Z. Zellforsch. 103, 34—47 (1970)

The Development of the Nucleolus of the Ovarian Nurse Cell of *Drosophila melanogaster*

CHARLES C. DAPPLES and ROBERT C. KING*
Department of Biological Sciences, Northwestern University
Evanston, Illinois, 60201, U.S.A.

Received September 27, 1969

Summary. A description is given of the development of the nucleoli of the ovarian nurse cells of *Drosophila melanogaster* during stages 7 through 10 of oogenesis. This developmental period lasts about a day, and during it the volumes of the nurse cell nucleolus, nucleus and cytoplasm all double once every 4—5 hours. The nucleolar bodies within the endopolyploid nurse cell nucleus grow until they form a thick network that is shaped like a shell whose outer boundary lies close to the inner surface of the nuclear envelope. RNA of nucleolar origin continually enters the cytoplasm. The nuclei of the nurse cells directly connected to the oocyte are most active in terms of DNA replication and RNA transcription. The nurse cells empty their cytoplasm into the oocyte which doubles its volume every 2 hours. The ribosomes stored in the ooplasm are derived almost exclusively from the nurse cell. The doubling time for the rDNA of the nurse cells is about 9 hours, and about 1,000 rRNA molecules are transcribed per rDNA cistron per hour during vitellogenesis.

Key-Words: Drosophila melanogaster — Oogenesis — Nucleolus — Vitellogenesis — rRNA transcription — Ovarian nurse cells.

In most advanced, holometabolous insects possessing polytrophic, meroistic ovarioles, the oocyte is one of a group of inter-connected cells. The other cells of the cluster, the nurse cells, function by synthesizing cytoplasm which they pour into the oocyte. In the fruit fly, *Drosophila melanogaster*, each egg chamber consists of an oocyte and 15 endopolyploid nurse cells surrounded by a follicular envelope one cell thick. The egg and its fifteen nurse cells are fourth generation descendants of a single cell, the germarial cystoblast (Koch, Smith, and King, 1967; Cummings and King, 1969).

Each sex chromosome of *Drosophila melanogaster* contains one nucleolus organizer. Ritossa and Spiegelman (1965) annealed rRNA to DNA isolated from adult flies containing one, two, three, or four nucleolus organizers in each somatic cell nucleus and found that saturation occurred at levels directly proportional to the number of nucleolus organizers. They reasoned that the nucleolus organizer region of the X and Y chromosomes contains the cistrons that transcribe both the 18S and the 28S rRNA molecules. Ritossa, Atwood and Spiegelman (1966) showed that no such proportionality occurred when tRNA was used in similar nucleic acid hybridization experiments. They concluded that the nucleolus organizer was not the site of transcription of this class of RNA molecules.

Von Borstel and Reckemeyer (1958) have demonstrated that fertilized eggs lacking sex chromosomes are still able to undergo ten to twelve mitotic divisions

* The authors are grateful to Mr. E. John Pfiffner for preparation of the model and the inked drawings and to Mrs. Birdeena C. Dapples for her conscientious assistance with the statistical analyses. We also appreciate the criticism of the manuscript by Dr. W. S. Klug. This research was supported by the National Science Foundation grant GB7457.

before dying. Therefore the ribosomes which function during this early embryonic period must have been added to the egg during oogenesis by cells possessing sex chromosomes in their nuclei. Koch, Smith and King (1967) have established that the nucleolus in the germinal vesicle breaks down during vitellogenesis. Therefore the oocyte nucleolus organizers can be a source of only a minute fraction of the total rRNA molecules found in the ooplasm. RNA synthesis goes on simultaneously in all nurse cell nuclei of an egg chamber (King and Burnett, 1959). Much of this nuclear RNA subsequently enters the cytoplasm (Zalokar, 1960). RNA of nurse cell origin continually enters the oocyte in a cytoplasmic stream, since tongues of intensely azure B-positive, nurse cell cytoplasm which protrude through the canals connecting adjacent nurse cells to the oocyte are often seen in sections (King, 1964).

In this paper we will describe the development of the nurse cell nucleus during the vitellogenic stages of *Drosophila* oogenesis and draw some conclusions as to rates at which nucleic acids are synthesized in nurse cell nuclei.

Materials and Methods

Oregon-R wild type *Drosophila melanogaster* were raised at temperatures ranging between 22 and 24° C upon David's medium (David, 1962) in a normal cycle of daylight and darkness. The ovaries studied were provided by mated, sexually mature females between 2 and 3 days old. The ovaries were dissected from etherized females submerged in *Drosophila* physiological saline (see Butterworth, Bodenstein, and King, 1965, p. 142 for the recipe) containing one drop of the wetting agent, Tergitol, per 100 ml. The ovaries were next transferred to a buffered (pH 7.4) hyaluronidase solution (150 USP units of enzyme per 7 ml 0.05 M cacodylate buffer). A three minute digestion at room temperature renders the tissue more permeable to fixatives and embedding media (Pallie and Pease, 1958). Subsequent to the digestion the ovaries were fixed for two hours in a solution consisting of one part 2% OsO_4 and one part 2% $K_2Cr_2O_7$ (both in cacodylate buffer). After six 10 minute washes in buffer, the ovaries were dehydrated rapidly through a series of water-acetone mixtures to 100% acetone. Next the ovaries were left for 24 hours in a mixture of equal parts propylene oxide and non-polymerized resin (Araldite-Epon, 1:1). The ovarioles were teased apart and individual chambers were placed in fresh resin in Beem capsules, and the resin was allowed to polymerize during three twenty-four hour periods at increasingly elevated temperatures (35, 45, and 60° C).

