Chromosoma (Berl) (1986) 94:319–328

CHROMOSOMA

Evidence for a particular mode of transcription in globular loops of lampbrush chromosomes of the newt *Pleurodeles waltlii*

M. Penrad-Mobayed, M.L. Bonnanfant-Jaïs, E. N'Da, and N. Angelier
Centre de Biologie Cellulaire, 67, rue Maurice Günsbourg, F-94200 Ivry-sur-Seine, France

Abstract. In amphibian lampbrush chromosomes, many loops have a specific morphology; this is the case for globular loops in the newt *Pleurodeles*. We have previously shown that the specific morphology of these loops is linked to an extreme compactness of the transcription products which make up their matrix. – We investigated RNA synthesis in this type of loop by carrying out autoradiographic and transcription inhibition studies. We also analysed the organization of transcriptional complexes in these loops in the electron microscope using spread preparations. These studies revealed the presence of several transcription units in the same loop and asynchronous variations in RNA synthesis in these transcription units. We propose and discuss several hypotheses in order to explain this asynchronous RNA synthesis. We also discuss these results in the context of loop morphology and transcription mode.

Introduction

Due to their large size and particular organization, amphibian lampbrush chromosomes constitute an excellent model for the study of transcription, both at the structural and molecular level (see review by Callan 1982). Lateral loops are the sites of transcriptional activity (Gall and Callan 1962; Izawa et al. 1963; Mancino and Barsacchi 1966; Snow and Callan 1969; Miller and Hamkalo 1972; Angelier and Lacroix 1975; Scheer et al. 1976; Angelier and Lavaud 1982); the newly synthesized RNA binds to proteins to form ribonucleoprotein (RNP) complexes (Sommerville 1973; Malcolm and Sommerville 1977).

The majority of loops, called normal loops, have a similar morphology (Callan 1963). Others, such as granular or globular loops, have a specific morphology which is related to the size and type of organization of their matrix components. These distinctive loops are always observed at a precise site along the chromosome axis and thus constitute real landmarks enabling the drawing of maps (Callan and Lloyd 1960; Mancino and Barsacchi 1965, 1966; Lacroix 1968). Recently, N'Da et al. (1986) have shown that the basic structure of the RNP matrix of both normal and landmark loops is a 30 nm RNP particle. Data obtained by scanning electron microscopy on chromosome spreads have shown that the specific morphology of the different kinds of loops is linked to a gradual packing of matrix components (Angelier et al. 1984; Bonnanfant-Jaïs et al. 1985, 1986). For Sommerville (1981), the existence of a morphological variability in landmark loops might be caused, in part at least, by differences in the level and the pattern of the transcriptional activity.

In order to ascertain whether special transcriptional activity can be observed in these landmark loops, we studied RNA synthesis in the globular loops of the newt *Pleurodeles waltlii*. These loops are most typical, due to the high degree of compaction of their matrix. Globular loops are always observed at the same place on 7 of the 12 bivalents in the *Pleurodeles* karyotype; they are characterized by the presence of several globules in their matrix (Lacroix 1968). We carried out autoradiographic and transcription inhibition studies in conjunction with analysis of transcription in the loops under the electron microscope, using a modified version of Miller's procedure (Angelier and Lavaud 1982). The results showed that globular loops exhibit a particular transcription mode.

Materials and methods

Incubation conditions for in vitro RNA synthesis. Large oocytes of the female newt *Pleurodeles waltlii* (stages V and VI described by Bonnanfant-Jaïs and Mentré 1983) were incubated at 18° C in Wolf and Quimby's medium (Gibco Biocult) which contained 250 µCi/ml of 5,6-3H-uridine (aqueous solution, sp. act., 42 Ci/mMol) and 250 µCi/ml of 5,6-3H-cytidine (aqueous solution, sp. act. 30 Ci/mMol, Amersham, UK). Incubation time ranged from 2 to 48 h.

Lampbrush chromosome preparations. Chromosome preparations were carried out as described by Gall (1954). Germinal vesicles of oocytes were isolated manually 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 $CaCl_2$. Each clean nucleus was transferred to the centrifugation chamber containing the same buffer and consisting of a circular glass slide with a central hole sealed by a coverslip. 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).

Autoradiography for light microscopy. Coverslips were detached from the slides and chromosome preparations were fixed for 30 min in 2% paraformaldehyde in phosphate

Offprint requests to: M. Penrad-Mobayed

buffer at pH 7.2 and then processed for autoradiography as described by Hartley and Callan (1978). To remove unincorporated precursors, chromosome preparations were washed with distilled water, treated for 5 min in freshly prepared ice-cold 5% trichloroacetic acid, washed in several changes of distilled water, dehydrated through an ethanol series, washed in xylene to remove paraffin wax and air-dried from acetone. Coverslips were dipped in NTB 2 emulsion (Kodak) diluted 1:1 with distilled water, left to expose at 4° C for 8 days and developed at 15° C in Kodak D19 developer. The chromosomes were stained as described by Gall (1981) with Coomassie blue (0.1% in 50% methanol, 10% acetic acid) for 10 min.

Autoradiography for electron microscopy. Chromosome preparations were fixed for 30 min in 2% paraformaldehyde in phosphate buffer at pH 7.2, post-fixed in a solution of 1% OsO_4, and dehydrated by passage through an ethanol series. Preparations were flat-embedded in araldite (Gounon and Karsenti 1981; Spring and Franke 1981). Ultrathin sections were processed and then dipped into L4 Ilford emulsion according to the technique of Larra and Droz (1970). After 10 weeks of exposure, autoradiograms were developed using a special procedure already described by Angelier et al. (1976). They were then stained with uranyl acetate and lead citrate and examined with a Philips 201 electron microscope (EM) at 80 kV.

