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SIZE UNIFORMITY OF RIBONUCLEOPROTEIN MATRIX PARTICLES IN LOOPS OF *PLEURODELES WALTLII* LAMPBRUSH CHROMOSOMES VISUALIZED BY ELECTRON MICROSCOPY

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SUMMARY

The different kinds of loops of lampbrush chromosomes were identified in phase contrast, then analysed by electron microscopy on thin sections. Examination at high magnification showed that the basic structure of the ribonucleoprotein (RNP) matrix of all kinds of loops is a 30 nm RNP particle. Furthermore, this study suggests that the morphological differences between the loops are due to the extent of aggregation of these particles.

INTRODUCTION

Lateral loops of lampbrush chromosomes are active in transcription, and the newly synthesized RNA molecules associate with proteins in a ribonucleoprotein (RNP) matrix (Gall, 1954; Callan & Lloyd, 1960; Gall & Callan, 1962). Many of these loops show a characteristic gross morphology related to the shape and density of their RNP matrix: granular, globular or 'dense matrix' loops form obvious landmarks, which permitted recognition and mapping of the lampbrush bivalents of many amphibian species (Callan & Lloyd, 1960; Mancino & Barsacchi, 1965, 1966; Lacroix, 1968).

Much of our knowledge about the structure of the lateral loops comes from light microscopic (LM) observations (Callan & Lloyd, 1960; Callan, 1963; Lacroix, 1968). Relatively few electron microscopic (EM) studies have been performed, and most of them concern the structure of normal loops, which are the most common (Miller & Beatty, 1969; Malcolm & Sommerville, 1974; Mott & Callan, 1975; Angelier & Lacroix, 1975; Scheer, Franke, Trendelenburg & Spring, 1976; Spring & Franke, 1981). A recent scanning electron microscopy (SEM) study by Angelier, Paintrand, Lavaud & Lechaire (1984) has provided the only available information on the structure of some of these typical loops.

We carried out a systematic study of the different kinds of loops occurring on lampbrush chromosomes of the newt *Pleurodeles waltlii*, using EM on thin sections at low and high magnification.

Key words: amphibian, lampbrush chromosomes, landmark loops, electron microscopy, RNP matrix.

MATERIALS AND METHODS

Chromosome preparations

In this study we used young female newts of the species *Pleurodeles waltlii* (Amphibia, Urodela) grown in our laboratory. Ovaries were removed from newts that had been anaesthetized in 0·1 % MS 222 (Sandoz). Germinal vesicles of large oocytes (stages V and VI; Bonnanfant-Jaïs & Mentré, 1983) were isolated by hand in a physiological medium containing 75 mM-KCl, 25 mM-NaCl, buffered to pH 7·2 with 10 mM-Tris·HCl, 0·01 mM-$MgCl_2$ and 0·01 mM-$CaCl_2$ (Gall, 1954), and each was transferred to a centrifugation chamber containing medium. In the chamber, the nuclear envelope was removed with needles and forceps, and the nuclear content was centrifuged (30 min, 1500 ***g***) onto the coverslip sealing the chamber. After centrifugation, the chromosomes were observed in phase contrast, identified, and photomicrographed; this procedure permits further recognition of the chromosome landmarks in EM and a comparison between the pictures obtained by LM and by EM.

EM on thin sections

Coverslips were detached from the slides and chromosome preparations were fixed briefly in a solution of 1 % glutaraldehyde buffered to pH 7·2 with 0·1 M-phosphate buffer, post-fixed in a solution of 1 % OsO_4, and dehydrated by passage through an ethanol series. Preparations were then flat-embedded in Araldite (Spring & Franke, 1981) and the assembly was left to polymerize at 60 °C for 48 h. The coverslips were then split from the Araldite blocks by placing the assembly in liquid nitrogen. The surface of each block was stained in methylene blue to mark the chromosome group, and examined under phase contrast. Using information obtained from previous light-microscopic observations, the chromosomes to be cut were chosen, and the block was trimmed down to a small area including the chromosomes of interest. Thin sections were cut on a Reichert OM-U3 ultramicrotome and picked up on copper grids. The sections were stained in a solution of uranyl acetate in 50 % ethanol, then in lead citrate. They were examined in a Philips 201 EM, operating at 80 kV.

