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Behaviour of centrosomes in early Tubifex embryos: asymmetric segregation and mitotic cycle-dependent duplication

Received: 17 July 1995 / Accepted in revised form: 21 September 1995

Abstract An antibody raised against a highly conserved peptide of γ-tubulin (Joshi et al. 1992) recognized a 50 kDa polypeptide in centrosomes in Tubifex embryos. Centrosomes labelled with this antibody are found at both poles of the first meiotic spindle and at the inner pole of the second meiotic spindle. At the transition to the second meiosis, there is no change in morphology of the centrosomes which are retained in the egg proper. In contrast, as the second meiosis proceeds from anaphase to telophase, centrosomes labelled with the antibody gradually become smaller, but are still recognized as tiny dots; each egg exhibits no more than one tiny dot. The first cleavage spindles exhibit a centrosome at one pole but not at the other. The spindle pole with a centrosome forms an aster which is inherited by the larger cell, CD, of the two-cell embryo; the centrosome-free spindle pole then becomes anastral and is segregated to a smaller cell AB. Centrosomes are present in the C and D cell lineages but not in the A and B lineages, at least up to the eighth cleavage cycle. During cleavage stages, centrosomes duplicate early in telophase of each mitosis, and their size changes in a cell cycle-specific fashion. Centrosomes which otherwise duplicate asynchronously in separate cells do so synchronously in a common cytoplasm. Centrosome duplication is inhibited by nocodazole but not by cytochalasin D. An examination of embryos treated with cycloheximide or aphidicolin also suggests that centrosome duplication during cleavages requires protein synthesis but no DNA replication per se. These results suggest that the centrosome cycle in Tubifex blastomeres is linked to the mitotic cycle more closely than is that in other animals.

Key words Centrosome cycle · Mitotic clock · Spindle organization · Cell lineage · Tubifex hattai

Introduction

The embryos of many kinds of animals generate a stereotypical pattern of cleavage during early embryogenesis. These divisions are regulated spatially and temporally to ensure not only equal distribution of genetic material but also proper segregation of developmental potential to daughter cells (Wilson 1925; Freeman 1979). The fundamental basis for the generation of cleavage patterns is not yet understood, however. There is no doubt that the elucidation of the mechanisms which determine the morphology, position and orientation of the mitotic apparatus is an important step towards an understanding of cleavage patterns (Freeman 1983).

The early development of an oligochaete Tubifex also consists of a stereotypical sequence of cell divisions (Penners 1922; Shimizu 1982, 1995a). The first cleavage is unequal, and produces a smaller AB cell and a larger CD cell. At the second cleavage, the CD cell divides into a smaller C and a larger D, and the AB cell into cells A and B of various sizes. From the third cleavage on, the quadrants A-D repeat unequal divisions three or four times, producing micromeres at the animal side and macromeres at the vegetal side; thereafter, the quadrants A, B and C divide equally at the sixth cleavage, and the D quadrant at the seventh cleavage. As Fig. 1 shows, the mitotic apparatus involved in these divisions exhibits various forms, each of which is specific to individual developmental stages and cell lineages. Apparently, differences in the form of the mitotic apparatus largely depend on the morphology of spindle poles and asters. Furthermore, it has been suggested that the inequality of divisions is correlated with the asymmetry of the mitotic apparatus organization (Penners 1922; Shimizu 1982); an asymmetrically organized mitotic apparatus is found in unequal divisions (Fig. 1a-d), whilst a symmetric mitotic apparatus is seen in equal divisions (Fig. 1e, f). We have previously postulated that the difference in spindle pole morphology might be, at least in part, a manifestation of a difference in organization of the microtubule-organiz-
bryo is coupled with the mitotic clock much more closely than is the case in other animals such as the sea urchin also suggest that the centrosome cycle in the other without a centrosome. The results reported here thereby establishes two cell lineages, one with and the other with poles, one of which is asymmetrically organized in association with the effects of various inhibitors on the organization of centrosomes in respect to the cell cycle, and (3) to examine the chemistry of embryos labelled with an antibody against g-tubulin (Joshi et al. 1992). We found that this antibody was able to visualize centrosomes clearly in whole-mount preparations of Tubifex embryos, allowing us to follow the behaviour of centrosomes in this animal. The objectives of this study were: (1) to characterize spindle poles in terms of centrosomes in four cell lines of the Tubifex embryo, (2) to describe morphological changes in centrosomes in respect to the cell cycle, and (3) to examine the effects of various inhibitors on the organization and duplication of centrosomes. We found that only a single centrosome is involved in the first cleavage, which thereby establishes two cell lineages, one with and the other without a centrosome. The results reported here also suggest that the centrosome cycle in the Tubifex embryo is coupled with the mitotic clock much more closely than is the case in other animals such as the sea urchin and Xenopus (Sluder et al. 1990; Gard et al. 1990).

