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NUCLEOLAR DNA ORGANIZATION IN POLYTENE NUCLEI OF DROSOPHILA

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A comparative study of mitotic and polytene chromosomes of *Drosophila* provides several interesting aspects. The differential replication of centric and pericentric heterochromatin of mitotic chromosomes during polytenization is now well documented[1-4]. The organization of the ribosomal cistrons (rDNA) in polytene nuclei is related to this unique behaviour of the heterochromatin since it is known that in *D.melanogaster* the rDNA is located on the heterochromatin of X and Y chromosomes[5,6] and that these blocks of heterochromatin do not replicate in polytene nuclei[3]. In the polytene nuclei of *Drosophila* a considerable amount of intranucleolar DNA is known to be present[7,8] and this DNA has been demonstrated to be complementary to the ribosomal RNA[9].

A number of questions pertaining to the organization of the rDNA in salivary gland nuclei remain to be understood. One is, how does the rDNA replicate in polytene nuclei when the surrounding blocks of heterochromatin do not replicate at all? Secondly, what is the physical relationship of the intranucleolar DNA with the chromosomal DNA in the polytene nuclei? We have initiated autoradiographic studies on the replication of intranucleolar DNA in several species of *Drosophila*; some of our preliminary results are presented here. The physical relationship of the intranucleolar DNA with the chromosomal DNA in these nuclei has also been analysed in the light of our current understanding of the correlation between mitotic and polytene chromosomes of *D.melanogaster*.

MORPHOLOGY OF INTRANUCLEOLAR DNA

We have studied the morphology of intranucleolar DNA by autoradiography after continuous labelling with ^{3}H-thymidine throughout the polytenic growth of larval salivary glands. For this purpose, freshly hatched 1st instar larvae were transferred to *Drosophila* food supplemented with ^{3}H-thymidine (5µCi/g of food) and the larvae grown in this

food at 24°C till late third instar stages. Salivary gland chromosome preparations from late third instar larvae were made and the radioactivity in polytene nuclei was localised by autoradiography. In this manner, the distribution of DNA in D.melanogaster, D.ananassae, D.kikkawai and D.pseudoobscura has been examined. In addition, in case of D.melanogaster, EM autoradiographic studies have also been done to localize DNA in the polytene nucleoli after feeding the larvae with ^{3}H-thymidine as above. The results of these studies have, in general, confirmed earlier morphological observations of Rodman[8] that even in a single species, the intranucleolar DNA may exist as a single clumped mass or in a diffuse manner over the entire nucleolus or in both forms in the same nucleolus. Two examples of EM autoradiographs of nucleoli of D.melanogaster are shown in fig.1. Similar results have been obtained with the LM autoradiography in all the other species examined. Judging from the patterns of silver grains in the autoradiographs, it may be said that in all the species studied, the DNA is distributed throughout the body of the nucleolus. In some nuclei, even when a clumped mass of labelled DNA is seen near the centre of nucleolus, grains may still be seen to be dispersed over the entire structure of nucleolus. In all the four species examined, instances were seen when a thread-like connection extended from the nucleolus to the chromocentre; in several cases this connective was seen to be labelled.

REPLICATION OF INTRANUCLEOLAR DNA IN LATE THIRD INSTAR SALIVARY GLANDS

In our laboratory, the replication patterns of intranucleolar DNA in salivary gland polytene nuclei of late third instar larvae of D.pseudoobscura and D.kikkawai have been studied[10,11]. Excised salivary glands from late third instar larvae of these two species were pulse labelled with ^{3}H-thymidine and the squash preparations of labelled salivary glands were autoradiographed. The object of the study was to find out the relationship between replication of intranucleolar and chromosomal DNA.

It has been seen that in D.pseudoobscura and D.kikkawai salivary gland nuclei, the labelling of nucleolus after a short pulse of ^{3}H-thymidine is usually dispersed over the nucleolus. But the intensity of the

labelling is variable in relation to the labelling patterns on chromosomes. As is well known, within the chromosomal DNA, well defined patterns of labelling on euchromatin and heterochromatin are discernible and these patterns are believed to be characteristic of different phases of the S-period of these nuclei[12]. However, when we compared the labelling over the nucleolus with different patterns of chromosomal labelling, no clearcut correlation between specific patterns of chromosomal labelling and the intensity of nucleolar labelling could be established. It was seen that in many nuclei with heavy labelling of chromosomes (continuous or discontinuous types), the nucleolus may have a heavy (more than 25-30 grains over nucleolar area), medium (15-25 grains) or a low (5-15 grains) labelling; even completely unlabelled nucleoli were also seen with this type of chromosomal labelling. Likewise, in nuclei with a low labelling of chromosomes, the nucleolar DNA could be labelled heavily, medium or low. Significantly, in many nuclei it was seen that the nucleolus shows low to medium incorporation of ^{3}H-thymidine, while the chromosomes are completely unlabelled. Some examples of the diverse types of nucleolar labelling seen in D.pseudoobscura are presented in fig. 2. It is clear from these examples that there is no correlation between the labelling patterns of nucleolus and chromosomes of a nucleus. It may be surmised from these data on the replication of intranucleolar DNA in late third instar salivary gland polytene cells of D.pseudoobscura and D.kikkawai, that the replication cycles ofthe intranucleolar and chromosomal DNA are not synchronised in these cells; they are independent.

