Centriole and centrosome cycle in the early *Drosophila* embryo

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**Summary**

Centriole and centrosome cycles were examined by indirect immunofluorescence and electron microscopy techniques in the early *Drosophila* embryo. The centrosomes, which are already divided at interphase, appear as compact spheres during prophase and metaphase, expand and flatten from anaphase to telophase and split into two units in late telophase. Centriole separation starts in late metaphase, becomes evident in anaphase and increases during telophase. Procentrioles appear during the following interphase.

Key words: centrosomes, mitosis, *Drosophila* embryo.

**Introduction**

Mitotic divisions in *Drosophila* occur with no discernible G1 or G2 phases (see Glover, 1988; Glover et al. 1989) and centrosome separation, which is generally considered to be a feature of late interphase in animal cells, is an early event in the mitotic cycle (Warn et al. 1987; Kellogg et al. 1988; Raff and Glover, 1988). Two centrosomes are always present above the interphase nuclei (Warn, 1986; Warn and Warn, 1986; Callaini and Anselmi, 1988). The centriole cycle also appears to be slightly modified. Huettner (1933) described the centriole cycle in *Drosophila* embryos from microscopic observation of material fixed with formalin–ethanol–acetic acid (1:1:1, by vol.) and stained with Heidenhain’s iron hematoxylin. He reported two centrioles near the nucleus during interphase and two widely separated centrioles recognizable at either pole of the mitotic spindle at anaphase. During late anaphase and early telophase the centrosomes remain more or less in the same position and during late telophase they begin to move apart. This description of the centriole cycle in the *Drosophila* embryo does not seem to have found a place in subsequent literature. Only recently were these observations taken up by Warn et al. (1987), who compared the centriole cycle as proposed by Huettner (1933) with centrosome division observations resulting from in vivo studies of *Drosophila* embryos injected with anti-tubulin antibodies. In the present study we used electron microscopy and the polyclonal antibody Rbl88, which specifically recognizes a centrosome-associated antigen in *Drosophila* (Whitfield et al. 1988), to follow centriole dynamics and to elucidate the centrosome cycle in *Drosophila*.

**Materials and methods**

**Collection of the embryos**

Embryos of *Drosophila melanogaster* (Oregon-R strain) were collected at 25°C on agar plates, dechorionated in a 50% commercial bleach solution and washed with distilled water. Following removal of excess of liquid by blotting on tissue paper, the embryos were processed for different preparations.

**Staging of the embryos**

The age of the embryos for immunofluorescence observations was determined according to Campos-Ortega and Hartenstein (1985) by direct observation of the embryos with interference contrast or by counting the somatic nuclei. The exact mitotic stage for electron microscopy preparations was determined as follows. The dechorionated embryos were treated with a solution containing 25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, in an equal volume of heptane (Zalokar and Erk, 1977). After 3 min the embryos were transferred for 30 min to a solution containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate to which 5% of sucrose was added and the vitelline membrane was removed with tungsten needles. Then the embryos were washed three times for 5 min in cacodylate buffer and incubated for 5 min with 1 μg/ml-1 of the DNA-specific dye Hoechst 33258 in cacodylate buffer. The embryos were temporarily mounted and observed with a Leitz Aristoplan microscope equipped with u.v. filter.

**Fluorescence microscopy**

The dechorionated embryos were fixed and their vitelline membrane was removed essentially as described by Warn and Warn (1986), except for a final fixation with acetone for 5 min. The embryos were then washed in phosphate-buffered saline (PBS) and incubated for 1 h in PBS containing 0.1% bovine serum albumin. For double labeling the embryos were incubated for 5 h at room temperature with the Rb188 antisera, which specifically recognizes a 185×10^3 M, antigen associated with the centrosome of *Drosophila* embryos (Whitfield et al. 1988) at a dilution of 1:400. The samples were then washed with PBS and incubated for 30 min at room temperature with a monoclonal antibody against α-tubulin (Amersham). After rinsing in PBS the embryos were incubated in secondary antibodies (goat anti-mouse rhodamine-conjugated IgG and goat anti-rabbit fluorescein-conjugated IgG at a dilution of 1:1000 each; Cappel, West Chester, PA), washed again in PBS and mounted in 90% glycerol containing 2.5% n-propyl gallate to reduce photobleaching (Giloh and Sedat, 1982). Fluorescence observations were carried out with a Leitz Aristoplan microscope equipped with fluorescein and rhodamine filters. Photomicrographs were taken with Kodak Tri-X pan film and developed in Kodak HC 110 developer for 7 min at 20°C.

