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Scope and Method of Study: This report has been undertaken in an attempt to obtain information on the structure, composition and function of the nucleolus. Information on the possible connections of the nucleolus with the mitotic cycle and cytoplasm was also obtained. Publications since the year 1959 were consulted. The materials used were chiefly periodicals and a few books.

Findings and Conclusions: Progress of research on the nucleolus has awaited improved techniques and new types of equipment. The structure of the nucleolus is described as a two component system. The nucleolus was seen to disperse during the mitotic cycle and to form on a particular chromosomal region. The possibility of nucleolar control on mitosis is indicated. The composition of the nucleolus is chiefly RNA and proteins. It may also contain certain enzymes and lipids. The synthesis of RNA in the nucleolus is generally accepted. The types are not agreed on. The transfer of RNA to the cytoplasm seems probable but the method is not apparent. Protein synthesis within the nucleolus is little, if any. Considering the conflicting information obtained, it is evident that nucleolar research has just obtained a good beginning.

ADVISER'S APPROVAL L. Herbert Blevens

RECENT STUDIES ON THE NUCLEOLUS

By

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CHAPTER I

INTRODUCTION

The nucleolus, found within the nucleus of the cell, was first described in 1781 by Fontana. Therefore, the presence of the nucleolus has been known for over a century and a half. The literature shows that a great amount of work has been done on this one component of the cell. Studies have been conducted to determine its structure, composition and functions. The aim of this paper is to review the recent literature published on the nucleolus. The literature, published since 1959 on the different phases of research on the nucleoli, was examined. Recent publications only were consulted because of improved techniques and newly invented equipment.

CHAPTER II

NUCLEOLAR STRUCTURE DURING INTERPHASE

The nucleolus, under the light microscopes, has been generally described as a structureless mass (7, 22). Under electron microscopy, the nucleolus was composed of two dense components; a central filamentous network, the necleolemma, and surrounding amorphous material called pars amorpha (7, 22). Davis (7) stated that the central material was composed of fibrous bundles 1000 to 2000 angstroms (A) in diameter which were composed of fibers 60 to 80 A in diameter. These bundles dissociated and swelled to a diameter of 150 A. Jacob and Sirlin (16) described the fully formed nucleoli as having a characteristic dual structure with a narrow dense periphery and a broader less dense internum. The fibrils and particles were present in both regions, and the difference in density reflects differences in the packing of the two structural elements. La Fontaine and Chouinard (22) described the nucleolemma as irregularly shaped patches of material which was more homogeneous and finer in structure than the pars amorpha. The packed and convoluted fibrils of the nucleolemma was 60 to 100 A in diameter. The pars amorpha was located in the peripheral portion of the nucleolus proper, on the surface of centrally located or large vacuoles (light areas) and in zones extending radially. This material appeared threadlike and had a diameter of 150 A.

CHAPTER III

CHANGES IN MORPHOLOGY OF NUCLEOLI DURING MITOSIS

La Fontaine and Chouinard (22) conducted a thorough study of the morphological changes of the nucleolus during the mitotic process using the electron microscope. During prophase, the nucleolus became quite irregular in shape with nucleolar indentations following the twists and bends of the chromosomes quite closely. The fibrillar material, nucleolemma, and granular material, pars amorpha, intermingled with the disappearance of the vocuoles. Before nuclear membrane breakdown, the nucleolar mass was indistinguishable from the nucleoplasm which was predominantly fibrillar in nature. La Fontaine and Chouinard (22) found that at early telophase, the electron microscope revealed the existence of coatings of a denser and finer material on the chromosomes than that associated with the spindle. The fibrillogranular material was in intimate contact with wavy contours of the chromosomes. The coatings of neighboring chromosomes merged filling part of the interchromosomal spaces and were composed of loosely arranged fibrillar elements of 60 to 100 A in diameter intermingled with dense 150 A granules. The interchromosomal masses enlarged and coalesced at a particular locus on the chromosomes forming a mature nucleolus. Jacob and Sirlin (16) called the earliest recognizable nucleoli "elementary bodies" which were located within the bands of the chromosomes. These bodies were less than 2000 A in diameter and composed

of 100 A fibrils (pair of 40 A fibrils) and 150 A particles.

