Little is understood about how the centrosome, a complex organelle and signaling hub consisting of hundreds of components, is assembled. In this issue of *Developmental Cell*, Conduit et al. (2014) shed light on this issue, showing that modification and recruitment of Centrosomin to the centrosome center creates a dynamic pericentriolar matrix.

The centrosome is a highly dynamic organelle. The two centrioles at its core duplicate once per cell cycle, creating perfect copies of their characteristic ninefold symmetric structure. The centrosome components that surround the centriole, the pericentriolar material (PCM), are in constant exchange with the cytoplasm, yet they manage to assemble into an organelle that is distinct from the cytoplasm without the benefit of a bounding membrane. Perhaps most impressively, the size and overall structure of the organelle are largely maintained even in early embryos of organisms that stockpile all of the components to make thousands of centrosomes. How then is the size of the centrosome determined, and how is centrosome assembly controlled in embryonic cells such that it does not occur all at once? An answer to this conundrum is provided by the findings of Conduit et al. (2014), in this issue of *Developmental Cell*. By focusing on the behavior of Centrosomin (Cnn), a linchpin of the PCM, these authors have identified a mechanism by which centrosomes are spatially and temporally organized.

The centrosome is an organelle of animal cells but is only one form of the more broadly conserved microtubule-organizing center (MTOC), a feature of all eukaryotic cells (Lüders and Stearns, 2007). Common to all MTOCs is the presence of γ-tubulin, a member of the tubulin superfamily that is specialized for nucleation of the microtubule polymer. In addition to γ-tubulin, each type of MTOC has its own constellation of associated proteins that are specifically concentrated there. In animal cells, several hundred proteins localize to the centrosome, as shown by a combination of microscopy and biochemistry (Jakobsen et al., 2011). Most of these proteins localize to the PCM, which was long described as an amorphous material, based on its non-descript, darkly staining appearance as viewed by electron microscopy. However, the application of new superresolution light microscopy methods, which can resolve the localization of individual PCM components, has revealed that the PCM is a layered structure with a core of a relatively small number of evolutionarily conserved proteins (Mennella et al., 2014). Most of these proteins have long coiled-coil domains consistent with the notion that they form a matrix of elongated fibers to which other components attach. Based on this model, a simple hypothesis for how assembly of the PCM is limited would be that there is a limiting component—one of the matrix proteins, for example. However, this is unlikely to be a generally applicable model, because in many embryos, there is sufficient quantity of all centrosome components to assemble thousands of centrosomes without new protein synthesis (Gard et al., 1990). A further complication of this simple view is that the PCM portion of the centrosome in most cycling cells dramatically increases in size at the transition to mitosis, in a process called centrosome maturation.

Conduit et al. (2014) address these issues head-on by examining the dynamic properties of Cnn in mitotic *Drosophila* embryo centrosomes. Cnn (and its mammalian ortholog, CDK5RAP2/CEP215) is known to be one of the proteins involved in the association of PCM with centrioles (see Kim and Rhee, 2014 for recent related work on the human protein). However, previous work from Conduit et al. showed that Cnn has a unique property compared with other PCM components: its rate of exchange between cytoplasm and centrosome is greater at the center of the PCM, near the centriole, than at the periphery (Conduit et al., 2010). This suggested that Cnn might load into the PCM at the centriole and then spread toward the periphery, but this observation could also be explained by differential rates of exchange at the two sites. In their new work, Conduit et al. (2014) use photoactivation to demonstrate that the load-at-center model is correct. Cnn tagged with the photoactivatable protein Dendra2 moved through the PCM after being activated at the center of the PCM. The fluorescent Cnn, derived from the center of the PCM, could be seen to detach from the PCM at the periphery in the form of filaments into the cytoplasm. Superresolution imaging of total Cnn in these mitotic centrosomes revealed a fibrous network centered on the centriole.

The imaging results suggest a model in which Cnn in the cytoplasm is altered at the centriole-PCM interface such that it incorporates into a PCM matrix of Cnn that is in flux from the center to the periphery. By this model, the size of the centrosome would be determined by two parameters, the rate of incorporation of Cnn at the center and the rate of removal of Cnn at the periphery. The authors found that, remarkably, Cnn is phosphorylated specifically at the centrosome during mitosis, and that this phosphorylation is likely carried out by the mitotic kinase Plk1, which has been implicated previously in mitotic centrosome maturation as well as other mitotic centrosome alterations. The phosphorylation sites lie in a region of Cnn with multiple phosphorylatable Ser/Thr residues. Mutation of these residues to prevent phosphorylation resulted in a Cnn protein that could be recruited to the center of the centrosome.
but did not migrate out from that site, resulting in defective centrosome maturation. In contrast, mutation of these residues to acidic residues to mimic the phosphorylated form resulted in a Cnn protein that aggregated to form multiple foci, independent of centrosomes. These foci could themselves nucleate and organize microtubules. Thus, the phosphorylation of Cnn by Plk1 stimulates the incorporation of Cnn at the center of the centrosome into a matrix that then moves outward toward the periphery. Consistent with this model, Plk1 is enriched at centrioles (Fu and Glover, 2012). The increased incorporation of Cnn and the accompanying increased flux of Cnn toward the periphery could in part explain the increase in PCM volume during mitotic centrosome maturation.

The results of Conduit et al. (2014) beautifully illustrate the power of assessing protein dynamics to derive mechanisms, as the flux of Cnn through the PCM is not apparent from the steady-state distribution of Cnn. From these results we have, for the first time, a plausible model for PCM assembly and size control that involves only the modification-dependent assembly of a single protein, Cnn. However, it leaves unanswered the questions of how removal of Cnn from the periphery of the PCM occurs and whether this is regulated in concert with the recruitment. It is also unclear how general this mechanism will be, since the vertebrate Cnn orthologs lack an obvious domain corresponding to the phosphodomain identified here, and because the involvement of Plk1 is unlikely to explain the interphase PCM assembly that occurs in most mammalian cells.

A particularly intriguing question is whether the mechanism described here for centriole-mediated recruitment of PCM might also be in play in other MTOCs. For example, in differentiated cells, the PCM is often lost from the centrioles and recruited elsewhere; in some cases this appears to be a direct ‘‘hand-off’’ of material (Feldman and Priess, 2012). This might be achieved by turning off Cnn recruitment at the centrioles and activating it (or another PCM assembly factor) at the new MTOC site. This problem might be amenable to new methods of identifying proximal relationships within complex structures such as the centrosome (Firat-Karalar et al., 2014), as part of the continuing quest to define the previously ill-defined microtubule-organizing material of eukaryotic cells.

REFERENCES


Motor neurons regulate neuromuscular junction formation by using agrin to stimulate acetylcholine receptor clustering and using acetylcholine to disperse unnecessary receptor clusters on muscle fibers. Wang et al. (2014) now report in this issue of Developmental Cell a critical role for caspase-3 in intracellular mechanisms of acetylcholine-induced dispersal.

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Caspase-3, Shears for Synapse Pruning

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http://dx.doi.org/10.1016/j.devcel.2014.03.010

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