

# Cytoskeleton: Centrosom-in absentia

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**Recent results challenge long-held assumptions that centrosomes are essential organizers of mitotic spindles, but suggest that they couple spindle behavior with developmental and cellular events, perhaps by nucleating astral microtubules which mediate interactions with other cytoskeletal components.**

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The centrosome is generally at the center of the cellular stage: it lies at the poles of mitotic spindles, and at the center of the interphase cytoskeleton. Furthermore, the centrioles, which are found within centrosomes and appear to nucleate and organize the more amorphous components of the centrosome, have an additional role as the basal bodies of flagella and cilia. More than a century ago, the positioning of the centrosome at the center of the cellular cytoskeleton was noted and it was inferred that the centrosome plays an organizing role, somehow modulating the diverse and dynamic cellular structures that shape, move and divide cells (reviewed in [1]).

These early proposals received dramatic support from cell and molecular biologists, who have shown that complexes of  $\gamma$  tubulin, which are concentrated at the centrosome, nucleate microtubules, the struts that give the cytoskeleton its overall order. Yet, there is reason to question whether the organizing role of centrosomes is essential. Some cells get along without them, including most cells of higher plants, certain cell lines and female germ cells of numerous species. Furthermore, it has been shown that chromatin can promote the organization of a bipolar spindle-like structure in the absence of centrosomes. Ultimately, a thorough genetic dissection will be needed to define the *in vivo* relevance of the centrosome itself and the roles of its components. Although we are only at the beginning of such analyses, recent studies [2,3] of the effects of mutations in a gene encoding a centrosomal component in *Drosophila*, a protein known as centrosomin, suggest that this approach will lead to new perspectives on the physiological functions of this intriguing organelle.

## Embryos need centrosomin

Centrosomin mutant (*cnm*<sup>-</sup>) fruitflies are viable but sterile [2,3]. Adult *cnm*<sup>-</sup> flies have centrioles, centrosomes and

bipolar spindles [4]. Hence, it appears that centrosomin is largely a dispensable gene. Molecular and phenotypic characterization of several *cnm* mutant alleles argues against, but can not completely dismiss, the caveats that persistence of maternally contributed centrosomin, or residual activity of the mutant alleles, might contribute to survival. Nonetheless, it appears that replication and segregation of centrosomes, and any essential mitotic function that centrosomes might have, can occur without zygotic centrosomin.

The real interest comes from the analysis of embryos from 'sterile' *cnm*<sup>-</sup> females. The female sterility is more appropriately termed a maternal-effect embryonic lethality, as *cnm*<sup>-</sup> mutant females make eggs that are fertilized and it is the embryos that die. In the *cnm*<sup>-</sup> embryos, staining for centrosomal proteins fails to detect the normal focus of staining for  $\gamma$  tubulin, CP-190 or CP-60. This suggests that centrosomes are either absent or defective in the absence of functional centrosomin. Centrosomes might be missing if centrosomin is required for the rapid amplification and/or successful segregation of centrosomes during the unusually rapid syncytial divisions of the early embryo. Alternatively, centrosomes might be present, but defective, if centrosomin is required for recruiting other centrosomal components during these cycles.

## Mitosis still happens in *cnm*<sup>-</sup> embryos!

Two questions of interest about the *cnm*<sup>-</sup> embryos are what happens to them, and what fails to happen? During the early syncytial mitoses of *cnm*<sup>-</sup> embryos, bipolar spindles form and these spindles successfully organize the chromosomes in a metaphase plate and segregate them in anaphase. The spindles are unusual, in that there are no asters around the poles. Furthermore, the poles are less focused than in a normal embryo and they lack, or have greatly reduced levels of, the centrosomal antigens. Hence, it appears that the spindles can form and function when centrosomes are either absent or defective [2,3].

These observations indicate that centrosomin is dispensable for elaboration of a passably functional mitotic spindle. It is, however, tempting to go beyond the normal genetic conclusion about the role of a gene product and draw inferences about the requirement for the centrosome in mitosis. Here there are important ambiguities in interpretation. Dispensability of a particular centrosomal component does not demonstrate dispensability of the centrosome. For example, mutations affecting a protein required for only a subset of centrosomal functions might produce a defective centrosome that still performs essential functions. This might be the case in the *cnm*<sup>-</sup> mutants. Only if the *cnm*<sup>-</sup>

defect causes a complete loss of the centrosome during the early syncytial cycles would we be able to interpret the phenotype in terms of the roles of the centrosome. At present, we can only say that a normal centrosome is not required, but, beyond the lack of staining for  $\gamma$  tubulin, CP-190 and CP-60, and the failure to organize asters, we do not yet know what the defects in the centrosomes might be.

