VARIABILITY IN MORPHOGENESIS

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Variability of development of the freshwater hydra was investigated at the metabolic level. The course of the morphological and metabolic indices with time showed considerable lack of coordination. The model investigation enabled the increase in variability to be correlated with the appearance of competence for differentiation in the system. The character of the final spatially-heterogeneous structure is determined not by the original data, but by the parameters of the developing system.

KEY WORDS: variability; competence for differentiation.

The phenomenon of increased variability was discovered comparatively recently during the study of morphogenesis. The nature of the phenomenon is that at the beginning of the process of formation of a new form variability increases significantly, but at the end of the process it virtually disappears. The term variability means differences in the properties of the same objects observed under the same conditions. Variability is manifested, for example, as a difference in the distribution of particular metabolites along the body of an object (hydra, in particular), and also as irregularity of the distributions themselves. At the end of the process the distributions become regular, monotonic, and virtually identical for the same objects.

The variability and equifinality of morphogenesis of several species of animals at the cellular and supracellular levels have been known for a comparatively long time [1, 3, 7] and new data have been added in recent years [2, 5]. It appears that morphogenesis can lead to the same end result by different and, generally speaking, undefined ways. At a certain (sometimes long) stage of its development, a living system behaves stochastically and possesses unpredictable behavior. Random external influences are considerably strengthened under these circumstances. However, at the end of the process a perfectly definite and nonstochastic picture emerges. This shows that the process of development cannot be determined at every stage and it raises the question of the ways by which the single final state is reached under these conditions.

The object of this investigation was to study variability of development of the freshwater hydra, not at the cellular, but at the metabolic level and, by the use of a mathematical model, to look for the conditions determining intermediate variability and ultimate equifinality.

EXPERIMENTAL METHODS

Polyps of the species Hydra attenuata Pall. were used. The polyps were cultured in an artificial medium [8]. Adult polyps and buds at the following stages of growth were investigated: protuberance, cylinder, bud with rudimentary, and with formed tentacles. The state of protein synthesis was determined by autoradiography with respect to incorporation of [^{3}H]leucine. The hydra was incubated in a solution of the labeled amino acid (concentration 20 μCi/ml medium, specific activity 72 mCi/mmole) for 2.5 h.

The number of tracks in the hypostomal region was counted in every second section, and in other parts of the body of the hydra every second to fourth section. The area of the chosen section was divided into four sectors. In each sector (separately in the ectoderm and entoderm) the tracks were counted on sample squares with an area of 288 μ^2. Graphs of the number of tracks against the number of the section, i.e., the position along the body axis, were plotted. To determine the statistical significance of differences in the concentration of tracks a variation series was plotted for each graph as follows: The abscissa was divided into small (1 to 3 sections) segments and the area below the graph calculated in each segment. The significance of the difference between this series and the variation series of the normal distribution was determined by the chi-square test

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TABLE 1. Significance of Differences between Individual Points in Zones of Local Differences and of Hypostomal Peaks

Significance	Graph					
	a		b		c	
	ectoderm	entoderm	ectoderm	entoderm	ectoderm	entoderm
$P>$	—	—	0,01	0,01	0,05	0,05

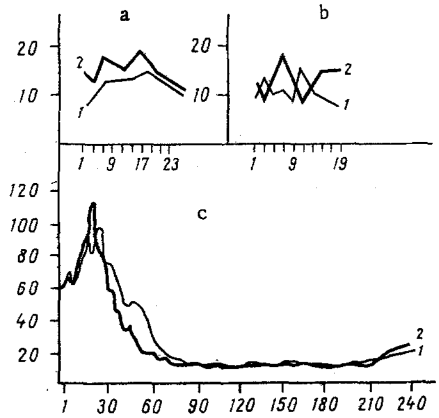

Fig. 1. Incorporation of [^{3}H]leucine into protein of developing bud. Abscissa, serial numbers of sections starting from apical end of bud; ordinate, mean number of tracks per section. 1) Entoderm; 2) ectoderm. a, b, c) Three main types of state of regional synthesis.

[4]. The significance of differences between individual points was determined by the Fisher–Student criterion.

For the model study of the phenomenon a mathematical system of Turingian type was used: This describes both the processes in each cell and processes of interaction between cells due, for example, to diffusion of metabolites. In many investigations devoted to systems of Turingian type [5, 9] it has been shown that such a model can describe both the spatially-homogeneous (structureless) state of the system and the heterogeneous (structured) state. The latter state arises when the parameters are in a certain ratio, when the homogeneous state becomes unstable (later this state will be interpreted as competent to form a structure). The model used in the investigation [6] has several important features which distinguish it from those examined previously. The main feature is as follows. Depending on the value of the parameters, the model can describe two qualitatively different situations, namely: The first corresponds to the case when each cell taken separately can function in only one stable, steady state (unisteadiness), the second corresponds to the case when each cell can function in one of two stable steady states (multisteadiness). The model enables the transition from unisteadiness to multisteadiness to be studied during a change in the parameters. Within the confines of the model this transition is interpreted as the appearance of competence for differentiation. This understanding of the term competence for differentiation differs from that generally accepted. The difference is due to the fact that within the confines of the model competence for differentiation and competence for the formation of a heterogeneous structure are separate, i.e., they require different conditions and cannot take place simultaneously. Experimentally these processes are difficult to distinguish from each other if attention is paid only to the change in form of an extended object: The appearance of heterogeneity of shape is usually interpreted simultaneously both as the appearance of a structure and as the appearance of differentiated cells, i.e., cells functioning according to different programs. These processes can be separated experimentally if attention is paid to the changes in shape and to behavior (distribution) of metabolites simultaneously. The most interesting state of development arises at the moment when the two competences mentioned above are superposed, i.e., when against the background of existing competence for the formation of a structure, competence for differentiation appears in the system.

A slow change of parameters, involving a qualitative change in the system, will be interpreted as the development of the system.

It was assumed in the investigation that external random disturbances act on the system.

EXPERIMENTAL RESULTS

Despite considerable variation in the distribution of the intensity of protein synthesis along the body axis at different stages of growth of the bud, three main types of the state of regional synthesis can be distinguished: 1) a state of absence of regional differences (this is observed in all stages of growth of the bud except a bud with formed tentacles; see Fig. 1a); 2) a state of local differences (revealed at the protuberance stage and detectable as far as the formed bud; Fig. 1b); 3) a state of a single hypostomal peak, characteristic of the adult hydra (Fig. 1c). In other words, there is considerable lack of coordination in time between the morphological and metabolic indices of formation of spatial organization. This lack of coordination is manifested as the absence of regional differences coupled with a state of local abrupt changes in the intensity of protein synthesis in the bud, alternating with a relatively steady state after the appearance of the hypostomal peak in the adult. In other words, variability increases at the beginning of growth of the hydra, but virtually disappears at its end.

The analogy with the dynamics thus revealed can be pursued by mathematical investigation of the phenomenon. It was discovered that the sensitivity of the system to external random disturbances increases during development at the time when competence for differentiation appears in the system. In that case the system acquires stochastic properties. This is manifested as the random appearance and disappearance of areas of differentiated tissue. In the case of multisteadiness, i.e., under conditions of high competence for differentiation, the spatially heterogeneous structure which arises becomes stabilized and variability disappears. One interesting result of the model investigation is an indication of the existence of correlation between the phenomenon of variability and the appearance of competence for differentiation in the system. The character of the eventual spatially heterogeneous structure under these circumstances is determined within the framework of the model not by the original data, but by the parameters of the system.

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