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ON THE SYNTHESIS OF CLEAVAGE CHROMOSOMES

BY THEOPHILUS S. PAINTER

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF TEXAS

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The question which has prompted the present inquiry is, "How is it possible for the chromosomes in rapidly segmenting eggs to synthesize so quickly the new material needed in the reduplication process prior to each cell division?" While it is not to be supposed that a full answer can be given to this question at present, nevertheless, if we consider modern ideas of protein and chromosome structure and behavior and some other facts scattered in the cytological literature of the past, there emerges a surprisingly simple and illuminating hint as to one of the ways this may be accomplished. Here it is proposed to draw attention to the pertinent evidence and some of its rather obvious or possible implications.

Chemists have shown that proteins are composed of long chain-molecules, each link of the chain being a chemical unit such as an amino-acid residue, a pyrimidine ring or the like, united with other structural units by rather simple bonds. Thus, two amino-acids may react to form a molecule of water and the "residues," as they are called, are linked by a "peptid" bond, and when many units are involved we have a "polypeptid" chain. When new molecules of a complex protein are formed the lattice of the old molecule is supposed to act as a form or mold, each unit of which, such as an amino-acid radical, attracts, or somehow receives from the surrounding medium, a unit like itself and in this way the new molecule is organized and built up. Such a method of formation allows us to understand how the high degree of specificity of proteins (and presumably of genes) is maintained. Viewed in this light, the reduplication 5 to 10 or more times of the same chromosome during 24 hours in segmenting eggs would involve the utilization of relatively large amounts of the constituent units of the nucleoproteins of the chromosomes and would be much more easily understood were we to assume that the egg cytoplasm is extremely rich in the structural units out of which nucleoproteins are made rather than to assume that these needed materials are synthesized anew, at some phase of the division cycle, out of more or less undifferentiated food material.

The early cytological literature is replete with descriptions of the various cellular mechanisms involved in the formation of yolk within the animal egg. In general there are two common methods, either through the agency of nurse-cells, or by the activity of the ovum itself. The cytological picture of the egg, in these two instances, is very different.

When the eggs grow at the expense of nurse-cells, the latter increase rapidly both in the size of the nucleus and the cytosome, and there is a very marked growth in the amount of chromatin as is shown by Feulgen's nucleal reaction. In the ovary of *Drosophila melanogaster*, for example,[4] the nurse-cell nuclei increase in diameter from 5 μ to 40 μ or more. This means that the volume of the original nuclei has been increased about 512 times. Judging from the figures of other investigators of nurse-cells (e.g., Gross,[1] or Jörgensen,[2]) this size increase is in no way unusual. As the growth of the ovum nears completion, the nurse-cell contents are usually absorbed by the egg either through a direct engulfing, as in many Diptera, or more indirectly by absorption through the egg wall, during which process the nurse-cell nucleus loses its chromaticity and dwindles in size along with the cell cytoplasm until the nurse-cell remains are shrivelled bits of débris which completely disappear. Thus directly or indirectly the nurse-cell contents are absorbed and large quantities of nuclear material enter the cytoplasm of the egg. An essentially similar condition obtains in the egg cells of many plants. Generally speaking, when nurse-cells function in yolk formation the egg nucleus remains small and stains very lightly.

When the formation of yolk is carried out by the egg itself, the egg nucleus, or germinal vesicle, invariably shows a great increase in size and this is accompanied by the formation of numerous nucleoli and often elaborate chromatic structures such as the so-called "lampbrush" chromosomes. After the formation of yolk is completed, the amount of chromatin which enters the first polar spindle is only a very small portion of the total amount visible when the germinal vesicle is at the height of its activity. Most of this chromatin, or its derivatives, is discarded into the egg cytoplasm, when the germinal vesicle breaks down, and is absorbed. Thus as regards nucleoproteins the situation is essentially similar to that in eggs nourished by nurse-cells.

In some animals both ovum and nurse-cells may function simultaneously or, as in the case of aphids, the summer eggs may be formed chiefly through the activity of a large germinal vesicle, while the yolk of the slow-growing winter eggs is due to the activity of nurse-cells. In the latter, the egg nucleus remains quite small and relatively achromatic.

The point of central interest for us is that by the cytological mechanisms employed in the growth of ova prior to fertilization, the cytoplasm of the egg is the recipient of large amounts of chromatin, or its derivatives, either through an engulfing or an indirect absorption of nurse-cells and their nuclei, or from the breakdown of the germinal vesicle.

