# Position of the nucleolus within the nuclei of pachytene spermatocytes of *Dromiciops australis* and *Marmosa elegans* (Didelphoidea-Marsupialia)

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*Summary.* The location of the nucleoli within the nuclei of pachytene spermatocytes, and their relation with the position of the nucleolar organizer region (NOR) was studied. It appears that a terminal NOR determines a peripheral location of the nucleolus, due to the position of the NOR over the synaptonemal complex and to the attachment of the nucleolar chromosome telomeres at the nuclear membrane.

During the meiotic prophase, mainly at pachytene, the nucleolus or nucleoli are located either peripheral or centrally within the nucleus, depending upon the species considered[2,3]. During this period, a progressive rearrangement of the nucleolar components takes place, resulting in segregated nucleoli such as those found in cells with low ribosomal RNA synthesis[4,5]. Under these conditions, the nucleolus is composed of 3 discrete components: the granular part, the fibrillar part, and the 'fibrillar center' (FC) or light zones[6]. Segregated nucleoli have been observed in plant meiocytes[7-9] and in pachytene spermatocytes[10,11]. It can be observed in pachytene spermatocyte, where segregated nucleoli are present along with synaptonemal complex (SC), that the nucleolar FC are intimately related to the SC[3,9,12]. These observations along with others[6,13] strongly suggest that the FC may constitute the nucleolar organizing region (NOR) of the chromosomes. We have previously demonstrated that the FC of the nucleolus in the spermatocytes of *Marmosa elegans* is associated with a defined SC and occupies the same relative position on the SC as that occupied by the secondary constriction in the short arm of chromosome $C_2$ pair[11]. These observations lead us to suggest that the FC is the NOR. This paper deals with the NOR position in mitotic chromosomes, and the relationships of the FC and nucleolus with the SC and nuclear envelope in spermatocytes of *D. australis* and *M. elegans*.

*Materials and methods.* 5 adult *Marmosa elegans* males and 2 of *Dromiciops australis* were captured in Los Ermitaños de Pichidangui (Province of Coquimbo) and the Valdivian forest (Province of Valdivia), Chile, respectively. Their furs and skulls are deposited in the collection of the Cytogenetics Unit, Department of Cell Biology and Genetics, University of Chile. Pieces of testis 1–2 mm in diameter were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.2, and post-fixed in 1% OsO4 in the same buffer. Embedding was carried out using Durcupan ACM (araldite). Random as well as serial sectioning (section thickness approximately 1000–1200 Å) were done using a Sorvall MT2b ultramicrotome. The sections were stained with uranyl acetate alone or double stained with uranyl acetate and lead citrate. The nuclei of spermatocytes were observed and photographed in a Siemens Elmiskop I or a Philips 300 electron microscope. From serial sections of whole nuclei or parts thereof, 3-D reconstructions were prepared in order to trace the SC and the chromosomal markers arranged along the SC, according to Wettstein and Sotelo methods[14]. Metaphase plates were prepared from bone marrow, and stained using the Ag-AS technique of Goodpasture and Bloom[15].

*Results and discussion.* Our observations confirm that the NOR is located on the secondary constriction of the short arms of chromosome pair $C_2$ in *M. elegans* (figure 1)[11]. It was also found that *D. australis* has 2 chromosome pairs $C_1$ and $C_2$ with NORs in a sub-terminal position on the short arms (figure 2)[3]. The Ag-AS technique identifies the chromosomal zones related to rRNA synthesis, as the stained material appears in the same chromosomal zones that hybridize with 18S and 28S RNA[15,16]. Electron microscopy of pachytene spermatocytes revealed 1 nucleolus in *M. elegans*[11] and 2 in *D. australis* (figure 3), invariably located at the periphery of the nucleus, near the nuclear envelope.

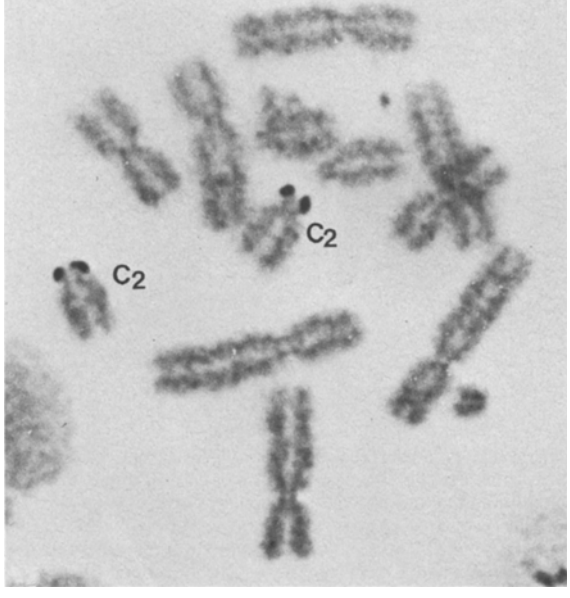



Fig. 1. *M. elegans* bone marrow metaphase plate. $C_2$ chromosome pair with positive Ag-AS reaction indicated.