Molecular chromatin spreads. Chromatin spread preparations wer made according to the method of Angelier and Lavaud (1982). This method, a modified version of the Miller procedure (Miller and Bakken 1972), used a special microcentrifugation chamber with two parts, a wide upper part containing a dispersal medium (either 0.1 M KCl, pH 9, or 0.05 M KCl, pH 9 or else 0.01 M borate buffer, pH 9.22) and a narrow lower part containing a sucrose formalin cushion (0.1 M sucrose, 1% formalin, pH 8.5). Germinal vesicles of oocytes were isolated in 0.1 M KCl, pH 7, and directly transferred into the upper part of the microcentrifugation chamber. Chromosomes were then isolated by opening the nucleus in the dispersal medium above the well of the chamber and immediately deposited onto a carbon-coated grid at the bottom of the chamber by centrifugation at 10° C through the sucrose formalin cushion (400 *g*, 5 min; 2,200 *g*, 15 min). Grids were then rinsed for several seconds in 0.4% Kodak photo-flo solution and air-dried; they were next stained with 1% phosphotungstic acid (PTA) or contrasted by tungsten tantalum (W.Ta) shadowing (rotative shadowing under an angle of 10°; thickness = 65 Å). They were observed with a Philips 201 electron microscope at 60 kV.

Transcription inhibition conditions. Oocytes were incubated at 18° C in Wolf and Quimby's medium containing actinomycin D (Calbiochem) at 50 µg/ml, or α-amanitin (Boehringer) at 100 µg/ml. Incubation time ranged from 1 to 6 h.

Results

RNA synthesis: labelling with ^{3}H-uridine and ^{3}H-cytidine and autoradiography

Autoradiographic study by light microscopy showed that all types of loops, including normal and landmark loops, were labelled. However, variations in the pattern of labelling were observed between globular loops and others.

After 2 h incubation of oocytes in medium containing tritiated cytidine and uridine, all types of loops were slightly labelled (Fig. 1a′). After 6 h of incubation, normal, giant and granular loops became strongly and uniformly labelled. In contrast, globular loops remained slightly and partially labelled, with no obvious polarity in silver grain concentration in their matrix, even after a long incubation time (24 h; Fig. 1b′). After a longer period of incubation (48 h) almost all of the globular matrix had become strongly labelled (Fig. 1c′).

Ultrastructural autoradiographic observations confirmed those previously described from light microscopy. Indeed, for a labelling period extending up to 24 h, only globular loops exhibited partial labelling; the others were uniformly labelled (Fig. 2a). Furthermore, EM autoradiographs showed that globules within the same loop could be either unlabelled, slightly labelled or strongly labelled (Fig. 2a, b). High magnifications clearly showed that, in labelled globules, silver grains were located in the dense central part as well as in the peripheral zone made up of more or less individualized fibrils (Fig. 2b).

Ultrastructural organization of transcriptional complexes in the globular loop matrix: chromatin spreads

Chromatin spreads of lampbrush chromosomes made according to the Miller procedure (Miller and Bakken 1972) did not permit us to analyse the overall organization of lampbrush chromosomes, nor to study the ultrastructural organization of transcriptional complexes in a precise type of loop. In contrast, after gradual disorganization of lampbrush chromosomes using Angelier and Lavaud's method (1982), it remained possible to identify the different kinds of loops along the chromosome axis and therefore to analyse their detailed organization at the molecular level. The degree of spreading of chromatin was dependent on the concentration of the dispersal medium (cf. Materials and methods and below).

Under moderate disorganization conditions (0.1 M KCl, pH 9), globular loops always appeared far less disorganized than granular, and especially normal loops (Fig. 3a); dense globules remained easily identifiable. More extensive disorganization (0.05 M KCl, pH 9) allowed us to observe both non-disorganized globules and globules in the process of disorganization. In the latter case, it was obvious that globules were due to a dense aggregation of lateral fibrils (Fig. 3b). When disorganization conditions (0.01 M borate buffer, pH 9.22) were acute, the globular matrix became more disorganized and displayed several more or less unfolded transcription units (TUs) (Fig. 4). These units exhibited a strong density of lateral fibrils extending from the loop axis and were distributed according to an increasing gradient of length (Figs. 4, 5). The units ranged from 3 to 10 µm in length and sometimes alternated with untranscribed regions. Many interconnections between fibrils of adjacent units were observed (Fig. 5).

Effects of actinomycin D or α-amanitin on lampbrush chromosome transcription

After a short time (1 h) of oocyte incubation with actinomycin D, lampbrush chromosomes showed modifications in

