrity of centrosomes, structures that rely on extensive microtubule interactions (5, 6). "We found that, if you don't have clathrin, you reduce ch-TOG at the centrosomes, and ch-TOG is less stable in the cell," Brodsky says. "So the simplest explanation is that clathrin helps these two microtubule-binding proteins—ch-TOG and TACC3—form a complex at the centrosome."

Brodsky thinks that clathrin's role at the centrosome, like its other functions in the cell, relies on its ability to oligomerize. "Clathrin is a remarkable-looking molecule with a unique trimeric configuration favoring self-assembly, so it provides multivalency to complexes," Brodsky says. "It's not surprising that this property is used in other situations in the cell. Everything clathrin does is due to its interactions as a multivalent protein."

Brodsky and colleagues now want to investigate how clathrin, ch-TOG, and TACC3 are targeted to centrosomes. Their initial studies suggest that clathrin might move to centrosomes on vesicles along a pathway shared by pericentrin and other important centrosomal proteins.

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4. Keppler, A., et al. 2004. *Methods.* 32:437–444.
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6. Gergely, F., et al. 2003. *Genes Dev.* 17:336–341.

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