heads of the natural filament were rather more distinct than has previously been described[6], but there was insufficient order to determine molecular organization. In contrast, tryptic treatment removed the heads to reveal considerable underlying detail. The most common components were the coiled structures (diameter ~ 13 nm) seen in the micrographs of treated natural and synthetic filaments (c–e). Top and bottom surface patterns are superimposed in the negatively-stained filaments. However their general appearance and seeming three-stranded nature is strong evidence for their consisting of 3 fibres (diameter 6–7 nm) wound with what appears as a right-handed screw, helix angle ~80°, helical repeat ~130 nm.

Another structural species observed in both natural (f) and synthetic (g) filament preparations were loose bundles of 8–10 fine strands held together by a high degree of intertwining. We consider these to be loosened forms of the tight coils seen in c–e. The diameter of these fine strands is 2–3 nm.

Although a left-handed coiling is evident amongst them neither this nor the helical repeat was able to be determined with certainty. The coiled structures are not artefacts formed from the digested material since the same forms are produced by digestion of thick filaments already attached to the grids.

The present information, by giving insight into the molecular organization of the backbone, restricts further, possibilities for head arrangements. It seems most probable the backbone is a triple coil, with a helical repeat of 130 nm. This strongly favours SQUIRE's[5] three-stranded 9/1 model over the 2- and 4-stranded arrangements. Further weight is given to this design, since seemingly 9 of the finer filaments make up the structure, presumably combined in 3 sets of 3 coils.

The information on coiling and filament numbers can be used in model building. Considering only the studded regions of the filament, the model shown in Figure h) brings together the present information and the X-ray data. In the model, the rope-like backbone is a right-handed tertiary coil of three secondary ones. Each of these in turn is a left-handed triple coil of 3 primary strands. The beads on the model surface are included to highlight the ordered relationship between strands. They are the likely points of emergence of H-meromyosin tangentially to the secondary filaments, and, depending on the coiling in the fine tertiary strands, closely in line with the model backbone. Alternative coilings are possible. For instance, if the secondary and tertiary strands in the model are wound in the same sense, the H-meromyosins would emerge approximately at right angles to the filament axis.

Without resorting to rigid proof, it is evident from the model that a strict relationship exists between the helical repeat (~130 nm) in the tertiary coil and the repeat in the triple coil of the secondary. In this respect, on winding together the 3 secondary filaments in the model, the 9/1 arrangement is achieved at the point at which resistance to further winding is first felt. This is because, with additional winding in the tertiary coil, the secondary coils are being forced to unwind.

On the basis of the model, the primary strands in the thick filaments are presumed to be formed from myosin molecules overlapping in the L-meromyosin region. Their rather regular width also favours coiling as this would hinder further side by side aggregation expected with strictly parallel alignment[10,11]. Assuming the various filaments to be circular in cross section, the 13 nm diameter of the backbone would reduce, by calculation to a diameter of the myosin tail of ~1.3 nm, rather less than the 2 nm that has been reported[12].

The proposed coiled structure gives equivalence to all the myosin molecules in the studded regions, without the need to propose a central supporting core of another protein[5]. The fact that synthetic filaments from the purified myosin form the same basic structures as the natural filaments, supports the non-existence of a core substance. A comprehensive report on the coiled nature of the thick filaments will appear later.

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[10] R. G. HARRISON, S. LOWEY and C. COHEN, J. molec. Biol. *59*, 531 (1971).

[11] M. YOUNG, M. V. KING, D. S. O'HARA and P. J. MOLBERG, Cold Spring Harbor Symp. quant. Biol. *37*, 65 (1973).

[12] S. LOWEY, H. S. SLAYTER, A. G. WEEDS and H. BAKER, J. molec. Biol. *42*, 1 (1969).

Gene Amplification in Oocytes of the Rove Beetle *Creophilus maxillosus* (Staphylinidae, Coleoptera-Polyphaga)[1]

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Summary. The extra-DNA body was found, for the first time, in oocytes of an insect with telotrophic ovary. Dispersion of this body is accompanied by an enormous increase of nuclear volume and production of multiple nucleoli. It is suggested that the extra-DNA contains a huge mass of nucleolus organizers.

It is known from experimental and descriptive studies that, at a given degree of ploidy, there is a close relationship between the size of an interphase nucleus on the one hand, and decondensation of chromatin and intensity of RNA-synthesis on the other[2,3]. This relationship seems to occur also in the prophase nuclei of growing oocytes. Thus, in insects with polytrophic or telotrophic ovaries, i.e. in the case when the growing ooctye is supplied with RNA produced by nurse cells, the oocyte chromosomes form more or less compact karyosphere, the nucleus is most often relatively small and RNA-synthesis in it is decreased in the same measure as its chromosomes are condensed[4–13].

