VISUALIZATION OF THE SEGREGATION STRUCTURE OF CYTOPLASM BY COMPUTERIZED MICROSCOPY

Ya. G. Dorfman and V. V. Istomin

UDC 612.613:612.64]-086.2:681.31

KEY WORDS: computerized microscopy; segregation structure; cytoplasm; yolk granules

Segregation or spatial separation of the component parts of the cytoplasm of the fertilized egg or, as the process is generally known, ooplasmic segregation, is one of the most important although least studied mechanisms of embryonic morphogenesis [7]. Even before the fertilized egg has divided into separate blastomeres, ooplasmic segregation marks the principal elements of spatial organization of the embryo. Direct interference with the distribution of the components of the egg by centrifugation or with the aid of a clinostat leads to marked development anomalies [1].

Ooplasmic segregation is characteristic of all species of animals. Processes similar to it continue throughout embryonic development. Segregation, aggregation, dispersion, and separation of the components of the cytoplasm into layers are well known during mitosis, during changes in the physiological state of pigmented, secretory, and nerve cells [2, 3], and in numerous pathological and repair processes. Segregation of the components of the cytoplasm as a rule is not just the simple segregation of mutually insoluble liquids. Discrete well formed cytoplasmic inclusions and organelles are actively separated and concentrated in different parts of the cell. Sometimes they are quite large and visible under the light microscope, but more often only concentrations and aggregates of them can be distinguished at the light-optical level. Not only organelles of different types, but also organelles differing from one another only in shape and size segregate.

Very labile segregation structures, statistical and hierarchical in nature, can be investigated by traditional morphological methods only in exceptional cases. They are invisible in the ordinary microscope, or with the magnifications necessary to resolve individual cytoplasmic inclusions, the field of vision is limited to a small part of the object. Many very important characetristics of texture, for example, the ratio of the size of particles to the volume in which they are distributed, are virtually impossible to estimate by eye. To visualize a segregation structure, i.e., to construct sufficiently detailed maps of the spatial distribution of textured parameters of the cytoplasm, numerous morphometric measurements are necessary. This type of investigation can be made at the present time only with the aid of modern automatic television image analyzers or, as they are still called, computerized microscopes.

EXPERIMENTAL METHOD

The drawing of maps of segregation structure requires serial analysis of the structure of an object on several three-dimensional scales. These can be distinguished naturally as follows: the scale of a single aggregate or concentration of particles; the scale on which characteristics of the microrelief of the cytoplasm can be determined, such as parameters characterizing a measure of its spatial heterogeneity; and finally, the scale on which the structure of the object as a whole can best be examined. This requires the object to be scanned under relatively low magnification, the local stereologic parameters measured, and on this bases, maps of segregation structure to be drawn.

Department of the Theory of Evolution, Faculty of Biology, Moscow State University. Laboratory of Clinical Neuromorphology, Research Institute of Clinical Psychiatry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 3, pp. 351-333, March, 1986. Original article submitted December 13, 1984.

0007-4888/86/0003-0367$12.50

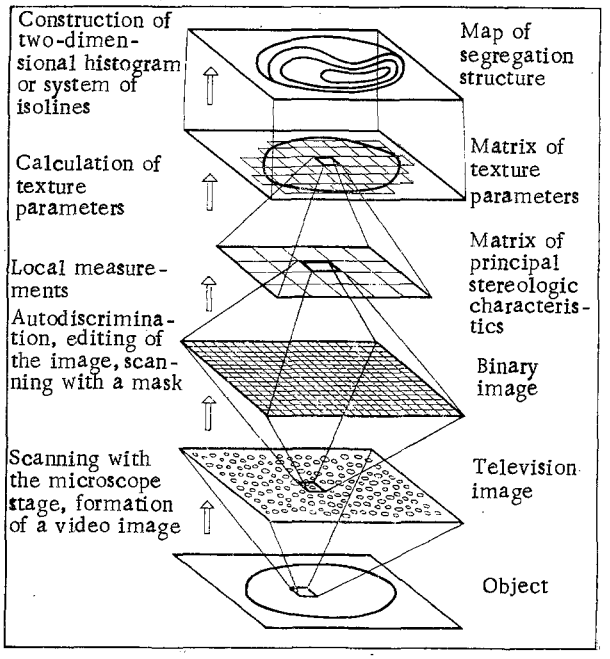

Fig. 1. Principal stages of Visualization of the segregation structure of cytoplasm (scheme).

