Scanning Electron Microscopy

Volume 1985
Number 2 *Part II*

Article 38

6-16-1985

Amphibian Lampbrush Chromosome Loops: Correlative Light Microscopy, Transmission Electron Microscopy, and Scanning Electron Microscopy Observations

M. L. Bonnanfant-Jais
Centre de Cytologie Expérimentale C.N.R.S. 67

E. N'da
Centre de Cytologie Expérimentale C.N.R.S. 67

M. Penrad-Mobayed
Centre de Cytologie Expérimentale C.N.R.S. 67

N. Angelier
Centre de Cytologie Expérimentale C.N.R.S. 67

SCANNING ELECTRON MICROSCOPY/1985/II (Pages 889-896)
SEM Inc., AMF O'Hare (Chicago), IL 60666 USA

0586-5581/85$1.00+.05

AMPHIBIAN LAMPBRUSH CHROMOSOME LOOPS : CORRELATIVE LIGHT MICROSCOPY, TRANSMISSION ELECTRON MICROSCOPY AND SCANNING ELECTRON MICROSCOPY OBSERVATIONS

M.L. BONNANFANT-JAIS* E. N'DA, M. PENRAD-MOBAYED and N. ANGELIER

Centre de Cytologie Expérimentale C.N.R.S.
67, rue Maurice Günsbourg 94200 IVRY-SUR-SEINE FRANCE

(Paper received January 18 1985, Completed manuscript received June 16 1985)

Abstract

The RNP matrix of landmark loops in Pleurodeles waltlii lampbrush chromosomes have been examined by light microscopy, transmission and scanning electron microscopy. This study shows that in normal loops as well as granular, globular and dense loops, the RNP matrices are composed of one basic structure : an RNP particle with a diameter of 30 nm. The scanning electron microscope study also clarified the spatial arrangement of the various types of RNP matrices. The specific morphology of the RNP matrices is due to i) the progressive packaging of transcription products and ii) a concomitant and gradual coiling of the loop axis.

KEY WORDS: amphibian - lampbrush chromosomes - landmark loops - RNP matrix.

*Address for correspondence
Centre de Biologie Cellulaire CNRS, 67, rue Maurice Günsbourg, 94200 IVRY-SUR-SEINE FRANCE

Phone no.: 1 672.18.00

Introduction

Amphibian lampbrush chromosomes are characteristic of the diplotene stage of female meiotic prophase. Their organization is well known : the DNA unfolds in the form of lateral loops distributed along the chromosome axis. The DNA of the loop axis is actively transcribed (Gall and Callan, 1962) and the nascent RNA associates with proteins of a cytoplasmic origin to form the ribonucleoprotein complexes (RNP) which make up the matrix of these loops. All loops have the same basic organization (Mott and Callan, 1975 ; Sommerville and Malcolm, 1976), but they differ in the organization and amount of their RNP matrix. Thus, a number of loops have a morphology which is highly characteristic. This is the case for "giant loops", and for so-called "granular" loops, which have been described in the genus Triturus (Callan and Lloyd, 1960 ; Mancino and Barsacchi,1965,1966) and in Pleurodeles (Lacroix, 1968). They are observed at very precisely reproducible sites along the chromosomal axis and constitute "landmarks" which help in identification of bivalents. These landmarks have enabled the establishment of lampbrush chromosome maps for a number of amphibian species (Callan and Lloyd, 1960 ; Mancino and Barsacchi, 1965, 1966; Lacroix, 1968). Their morphological characteristics are stable and inherited, and thus they may be considered as reflecting a specificity of information localized at the level of loop DNA. Nonetheless, the biological significance of the information transcribed at the level of the lateral loops, and especially its possible role during development has not yet been elucidated. The structural organization of the landmarks also remains to be determined.

Indeed, most of the information concerning their structure has been obtained from observations carried out with the light microscope (Callan and Lloyd, 1960 ; Callan, 1963 ; Lacroix, 1968). Ultrastructural studies currently available mainly concern the structure of the matrix of so-called "normal" loops, that is, the most frequently found loops. This is true for observations performed on thin sections (Callan, 1982 ; Malcolm and Sommerville, 1974 ; Mott and Callan, 1975 ; Spring and Franke, 1981) or by chromosome spreading according to the Miller and Beatty

(1969) technique (Angelier and Lacroix, 1976; Scheer et al, 1976). Very few studies using scanning electron microscopy have provided information on the ultrastructure of several of these landmarks (Bakken and Graves, 1975 ; Angelier et al, 1984).

We thus decided to carry out a systematic ultrastructural analysis of landmark loops of lampbrush chromosomes from Pleurodeles oocytes. We have employed thin sectioning and scanning electron microscopy using procedures what have been successfull developed for Pleurodeles oocytes (Spring and Franke, 1981 ; Angelier et al, 1984).

Materials and Methods

Chromosome preparation. Female newts of the species Pleurodeles waltlii (Amphibia. Urodela) were used. Ovaries were removed from newts that had been anesthetized in 0.1 % MS 222 (Sandoz). Germinal vesicles of large oocytes 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). Single clean nuclei were each transferred to a centrifugation chamber containing medium and consisting of a circular glass slide with a central hole sealed by a circular coverslip (diameter 12 mm) glued to the underside of a slide. In the chamber the nuclear envelope was removed with needles and forceps, and the nuclear contents were centrifuged (30 min 1,500 g) onto the coverslip. After centrifugation, the chromosomes were observed in phase contrast with an inverted microscope (Carl Zeiss). Bivalents were identified photomicrographed, and processed for SEM.

