19 V 16

The Nucleolus: its place in the cell.

A review of the literature and an account of some research by Joshua Lederberg

Zoology 1. Paper

December 29, 1941.

to H. B. Steinbach.

The work mentioned by the author was done at the American Institute Service Laboratory and in the Histology Laboratory, Zoology Dept., Columbia University, to the directors and supervisors of which grateful acknowledgement is made.

The Nucleolus: Bibliography

1. Sharp, L.W. 1934 Introduction to Cytology , Wiley.

2.

The cell was just discovered as a structural unit of plants by Hooke in 1665, but it was not until the 1820's and "30's" that Dutrochet, Schleiden and Schwann formulated the cell theory as we recognize it today. In 1831, Robert Brown published the first clear description of the nucleus, shortly after which this body was recognized as a universal component of the cells of higher organisms. We can date the modern science of cytology from this time. . . (1)

The remainder of the 19th Century was occupied largely with ascertaining precisely what the morphology of cells consisted of. In the 1880's, Flemming, Altmann and Strasburger demonstrated what are now known to be mitochondria, and universally present in animal and plant cells. In 1898, Golgi discovered the argentophil, osmophil Golgi apparatus, which has since been found generally in animal cells, and to which various homologues have been suggested in plants and protozoa.

Chromosomes were first seen in 1848 by Hofmeister, but their place in cell-division, or the conception of mitosis, appeared only in the 1880's. Under the impetus of genetic researches and the formulation of the chromosome theory of heredity most modern cytology is the cytology of the chromosomes. Mitochondria and the Golgi material have also been studied inten-

sively. Although there can not yet be any definiteness about the matter, the Golgi and the chondriome are widely felt to have an important bearing on the secretory processes of the cell. In spermatogenesis, they have been shown to play important and specific parts, the Golgi organizing the acrosome of the sperm head and the mitochondria forming the substance of the middle piece. (2.), (3.)

delete

There are three items of the cell that I have not yet mentioned. These are, firstly, the cytoplasm or hyaloplasm which has no visible structural differentiations in the typical cell. There are also, of course, various inclusions such as plastids, vacuoles of various sorts, blepharoplasts, contractile structures, fibrillae for nervous coordination, cell wall, and others, but we cannot concern ourselves here. Secondly, there is the problem of the external environment of the cell, which was studied by Carrel at the Rockefeller Institute, and thirdly, and the phase that this paper discusses, there is the nucleolus, which receives scant enough attention in the textbooks and

delete

delete not so the literature.

It is difficult to give a comprehensive definition of this structure, as it shows some diversity. However we can call it a body, found within a "resting" nucleus, that can be differentially stained from the chromosomes, makes no direct contribution to

or the chromosomes
the achromatic figure, in ~~mitosis~~ division, and generally disappears in ~~the~~ metaphase of ~~a mitosis~~. In this discussion, ~~this shall be the only use of~~ the word 'nucleolus', ~~and it~~ is substantially identical with 'plasmosome.'

The nucleolus is almost ubiquitously associated with nucleus. Wherever these are found, some nucleolus-like structure is commonly found also. Where there is no formed body corresponding to the nucleus, ~~we can~~ no nucleolus can be recognized. Thus the lower plants — bacteria, Cyanophyceae, are excluded. In Protozoa and in other lower plants, there is no true nucleolus, the nuclear inclusion being either a karyosome, contributing to chromosomes, or an intra-nuclear division center. In some instances, there is an amphi-nucleolus which is a double structure, being composed of a plasmosome and a condensed chromosome. Such bodies are frequently found in insect spermatocytes. (4). True nucleoli appear consistently in almost all cells of higher organisms.