RESULTS

Identification of the various landmarks by EM on thin sections

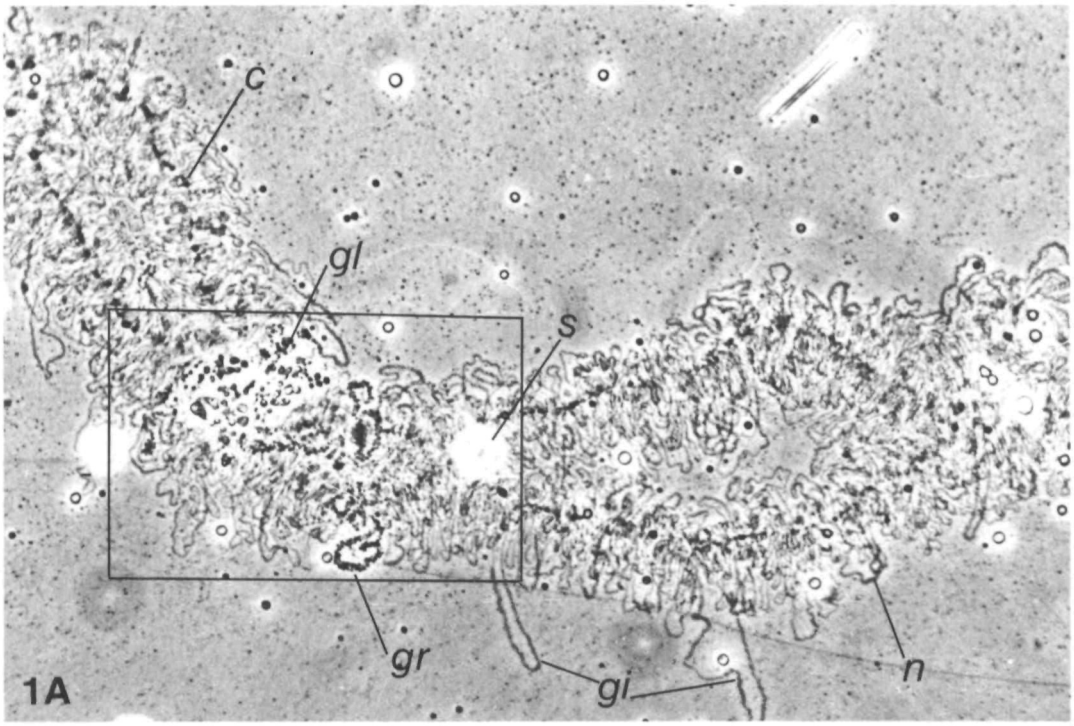
The procedure described above (see Materials and Methods) permitted the observation of the same bivalent by LM and by EM. Fig. 1 presents one of the 12 bivalents from *P. waltlii* oocyte karyotype, observed by LM (Fig. 1A) and by EM (Fig. 1B); this bivalent is characterized by several landmarks: a median sphere, granular and globular loops. Comparison between the pictures obtained by LM and by EM obviously showed that chromosome features were not altered in the course of their preparation for observation by EM: the chromosomes exhibited exactly the same topography in LM and in EM.

Landmark loops were identified on thin sections at low magnification, then in detail at very high magnification.

Normal and giant loops

Normal loops were the most frequent along the chromosome axis; they were 10–50 μm long.

