Centrosomes: ***Sic transit gloria centri***

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Centrosomes are thought to ensure spindle bipolarity and thus correct chromosome segregation during mitosis, but recent studies indicate that somatic cells have an alternative mechanism that enables them to form a bipolar spindle and segregate chromosomes independently of centrosomes.

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The role of the centrosome in cell organization has fascinated cell biologists since it was first viewed by light microscopy. As the microtubule-organizing center of the cell, the centrosome has been implicated in many microtubule-dependent functions. During mitosis, a duplicated centrosome is thought to be necessary to ensure the bipolarity of the mitotic spindle, a structure required for chromosome segregation during mitosis that consists largely of microtubules extending from the spindle poles towards the chromosomes [1] (Figure 1). Although numerous experiments have given support to this model, there are, however, some exceptions to this requirement for centrosomes: a few cell types lack centrosomes and can still assemble a bipolar spindle. Two recent studies now indicate that all cells may have the ability to assemble a bipolar spindle in the absence of centrosomes [2,3].

During metaphase of mitosis, duplicated sister chromatids align at the center of the mitotic spindle, equidistant between the two spindle poles. At anaphase, the sister chromatids split and move to opposite poles. Bipolarity of a mitotic spindle is therefore an essential feature of chromosome segregation, and mechanisms must be in place to ensure that a spindle has two and only two poles. The centrosome is at the center of most animal spindle poles. The definition of a centrosome has long been a topic of discussion, but the simplest description is a pair of centrioles surrounded by pericentriolar material, which is required for microtubule nucleation. Every cell inherits one centrosome after cell division; this centrosome divides and each of the daughter centrosomes becomes incorporated in one spindle pole (Figure 1). This cycle of centrosome duplication suggests an obvious model for spindle bipolarity. After duplication, each of the two centrosomes nucleates microtubules, which become captured by the chromosomes themselves (Figure 1). Thus, the two centrosomes, by forming the spindle poles, ensure bipolarity [4]. Support for this mechanism has come from experiments in which the number of centrosomes is varied (see [5], for example). In these cases, the number of spindle poles always corresponds to the number of centrosomes.

It has long been known that there are some exceptions to the rule that a centrosome is required to direct the formation of a spindle pole. The most notable example is in the plant kingdom, where a spindle pole does not contain obvious centrosomes, as defined by the presence of a centriole pair [6]. Furthermore, in animal cells, centrosomes are missing in female germ cells undergoing meiosis. These observations suggested that there might be alternative mechanisms to ensure spindle bipolarity, and this idea is supported by studies in *Xenopus* eggs and extracts [7,8]. In *Xenopus* extracts, the addition of chromatin on beads is sufficient to generate spindles in the absence of centrosomes [7]. In these extracts, microtubules nucleate locally around chromatin and the nucleated microtubules are then organized into two poles by the action of different microtubule-dependent motor proteins. It subsequently became clear that such a pathway of spindle assembly is used for meiosis in the female germ cells of many organisms [9]. This type of mechanism differs fundamentally from the mechanism proposed for spindle formation in the presence of centrosomes. Centrosomes provide the inherent bipolarity and the source of microtubules, whereas in the absence of centrosomes, randomly nucleated microtubules are organized into bipolar structures by a self-assembly process using microtubule-based motors [1]. Therefore, in cells lacking centrosomes bipolarity is a consequence of the self-organization properties of microtubules and motors.

