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A comparative study of mitotic and polytene chromosomes of Prochilodus 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 clusters (RNA) in polytene nuclei is related to the unique behavior of the heterochromatin since it is known that in *D. melanogaster* the RNA is located at the heterochromatin of X and Y chromosomes [5,6] and that these blocks of heterochromatin do not replicate in polytene nuclei [7]. In the polytene nuclei of *Prochilodus*, a considerable amount of intranuclear RNA is known to be present [7,8] and this RNA has been demonstrated to be complementary to the ribosomal RNA [9].

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

**Morphology of intranuclear RNA**

We have studied the morphology of intranuclear RNA by autoradiography after continuous labeling with 3H-thymidine throughout the polytenic growth of larvae' salivary glands. For this purpose, recently hatched 1st instar larvae were transferred to *Prochilodus* food supplemented with 3H-thymidine (50μCi/g of food) and the larvae grew in this...
food at 21°C till into third instar stages. Salivary gland chromosomes preparations from late third instar larvae were used and the radio-
activity in polytene nuclei was localized by autoradiography. In this
manner, the distribution of DNA in B. neglecta, B. aequovaniformis, B. blake-
rudi and B. pennsylvanica has been examined. In addition, in case of
B. neglecta, no autoradiographic studies have also been done to
localize DNA in the polytene nuclei after feeding the larvae with
3H-thymidine as above. The results of these studies have, in general,
confirmed earlier morphological observations of Dobzansky (6) that even
in a single species, the intranucleolar DNA may exist as a single
densed mass or a diffuse mass over the entire nucleus or in both forms in the same nucleus. Two examples of BN autoradiographs of
nucleoli of B. neglecta are shown in fig. 1. Similar results have
been obtained with the BN autoradiography in all other
species examined. Judging from the patterns of silver grains in the
autoradiography, it may be said that in all the species studied,
the DNA is distributed throughout the body of the nucleus. In some
nuclei, when a detailed 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 chromosome; in several cases this connection was
seen to be labelled.

EXPLANATION OF INTRANUCLEOLAR DNA IN LATE THIRD INSTAR SALIVARY GLANDS

In our laboratory, the localization patterns of intranucleolar DNA
in salivary gland polytene nuclei of late third instar larvae of
B. neglecta and B. blake-rudy have been studied (10, 11). Recent
salivary glands from late third instar larvae of these two species
were pulse labelled with 3H-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 inter-
nucleolar and chromosomal DNA.

It has been seen that in B. neglecta and B. blake-rudy salivary gland
nuclei, the labelling of nucleolus after a short pulse of 3H-thymidine
is usually dispersed over the nucleolus. But the intensity of the
labelling is variable in relation to the labelling patterns on chromocenes. As is well known, within the chromocenial DNA, well defined patterns of labelling on euchromatin and heterochromatin are discernible and these patterns are believed to be characteristic of different stages of the S-period of these nuclei (12). However, when we compared the labelling over the nucleus with different patterns of chromocenial labelling, no clearcut correlation between specific patterns of chromocenial labelling and the intensity of molecular labelling could be established. It was seen that in many nuclei with heavy labelling of chromocenes (continuous or discontinuous type), the chromosomes may have a heavy (more than 25-30 grains over molecular area), medium (15-25 grains) or a low (5-15 grains) labelling; even completely unlabelled molecules were also seen with this type of chromocenial labelling. In nuclei in nuclei with a low labelling of chromocenes, the molecular DNA could be labelled heavily, medium or low. Significantly, in many nuclei it was seen that the chromosomes were low to medium incorporation of 3H-thymidine, while the chromosomes are completely unlabelled. Some examples of the diverse types of molecular labelling seen in D.pseudosubterraneum are presented in fig. 2. It is clear from these examples that there is no correlation between the labelling patterns of nuclei and chromosomes of a molecule. It may be surmised from these data on the replication of intranuclear DNA in late third instar salivary gland polytene cells of D.pseudosubterraneum and D.ribauti, that the replication cycle of the intranuclear and chromocenial DNA are not synchronized in these cells; they are independent.