Transmission electron microscopy. During the entire preparation the chromosomes were kept in the centrifugation chamber. The lampbrush chromosomes were fixed for 20 mm with 1 % glutaraldehyde, buffered to pH 7.2 with 0.1 M phosphate buffer. After postfixation with 1 % osmium tetroxide, the samples were dehydrated in a graded series of ethanol. After the last dehydration step, the coverslip was carefully removed from the centrifugation chamber. A capsule filled up with araldite was turned over the sample. After polymerisation of the resin, the coverslip was removed from the embedded specimen by immersion in liquid nitrogen, thereby leaving an araldite block in which the lampbrush chromosomes were positioned at the surface. This surface of the araldite block can be stained with 1 % methylene blue solution which permits identification of each of the lampbrush chromosomes. Thin sections were prepared on an ultramicrotome (Om U_3, Reichert Jung) using diamond knives and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips 201 transmission electron microscope operated at 80 kV.

Scanning electron microscopy. Fixation and postfixation conditions were identical for TEM and SEM studies. For SEM, lampbrush chromosomes were dehydrated in a graded series of ethanol, then in a graded series of acetone containing absolute ethanol. The samples were dried at the critical point in a Balzers CPD 010 apparatus using Liquid CO_2 as the intermediate fluid. The specimen were immediately transferred to a vacuum evaporator (Edwards S 15 PA), sputter coated and maintained at a pressure of 10^{-1} Torr for several minutes. After evacuation purified argon was admitted into the bell jar to a pressure of 7.10^{-2} Torr. Samples were shadowed with gold for 60 s at 20 mA and examined with a Philips 505 scanning electron microscope.

Results

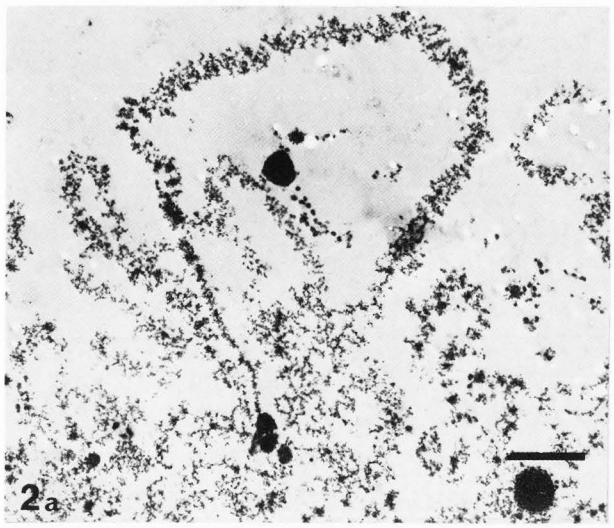
Normal loops. Under the phase contrast microscope the overwhelming majority of the lateral loops of the chromosomes conform to one pattern: such loops are termed "normal". Their polarized transcription units are seen to carry a finely structured RNP matrix (fig. 1).

Electron microscopy. In thin section through embedded normal preparation the particulate texture of normal loop matrix RNP is immediately apparent (fig. 2). Along the loop axis, each transcript consists of particles some 30 nm wide connected to one another like a string of beads as described by Mott and Callan (1975) (fig. 2). These RNP matrix particles are remarkably uniform in size throughout the length of the RNP loop matrix of all normal loops. Numerous connections occur between adjacent transcripts.

Scanning electron microscopy. A precise view of spatial arrangement of the transcripts on the loop axis is afforded by study with the scanning electron microscope (fig. 3). At high magnification, SEM provides confirmation of the linear disposition of the RNP matrix particles along the transcript, as observed with EM. These particles show the same uniformity of size, of the order of 50 nm in diameter. The difference in apparent size of thin sections (30 nm) as compared with SEM preparations (50 nm) is due to the gold coat (20 nm).

The RNP transcripts on normal loops show a helical coil around the loop axis (fig. 3). With EM, where loops happen to be tangentially disposed in relation to the plane of sectioning, this helical arrangement of RNP transcripts can be recognized ; in these cases the loop axis is not apparent (fig. 4).

Granular loops. Observations in phase contrast show different kinds of granular loops ; the RNP matrix of such loops appears to be much thicker than that of normal loops and is composed of granules varying in size from one granular loop to another (0.5 to 1 µm). All granular loops present a matrix markedly polarized with a thin and a thick insertion onto the chromosome axis (fig. 1). When the thin insertion is devoid of granules, the matrix is similar to that of a normal loop; in this type of loop, the RNP matrix is arranged in granules that increase in size (0.5 µm to 1 µm) onto the chromosome axis. In granular loops that show small RNP matrix granules at the level of thin insertion on the chromosome axis, the granules can reach a size of 3 µm at the thick insertion site: therefore this area of the loop presents a globular aspect.