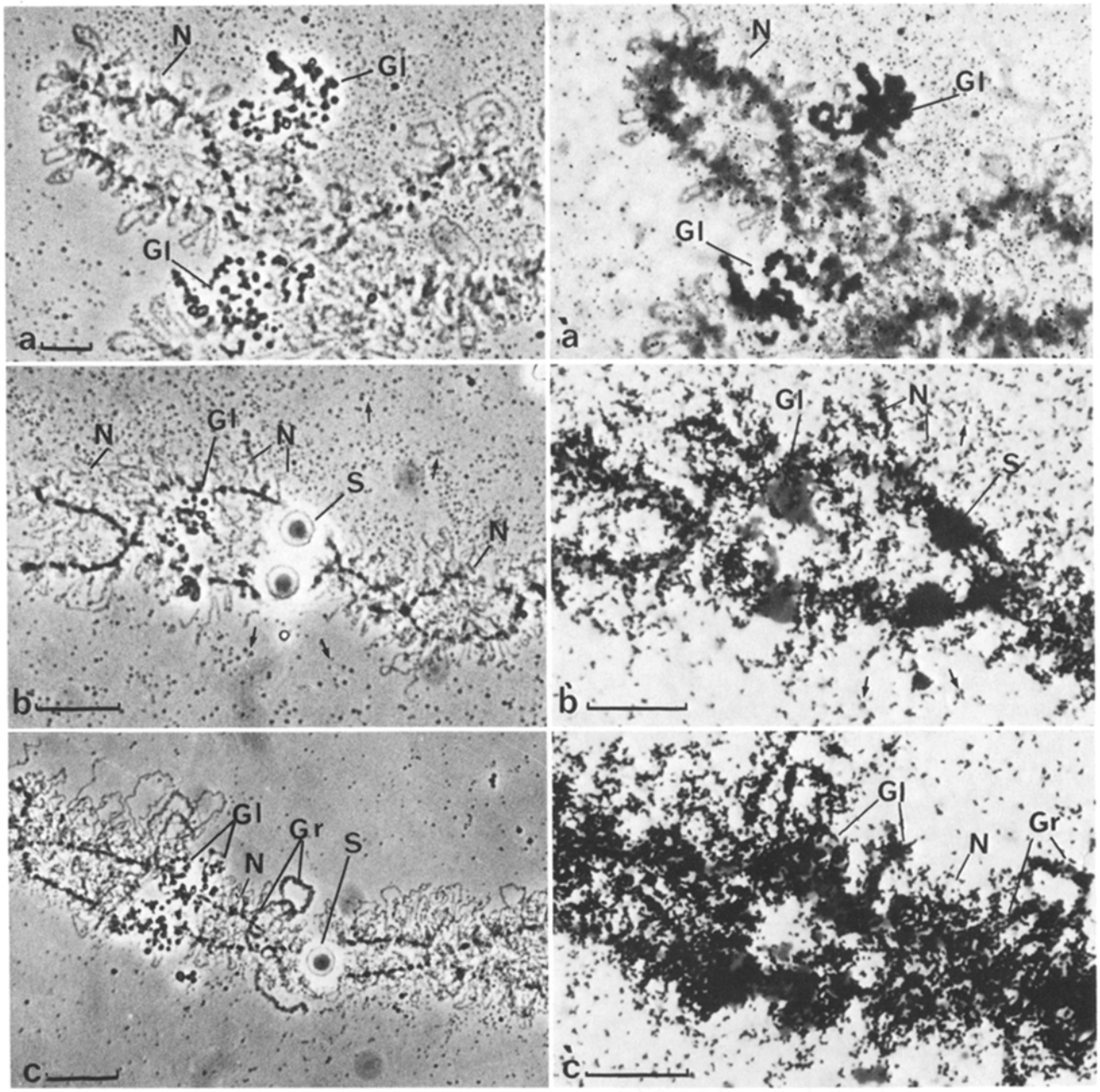


Fig. 1 a–c. RNA synthesis. Light microscope autoradiographs of lampbrush loops after oocyte incubation with tritiated cytidine and uridine. **a, a′** After 2 h of incubation. **a** Before autoradiography; **a′** after autoradiography. Slight labelling is observed both with normal loops and with globular loops. **b, b′** After 24 h of incubation. **b** Bivalent XI before autoradiography; **b′** after autoradiography. Globular loops ae partially and slightly labelled, in contrast to other loops which appear uniformly and strongly labelled. Note the high labelling of the free ribonucleoprotein particles in the nuclear sap (*arrows*). The sphere deprived of RNA and rich in proteins is devoid of any labelling. **c, c′** After 48 h of incubation. **c** Bivalent XI before autoradiography; **c′** after autoradiography. Globular loops appear strongly labelled. *Gl* globular loop, *Gr* granular loop; *N* normal loop, *S* sphere. Bars represent 10 μm in **a** and **a′**, 20 μm in **b** and **c** and 10 μm in **b′** and **c′**

their features: they were devoid of all normal loops, whereas landmark loops (granular and globular) remained developed (Fig. 6a). After 2 h of incubation, chromosomal axes were extremely condensed and foreshortened, and only globular loops remained visible, while the other landmark loops were retracted (Fig. 6b). However, the globular loops became far less developed as the time of incubation with actinomycin D increased, so that after 6 h, retraction of globular loops was complete (Fig. 6c).

The same results were obtained with α-amanitin.

Discussion

The initial conclusion which can be drawn from these studies is that globular loops are the site of RNA synthesis, as previously demonstrated for other loops in other species (Gall and Callan 1962; Izawa et al. 1963; Mancino and Barsacchi 1966; Hartley and Callan 1978). Indeed, this transcriptional activity is proved by the incorporation of RNA precursors into the globular matrix. Further proof is provided by the visualization of transcription complexes

