Fine structure of differentiated chromosome segments during the meiosis of pollen mother cells of Allium cepa

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Summary. Differentiated chromosome segments were observed during studies of pollen mother cells of A. cepa during the first meiotic division. Their structure is like those of the nucleolar organizing region (NOR) described in A. cepa microspores[7]. It is suggested that these differentiated chromosome segments correspond to the secondary constrictions seen under the optical microscope.

The primary and secondary constrictions are well-defined regions of the chromosome, constituting constant morphological characteristics. Under the optical microscope, chromosomes usually show a secondary constriction which is known to correspond to the nucleolar organizing region[1]. On the ultrastructural level, these constrictions are seen rather as a differentiation of the chromosome in a well-defined segment. There are only a few studies on the secondary constrictions of mitotic chromosomes[2,3] and practically none on meiotic chromosomes.

In the course of ultrastructural research on Allium cepa anthers fixed in glutaraldehyde-osmium, embedded in epon and contrasted with uranyl-lead as usual, chromosome segments were observed in pollen mother cells during first meiotic division, their structure always showing the same particularities. The morphological analysis of this structure and its possible functional significance are the object of this study.

Results and discussion. In addition to primary constrictions (kinetochores), other types of differentiated segments are quite frequently formed in metaphase chromosomes (figures 1 and 2). In the cavities or cups formed by the chromatin, we find a more or less spherical, heterogeneous structure, 0.8 to 1 μm in diameter, made up of areas of higher electronic density enclosed in an area of lower density. The dense zones are more or less rounded, their diameters ranging from 600 to 800 Å. When greatly magnified, they are seen to be made up of tightly packed filaments of about 100 Å with the same characteristics as the adjacent chromatin (figure 4). The less dense areas show a far less concentrated filaments of about 90–100 Å or possibly less. A close relationship is occasionally seen between the dense zones and the neighbouring chromatin (figure 3). This heterogeneous structure may be equally well found on opposite sides of the bivalent during the first metaphase (figure 3). It is already known that A. cepa has only a pair of nucleolar chromosomes for each

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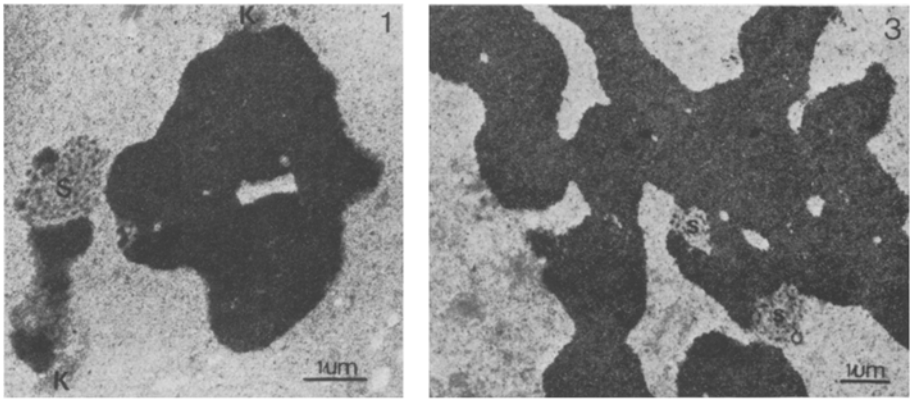

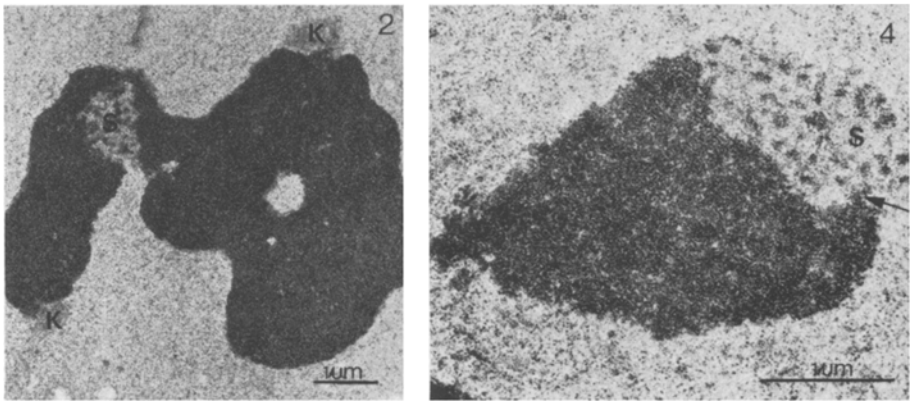

Fig. 1 and 2. 2 serial sections of a metaphase chromosome during the first meiotic division. *K* Kinetochore; *S* heterogeneous structure located in a chromosome differentiation. × 12,000.

Fig. 3. Metaphase I. The heterogeneous structure is located on opposite sides of a chromosome constriction. × 9000.