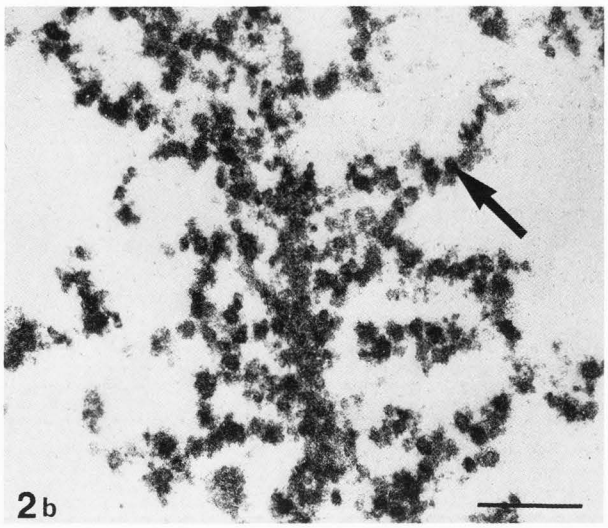
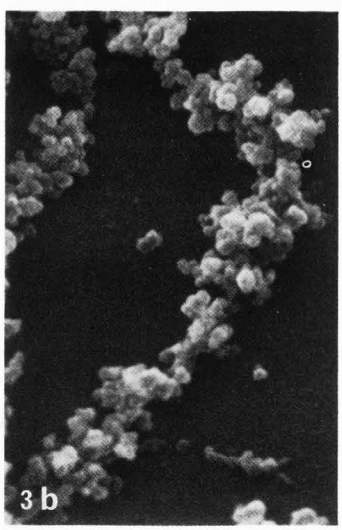
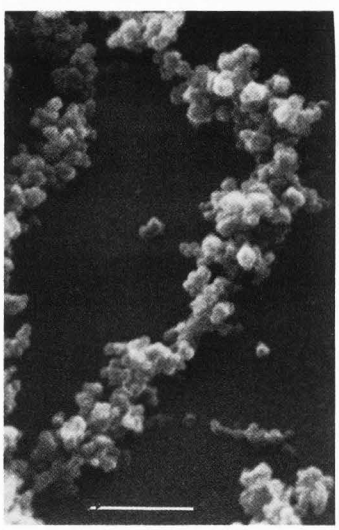
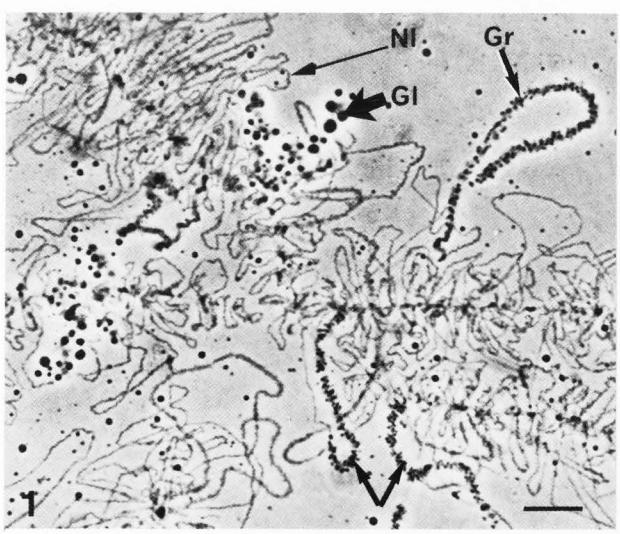


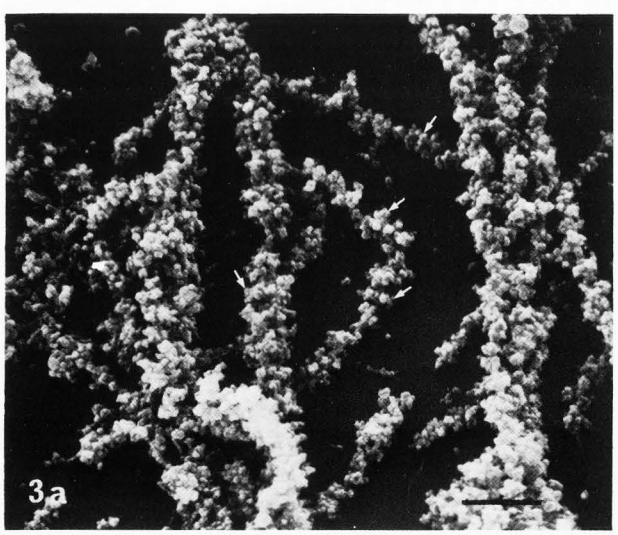
Fig. 2 a, b : Normal loop ultrastructure. a : normal loop with a polarized matrix. b : detail of the normal loop axis carrying transcripts ; matrix appears to be composed of particles (arrow) connected to one another. Note the uniformity of particle size. Bars = 2 µm in a ; 2500 A in b.

Fig. 1: Phase-contrast light micrograph. Homologous chromosomes show lateral loops arising from their axis: normal loops (Nl) granular loops (Gr) and globular loops (Gl). Note the obvious polarity of granular loops (black arrows). Bar = 5 µm.
Fig. 3a: Normal loops viewed by SEM. b: Detail (stereo-view). Note the linear disposition of the RNP matrix particles and the helical coil of RNP transcripts (arrows). Bar = 1 µm in a; 0.5 µm in b.









A transmission electron microscope study of the granular loops reveals a matrix composed of RNP particles (30 nm in diameter) aggregated into dense bodies which correspond to the granules seen in the phase contrast microscope (fig. 5). The matrix of the granular loops has the same fundamental organization as normal loops : adjacent transcripts composed of particles in linear arrangement are seen to form granules of various size.

This is further supported by observations carried out in the scanning electron microscope at high magnification : the granules are composed of particles which are similar to those found in the RNP matrix of normal loops. Associated transcripts conserve their helical coil around the loop axis and in addition to this coiling there is a coiling of the loop axis itself (figs. 6 and 7). In granular loop carrying voluminous granules, the coiling of the loop axis is obvious; in this case, the granules are closely packed.

Globular loops.

Phase contrast microscopy.

