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SHORT COMMUNICATION

LOCATION OF RIBOSOMIC CISTRONS IN THE SALIVARY GLAND CELLS OF *D. mulleri, D. arizonensis* AND THEIR HYBRIDS*

Hermione E. M. de C. Bicudo[1] and Richard H. Richardson[2]

The morphological association of chromosomes with nucleoli was previously studied in the salivary gland cells of *Drosophila mulleri, D. arizonensis* and their hybrids (Bicudo and Richardson, 1977). Intraspecifically the X chromosome is attached to the nucleolus in males and females of both species. However, in the hybrid females a single X chromosome appears associated with the nucleolus, whereas in male hybrids there is amplification of one microchromosome and attachment of this amplified microchromosome to that organoid. Since morphological markers showed that the X chromosome associated with the nucleolus in hybrid females is originated from *D. arizonensis* and there is also indication that the microchromosome amplified in hybrid males is derived from the same species, the observations described led the authors to the following conclusions: (1) both the X chromosome and the microchromosome of *D. arizonensis* carry rDNA cistrons; the X chromosome operates intraspecifically as the main nucleolar organizer and the

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[1] Universidade Estadual Paulista "Julio de Mesquita Filho", Rua Cristovão Colombo, 2265, São José do Rio Preto, SP, Brasil.

[2] Department of Zoology, The University of Texas at Austin, Austin 78712, USA.

microchromosome as a secondary nucleolar organizer. (2) In hybrids between both species there is a dominance of the regulatory system of the nucleolar organizer of *D. arizonensis* over that of *D. mulleri;* since hybrids of both species are obtained only in the direction of crosses involving *D. mulleri* females and *D. arizonensis* males, in male hybrids, in which the X chromosome derived from *D. arizonensis* is absent, the secondary nucleolar organizer (microchromosome) apparently derived from the same species is amplified and activated to operate as the main nucleolar organizer. This amplification seems to be caused by additional polyteny of the entire microchromosome.

Although the association of chromosomes with nucleoli has been interpreted since McClintock (1934) as indicative of nucleolar activity, in the present study we intended to obtain additional support for the previous data on the location of ribosomal cistrons of these flies by using the *in situ* hybridization technique (Gall and Pardue, 1969). The desired confirmation was mainly concerned with the fact that typically *Drosophila* species have nucleolar organizer regions (NORs) confined to the sex chromosomes, and in the present case one autosome (the microchromosome pair) is also involved.

The *in situ* hybridization technique, which employs labelled ribosomal RNA and subsequent autoradiography, has been extensively used for identifying NORs, and is considered the most secure way of doing it. In the present study chromosome preparations of both species and hybrids were made from salivary glands of third instar larvae dissected in Demerec solution, transferred to 3:1 ethanol-acetic acid for 2 minutes, transferred to 50% lactic acid solution for 3 minutes, transferred to 45% acetic acid and squashed immediatelly under a coverslip in the same solution. Gelatinized slides were used and squashing was done as indicate by Atherton and Gall (1972). The slides were frozen in liquid nitrogen for removing the coverslip, placed for 15 minutes in 3:1 ethanol-acetic acid and for 10 minutes, 5 minutes and 12 hours, respectively, in 70%, 95% and 100% ethanol. The slides were then air dried. *In situ* hybridization with I^{125} iodinated 18S and 28S rRNA was essentially performed as described by Wimber *et al.* (1974). *Drosophila melanogaster* rRNA, kindly furnished by Dr. Dale M. Steffensen, was used. The specific activity was about 7×10^6 dpm/μg, and the incubation at 30°C in 50% formamide-2XSSC lasted 22 hours. Slides were developed after 90 days.

The observations are shown in Figure 1 A-F. The silver grains indicative of hybridization were predominantly found in the nucleoli of males and females of both species and hybrids. Similar observations were previously