It is to be noted, however, that Redman (6) had studied replication of intranuclear DNA in salivary glands of late third instar larvae of D.pseudosubterraneum and had concluded that "in general, label over the nuclei is parallel density of label over the related chromosomes? The reasons for the discrepancy between our data and those of Redman (6) are not clear, but we are now reassessing the replication of intranuclear DNA in D.pseudosubterraneum salivary gland polytene nuclei. In this context, it is significant to note that in polytene nuclei of a chromosomal, Rettig, Jacob and Danilessi (13) have demonstrated an
Fig. 1 EM autoradiographs of salivary gland polytene nuclei of D. melanogaster after feeding of larvae with $^{3H}$-thymidine. In $\textbf{A}$, the nucleolus shows a central mass of debris which is densely labeled; the remaining zonular areas in this section are nearly free of any label. $\textbf{B}$ shows a nucleolus where the labeling is dispersed over a wider area of nucleolus. $\textbf{C}$ is a chromosome (Post heterochromatin); $\textbf{CH} = \text{chromosomes regions}; \text{CH} = \text{cytoplasm}; \text{nuc} = \text{nuclear envelope}; \text{nu} = \text{nucleolus}$.
Fig. 2. LM autoradiographs of scratch preparation of salivary gland of D. pseudobscura after a 20 min in vivo pulse labelling with $^{3}H$-thymidine. 1a shows interband type of chromosomal labelling with nucleoli unlabelled; in 1b, c and d, chromosomes show medium to heavy continuous type of labelling; the nucleolar labelling in 1b is low, medium in 1d and heavy in 1c. 1e shows chromatids with heavy discontinuous labelling but completely unlabelled nucleoli; in 1f and g chromosomes have low discontinuous labelling but in f, the nucleoli is heavily labelled while in 1g, it is low. 1h and 1i are two instances where the chromatids are completely unlabelled and yet, the nucleoli shows distinct labelling. In 1b, the medium is 1b.
in-vivo examination of molecular and chromosomal RNA in such the same way as our present results now in P. aerugonosa and P. fluorescens. In P. aerugonosa, polyribosomes exist too, heavier and bolder, have biochemically demonstrated an independent control of RNA replication; it now needs to be confirmed autoradiographically. It seems likely that the replication pattern of intramolecular RNA in 8aerugonosa would not be much different from that in other species. The apparently independent replication of intramolecular RNA becomes significant in view of the demonstration of independent replication of RNA in polyribosomes of 8aerugonosa (1,29,15) and in species of P. fluorescens (22). Under-examination of RNA in polyribosome models of P. aerugonosa and P. fluorescens 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 polyribosome cells have shown an under-replication of these sequence of RNA (14-16).

Obviously, if RNA is not replicating as may time as the chromosomal RNA in polyribosome cells, the replication cycles of the two are expected to be independently regulated. There are several possible ways in which the under-replication of RNA in polyribosome cells may be brought about. Gautheret and Larr (14) have suggested that this may occur either through a slow replication of viral electrons compared to the chromosomal RNA or there may be a different replication of the different types of viral electrons within the cycle of replicative ribosomal electron, chromosomal and viral (22) have considered a differential replication to be more likely. However, further studies are needed to elucidate this point.

PHYSICAL RELATIONSHIP OF INTRAMOLECULAR RNA WITH CHROMOSOMAL RNA

In 1954, several workers have attempted to identify the locus of molecular organization in the polyribosome of P. aerugonosa (12,17).

Although the exact site has not yet been determined, consensus has been that in the salivary gland model of P. aerugonosa, the band 250 or 230 of Brand's map (18) of the E. coli chromosome, correspond to the molecular organizer region of the polyribosome RNA and E. coli chromosome (19).