The RNP matrix of these loops does not display any obvious polarity. It is composed of globules 3 to 4 µm in diameter (fig. 1).

Transmission electron microscopy.

The globules are electron-dense bodies leaving the tips of protruding transcripts visible. The transcripts surrounding the dense globules are similar to those observed in the normal and granular loop RNP matrices : i.e, they are composed of interconnected 30 nm particles (fig. 8). The loop axis is not obvious on thin sections. This suggests that the globules result from a tight packaging of usual RNP transcripts. In some cases, the RNP matrix forms a true sleeve around the loop axis (fig. 8).

Scanning electron microscopy.

Globular loops do not stretch out of the chromosome axis as do normal and granular loops. SEM views reveal a considerable coiling of the globular loop axis. The globules are disposed very close to each other and arranged into a spiral pattern (fig. 9). Numerous transcript connections take place between adjacent globules. In some supercoiled loops, globules fuse and the RNP matrix becomes continuous (fig. 9). The surface of the globules appears to be composed of RNP particles similar to those observed on RNP transcripts of normal and granular loops. This corroborates the observations made by transmission electron microscopy that showed the uniform size of the RNP particles.

Dense matrix loops. These loops are observed only on two of the twelve bivalents making up the Pleurodeles waltlii karyotype (Lacroix, 1968).

Phase contrast microscopy.

The morphology of these landmark loops is highly variable ; sometimes their RNP matrix appears to be composed of globules (0.05 to 0.1 µm) more often the complete coalescence of the RNP matrix throughout the loop axis makes them appear as short small lobes (fig. 10).

Transmission electron microscopy.

The usual loop pattern is not recognizable because of the high degree of compaction of the RNP matrix. These loops are homogeneously composed of 30 nm RNP particles (fig. 11).

Scanning electron microscopy.

The axis of the dense loops is strongly coiled and the RNP matrix is twisted (fig. 12). The thickness of the matrix is uniform over most of the length of these loops. At high magnification, the matrix is made of tightly packaged RNP particles and forms a continuous sheath around the loop axis.

Discussion

Amphibian lampbrush chromosomes have been studied by classical transmission electron microscopy (TEM) and by high-voltage electron microscopy (HVEM) on thin and thick sections (Malcolm and Sommerville, 1974 ; Mott and Callan, 1975 ; Spring and Franke, 1981), and by scanning electron microscopy (SEM) (Angelier et al, 1984). However, correlative LM, TEM and SEM observations of lampbrush chromosomes have never been carried out, probably because it is difficult to identify the bivalents at the same time in LM, TEM and SEM.

Identification of bivalents in LM, TEM and SEM. In Pleurodeles waltlii the observations of lampbrush chromosomes by phase contrast microscopy led to a classification of the lateral loops according to the aspect of their matrix. Detailed maps have been established allowing the identification of bivalents in oocyte (Lacroix, 1968).

The flat embedding procedure used in this study, allows the observation of the same chromosome both by phase contrast microscopy and electron microscopy. Furthermore, the bivalents are easily identified by SEM at low magnification. Thus a given lateral loop can be studied by phase contrast microscopy, transmission and scanning electron microscopy. This provides additional information and allows one to establish correlations between different aspects of the fine chromosomal organization.

SEM observations can be performed at high magnifications equivalent to those used in TEM. Stereo views are very useful to describe the precise spatial arrangement of fine structures. This is extremely difficult in TEM, even with serial thin sections.

Fundamental organization in the RNP matrix. The electron microscopy study of the different types of loops has revealed the particulate nature of the RNP matrix of the loops. Whatever the morphology of the RNP matrix (granular, globular or dense), the transcripts seem to be made of interconnected RNP particles. These particles have a remarkably uniformity in size of 30 nm, throughout the matrix of normal, granular, globular and dense loops.

These observations corroborate those of Miller (1966), Malcolm and Sommerville (1974), Mott and Callan (1975), Spring and Franke (1981). These authors described the existence of such RNP particles in the matrix of most lateral loops. However, they did not point out the presence of the same particles in all types of loop matrix. The present study clearly shows that the fundamental subunit found in the matrix of all types of loops is a 30 nm RNP particle.

The uniformity of the size of the RNP particles in different types of loops is the predominant morphological feature of the RNP matrix.