Perhaps the clearest appearance of the nucleoli exists in the somatic cells of higher plants where they are usually by far the most prominently staining objects in the cell. Many special fixation-staining methods are applicable to the morphological study of nucleoli, of which only a few need be mentioned. They stain with acid fuchsin preferentially in

Delete and give account of Protozoa separate. Classification for inclusion

the Champy-Kull method, or in acid fuchsin-methyl green. A very pretty (aesthetically) picture is given by the Flemming triple stain where they take safranin in contrast to chromosomal violet. They react negatively to the Feulgen Reaction, for 'nuclein acid', but otherwise stain well in basic dyes. This is particularly the case in Allium cepa root-tips, on which the author has worked. (5) The nucleoli of oöcyte germinal vesicles represent a special case (6, 7) which will be discussed later.

unimportant / (see Wilson's account)

Hofmeister (8) in 1848 was the first to report the dissolution of the nucleolus in cell division in Tradescantia. Since that time this phenomenon has been noted repeatedly; ~~so that~~ cases of persistent nucleoli are exceptional. Zirkle (9) offers some magic evidence for the inflow of the nucleolus substance into the core of the chromosomes, in Zea mays root-tips, using special fixatives. He also describes the extrusion of some nucleolar fragments in metaphase. In Pinus strobus cambium and root-tips (10) he describes again the flow of nucleolus into spireme, but here all remnants of the nucleolus disappear before the orientation of the figure into an equatorial plate. Inclusions in the living nucleolus are also reported.

some exception / of untrue

Fiking (11) questions this interpretation. In Rumex

scutatus typicus, tetraploid, he reports fusion of nucleoli in pre-meiotic phase. At no time is a definite, fixed connection with the spireme seen. As different fixatives give a different picture of the nucleolar inclusions, these are considered artifacts. Between diakinesis and Metaphase I the nucleolus enlarges and becomes more diffuse, giving the appearance of solution in the karyolymph. It has disappeared by the time of appearance of the multipolar spindle. It is pointed out that for the nucleolus to make a direct contribution to all the chromosomes requires a continuous spireme thread which does not exist. Citing Meyer (?) on parenchyma of Galtonia candicans, he states that the nucleolus diminishes in emaciation, and gives as its function only that it is merely ergastic material.

The author would like to point out at this junction that Zickle's material is somatic Zea and Pinus, while Eiby's is meiotic Lumex.

An interesting deviation has been demonstrated by Frankel (12) in the meiotic divisions of certain species of Fritillaria. In ~~Fool~~ F. obliqua, in the earliest meiotic prophases, the nucleolus is a cap-like structure on the periphery of the reticulum, just inside the nuclear membrane. The suggestion is of strong pressure on the nucleolus 3 of the

bivalent chromosomes may be attached, probably corresponding to 3 pairs of "SAT chromosomes" (13) in the somatic prometaphase. At pro-metaphase, the nucleolus globularizes. This is evidently a sudden process ~~as~~ related to the release of the pressure when the nuclear membrane dissolves. The chromosomal attachments thenceforth are lost. The nucleolus persists, may fragment somewhat, and is randomly distributed in the sporogenetic divisions. The somatic divisions of this species show the typical picture. Similar behavior seems to be exhibited by F. plurifola, but non-cap nucleoli are more frequent. In F. meleagris contorta, no prophase material was available, but two persistant nucleoli were seen at metaphase I. In early Telophase I of F. citrina and other forms, globules form close to the distal ends of the anaphase chromosomes as they approach the poles. These swiftly disconnect from the chromosomes, are not visible in Prophase II, but reappear in Telophase II, not showing in the tetrads. In somatic mitosis, none of this is seen. After osmic fixation, both nucleoli and telophase globules react + to the Feulgen. There is no evidence of matrix swelling as McClintock's theory (14) would seem to demand.

These phenomena clearly represent a divergent and atypical case in which the nucleoli are not equivalent to the usual type and history. Their significance will be touched upon

below.

Another aberrant is noted in rice. Selim (15) working on various varieties of Oryza sativa L., particularly Rochwitta, whatever that means, gives the following account of nucleolar changes in the meiotic divisions

In prezygotene, there is ordinarily a single nucleolus or perhaps two on opposite sides of the nucleus. In prophase, during reticular contraction and spireme formation, the nucleolus buds off a secondary one which remains attached. Some races vary. The same phenomenon ensues in the megasporocyte divisions. The following figures are given for the frequency of this phenomenon.