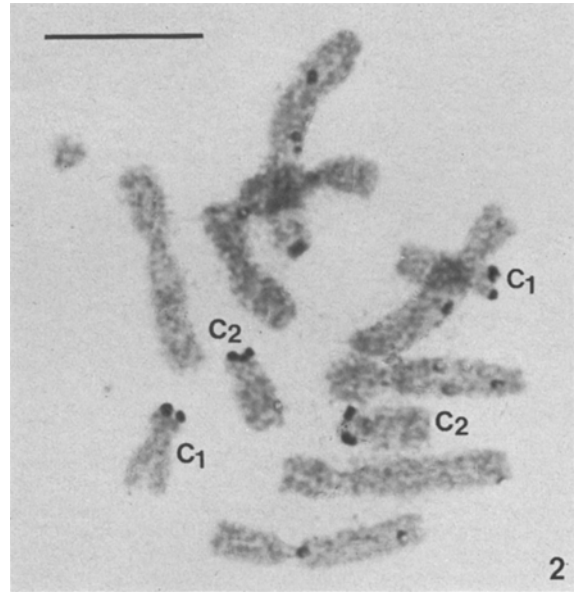
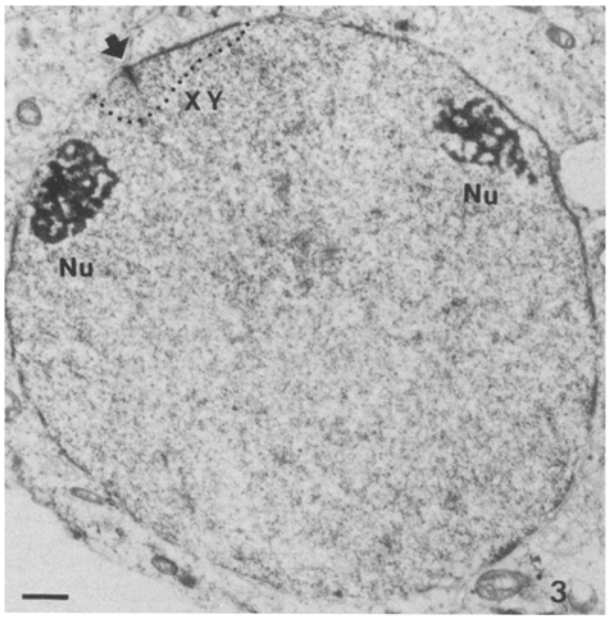
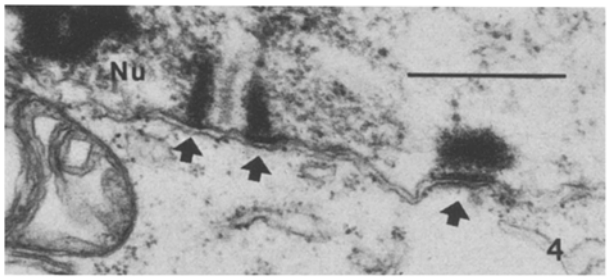
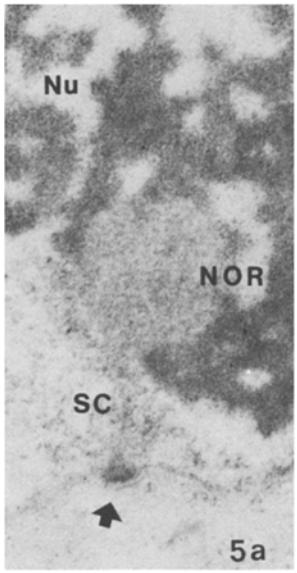
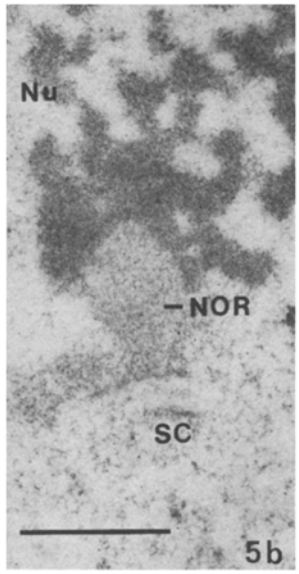