Fig. 4. The heterogeneous region shows dense zones embedded in a less dense area. The dense areas are seen to be connected to the chromatin (arrow). × 24,000.

diploid set[4–6]. Since in the numerous different sections examined, never has more than one pair of these structures for diploid chromosome complement been observed, it is tempting to speculate that this structure could correspond to the nucleolar organizing region (NOR), which is visualized as a secondary constriction at the optical level. Moreover, the similarity between the characteristics of this structure found during meiotic division and those of the NOR described in A. cepa microspores[7], suggest that we are looking at the same region through the different stages of meiosis.

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Efficient methods for isolation of X-linked male sterile mutations in Drosophila melanogaster[1]

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Summary. Three different mating systems based on the production of virgin females in F_1 and the elimination of undesired males in F_2 are proposed for an efficient isolation of X-linked male sterile mutations in Drosophila melanogaster.

Male sterile mutations in Drosophila melanogaster provide a useful tool to study the control of sperm development at the gene and chromosomal level[3–5]. At least for the X chromosome, the large majority of mutations are known to interfere with spermatogenesis: in 180 from 192 mutant stocks tested, no mature motile sperm was found[6]. Some additional use of X-linked male sterile mutations can be inferred from the findings about the remaining 12 mutant stocks: they showed abnormalities in mating behavior[4], sperm transfer[5], acrosome reaction[5] or only a low number of weakly motile sperm was produced[6].

We are interested in mutations affecting the male accessory gland (paragonial) proteins. They should enable us to clarify the functional significance of these proteins. We assume that some of them are sterile and might belong to the group of mutations which interfere with sperm transfer. From the above data, only a very low frequency of such mutations could be expected. An economic screening system should facilitate our project.

Our system is based on Muller's standard procedure for detecting X-linked recessive lethals and sterile factors[7,8]. We found that it was desirable to eliminate or simplify the following time-consuming steps: first, the collection of virgin females in F_1, and second, the test for fertility with F_2 males bearing a mutagenized X chromosome. Figure 1 presents the mating scheme which has been realized by us. The Killer-prune system was utilized to produce virgin females in F_1: all flies which bear a Killer-prune (K-pn) allele and are homozygous or hemizygous respectively for prune (pn) die as larvae[9,10]. To simplify the test for fertility, we introduced a lethal factor into the Muller-5 (M-5) balancer. As a consequence, only those males which carry a mutagenized X chromosome emerge in the F_2. Thus, the test for fertility can easily be undertaken by transferring the offspring into fresh

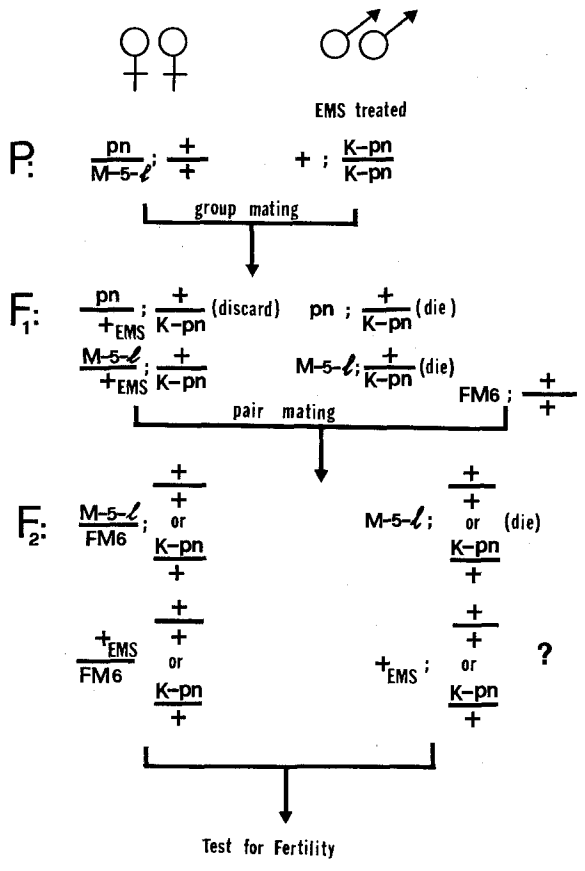

Fig. 1. Mating scheme 1 for isolation of X-linked male sterile mutations in Drosophila melanogaster. The Killer-prune system and Muller-5 balancers were combined in such a way that in F_1 all males died, and in F_2 those not bearing a mutagenized (EMS treated) X chromosome were eliminated. The test for fertility could be carried out simply by transferring all male-producing F_2 cultures into fresh vials and watching for larval growth survival. In case a mutation was found, $+_{EMS}$/FM6 females were crossed to FM6 males for maintaining the mutant stock. M-5-l, Muller-5 balancer (In(1)$sc^{s1}r sc^{8R}$+S, $sc^{s1}sc^{8}w^{a}$B) carrying a lethal. It was produced as follows: prune (pn) females were crossed to mutagenized M-5 males. F_1 females were set up in single cultures with pn males; FM6, First Multiple 6 (In(1)FM6, $y^{31d}sc^{8}$dm B); K-pn, Killer-prune.

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