THE CHROMOSOMES OF SOME LOWER CHORDATES*

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Abstract. The chromosomes of three species of lower chordates were examined using a squash technique on small pieces of testis. Ciona intestinalis, a tunicate of the order Enterogona, has fourteen pairs of minute chromosomes. Styela plicata, a tunicate of the order Pleurogona, has sixteen pairs of chromosomes whose total size is approximately twice that of the Ciona chromosomes and about 10% of that of a typical mammalian complement. The hagfish, Eptatretus stoutii, of the suborder Myxinoidea, order Cyclostomata, has twenty-four pairs of chromosomes and what appear to be one to four small supernumeraries in some animals. The hagfish chromosomes are large, approaching the size of a typical mammalian complement. These size relationships agree in general with a concept of a small ancestral vertebrate genome which evolved into the larger present day genomes through a series of duplications of genetic material.

Introduction

It has been shown that fishes belonging to the class Pisces are diverse groups with regard to their DNA contents (Ohno and Atkin, 1966). The lungfish (Lepidosiren paradoxa, 2n = 38), representing the order Dipnoi of the subclass Crossopterygii, was similar to Caudata (Salamanders) in having an enormous amount of DNA, in this case 3540% of the typical complement in placental mammals. Among ray-finned fishes, the trout (Salmo iridesus, 2n = 60 ±), belonging to the order Isospondyli, resembled Crocodilia (a caiman) and Chelonia (a turtle and a tortoise) in having a DNA value which was 80% of that in mammals. The goldfish (Carassius auratus, 2n = 102 ±), of the order Ostariophysii, resembled Squamata (both snakes and lizards) on one hand and Aves on the other hand in having an amount of DNA equal to about 50% of that in mammals. The lowest DNA value, 20% of that in mammals, was possessed by members of the orders Heterosomata (sole, turbot) and Microcypriini (swordtail). On the basis of this evidence these authors postulated that a series of gene duplications took place while vertebrates were still aquatic forms and that evolution of terrestrial vertebrates was polyphyletic. The fishes with the DNA value which was only 20% of that of mammals were regarded as having retained the original vertebrate genome.

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There has been much discussion and disagreement regarding the relationship of living species of protochordates and lower vertebrates to possible ancestral chordate, and especially vertebrate, stocks. For example, Berrick (1955) and Carter (1957) disagreed on the place of the tunicates, especially Ciona, in chordate evolution. Still, there is no doubt that these lower forms do share a common ancestry with the vertebrates whose chromosomes were studied in the previously mentioned work. Therefore, it seems desirable to provide similar descriptions of chromosomes and DNA contents in some of the lower chordates.

The present study describes the chromosome complements of two species of tunicates belonging to two different orders and of one species of hagfish.

Material and Methods

The following species were studied:

1. Ciona intestinalis is a tunicate belonging to the Order Enterogona, Class Asciidiacea. It is found in shallow water on pilings and on ship bottoms. It has a worldwide distribution, and is essentially an annual species. Berrick (1955) considers it to be an extremely primitive tunicate.

2. Styela plicata is a tunicate belonging to the Order Pleurogona, Class Asciidiacea. It is widely distributed on the coasts of the warmer parts of the Atlantic, Pacific, and Indian Oceans and in the Mediterranean in much the same conditions as Ciona. Its suborder, Stolidobranchiata, is very large and is considered the most highly specialized group of Ascidians (van Name, 1945).

3. Eptatretus stoutii is a hagfish, suborder Myxinoidea, of the Order Cyclostomata Class Agnatha. This is a Pacific coast species inhabiting non-sandy ocean bottoms, of the continental shelf from Alaska to Baja California, at depths of 60 to 1800 feet. The hagfishes are generally considered to be the most primitive living vertebrates.

In none of these three species is there any large accessible mass of mitotically active adult tissue such as the spleen or bone marrow of higher vertebrates. The testis provides the only readily available source of either mitotic or meiotic chromosomes. Chromosome preparations from testis were made following the method described by Ohno (1965).

Chromosome squashes were prepared from the testis of colchicinized animals, small pieces of testis being pretreated in distilled water and then fixed in methanol-acetic acid (3:1). The preparations were then hydrolyzed in warm 1 N HCl and stained in Giemsa. Because each of these three species is essentially isotonic with sea water, the colchicine was dissolved in sea water for injection. For the same reason two changes of double distilled water were used during the hypotonic pretreatment before fixation.

Measurements of the total chromosome area per mitotic metaphase were made for each species by using a polar planimeter on traced projections from Polaroid 46 L projection film. This technique appears useful for larger complements, but the final magnification necessary to bring the tunicate chromosomes into the size range needed for desirable accuracy by the planimeter was close to 12,000 X. At this magnification the edges of the chromosomes were fuzzy. Thus only relatively uncertain estimates were obtained for these three species and for human mitotic metaphase chromosomes measured at the same magnification.

The relative DNA values of these species are being measured by Dr. N. B. Atkin, using the same technique previously applied to higher vertebrates (Atkin, Mattingson, Bečak, and Ohno, 1965; Ohno and Atkin, 1966).