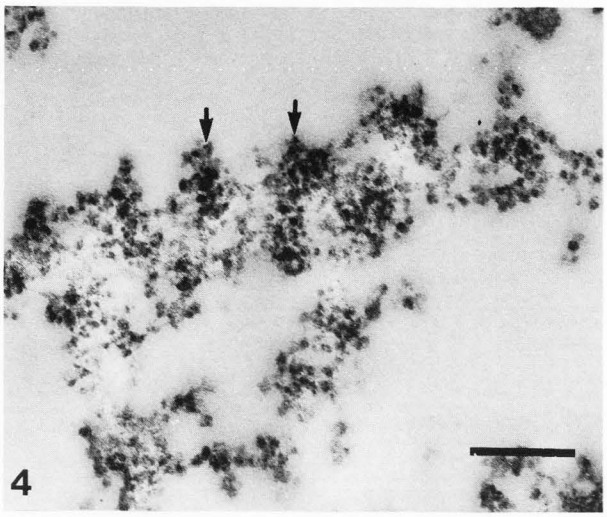
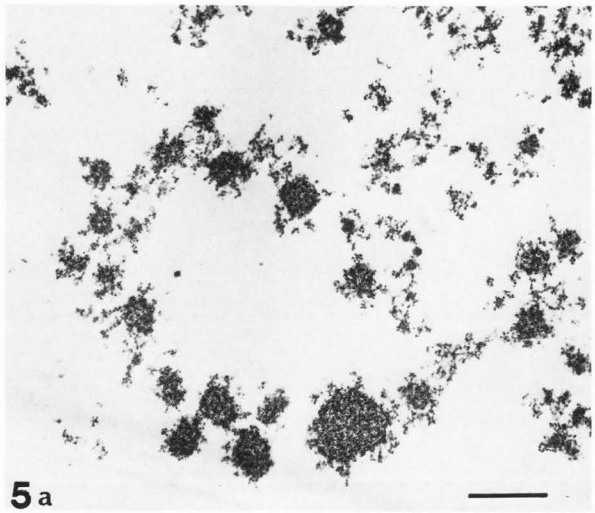
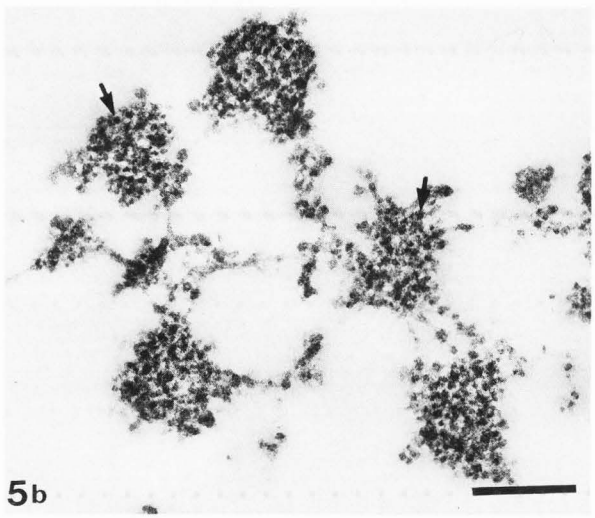


Fig. 4 : Normal loops : helical coil of RNP transcripts observed by TEM (black arrows). Bar = 2500 A.





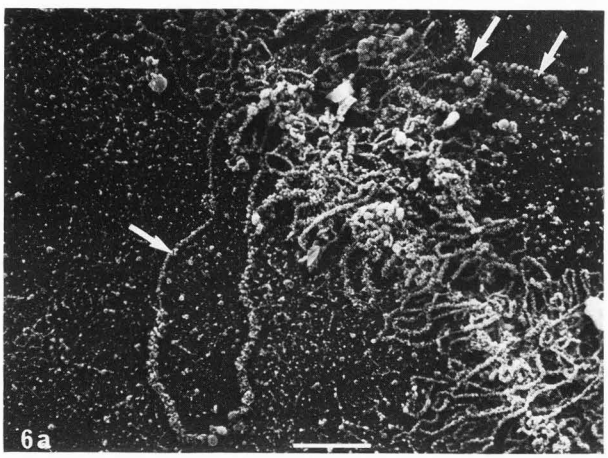
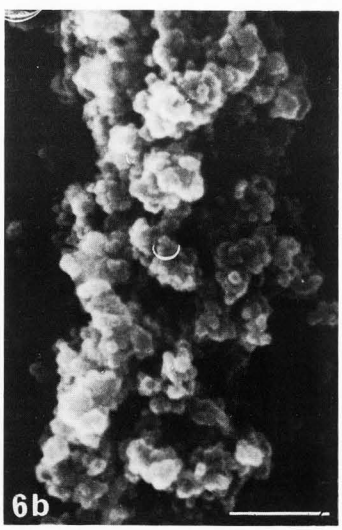
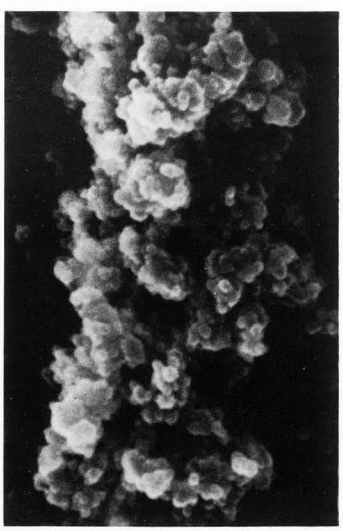


Fig. 5: Granular loop observed by TEM. a: matrix is composed of granules of increasing size. b: granules appear as dense aggregates of RNP particles of uniform size (arrows). Bars = 1 μm in a; 2500 A in b.
Fig. 6: Granular loop viewed by SEM, a: bivalents show different types of granular loops (arrows). b: stereo-view of the matrix; granules appear to be composed of particles identical to those of the matrix of normal loops. Note the helical disposition of granules. Bars = 10 μm in a; 0.5 μm in b.