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Electron microscopy
For transmission electron microscopy observations the embryos at the desired mitotic stage were fixed in the triadeholde solution of Kalt and Tandler (1971) for 2h. After rinsing in 0.1M cacodylate buffer, pH7.2, the embryos were postfixed in 1% osmium tetroxide for 2h and dehydrated in a graded series of ethanol and bulk-stained in 1% uranyl acetate for 1h. After treatment with propylene oxide the embryos were embedded in an Epon–Araldite mixture and polymerized at 60°C for 48h. Random and serial sections cut using an LKB Nova ultramicrotome and a diamond knife (Diatome Ltd, Switzerland) were collected on copper grids and stained with uranyl acetate and lead citrate. Sections were observed and photographed with a Philips EM 400 electron microscope.

Results

Immunofluorescence observations
During prophase the centrosomes, which appear as compact spheres, move to the opposite sides of the nuclei and begin to form the mitotic spindle. The microtubules form weak asters immediately surrounding the centrosomes (Fig. 1A).

At metaphase the centrosome material is condensed at opposite poles of the mitotic apparatus and the microtubules give rise to metaphase figures with a small aster and a distinct midzone (Fig. 1B). The separation of the sister chromatids and their shifting toward the poles, which occurs in early anaphase, is marked by a further elongation of the spindle and aster microtubules, in contrast to what was seen in metaphase, in which relatively long microtubules radiate toward the periphery of the spindle (Fig. 1C). Spindle lengthening ends in late anaphase and the interzonal microtubules gradually diminish in density and become progressively fainter near the poles (Fig. 1D). During early anaphase the centrosomes gradually lose their compactness and in late anaphase organize in ovoid plates perpendicular to the longitudinal axis of the mitotic apparatus (cf. Fig. 1C and D).

Telophase is characterized by the gradual breakdown of the interzonal microtubules, indicated by a reduction in the staining, and by the growth of the astral microtubules. During late telophase the spindle microtubules are reduced to a small central fluorescent area, including an interzone region of reduced fluorescence, the midbody (Fig. 1E). The centrosome material, recognized by the Rb188 antibody, splits into two units (Fig. 1E).

During early interphase the centrosomes are already divided and organize the microtubule network in the space between the nucleus and the plasma membrane (Fig. 1F). Centrosome material, as recognized by the Rb188 antibody, is loosely arranged near the nuclear surface. When interphase progresses the centrosome material is more densely associated and the centrosomes are seen as two widely separated spheroidal structures (Fig. 1F).

Electron microscopy observations
As soon as prophase begins, each centrosome contains parent and daughter centrioles, closely juxtaposed and in perpendicular orientation to each other (Fig. 2A). The centrioles are surrounded by clouds of electron-dense material from which microtubules originate (Fig. 2A). As prophase progresses the centrosomes continue to migrate to their proper sites at the opposite poles of the nuclei.

Fig. 1. Indirect immunofluorescence of whole-mount Drosophila embryos during nuclear cycle 10 (A,B,C,D,E) and nuclear cycle 11 (F) double stained for microtubules (MTs, left) and centrosomes (Cent) (middle and right, for detail). (A) Prophase. The mitotic apparatus begins to form (left). Centrosomes appear as compact spheres (middle and right). (B) Metaphase. The mitotic spindle is well developed but the asters are very small (left). The centrosomes are quite compact (middle and right). (C) Early anaphase. Aster microtubules grow (left). The centrosomes gradually lose their compactness (middle and right). (D) Late anaphase. The spindle elongates and microtubule staining diminishes at the poles (left). Centrosomes expand and enlarge into irregular ovoid plates (middle and right). (E) Telophase. Aster microtubules enlarge again and spindle microtubules reduce (left). Centrosomes expand further and split into two units (middle and right). Arrows and arrowheads indicate midbodies and divided centrosomes respectively. (F) Early interphase. The midbodies have disappeared and the microtubules have reduced in length (left). The already divided centrosomes gradually condense (middle and right). Bars, 10 µm.

When the chromosomes have reached metaphase, each spindle pole has a pair of mutually perpendicular centrioles (Fig. 2B). During late metaphase, the daughter centrioles, equal in length to the parent centrioles, are still adjacent to their parents but the angle between them is no longer a right-angle (Fig. 2C). This suggests that they are starting to move away from each other.

In anaphase the centrosomes contain parent and daughter centrioles, which are almost always disoriented and separated by several micrometers (Fig. 2D).