Fig. 1. A,B. Bivalent 11 of *Pleurodeles* oocyte showing lateral loops extending from chromomeres (*c*). This bivalent is characterized by a median sphere (*s*) and by landmark loops: giant (*gi*), granular (*gr*) and globular (*gl*), distinct among the normal loops (*n*). A. Bivalent 11 in phase contrast; ×1000. B. Part of the same bivalent (framed in A) observed by EM on thin section. Globules can fuse to form sleeves (arrows); ×2000.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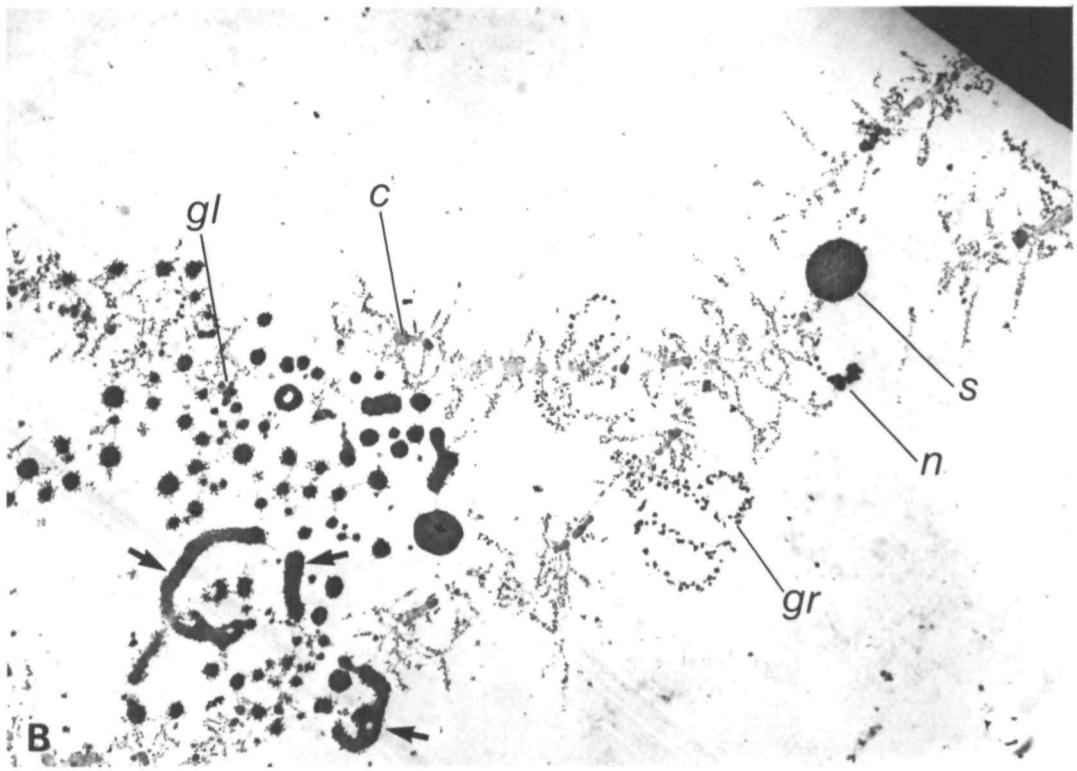
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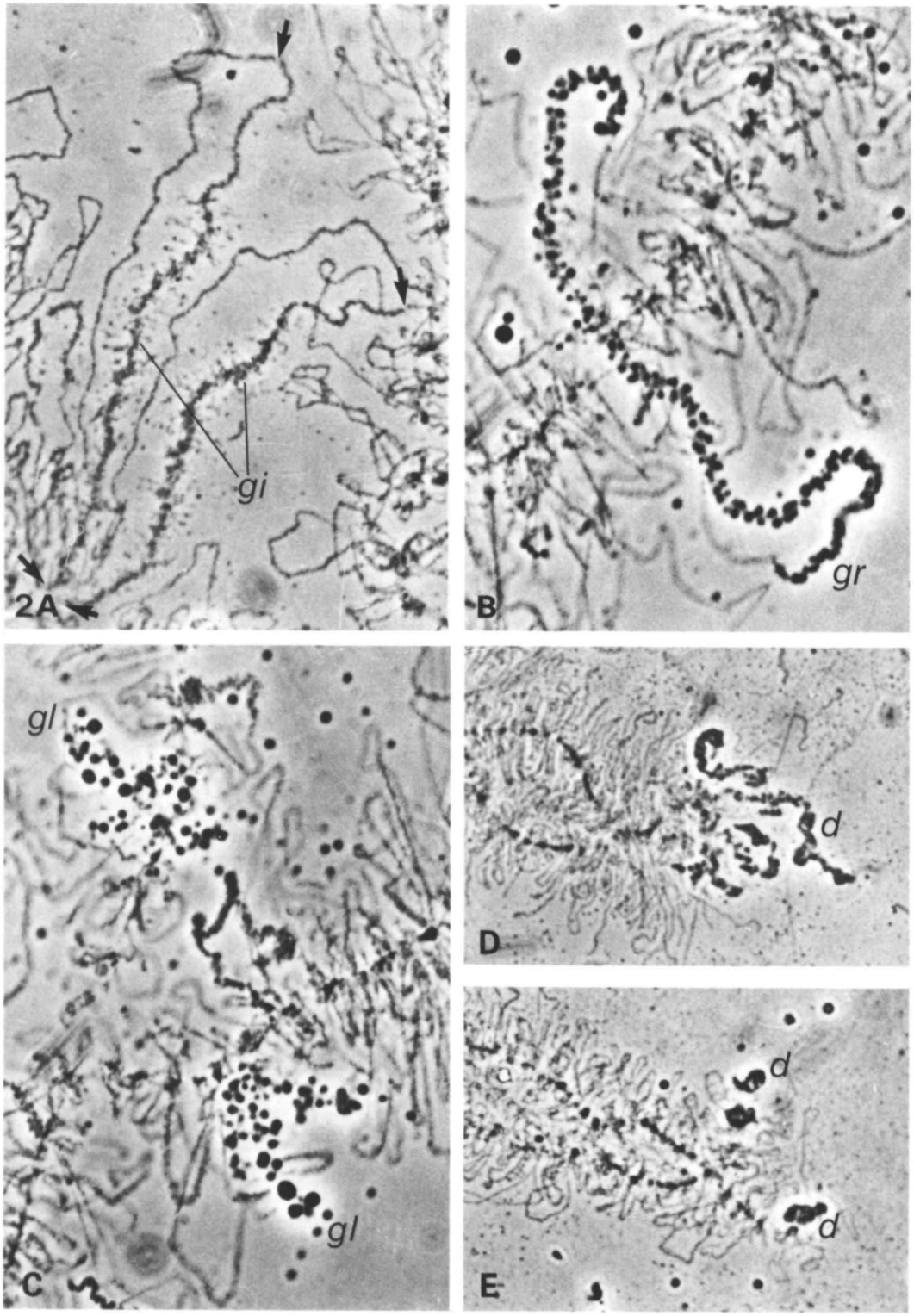
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d
E