CHAPTER IV

SOURCE OF NUCLEOLAR MATERIAL

The disappearance and reappearance of nucleoli in dividing cells is an active area of research. It is generally agreed that the nucleolar material disappears because of dispersion of the fibrillogranular material during prophase. The origin of the reappearing nucleolus, however, is an area of disagreement. Two theories are that the nucleolus formed in telophase is due to an accumulation of premitotic nucleolar material or to an accumulation of material synthesized by early and midtelophase chromosomes. Martin (25) demonstrated by interferometry that the sum of dry masses of the postmitotic nucleoli was equal to the pre-mitotic nucleoli of cells of Strelitzia reginae Banks. He concluded that the nucleolar material was of pre-mitotic origin. Harris (14) conducted an experiment also showing the continuity of nucleolar material in mitosis. He used tritium labelled valine. Connective tissue cells of rat heart which had been exposed to tritium labelled valine were transferred to non-radioactive medium containing unlabelled valine and incubated in this medium until they had all undergone mitosis. The autographs of the daughters showed labelled nucleoli. If the molecular protein had been synthesized de nova during or after mitosis, the nucleolus would have been unlabelled. Harris (15) concluded that the reappearance of the nucleolus was due to aggregation of pre-mitotic material. La Fontaine and Chouinard (22) presented the following evidence for persistence of

nucleolar material: 1) the nucleolar material of disintegrating and non-disintegrating material are of the same composition, 2) during disintegration nucleolar material was seen merging with nucleoplasm, and 3) when all formed elements of nucleolus have disappeared from view, the nucleoplasm appeared homogeneously filled with a larger number of granules (150 A) previously associated within the nucleolus. Further evidence could be the possible compactness of late prophase chromosomes; if this developing compactness was due to adhesion of the nucleolar material to the chromosomes. La Fontaine and Chouinard (22) found evidence for the existence of a matrix substance within late prophase, metaphase, and anaphase chromosomes. The evidence stated was as follows: 1) longitudinal sections of metaphase chromosomes appeared homogeneously dense and of uniform fine fibrillar structure in spite of the fact that their wavy contours strongly suggested the presence of coiled chromonemata within their mass and 2) transverse sections of metaphase chromosomes clearly showed a Feulgen-negative chromatid core. Achromatic matrix filled the central core as well as the spaces between the coiled chromonemata of metaphase and anaphase chromosomes. However, La Fontaine and Chouinard (22) argued against the chromosomal matrix as the source for the reappearing nucleolus. Their reasons were as follows: 1) the amount of matrix material was not sufficient to account for the relatively large quantity of prenucleolar material that accumulated in the interchromosomal spaces from early to midtelophase, 2) the small number of 150 A granules observed within the condensed early telophase could not account for the large number of similar granules found in the prenucleolar material and 3) the compactness of the telophase chromosomes did not change to the

extent expected if most of this material was transferred to the forming nucleolus. La Fontaine and Chouinard (22) argued for the synthesis of prenucleolar material (RNA and proteins) by early and midtelophase chromosomes. Evidence for synthesis was as follows: 1) prenucleolar material appeared intimately associated with the surface of the chromosomes generally called the nucleolus organizer, 2) prenucleolar material exhibited a fine structure and staining characteristics similar to that of the material found on the surface of telophase chromosomes and 3) continued release of prenucleolar material synthesized within the chromosomes could account for both the large amount of such material observed within the interchromosomal spaces and for the fact that the chromosomes remained quite compact during early and midtelophase. Jacob and Sirlin (16) envisaged two possible derivations for the fibrils found in their "elementary bodies" both depend on the synthesis of nucleolar material by chromosomes. The first possibility was that certain chromosome fibrils initiated the formation of the nucleolar material and became part of it. The second hypothesis was that the nucleolar material consisted of RNA newly synthesized in the genes concerned. Evidence against the first possibility was the lack of DNA and no apparent disruption of the chromosomes. They suggested that the second hypothesis was more likely. Woodward, Rasch and Swift (39) using specific precursors and stains from DNA, RNA and proteins determined the rate of incorporation (precursors) and rate of change in amounts of the various components (stains) within parts of the cell. The data indicated four divisions of the mitotic cycle; three were considered anabolic and one predominantly catabolic. The three anabolic periods were: 1) telophase to post-telophase during which there were high rates

