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DNA Values of Four Primitive Chordates*

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Abstract. DNA values lower than the lowest value of the vertebrates were found for a urochordate and a cephalochordate. On the assumption that the genomes of surviving primitive chordata reflect the status of the genomes possessed by ancient organisms from which vertebrates eventually evolved, it is suggested that vertebrates started from an organism with a very small amount of DNA. Two cyclostomes, on the other hand, showed relatively high DNA values suggesting that the increase in DNA both by regional duplication of chromosomal segments and by polyploidization began to occur before vertebrates developed the jaw. The DNA values relative to the human female leucocyte value were: *Ciona intestinalis:* 6%; *Amphioxus lanceolatus:* 17%; *Lampetra planeri:* 38%; *and Eptatretus stoutii:* 78%.

Introduction

We have previously suggested (OHNO and ATKIN, 1966) that in the course of vertebrate evolution different degrees of gene duplication both by regional duplication of small chromosome segments and by polyploidization occurred to the ancestral genome during the aquatic stage, before the establishment of the chromosomal sex-determining mechanism; this was in keeping with the varying DNA values found among 8 species of fishes. The lowest value, about 20% of the mammalian value, was found for the swordtail (*Xiphophorus helleri*), the hornyhead turbot (*Pleuronichthys verticalis*) and the fantail sole (*Xystreurys liolepsis*). In a more recent study (OHNO, MURAMOTO, CHRISTIAN, and ATKIN, 1967), similar low values were found for two species of barb (*Barbus tetrazona* and *Barbus fasciata*). It was postulated that these species having 20% of the mammalian value had retained the original vertebrate genome.

In view of the uncertainty with regard to the position of primitive chordates, such as those belonging to the subphyla *Cephalochordata* and *Urochordata*, and *Vertebrata* of the class *Cyclostomata*, in vertebrate evo-

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lution, data on their chromosomes and cellular DNA content are of particular interest. TAYLOR (1967) has recently reported observations on the chromosomes of two tunicates, *Ciona intestinalis* and *Styela plicata*, and on a cyclostome, *Eptatretus stoutii*. In this paper we have supplemented TAYLOR's observations with estimations of the DNA values of four lower chordates.

Materials and Methods

The species studied were:

1. *Ciona intestinalis* (sea squirt), subphylum *Urochordata*.
2. *Amphioxus lanceolatus* (lancelet), subphylum *Cephalochordata*.
3. *Lampetra planeri* (brook lamprey), subphylum *Vertebrata*, superclass *Agnatha*, class *Cyclostomata*.
4. *Eptatretus stoutii* (hagfish), subphylum *Vertebrata*, superclass *Agnatha*, class *Cyclostomata*.

The methods of preparation and the microspectrophotometric technique were the same as those used in previous studies (ATKIN, MATTINSON, BEÇAK and OHNO, 1965; OHNO and ATKIN, 1966; OHNO, MURAMOTO, CHRISTIAN and ATKIN, 1967).

Preparations were made of erythrocytes from the two cyclostomes and of the pharynx from the other species. Air-dried smears of hagfish erythrocytes were prepared in California and sent by air mail to Northwood where control cells were added. Air-dried smears of lamprey erythrocytes were made in Northwood, control cells being added two days later. Smears of the other materials were prepared in Northwood by tapping out the fresh tissues in a few drops of Earle's solution on coverslips followed by fixation by freeze-substitution. Human small lymphocytes were added to all the preparations immediately before fixation by freeze-substitution. The control cells were small lymphocytes from tonsillar tissue removed by tonsillectomy from female patients.

Results

The DNA values of the four species are shown in the Table. It can be seen that the values extend over a wide range. The hagfish, *Eptatretus stoutii*, has a value which is almost 80% of the human value. The diploid chromosome number of this species has been shown to be 48 (TAYLOR, 1967). The lamprey, *Lampetra planeri*, has a DNA value approximately half that of the hagfish. It is of interest to note that the diploid chromosome complement of a fresh water lamprey of Japan, *Entosphenus reissneri*, has been reported to contain 94 to 96 minute chromosomes (NOGUSA, 1960). The lancelet, *Amphioxus lanceolatus*, representing *Cephalochordata* has 17% of the human value, i.e. a little under half the lamprey's value. Although the diploid chromosome number of this species is unknown, *Branchiostoma belcheri* of Japan has been found to contain 32 chromosomes in the diploid complement (NOGUSA, 1960). The sea-squirt, *Ciona intestinalis*, has only 6% of the human value; its diploid chromosome number is 28, the chromosomes being very small (TAYLOR, 1967).

Table

	Cell type	No. of cells measured[a]	DNA value relative to human female leucocyte value and standard error
Ciona intestinalis	Epithelium (pharynx) Leucocytes (peripheral blood)	86 (37) 22 (16)	0.056 ± 0.004
Amphioxus lanceolatus	Epithelium (pharynx)	34 (30)	0.167 ± 0.004
Lampetra planeri	Erythrocytes	40	0.384 ± 0.009
Eptatretus stoutii	Erythrocytes	50	0.781 ± 0.012

[a] The figures in brackets indicate the number of individual measurements, where some of the cells were measured in groups of 2 or more. Approximately the same number of control cells were also measured; the control cells were human small lymphocytes from tonsillar tissue removed by tonsillectomy from female patients.

Discussion

Measurement of the chromosome area suggested that *Ciona intestinalis* had a diploid DNA content of only 5% of that of placental mammals (Taylor, 1967); the DNA value of 6% of the human value is in good agreement with this. Taylor (1967), on the other hand, found that chromosome area measurements on the hagfish, *Eptatretus stoutii*, indicated an amount of genetic material approaching that of placental mammals; this has been confirmed by our DNA determinations which gave a value of 78% of the human value. The high value in this species is in agreement with electrophoretic studies which suggested that the hagfish genome contains as many duplicated gene loci as do the mammalian genome with regard to those coding for subunits of hemoglobin and lactate dehydrogenase (Ohno and Morrison, 1966; Ohno, Klein, Poole, Harris, Destree, and Morrison, 1967).

The DNA value of 17% found for the lancelet, *Amphioxus lanceolatus*, though higher than that of *Ciona intestinalis*, is slightly lower than the lowest value found in our studies of Vertebrata. The value of 20% found in certain fishes was the lowest (see Introduction). It is of interest that, according to Young (1950), *Amphioxus* "shows us a stage very like that through which the ancestors of the true fishes evolved".

The results suggest that the genome of an ancient organism which served as a foundation stock of vertebrates probably contained a very small amount of DNA, and that at the initial stage of evolution a series of gene duplications both by regional duplication of chromosomal segments and by polyploidization occurred. This would account for the wide range of DNA values presently found for the primitive *Chordata* and *Cyclostomata*.

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