Conversely, a mitotic requirement for a particular centrosomal component does not demonstrate a requirement for the centrosome. Consider an essential mitotic protein that is normally at the centrosome but does not have to be localized to the centrosome for its function. Mutations inactivating this protein would prevent mitosis, but this is a direct result of the loss of the protein's essential function and does not imply an essential mitotic role for the centrosome. Indeed, mutations in another gene might disrupt the centrosome and disperse the essential mitotic factor without interfering with mitosis. More detailed analysis of mutations of centrosomal components should eventually give us a fairly complete view of the role of the centrosome. But for now, because of these caveats, our impression of the roles of the centrosome relies heavily on cell biological studies.

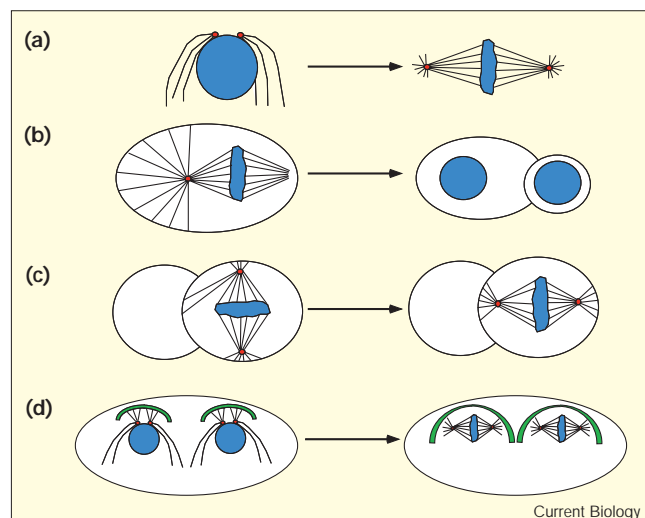
Cell biological investigations have given apparently discordant results with respect to the dispensability of the centrosome for mitosis. The removal of centrosomes in tissue culture cells, sea urchin and grasshopper meiocytes blocked mitosis or the elaboration of a spindle [5–7]. In contrast, mitosis was seen to proceed in unfertilized *Sciara* (insect) embryos in the absence of centrosomes and in a *Chlamydomonas* (green alga) mutant that lacks centrioles [8,9]. The various different results might depend on the biological system (see below) or perhaps on the type of experiment. For example, an experimental approach involving removal of a centrosome might remove essential mitotic factors that are localized at the centrosome, while an experiment involving disruption of a centrosome might delocalize essential mitotic factors. While elimination of mitotic factors would result in mitotic defects, dispersal might not.

More detailed analyses of the *cnm*<sup>-</sup> embryos and other mutants should define the roles of centrosomes and of various centrosomal components in *Drosophila*. It is not clear, however, whether we should expect a clear and universal answer to the question of the dispensability of the centrosome. As evolution has preserved it for millennia in some lineages and abandoned it in others, it might well be that mitosis will rely extensively on the centrosome in some organisms, while using centrosome-independent modes of spindle assembly and function in others.

#### Cytoskeletal derangements occur in *cnm*<sup>-</sup> embryos

Although *cnm*<sup>-</sup> embryos succeed at mitosis, they do not succeed at early embryonic development. The early mitotic

Figure 1



The role of the centrosome that is most often emphasized is to organize the spindle, but the mitotic role that is most specific to the centrosome might be to coordinate spindle behavior with developmental and cellular events. (a) At the transition from interphase to mitosis, centrosomes nucleate asters of microtubules which contribute to elaboration of the mitotic spindle. (b) In the *Tubifex* embryo, elaboration of the first mitotic spindle uses only one centrosome to produce a monoastral bipolar spindle. The monoastral spindle is acentrically positioned within the cell and division is asymmetric. Introduction of a second centrosome produces a biastral spindle and results in symmetric division. (c) In the *C. elegans* embryo, astral microtubules orient the mitotic spindle by interacting with a specific cortical site. (d) In blastoderm *Drosophila* embryos, centrosomes induce actin caps apical to the nuclei, and these are dynamically restructured to create a cage that serves to keep neighboring nuclei and spindles apart. Blue represents DNA; the orange dot indicates the centrosome; green is actin; and the line segments are microtubules. The diagrams are purely schematic and not in proportion.

cycles of normal *Drosophila* embryos are abbreviated cycles that lack the gap phases — G1 and G2 — and cytokinesis. The exponentially expanding population of nuclei undergo a stereotyped program of movements within a syncytial cytoplasm. A shell of evenly spaced nuclei expands outward and reaches the cortex of the syncytial embryo at cycle 9. Upon reaching the cortex of the embryo, each nucleus organizes an actin cap on its apical side, and a cytoskeletal cage appears to surround each nucleus, replicate with it and insulate it from the adjacent nuclei (Figure 1d).