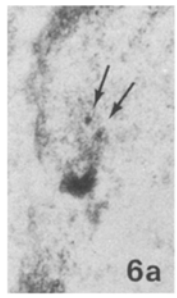

Fig. 2. *D. australis* bone marrow metaphase plate. $C_1$ and $C_2$ chromosome pairs with positive Ag-AS reaction indicated. Bar = 10 µm.

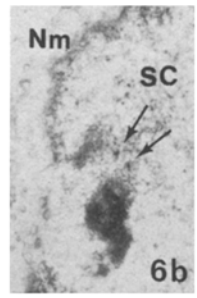


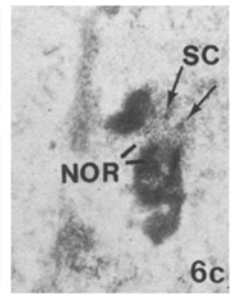



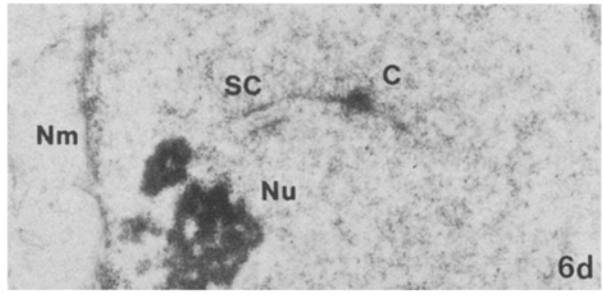



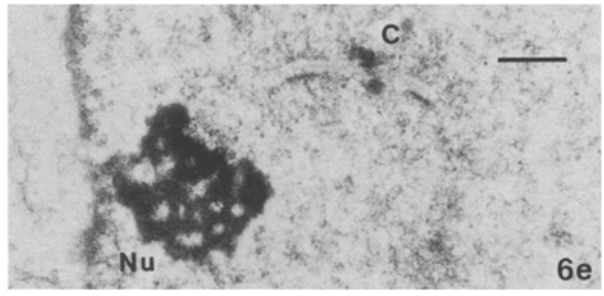



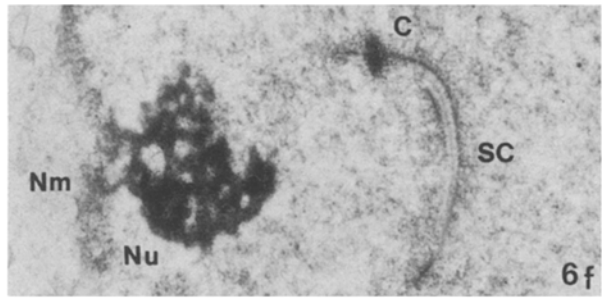



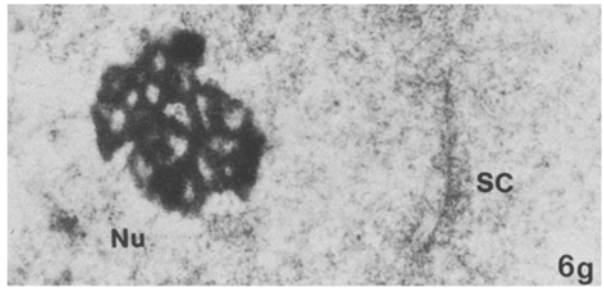




Fig.3–6. *Abbreviations:* C. centromere; Nm, nuclear membrane; NOR, nucleolus organizer region; Nu, nucleolus; SC, synaptonemal complex; XY, sex bivalent. *3 D.australis* spermatocyte with 2 nucleoli. Arrow indicates the attachment of 1 sex chromosomes to the Nm. Bar = 1 µm. *4 D.australis* spermatocyte. The 1st 2 arrows indicate the attachment to the Nm of a nucleolar bivalent; the 3rd arrow indicates the attachment of one of the sex chromosomes to the Nm. Bar = 0.25 µm. *5 M.elegans* spermatocyte. *5a* Arrow indicates the attachment to the Nm of the nucleolar chromosome. The NOR and the nucleolar material are visible. *5b* The NOR is intimately related to the lateral component of the SC and to the nucleolar material. Bar = 0.25 µm. *6 D.australis* spermatocyte: serial sections of one of the nucleolar bivalents. *6a* Arrows indicate the attachment to the Nm. *6b* and *c* The SC transversally sectioned. The NOR can be see around the SC. *6d, e* and *f*. The SC becomes more horizontal. The C is clearly visible. *6f* and *g* Part of the long arms of the nucleolar bivalent. Bar = 0.25 µm.