	2 nucleoli.	?	1 nucleolus.	
Zygotenes	535 = 88.2%	34 = 5.7%	37 = 6.1%	(1)
Leptonema	356 = 95.5%	9 = 2.2%	10 = 2.3%	(2)

The conclusion might be drawn that there is a ~~feathering between~~ fusion of nucleoli between leptonema and zygotenes. This is a substantiated phenomenon, but the figures have statistically are rather insignificant. The author calculates σ = ~~.02%~~ 5.74%. Inasmuch as there is a difference of only 7.7% in the di-nucleolate forms, which could be reduced to 2% if all of the doubtful cases in (1) happened to be dinucleolate, etc..., the statistical significance of these figures is very small, being of the order of 10, indicating

that there is only about 1 chance in three that this difference is not due to chance sampling alone.

Selin suggests that the primary nucleolus contributes to the spindle, and the secondary to the substance of the chromosomes.

For a fairly modern review of the nucleolus, Gates (16) can be consulted. An exhaustive review of the early works [19th Century] is given in Montgomery (17), also Sedford (7).

In Montgomery, evidence is presented for the movement, fusion and division of nucleoli; this is evident in oöcytes. Because of the rather aberrant behavior of nucleoli in oöcytes, it may be that these nucleoli are not homologous to those found in plant material. Sedford describes ~~the budding of the nucleolus (in Patella and Lymnaea oögenesis) to produce another~~ the extrusion of nucleolar oxyphilic material into the cytoplasm in Lymnaea oöcytes. The nucleolus differentiates into a "basophil" and an "oxyphil" set, the latter being extruded and possible have some rôle in yolk-formation. At the end of oögenesis, the basophil part fragments and distributes through the cytoplasm. A correlation is noted between size of nucleolus and cell-activity. The early (1900-1930) works on animals does not contribute much to the connection between chromosomes and nucleolus. Connections in plants material have long been noted. The situation in most animal cells is believed now to follow that in plants, as elucidated in part by McClintock. (v. infra)

Interaction in some form or another between nucleoli and chromosomes has been suspected by many observers for many years, although others, as Fikry have questioned it. Perhaps the most striking and obvious relationship concerns the nucleolar number, particularly in polyploid plants. In 1927, De Mol (18) published some observations on diploid and triploid hyacinths. It was noted in the root tips of many varieties that a diploid nucleus was associated with a maximum of two nucleoli, a triploid three. His interpretation was that there was a relationship to the entire genome, and is in part superseded by modern developments. Certain exceptions were noted in the matter of nucleolar number which seem temptingly susceptible to analysis on the bases of deficient or supernumerary individual chromosomes, today.

~~1931~~, Navaschin (cited in Sharp, original inaccessible) observed in Galtonia candicans that the chromosome satellites lie on the surface of the nucleolus in late somatic prophase. In 1931, (13) Heitz showed in somatic Vicia faba the equivalence of an SAT region (sine acido thymonucleo, because it was believed to be achromatic to Feulgen) to the separation of a chromosome.

More recently, McClintock (14, and personal communication) has shown that, in Zea, there is a definite, chromatic, nucleolar organizing region on chromosome VI. The chromosomes in Zea

completely formed genome, no distinct nucleoli develop, but a large number of nucleolus-like bodies develop along the superficial matrix of the chromosomes, evidently by the coalescence of this substance, the "chromosomal matrix". There are two larger bodies at the organizers of the chromosomes, however.

In other X-rayed material in which the organizer is entirely lost, a similar situation prevails except that there are no ~~two~~ larger bodies representing organizer activity.

The evidence is rather definite and indicates that the number of nucleoli that develop is ~~proportional~~ dependent on the number of organizer loci and a fairly complete chromosomal complement. This evidence is confirmed in somatic (root-tip) material. In normal plants there may be two nucleoli in the telophase, but as Heitz had already shown, (v. Sharp), when nucleoli develop in proximity in the telophase they may fuse. The maximum number of nucleoli, then, reflects the number of organizer loci.