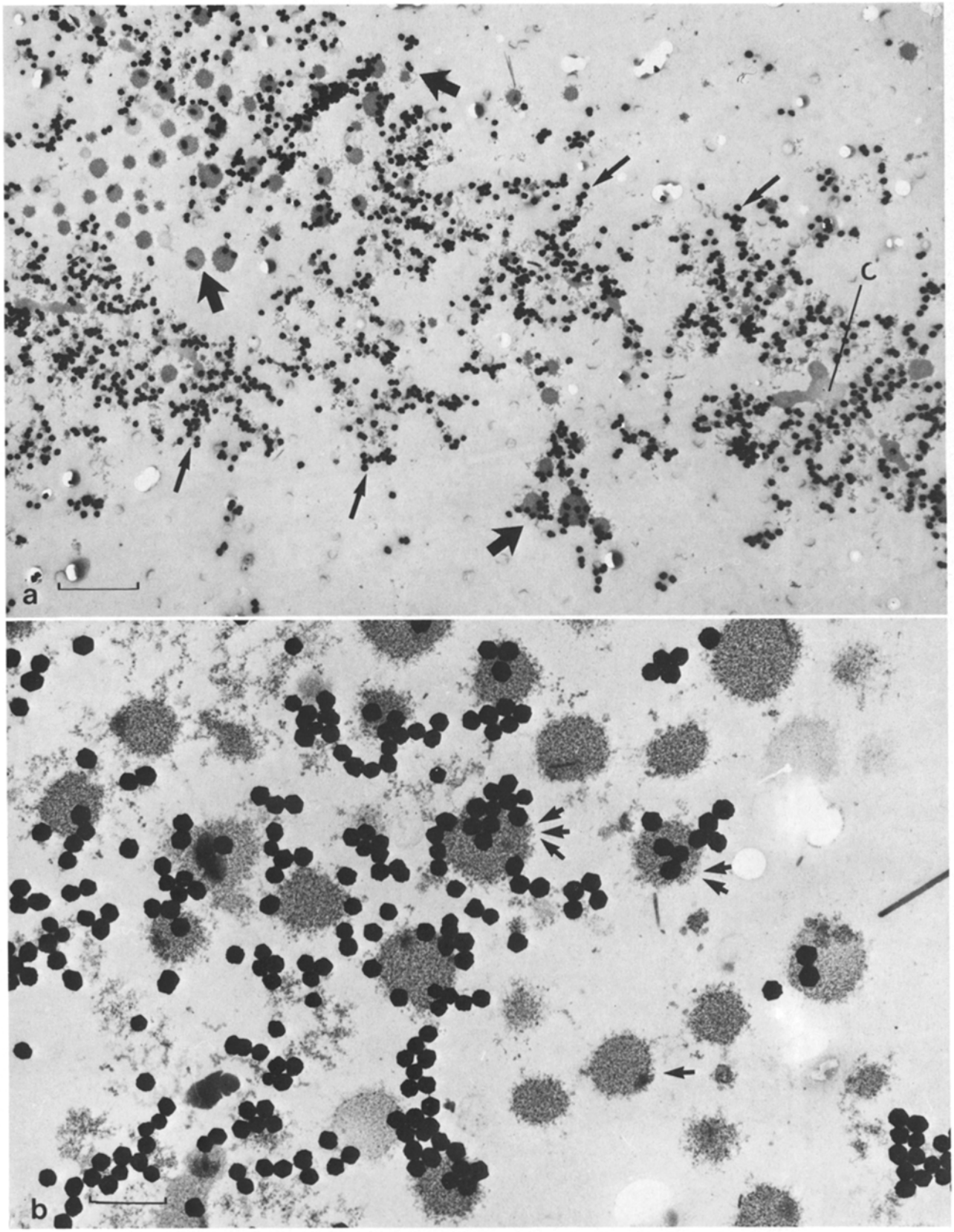


Fig. 2a, b. RNA synthesis. Electron microscope autoradiographs of lampbrush loops after 24 h of oocyte incubation with tritiated cytidine and uridine. **a** Thin section of one bivalent exhibiting normal and globular loops. Note the heterogeneous labelling of globular loops (*thick arrows*) compared to normal