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The Nucleolus.

a report by
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While, in recent years, cytologists have been able to ascribe some probable function and unity of form to most of the components of cells in different species, the nucleolus remains one of the least explicable problems of cytology today. This body is a conspicuous, typical part of the nucleus of most cells of higher animals. In different forms and situations, however, it reveals a diversity that is not equalled by other cell components.

Inasmuch as the nucleolus undergoes a typical cycle in mitosis, disappearing at the metaphase and reappearing at the very early anaphase, a link between the chromosomes and the nucleolus has long been suspected. The following papers are indicative of the early approach to the problem:
Zirkle, 1928a, 1931; Filzer, 1930; Montgomery, 1899; de Mol, 1927.

The work of Zirkle, Filzer and de Mol, is out-dated and superseded by more modern developments; Montgomery's is an exhaustive review, largely historical, of the nucleolus up to 1898. The author does not regard it as essential to discuss the historical aspect of nucleolar research. On that point, Sharp, 1934, Hofmeister, 1848, might be consulted. Gates (1937) reviews the more modern work.

Modern work on a number of species of animals and plants indicates a close connection between the nucleolus and a secondary constriction of a chromosome. Heitz (1931a+b) has shown that the position and number of the nucleoli in various varieties of Vicia follow the position and number of the secondary constrictions of particular chromosomes in the ~~resting nuc~~ reconstructing nucleus in telophase. More recently, McClintock (1934) has described the formation of the nucleolus at a chromatic region setting off a satellite - constriction in Zea mays PMC's. Furthermore, with the assistance of X-Rays, a reciprocal translocation was secured between the 6th and the 9th chromosomes, such that the break in chromosome 6 occurred through the ~~achr~~ chromatic region. The number and position of the nucleoli in the products of subsequent crosses depended on the number and position of such regions, wherefore it is called the "nucleolus organizer". When no organizer is present, no single nucleolus is formed, but a number of "nucleolus-like" bodies appear to have formed by the coalescence of matrix along the length of the chromosomes. Also, when a nucleolus is present, the extent of chromosomal matrix is inversely proportional to the extent of the nucleolus. McClintock suggests, therefore, that the nucleolus has some close relationship to the chromosomal matrix. Mensinkai (1939) who confirms this process in many species of Allium, attempts to give a chemical basis for this relationship. At the present stage of our knowledge of the chemistry of nucleolus and matrix, any conclusions are very tentative indeed.

This relation between "constriction" and nucleolus is confirmed for many other organisms (see, for example, Grasse + Lesperon "'38".) In prophase, presumably, nucleolus contributes to matrix. (?)

A few instances have been described where the mitotic history of the nucleolus apparently does not conform with the account given for Zea. It must be emphasized first of all that no satisfactory description of the prophase history of the nucleolus is available. It appears to fragment, dissolve, become extruded into the cytoplasm, and disappear. See, for instance, Francini ("1938", a, b.), and Zirkle, 1928.

Frankel (1937), describes an 'aberrant' case in the meiosis in a few strains of certain species of Fritillaria. In F. obliqua, the nucleolus, instead of a spheroid more or less centrally located, is a cap on the periphery of the interphase reticulum, just inside the nuclear membrane. 3 chromosomes may be attached to it, presumably the 3 which show secondary constrictions in somatic metaphase. Before metaphase I, the nucleolus becomes globular and separates from the chromosomes. The appearance suggests the sudden removal of the pressure exerted by the nuclear membrane. This nucleolus persists, may fragment, and is distributed at random to the tetrad of spores. F. plurifolia exhibits somewhat similar behavior, but a smaller proportion of "cap nucleoli" are seen.

In other species, particularly F. citrina, the telophase is peculiar. Globules appear at the ends of the chromosomes, looking like nucleoli, and separate as the figure polarizes. These disappear for Prophase II, reappear at Telophase II. After "osmic" fixation, these globules as well as the regular nucleoli, are positive to Feulgen-Nuclealfarbung, indicating chemical similarities to chromosomes. There is no evidence of matrix coalescence as McClintock's theory demands

In another genus, Oryza sativum (rice) Selim ('30) also finds peculiar behavior in the meiosis of some strains. In early prophase, the nucleolus buds off a secondary nucleolus which remains attached. Different races vary in the details. Similar phenomena occur in megasporocyte divisions. Selim regards (without great foundation) that the primary nucleolus contributes to the achromatic figure and the secondary to the substance of the chromosomes. In plants, the nucleoli appear to remain quiescent in the inter-mitotic phase, and do not show much specialization.