A Leitz Fernandez-Moran microtome equipped with a glass knife was used to cut 1 μ serial sections which were mounted on albuminized slides. Sections were stained for one hour at 60° C in an aqueous 0.025% solution of azure B at pH 9. This dye binds to all nucleic acids, proteins, and lipids bearing negatively charged groups at pH 9 (King, 1960). The sections were photographed at a magnification of 1,000 using a Leitz Ortholux microscope equipped with an Orthomat automatic 35 mm camera loaded with Kodak high contrast copy film, and 8×10 enlargements were made of the negatives.

Kodak diffusion sheets, 8×10 inches, were punched to fit into a standard three-ring notebook, and the photograph representing the center section through the chamber to be reconstructed was chosen and traced with India ink on a diffusion sheet in the notebook. The photograph representing the next section below the one just traced was placed along with a blank sheet of diffusion paper beneath the tracing and the photograph orientated with the tracing. The first tracing was then flipped over in the notebook, and the second tracing was completed. This procedure was followed until all photographs of both lower and upper sections had been traced. The result was a notebook containing a series of tracings of sections, all of which were properly oriented with respect to one another.

The nucleoli were reconstructed in the following manner. Beginning at the bottom of a stack of orientated tracings a single nucleolar body was chosen and colored in on the bottom tracing, using a colored pencil. The next tracing was placed on top, and the corresponding nucleolar body was given the same color. This procedure was continued until the other end

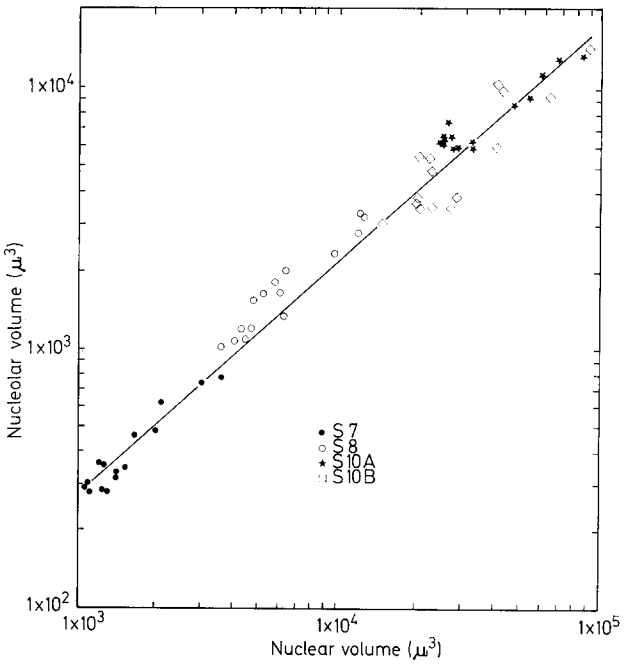

Fig. 1. The relation between the nucleolar volume and nuclear volume (both expressed logarithmically) for nurse cells from egg chambers at different developmental stages. The slope for the line of best fit is 0.98

of the nucleolar body was reached. Then a second color was chosen, and the procedure was carried out on another nucleolar body in the same nucleus, and so forth until all the nucleolar bodies within a given nucleus were colored in. In this way it could be determined, if a single reticulate nucleolus extended throughout the nucleus, or if there were several unconnected nucleoli within a single nucleus. This method of coloring allows one to readily determine the spacial interrelationships of various nucleolar bodies which would not be otherwise obvious in sections.

The area of the nucleus and the nucleolus on each tracing was measured using a compensating polar planimeter. Once all the data were assembled for a given nucleus, the volume of the nucleus and its nucleolus could be computed knowing the thickness of each section. The data for all nurse nuclei from the chambers chosen for study were eventually assembled, and a double log plot was made relating the nucleolar and nuclear volumes of nuclei of a variety of sizes. Volumes of the oocyte and nurse subchambers were calculated by assuming that the subchambers were half spheroids of appropriate dimensions. Lines of best fit were calculated using the method of least squares.

An LKB Ultrotome equipped with a glass knife was used to cut 75 mμ thick sections of nurse cell nuclei, and these were mounted on Formvar-coated, copper mesh grids and stained with uranyl acetate and lead citrate. Such sections were viewed and photographed with a Hitachi HS-8 electron microscope.

Results

Volume Measurements

The results of the volume measurements are plotted in Fig. 1. See Cummings and King (1969) for the staging criteria and estimates as to the minimum time spent in each stage. The volume of the nurse cell nucleus increases from a little