loops (*thin arrows*). **b** Thin section of one globular loop at high magnification showing that globules are strongly labelled (*3 arrows*), slightly labelled (*2 arrows*) or unlabelled (*1 arrow*). **c** chiasmata. Bars represent 4 μm in **a** and 0.9 μm in **b**

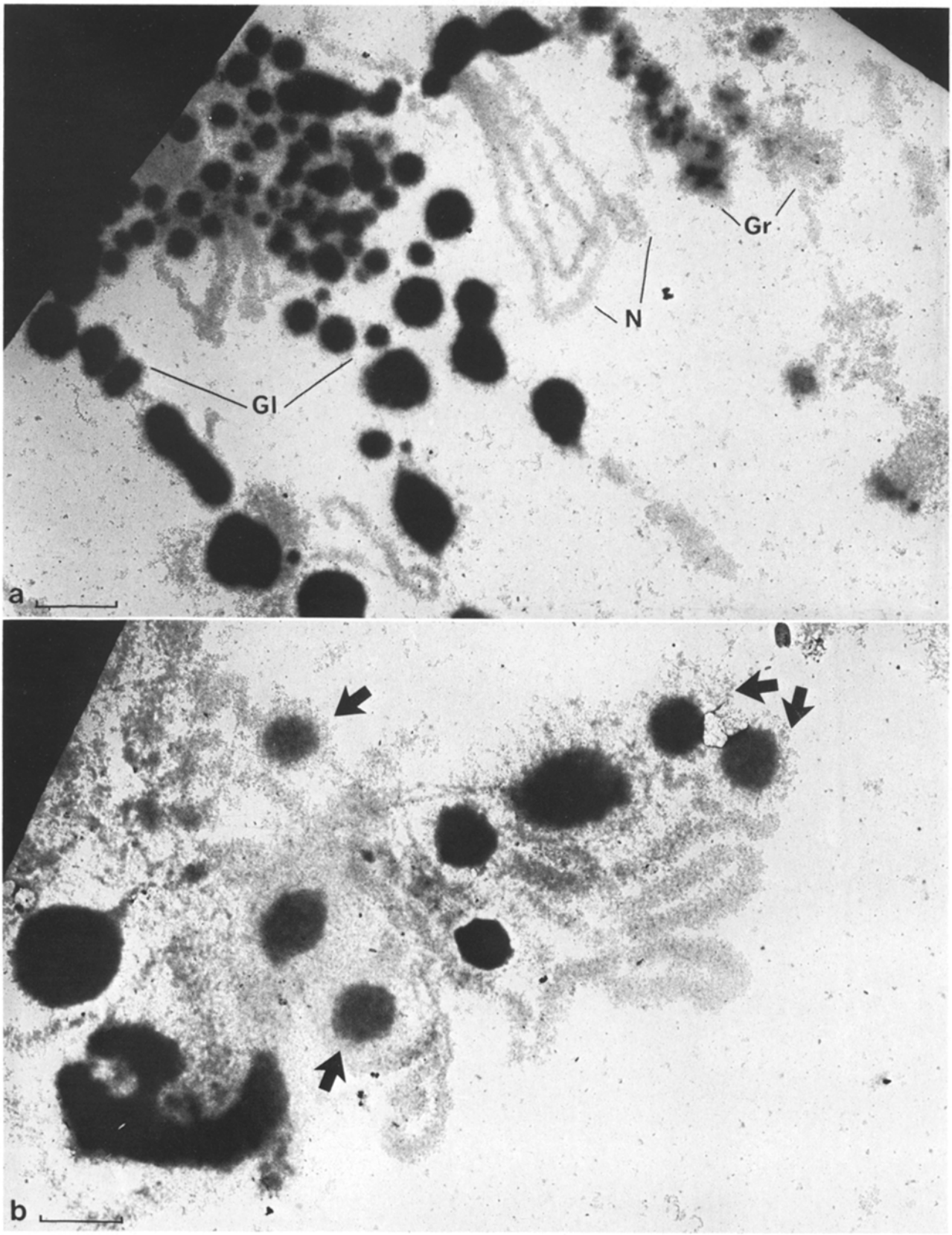
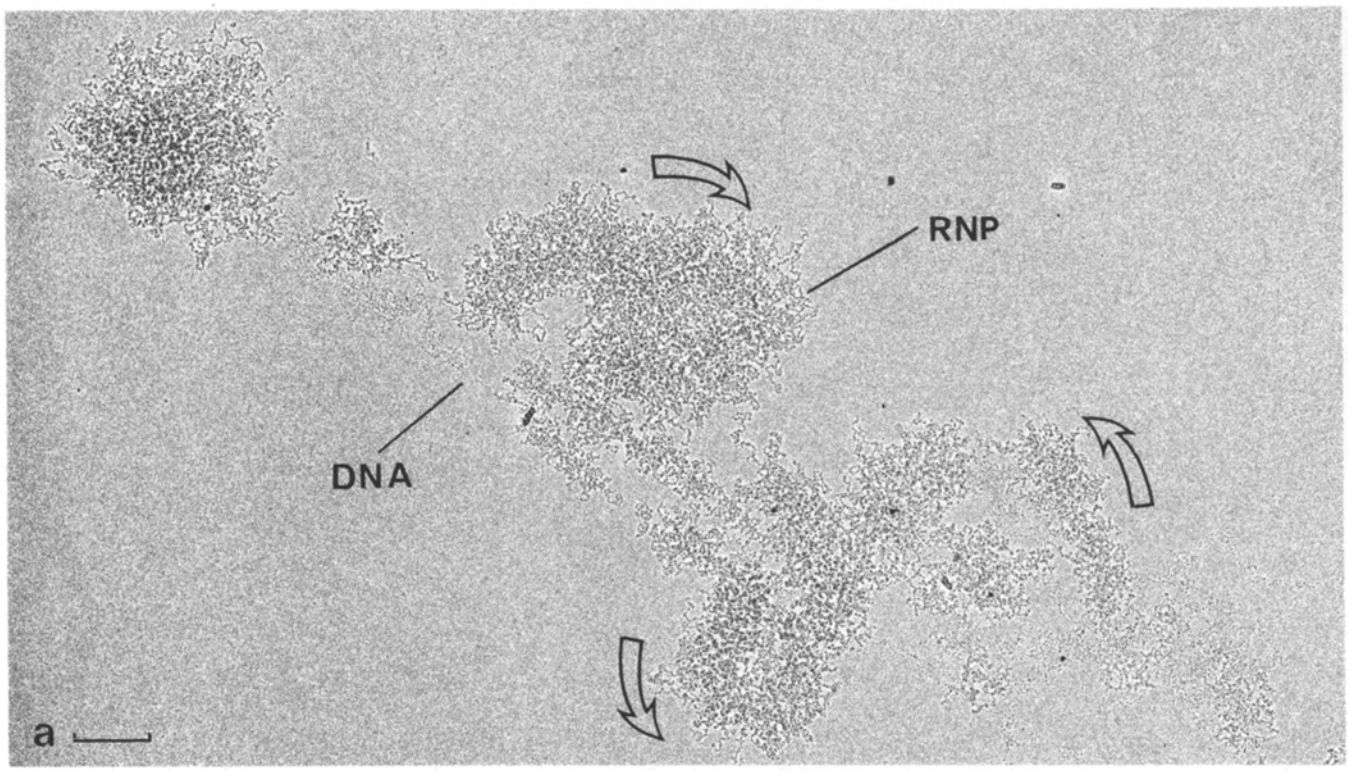