In animal material, there are also many peculiar specializations, but these occur in "resting" stages. There are three well-known instances where the nucleolus may contribute directly to the growth or secretion of cells. These are the developing oöcytes in many gastropods — e.g., Limnaea or Patella; in the spinning silk glands of many larval insects, and in the keratinizing layers of the dermal epithelium. However, these instances are by no means thoroughly established or generally accepted (Wilson, '25, ~~pg~~. p. 346, et. al).

Ludford has worked on the egg development in Lymnea. Here, the "typical" nucleolus of the early oogonium differentiates into a basophil and an oxyphil set. The latter is extruded, and in conjunction with Golgi and chondriosomes play some role in yolk-sphere formation. (Ludford, '22.) At the "end" of oögenesis the basophil part fragments and distributes ~~the~~ throughout the cytoplasm. He considers there is a correlation between the size of the nucleolus and the degree of cell activity. Similar phenomena are noted in many other sorts of oöcyte material. Wilson ('25) p. 271 cites Jorgensson on various vertebrates where the chromosomes of the germinal vesicle enlarge, become less basophilic (although this is not to be imagined as the vertebrate case. and assume a "lampbrush" form. The nucleolus than becomes basophilic, seeming to act as a reservoir of chromatin, although there is no direct chemical evidence for this. There are scattered references of uncertain significance, in the literature, reporting the reaction of similar nucleoli to Feulgen. This question is still open. In certain fish, the basichromatic nucleoli spin out into chromosome-like segments, and at one time were mistaken.

for such. Also, in some cases, the nucleolus persists after the breakdown of the germinal vesicle, and thus must contribute to the egg cytoplasm. This breakdown is the initiation of egg maturity; it is necessary before fertilization can ensue. The significance of the nucleoli here is speculative.

Nakahara (1917) is one of many authors who have examined nucleoli in silk glands. He concludes that silk (fibroin) is composed of extruded nucleoli. In these cells, the nucleus grows to a large size and branches so that a large surface is presented. There is a figure in Wilson, p. 86. At the same time, the nucleolus divides many times so that there are fragments throughout the nucleus. These particles seem to pass out of the ~~cell~~ nuclear wall into the cytoplasm, where the staining reaction changes. The silk in the gland lumina has a similar reaction. The material worked on was Pieris rapae (a caterpillar) and Neuronica postica Walker (the caddis-fly larva.) The nature of this extrusion seems to demand further confirmation. Observations on live material would be most instructive.

Ludford (1924) has described the role of extruded nucleoli, Golgi and mitochondria in the formation of "keratohyalin" and keratin in the mouse epidermis. See also Cowdry (1928.)

This sort of work should demonstrate that we have not yet reached a unified conception of nucleolar function. In some instances the nucleolus appears to have some role in secretions. It has been proposed that the chromosomes exert their genetic effects on cytoplasm through extruded nucleoli. But these, of course, are all speculations. Throughout any discussion of nucleolar fragmentation, one must keep in mind that the chondriosomes in material inadequately prepared, may simulate nuclear products. In any case, extreme caution must be used in any nucleolar interpretation.

In most animals and plants, of course, the nucleolus has not been so closely studied. It is probable that their usual history is that outlined by McClintock in relation to the Zea chromosomes.