Giant loops (Fig. 2A) exhibited the same kind of RNP matrix as normal ones, from which they differed only in their exceptional length, about 100 μm; this matrix usually showed one, and sometimes several transcription units, the polarity of which could be observed in LM (Fig. 2A).

Examination of normal or giant loops in EM at low and high magnification (Figs 3A, 4A) showed that their RNP matrix was composed of fibrils extending radially from the loop axis. These fibrils were associated with RNP particles 20–30 nm in diameter (Fig. 4A).

Granular loops

In phase contrast, the matrix of granular loops was much thicker than that of normal loops and was composed of 1–3 μm granules, presenting a zig-zag arrangement (Fig. 2B). This matrix usually showed an obvious polarity related to a slow or rapid increase in the size of its granules (Fig. 2B).

In EM, the granules still presented a zig-zag arrangement in the matrix of the granular loop (Figs 3B, 4B). At high magnification, (Fig. 4B), each granule appeared to be composed of small bodies 30 nm in diameter, identical in size to those previously observed in the RNP matrix of normal loops. At the periphery of some granules, these 30 nm particles exhibited a linear disposition recalling that observed in the matrix of normal loops (Fig. 4B).

Globular loops

In phase contrast, the RNP matrix of globular loops was composed of very dense globules, 3–4 μm in diameter (Fig. 2C); the polarity of this matrix was not always obvious. In EM, the globules showed a very dense central region, from which RNP fibrils extended forming a less-compact peripheral region (Figs 3C, 5A). At high magnification these fibrils bore the small particles, of 30 nm diameter, already observed in the matrix of normal or granular loops (Fig. 5A); RNP fibrils from adjacent globules generally developed important interconnections (Fig. 5A).

Globules were often fused in a more or less extended sleeve around the loop axis (Fig. 1B).