The facts cited have been known to cytologists for a long time but it is only recently that we have gained a clear insight into the nature of the changes in the chromosomes which go hand in hand with any great increase in nuclear size.

In larval tissues of insects, growth is often accomplished by an increase in nuclear and cell size rather than by cell division. Accompanying this increase in nuclear size Geitler[3] has shown that there is a series of intra-nuclear chromosome divisions so that ultimately these larval somatic cells reach a high degree of polyploidy. For example, the large lobed nuclei in the salivary gland of *Gerris* have either 1024 or 2048 complete sets of chromosomes, oenocytes are 128-ploid and nuclei in the septum walls of the testis are commonly 16-ploid. In Diptera, the salivary gland chromosomes exhibit a special type of polyploidy in which the chromatids (after somatic synapsis) remain closely associated together.

Recently the writer and E. R. Reindorp[4] made a study of the nurse-cells in the ovary of *D. melanogaster* and we found very clear evidence for a series of intra-nuclear chromosome division cycles going hand in hand with an increase in the size of nurse-cell nuclei so that by the time a diameter of 40 μ is reached there are probably 512 haploid sets of chromosomes present in each nurse-cell. Since there are 15 nurse-cells associated with each egg, in the fruit fly, and these are all eventually absorbed by the egg cytoplasm, it is obvious that prior to fertilization and cleavage the ovum receives the materials of thousands of homologous chromosomes.

No one has as yet made a study of the growth of germinal vesicles to determine if intra-nuclear division cycles occur here, but our modern cytological outlook compels us to infer that the great increase in the chromaticity of these nuclei is due to some sort of reduplication of the constituent chromosomes and there is much evidence already which suggests that "lampbrush" chromosomes may not be simple pachytene chromosomes, but chromosome aggregates. In the first place, if one goes back to Rückert's original description of how lampbrush chromosomes are formed in, for example, *Pristiurus*,[5] we are confronted with the fact that a typical pachytene thread, 10 μ long and 0.5 μ in diameter grows into a structure 100 μ long and 10 μ in diameter. During this growth Rückert says that the "Mikrosomen" (chromomeres) put out side branches which lie at right angles to the long axis; these side branches are chromomeric in structure and, were one of them to lie separately, it would be considered a single chromosome. These side branches would account for the increase in the breadth of the chromosomes but not their ten-fold increase in length. A second and very suggestive fact is that in the nurse-cells of *D. melanogaster*, at a definite point in the intra-nuclear division cycle of large nuclei, the homologous chromatids, derived from the repeated divisions of the progeny of a single chromosome, form a hairy-caterpillar-like aggregate which is strikingly similar in form to the lampbrush chromosomes. There is a tendency for the separate chromatids to lie parallel to each other in these aggregates, and were one to transplant such an aggregate to a vertebrate egg undoubtedly it would be called a "lampbrush" chromosome. We are now making at my laboratory a study of the way typical lampbrush chromosomes are formed, but in the meantime it seems reasonably safe to conclude, in the light of all the evidence, that the growth of the germinal vesicle in eggs is accompanied by some sort of reduplication of the constituent chromosomes. As I have pointed out, only a very small part of the chromatin in the germinal vesicle enters the first polar spindle, and the remains of the germinal vesicle are absorbed by the egg plasm. This means that the material from thousands of chromosomes is set free in the cytoplasm, and just as in eggs with the nurse-cell mechanism, this would be available for use by the cleavage chromosomes.

There arises now the question: What happens to the chromosomes (or their derivatives) when they enter the egg cytoplasm, either by the nurse-cell or the germinal vesicle route? That they do not persist as visibly organized structures is well established cytologically and since the most diverse types of eggs have been tested by Feulgen's nucleal stain, with negative results, it appears that nucleic acid, as such, does not persist. On the other hand, there is reason to believe that the constituent proteins and nucleoproteins of the chromosomes do persist in a partially broken down form, because a number of chemical analyses have shown, in diverse eggs, the presence of large amounts of substances closely related to nucleic acid. Thus, in the mature egg of *D. melanogaster* Caspersson[6] has shown by his ultra-violet photo-electric method, that in the egg cytoplasm there is a high concentration of substances containing the pyrimidine ring. And Caspersson and Schultz[7] report that in the eggs of XX females there is appreciably less of this pyrimidine ring bearing material than in XXY eggs. This is in line with what we would expect in view of the fact that this substance is derived from the breakdown of nurse-cell chromosomes. Also in a number of marine and other eggs, many of which are of the germinal vesicle type, Brachet[8] and other workers have found a high concentration of nucleotides which we can understand in view of the large amount of nuclear material which is set free in the egg cytoplasm by the breakdown of the germinal vesicle.