A scheme illustrating the principal stages of visualization of segregation structures is given in Fig. 1. The complex system of morphometric measueements and numerical analysis of the data thus obtained are only the preliminary stages of visualization of structure. The procedure described was realized in the form of a set of programs for the Leitz TAS television analyzer, by means of which the construction of maps of segregation structure can be completely automated. Stages of scanning, automatic focusing, automatic discrimination, automatic searching and selection of visual fields, and also preliminary editing of the image are included as elements in the program. At the beginning of work the region of the preparation to be analyzed, the scale of averaging, one or more granulometric characteristics relative to which the maps are drawn, and the volume and shape of presentation of information supplementary to the maps may be decided.

Since the work must be done at two levels of resolution in order to obtain maps based on parameters of heterogeneity of the spatial distribution of organelles, it was natural to divide the scanning process into two stages: rough, with a step equal to the field of vision of the instrument, namely 184 μ corresponding to a magnification of 25, and fine, scanning the field of vision with a mask. Usually the field of vision was divided into 8 × 8 square fields, in which elementary stereologic parameters were measured: the specific surface area of the particles, their number, their mean volume, and volume concentration. On the basis of the resulting matrix of primary stereologic parameters, characteristics of texture were calculated, such as the mean value of the measured parameters in a large field of vision, the coefficient of variation of these parameters, the mathematical expectancy of the granulometric characteristics in neighboring fields of measurement, and so on. Thus for the section as a whole, 24 different numerical maps can be drawn simultaneously.

In the next stage of visualization the computerized microscope is programmed to synthesize images according to the numerical map. A system of isolines is plotted, after which, on an appropriate scale, it is superposed on the image of the object under low power, and led out to display units for analysis and photography. In this way the distinguishing features of segregation structures can be correlated with traditional elements of morphologic structure. An atlas of the maps thus produced is stored on magnetic tape or disk.

At all stages of the work, traditional algorithms of mathematical morphology are used for image conversion [6].

EXPERIMENTAL RESULTS

By means of the set of programs devised, segregation structures of cleaving eggs of the frog *Rana temporaria* were investigated and, in particular, the formation of dorsoventral polarity of the embryo. Semithin Epon sections (1 μ), stained with Fast green, were used.