Fig. 3a, b. Chromatin spread observed on an electron microscope grid mesh. **a** Region of one chromosome isolated in 0.1 M KCl at pH 9 and exhibiting globular, granular and normal loops. Note that globular loops are far less disorganized than the others. **b** Region of one chromosome isolated in 0.05 M KCl at pH 9 and exhibiting one globular loop. Globular components are more or less disorganized and are due to a dense aggregation of ribonucleoprotein fibrils (*arrows*). *Gl* globular loop, *Gr* granular loop, *N* normal loop. Bars represent 5 μm in **a** and 4 μm in **b**



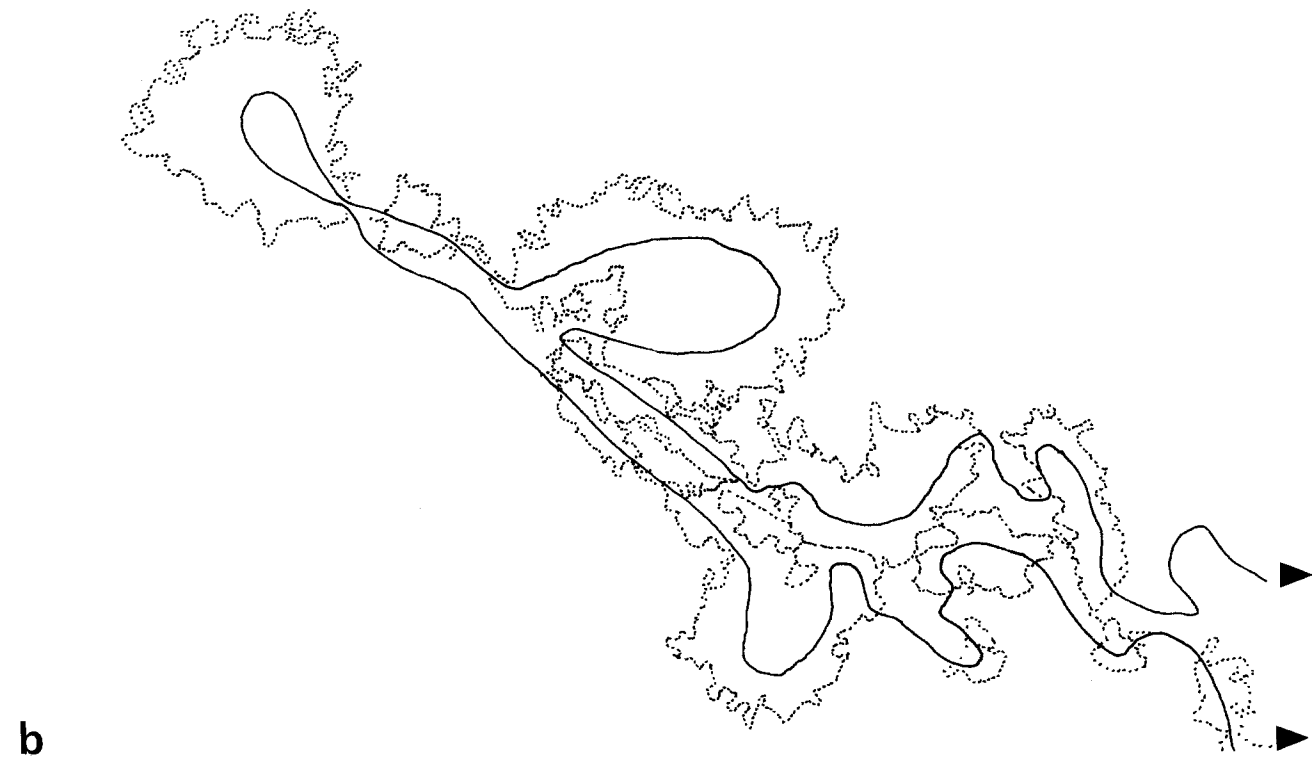


Fig. 4. **a** Chromatin spread of a globular loop isolated in 0.01 M borate buffer at pH 9.22: overview of the loop matrix showing several transcirptional units (TUs), more or less unfolded. In the unfolded TUs (*arrows*) the ribonucleoprotein (*RNP*) fibrils are distributed along the deoxyribonucleoprotein (*DNP*) axis according to an increasing gradient from the thin to the thick extremity of the TU. Note that the polarity of the RNP fibrils can be inverted from one TU to another as indicated by *arrows*. **b** Schematic representation of the globular loop shown in **a.** *Thick line* represents the DNP loop axis and *dots* represent the border line of the TUs. *Arrowheads* indicate insertions of the loop on the chromosomal axis. Bar represents 1 μm in **a**

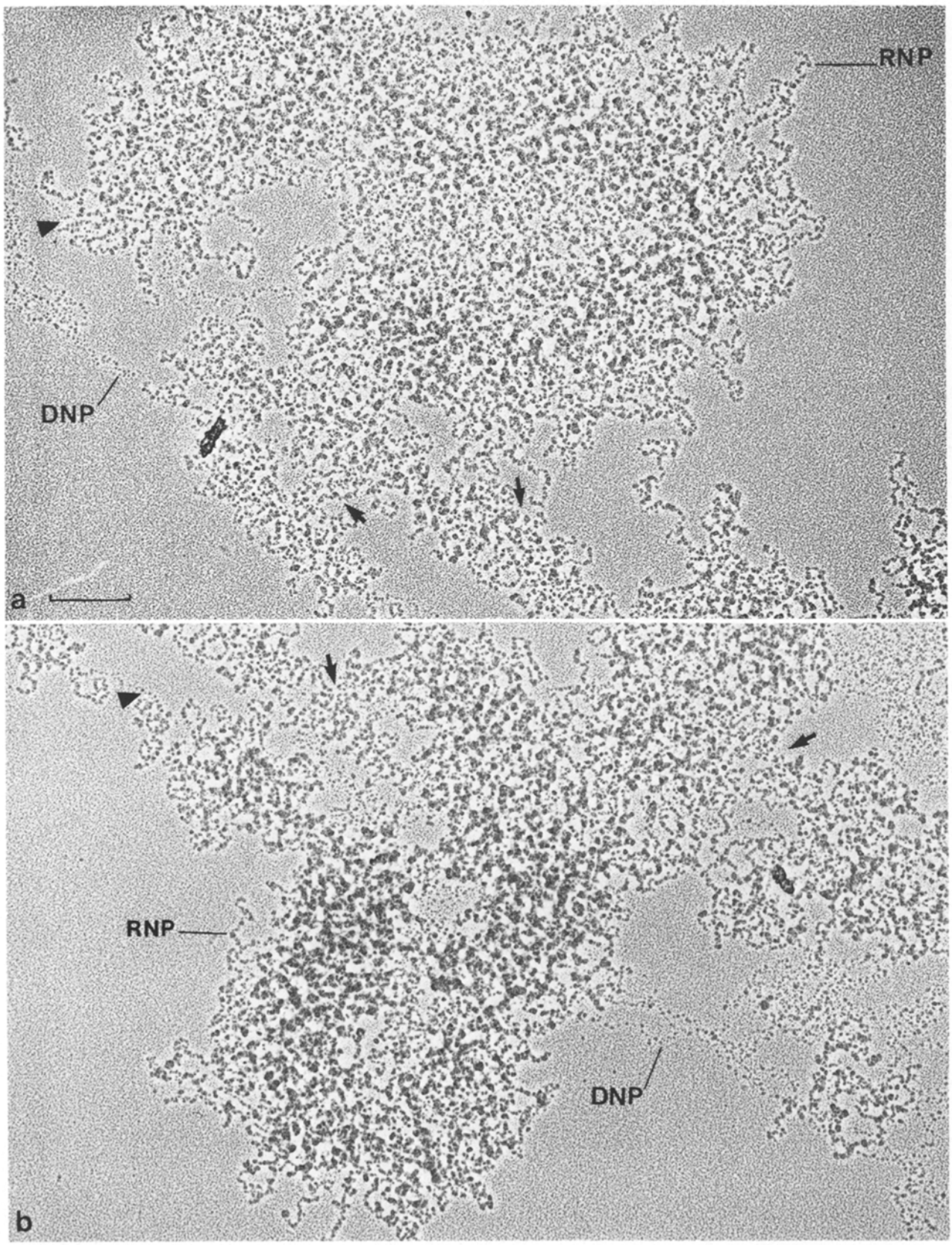


Fig. 5a, b. Chromatin spread of globular loops isolated in 0.01 M borate buffer at pH 9.22. **a** and **b**, high magnification of two of the transcriptional units (TUs) shown in the Fig. 4. *Arrowheads* indicate the thin extremity of these TUs. Note the high density of ribonucleoprotein fibrils (*RNP*). Between the TUs, the deoxyribonucleoprotein axis (*DNP*) has nucleosomal configuration. *Arrows* indicate interconnections observed between RNP fibrils of adjacent units. Bar represents 0.5 μm in **a** and **b**