Plants heterozygous for this interchange ($6\cdot9, 6^9\cdot9^6$) show 3 nucleoli, 2 large and 1 small, evidently from the regions in $6, 9^6$ and 6^9 respectively. Plants homozygous for this interchange show 4 nucleoli, representing the 2 "6^9" and the 2 "9^6" regions. In Zea, there is ordinarily one pair of SAT chromosomes. Thus, the nucleolus is shown to

have been mapped out genetically to some extent and can be distinguished by the position of spindle fiber attachments, size, and chromatic knobs. In prophase, the chromosome is always in contact with this organizer, while the satellite may be some distance away on the surface of the nucleolus

By means of X-Rays, McClintock was able to procure plants in which there was a reciprocal translocation with chromosome 9, the break in 6 being across the chromatic nucleolar organizer. Both parts of the organizer now functioned independently. The part that is more proximal to the achromatic region seemingly is more active as larger nucleoli develop in connection with chromosome 9 than 6, although 6 has the larger part. The findings are as follows, with respect to the situation in spores to which different chromosome combinations were distributed in the reduction divisions of a plant heterozygous for the interchange, i.e., whose genotype was $6-6^9, 9-9^6$: 6 is the normal chromosome, 6^9 is the trans-located.

I. 6-9. This is normal, and produces one nucleolus at 6

II. $6^9 \cdot 9^6$ This has a complete complement, but with a divided organizer, one at 6 one at 9 and produced nucleoli correspondingly.

III. $6^9 \cdot 9$ This is missing the translocated portion of 6, but contains only one organizer and produces one nucleolus accordingly.

IV $6 \cdot 9^6$ This is missing the rather large translocated portion of 9, and has two organizers. Presumably because of the in-

have a direct morphological relationship to one of the chromosomal elements – a chromatic region set off by an 'SAT' region, at the other end of which may be a satellite, or which may be merely a 'secondary constriction'.

The formation of nucleolar-like material from the matrix leads McClintock to suggest that the nucleolus is formed by the confluence of this matrix, or that there is certainly a close relationship between matrix and nucleolus. This is particularly well-shown when the organizer is inactive, and nucleolus-like bodies form as droplets or chromosomes "from matrix." The behavior of this substance suggests that nucleolus-organization is a means of distributing "chromatin" to reform the metabolic nucleus, and that "complete release of the matrix from the chromatin is necessary before the chromatin can function properly in metabolism."

This process of nucleolus formation has been confirmed more or less exactly in Allium (19), in Tradescantia (a beetle), (20), to cite two references, and by many observers in both plants and animals, particularly insects. However, not sufficient work has been done to show how general this process is. It is, however, quite clear that in many instances the chromosomes do make a definite contribution and control to the nucleolus.

Nucleoli in plant and animal cells present such a diversity of form that it may not be possible to rationalize them all into a

single unified conception of this structure. In plant root-tips, the nucleolus is relatively "basophilic"; in animal material they are more characteristically "oxyphilic" — except in the case of oöcytes already cited. Another disturbing factor is their discontinuity. In regularly dividing cells, there may be a certain similarity in nucleolar positions between adjacent cells. This, however, is a manifestation of the continuity of the chromosomes and thence of the nucleolar organizer.

No positive conclusion can yet be drawn as to the physiology of the nucleolus. Its metabolism is a mystery; its chemical composition hardly less so. Its morphological history, however, is not consistent. In the cases already cited, there has been evidence for extrusion of nucleolus into oöplasm, possibly in yolk formation. It is still somewhat open to doubt whether these changes actually do take place, but certainly there is sufficient evidence to show that the oöcyte nucleolus is rather anomalous. Furthermore, Nabahara (31) has described a similar extrusion in nucleoli of ~~sp~~ silk gland cells of a catapiller (Pieris rapae) and of caddis-fly larvae (Neuronia postica Walker), but here the evidence is even more obscure. Some great chemical change in the nucleolar substance is presumed to account for changes in stain after the "nucleoli" have extruded. This "nucleolar" substance is regarded as part of the substance of the secreted silk. In view of the uncertainty regarding the

nature of these nucleoli even when intra-nuclear, this account must be held in abeyance until further evidence of the history of these "nucleoli" is presented.

