study may be summed up as follows: After treatment with alkali-urea, three size orders of chromatin fibrils can be recognized in salivary gland chromosomes: (1) 20 Å-thick fibrils, and sometimes thinner ones, which are usually found in well-stretched axial fibrils and sometimes in loop fibrils, though mostly in the background. They supposedly represent bare DNA. (2) 30 Å fibrils, which exist both in the axis and loops. They supposedly represent DNA-histone fibrils. (3) 100 Å-thick fibrils, which exist mostly in unstretched parts of loops, and evidently represent secondarily-coiled DNA-histone fibrils of the second order. The fibrils which are somewhat thinner (about 70 Å or even less) can be observed sometimes in divided segments and in well-stretched regions of 100 Å fibrils. In thin sections of untreated salivary gland chromosomes, both the band regions and the unstretched interbands consists of about 100 Å fibrils (actually 50–150 Å thick). In bands, however, fibril coiling is clearer and fragments of higher orders of coiling can be observed (cf. also Sorsa and Sorsa 1967, 1968 a, b).

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C. B. Gillies and Beal B. Hyde:
Intranucleolar bodies in maize pachytene microsporocytes
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The ultrastructure of the nucleolus in meiosis shows segregation into granular and fibrillar regions as it does in mitosis. Nevertheless, several inclusions peculiar to meiotic nuclei have been reported. Stockert et al. (1970) and Giménez-Martin and Stockert (1970) described several types of vacuolated globules or dark knobs which were usually present in, or associated with the nucleolus of Allium cepa pachytene meiocytes. The compact globuli were reported to be continuous with the lateral elements of the synaptonemal complex. Compound lamellate bodies consisting of periodic layers of dense material which were associated with homogeneous, apparently RNA containing granular bodies, have been described in maize microspores from plants with genetically disturbed nucleolus organizers (Swift and Stevens 1966). Bell (1968) found bundles of fibrils in the nucleolus of primary archegonial cells of Pteridium, and suggested they were related to the nucleolus organizers. Structures resembling the central region of the synaptonemal complex have been found in early prophase I nucleoli of the Ascomycete Neotyella by Westergaard and von Wettstein (1970) and the Myxomycete Echinostelium by Haskins et al.

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(1971), leading the authors to suggest that the nucleolus has a role in synaptonemal complex formation.

In the course of examination of synaptonemal complexes in pachytene microsporocytes of maize, the nucleolar inclusions described below were noted.

Materials and methods

*Maize* (*Zea mays* L.) plants heterozygous for either inversion 3b or inversion 4a, both with and without abnormal chromosome 10, and also plants having a transposition of part of chromosome 3 into chromosome 9 (Tp9N9/Df3N3) were grown in the phytotron at the Royal College of Forestry, Stockholm. The inversion stocks all had closely related genetic backgrounds (KYS) which differed from the transposition stock.

Anthers from main spikes which were at pachytene were fixed for one hour in 4% formaldehyde, followed by two hours in a mixture of 2% formaldehyde and 3% glutaraldehyde, all fixatives in 0.1 M cacodylate buffer + 4% sucrose. Anthers were then either dehydrated, stained for 15 or 19 hours in 1% alcoholic phosphotungstic acid (PTA) at 4°C (SHERIDAN and BARRNETT 1969), embedded and sectioned, or else post-fixed for one hour in 2% OsO₄, dehydrated, embedded and sections stained in the normal manner with uranyl acetate and lead citrate. Sections were examined and photographed with either a Zeiss EM9A or a Siemens Elmiskop IA.

Results and discussion

The pachytene stage is characterised by the presence of synaptonemal complexes (MOSES 1968). In the transposition stock the relatively homogenous pachytene nucleolus often had vacuoles of varying size, and in some of these were found striated bodies (Fig. 1, 2), one per nucleolus. The bodies were composed of parallel rods about 100–200 Å wide (Fig. 2) which often converged at the ends to give a fusiform shape (Fig. 1). In cross section the bodies were seen to be composed of a bundle of regularly spaced rods separated by fine fibres or amorphous material (Fig. 3). The spacing between rods was 450–600 Å. From measurements of random sections and serial sections of two nuclei the bodies were found to be 0.4 to 0.86 μ long, and 0.15 to 0.4 μ wide. In one case a striated body was found in a lacuna between the nucleolus and the nuclear membrane.