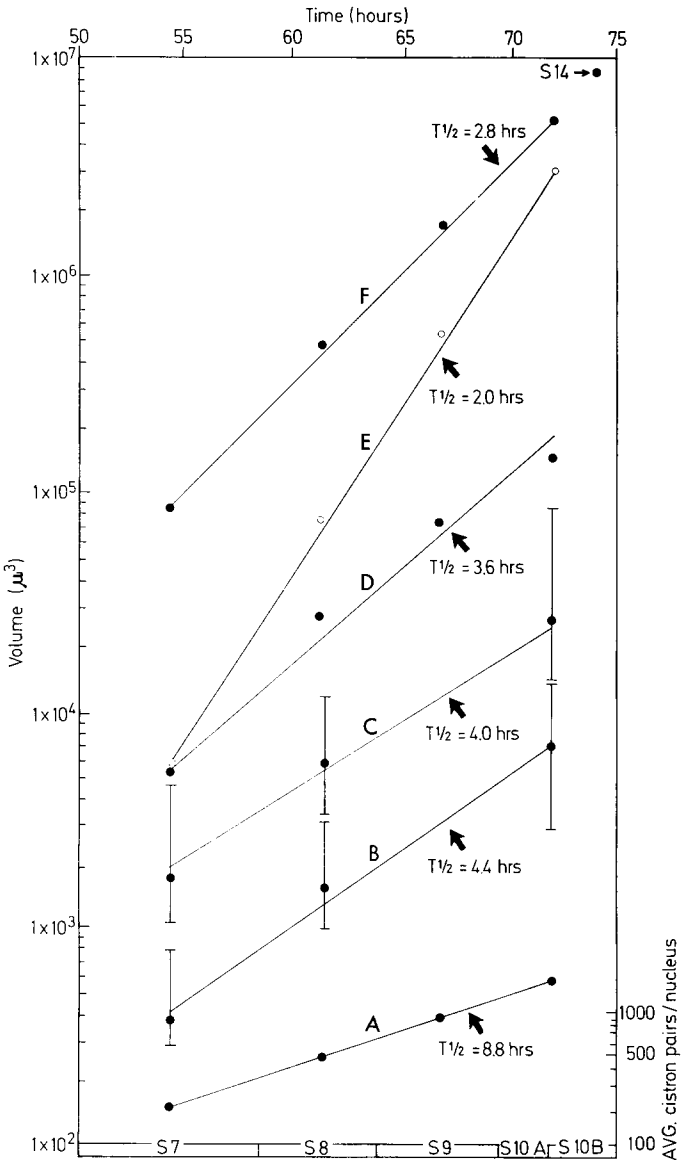

Fig. 2. The relation between volume (expressed logarithmically) and time for various components of an egg chamber. Line *B*, growth in nucleolar volume for the average nurse cell. Line *C*, growth in nuclear volume for the average nurse cell. Line *D*, growth in protoplasmic volume for the average nurse cell. Line *E*, growth in protoplasmic volume for the oocyte. Line *F*, growth in the volume of total protoplasm of the 16 sister cells. Line *A* shows the relation between the number of pairs of rDNA cistrons per average nurse cell nucleus and time. *S*, stage; $T^1/_2$, doubling time. The brackets include the largest and smallest nurse cell nuclei or nucleoli for a given stage

over 1×10^3 μ^3 at the beginning of stage 7 to nearly 1×10^5 μ^3 at the end of stage 10B, in a period that according to David and Merle (1968) occupies 25 hours under optimal conditions. The nucleolus increases in volume from 3×10^2 μ^3 at

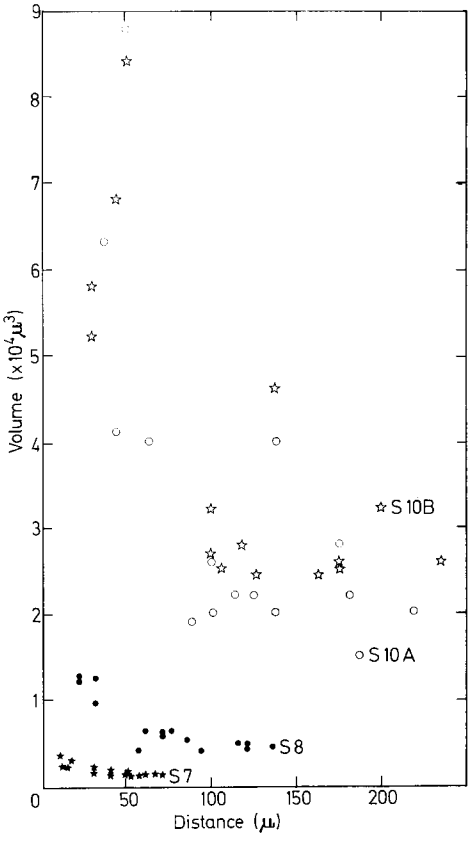

Fig. 3. The relationship between nurse cell nuclear volume and the minimum distance from the center of the nurse nucleus to the surface of the oocyte. *S*, stage

stage 7 to 1.5×10^4 μ^3 at stage 10B. The line of best fit for these data has a slope very close to unity, which indicates that the nucleolus is increasing in volume at a rate similar to that of the nucleus as a whole.

When average volumes for the nuclei and nucleoli of each population of nurse cells making up the stage 7, 8 and 10 chambers are computed and are plotted against the midpoints of each stage in time, one obtains a graphic representation of the rates of increase in nuclear volumes. On the same drawing (Fig. 2) are plotted the data for the cytoplasmic volume of the average nurse cell as a function of stage. Each value is computed by taking $^1/_{15}$ of the volume of the nurse subchamber from a chamber of defined stage. The data for the increase in ooplasm as a function of stage and of total protoplasm as a function of stage are also plotted. The latter values are computed by summing the volumes of the protoplasm of the oocyte and of all nurse cells in the chamber. Doubling times were determined from the lines of best fit drawn through each series of points. The doubling times for the volumes of the nurse cell nucleolus, nucleus and cytoplasm are all in the neighborhood of 4 hours, whereas the ooplasm doubles every 2 hours and the cytoplasm of the 16 cell cluster as a whole doubles every 3 hours during stages 7

