THE CHROMOSOMAL COMPLEMENT OF BLASTOMERES IN ARBACIA PUNCTULATA*

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Abstract. The diploid chromosomal complement of blastomeres of Arbacia punctulata is composed of 44 units. It includes two chromosomal pairs with distinctive structural features: the Nos. 1 which are long and subacrocentric, and the Nos. 2, somewhat shorter and submediaocentric. The remaining short chromosomes, although structurally similar to each other, may be divided into three recognizable groups.

Introduction

Tissue cultures of mammalian cells and rapidly growing rootlets of various plants have frequently been employed in studying chromosomal structure and mechanisms and in assaying the ability of various chemical, physical, and viral agents to break chromosomes. The marine embryos, some of which have already been examined extensively from aspects such as mitosis, metabolism, and biochemical processes associated with differentiation, and in which there are available many rapidly dividing cells, remain essentially unexplored as material for the in vitro study of chromosomal behavior, structure, function, and reduplication. During an investigation of various salt water species to be used for such purposes, the somatic cell complement of Arbacia punctulata was determined (German, 1964). The modal number was found to be different from that reported in the earlier literature (Tennent, 1912; Matsui, 1924; Morgan, 1927; Harvey, 1940 and 1956). Furthermore, the animal's karyotype had not been accurately described. The following report concerning the complement of this sea urchin may contribute to further use of this animal in scientific study.

Material and Methods

Adult specimens, collected in the Cape Cod waters between Nobska Point and Falmouth, Mass., were maintained in the laboratory in running sea water and used repeatedly as sources of gametes (Harvey, 1954). To obtain gametes, an animal was removed from the water to the laboratory bench, where two lead electrodes were

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applied to the aboral aspect of its test about 1.5 cm apart near the gonopores. The flow of 10 volts alternating current resulted in the immediate shedding from the animal of either reddish-brown eggs or white sperm. The gametes were aspirated with small glass pipettes. Eggs were transferred to sea water in a covered Petri dish; the number of eggs used was adequate to produce a visible light brownish covering on the bottom of the dish. Two drops of sperm were transferred from the animal to a test tube containing 15 ml of sea water. Five drops of this slightly cloudy suspension of sperm were then transferred to the egg dish, which was agitated a few times to diffuse the sperm among the eggs. The entire procedure took place at room temperature with water near 21°C. The progression of fertilization, cleavage, and early development was observed with phase contrast microscopy. There was almost 100% fertilization, and many-celled blastulae were present by 5–6 hours; if left undisturbed, these hatched, formed gastrulae, and finally became active plutei.

For chromosomal preparations, colcemid was added 5 hours after fertilization to make a final concentration of 0.6 mg per 100 ml sea water. From one to sixty minutes later they were transferred to a conical test tube, centrifuged gently for one minute, and then transferred to 2–3 ml of sea water deficient in calcium and magnesium which contained a colcemid concentration of 0.16 mg % and a trypsin concentration of 0.16 g%. The tube was warmed by holding it in the hand for ten minutes, and the embryos were pipetted up and down gently in order to disperse the cells. Because of the delicacy of the blastomeric membranes some cell breakage was inevitable, although with gentle handling this was considerably reduced in the later experiments. When the suspension of blastomeres was achieved, the tube was centrifuged gently, and the cells were resuspended in 1.0 ml sea water. Two volumes of distilled water (2.0 ml) were slowly added to the cell suspension (Hsu and Pomerat, 1953). After twenty minutes the suspension was centrifuged at 350 RPM for five minutes. The supernatant fluid was removed, and the cells were fixed by the addition of 3 ml of acetic methanol (1 part glacial acetic acid and 3 parts anhydrous absolute methanol). Repeated aspirations with a small glass pipette and bulb effectively converted strands and clumps into a cellular suspension. This was then centrifuged at 500 RPM for three minutes, the fixative was decanted, and a few drops of fresh fixative were added to produce a slightly cloudy suspension of cells. Onto the surface of a cold, wet microscope slide were placed 2–3 drops of this suspension, and the slide was dried at once by fanning and gentle heating. The dried slide was allowed to stand overnight in a jar of freshly filtered 1% acetic orcein and then was passed through alcohols to xylol, following which the cells were mounted with Permount under a cover glass.

Results

In some preparations the blastulae (Fig. 1) were not adequately dispersed into single cells and the chromosomes lay in multiple planes. However, overly vigorous handling resulted in rupture of many cells and disruption of their chromosomal complements. The metaphase chromosomes of a single cell were often quite widely separated. Many hundreds of cells in various stages of division were found on each microscope slide, with c-metaphases in great abundance. In addition discrete, non-dividing nuclei of various sizes were present. The cytoplasmic background appeared dense and was sometimes heavily stained with the orcein. The metaphase chromosomes were longer when the colcemid treatment was