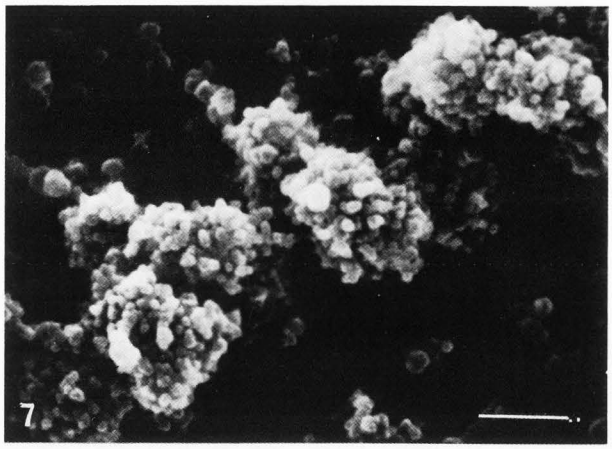
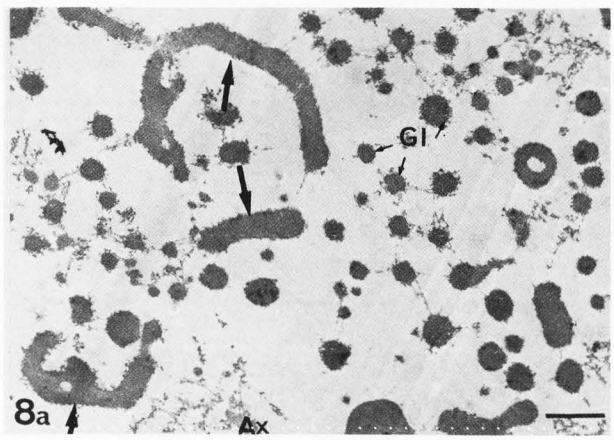


Fig. 7 : Granular loop viewed by SEM. Loop axis shows an obvious helical coiling ; voluminous granules are brought close together. Bar = 0.5 µm.



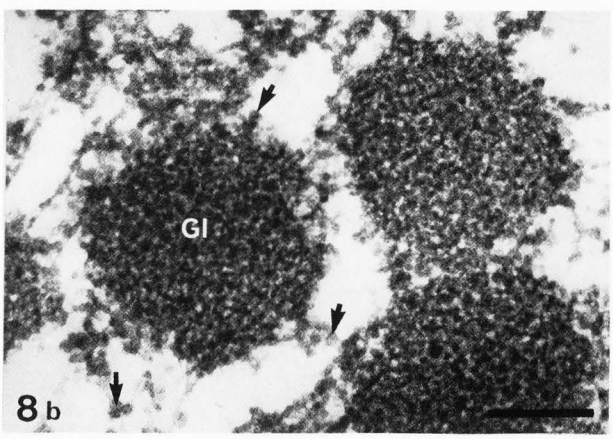
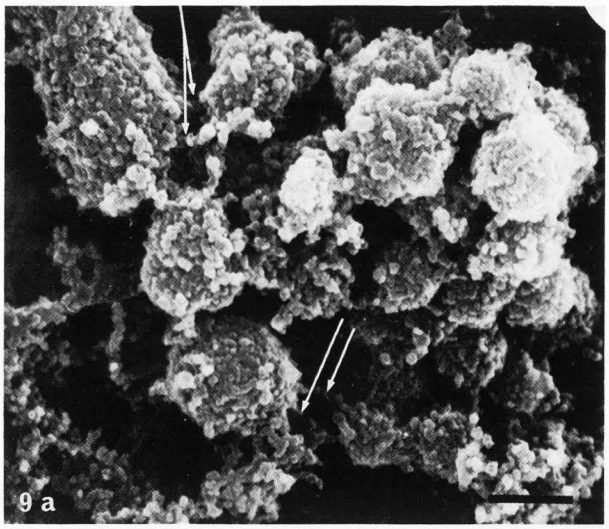


Fig. 8 : Ultrastructure of a globular loop. a : globules appear as dense bodies connected to one another. In some parts of the loop globules are fused (arrows). Ax : loop axis. b : at high magnification, globules (Gl) appear to be composed of particles identical to those of the normal and granular loops (arrows). Note the numerous interrelations between granules. Bars = 2 µm in a ; 2500 A in b.



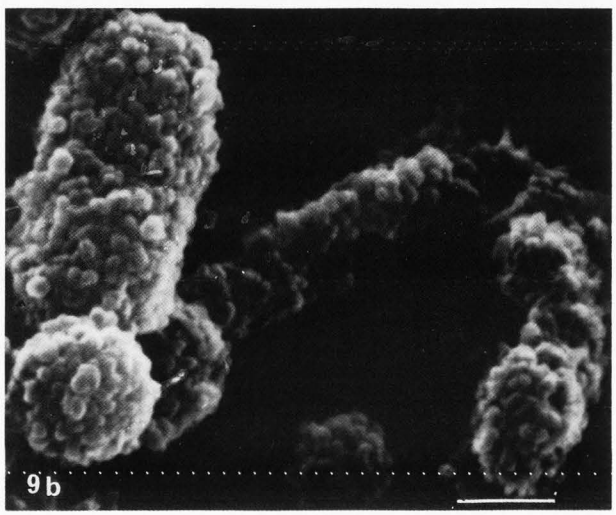


Fig. 9 : Globular loop observed with SEM. a : note the spiral disposition of the globules and the numerous interrelations between them (arrows). b : globules appear to be composed of particles uniform in size ; globules are fused into a sleeve around the loop axis. Bars = 1 µm in a ; 0.5 µm in b.

Mott and Callan (1975) suggested that this uniformity in size expresses uniformity in molecular weight between associated proteins and nascent RNA. Malcolm and Sommerville (1977) have shown that polypeptides associated with nascent RNA have heterogeneous molecular weight.

Mode of compaction of the RNP matrix in different types of loops.

Evidence that the different types of RNP matrices observed on normal, granular and globular loops are linked to a progressive compaction of transcript products. SEM examination of the different types of loops shows the process of progressive compaction of the matrix components. This compaction results both from a progressive packaging of the transcripts and from an increased coiling of the loop axis.