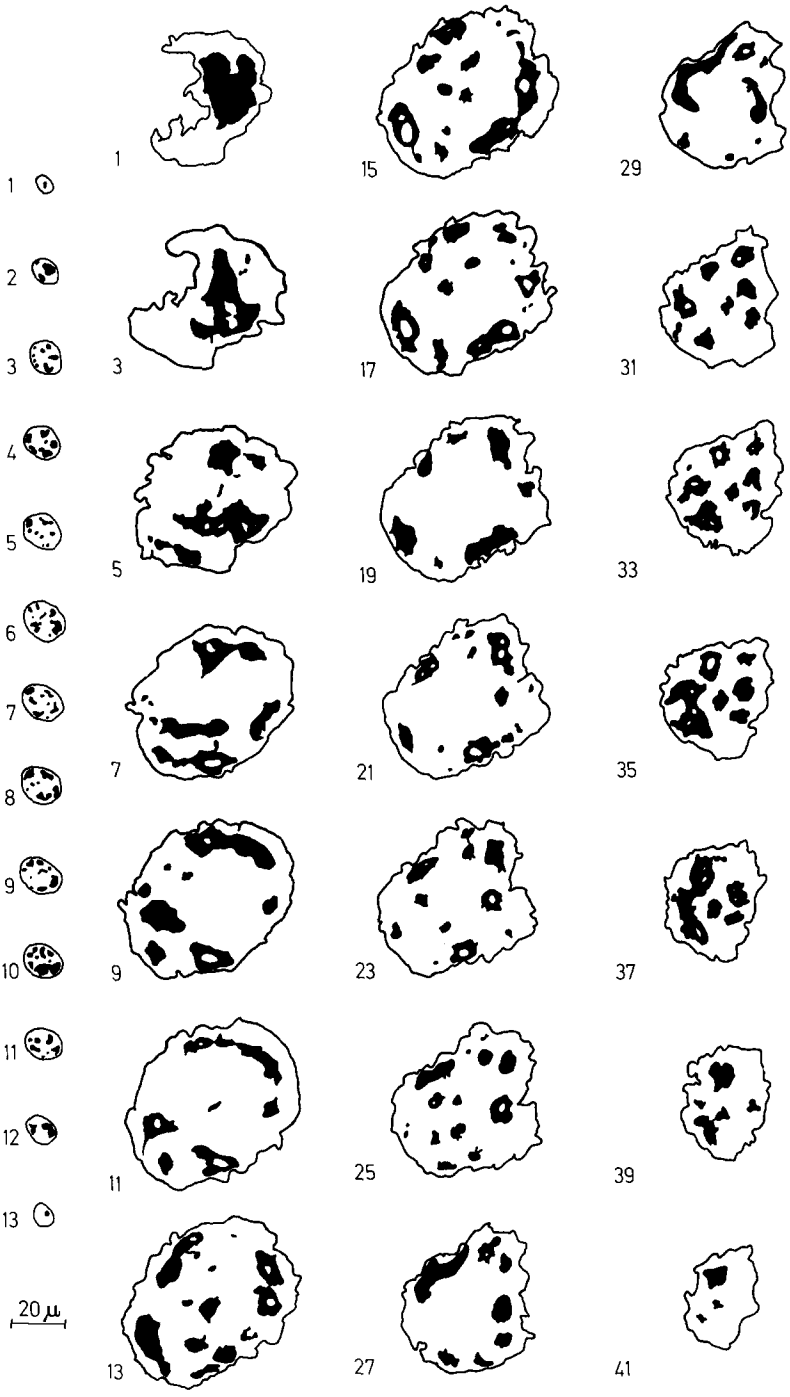

Fig. 4. Nurse cell nuclei as seen in serial sections. The left column shows all 13 sections from a stage 7 nurse cell nucleus. The remaining 3 columns show every other section from a stage 10B nucleus. The stage 7 nucleus is the smallest and the stage 10B nucleus the largest in the sample of 60 nuclei presented in Fig. 1

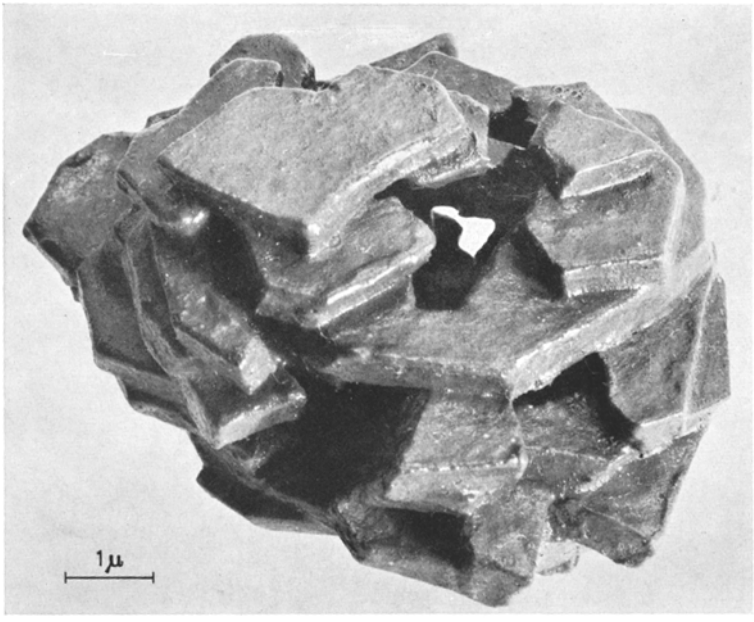

Fig. 5. A model of a nurse cell nucleolus from a stage 9 nucleolus. Since it is very difficult to cut from thick cardboard sheets the intricate, convoluted shapes taken by the nucleolar projections, the model shows squared off edges that are not true to life

through 10. By the end of stage 10B the total protoplasmic volume is equivalent to that of the mature (stage 14) oocyte. As far as the nurse cells are concerned the doubling times give an *underestimate* of the synthetic rates, since the products exported are not included in the data. Thus the nuclear RNA that has entered the nurse cell cytoplasm is not recorded in the nucleolus measurements, and the nurse cell cytoplasm that has entered the oocyte is not recorded in the measurements of nurse cytoplasm.

In a given chamber the volume of the nurse cell nuclei often vary depending on the distance of the cell to the oocyte. The relationships between nurse cell nuclear volume and distance from the oocyte for stage 7, 8, 10A and 10B chambers are presented in Fig. 3. Inspection of the figure shows that the polarized distribution of nuclear sizes becomes more pronounced as development proceeds. However, as early as stage 7 two of the nurse cells adjacent to the oocyte have nuclei whose volumes are double those observed for the other 13 nuclei. All nuclei continue to grow, and by stage 8, four nuclei adjacent to the oocyte are each at least twice as large as any of the other nuclei in the nurse chamber. In stage 10 chambers one again sees that the 3 or 4 nuclei closest to the oocyte are relatively gigantic.