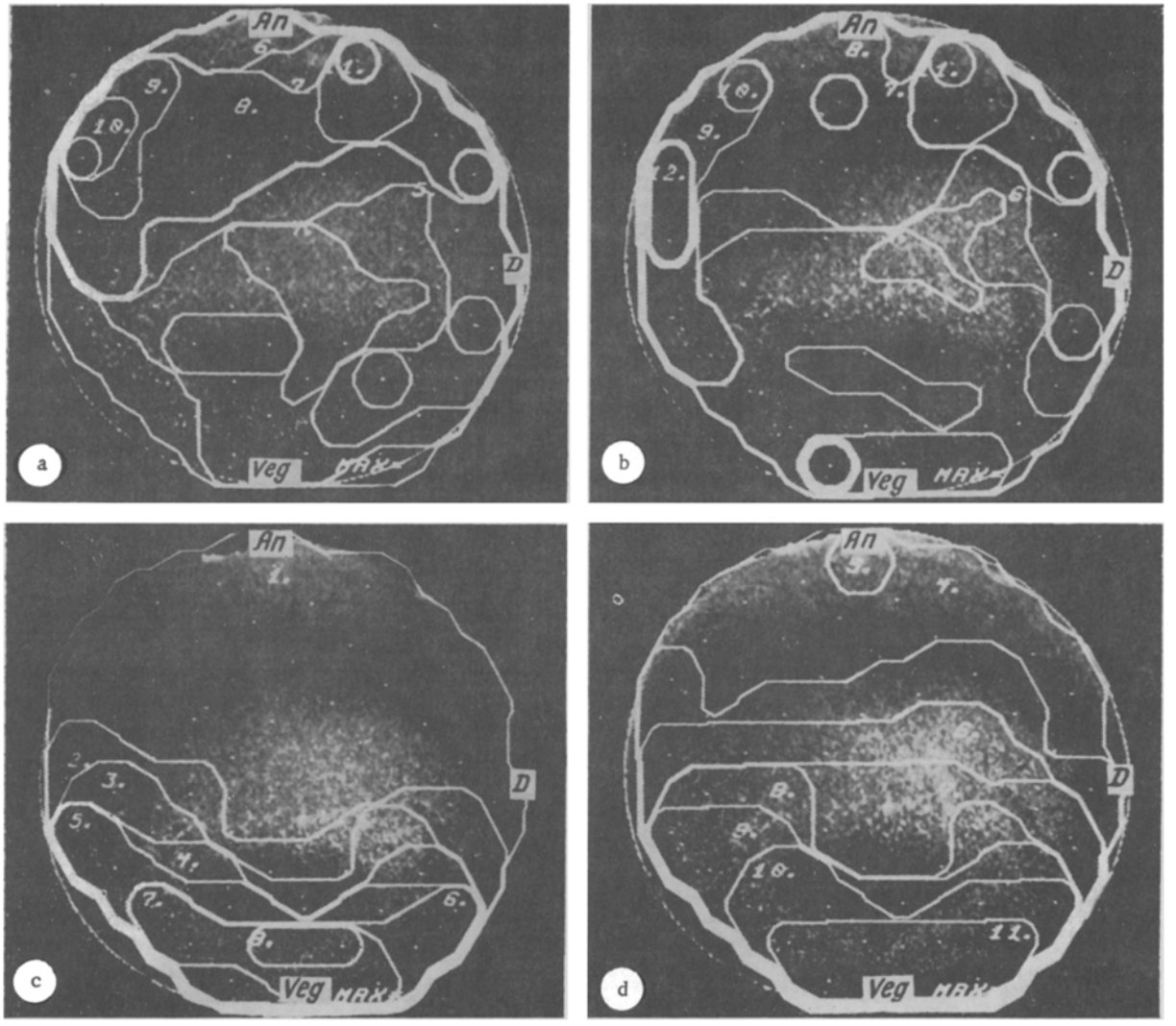

Fig. 2. Maps of segregation structures of cytoplasm of a fertilized egg of *Rana temporaria*, drawn from characteristics of spatial distribution of yolk granules (the gray crescent stage, 1 h 10 min before the beginning of cleavage, τ_0 = 75 min, sagittal plane). Calculation of textural parameters: field 184 × 184 μ, scanning step 180 μ; measurements: field 23 × 23 μ, scanning step 23 μ, resolution 0.72 μ. An) Animal pole, Veg) vegetal pole, D) dorsal aspect of egg. a) Distribution of local coefficient of variation C_{vl} of mean volume of yolk granules V_{st}. Neighboring isolines correspond to values of C_{vl} differing by 9% (Δ = 9%); b) distribution of parameter of local spatial nonhomogeneity LT of volume concentration of yolk S (Δ = 32 units); c) distribution of mean volume of yolk granules $\overline{V}_{st}$, (Δ = 20.4); d) distribution of mean volume concentration of yolk granules $\overline{S}$ (Δ = 3.6%).

In this paper we give only four maps of the spatial distribution of yolk granules in the sagittal plane of the egg of *Rana temporaria* at the gray crescent stage. It will be clear that maps drawn on the basis of different textural characteristics will provide mutually complementary information (Fig. 2). In particular, the distribution of mean values of stereologic parameters differs sharply from that of the parameters of heterogeneity.

The multiplicity of segregation structures is thus a matter of principle. By changing the image transformation function at any of the stages of visualization, we can as it were change the angle of examination of the object, and observe it under a different magnification or with a different optical system. In future, we consider, replacement of one visualization system by another by the microscopy will be just as easy as it is now to change the magnification of a microscope or filter.

It will be clear from the maps that at the gray crescent stage the dorsoventral polarity of the egg is manifested not only as asymmetry of arrangement of granules of different sizes, but also as the unique shape of the regions of increased heterogeneity of the cytoplasm (see [4]). The measure of spatial heterogeneity of distribution of the organelles in the cytoplasm correlates well with the morphogenetic activity of the corresponding region of the embryo. This same characteristic is closely linked with the physiological state of cells of the adult organism [2]. Double and even triple isolines bound regions with sharply different textural characteristics. It is these strong segregation boundaries that mark out the principal morphological structures of the embryo [5].

Segregation structures of the cytoplasm are evidently a connecting link between biochemical and morphological levels of biological organization. It is apparent that the use of computerized microscopes in order to visualize them will provide new data on the nature of morphogenetic processes.

LITERATURE CITED

1. Ya. G. Dorfman and V. G. Cherdantsev, Ontogenez, 8, No. 3, 238 (1977).
2. S. L. Zaguskin, L. E. Namirovskii, A. V. Zhukotskii, et al., Tsitologiya, No. 8, 982 (1980).
3. N. K. Kol'tsov, Organization of the Cell [in Russian], Moscow–Leningrad (1936).
4. J. Gerhardt, G. Ubbels, S. Black, et al., Nature, 292, 511 (1981).
5. N. Naktzuji, W. Roux's Arch. Dev. Biol., 178, 1 (1975).
6. J. Serra, Image Analysis and Mathematical Morphology, London (1982).
7. T. Shimizu, W. Roux's Arch. Dev. Biol., 191, 246 (1982).