Dense matrix loops

Called 'D-type-loops' by Lacroix (1968), these loops occurred on two of the twelve bivalents of the *P. waltlii* oocyte karyotype. In phase contrast, the matrix of these loops presented a variable aspect related to the extent of fusion of its elements. Sometimes, this matrix appeared to be composed of discrete granules of 0·5–1 μm

Fig. 2. A–E. Landmark loops observed in phase contrast. A. Pair of giant loops (*gi*) the matrix of which exhibits at least one obvious transcription unit (arrows); ×3300. B. Pair of granular loops (*gr*) showing an evident polarity of their matrix. Note the zig-zag arrangement of the granules; ×3100. C. Homologous stacks of globular loops (*gl*). The individual axis of each loop is difficult to define; ×3000. D,E. Homologous pairs of dense loops (D), the matrix of which can be composed of small granules (D), or reduced to a few lobules (E); ×1500.

(Fig. 2C), but in cases of extreme fusion of these granules, the matrix was reduced to a few dense lobules (Figs 2D, 3C).

In EM, the matrix of these loops showed 30 nm particles, aggregated in dense, irregular-shaped bunches, separated from each other by wide vacuoles (Fig. 5B). The usual linear arrangement of the RNP particles was not recognizable in this matrix.

DISCUSSION

Unique basic structure of the RNP matrix: 30 nm RNP particles

Our observations in EM on thin sections clearly showed that the RNP matrix of all loops, whatever their gross morphology, was composed of RNP particles of constant size, about 30 nm. These 30 nm particles were found in the matrix of the so-called normal loops as well as in the dense aggregates that form the matrix of granular, globular and dense matrix loops.

These results agree with those of Miller (1965), Malcolm & Sommerville (1974), Mott & Callan (1975), Spring & Franke (1981), who described the same particles in the EM. However, none of these authors has shown that they occurred in all types of loops. Our systematic study demonstrates that the basic structure of the RNP matrix of all types of loops is a 30 nm RNP particle.

In the matrices of normal, granular and globular loops, the 30 nm particles were organized in fibrils. However, probably because of the very high degree of compaction of the transcription products, such a linear disposition could not be observed in the dense matrix loops.

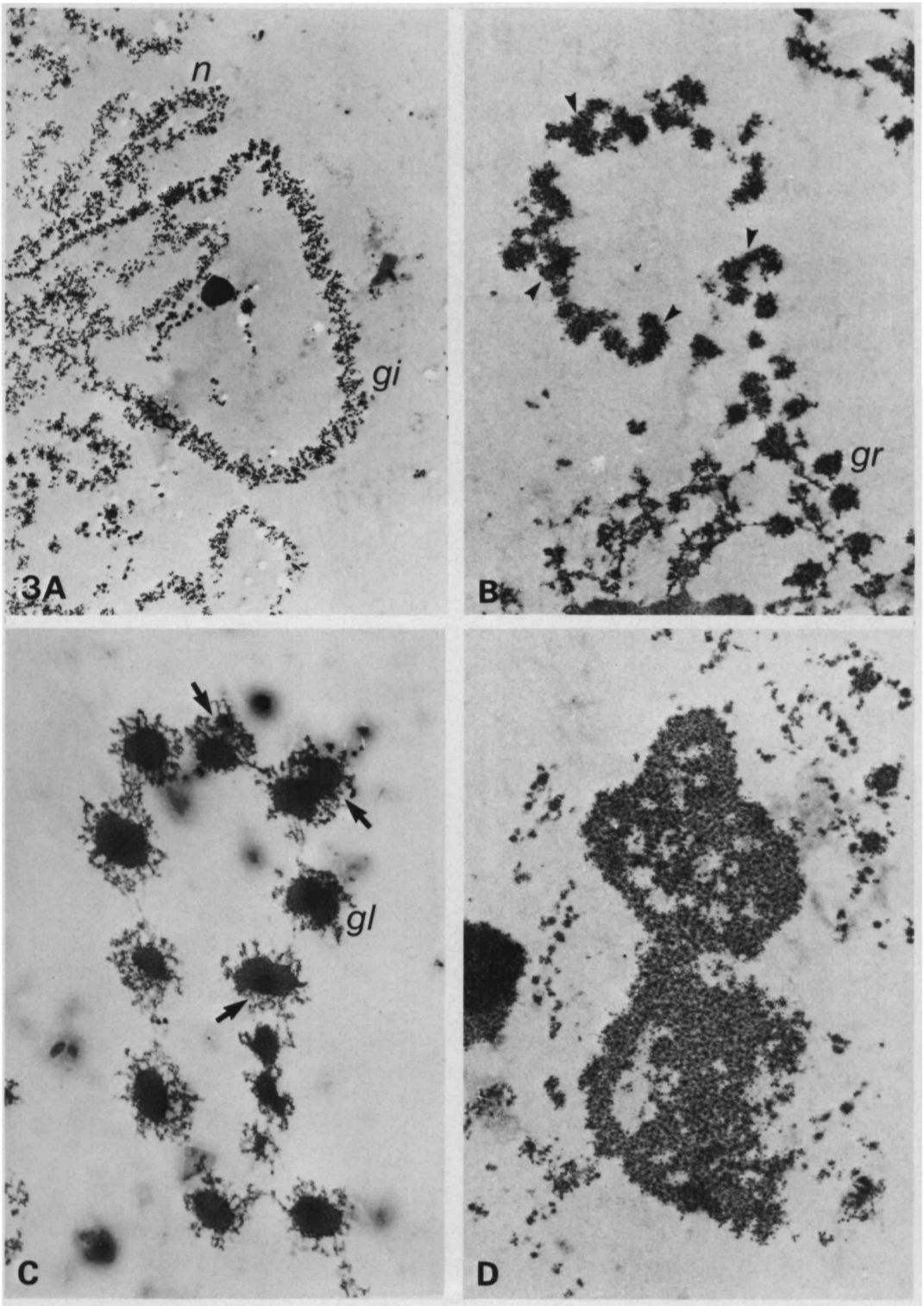
The diameter of the RNP particles is remarkably constant, not only all along a single loop, but also from one loop to another, and from one kind of loop to another. Mott & Callan (1975) suggested that this uniformity in size reflected an identity in the molecular weight of the proteins that associate with the transcripts. But Malcolm & Sommerville (1977) showed, on the basis of biochemical data, that unlike the strictly limited number of histones associated with the DNA, the non-histone proteins that associate with the transcripts include a great number of polypeptides, heterogeneous in molecular weight and in charge. At the present time, the origin of this uniformity in size remains unexplained.