That aberrations are not limited to animals is shown by the papers of Selivin (15) and Frankel (12). Most of these deviations occur in the meiotic phase or where highly specialized cells are concerned. The keratinization and pigmentation of skin are cited in (32) as another example. See also, Lindford, 1924 (33), in which extruded nucleoli and mitochondria and Golgi are seen to be somehow interrelated in the formation of "keratohyalin" and keratin.

Enough has been presented to show that, although there is a fundamental ground-plan for the formation and nature of nucleoles in cells just after a mitotic or meiotic phase, — the formation in relation to chromosomes — in cells that have become highly specialized, the nucleolus may assume structural and functional characters so far removed from the fundamental that we can question their homology with it.

Very little has been done on the chemical relations of the nucleoli. The present author is now investigating the "isoelectric point" of nucleolar and chromosomal substances in fixed onion root tip in an effort to find some relation. However, as Cohn (34) points out: "a physical chemist defines the iso-electric point as that where minimum dissociation takes place.

A protein occurring in a solid form as in sections of fixed tissue can hardly be expected to dissociate and, accordingly, it is a question whether it can have p can properly be said to have an isoelectric point."

The chemistry and physics of the nucleus are obscure. Evidence has accumulated that it is the heaviest component of the nucleus. In many eggs, it is influenced by gravity to stay at the bottom of the nucleus. In particular, the result of centrifuging (see 35) show that it is probably somewhat denser than the chromosomes. A general discussion of the results of centrifuging is given in (36). In tissue culture, the nucleoli are mobile and may divide and fuse again. (36.). In the insect (Dissosteira Carolina), Chambers (38) has found that the nucleolus can readily be moved through the nuclear "fluid" without hindrance.

In the course of an attempt to rationalize fixation images, Zirkle (21-26) found that certain fixatives were followed by the staining of nucleoli with Iron-Hematoxylin, and others not. Particularly, acetic acid causes non-stainability; formalin causes a large degree of staining. Mixtures may be diffused differentially to give the effects noted. The author has preparations of Allium root tip fixed in formalin and acetic acid. Some of the central cells have stained nucleoli. In an appreciable number of cases, there are nuclei with two nucleoli of which one may be stained and the other not!

It is too early to draw any conclusions on this sort of thing; the author is working on it now. However, there is little question that all the nucleoli in meristem root tip cells are equivalent, and "good" plasmosomes. Quantitative data on diffusion rates in guinea pig liver are given in (27). Toolstouchov (28) has done some work on the chemical effects (on isoelectric point) of fixatives that may be a starting point for similar work on the nucleolus. Shinke and Shigeniaga (39) give histo-chemical evidence for the presence of lipoids (solvents for Sudan III) in the nucleolus and chromosomes, and cytoplasm, reticulum of the resting nucleus, phragmoplast, with a little question as to spindle fibers. This may be more a demonstration of the non-specificity of the reaction than of the general occurrence of fats in any concentration. Millon's reagent is used as a test for proteins and turns out to be indefinite. Feulgen is negative. This is interpreted as + for lipoids ? for proteins and − for "thymonucleic acid." ~~Hillon's reagent is a test for carbohydrates rather than proteins and is used only because many proteins have carbohydrate substituents~~. These tests are only very vague and far from specific. On this basis, the far-fetched speculations of Mensinkai (19) may be dismissed. Francini (29) in an inaccessible paper notes a positive reaction to Ruthenium Red (see 34, page 187.) in nucleolus of the orchid Paphiopedilum spicerianum. This is interpreted as indicating the presence of a pectin or other polysaccharide. In prophases these material is dissolved in the karyolymph. The equivalence

of nucleolus with spindle is suggested. This may be a recurrence of many fantastic ideas that appeared in the past.

By now, we should be more firmly convinced than ever of the hazy situation respecting nucleoli. They must perform some important functions within the typical cell – what it is we cannot say. As cells specialize, they specialize also.

But time is not for speculation or baseless guessing but for further experimental elucidation in the laboratory.

Joshua Lederberg

12/23/41.