Hence, a direct relationship exists between NOR number in mitotic chromosomes and the number of nucleoli in the spermatocytes of these species. As in *M. elegans*, 1 *D. australis* nucleolus was found to show a persistent association with the XY bivalent, being either contiguous (figure 3) or very close (figure 4). Serial sections in both species of spermatocyte nuclei[3,11] showed that the nucleoli are segregated and that the FC is intimately associated with lateral elements of the SC and with the nucleolar material (figure 5,b). The SC crosses through the FC and can be followed up to its insertion on the nuclear membrane (figure 5,a). As previously observed in spermatocyte nuclei of *M. elegans*, the SC that traverses the FC and extends from the nuclear membrane to the centromere, corresponds to the short arms of the bivalent formed by the chromosome $C_2$[11]. Using the same markers, 2 SC segments of different lengths have been found in *D. australis*. These segments are equivalent to the short arms of the $C_1$ and $C_2$ pairs in this species[3], as judged by the segment lengths of the SC involved and by the position of the markers (figure 6). It appears therefore that the sequence of telomere-NOR-centromere of the short arms of the mitotic chromosome corresponds to the proposed sequence in this work for meiotic chromosomes: insertion on the nuclear membrane-FC-centromere, along the SC. During pachytene, it is known[12] that paired bivalents are inserted on the nuclear membrane by their telomeric ends (figure 4) and that the SC extends along the whole length of the chromosomal pair[14]. If the NOR position in the mitotic chromosomes of these species is subterminal, as shown by the Ag-AS technique, then a similar position for the NOR is to be expected in meiotic chromosomes. Our observations show that the FC position on the SC is also subterminal. If we consider: a) the relationships between the FC with SC and with the nucleolar material[3,9,11,12], b) the ultrastructural resemblance of the FC to secondary constrictions as revealed by electron microscopy of mitotic chromosomes[17,18], and c) the other morphological data presented in this work, we are lead to conclude that the FC of the spermatocyte nuclei herein discussed, corresponds to the NOR zone. The nucleolar morphology is highly variable and its organization depends upon the stage in the cell cycle[2] as well as on the rRNA synthetic activity that takes place in this nuclear organelle[6,13]. In both species, the spermatocyte nucleolus displays a similar structure and organization. It has an irregularly elongated comma shape, with a thickened tip that includes the NOR, the fibrillar part and a fraction of the granular part; and a caudal end composed mainly by trabecules of the granular part. The nucleolus is associated with the SC by its thickened end, whereas its caudal one penetrates deep into the nucleus, approximately following the pathway of the nucleolar SC. Obviously, in order to ascertain the true position of the nucleolus within the nucleus, the NOR position should be considered. As the position of the nucleolus in spermatocyte nuclei depends upon the position of the NOR[2,3], and as the latter is adjacent to the telomere inserted on the nuclear membrane, it follows that the position of the nucleolus must be peripheral and close to the nuclear envelope in both species. It is probable that the above criteria, and rationale of nucleolus location in spermatocyte nuclei of *M. elegans* and *D. australis*, may also be valid for the same cell types in other animal species and for plant meiocytes.

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## Sexual selection, *Drosophila* age and experience

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*Summary. Drosophila* females modify their choice of mates after an initial mating experience. The altered choices correspond to selective pressures within strains *(D. pseudoobscura)*, semispecies *(D. paulistorum)*, and full species *(D. melanogaster* and *D. simulans)* and indicate a learned component in sexual selection.

*Drosophila* females are known to mate repeatedly both in the laboratory and in the field[1]. We have shown that prior mating experience leads to altered choice of mates in subsequent multiple choice experiments[2]. *Drosophila pseudoobscura* Arrowhead females, for example, show a statistically significant preference for orange-eyed Standard males (autosomal recessive, or; Arrowhead and Standard represent different karyotypes) after an initial mating experience with males of this karyotype. We have further shown that this change in preference resembles learning in that it is subject to disruption by cyclohexamide, a protein synthesis inhibitor, in ways analogous to those reported for mice and