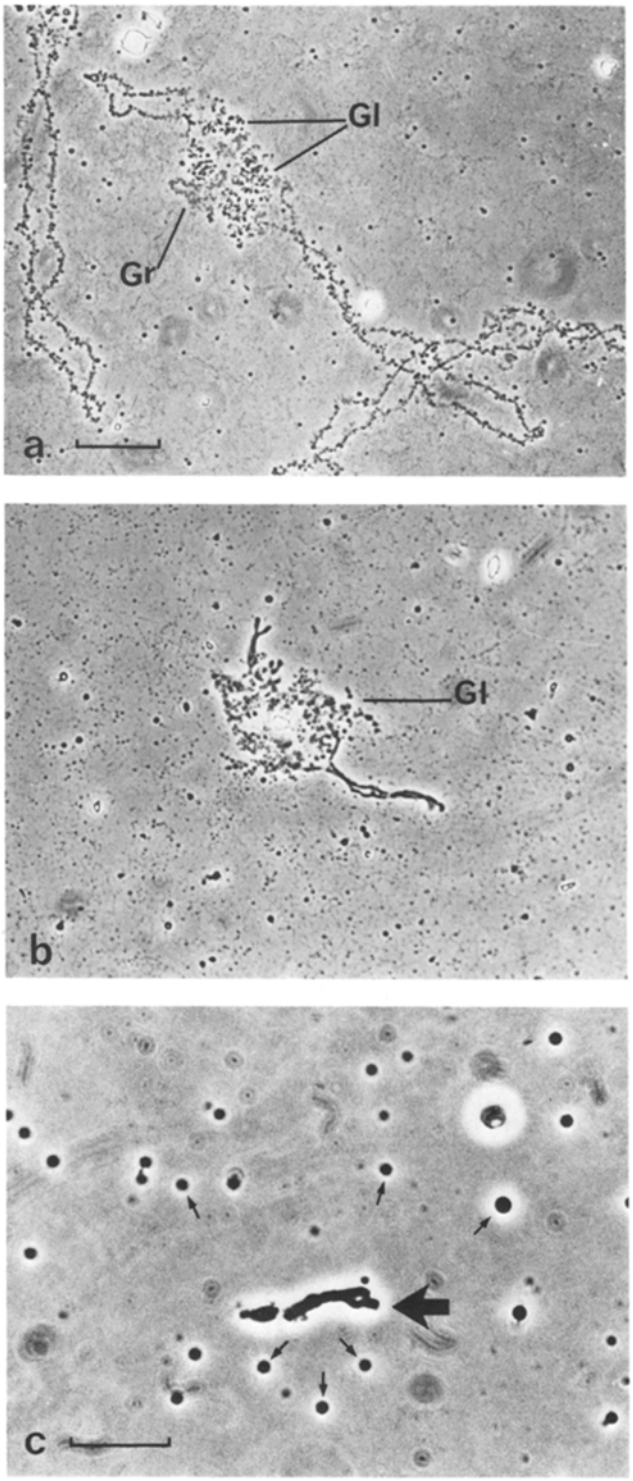


Fig. 6a–c. Inhibition of RNA synthesis in lampbrush chromosomes. Incubation of oocytes with actinomycin D at 50 μg/ml. **a** After 1 h of incubation. Bivalent VII observed in phase contrast. Normal loops are retracted, whereas granular and globular loops are still developed. **b** After 2 h of incubation. The same bivalent. Chromosomes are foreshortened and only globular loops are visible. **c** After 6 h of incubation. All bivalents exhibit the same features as this one (*thick arrow*). They are extremely foreshortened and are devoid of any type of loops. Note the numerous ribonucleoprotein particles in the nuclear sap (*small arrows*). *Gl* globular loop, *Gr* granular loop. Bars represent 40 μm in **a** and **b**, and 20 μm in **c**

in the matrix of these loops. This RNA synthesis is also indirectly confirmed by the retraction of globular loops in the presence of actinomycin D or α-amanitin. These two drugs inhibit RNA synthesis through different mechanisms: actinomycin D interferes with RNA polymerase by binding to DNA (Reich et al. 1967), while α-amanitin binds directly to RNA polymerase molecules (Cochet-Meilhac et al. 1974). It has been shown that the extension of lampbrush loops is linked to their transcriptional activity, since blocking transcription causes the retraction of loops into chromomeres (Snow and Callan 1969; Sommerville 1973; Scheer et al. 1979). However, in our transcription inhibition study, the retraction of globular loops was slow and was always the last to occur. This point will be discussed later. Snow and Callan (1969) and Mancino et al. (1971) noticed the same behaviour in the lumpy loops, giant fusing and giant granular loops of *Triturus cristatus*.

The second conclusion is that the globular loop matrices appear to be made up of several TUs, since several globules were observed in the same loop and these globules correspond to TUs as revealed by our macromolecular spread analysis. Indeed, the chromatin spreading displays several units of growing transcripts with their own initiation sites, alternating with untranscribed regions. The presence of several TUs within a single loop has previously been described in amphibian lampbrush chromosomes (Angelier and Lacroix 1975; Scheer et al. 1976) but these observations were never related to a precise type of loop. If, under moderate conditions of spreading, the configuration of the TUs is highly characteristic of the globular matrix, the growing transcripts of these TUs do not seem to exhibit a specific secondary structure as was the case for transcripts of some Y chromosomal lampbrush loops ("nooses", "threads" or "pseudonucleolus") in *Drosophila* spermatocytes (Grond et al. 1983; De Loos et al. 1984; for a general review, see Hennig 1985).

The final conclusion is that RNA synthesis occurs in the different TUs of the same loop with asynchronous variations. This conclusion is provided essentially by two autoradiographic data. First, globular loops displayed a particular pattern of incorporation: they were slightly and partially labelled after a period (24 h) when the other kinds of loops appeared uniformly and strongly labelled. They became strongly labelled after a longer incubation period (48 h). Moreover, when globular matrices were partially labelled, with globules strongly labelled, slightly labelled or unlabelled, no obvious polarity was observed at the level of their labelling. Therefore, this pattern of labelling did not seem to be sequential, as was the case with the giant granular loops of *T. cristatus* (Gall and Callan 1962) and the giant fusing loops of *T. marmoratus* (Nardi et al. 1972). These data, combined with those obtained by macromolecular spread analysis, demonstrate that globules do not simultaneously incorporate tritiated precursors, although they represent TUs. This would mean that all globules are indeed the site of RNA synthesis, but such synthesis occurs along these different TUs with variable speeds; it might be slowed down or even arrested. We can assume that this variation might be caused by: (1) a low rate of RNA polymerase molecule attachment to the initiation site, leading to a low density of RNP fibrils along the TUs; (2) a slow rate of RNA polymerase movement along these units, or (3) arrest of RNA polymerase movement without release of RNP fibrils. The first two hypotheses have also been

formulated by Hartley and Callan (1978) in order to explain the low rate of RNA synthesis in the giant lampbrush loops of *Notophthalmus viridescens*. Data obtained by our macromolecular spread analysis allow us to rule out the first hypothesis, since a strong density of RNP fibrils was observed in all TUs of globular loops. The third hypothesis, i.e. the transcription activity phase and the transcription arrest phase occurring successively in the same unit, seems to be more plausible than the second hypothesis for explaining the slow retraction of globular loops observed in our transcription inhibition experiments using actinomycin D or α-amanitin. Indeed, in these loops, only TUs in the activity phase would be affected by inhibitors, while units in the arrest phase would be affected once they were back in the activity phase. If this hypothesis is correct, then all units of the same loop must undergo these two phases during a maximal time of 6 h (the period needed to obtain complete retraction of globular loops after treatment by actinomycin D). This period appears to be very short compared to that (48 h) required to obtain total labelling of these loops, but it can be accommodated by the fact that, in the *Pleurodeles* oocyte, the pool of endogenous nucleotides is not saturated by exogenous precursors, even after 50 h of incubation (Denoulet et al. 1977). The existence of asynchronous variations in transcriptional activity has also been reported in the nucleolar TUs by Angelier et al. (1979).