When the nuclear envelope begins to re-form around the decondensing chromosomes at telophase, the centrioles continue to move apart. Fig. 2E shows the ultrastructure of a spindle region during late telophase. This appears as an area devoid of yolk granules and mitochondria. In the region between the two re-forming nuclei, small dense bodies are visible, each composed of several densely packed microtubules covered with an accumulation of electron-dense material. To confirm whether the pairs of fluorescent structures observed at the pole of the telophase spindles with Rb188 antibody contain only one full-sized centriole and no procentrioles, the polar region was analyzed ultrastructurally by serial sections. Random or incomplete sections would not be adequate because of the small size and the position of the procentrioles at the time of their appearance. Serial sections through one pole of the spindle show two full-sized centrioles separated from each other by several micrometers (Fig. 2F, G and H). These centrioles measure 0.20 µm in diameter, 0.15 µm in length, and consist of the usual nine peripheral triplets of microtubules. Sections above and below those shown in Fig. 2F and H contain no additional centrioles.

Once the nuclear envelope has completely re-formed, the centrosomes have migrated to a position between the nuclear envelope and the embryo surface (Fig. 2I). The centrosomes have enlarged and consist of several small clouds of electron-dense material surrounding the centriolar region (Fig. 2J). The procentriole, a small linear dense body with no internal organization, is visible near an extremity of the parent centriole wall (Fig. 2K). The procentrioles keep elongating during prophase and reach the length of the parent centriole at the beginning of metaphase. The structure of the procentrioles changes during this time and the microtubular triplets become visible.
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Discussion

The centriole cycle in *Drosophila* as proposed by Huettner (1933) raises two main questions. The first concerns the position and number of centrioles observed during the different stages of mitosis, and the second the time of centriole duplication. These questions, unresolved by conventional light microscopy, may be answered by ultrastructural analysis. The present results suggest a centriole cycle that is slightly modified with respect to

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those proposed by Huettertner (1933) and more recently by Staafstrom and Staehelin (1984). Two centrosomes are observed at the beginning of the mitotic cycle, each consisting of parent and daughter centrioles, which migrate to the opposite poles of the nucleus to form the mitotic spindle. At metaphase the centrosomes contain gradually lose their orthogonal arrangement in the transition to anaphase. At telophase the centrioles move apart, and during interphase they undergo duplication. The procentriole maturation follows the conventional pathway observed in other animal cells (see Vorobjev and Nadezhhdina, 1987).

The distribution of the centrosome-associated antigen recognized by the Rb188 antibody changed during the mitotic cycle. Combined immunofluorescence and electron microscopy observations suggested a close relationship between pericentriolar material and centrioles in the assembly of functional spindles. When parent and daughter centrioles were orthogonally arranged in prophase and metaphase, the centrosomes appeared as compact spheres. From late metaphase the centrioles lost their orthogonal arrangement and moved away from each other until late telophase. At this time the centrosomes spread in a flat ovoid plate and divided into two separate units each containing only one centriole. The splitting of the centrosomal material at telophase in Drosophila was recently also confirmed with photobleaching of rhodamine and fluorescein protein conjugates by Nadezhhdina (1987).

The Drosophila centrosome cycle reported here differs from the special case of mitosis in sea urchin eggs, in which the centrosome divides before the nuclear envelope has formed (Pawelewlt et al. 1984, 1987). However, in sea urchin eggs parent and daughter centrioles shift and duplicate in telophase (Sluder and Rieder, 1985), whereas in the Drosophila embryo the shifting of the centriole pairs is an event that is distinct from centriole replication and occurs at different times during the mitotic cycle. Serial sections demonstrated that in late telophase the centrosomes have already separated into two solitary centrioles, each surrounded by an aster of microtubules, and the procentrioles appear later in interphase. These ultrastructural observations are in agreement with the findings in mercaptotethanol-treated sea urchin eggs in which centriole replication is not a prerequisite for the splitting of daughter centrioles (Sluder and Rieder, 1985).

Centriole replication in the early Drosophila embryo is not a prerequisite for the splitting of the centrosome-associated antigen recognized by the Rb188 antibody. This centrosomal material splits into two separate units, each containing only one centriole and able to nucleate microtubules. However, centriole pairs are apparently needed to start regular mitotic progression. This agrees with the observations of Sluder and Rieder (1985) that the reproductive capacity of sea urchin centrosomes is correlated with the number of centrioles they contain and that only centrosomes with centriole duplexes reproduce between mitosis and form regular mitotic spindles (Sluder et al. 1989).

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References