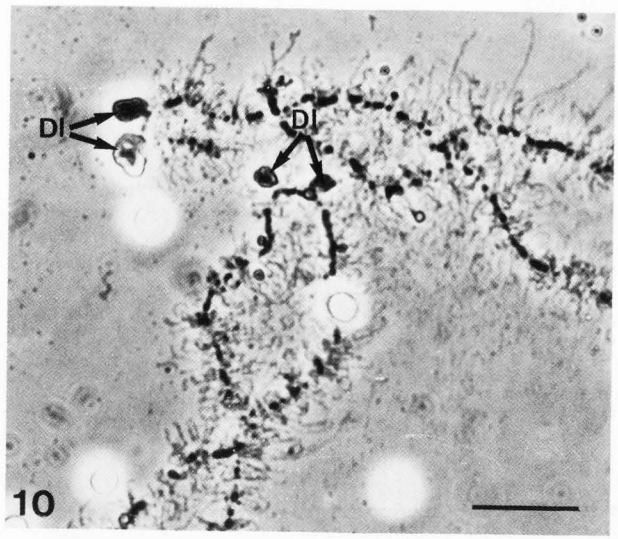
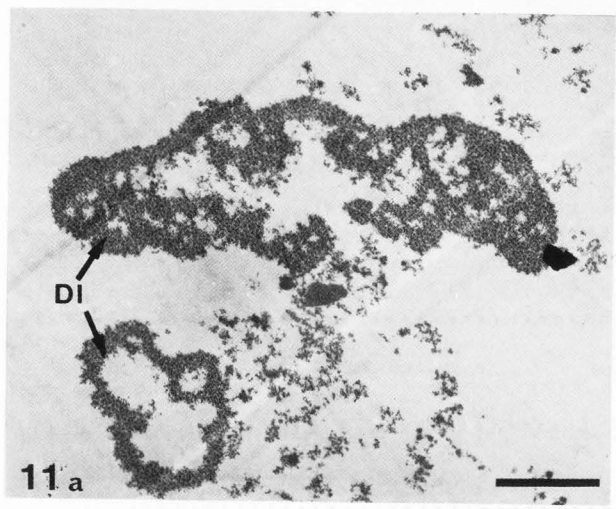
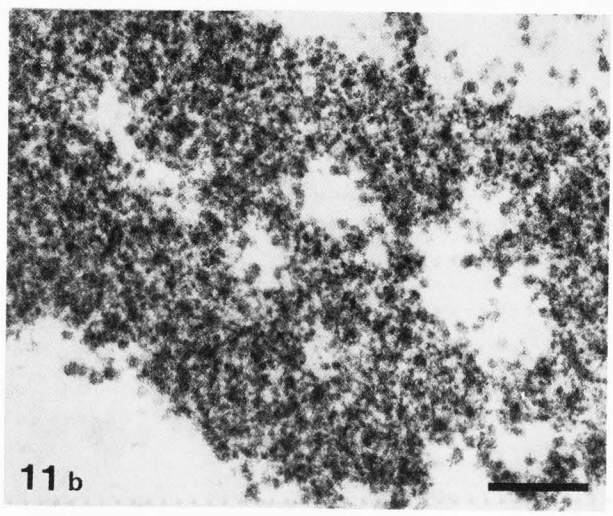
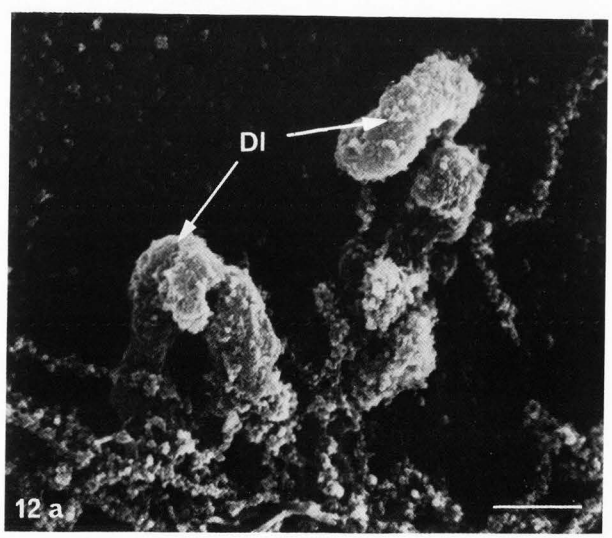


Fig. 10: Dense matrix loops (Dl) observed in phase contrast. Bar = 10 µm.

Fig. 11 : Ultrastructure of the dense loops. a : dense loop (Dl) show a dense compact matrix. b : structure of the matrix is homogeneous and composed of particles uniform in size. Bars = 2 µm in a ; 2500 A in b.







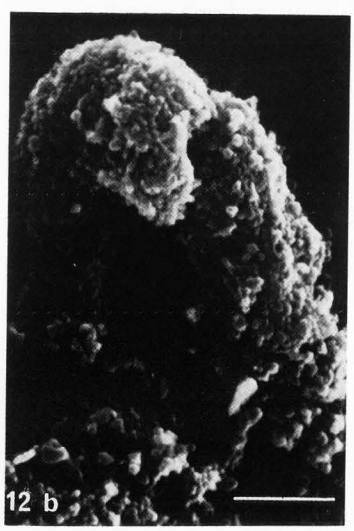


Fig. 12 : Dense loops viewed by SEM. a : two different aspects of the dense loops (Dl) : the right one shows a twisted aspect, the left one appears as a short compact lobe. b : the matrix is composed of tightly packaged RNP particles. Bars = 2 µm in a ; 1 µm in b.