Qualitative Changes. The morphology of the nurse cell nucleolus alters during the developmental stages studied. Drawings of sections through nuclei representative of early stage 7 and late stage 10B are presented in Fig. 4. These nuclei differ in volume by a 100-fold factor, and their nucleoli have volumes differing by at least a 50-fold factor. The stage 7 nucleolus is composed of a series of fingerlike

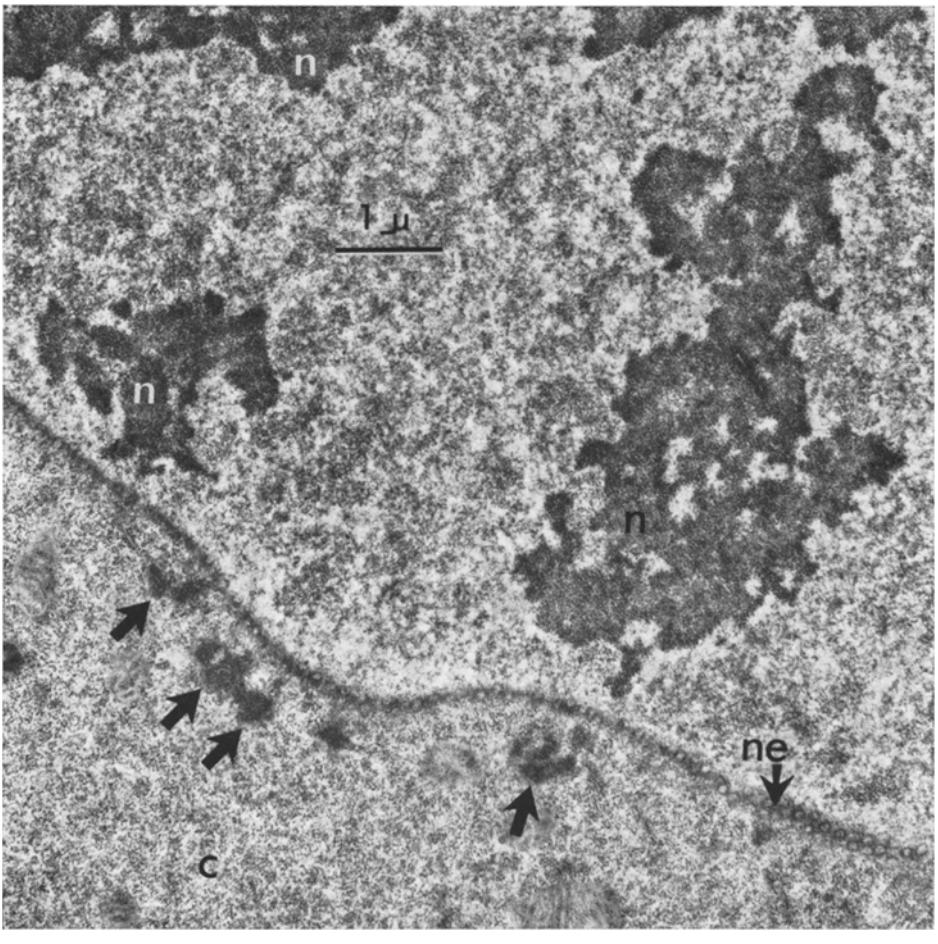

Fig. 6. An electron micrograph of a portion of a stage 8 nurse cell nucleus. Arrows point to clouds of ribosomal particles which are thought to have been recently liberated from the nucleus. *c*, cytoplasm; *n*, nucleolar processes; *ne*, annulate nuclear envelope

projections, most of which are interconnected. As development proceeds the nucleolar fragments seen in each section increase in size. It is obvious that these fragments are not randomly distributed throughout the nucleus, but instead are closer on the average to the surface than to the center of the nucleus. Fig. 5 presents a photograph of a three dimensional model of the nucleolus from a stage 9 nurse cell. It would be representative of a stage in nucleolar development midway through the sequence plotted in Fig. 1. While the model is rather crude, it demonstrates that the vast majority of all nucleolar segments are interconnected. They form a thick network shaped like a spherical shell whose outer boundary lies close to the inner surface of the nuclear envelope. In some of the sections passing through larger nucleolar bodies, spherical, non-staining areas are observed (see Fig. 4 — sections 15 and 17, for example). Serial reconstructions demonstrate