It is to be noted, however, that Rodman[8] had studied replication of intranucleolar DNA in salivary glands of late third instar larvae of D.melanogaster and had concluded that "in general, label over the nucleolus parallels density of label over its related chromosome set". The reasons for the discrepancy between our data and those of Rodman[8] are not clear, but we are now reexamining the replication of intranucleolar DNA in D.melanogaster salivary gland polytene nuclei. In this context, it is significant to note that in polytene nuclei of a Chironomid, Smittia, Jacob and Daniellì[13] have demonstrated an

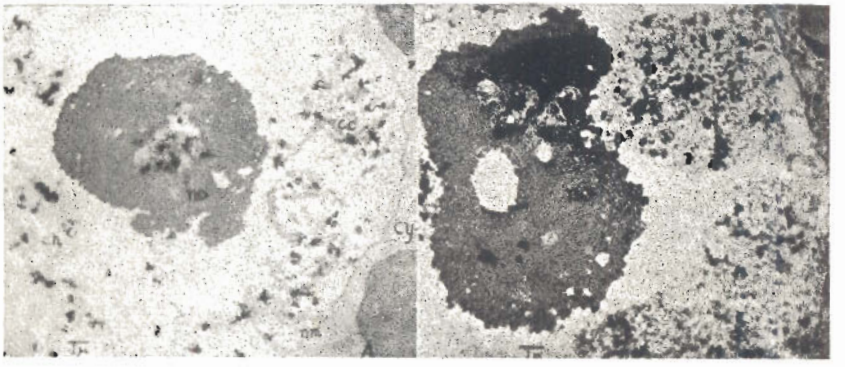

Fig. 1. EM autoradiographs of salivary gland polytene nuclei of *D. melanogaster* after feeding of larvae with ^{3}H—thymidine. In 1 A, the nucleolus shows a central mass of fibrils which is heavily labelled; the remaining nucleolar areas in this section are nearly free of any label. Fig. 1 B shows a nucleolus where the labelling is dispersed over a wider area of nucleolus. cc = chromocentre (beta heterochromatin); ch = chromosome regions; cy = cytoplasm; nm = nuclear envelope; nu = nucleolus.