Fig. 1-7 Schematic representations of Tubifex embryos from the first cleavage through the formation of the teloblasts. All embryos are viewed from the animal pole (i.e. the future dorsal side). The anterior end of the embryo is up. Short lines straddling the blastomeres in d and f are drawn to indicate sister cells just produced and the direction of cell divisions. Mitotic apparatuses are also shown diagrammatically in some cells in a, b, c and e; a bundle of three parallel lines represents a metaphase spindle, and a thick line crossing this bundle indicates chromosomes aligned at the metaphase plate. a One-cell stage. The mitotic apparatus for the first cleavage possesses an aster at one pole but not at the other. b Two-cell stage. The smaller cell AB exhibits an anastral spindle. In the larger cell CD, the mitotic apparatus possesses asters at both poles, one of which is asymmetrically organized in association with the cleavage membrane. c Four-cell stage. In the largest cell D, asters develop differently between the poles. d Eight-cell stage. The first quartet of micromeres are formed at the animal side of the embryo. e Twenty-five-cell stage. 2d111 is derived from the second micromere (2d) of the D lineage. The mitotic apparatus in the fourth micromere 4d is organized symmetrically. f Seventh cleavage. Formation of ectodermal teloblast precursors (NOPQ) and mesodermal teloblasts (M). (EP endodermal cell derived from 4D).

Materials and methods

Fertilized eggs and embryos of the freshwater oligochaete Tubifex hattui were obtained as previously described (Shimizu 1982). For the experiments, all eggs and embryos were fixed from their coelomes. Ovisac eggs were obtained according to Shimizu (1982), except that ovisacs were dissected out of mature worms in a microtubule-stabilizing solution (MTS solution; 30 mM KCl, 5 mM MgCl2, 5 mM EGTA, 10 mM Pipes, pH 6.9); eggs were taken out of ovisacs in the same solution. Unless otherwise stated, all experiments were carried out at 19-20°C.

Whole-mount immunocytochemistry

Eggs and embryos were rinsed briefly in MTS solution and fixed in the same solution containing 3.5% formaldehyde and 0.9% Triton X-100 for 60 min. After the removal of vitelline membranes in MTS solution, fixed eggs and embryos were immersed in methanol/dimethyl sulfoxide (DMSO; 4:1) overnight, placed in methanol containing 10% H2O2 for 24 h, and stored in methanol at -20°C until use.

Immunocytochemical whole-mounts of fixed eggs and embryos were prepared according to Shimizu (1993), with some modifications. A rabbit polyclonal antibody (kindly donated by Dr. H. C. Joshi) raised against a highly conserved peptide of γ-tubulin (Joshi et al. 1992) was used as a primary antibody. A secondary antibody was goat anti-rabbit IgG antibody conjugated to horse radish peroxidase (HRP; Tago, Inc.). Fixed eggs and embryos were incubated with the primary (1:4,000 diluted) and secondary (1:2,000 diluted) antibodies for 48 h at 4°C.

Immunoblotting

After being rinsed in 50 mM TRIS/HCl (pH 6.9) containing a cocktail of protease inhibitors (0.2 μg/ml aprotonin, 1 μg/ml leupeptin, 1 μg/ml puromycin and 50 μg/ml phenyl methyl sulfonyl fluorid e), one to four-cell stage embryos (~1,000) were homogenized and fractionated into clear cytoplasm and yolk granules by centrifugation (10,000 g, 10 min) at 4°C. The clear cytoplasm was mixed with the same volume of double-strength sodium dodecyl sulfate (SDS) sample buffer (Laemmli 1970) and boiled for 3 min. Proteins were separated on a 12.5% acrylamide gel and transferred electrophoretically onto nitrocellulose membranes. Nitrocellulose blots were incubated for 6 h with a blocking solution consisting of phosphate-buffered saline (PBS), 2% bovine serum albumin (BSA), 3% dry skimmed milk, 0.1% Tween-20 and 0.1% sodium azide. Blots were then incubated with either the rabbit anti-γ antibody or the mouse monoclonal antibodies to α- and β-tubulin (1:2,000, 1:1,000 and 1:1,000, respectively, in the blocking solution) for 24 h at 4°C. The primary antibodies were detected by HRP-conjugated goat anti-rabbit or anti-mouse secondary antibody (1:500 in the blocking solution without sodium azide) for 6 h. Colour development of the activity of HRP was carried out according to Shimizu (1993).

Blot affinity-purification of antibodies

Antibody purification using nitrocellulose blots as an affinity matrix was carried out according to Smith and Fisher (1984), with some minor modifications. After 5-10 aliquots of the protein sample and the mixture of prestained molecular weight markers (Bio-Rad) were loaded onto alternate lanes of a 12.5% gel, electrophoresis, protein transfer to nitrocellulose membranes, blocking of blots, and incubation with the anti-γ antibody were carried out as described above. Blots were rinsed in PBS containing 0.1% Tween-20. By referring to the positions of prestained molecular weight markers, we excised regions corresponding to bands of 50, 64 and 79 kDa (see Fig 2a) from the blot and transferred them to microtage tubes. (The remaining parts of the blot were incubated...