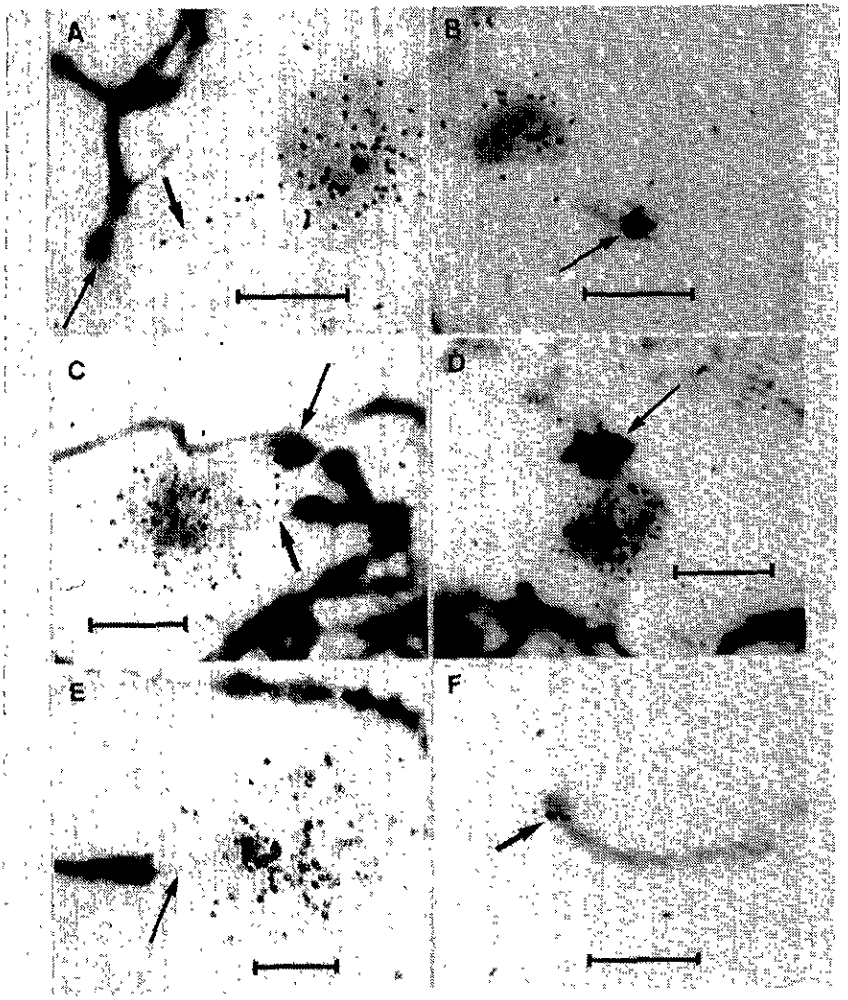

Figure 1 - 1A-F. *In situ* hybridization with ^{125}I rRNA. A. *D. arizonensis* female cell showing silver grains on the nucleolus, the microchromosome (arrow) and on the threads which arise from the microchromosome. B. *D. mulleri* female cell. Grains are present on the nucleolus but are absent from the thread (arrow) which associates the X chromosome with this organoid. C. *D. mulleri* male cell. A very rare observation among the cells examined: grains (arrow) on the X chromosome. D. Hybrid female cell. Grains are present on the nucleolus and on the thread (short arrow) which attaches it to the microchromosome (long arrow). E. Hybrid female cell. Grains are present on the nucleolus and on the thread which associates it with the microchromosome (long arrow) but they are absent from the thread (short arrow) which attaches one X chromosome to the nucleolus. F. Hybrid male cell. Amplified microchromosome (arrow) associated with the nucleolus which exhibits a greater amount of silver grains than that observed in the parental or hybrid female nucleoli.

made in salivary gland cells of *D. hydei* by Pardue *et al.* (1970). These authors used *Xenopus* rRNA for hybridization and grains were exclusively present inside the nucleolus in spite of the well established presence of NORs in the X chromosome (Heitz, 1934 in Pardue *et al.*, 1970). Our present observations differed from those in *D. hydei* in that a small number of grains was also observed on the proximal ends of the microchromosomes and in the threads joining them to the nucleolus, in males and females of both species and hybrids. Since the slides were clean of background and this location of grains was found in several cells, these observations may be considered to support the previous conclusion on the presence of ribosomal cistrons in the microchromosome of these flies.

Grains on the X chromosome were only observed in 3 cells. Another apparently meaningful finding was that grains were clearly more numerous in some nucleoli of male hybrids than in the nucleoli of males and females of the parental species and hybrid females. This observation may indicate that the additional polyteny of one of the microchromosomes in hybrid males also involves amplification of the rDNA cistrons. However, this aspect deserves further studies involving quantification of rDNA cistrons in the X chromosome and microchromosomes of parental species and hybrids.

The finding of rDNA cistrons in the microchromosomes of the species studied has, in addition to the concern with their nucleolar organizing activity, a more general evolutionary implication since it constitutes another example of genetic homology supporting the view of a common phylogenetic origin for the X chromosome and the microchromosome in *Drosophila* (short review in Hochman, 1976).

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