While nuclei do move in the *cnm*<sup>-</sup> embryos, the movements are imprecise, the nuclei are not very evenly spaced and they fail to organize the actin cytoskeleton when they arrive at the surface [3]. Additionally, the actin-rich transient 'cleavage furrow' that usually separates adjacent mitotic figures is absent (Figure 1d). At this point, mitoses are generally defective, at least in part

because of shared poles between adjacent spindles [2,3]. Fusion of adjacent spindles is a phenotype that has been observed previously in mutants that are defective in the spacing of the syncytial nuclei, so it might be secondary to defects in the organization of the cytoskeletal cages.

The phenotype of the *cnm*<sup>-</sup> embryos is complex and might include defects that are only distantly connected to the primary defect caused by loss of centrosomin. Despite the complexity, the failure of the cytoskeletal restructuring that is ordinarily coordinated on arrival of a nucleus at the embryo surface suggests that the centrosomin plays an important role — direct or indirect — in this restructuring. A role for the centrosome in the restructuring of the cortical cytoskeleton had previously been suggested on the basis of the phenotype of *gnu* mutant embryos [10]. Despite a failure of nuclear divisions, the centrosomes of *gnu* embryos replicate and migrate to the surface of the embryo. Upon reaching the cortex of the embryo, each centrosome organizes a cytoskeletal cage resembling that induced when a nucleus accompanies the centrosome. These observations suggest that centrosomes can organize the actin cytoskeleton.

The absence of the normal reorganization of the cortical cytoskeleton in *cnm*<sup>-</sup> embryos demonstrates that centrosomin is required for this reorganization, a finding that is most simply explained if centrosomin is required to produce centrosomes with a normal ability to modify the cortical cytoskeleton. A mutation in the gene *nuclear fall out (nuf)* also results in defective reorganization of the cortical cytoskeleton, but does not obviously disrupt the centrosomes [11]. As the Nuf protein is localized to centrosomes, it is a candidate for being a factor that mediates centrosomal reorganization of the cytoskeleton. Consequently, it would be interesting to know if Nuf is recruited to defective centrosomes in *cnm*<sup>-</sup> embryos.

### Extrapolations

Ordinarily, the poles of mitotic spindles are associated with a bushy sphere of astral microtubules. The spindles in *cnm*<sup>-</sup> embryos lack asters. Indeed, it is a general finding that spindles that lack centrosomes lack asters. It seems reasonable to infer that the presence of the aster depends on the ability of the centrosome to nucleate the radial microtubules, while the presence of the central part of the spindle can be independent of centrosomes because chromosomes can promote microtubule assembly. Asters appear to have roles in the coordination of mitosis with events of development. Astral microtubules orient mitotic spindles in embryos of the nematode *Caenorhabditis elegans* by interacting with a specific site; they position the mitotic apparatus in *Tubifex*; and they also appear to organize the cytoskeletal cages that allow autonomous behavior of syncytial nuclei in *Drosophila* (Figure 1) [12–14]. Given the extraordinary choreography of nuclear movements, spindle

rotations and asymmetric divisions during development, loss of asters might cause rather diverse phenotypes.

Much needs to be done to define roles for the centrosome and the various components that localize at this structure. In particular, we need more complete characterization of the poles of spindles in mitoses that appear to lack centrosomes, and more detailed analysis of the progression of mitosis. It would, for example, not be surprising if centrosomes ordinarily promote the elaboration of a spindle and that prophase is prolonged in acentrosomal mitoses [15]. Despite the limitations of the presently available data, they do suggest a modification of a perspective that has prevailed for a century. It may well be that the centrosome plays only a subsidiary or redundant role in the elaboration of mitotic spindles, and that its unique role in mitosis is to promote the formation of spindle asters. These spindle asters appear to be used during development to manipulate the spindle alignment, and to be used during mitosis to coordinate other cytoskeletal changes with the dynamic spindle architecture.

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