of accumulation of cytoplasmic and nucleolar RNA and nucleolar and chromosomal total protein, 2) post-telophase to preprophase characterized by minimal accumulations of cytoplasmic RNA and chromosomal and nucleolar total protein and RNA accompained by a diphasic synthesis of DNA with a peak at mid-interphase and a minor peak just before prophase and, 3) pre-prophase to prophase in which there were again high rates of accumulation of cytoplasmic RNA and nucleolar and chromosomal total protein and RNA. The catabolic phase included the rest of the mitotic cycle during which there were marked losses of cytoplasmic RNA and chromosomal and nucleolar total protein and RNA. Woodward, Rasch and Swift (38) concluded that much of the mitotic process involved a utilization and segregation of materials synthesized during the preceding interphase. He did not draw any conclusions concerning the close correlation between nucleolar and chromosomal accumulations.

CHAPTER V

SITE OF NUCLEOLAR FORMATION

The contact between the nucleolus and chromosomes has already been indicated from the works of La Fontaine (22), Davis (7) and Jacob (16). The portion of the chromosome at which the nucleolus forms and enlarges is generally called the nucleolar organizer. Ohno, Weiler and Stenius (29) noted that only the largest acrocentric pair of autosomes of Cavia cobaya have the inherent capacity to form a nucleolus. However, only one member of the pair in any nucleus demonstrated a SAT-zone (nucleolar organizer) on its second arm and actually participated in nucleolus formation. Under the electron microscope this zone appeared as a secondary constriction on the arm of the chromosome. Kahn (18) noted that a mutation of Xenopsis laevis found by Fischberg and Wallace (11) which resulted in the reduction of nucleolar number also resulted in the reduction of secondary constrictions in mitotic chromosome complements. The complement of wild-type animals included a pair of chromosomes both of which posses a secondary constriction. In heterozygous (mutant) animals only one member of the pair had a secondary constriction. He correlated the secondary constrictions with number of nucleoli formed. Jain (17) found that the inactivation of the nucleo-organizing region in Lolium does not prevent the formation of nucleoli. The nucleoli are formed on other chromosomes which have not been inactivated.

CHAPTER VI

POSSIBILITY OF NUCLEOLAR CONTROL ON THE MITOTIC CYCLE

The relationship between the nucleolus and mitotic cycle as such has been studied in the past five years. Gaulden (12) showed that irradiating the nucleolus of the neuroblast of the embryo of the grasshopper, Chortophaga virdifasciata, with an ultraviolet beam resulted in permanent cessation of mitotic progress in cells at stages from late telophase to the middle of midprophase. Half the cells with the nucleoli irradiated in the latter part of midprophase and all those irradiated in late and very late prophase divide. Irradiation of a non-nucleolar portion of the nucleus at any stage did not usually prevent division but did cause some retardation in mitotic rate. Jacob and Sirlin (16) noted that in works of others such as La Fontaine (22) and Kahn (18) that the nucleoli form at specific chromosome loci. However, in Bradysia mycorum Frey, they observed the occurrence of unorganized, multiple nucleoli. This species could be considered a primitive form assuming an evolutionary acquisition of an organizer. This would indicate a coparticipation of many genes in nucleolar formation. They further stated that this implied that the nucleolus functions as an intermediary in the transfer of information from genes. Fabbri (10) further supposed that in the nucleolus (perhaps in the nucleolemma) might be located two different ribonucleic unities, analogous to the chromosomicgener, which would supervise the principle vital activities of the cell. The hypothesis was formulated that the mitotic phenomenon was initiated by

these unities, being afterwards by then controlled. Each species has its own set.