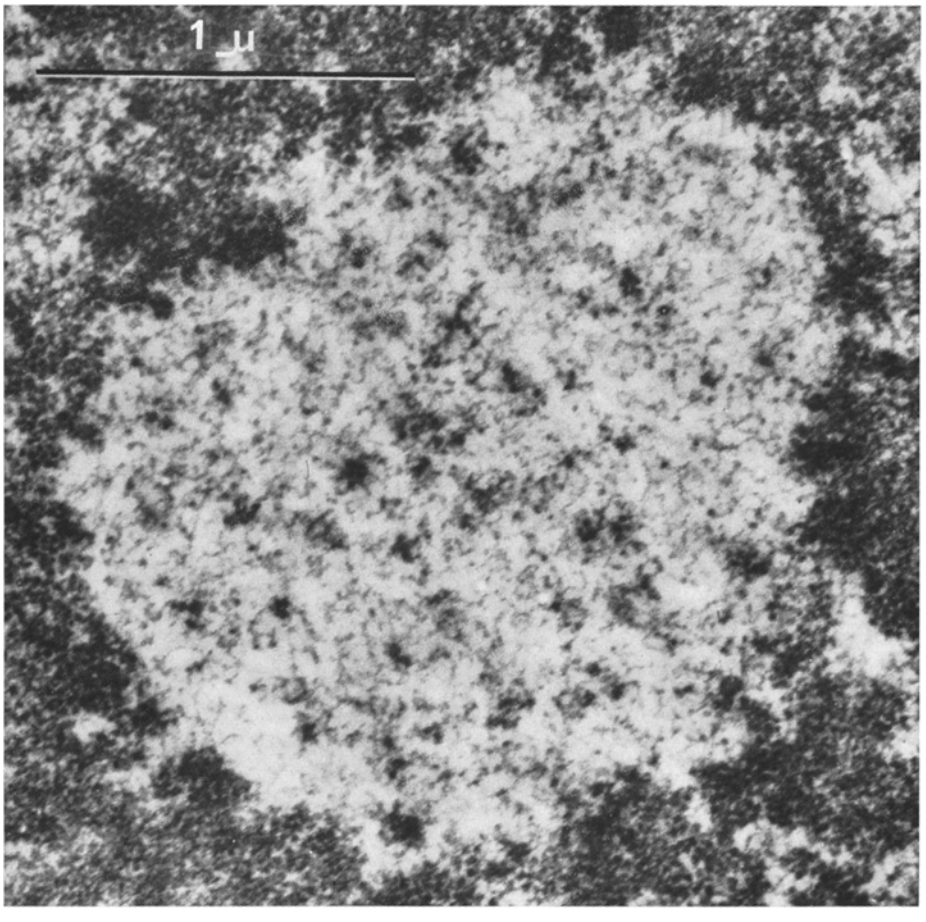

Fig. 7. A higher power electron micrograph of a nucleolar vacuole from stage 10B nurse cell nucleolus showing the fibrillar network within

that these azure B-negative regions are entirely surrounded by nucleolar material. The areas in question do bind fast green at pH 2 and therefore presumably represent protein-containing droplets embedded in a ribonucleoprotein matrix. The concentration of these intranucleolar droplets increases with time and reaches a maximum in stage 10B. The average stage 7 nurse nucleolus makes up 25.3 $\pm$0.8% of the nucleolus and is composed of 2.0 $\pm$ 0.3 bodies. The stage 10B nurse nucleolus makes up 19.4 $\pm$ 1.2% of the nucleolus and is made up of 4.9 $\pm$ 0.5 bodies. It follows that as vitellogenesis proceeds the nucleolus fragments and becomes smaller relative to the nucleus as a whole.

Under the electron microscope the majority of the nurse nucleolus is seen to consist of a dense feltwork of fibers lying in an amorphous, less dense matrix. However, still denser patches of material are scattered throughout the nucleolus, and these patches generally lie in the nucleolar periphery. Such dense areas consist of closely packed fibrils similar to those found within the majority of the

nucleolus together with particles of ribosomal dimensions (Fig. 6). Sections through the intranucleolar "droplets" (Fig. 7) show that these areas contain a reticulum of fibrils.

Discussion

The nuclei of the nurse cells undergo striking changes in size, shape, and DNA content which reflect changes in the synthetic activities of these cells during oogenesis. Nurse cell chromosomes undergo a series of endomitotic doublings, the first of which presumably occurs in the germarium, and seven or eight more doublings occur within the vitellarium. The replication of DNA in a population of nurse cell nuclei from a given chamber is asynchronous (King and Burnett, 1959). As the chromosomes replicate they first show somatic pairing. Homologous chromosomes are synapsed and attached at their centromeric regions to a single nucleolus (Hsu and Hansen, 1953). The nurse chromosomes next elongate by uncoiling and concurrently undergo a series of endomitotic doublings which produce polytene chromosomes whose multiple strands become more and more loosely associated as the nucleus grows. By stage 6 the homologous chromosomes have dissociated completely, and all nuclei become filled with a jumbled mass of Feulgen-positive threads (see Klug, Bodenstein, and King, 1968, their Fig. 1).

Jacob and Sirlin (1959) estimated the relative ploidy values for the nuclei of *Drosophila* nurse cells at various developmental stages. However, it is likely that all these values are underestimates (Koch and King, 1966). Doubling the Jacob and Sirlin estimates brings them into harmony with those reported by Schultz (1956). The values we use in further calculations are listed in Table 1 of the paper by Cummings and King (1969). According to Ritossa and Spiegelman (1965) about 0.27% of the DNA in the haploid genome of *Drosophila melanogaster* is used to transcribe the 18S and 28S RNA molecules of ribosomes. From this datum they calculated that there are approximately 130 cistrons for the 18S rRNA and an equal number for the 28S rRNA in each sex chromosomes. By multiplying 130 by the number of haploid chromosome sets in the average nurse cell nucleus for a given stage, one can obtain the number of rDNA cistron pairs per average nurse cell nucleus for each of the vitellogenic stages of oogenesis, and such data are presented in Fig. 2. Note that the doubling time for nurse cell DNA is 8.8 hours, roughly twice that required to double the volume of the nucleolus. Data from other experiments (Chandley, 1968) suggest that the time required for DNA replication is about 0.5 hours. If one assumes that transcription from rDNA cannot take place until all DNA is replicated, then the rDNA cistrons in the nurse nuclei have approximately 7.5 hourly transcription periods between replications.

Klug, King and Wattiaux (1970) have calculated that there are approximately 2×10^{10} ribosomes in a stage 14 oocyte. Each ribosome contains one 18S and one 28S RNA molecule. Let us assume that the vast majority of these ribosomes were synthesized in the nurse cells during stages 7 through 10B. We can estimate the total number of rDNA cistron pairs in the fifteen nuclei in a given stage and multiply this by the time interval available for transcription in this stage. Adding the data together for stages 7 through 10B, we come up with a value of approximately 2×10^7 cistron pair hourly transcription periods. Thus in an hour about

1,000 rRNA molecules are transcribed per rDNA cistron. A value of 600 rRNA molecules per hour is cited by Klug, King and Wattiaux. This calculation differs in that the time spent in stages 11—14 is included, the time required for replication is not subtracted, and an overall DNA value of 512 is used for all nuclei in all stages. If we assume that transcription of a second rRNA molecule cannot occur until the first has detached from the rDNA cistron, then we can calculate the time for transcription of one rRNA molecule as $^1/_{1000}$ hr or 3.6 seconds. This may be an underestimate, since more than one rRNA may be transcribed simultaneously. Thus, if three were transcribed at a time, 11 seconds would be required to complete one rRNA molecule.