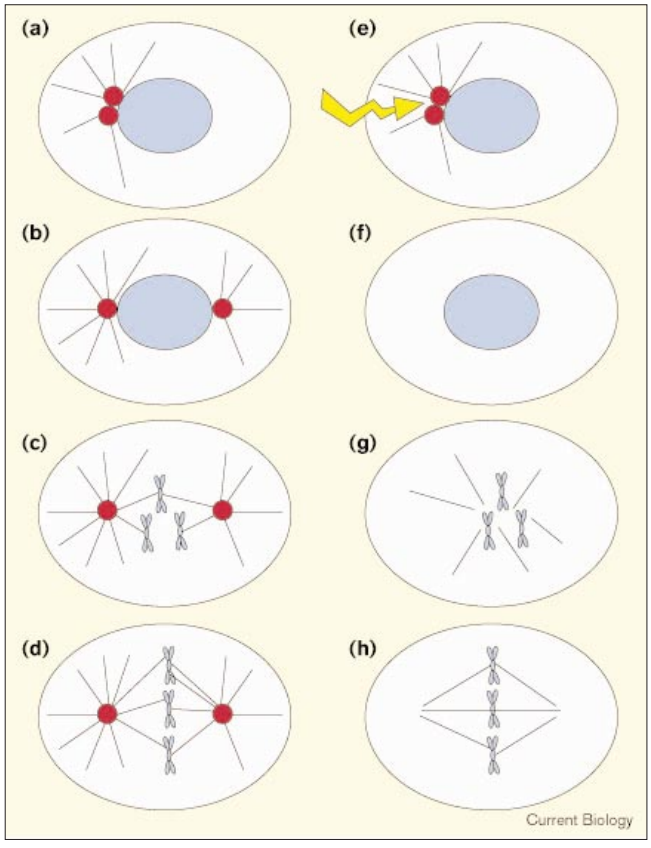
The two recent studies address whether the ability to form a bipolar spindle in the absence of centrosomes is confined to a few special cases or whether such a mechanism exists in all animal cells. Each study has employed a different procedure to remove centrosomes from the cell before following the pathways of spindle assembly. In one study, Khodjakov *et al.* [2] used a laser to ablate the centrosome in a monkey fibroblast cell line. The centrosome was labeled with green-fluorescent protein (GFP) to delineate its boundary, and the laser was focussed on the centrosome before cell division to completely ablate it. The results show clearly that in this cell line, a bipolar spindle can still assemble in the absence of a duplicated pair of centrosomes. Furthermore, these cells proceed through mitosis to segregate their chromosomes, indicating that the spindles

are fully functional. In a second study, Bonaccorsi *et al.* [3] used a mutant, *asterless*, which prevents centrosome assembly in *Drosophila* neuroblasts. The authors show that spindles form perfectly in neuroblasts from an *asterless* mutant, and a pathway reminiscent of centrosome-independent spindle assembly in *Xenopus* egg extracts is used.

Although these experiments do not show that spindles can assemble in a centrosome-independent manner in all cell types, they do show that in somatic cells it is possible to assemble spindles without centrosomes. Therefore, it seems as if the ability to form a bipolar spindle without centrosomes is present throughout complex eukaryotes, although it may not necessarily be used in all cases.

It is still unclear from these experiments whether the non-centrosome mechanism is used in the presence of centrosomes, or whether the non-centrosome pathway is a neomorphic mechanism that arises after the centrosomes are ablated. However, the important implication is that if spindles can assemble without centrosomes, why have animal cells evolved a mechanism that involves centrosomes? Part of the answer may lie in the fact that the centrosome is an important interphase structure. For example, in many cells, the centrosome forms the basal body, which is required to organize the flagellum. The centrosome is also important for ensuring that microtubles emanate from one place to mediate certain aspects of membrane traffic; for instance, in many cell types the Golgi apparatus forms at the centrosome, so that proteins moving along microtubules from the endoplasmic reticulum reach the region of the centrosome and thus the Golgi apparatus. It is also likely that the duplication of the centrosome is important in providing a means to partition membrane components at mitosis. At prophase, the membrane compartments tend to fragment and concentrate at the spindle poles [10]. The duplicated centrosomes have always separated by prophase, so the fragmented membranes partition between the two centrosomes. As each daughter cell inherits one centrosome, an equal partitioning of membranes independently of the size of the daughter cells is ensured.