The RNP transcripts show a helical arrangement around the loop axis ; this is clearly observed on normal and granular loops. As described in the SEM study, the granules of the granular RNP matrix are made of tightly associated transcripts. Obvious connections between adjacent transcripts are also observed by EM, on thin sections.

In addition to this packaging of the matrix components, the progressive coiling of the loop axis brings the granules closer to each other. Observations of the granular and globular matrices provide evidence that their increasing size is correlated with an increasing coiling of the loop axis. The dense loop axis exhibits an extreme coiling which goes along with the maximum degree of compaction of its matrix components.

This compaction could be the result of molecular interactions between the RNP of the same transcript or of adjacent transcripts which lead to the formation of aggregates of increasing size. According to Malcolm and Sommerville (1977) these interactions occur at different level : within the RNA molecule or through RNA-protein interactions and protein-protein interactions. The coiling of the loop axis would be due to interactions between adjacent aggregates of transcription units.

The biological significance of the compaction of the matrix components remains to be elucidated.

Acknowledgements

This work was supported by the French CNRS (ATP 950 605).

References

ANGELIER N, LACROIX JC (1975). Complexes de transcription d'origine nucléolaire et chromosomique d'ovocytes de Pleurodeles waltlii et P. poireti. Chromosoma 51 : 323-335.

ANGELIER N, PAINTRAND M, LAVAUD A, LECHAIRE JP (1984). Scanning electron microscopy of Amphibian lampbrush chromosomes. Chromosoma 89 : 243-253.

BAKKEN AH, GRAVES B (1975). Visualization of the tertiary structure of lampbrush chromosomes with the scanning electron microscope. J. Cell Biol. 67 : 17a.

CALLAN HG (1963). The nature of lampbrush chromosomes. Int. Res. Cytol. 15 : 1-34.

CALLAN HG, LLOYD L (1960). Lampbrush chromosomes of crested newts Triturus cristatus Laurenti. Philos. Trans. R. Soc. B 243 : 135-219.

CALLAN HG (1982). Lampbrush chromosomes. Proc. R. Soc. Lond. B 214 : 417-448.

GALL JG (1954). Lampbrush chromosomes from oocyte nuclei of the newt. J. Morphol. 94 : 283-351.

GALL JG, CALLAN HG (1962). ^{3}H-uridine incorporation in lampbrush chromosome. Proc. Natl. Acad. Sci. 48 : 562-570.

LACROIX JC (1968). Etude descriptive des chromosomes en écouvillon dans le genre Pleurodeles (Amphibien, urodèle). Annls. Morphogen. 4 : 383-396.

MALCOLM DB, SOMMERVILLE J (1974). The structure of chromosome-derived ribonucleoprotein in oocytes of Triturus cristatus carnifex (Laurenti). Chromosoma 48 : 137-158.

MALCOLM DB, SOMMERVILLE J (1977). The structure of nuclear ribonucleo-protein of amphibian oocytes. J. Cell Sci. 24 : 143-165.

MANCINO G., BARSACCHI G (1965). Le mappedei chromosomi "lampbrush" di Triturus (Anfibi, Urodeli). I. Triturus alpestris apucenus, Caryologia 18 : 637-665.

MANCINO G, BARSACCHI G (1966). Le mappedei chromosomi lampbrush di Triturus (Anfibi, Urodeli). II. Triturus Helvetieus helveticus. Rev. Biol. 19 : 311-351.

MILLER OL (1966). Fine structure of lampbrush chromosomes. Natl. Cancer Inst. Monogr. 18 : 79-99.

MILLER OL, BEATTY BR (1969). Visualization of nucleolar genes. Science 164 : 955-957.

MOTT MR, CALLAN HG (1975). An electron microscope study of the lampbrush chromosomes of the newt Triturus cristatus. J. Cell Sci. 17 : 241-261.

SCHEER U, FRANKE WW, TRENDELENBURG M, SPRING H (1976). Classification of loops of lampbrush chromosome according to the arrangement of transcriptional complexes. J. Cell Sci. 22 : 503-519.

SOMMERVILLE J, MALCOLM DB (1976). Transcription of genetic information in amphibian oocytes. Chromosoma 55: 183-208.

SPRING H, FRANKE WW (1981). Transcriptionally active chromatin in loops of lampbrush chromosomes at physiological salt concentrations as revealed by electron microscopy sections. Eur. J. Cell Biol. 24: 298-308.

Discussion with Reviewers

H.C. Macgregor: The paper makes no mention of chromomeres, yet a comparison of the appearance of these in the LM and the SEM would be very valuable. They are an important component of the chromosomes and it would be particularly interesting to see, at the SEM level, how the loops are "inserted" into the chromomeres. It would also be interesting to see the surface organisation of chromomeres.

Authors: Chromomeres have been already described in the recently published SEM work from our group (Angelier et al., 1984).

T.D. Allen: Have the authors considered, or attempted any alternative medium to the one quoted for extraction of the lampbrush chromosomes, in case there is a variation induced?

Authors: Different media have been already attempted for extraction of lampbrush chromosomes in different species as Triturus cristatus (Callan and Lloyd, 1960), Pleurodeles waltlii and Pleurodeles poireti (Lacroix, 1968). This medium has proved to be the best for preserving the lampbrush chromosome structure.

Additional Reference

Lacroix JC (1968). Variations expérimentales ou spontanées de la morphologie et de l'organisation des chromosomes en écouvillon dans le genre Pleurodeles (Amphibian, Urodèle), Am. Embr. Morpho., 1, 205-248.