Ultrastructure and \(^3\)H-Thymidine Incorporation by Chromosome Vesicles in Sea Urchin Embryos

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Abstract. The ultrastructural features of chromosome vesicle formation in early sea urchin embryos and chromosome vesicle uptake of tritiated thymidine is described. Envelopes which resemble typical nuclear envelopes form around the condensed anaphase chromosomes. In late anaphase or early telophase, the chromosomes swell and decondense and it is at this time when tritiated thymidine is incorporated. This study shows that DNA synthesis in the rapidly dividing cells of early sea urchin embryos occurs in chromosome vesicles which form during anaphase.

Introduction

In spite of their minuteness, individual chromosome vesicles, also known as karyomers, were identified in various eggs during cell division almost 100 years ago (see Richards, 1917, and Wilson 1925, for reviews). These chromosome vesicles are mitotic chromosomes which are converted into tiny nuclei during anaphase which then fuse to form a telophase nucleus. This process was described in the large cells of the early cleavage stages which are favorable for light microscopical study. In Crepidula (Conklin, 1901) and Fundulus (Richards, 1917), the apparent incomplete fusion of the chromosome vesicles during mitosis was used to support the hypothesis that chromosomes were the basis for genetic continuity.

The first report of chromosome vesicles in sea urchins appears to be by Wilson (1900) in Toxopneustes and Boveri (1901) in Echinus zygotes. Their drawings clearly indicate that the mitotic chromosomes in late anaphase were already decondensing. Although subsequent workers have noted their presence, studies on these vesicles have been relatively limited. In a detailed ultra-structural study, Harris (1961) examined Stronglylocentrotus purpuratus embryos in the 16 to 32 cell stage and elegantly illustrated well-formed chromosome vesicles with typical nuclear envelopes and pores. Some of the figures suggested the formation of the envelope during late anaphase but the osmium tetroxide fixation...
used did not allow a detailed analysis of the steps of chromosome vesicle formation. Merriam (1961) described the ultrastructure of karyomere formation in Chaetopterus zygotes by vesiculation of the nucleus prior to chromosome condensation. In telophase these vesicles fused to form daughter nuclei. More recently, Longo (1972) published excellent electron micrographs illustrating the ultrastructural features of cleavage in the Arbacia zygote and described the formation and fusion process of chromosome vesicles.

During the course of attempting to study the ultrastructural features of the cleavage stages in Arbacia punctulata, we obtained embryos which were favorably preserved to study chromosome vesicles. These observations were first made in 1968 (Goodenough et al., 1968 abstract). Subsequently, additional samples of Arbacia as well as Hemicentrotus pulcherrimus were studied. Although the structural preservation of the latter species was less favorable, the essential features reported for Arbacia seems to be very similar in Hemicentrotus. The autoradiographic portion of this study is an extension of the recent report on the cell cycle of Hemicentrotus by Dan et al. (1980).

Materials and Methods

The sea urchin Arbacia punctulata was obtained from the supply department of the Marine Biological Laboratory, Woods Hole, Massachusetts and Hemicentrotus pulcherrimus from the Misaki Biological Laboratory, Misaki, Japan. Gametes were obtained by injecting 0.5 M KCl or by electroejaculation and fertilized as needed. At appropriate intervals, the embryos were fixed and processed for electron microscopy.

After numerous trials with a wide spectrum of fixation techniques, the fixative selected contained 2% glutaraldehyde, 2% formaldehyde, and 0.02% trinitroresorcinol or trinitrophenol (picric acid) in 0.1 M cacodylate buffer pH 7.4 (Ito and Karnovsky, 1968). The sea urchin embryos of the desired developmental stages suspended in about 1 ml of sea water were pipetted into about 5 ml of the fixative at room temperature, 18-22°C. Some samples of fixative contained 0.2% lanthanum nitrate. After 1-2 h in the fixative, the eggs were washed with sea water and osmicated with 1% OsO4 in sea water. The specimens were dehydrated in a cold ethanol series and embedded in Epon 812 or a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead and observed by transmission electron microscopy.

The autoradiographic procedure used in this study has been described previously (Dan et al., 1980). Developing Hemicentrotus pulcherrimus zygotes in sea water at 15°C were incubated with 3H-thymidine (sp. act. 18-28 Ci/m mole, Radiochemical Centre, Amersham, England) at a concentration of 10 μCi/ml for 18 min prior to the predicted cleavage time. Samples of embryos were fixed in 10% formalin in sea water at 3 min intervals up to and 3 min beyond the initiation of cell cleavage. Paraffin sections of the samples were treated with 5% trichloroacetic acid for 30 min at 4°C, stained with pararosanilin-Schiff (Böhm, 1972) after hydrolysis in 5 N HCl for 30 min at room temperature, and coated with Sakura NR-M2 autoradiographic emulsion. The autoradiographs were developed for analysis after 48 hr exposure.

Observations

I. Electron Microscopy

The metaphase chromosomes of dividing sea urchin zygotes appear as dense aggregates of granules and filaments in all cleavage stages examined. The mitotic spindle contains numerous microtubules and ribosomes and a moderate number of spherical and irregular shaped vesicles about 100 to 400 nm in diameter.