CHAPTER VII

CHEMICAL COMPOSITION OF NUCLEOLI

The composition of the nucleolus is essentially ribonucleic acid (RNA) and proteins. The nucleic acid component had been indicated by the ultraviolet absorption spectrum of the nucleolus (14). The action of ribonuclease on nucleoli substantiated the presence of RNA (14). Birnstiel and Hyde (3), by using L-leucine C^{14} identified three classes of proteins. The fractions were weak saline extractable proteins, basic, non-histone proteins and residual proteins. He further noted that the amino acid incorporation rate of the nucleolus was higher than the chromatin rate and was, in addition, deoxyribonclease (DNAse) insensitive. This indicated that the nucleolar incorporation was not due to chromatin containination. Tewari and Bourne (38) subjected sections of dorsal root spinal ganglion cells of rats to histochemical tests for various enzymes. The nucleoli were found to contain adenosine triphosphatase (ATPase), glucose-6-phosphatase, specific cholinesterase, alkaline phosphatase, and succine dehydrogenase. The intensity of these enzymes varied in the nucleoli of different cells and this variation appeared to be related to nucleolar activity. When the nucleolus was in contact with the nuclear membrane, the nucleolus was ATPase positive with ATPase clumps appearing in the cytoplasm. Glucose-6-phosphatase demonstrated positive nucleolar then nuclear activity. When the cytoplasm was showing activity, the nucleus and the nucleolus were negative.

Specific cholinesterase was localized only in the peripheral part of the nucleolus. Alkaline phosphatase distribution patterns showed that it can be scattered throughout the whole nucleolus, concentrated in granules (intranucleolar bodies) or localized in the periphery of the nucleolus. Succine dehydrogenase and alkaline phosphatase showed similar concentration patterns. The intranucleolar bodies found only in nucleoli located centrally are composed of lipid as well as RNA, with one or the other dominating. The lipid material was considered unsaturated. Brown (5), however, performed cytochemical tests on Triturus oocyte nucleoli which gave no indication of lipids or alkaline phosphatase. Brown (5) and Tewari (38) agreed that the nucleoli contained no deoxy-ribonucleic acid (DNA), phospholipids, or acid phosphatase. Tewari (38) did not detect cytochrome oxidase. Birnstiel, Fleissner and Borek (4) associated another enzyme, RNA methylase, with the nucleolus.

CHAPTER VIII

RIBONUCLEIC ACID SYNTHESIS BY NUCLEOLI

The site of RNA synthesis is almost completely associated with the nucleolus. Prescott and Bender (32) used an RNA precursor, tritiated uridine, in the medium of mammalian tissue cells. The radioautographs showed a greater incorporation of the precursor in the nucleolus than any other region of the cell. Koulish and Kleinfield (21) injected rats intraperitoneally with tritiated cytidine. The rats were sacrificed and the liver tissue examined for RNA turnover. In the liver parenchymal cells of the treated rats, the nucleolus showed a 14-fold increase in volume, 25-fold increase in RNA content and a 30-fold increase in grain count per total structure. In comparison, the volume of the nucleus increased 2-fold and its total grain count increased 3-fold. It was also noted that the incorporation curves for the nucleolus and non-nucleolar nucleus contained two distinct turnover fractions, one being rapid and the other slow. Both fractions increased, but remained proportional to the control. Many experiments have been conducted recently on the nucleolus by using microbeam irradiation. Seed (33) irradiated one of each pair of twin daughter cells from mouse heart fibroblast with a 2.5 micron x-ray microbeam. The other was used as a control. The irradiated nucleolus showed a slight darkening momentarily under a phase contrast microscope. The cells with irradiated nucleoli showed a significant decrease in nucleic acid

absorption range. The un-irradiated twin cells and cells which had been irradiated in the nuclear sap region showed no appreciable change in absorption in the nucleic acid range. Perry (30) conducted a similar experiment. The nucleoli of HeLa tissue cells were irradiated with a 2.2 micron microbeam of ultraviolet radiation. Controls included cells not irradiated and others irradiated in an area near the nucleolus. The nucleoli of the irradiated cells showed approximately two-thirds decrease in absorption activity in the nucleic acid range. Montgomery and Huxley (28) found also that ultraviolet microbeam irradiation of the nucleoli of living cells resulted in a marked loss of ultraviolet material from the nucleoli in approximately four to six hours. In the next six or seven hours no detectable change occurred in the ultraviolet absorption image of the cell or in the visible light image of the cell. It was also noted that microscopically detectable cell functions and types of motion continued in unaltered fashion.

