Distribution of repetitive DNA sequences in the polytene chromosomes of *Rhynchosciara americana*[1]

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Summary. The distribution of fast, intermediate and slow renaturing fractions of *Rhynchosciara americana* DNA was examined in the polytene salivary gland chromosomes by in situ hybridization. Heterochromatic areas readily hybridized but hybrid formation in the euchromatin depended more on the repetitiveness of the RNA probe.

In a previous publication[4], we have shown that the salivary gland DNA of *Rhynchosciara americana* can be fractionated into fast, intermediate and slow renaturing fractions. Here, we report on the cytological distribution of these 3 fractions in the polytene salivary gland chromosomes of *R. americana* as determined by the technique of in situ molecular hybridization.

Materials and methods. Salivary glands were dissected from 4th instar larvae and fixed in ethanol:acetic acid (3:1) for 30 min. Squash preparations were made in 45% acetic acid using only the anterior regions[5]. Alternatively, squash preparations were prepared using isolated salivary gland nuclei[6] as a source of chromosomes.

DNA was isolated from dissected salivary glands[7] and fractionated into 3 reassociation classes[4]. The fast renaturing fraction contained highly repetitive nucleotide sequences reassociating by a maximum C_0t-value of 0.018. The intermediate fraction was comprised of sequences reassociating between C_0t-values of 0.042–0.18. The slow renaturing fraction consisted of those sequences which reannealed at C_0t-values greater than 1.

Complementary RNAs (cRNAs) were transcribed from total DNA and from the fast, intermediate and slow renaturing fractions[8]. The specific activity of the cRNAs was calculated to be about 7×10^6 cpm/μg. Cytological hybrids were prepared to each of the cRNAs[9]. For hybridization, 30 μl of $2 \times$ SSC containing $6\text{–}7.5 \times 10^3$ cpm of radioactive cRNA were used. Incubations were carried out at 65 °C for 3 h.

Results. In situ molecular hybrids were prepared using cRNAs to total DNA and to the fast, intermediate and slow renaturing fractions. With all of the cRNAs used, the majority of the silver grains was always found over the heterochromatic regions of the chromosomes. Hybridizations with cRNA to the slow renaturing fraction showed relatively few silver grains over the euchromatic arms as

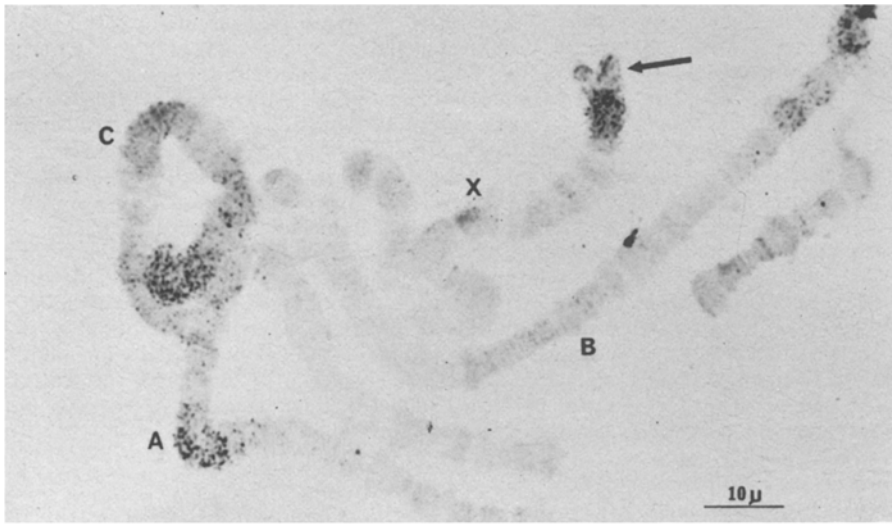

Fig. 1. In situ molecular hybridization of the polytene salivary gland chromosomes (A, B, C and X) of *R. americana* with cRNA to the slow renaturing fraction. Autoradiogram was exposed for 10 weeks. Arrow points to primary nucleolus organizer region.

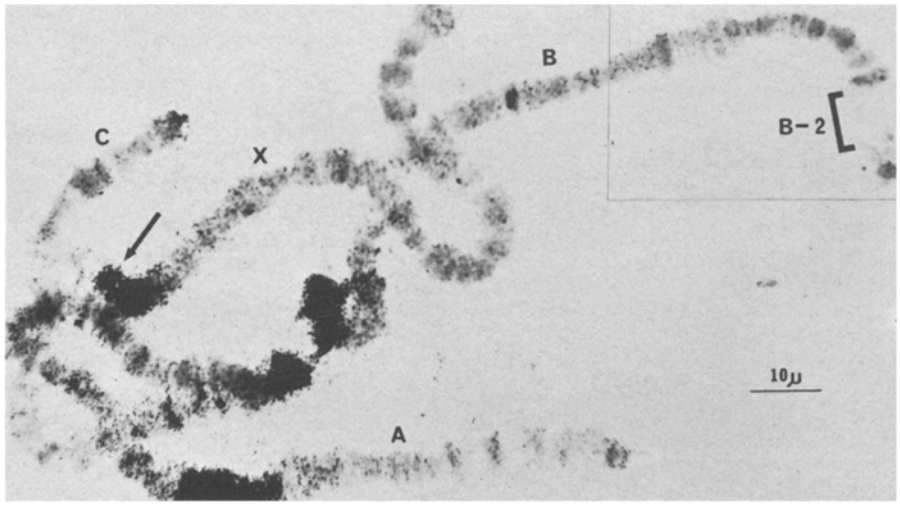

Fig. 2. In situ molecular hybridization of the polytene salivary gland chromosomes (A, B, C and X) of *R. americana* with cRNA to the intermediate renaturing fraction. Autoradiogram was exposed for 5 weeks. Arrow points to primary nucleolus organizer region. Bracket indicates the DNA puff region B-2.