Fig. 3. A–D. Electron micrographs of thin-sectioned landmark loops. A. Giant loop (*gi*) showing a strikely polarized matrix. *n*, normal loop; ×7000. B. Granular loop. Note the zig-zag disposition of the granules (*gr*), which can fuse together in some parts of the loop matrix (arrows); ×10 000. C. Globular loop. Each globule (*gl*) presents a dense centre and a less-compact peripheral region. Adjacent globules can fuse to form large ones (arrows); ×10 500. D. Dense matrix loop exhibiting a vacuolated aspect; ×5000.

Figs 4, 5. Details of thin-sectioned lateral loops; 30 nm particles (black arrows) occur in the matrices of all kinds of loops. RNP fibrils of adjacent granules (Fig. 4B) or globules (Fig. 5A) develop important interconnections (white arrows).

Fig. 4. A. Portion of normal loop matrix, showing RNP fibrils extending from the loop axis (*ax*); ×90 000. B. Portion of the granular loop in Fig. 3B; ×40 000.

Fig. 5. A. Portion of the globular loop in Fig. 3C; ×42 000. B. Portion of dense loop matrix. No linear arrangement of the 30 nm particles can be recognized; ×110 000.


n
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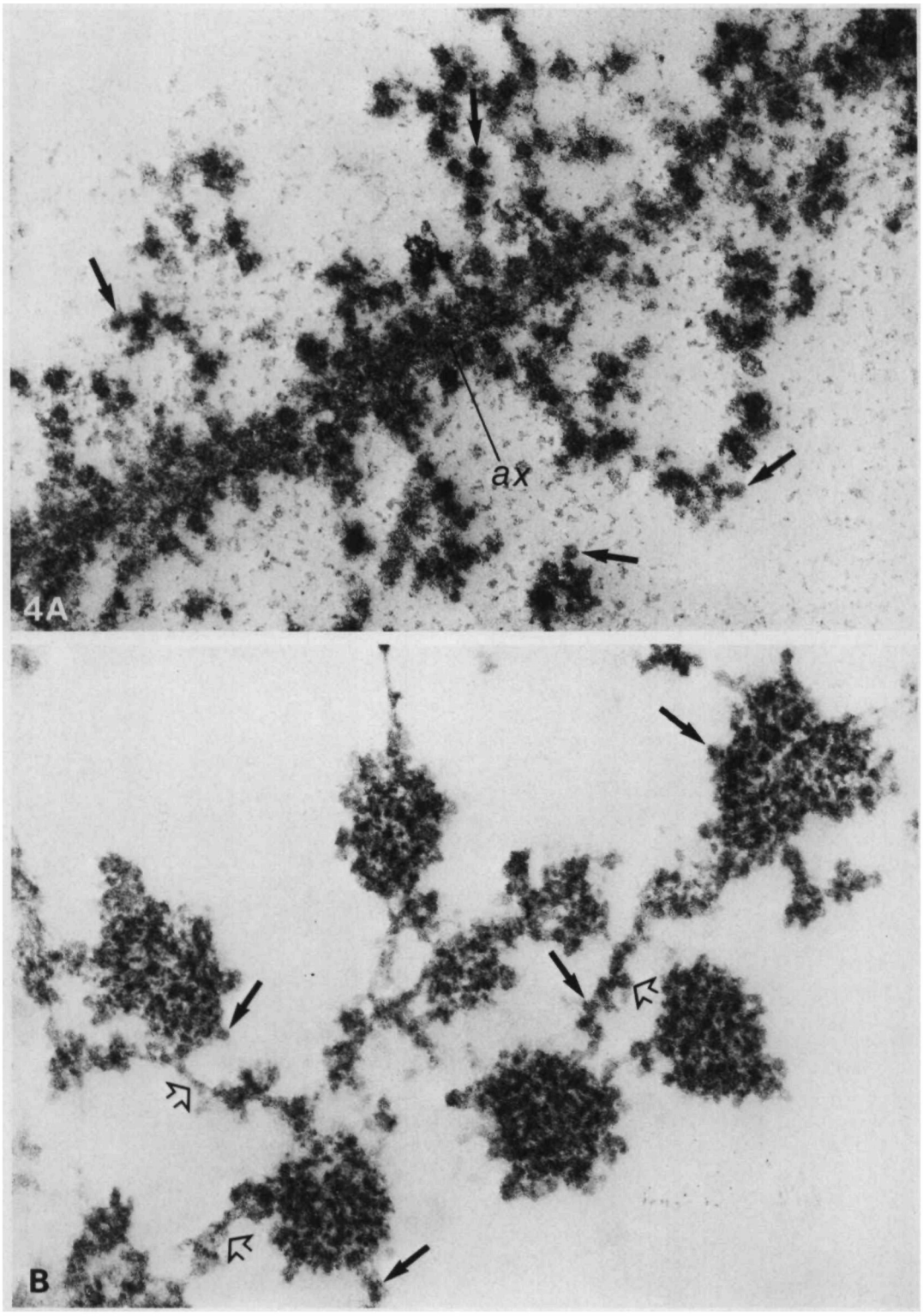