CHAPTER IX

TYPES OF RIBONUCLEIC ACIDS FOUND IN NUCLEOLI

Further studies on the RNA of the nucleolus has in some instances resulted in a division of the RNA content into various types. Sirlin, Jacob and Kato (35), using cells of salivary glands of fully grown larvae of Smittia chironomidae, treated the nuclei in situ with various inhibitors and then exposed the nuclei to tritiated uridine. Actinomycin blocked nucleolar RNA synthesis. Actinomycin blocks DNA primer which indicates that nucleolar RNA depends on a normal contribution of DNA primed chromosomal-RNA. Another inhibitor, TRB (4, 5, 6 or 5, 6, 7-trichloro-1-beta-D-ribofuranosylbenzimidazole), resulted in blockage which suggested, in addition, an innate nucleolar-RNA (nu-RNA) turnover. This particular turnover subsisted for sometime after the chromosomal-RNA (ch-RNA) contribution which provided primer for the innate turnover had been blocked. The experimentors stated that ch-RNA had access to the nucleolus and there it primes nu-RNA turnover. The net synthesis of RNA in the nucleolus included self-primed ch-RNA synthesis and/or ch-RNA primed nucleolus-modified synthesis of a nu-RNA. Georgiev and Mantieva (13) considered the nucleolus only in association with chromatin material. The nucleolo-chromosomal RNA of rat liver, Ehrlich ascites and carcinoma cells were separated into two high molecular weight RNA components. The nucleotide composition, physiochemical

properties and P^{32} incorporation patterns indicated one fraction of the adenine-uracil type and one of the guanine-cytosine type. The adenine-uracil type was considered the ch-RNA and the guanine-cytosine type was considered the newly formed ribosomal RNA in the nucleolus. Sirlin, Kato and Jones (34) incubated nuclei of salivary glands of Smittia chironomidae with tritiated amino acids precursors and tritiated pseudoruidine. Cytidylic acid and adenylic acid turnover was associated with a nucleolar transfer RNA (t-RNA). Pseudouridine incorporation ceased when the cell reached maximum size. Pseudouridine was suspected of being incorporated into t-RNA also. Guanylic acid and uridylic acid are mostly incorporated internally into a second type of nucleolar RNA. Birnstiel, Fleissner and Borek (4) incubated sub-nuclear units in the presence of methyl-free t-RNA obtained from Escherchia coli. The RNA methylase activity was almost completely associated with the nucleolar fraction. Incorporation of methyl-C^{14} was maximal in the nucleoli. Incubation of the nucleoli with ribonuclease resulted in a ninety per cent reduction in incorporation rate. The ribosomal RNA resembling other nucleolar RNA did not incorporate methyl-C^{14}. Maggio, Siekevitz and Palade (25) in their studies of guinea pig liver isolated one RNA fraction from the nucleolus and two others from the nucleoplasmic subfractions. Nucleolar RNA was characterized as insolubile in salt solutions, having a high quanosine phosphate (GMP) and low adenosine phosphate (AMP) content and a high turnover rate. They associated the other two RNA's with the nucleoplasm only.

CHAPTER X

TRANSFER OF RIBONUCLEIC ACID TO CYTOPLASM

The possibility of nucleolar material being transferred from the nucleolus to the cytoplasm has been studied extensively. In one such study, adult mice were given a single subcutaneous injection of tritiated cytidine then the prepared tissues treated with DNAse. In liver sections, the nucleoli showed the highest radioactive concentration with an apparent migration of radioactivity with time from the nucleolus to the cytoplasm (23). Amando and Leblond (1) stated that the nucleolar RNS behaved as the precursor of the cytoplasmic RNA. It was noted that the cytoplasmic RNA time curve reached a maximum about the point where it cuts the time curve of the nucleolus. Perry and fellow workers (9, 30, 31) in their work on the tritiated cytidine incorporation by HeLa cells, found that by irradiating the nucleolus the cytoplasmic incorporation rate was reduced by sixty-five per cent. The large dependence of cytoplasmic incorporation on the nucleolus was attributed to the RNA being synthesized in the nucleolus and moved to the cytoplasm. They further noted the dependence of nuclear (other than nucleolus) incorporation on the nucleolus. This dependence was contributed to the RNA in transit to the cytoplasm. This was supported by the fact that the nucleus was relatively independent of the nucleolus at short incubation times and attained a constant dependence at about two hours--just prior to the time the cytoplasm began to be significantly labelled.