The distribution of nuclear volumes among the population of 15 nurse cells in chambers in stages 7 through 10B (Fig. 3) shows a polarity, since the volume increases as the distance between the oocyte and the nurse cell decreases. The volume of a *Drosophila* nurse nucleus also seems to be correlated with the pattern of ring canal interconnections as well as the nearness of the cell to the oocyte. Brown and King (1946, see their Fig. 9) found that the four nurse cells connected directly to the stage 10A oocyte, i.e., cells 2, 3, 5, and 9, posses nuclei with the largest volumes. Of these four cells the one having the greatest nuclear volume (cell 9) has no other nurse cells connected to it. Conversely, the cell with the smallest nuclear volume is nurse cell number 2 which is connected to three nurse cells. Of all the nurse cells in the chamber, number 10 possesses the smallest nucleus, and, of all nurse cells, this one occupies the most central region of the nurse chamber and has less of its surface in contact with the follicular epithelium than any other nurse cell. Brown and King suggested that in a given nurse cell the volume of the nucleus, which we now know is proportional to the degree of endopolyploidy and to the nucleolar volume, increases as a function of the rate at which products synthesized by the cell are removed. Thus, the greater the area of the cell's surface which is in contact with the squamous follicle cells (through which extraovariolar nutrients must pass), the faster the nurse cell will receive precursors, and the closer the nurse cell is to the oocyte, the faster its synthetic products will be removed. Also, if other nurse cells discharge their products into the nurse cell in question, the concentration of products will rise in this cell. Thus, the rate at which products are drained from the cell is also a function of the number of anterior nurse cells connected to it. According to this hypothesis cell 9 should show the greatest nuclear volume because (1) it receives an abundant supply of nutrients through the relatively large surface area it presents to the attenuated follicle cells, (2) it empties its products directly into the oocyte, and (3) it receives no synthetic products from adjacent nurse cells. Of the nurse cells connected directly to the oocyte, cell 2 has the smallest nucleus, and it receives products from *seven* other nurse cells. There is a reason in addition to the one just cited as to why cell 2 should lag behind cells 9, 5, and 3 in replicating its DNA. Since cell 2 is one of the two pro-oocytes, it spends its time synthesizing a synaptonemal complex while the other 14 cells are undergoing at least two replication cycles (Koch, Smith and King, 1967). Thus by the time cell 2 switches to the developmental pathway characteristic of nurse cells, its 14 companions have a considerable head start. In Jacob and Sirlin's studies (1959)

the "three most posterior" nurse cells were shown to synthesize their DNA more rapidly than other nurse cells, since these posterior nuclei underwent more DNA replications and reached their maximum DNA values before other nurse cells. It is probable that the posterior nurse cells studied by Jacob and Sirlin were 9, 5, and 3. There are reports in other species of Diptera where the nurse cells closest to the oocyte have particularly large nuclei (Detinova, 1962; Odhiambo, 1968).

In *Drosophila melanogaster* at still later stages of oogenesis some breakdown of nurse cell DNA takes place (Jacob and Sirlin, 1959). Again it is the most posterior nuclei which are first effected. This polarized breakdown of DNA, beginning with the nurse cells nearest the oocyte, can be induced prematurely by feeding females the folic acid antagonist, aminopterin (King and Sang, 1959). The significance of this polarized DNA catabolism is unknown. A polarity is also seen in the abnormal chambers produced by females homozygous for *fes* (King, 1969). It is the nurse cells nearest the oocyte which possess banded, polytene chromosomes in their nuclei.

Cytochemical studies on the nucleoli of *Drosophila* nurse cells have been reported by King (1960). RNA-ase treatment removes the nucleolar material which stains with azure B and trypsin extracts fast green-stainable material. Polysaccharides and phospholipids are also demonstrable in the nucleolus. Electron microscopic histochemical analyses have shown that both the fibrillar and particulate components of nucleoli are ribonuclease-sensitive, while the amorphous, matrix material in the nucleolus is susceptible to digestion by proteases (Marinozzi, 1964). Our studies demonstrate that as the nucleolus reaches its maximum volume, it seems to produce fibrous proteins which are segregated into intranucleolar droplets.

The provision of the *Drosophila* oocyte with fifteen highly polyploid nurse cells serves to multiply the rDNA available for transcription by thousands of times. Methods for the selective replication of oocyte rDNA have evolved in other species which are not provided with nurse cells (Allen and Cave, 1968; Davidson, 1968; Gall, 1969; Miller and Beatty, 1969). Various species of dytiscids seem to represent an intermediate case. In such water beetles *both* the oocyte and the nurse cell nuclei synthesize RNA which eventually enters the ooplasm. The oocyte nucleus contains a mass of extra chromosomal DNA and appropriate hybridization experiments (Gall, Macgregor, and Kidston, 1969) show that there is an amplification of rDNA in the ovary. However, the extra chromosomal DNA contains much DNA which is not complementary to rRNA.

Recent studies by Ribbert and Bier (1969) suggest that the nurse cells of *Calliphora erythrocephala* contain multiple free nucleoli, each with its own rRNA synthesizing templates. In the case of *Drosophila melanogaster* we have always assumed that the multiplicity of nucleoli is the result of polyploidization followed by separation of individual chromatids with each separate nucleolus organizer forming its own nucleolus. Evidence in support of this concept comes from studies of the polytene chromosomes seen in the nuclei of nurse-like cells in the ovaries of females homozygous for the female sterile gene, *fes*. Here a single nucleolus is formed of dimensions similar to those found in the larval salivary nuclei (King, 1969).