The behaviour of oocyte nuclei deviating from the rule of restricted RNA-synthesis in the polytrophic ovary is conditioned by the presence of a considerable quantity of extrachromosomal DNA[6,14]. In such cases, inspite of the fact that the oocyte chromosomes form a karyosphere, the volume of the nucleus increases considerably and the nucleus is very active in the process of RNA-synthesis.

It is the extrachromosomal DNA which occurs in the state of an extreme decondensation during the period of oocyte growth that is responsible for RNA-synthesis in the oocyte nucleus at that stage[6,14]. From morphological and cytochemical studies[6,14,15], as well as from RNA-DNA hybridization experiments[16], it is concluded that the extrachromosomal DNA in the oocyte nuclei of insects with polytrophic ovaries contains copies of the nucleolar organizer and hence codes for ribosomal RNA.

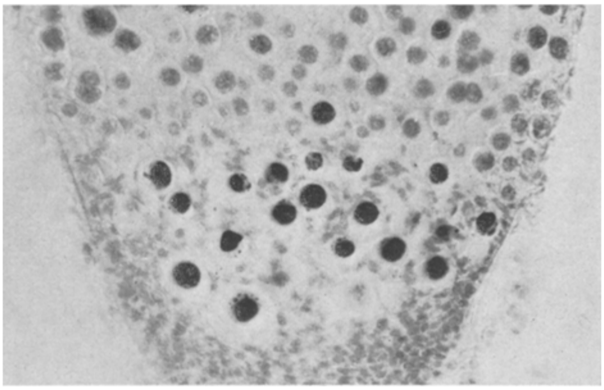
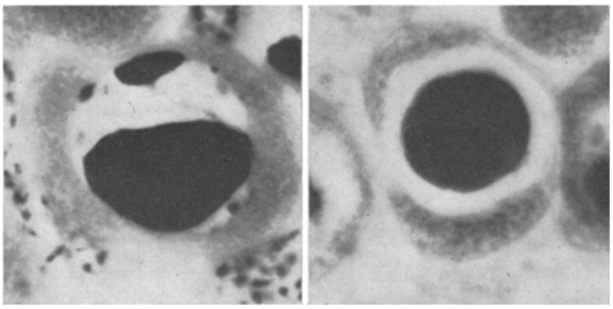


Fig. 1. Longitudinal section through the distal region of the germarium. Each nucleus in young oocytes, which are grouped in this part of ovarioles, contains a compact heterochromatic body of extrachromosomal DNA. Feulgen. × 240.



Figs. 2 and 3. Pachytene oocytes from the distal region of germarium, stained with Heidenhain's hematoxylin (left) and azure B (right). In Figure 2, in the nucleus, in addition to the conspicuous cap and two smaller bodies of extrachromosomal DNA, faintly stained chromosomes are also clearly visible. Figure 2, × 1600; Fig. 3, × 1800.

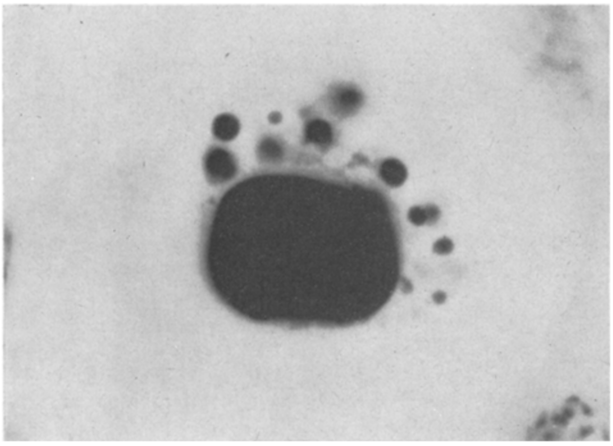


Fig. 4. Early previtellogenic oocyte. The nuclear extrachromosomal DNA begins to fragment, giving off from its surface numerous spherical bodies. Feulgen. × 2000.

