METHODS

Changes in the Histone/DNA Ratio During the Development of a Multicellular Organism

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 12, pp. 695-698, December, 1996
Original article submitted September 20, 1995

Changes in the histone/DNA ratio are studied in the star-fish. It is found that the development of a multicellular organism is based on the principle of nonequivalence of the sister cells which is described by the distribution of Polya. It is postulated that the development of an organism obeys the laws of mathematics.

Key Words: *division; blastomere; development; principle of nonequivalence of the sister cells*

In a multicellular organism, all cells originate from the same precursor, the fertilized oocyte. During ontogeny they differentiate to become specialized cells performing particular functions.

What is the mechanism responsible for cell differentiation? Presumably, it is based on the principle of nonequivalence of the sister cells which was formulated in the 19th century [11]. According to this principle, the sister cells formed as a result of division of the mother cells are not identical (nonequivalent) in the realization of their genetic programs [3], implying that metabolic processes in these cells are asynchronous and one cell controls the development of the other.

There is considerable evidence supporting this principle. For example, the majority of mitoses in binuclear cardiomyocytes of 5-14-day-old mice are asynchronous [5], i.e., one nucleus is in the interphase, while the other is in the prophase or metaphase. Radioautography and cytophotometric analyses of DNA synthesis in the *Allium* meristoma revealed several types of labeled cells [8]. There were cells with one nucleus in the G_1 phase, while the other nucleus was in the S phase. In numerous cells one nucleus was in the initial stage of replication, while the other was at the end of this process. In was demonstrated that in the early replicating binuclear cells of *Allium* the G_2 is longer that in the late replicating cells [9]. The asynchronism of processes in the sister cells was confirmed by others [6,9].

In order to verify the principle of nonequivalence of the sister cells we studied the early division stages in the echinoderm embryo using the coefficient F_{fagen} to evaluate the functional activity of cell genome [7]. This method is based on cytophotometric analysis of cell population after staining with naphthol yellow S (for histones) and by the method of Feulgen (for DNA). The coefficient K_{fagen} is calculated as the histone/DNA ratio, where histone and DNA are the light absorbances of nuclei stained with naphthol yellow S and after Feulgen.

MATERIALS AND METHODS

The study was carried out on zygotes and dividing embryos of the star-fish *Patiria pectinifera*. The embryos were fixed in 10% neutral formalin in sea water. Before staining, the blastomeres were mounted on a glass slide using a 0.1% solution of polylysine (1 mg/ml). After 10-15 min, a polylysine film formed on the slide, and extra solution was aspirated with a

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0007-4888/96/0012-1251$15.00

pipette. The slide was rinsed several times with bidistilled water. A drop of the embryo suspension was transferred onto the polylysine film and covered with a polyethylene film and a coverslip. The preparation was placed on black paper, and the embryo was destroyed under a light microscope by pressing the coverslip with forceps so that the blastomeres were located in the same plane and were well seen. The preparations were air-dried, washed for 30 min with tap water and for 10 min with distilled water, hydrolyzed with 5 N HCl at 37°C for 18 min, washed with cold 1 N HCl, and stained with Schiff's reagent (pH 2.7) for 1.5 h. Preparations of embryos from zygote to the 4th cleavage were stained at the same time, washed with sulfur water (10 min, 3 times), warm tap water (20 min), and distilled water (10 min), stained with naphthol yellow S for 35 min, rinsed three times with tert-butanol (2-methyl-2-propanol), and dried for 24 h at 37°C in a thermostat.

The preparations were analyzed in a Univar microspectrophotometer. Light absorbance after staining with Feulgen's reagent was measured at a wavelength of 575 nm and, after staining with naphthol yellow S, at a wavelength of 445 nm. The results were analyzed using Student's *t* test.

RESULTS

As shown in Table 1, K_{fagen} of the blastomeres formed after the first cleavage are statistically different, i.e., according to K_{fagen}, the distribution of the blastomeres is 1:1. Consequently, these sister cells are nonequivalent by realization of genetic program. Since a multicellular organism is a living system consisting of cells derived from the two sister cells, the possibility that this organism develops according to the principle of nonequivalence of the sister cells cannot be ruled out. The differences between the sister cells do not disappear after the first cleavage. After the second cleavage, the blastomeres distribute as follows: one blastomere with the minimal K_{fagen}, two blastomeres with significantly higher K_{fagen}, and one blastomere with the maximum K_{fagen}. This distribution can be written as 1:2:1. After the 3rd cleavage, cells are distributed as 1:3:3:1 and after the 4th cleavage the distribution is 1:4:6:4:1.

After arranging the distributions is rows and bearing in mind that the development of a multicellular organism starts from the zygote, the distributions of cells according to K_{fagen} can be written as follows:

zygote	0
first cleavage	1:1
second cleavage	1:2:1
third cleavage	1:3:3:1
fourth cleavage	1:4:6:4:1

This distribution can be described with the Pascal triangle [2], which in general terms is the distribution of Polya [4].

Thus, the distribution of blastomeres according to K_{fagen} obeys mathematical laws, i.e., these laws can be used for analysis of the living matter.