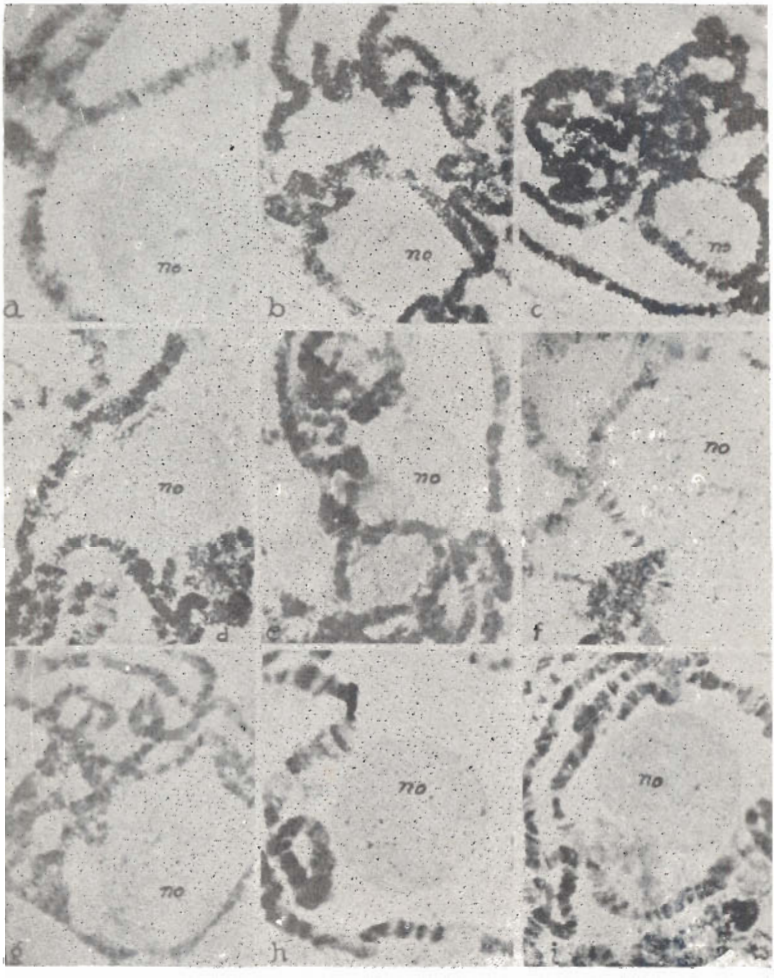

Fig. 2. LM autoradiographs of squash preparations of salivary glands of *D. pseudoobscura* after a 20 min *in vitro* pulse labelling with ^{3}H–thymidine. 2a shows interband type of chromosomal labelling with nucleolus unlabelled; in 2b, c and d, chromosomes show medium to heavy continuous type of labelling, the nucleolar labelling in 2b is low, medium in 2d and heavy in 2c; 2e shows chromosomes with heavy discontinuous labelling but completely unlabelled nucleolus; in 2f and g chromosomes have low discontinuous labelling but in f, the nucleolus is heavily labelled while in 2g, it is low; 2h and 2i are two instances where the chromosomes are completely unlabelled and yet, the nucleolus shows distinct labelling, low in 2h and medium in 2i

independent replication of nucleolar and chromosomal DNA in much the same way as our present results show in D.pseudoobscura and D.kikkawai. In D.melanogaster polytene nuclei too, Spear and Gall[14] have biochemically demonstrated an independent control of rDNA replication; it now needs to be confirmed autoradiographically. It seems likely that the replication patterns of intranucleolar DNA in D.melanogaster would not be much different from that in other species.

The apparently independent replication of intranucleolar DNA becomes significant in view of the demonstration of under-replication of rDNA in polytene nuclei of D.hydei[15]; D.melanogaster[14] and in some species of Rhynchosciara[16]. Under-replication of rDNA in polytene nuclei of D.pseudoobscura and D.kikkawai has not yet been demonstrated, but it may safely be assumed that this occurs in these species as well since in all the species examined so far, polytene cells have shown an under-replication of these sequences of DNA[14-16]. Obviously, if rDNA is not replicating as many times as the euchromatic DNA in polytene cells, the replication cycles of the two are expected to be independently controlled. There are several possible ways in which the under-replication of rDNA in polytene cells may be brought about. Gambarini and Lara[16] have suggested that this may occur either through a slow replication of rDNA cistrons compared to the euchromatic DNA or there may be a differential replication of the different types of rRNA cistrons within the cluster of repetitive ribosomal cistrons. Gambarini and Lara[16] have considered a differential replication to be more likely. However, further studies are needed to elucidate this point.

PHYSICAL RELATIONSHIP OF INTRANUCLEOLAR DNA WITH CHROMOSOMAL DNA

In past, several workers have attempted to identify the locus of nucleolar organizer in the polytene X-chromosome of D.melanogaster[5,17]. Although the exact site has not yet been ascertained, consensus has been that in the salivary gland nuclei of D.melanogaster, the band 20B or 20C of Bridges' map[18] of the X-chromosome, corresponds to the nucleolar organizer region of the mitotic X- and Y-chromosomes[19]. It has been claimed that a Feulgen-positive connective extends from

the band 20B or 20C on the polytene X-chromosome to the nucleolus and this has been taken to be indication of this band being the NO (nucleolar organizer) region. However, this presumed location of the NO region on section 20 of the polytene X-chromosome of D.melanogaster is questionable in light of our present understanding of homologies of different segments of the X-chromosome in mitotic and polytene cells of D.melanogaster. The suggested homologies of the X-chromosome in mitotic and polytene nuclei is presented in fig.3. On this model, the section 20 of Bridges' map, which hitherto has often been suggested to be representing the centric heterochromatin of mitotic X-chromosome[5], actually represents the euchromatin immediately next to the heterochromatin of the mitotic X-chromosome. This point has also been recently emphasised by Gall[20] and Lefevre[21] and this interpretation finds support from our[3] earlier EM autoradiographic observations on the replicative organization of the chromocentre heterochromatin in polytene nuclei of D.melanogaster. However, at the moment, the possibility that the section 20 of Bridges' map corresponds partly to the beta-heterochromatin, can not be ruled out.