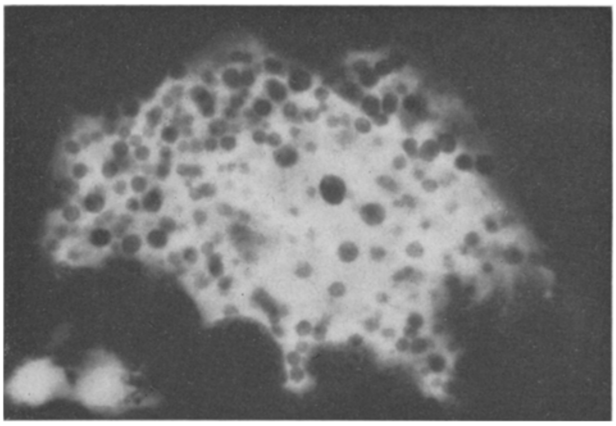


Fig. 5. Section through a growing oocyte, showing enlarged and irregularly shaped germinal vesicle which contains many multiple nucleoli. The extrachromosomal DNA, owing to the high degree of its dispersion, is not detectable with staining reactions at this stage. Methyl green pyronin. × 1300.

It has recently been demonstrated that the synthetic activity of extrachromosomal DNA occurring in whirligig beetles is accompanied by an immense increase in the size of oocyte nuclei. Thus, during the previtellogenetic period of oocyte growth, the nucleus undergoes a particularly strong elongation and attains the length of about 500 μm. In addition, the surface of the oocyte nucleus increases as a result of wrinkling of the nuclear membrane and formation of numerous nuclear processes, which penetrate the adjacent ooplasm at a distance of about 10 μm[17].

In the light of what has been said above, it seems that the size and morphology of the oocyte nucleus can serve as an indication of gene amplification in various insect groups. In particular it would be interesting to check the validity of this supposition with regard to insects with telotrophic ovaries, in which the process of gene amplification has not so far been recorded.

A common representative of rove beetles, *Creophilus maxillosus*, was chosen as the object of our study. In this choice the authors were guided by a short report[18], stating that the oocyte nuclei of an undefined species of Staphylinid beetles are of a particular irregular shape.

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[1] This research was supported in part by funds from the Cytobiology Committee of the Polish Academy of Science.

[2] J. B. Gurdon, Symp. Soc. exp. Biol. *24*, 369 (1970).

[3] H. Harris, *Cell Fusion* (Clarendon Press, Oxford 1970).

[4] K. Bier, J. Cell Biol. *16*, 436 (1963).

[5] K. Bier, Zool. Jb. Physiol. *71*, 371 (1965).

[6] K. Bier, W. Kunz and D. Ribbert, Chromosoma *23*, 214 (1967).

[7] K. Bier, W. Kunz and D. Ribbert, *Chromosomes Today* (Eds. C. D. Darligton and K. R. Lewis, Oliver and Boyd, Edinburgh 1969), vol. 2, p. 107.

[8] P. S. Ramamurty, Naturwissenschaften *50*, 383 (1963).

[9] W. Engels, Insect. Soc. *15*, 271 (1968).

[10] W. Engels, Zool. Anz. Suppl. *30*, 30 (1970).

[11] U. Mays, Z. Zellforsch. *123*, 395 (1972).

[12] J. Büning, Z. Zellforsch. *128*, 241 (1972).

[13] S. L. Ullmann, J. Embryol. exp. Morph. *30*, 179 (1973).

[14] M. N. Gruzova, Z. P. Zaichikova and I. I. Sokolov, Chromosoma *37*, 353 (1972).

[15] K. Kato, Expl Cell Res. *52*, 507 (1968).

[16] J. G. Gall, H. C. MacGregor and M. E. Kidston, Chromosoma *26*, 169 (1969).

[17] B. Matuszewski and P. Hoser, Experientia *31*, 431 (1975).

[18] Ch. Soyer, C. r. heb. Soc. Biol., Paris *63*, 158 (1907).

In connection with the considerations just presented, it was most exciting to observe that, in the female germ cells of *Creophilus*, the microscopical effect of the process of gene amplification is very distinct. It was found that the nuclei of all young oocytes, which in the ovarioles of adult females are grouped in the distal part of the germarium, contained, in addition to the chromosomes, extrachromosomal DNA (Figure 1) in very large amount. In the pachytene nuclei, the extrachromosomal DNA occurs as a more or less spherical, compact, heterochromatic body, about 10 µm in diameter (Figures 2 and 3). Morphological and autoradiographic examination with the use of ^{3}H-thymidine emphasized that the process of gene amplification was concluded in this species before the adult insect emerged or at the earlier stages of germ cell development.