Interestingly, one cell with K_{fagen} similar to that of the zygote 0.97 (0.96÷0.98) is present during all cell divisions (Fig. 1). We believe that this cell is the unique stem (pluripotent) cell in the multicellular organism. The K_{fagen} coefficients of all other cells are statistically higher than that of the stem cell, indicating different functional activities of their genomes associated with activation of histones. It was reported that before division the content of H1 histone in the sea-urchin oocyte increases [12]. The 2nd cleavage

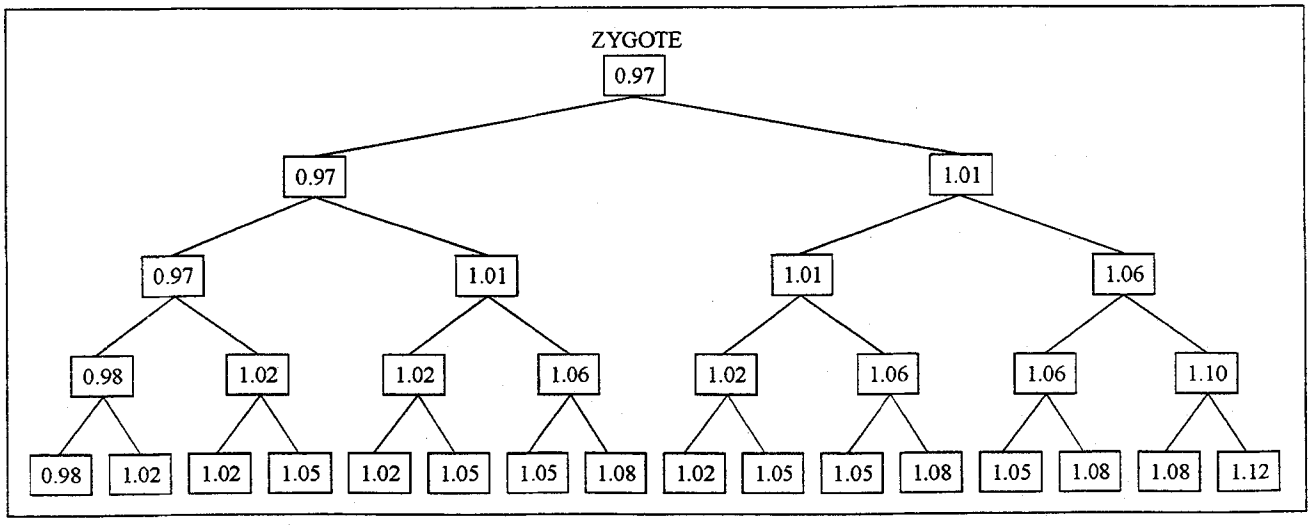

Fig. 1. Distribution of blastomeres according to K_{fagen} during the I—IV cleavage of a star-fish embryo.

TABLE 1. Changes in the Histone/DNA Ratio (K_{fagen}) During the First—Fourth Cleavage of the Star-Fish

Blastomere No.	Cleavage*			
	I	II	III	IV
1	0.97 (0.96÷0.98)	0.97 (0.96÷0.98)	0.98 (0.97÷0.99)	0.98 (0.97÷0.99)
2	1.01 (1.00÷1.02)	1.01 (0.99÷1.03)	1.02 (1.01÷1.03)	1.02 (1.01÷1.03)
3		1.01 (0.99÷1.03)	1.02 (1.01÷1.03)	1.02 (1.01÷1.03)
4		1.06 (1.04÷1.08)	1.02 (1.01÷1.03)	1.02 (1.01÷1.03)
5			1.06 (1.05÷1.07)	1.02 (1.01÷1.03)
6			1.06 (1.05÷1.07)	1.05 (1.04÷1.06)
7			1.06 (1.05÷1.07)	1.05 (1.04÷1.06)
8			1.10 (1.08÷1.12)	1.05 (1.04÷1.06)
9				1.05 (1.04÷1.06)
10				1.05 (1.04÷1.06)
11				1.05 (1.04÷1.06)
12				1.08 (1.07÷1.09)
13				1.08 (1.07÷1.09)
14				1.08 (1.07÷1.09)
15				1.08 (1.07÷1.09)
16				1.12 (1.10÷1.14)
Distribution of blastomeres according to K_{fagen} at different stages of ontogeny	1:1	1:2:1	1:3:3:1	1:4:6:4:1

Note: *The number of embryos analyzed: I cleavage, 58; II, 56; III, 38; IV, 24. The confidence interval is given in parentheses.

is characterized by an increase in the H2a and H2b fractions, while the content of H3 histone rises in the blastula, and the content of H4 histone is increased in the gastrula [1]. It seems reasonable to suggest that the three blastomeres with $K_{fagen}=1.02$, which is close to that of the stem cell, are stem cells with a limited potency (committed cells). Presumably, they are the "ancestors" of erythro- and lymphopoietic cells. Cells with the maximum K_{fagen} have the highest degree of differentiation at this stage of development of a multicellular organism.

Thus, the results of the present study indicate that the ontogeny of a multicellular organism proceeds according to the principle of nonequivalence of the sister cells that is described by the distribution of Polya.

If the development of a living matter is governed by mathematical laws, the amount of cells at a given stage of ontogeny can be calculated. Modification of these cells can change the organism's ontogeny.

We are grateful to Dr. L. E. Martynova for helpful consultations and star-fish embryos.

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