The evidence, then, indicates that in the cytoplasm of all eggs there are the products of thousands of maternal chromosomes. Just in what form the constituent proteins and nucleoproteins exist is a matter for the biochemist to determine. In the meantime, it seems reasonable to conclude that the rapid building up of the cleavage chromosomes is possible in the segmenting egg because the synthesis is more in the nature of a reassembling of already existing materials, such as nucleotides, etc., under the guidance of the active chromosomes, rather than an actual synthesis of the building blocks from relatively simple substances.

The presence of materials derived from a very large number of maternal chromosomes and genes in the cytoplasm of eggs not only allows us to understand the rapid reduplication of the cleavage chromosomes but also gives us a simple explanation for certain types of cytoplasmic or matroclinus inheritance. We commonly think of genes as forming specific substances which react with other cellular constituents to produce, in the end, phenotypic expressions. Furthermore, Ephrussi[9] has shown that substances produced early in ontogeny may persist and affect structures developed in late larval life. This being true we might anticipate that the presence of large amounts of material derived from the maternal chromosomes and genes might sometimes affect the F_1 phenotype irrespective of the genotype of the latter. Many different cases of matroclinus inheritance, especially those which deal with larval characters, seem best understood in this light, and adult characters may occasionally be affected. Thus Noujdin[10] finds that the presence of a *Y* chromosome in the female fruit fly tends to suppress mottling and that all the progeny of such *XXY* females also show the same suppression no matter what their chromosome and genetic constitution may be. Since all of the eggs of an *XXY* female receive from their nurse-cells thousands of *Y* chromosomes which enter the egg cytoplasm we may assume that products of these *Y*'s persist and either quantitatively or qualitatively function to suppress the mottling. Since, however, the eggs of the F_1 females receive from their nurse-cells the nuclear products of their own genotype, the matroclinus suppression of mottling would persist only through the one generation.

Attention must also be given to the possible influence which the maternal chromosome products in the egg cytoplasm may play in development in general and especially in so-called parthenogenic merogony. The fact that Mrs. E. B. Harvey[11] has succeeded in stimulating enucleated egg fragments to develop in some instances to a morula stage would seem to minimize the importance of the chromosomes and genes in early development. Such a conclusion does not necessarily follow because it is quite possible that in the absence of a normal nuclear set-up, the genes, and possibly the division centers, brought into the egg by the nurse-cell or germinal vesicle route, may function in this type of abortive development.

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A NEW INHERITED CHARACTER IN MAN

BY A. H. STURTEVANT

WM. G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

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Observations on more than 280 human subjects show the existence of two fairly distinct classes with respect to the ability to turn up the lateral edges of the tongue. In typical positive cases the edges can be rolled together over a considerable portion of the distal area of the tongue, while the organ is slightly protruded. In negative cases there is no turning up of the edges at all. A few intermediates have been encountered; and in numerous cases the ability, at first absent, has been acquired by practice. This latter phenomenon is most frequent in children, only one clear case having been found in an adult—and here prolonged efforts were necessary, whereas in children a few hours are sometimes enough. One man reports that he learned the trick as a child, but now has forgotten it and can no longer do it. It should be added that some children, like most negative adults, appear to be unable to learn. In the data that follow, all cases where the ability was at first absent are entered as negative.

Another complication encountered is that a few children are unwilling to show whether they possess the ability or not. In one of these cases the child later said that this unwillingness was due to embarrassment because the ability was absent. The few (two) remaining such individuals have been entered in the tables as negatives.

The ability evidently has no relation to sex, as is shown by table 1:

TABLE 1

	POSITIVE	NEGATIVE	TOTAL	PER CENT NEGATIVE
Female	88	43	131	32.8 ± 4.1*
Male	95	56	151	37.1 ± 3.9*
Total	183	99	282	35.1 ± 2.8*

* Standard error.