References

Allen, E. R., Cave, M. D.: Formation, transport, and storage of ribonucleic containing structures in oocytes of *Acheta domesticus* (Orthoptera). Z. Zellforsch. **92**, 477—486 (1968).

Borstel, R. C. von, Reckemeyer, M. L.: Division of a nucleus lacking a nucleolus. Nature (Lond.) **181**, 1597—1598 (1958).

Brown, E. H., King, R. C.: Studies on the events resulting in the formation of an egg chamber in *Drosophila melanogaster*. Growth **28**, 41—81 (1964).

Butterworth, F. M., Bodenstein, D., King, R. C.: Adipose tissue of *Drosophila melanogaster*. I. An experimental study of larval fat body. J. exp. Zool. **158**, 141—154 (1965).

Chandley, A. C.: Studies on oogenesis in *Drosophila melanogaster* with ^{3}H-thymidine label. Exp. Cell Res. **44**, 201—215 (1966).

Cummings, M. R., King, R. C.: The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster*. I. General staging characteristics. J. Morph. **128**, 427—442 (1969).

David, J.: A new medium for rearing *Drosophila* in axenic conditions. Drosophila Information Service **36**, 128 (1962).

— Merle, J.: A re-evaluation of the duration of egg chamber stages in oogenesis of *Drosophila melanogaster*. Drosophila Information Service **43**, 122—123 (1968).

Davidson, E. H.: Gene activity in early development. New York: Academic Press 1968.

Detinova, T. S.: Age-grouping methods in Diptera of medical importance. Wld Hlth Org. Monogr. **47**, Geneva, 191 pp. (1962).

Gall, J., Macgregor, H., Kidston, M.: Gene amplification in oocyte of dytiscid water beetles. Chromosoma (Berl.) **26**, 169—187 (1969).

Gall, J. G.: The genes for ribosomal RNA during oogenesis. Genetics **61**, Suppl. 1 (Part 2), 121—132 (1969).

Hsu, W. S., Hansen, R. W.: The chromosomes in the nurse cells of *Drosophila melanogaster*. Cytologia (Tokyo) **18**, 330—342 (1953).

Jacob, J., Sirlin, J. L.: Cell function in the ovary of *Drosophila*. I. DNA. Chromosoma (Berl.) **10**, 210—228 (1959).

King, R. C.: Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. Growth **24**, 265—323 (1960).

— Studies on early stages of insect oogenesis. Symposium of the Royal Entomological Soc. of London; No 2; Insect Reproduction, ed. K. C. Highnam, p. 13—25. 1964.

— The hereditary ovarian tumors of *Drosophila melanogaster*. Nat. Cancer Inst. Monogr. **31**, 323—345 (1969).

— Control of oocyte formation by female-sterile (*fes*) *Drosophila melanogaster*. Nat. Cancer Inst. Monogr. **31**, 347—349 (1969).

— Burnett, R. G.: An autoradiographic study of uptake of tritiated glycine, thymidine, and uridine by fruit fly ovaries. Science **129**, 1674—1675.

— Sang, J. H.: Oogenesis in adult *Drosophila melanogaster*. VIII. The role of folic acid in oogenesis. Growth **23**, 37—53 (1959).

Klug, W. S., Bodenstein, D., King, R. C.: Oogenesis in the *suppressor of Hairy-wing* mutant of *Drosophila melanogaster*. I. Phenotypic characterization and transplantation experiments. J. exp. Zool. **167**, 151—156 (1968).

— King, R. C., Wattiaux, J. M.: Oogenesis in the *suppressor of Hairy-wing* mutant of *Drosophila melanogaster*. II. Nuclear morphology and *in vitro* studies of RNA and protein synthesis. J. exp. Zool. (in press) (1970).

Koch, E. A., King, R. C.: Studies of the *fes* mutant of *Drosophila melanogaster*. Growth **28**, 325—369 (1964).

— — The origin and early differentiation of the egg chamber of *Drosophila melanogaster*. J. Morph. **119**, 283—304 (1966).

— Smith, P. A., King, R. C.: The division and differentiation of *Drosophila* cystocytes. J. Morph. **121**, 55—70 (1967).

Marinozzi, V.: Cytochimie ultrastructurale du nucléole-RNA et protéines intranucléolaires. J. Ultrastruct. Res. **10**, 433—456 (1964).

Miller, O. L., Jr., Beatty, B. R.: Visualization of nucleolar genes. Science **164**, 955—957 (1969).

Odhiambo, T. R.: Ring-canals and intercellular communication within the egg-chamber of a Tsetse fly species. Experientia (Basel) **24**, 738—740 (1968).

Pallie, W., Pease, D. C.: Prefixation use of hyaluronidase to improve *in situ* preservation for electron microscopy. J. Ultrastruct. Res. **2**, 1—7 (1958).

Ribbert, D., Bier, K.: Multiple nucleoli and enhanced nucleolar activity in the nurse cells of the insect ovary. Chromosoma (Berl.) **27**, 178—197 (1969).

Ritossa, F. M., Atwood, K. C., Spiegelman, S.: On the redundancy of DNA complementary to amino acid t-RNA and its absence from the nucleolar organizer region of *Drosophila melanogaster*. Genetics **54**, 663—676 (1966).

— Spiegelman, S.: Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. nat. Acad. Sci. (Wash.) **53**, 737—745, (1956).

Schultz, J. The relations of the heterochromatic chromosome regions to the nucleic acids of the cell. Cold Spr. Harb. Symp. quant. Biol. **21**, 307—328 (1956).

Zalokar, M.: Sites of ribonucleic acid and protein synthesis in *Drosophila*. Exp. Cell Res. **19**, 184—186 (1960).

Dr. Robert C. King
Department of Biological Sciences
Northwestern University
Evanston, Illinois, 60201/U.S.A.

Dr. Charles C. Dapples
Department of Biology
Rocky Mountain College
Billings, Montana, 59102/U.S.A.