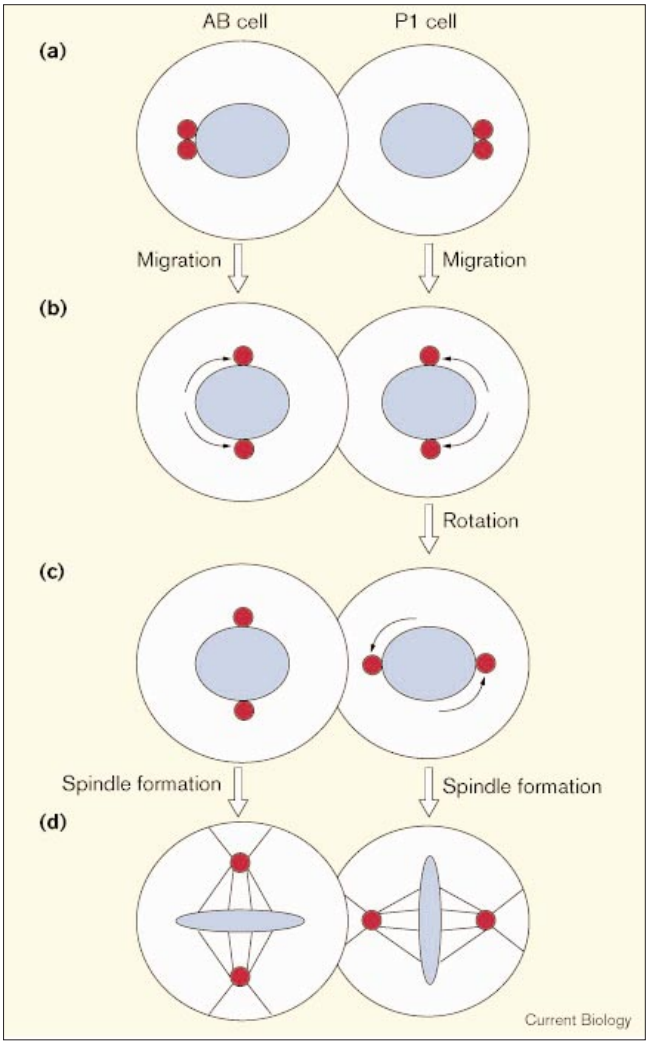
Figure 1



Spindle assembly in cells containing centrosomes and in cells where the centrosomes have been ablated. **(a–d)** Untreated cells. **(e–h)** Cells in which the centrosomes have been irradiated. (a) In untreated cells, each daughter cell inherits a single centrosome, which duplicates and (b) the two centrosomes move apart from each other. (c) The nuclear envelope disassembles to initiate spindle assembly, with each of the two centrosomes acting as a spindle poles, and finally (d) a bipolar spindle is assembled. (e) Centrosomes labeled with GFP are irradiated with a laser, thus (f) there are no foci of microtubule assembly. (g) As the nuclear envelope breaks down, microtubules nucleate around chromatin in a random array, but (h) subsequently reorganize to form a bipolar mitotic spindle by the self-organization of microtubules and motor proteins. The red dots indicate centrosomes, blue indicates DNA, and the lines represent microtubules.

In addition, centrosomes play an important role in mitosis besides spindle assembly. By nucleating astral microtubules, they provide a mechanism for positioning mitotic spindles. Correct spindle position is an important feature of many cell divisions. In most polarized cell types, the spindle must have a definite orientation in order to enable cells to be correctly positioned after cell division, and to ensure that the cleavage axis of the cell lies along the axis of polarity of the embryo. This process has been extensively studied in the early embryos of *Caenorhabditis elegans* [11]. Here, the centrosomes have defined patterns of movement, and these can be shown to determine the position of the spindle [12] (Figure 2). In other words, the final position of the centrosomes before nuclear envelope disassembly determines the position of the spindle poles. The position of the centrosomes must therefore be the dominant factor in determining the position of the spindle pole, even in systems in which spindles can form in a centrosome-independent manner. Such dominant activity of centrosomes has indeed been described in *Xenopus* egg extracts. As mentioned above, spindles form in these extracts in the absence of centrosomes by the self-organization of microtubules and motor proteins. However, when centrosomes are present, they determine the position of the spindle poles [13]. Thus, centrosomes provide dominant sites of spindle-pole assembly and direct the position of the spindle pole in the cell.

Figure 2



Centrosome movements in early *C. elegans* embryos determine the orientation of spindle axes. **(a)** At the two cell stage, each of the two cells (termed AB and P1) have inherited a centrosome which has duplicated. **(b)** These two centrosomes then migrate apart from each other. **(c,d)** In the AB cell, the centrosomes now lie on the transverse axis of the embryo, and the spindle forms between the centrosomes on this transverse axis. In the P1 cell, the centrosomes rotate onto the longitudinal axis of the embryo after they have migrated apart. Thus the spindle assembles on the longitudinal axis, perpendicular to that of the AB cell.

In conclusion, from the recent experiments on the role of centrosomes in spindle assembly it seems increasingly likely that, rather than having evolved for the process of spindle assembly itself, centrosomes provide the information to the cell about where to build microtubule-based intracellular structures.

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