Fig. 4. For legend see p. 22

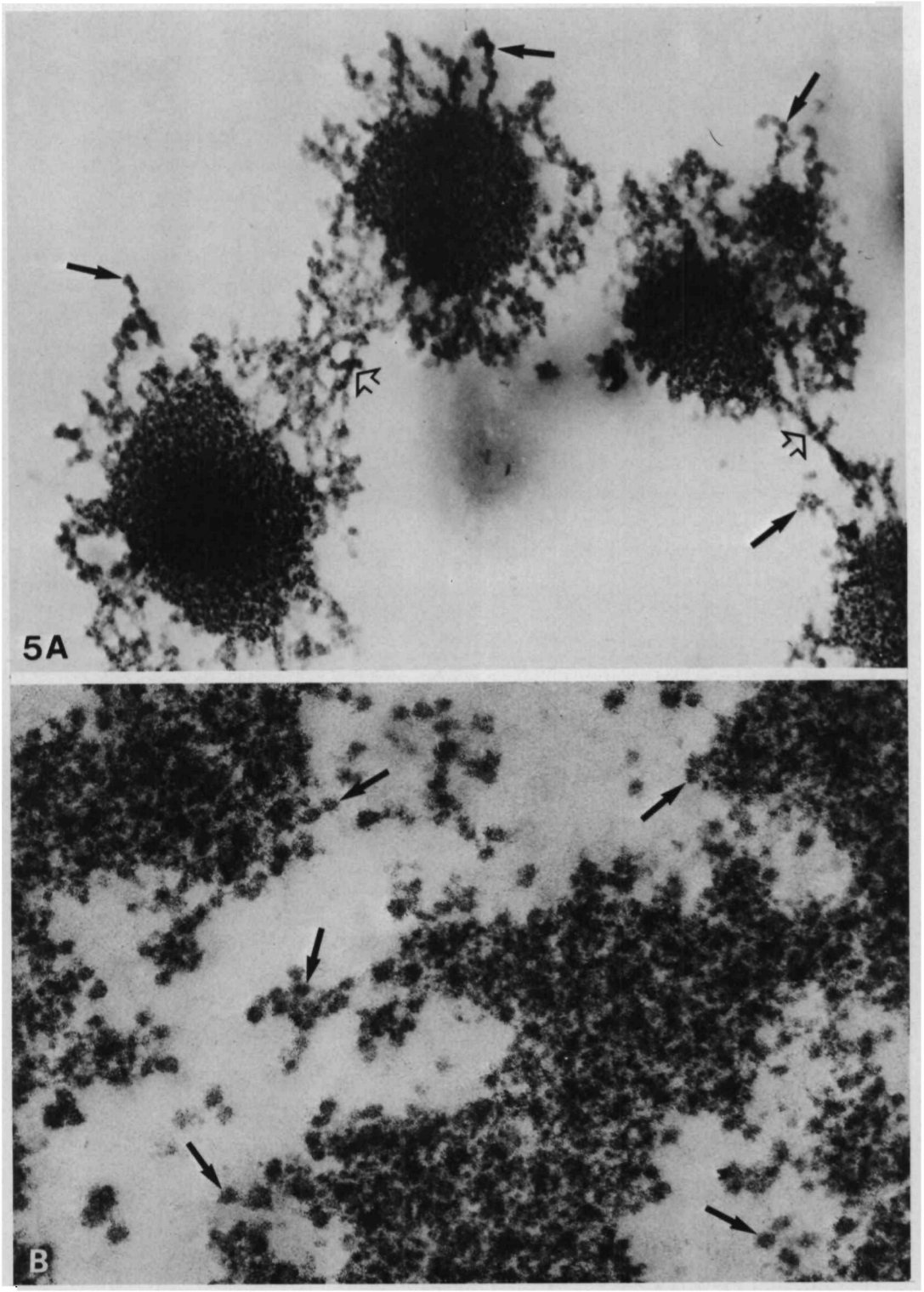

Fig. 5. For legend see p. 22

Morphological significance of the landmark loops

According to Malcolm & Sommerville (1974), the formation of RNP particles implies three kinds of molecular interactions. First, interactions occurring within each RNA molecule are responsible for the apparent foreshortening of DNA involved in transcription. The second kind of interaction is represented by the association of nuclear proteins with the transcripts. Immunological studies on lampbrush chromosomes provided good evidence for sequence-specific binding of proteins to nascent transcripts (see review by Sommerville, 1981; Lacroix *et al.* 1985). Thirdly, salt dissociation experiments (Malcolm & Sommerville, 1974) show that protein–protein interactions occur in the formation of RNP particles; these interactions involve forces much greater than those required for protein–RNA binding. Nuclear proteins binding to the RNA molecules immediately after transcription may have a protective effect. Moreover, as they probably stabilize the RNA secondary structure, protein–protein interactions may favour the foreshortening of RNP fibrils, the result being a minimal spatial encumbrance.

From the pictures we obtained in EM on thin sections, we believe that protein interactions also occur between adjacent RNP fibrils, enabling the formation of more or less tight aggregates in the matrix of the different kinds of loops. The morphological differences observed are due to a variable degree in the compaction of the transcription products. This is in good agreement with the SEM data previously reported by Angelier *et al.* (1984). We conclude that the matrix of the different types of loops of lampbrush chromosomes presents the same basic organization in EM.

Concerning the biological significance of these landmark loops, further investigations using other techniques will be necessary to elucidate the way in which their morphological characteristics are related to genetic information located in the loops.

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