Pachytene nucleoli of the inversion stocks tended to be more segregated into granular and fibrillar components than the transposition nucleoli. Both inversion stocks regardless of the presence of abnormal chromosome 10 had a dark, hollow, roughly spherical or ovoid body located in the periphery of the nucleolus (Fig. 4–6). Included in the hollow interior of this dark body was a striated fusiform structure (Fig. 4, 5). The dark body consisted of a fine grained, dense, homogeneous matrix which is more intensely stained with uranium/lead than the fibrillar region of the nucleolus (Fig. 4, 5). With alcoholic PTA, which stains basic protein (SHERIDAN and BARRNETT 1969), the dark body was also more intensely stained (Fig. 6). From random sections of five different nucleoli, and serial sections of seven other nuclei, the dimensions of the dark body were found to range from 1.6 × 1.2 μ to 3.0 × 2.0 μ, with a mean size of 2.3 × 1.8 μ. The hollow interior ranged from 0.7 × 0.4 μ to 1.6 × 1.15 μ, with a mean size of 1.2 × 0.84 μ.

The fusiform striated inclusions of the dark bodies were often found in cross section to contain a hollow centre. The striations resulted from two or three concentric rows of connected rods (Fig. 6). The rods were similar to those described above in the transposition stock, and the spacing was again 500–600 Å. The fusiform striated bodies varied from 0.3 to 0.55 μ in width at their broadest. They always appeared attached to the dark body at one or both ends, and in some cases were appressed to the inside of the dark body along their entire length (Fig. 4, 5). The rods stained as intensely as the dark body with either uranium/lead or PTA.

In none of the pachytene nuclei examined was the dark body associated with chromosomes or the synaptonemal complex, and serial sections showed that the dark body was not associated with the nucleolus organizer (Fig. 4). Thus the dark body described here appears similar to that found in *Allium* pachytene nucleoli by GIMÉNEZ-MARTIN and STOCKERT (1970), but there is no evidence of the association with the synaptonemal complex found in *Allium* (STOCKERT et al. 1970). In addition, the vacuole of the *Allium* nucleolar globulus did not contain any inclusions. The
Fig. 1–6. — Fig. 1–3. Fusiform striated bodies (S) lying in vacuoles (V) in nucleoli (Nu) from transposition plants. — Fig. 1 and 2. Striated bodies in longitudinal section. — Fig. 3. A striated body in cross section. — Fig. 4–6. Hollow dark bodies (D), lying in the periphery of nucleoli (Nu) in inversion stocks, and containing fusiform striated bodies. — Fig. 4 and 5. Striated bodies in longitudinal section. The arrow in Fig. 4 shows the position of the nucleolus organizer in later serial sections. — Fig. 6. A cross sectioned striated body consisting of two concentric rows of rods. — E the nuclear envelope, N nucleoplasm, V nucleolar vacuoles. Fig. 1–5 uranium/lead stain, Fig. 6 alcoholic PTA stain. Fig. 1 and 4 × 10,000. Fig. 2 × 60,000. Fig. 3 and 5 × 29,000. Fig. 6 × 22,000.
nucleolar body we describe may be the same as that found in maize pachytene by Pollister and Ris (1947) using light microscopy. Isolated observations we have made reveal the presence of the hollow dark body at zygotene. Dark bodies which appeared solid in section were also seen in a central nucleolar vacuole, and external to the nucleolus seemingly associated with chromatin, in two diplotene — diakinesis nuclei. It is possible that the dark body is expelled from the nucleolus after pachytene. A similar nucleolar dark body which formed at leptotene and was expelled into the nucleoplasm in early pachytene, was reported in light microsce studies of Lilium megasporocytes by Johansen and Flint (1959).

The fusiform bodies in both the transposition and the inversion stocks have certain similarities with the compound lamellate bodies described in maize microspores by Swift and Stevens (1966), which, however, appeared to consists of layers rather than rods, and had different periodicity. Swift and Stevens suggested that the associated granular bodies which appeared to contain RNA, were not derived from the nucleolus organizer. Rasmussen (1973) has found unordered accumulations of large numbers of rods, with similar dimensions to those composing the fusiform bodies, throughout the primary spermatocyte nucleoli of Drosophila melanogaster, an organism having no crossing over or synaptonemal complex formation in the male meiosis. The periodicity of the rods in maize striated bodies (500–600 Å) is approximately half that of the central region of the synaptonemal complex. In this respect the striated bodies are similar to the composite bodies described by Wettstein and Sotelo (1965) in Acanthopachybus meiotic prophase.

The striated bodies may be involved in the formation of the central region of the synaptonemal complex (Haskins et al. 1971; Stockert, et al. 1970; Westergaard and von Wettstein 1970), particularly if the dark body enclosing them in inversion stocks is in fact expelled from the nucleolus post pachytene. However, the possibility that both structures are special abnormalities associated with the particular genetic backgrounds of the stocks used, cannot at present be ruled out. Further studies of other stocks are necessary to determine if either body is present at pachytene.

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