Something is known of the physical situation of nucleoli. Macklin, cited in Ludford '22, reported that they are active bodies, continually joining and fusing, in tissue-culture material. Chambers, 1924, on grasshopper spermatocytes (Dissosteira carolina) using the micromanipulator, has found that the nucleolus can be readily moved in the cell, so that it is not firmly bound to any nuclear structure here. Gatenby (1938) gives a general account of ultra-centrifuge results, but see particularly Beams, et al., '37. After ultracentrifugation of Helix aspersa, and other species, spermatocytes, the nucleolus was found the heaviest objects in the nuclei, with the chromosomes follow. As in Allium cepa,

√the reference for which I cannot recall, the nucleolus may break through the nuclear membrane. Superficially, this is not in accordance with the supposed lipoid nature of the nucleolus (see infra) as lipoids are ordinarily less dense than water or "protoplasm". Incidentally, the mitochondria are the densest, the Golgi and fat globules the lightest objects in cells. This density, implying ~~a~~ high concentration of its components is in accordance with the high stainability of the nucleolus even where a differential stain is not used (Lederberg, unpub.)

In the chemistry of the nucleolus, the most obscure mystery of nuclear biochemistry resides. Zirkle ('28, '29, '33a, b, c, d) reports variations in staining of the nucleolus by iron hematoxylin after different fixatives. Underhill's data (1932) may be useful in interpreting the antagonistic effects of different fixatives in a mixture. These data concern the penetration of fixatives into pieces of guinea pig liver. The author's experience would confirm the list of relative penetrability given in Underhill's paper, except that his figure for formalin is far too low when applied to onion root-tip.

The author, at another laboratory, has investigated, to some extent, the effects of fixatives, separately and in mixtures, on onion root tips. (Lederberg, unpub.) This properly would form the subject of another report. It has been found that acetic acid fixes nucleoli, so ~~that~~ they do not stain with Fe-Hem; formalin so that they do. ~~not to stain Fe-Hem, formalin does.~~ In mixtures of the two, the acetic fixation (structurally) is evident, but some nucleoli in some nuclei stain. In some instances one nucleolus of two in a nucleus may stain, the other not, which proves nothing — yet, except that fixation produces erratic results. The subject is now being re-investigated. In connection with this, I am also studying the effects of various reagents on the apparent iso-electric points of the nucleolar proteins. Inasmuch as Bouin's fixative confers an apparent isoelectric point near 2.5 on the onion root nucleoles, there probably is some ampholyte present, undoubtedly a protein or proteins. This protein is not nucleoprotein, as it is negative to Feulgen (author's, unpub.), as seems generally to be the case with typical nucleoli. The author believes that it is this IE P which determines the stainability of the nucleolus. The lower it is, the greater should be the stainability. This investigation is now proceeding. The method essentially is Tolstoouhov's.

Other authors — Mensinkai, 1939; Shinke and Shigenaga, 1933; Yasui, 1939; — claim the presence of lipoid substances in nucleoli. Inasmuch as lipoids are universally present in protoplasm, we cannot yet claim an especial proportion in nucleolus; none of the investigators claim this. Indeed, Shinke + Shigenaga find fats in "chromosomes, cytoplasm, nucleolus, nuclear ~~lym~~ ~~lymph~~ reticulum, and probably spindle fibers". There certainly is no basis, as Mensinkai proposes — for the formulation of the chemical cycle whereby chromosomal matrix, nucleoprotein is converted into nucleolus, lipoid with a protein surface so it can be electrically charged. Francini, 1938, ~~find~~ in some orchids, finds a red coloration with Ruthenium chloride, interpreting this as a positive test for pectin~~s~~ or other polysaccharide. The author is continuing such histochemical tests in the Columbia histology laboratory. If, in Allium cepa, there is a protein in the nucleolus, as would seem, he hopes to find from specific amino acid tests, IEP changes after formalin and HNO_2 treatment, and similar analytical methods, something of its nature and structure. It is inaccessible to analysis in the living cell because of its location and size. In all, this subject has remained one of the most obscure in cytology and biochemistry.

Abbreviations of Journals listed:

Ann Bot	Annals of Botany
B.A.	Biological Abstracts
JRMS	Journal of the Royal Microscopical Society
QJMS	Quarterly Journal of the Microscopical Society
J.M.	Journal of Morphology
St. Tech.	Stain Technology
Zeits. Zellf. Mikr. Anat	Zeitschrift für Zellforschung und Mikroskopische Anatomie.

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