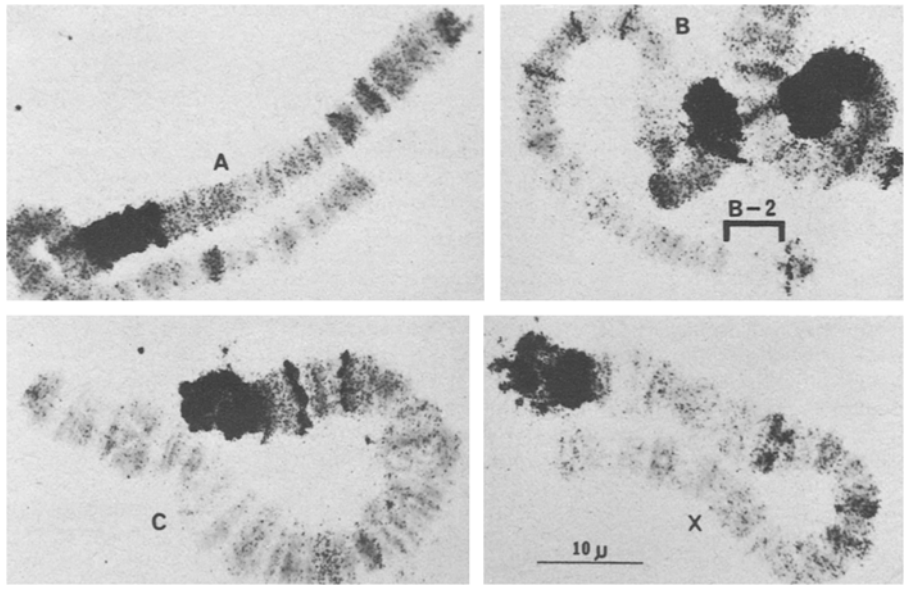

Fig. 3. In situ molecular hybridization of the polytene salivary gland chromosomes (A, B, C and X) of *R. americana* with cRNA to the fast renaturing fraction. Autoradiogram was exposed for 5 weeks. Bracket indicates the DNA puff region B-2.

compared to the labelling of the pericentromeric heterochromatin (figure 1).

Preparations hybridized with cRNA to the intermediate renaturing fraction showed more intense label over both the heterochromatic and euchromatic regions of the chromosomes (figure 2). The intensity of label differed over the pericentromeric heterochromatin of the various chromosomal types and many regions contained distinctly labelled bands. In addition, the primary nucleolus organizer region on the short arm of the X-chromosome (see arrows, figures 1 and 2) showed considerably more label than in the slow fraction-cRNA hybrids. The labelling pattern observed in the fast fraction-cRNA hybrids was very similar to that of the intermediate fraction hybrids (figure 3). Many bands were labelled with both fast and intermediate fraction-cRNA. However, several bands were more intensely labelled with fast fraction-cRNA including a band adjacent to DNA puff region B-2. Complementary RNA transcribed from total DNA hybridized intensely with all chromosomes and yielded a distinctly banded labelling pattern.

Discussion. The hybridization patterns found using the different cRNAs indicate that there are large concentrations of repetitive DNA sequences in the pericentromeric heterochromatin as well as in heterochromatic areas interspersed in the euchromatic areas of the polytene salivary gland chromosomes. These results were expected, in part, since repetitive satellite DNAs have been demonstrated to occur in the heterochromatic regions of a variety of organisms[10]. However, in *R. americana,* as has been shown in *R. hollaenderi*[11], there were differences in the distribution of silver grains over the heterochromatic regions when hybridizations were carried out using cRNAs to different repetitive DNA classes. These differences suggest that heterochromatic areas contain more than one type of repetitive DNA, which belong to several repetitive classes. Furthermore, the differences in intensity of labelling of the pericentromeric heterochromatin of the 4 chromosomes, after hybridization with fast, intermediate or slow renaturing fraction-cRNA, suggest that these regions contain varying amounts of different repetitive DNAs characteristic of the individual chromosomal types. It is difficult, however, to establish more precisely the degree of differential distribution of repetitive sequences because of the possible cross-contamination of the DNA fractions inherent in the fractionation procedure.

Also of interest were the hybridization patterns over the primarily euchromatic chromosomal arms. The observation of distinctly labelled bands after hybridization with both the fast and intermediate fraction-cRNA indicates that numerous bands, scattered over many areas of the chromosomes, contain repetitive DNA sequences of differing degrees of repetitiveness. This finding is in good agreement with the demonstration of bands containing repetitive DNA on the euchromatic arms of the polytene chromosomes of *R. hollaenderi*[11] and *Drosophila*[12–14]. Some of the bands which hybridized very distinctly with fast fraction-cRNA are also known to contain A-T-rich DNA sequences[15].

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