If the most basal banded region (section 20) of D.melanogaster polytene X-chromosome corresponds to the euchromatic region of mitotic X or even to the beta heterochromatin, it becomes obvious that the NO region can not be located in this segment. This, in fact, may explain the failure of even most extensive cytogenetic analysis[5] to unequivocally locate the NO region on one of the bands of section 20 of polytene X-chromosome of D.melanogaster. In this context, the results of in situ hybridisation studies by Pardu et.al.[9] to locate the site of rRNA cistrons in Drosophila polytene nuclei, are significant. It was noted that none of the chromosome bands hybridised any rRNA; rather all detectable hybridisation occurred with the intranucleolar DNA. This would imply, as Pardue et.al.[9] also conclude, that in late third instar larval salivary glands, all rRNA cistrons are exclusively located within the nucleolus. Accordingly, in the present model (fig.3), the replicating NO region in polytene nuclei is shown to be in the nucleolus. In view of the suggested linear

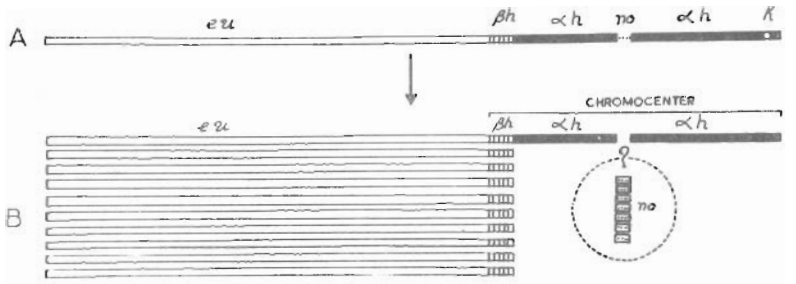

Fig. 3. Diagrammatic representation of the presumed linear homologies of the different regions of the X-chromosome in mitotic (A) and polytene (B) nuclei of D.melanogaster. Mitotic metaphase X-chromosome shows nearly terminal centromere (k) and almost 1/3 of proximal segment is heterochromatic; bulk of this is presumed to be alpha-heterochromatin (∝h) and a small segment at the junction of X-heterochromatin and euchromatin to be beta heterochromatin ($^{\beta}$h). Nucleolar organizer region (no) is located midway between the alpha heterochromatin. During polytenization in larval salivary glands (B), the euchromatin and beta-heterochromatin replicate upto 10 times, the alpha-heterochromatin does not replicate at all. The NO region (rDNA) replicates only 6-7 times[14,15] within the nucleolus. The section 20 of Bridges' map of polytene X-chromosome is believed to represent the basal part of euchromatin of mitotic X, or possibly the beta heterochromatin and, therefore, can not be the site of NO region in polytene nuclei as suggested by earlier workers[5,17]. However, it not known, how the rDNA located within the nucleolus maintains its continuity, if at all, with the unreplicating alpha-heterochromatin.

homologies of different segments of mitotic and polytene X-chromosome in D.melanogaster, the DNA connective thread from the nucleolus would be expected to extend to the chromocentre region (alpha-heterochromatin). In fact, we have seen in favourable preparations of D.melanogaster polytene chromosomesthat thread-like connective extends in some nuclei from within the chromocentre heterochromatin to the nucleolus. In D.pseudoobscura, D.kikkawai and D.ananassae also a ^{3}H-thymidine labelled thread-like connective has been seen between nucleolus and the chromocentre heterochromatin.

Now it remains to be seen as to how the rDNA located within the nucleolus[9] and replicating a few times, maintains its continuity with the DNA in the alpha-heterochromatin which does not replicate at all[3]. Kavenoff and Zim[22] have provided convincing evidence that in each mitotic chromosome of Drosophila, one continuous DNA molecule extends from one end to the other. Does this imply that a linear continuity of DNA molecules is maintained in polytene chromosomes as well? In view of the already described non-replication of the alpha-heterochromatin[2,3] in polytene nuclei, continuous DNA molecules extending from oneto the other end of the polytene chromosome are not feasible. In addition, rDNA also replicates a few times less than the euchromatin[14,15] and in D.melanogaster, the rDNA is separated from the replicating beta heterochromatin by a non-replicating alpha-heterochromatin segment (fig.3). Laird[23] has suggested a very interesting model of the polytene chromosomes in which multi-replication-fork sites are assumed to be present at the junction of replicating and under-replicating or non-replicating DNA regions; in this manner a kind of continuity of DNA molecules in polytene chromosomes is also presumed to be maintained. Alternatively, it is also possible that there are actual discontinuities in the polynucleotide chains at such junctions. But since DNA connections are often seen to extend from within the nucleolus to the chromocentre, it is likely that some kind of connection between under-replicating rDNA and non-replicating alpha-heterochromatin is maintained. It should be pointed out that in the model presented in fig.3, a distinction has not been made between a slow replication of rRNA cistrons and a differential replication of rRNA

copies as suggested by Gambarini and Lara[16]. Further studies, utilizing various modern techniques are expected to throw light on the control of the these interesting examples of differential replication of different segments of the same chromosome. Finally, it may be said that polytene chromosomes still offer exciting possibilities to understand the structural and functional aspects of eukaryotic chromosome.

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