It has been claimed that a polyribosome-connective extends from
the band 208 of 206 on the polytene X-chromosome to the nucleolus and this has been taken to be indicative 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-chromosomes in situ and polytene cells of D. melanogaster. The suggested homologies of the X-chromosomes in situ and polytene nuclei is presented in Fig. 3. On this map, the section 20 of Bridges' map, which kitten has often been suggested to be representing the centric heterochromatin of situ X-chromosome (5), actually represents the subchromatin immediately next to the heterochromatin of the situ X-chromosome. This point has also been recently emphasized by young (8) and Lefevre (9) and this interpretation finds support from the earlier autoradiographic observations on the replicative organization of the chromocenter 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 band region (section 20) of D. melanogaster polytene X-chromosomes corresponds to the subchromatic region of situ 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-chromosomes of D. melanogaster. In this context, the results of in situ hybridization studies by Purdie et al. (9) to locate the site of XRNA cistrons in Drosophila polytene nuclei, are significant. It was noted that none of the chromosomes bands hybridized any RNA; rather all detectable hybridization occurred with the inter- molecular DNA. This would imply, as Purdie et al. (9) also conclude, that in late third instar larval salivary glands, all RNA cistrons are exclusively located within the nucleolus. Accordingly, in the present study (fig. 3), the replicating NO region in polytene nuclei is shown to be in the nucleolus. In view of the suggested linear
Fig. 3. Diagramatic representation of the presumed linear homologies of the different regions of the X-chromosome in mitotic (a) and polytene (b) nuclei of *Drosophila melanogaster*. Mitotic metaphase X-chromosome shows nearly terminal centromere (h) and almost 1/3 of proximal segment is heterochromatin; bulk of this is presumed to be alpha-heterochromatin (c) and a small segment at the junction of X-heterochromatin and euchromatin to be beta heterochromatin (Pa). Posterior organelle region (m) is located midway between the alpha heterochromatin. During polytenization in larval salivary glands (b), the euchromatin and beta-heterochromatin replicate up to 10 times, the alpha-heterochromatin does not replicate at all. The NO region (ZDNA) replicates only 6-7 times*[(14,15)]* within the molecule. The section 50 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 is not known how the ZDNA located within the molecule maintains its continuity, if at all, with the unreplicating alpha-heterochromatin.
localities of different segments of mitotic and polytene X-chromosomes in Drosophila. the DNA connection thread from the molecule would be expected to extend to the chromocentre region (alpha-betachromatin). In fact, we have seen in favourable preparations of Drosophila polytenes, where the DNA connection thread-like structure extends from a molecular local in the chromocentre to the nuclei. In Drosophila, Brachyrhina and Apis mellifera, also the nuclear labelled thread-like connection has been seen between molecules and the chromocentre heterochromatin.

Now it remains to be seen as to how the DNA located within the molecule (9) and replicating a few times, maintains its continuity with the DNA in the alpha-betachromatin which does not replicate at all (1). Keveroff and Mostow (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-betachromatin (23) in polytene nuclei, continuous DNA molecules extending from one end to the other end of the polytene chromosomes are not feasible. In addition, Drosophila also replicates a few times less than the euchromatin (24) and in Drosophila, the DNA is separated from the replicating beta-hetachromatin by a non-replicating alpha-betachromatin segment (fig. 3). Lauter (25) 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 polytene DNA chains at such junctions. But since DNA connections are often seen to extend from within the molecule to the chromocentre, it is likely that some kind of connection between under-replicating DNA and non-replicating alpha-betachromatin is maintained. It should be pointed out that in the model presented in figs. 3, a distinction has not been made between a slow replication of DNA constituents and a differential replication of DNA
Further studies, utilizing various modern techniques are expected to throw light on the control of the three interacting examples of differential replication of different segments of the same chromosome. Finally, it may be said that polyten chromosome still offer exciting possibilities to understand the structural and functional aspects of eukaryotic chromosomes.

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EXPERIMENTAL