At the beginning of oocyte growth, small nucleoli appear on the surface of the extrachromosomal DNA body and also inside it, in the spaces which look like vacuoles in preparations stained with the Feulgen method. The extrachromosomal DNA body soon begins to fragment (Figure 4), and within a short time it resolves into a network of very fine, Feulgen positive fibrils which are evenly distributed throughout the nucleus. This dispersion of highly condensed extrachromosomal DNA is accompanied by a rapid increase in volume, and drastic changes in the form of the oocyte nucleus. Thus, the nucleus, originally spherical, begins to form numerous ameboid processes which enormously increase its surface. At the later stages of oocyte growth, no doubt as a result of decreasing concentration of the extrachromosomal DNA, it is no longer possible to detect it with the Feulgen method.

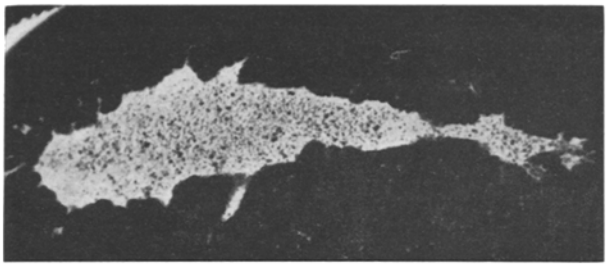
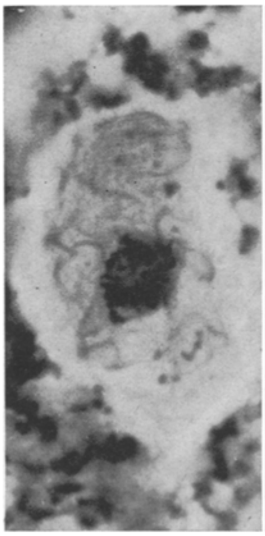


Fig. 6. Greatly elongated oocyte nucleus with numerous lateral processes; the whole nucleus is occupied by a finely granulated nucleolar material. Azure B. × 160.



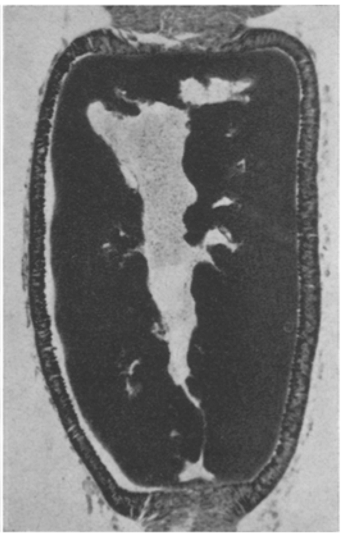


Fig. 7. The karyosphere situated within a particular region of the nucleus fibrillar or lamellar in structure and free of the nucleolar material. Chromosomal nature of the karyosphere is clearly visible. Heidenhain's hematoxylin. × 1500.

Fig. 8. Longitudinal section of late previtellogenic follicle. Note the greatly enlarged oocyte nucleus with many processes and ramifications, some of which almost reach the oocyte surface. Methyl green pyronin. × 125.

The beginning of dispersion process of extrachromosomal DNA coincides with the appearance of many true nucleoli (Figure 5). As this process proceeds, the number of nucleoli increases, while their dimensions decrease (Figure 6). At the further stages of oocyte growth, almost the whole nucleus becomes evenly filled with a basophilic, RNA-containing substance which, on close examination, appears to be composed of very small granular nucleoli, as in the case of oocyte nuclei of Dytiscidae[6], Chrysopidae[14], and Gyrinidae[17].

This unusually great increase of the nuclear volume and the mass production of multiple nucleoli suggests that active RNA synthesis takes place in the oocyte nucleus of *Creophilus* during previtellogenesis. This is the case recently demonstrated by autoradiographic experiments[19] with the use of ^{3}H-uridine. Since, during the whole period of previtellogenesis, the oocyte chromosomes form a more or less compact karyosphere located inside a small area of the nucleus (Figure 7), it appears evident that it is the decondensed extrachromosomal DNA that is active in the RNA synthesis. Moreover, on the basis of cytological data presented in this paper, one can also suggest that at least the bulk of extrachromosomal DNA in oocytes of *Creophilus* contains genes for ribosomal RNA.

At late previtellogenesis, the oocyte nucleus of *Creophilus* attains immense dimensions and a shape not recorded in the oocytes of other insect groups (Figure 8). For instance, in oocyte about 870 µm long, the length of the nucleus is about 700 µm and its width 160 µm in the largest, central part. There is evidence from autoradiographic studies[19] that the extrachromosomal DNA in *Creophilus* is still very active in RNA synthesis during that stage of oocyte growth.

The data presented are preliminary results of current investigations.

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[19] M. KLOC, in preparation.