The residuum of approximately thirty-five per cent cytoplasmic and seventy per cent nuclear (other than nucleolus) activity was considered independent of the nucleoli. Chipchase and Birnstiel (6) discovered that P^{32} - labelled pea ribosomal RNA hybridizes with the whole genomal DNA of pea seedlings and not with DNA of heterologous origin (Escherchia coli). RNA of heterologous origin would not combine with pea DNA. The complex formed between P^{32}- labelled ribosomal RNA and pea DNA was attributed to base sequence complement. Nucleolar RNA competed with ribosomal RNA for DNA stretches complementary to the latter. Chipchase and Birnstiel concluded that nucleolar RNA was similar to cytoplasmic ribosomal RNA in base composition, sedimentation rate and also in base sequence. This indicated that the nucleolar RNA included ribosomal RNA. The protein in nucleoli resembled that found in the ribosomes as shown by amino acid composition. The experimentors suggested the possibility that the protein in nucleoli resembling ribosomal protein complexed with the newly synthesized ribosomal RNA near the chromatin and was transferred to the nucleolar periphery. These nucleolar ribosomes are unfinished ribosomes because of the inability to function as such. Edstrom and Gall (8) isolated nuclei from Triturus oocytes. The base composition of ribonucleic acids were determined by microelectrophoresis. It was found that nucleolar and cytoplasmic RNA's are similar in composition and both are of the guanine-cytosine rich type. Nucleolar RNA is then evidently a precursor of ribosomal RNA because of correlation in base ratios of the two. McMaster (27) studied autoradiographs obtained by the incorporation and retention of adenine-8-C^{14} and of $P^{32}O4$ by nucleolar, chromosomal and cytoplasmic RNA of Drosophilia salivary

glands. Radioisotope concentrations were determined from autoradiographs by grain counting and RNA concentrations by microphotometry after basic staining. The relationship between rates of adenine incorporation and RNA accumulation was used to obtain estimates of turnover rates. Nucleolar incorporation patterns indicated a complete turnover of the fraction in an hour or less, but there was no corresponding cytoplasmic turnover. McMaster stated that the nucleolar turnover was due to degradation of RNA within the nucleolus rather than to movement of intact molecules from the nucleolus. It was further stated that different ultimate precursors are indicated for nucleolar and non-nucleolar RNA because the nucleolar precursor was labelled before the precursor of non-nucleolar RNA. Koulish and Kleinfield (21) stated also that there was no passage of formed nucleolar RNA into the cytoplasm. This statement was based on the normal turnover of cytoplasmic RNA compared to the increased nucleus and in particular nucleolar turnover due to intraperitoneally injected tritiated cytidine in rats.

CHAPTER XI

MECHANISMS FOR TRANSFER OF RIBONUCLEIC ACID

A relationship between one type of nucleolar RNA and ribosomal RNA has been repeatedly found by different individuals. It has been stated that the nucleolar RNA is a precursor of the ribosomal RNA. This possibility indicates that the nucleolar RNA must be somehow transferred from the nucleus through the nuclear membrane to the cytoplasm. Recent studies have resulted in a variety of hypothesis. Beams and Kessel (2) studied the ovaries of species of Cambarus, Orconetes and Procambarus using the electron microscope. They found the nucleoli to be arranged in an irregular pattern adjacent to the nuclear membrane. Furthermore, immediately outside the nucleus, they observed clusters of dense masses of granules very similar to the nucleoli material inside. Beams and Kessel suggested that the nucleolar threads passed through pores in the nuclear membrane. In a later work, Kessel and Beams (19) described the extrusion of nucleoli in oocytes of Thyone briareus. They observed a large number of nucleoli in the peripheral region of the nucleus. Some nucleoli were flattened against the nuclear membrane. A conical evagination of the nuclear membrane. A conical evagination of the nuclear membrane appeared with the dense nucleolar material located in the cone. The nuclear envelope progressively stretched as the greater part of the nucleolus became enclosed within the evagination. When the nucleolus was

completely separated from the nucleus, the nuclear membrane seemed to dissolve. They also noticed the nucleolar material breaking down into smaller masses. They hypothesized that these masses could become the ribosomes. Szollosi and Ris (37) also observed the extrusion of nucleoli from both male and female pronuclei of rat eggs. After sperm penetration, large perinuclear lacunae developed. Electron-opaque masses formed near the inner nuclear membrane. These masses protrude into the cytoplasm carrying the nuclear membrane. These masses remained trapped or were released into the cytoplasm. Tewari and Bourne (38) observed in the spinal ganglion neuron of the rat that the nucleoli near the inner nuclear membrane were surrounded by mitochondria. They suggested the possibility that enzymes of the mitochondria dissolved the nuclear membrane allowing the nucleoli to move into the cytoplasm. Kordan and Morgenstern (20) studied sections of proliferating fruit tissue of Citrus medica L. and Citrus aurantium L. They observed an interaction between bodies in the cytoplasm and nuclei of the growing cells resulting in the transfer of nucleolar bodies from the nuclei to the cytoplasmic structures. In a normal non-growing cell, the cytoplasm contained granules which were dumbbell-shaped with the lobes being of approximately the same size referred to as B-1 bodies. However, in a growing cell, the cytoplasm, in addition, contained larger granules which were also dumbbell-shaped with one lobe larger than the other called B-2 bodies. The B-2 bodies were shown to be derived from the B-1 bodies by an increase in size of one lobe. The nucleus of the growing cell was observed to be encircled by B-1 and B-2 bodies. The smaller lobe of the B-2 bodies united with the nuclear membrane and the nucleolar apparatus entered the B-2 bodies. The

larger lobe continued to increase in size and received the nucleolar material. The B-2 body then separated from the nucleus. The nucleolar material was then extruded into the cytoplasm in the form of circles adjacent to one another and interlaced by a spiral chain. The B-1 bodies encircled the extruded nucleolar material and removed the nucleolar satellites from the spiral strand. The fate of the B-2 shell and B-1 bodies containing the nucleolar apparatus were undetermined. The entire process appeared to be a mechanism of transferring nuclear material to the cytoplasm of the cell without allowing direct contact between the cytoplasm and nucleoplasm to occur during the exit of nucleolar material.

CHAPTER XII

PROTEIN SYNTHESIS WITHIN THE NUCLEOLUS

The possibility of protein synthesis by the nucleolus has been referred to in the work by Birnstiel and Hyde (3). They found that pea nucleoli incorporation rate was greater than the chromatin fraction, Since chromatin was less active in amino acid incorporation than was the nucleolar fraction and was, in addition, DNAse sensitive, it was concluded that amino acid incorporation was not due to chromatin contamination. They found three types of proteins as mentioned before. Tewari and Bourne (38) in their research on the nucleoli of spinal ganglion neurons of rat found evidence for exzymatic proteins. They suggested that some of these enzymes were synthesized in the nucleolus. For instance, the pattern of ATPase concentration in the nucleolus indicates its synthesis in this region. Leblond and Amando (23) incubated purkinje cells of rats with tritiated-leucine. The radiographs showed heavy activity in the cytoplasm and some activity in the chromatin region and nucleoplasm. However, very little if any incorporation occurred in the nucleolus. Therefore, little protein synthesis occurs in the nucleolus. Prescott and Bender (32) using tritiated precursors found no indication of protein synthesis in mammalian tissue culture cells.

CHAPTER XIII

CONCLUSION

One prominent feature of the literature consulted was the conflicting nature of the data. Also, it is noted that new equipment, such as the electron microscope eliminates some of these conflicts. The structure of the nucleolus was found to be composed of two main divisions. The significance of the two divisions remains to be determined. The disappearance and reappearance of the nucleolus during the mitotic cycle resulted in two hypotheses concerning its origin. One hypothesis states that the nucleolus forms by accumulation of premitotic material. The other states that is is synthesized by the midtelophase chromosomes. The formation of the nucleolus on a particular chromosomal region was indicated but seemed not necessary in every case. The possibility of nucleolar control over the mitotic cycle was indicated. The chemical composition of the nucleolus is an area of controversy. It is, however, generally agreed that the nucleolus contains RNA and proteins. The nucleolus is considered one site of RNA synthesis. The types of RNA included within the nucleolus, however, is not agreed on. The transfer of nucleolar material to the cytoplasm was indicated by a number of experiments showing the similarity between nucleolar material and ribosomal material. The mechanism of transfer of the nucleolar material ranges from a simple molecular escape through the nuclear membrane to a complex cycle of transfer involving

cytoplasmic bodies. Synthesis of proteins within the nucleolus was indicated by some of the research, but not by all. It is quite obvious that more research is required on the nucleolus.

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