In summary, the study of RNA synthesis in the globular loops of the newt *Pleurodeles* reveals a particular transcription mode. Therefore, it seems unlikely that the specific morphology of these landmark loops corresponds to the storage of transcription products, as previously suggested by Angelier et al. (1984). On the contrary, our results corroborate the interpretation of Sommerville (1981), who suggested that the morphological variability of loop matrices might be due, in part, to a particular transcription pattern. However, the question of whether there is a direct relationship between matrix morphology and a particular transcription mode remains to be elucidated.

Acknowledgements. We would like to thank Professor H.G. Callan for his helpful suggestions and criticisms. This work was supported by the "Centre National de la Recherche Scientifique" (ATP 950605).

References

Angelier N, Lacroix JC (1975) Complexes de transcription d'origines nucléolaire et chromosomique d'ovocytes de Pleurodeles waltlii et P. poireti. Chromosoma 51:323–335

Angelier N, Lavaud A (1982) Improvements in the ultrastructural approach of lampbrush chromosomes of amphibians. IX. Congress of the ISDB, Basel, Switzerland, August 1981. In: Burger A, Weber MM (eds) Embryonic development. 85A: Genetic aspects. Alan Liss, Inc, New York, pp 187–198

Angelier N, Bouteille M, Charret R, Curgy JJ, Delain E, Fakan S, Geuskens M, Guelin M, Lacroix JC, Laval M, Steinert G, Van Assel S (1976) Detection of nucleic acids by means of ultrastructural autoradiography. J Microscopie Biol Cell, 27:215–231

Angelier N, Hemon D, Bouteille M (1979) Mechanisms of transcription in nucleoli of amphibian oocytes as visualized by high resolution autoradiography. J Cell Biol 80:277–290

Angelier N, Paintrand M, Lavaud A, Lechaire JP (1984) Scanning electron microscopy of amphibian lampbrush chromosomes. Chromosoma 89:243–253

Bonnanfant-Jaïs ML, Mentré P (1983) Study of oogenesis in the newt Pleurodeles waltlii M.I. Ultrastructural study of the different stages of oocyte development. J Submicrosc Cytol 15:453–478

Bonnanfant-Jaïs ML, N'Da E, Penrad-Mobayed M, Angelier N (1985) Amphibian lampbrush chromosome loops: correlative light microscopy, transmission electron microscopy and scanning electron microscopy observations. Scan Elec Microsc II:889–896

Bonnanfant-Jaïs ML, N'Da E, Penrad-Mobayed M, Angelier N (1986) Structure of landmark loop ribonucleoprotein matrices in Pleurodeles waltlii lampbrush chromosomes visualized by scanning electron microscopy. J Cell Sci 81:29–42

Callan HG (1963) The nature of lampbrush chromosomes. Int Rev Cytol 15:1–34

Callan HG (1982) Lampbrush chromosomes. Proc R Soc Lond [Biol] 214:417–448

Callan HG, Lloyd L (1960) Lampbrush chromosomes of crested newts Triturus cristatus Laurenti. Philos Trans R Soc Lond [Biol] 243:135–219

Cochet-Meilhac M, Chambon P (1974) Animal DNA-dependent RNA polymerases: 11. Mechanism of the inhibition of RNA polymerases B by amatoxins. Biochim Biophys Acta 353:160–184

De Loos F, Dijkhof R, Grond CJ, Hennig W (1984) Lampbrush chromosome loop-specificity of transcript morphology in spermatocyte nuclei of Drosophila hydei. EMBO J 3:2845–2849

Denoulet P, Muller JP, Lacroix JC (1977) RNA metabolism in amphibian oocytes. I. Chromosomal premessenger RNA synthesis. Biol Cell 28:101–108

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 USA 48:562–570

Gall JG, Stephenson EC, Erba HP, Diaz MO, Barsacchi-Pilone G (1981) Histone genes are located at the sphere loci of newt lampbrush chromosomes. Chromosoma 84:159–171

Gounon P, Karsenti E (1981) Involvement of contractile proteins in the change in consistency of oocyte nucleoplasm of the newt Pleurodeles waltlii. J Cell Biol 88:410–421

Grond CJ, Siegmund I, Hennig W (1983) Visualization of a lampbrush loop-forming fertility gene in Drosophila hydei. Chromosoma 88:50–56

Hartley SE, Callan HG (1978) RNA transcription on the giant lateral loops of the lampbrush chromosomes of the American newt Notophthalmus viridescens. J Cell Sci 34:279–288

Hennig W (1985) Spermatogenesis in Drosophila hydei. Adv Genet 23:179–234

Izawa M, Allfrey VG, Mirsky AE (1963) The relationship between RNA synthesis and loop structure in lampbrush chromosomes. Proc Natl Natl Acad Sci USA 49:544–551

Lacroix JC (1968) Etude descriptive des chromosomes en écouvillon dans le genre Pleurodeles (Amphibien, Urodèle). Ann Embryol Morphol 1:179–202

Larra F, Droz B (1970) Techniques autoradiographiques et leurs applications à l'étude du renouvellement de constituants cellulaires. J Microscop 9:845–880

Malcolm DB, Sommerville J (1977) The structure of nuclear ribonucleoprotein of amphibian oocytes. J Cell Sci 24:143–165

Mancino G, Barsacchi G (1965) Le mappe dei cromosomi "Lampbrush" di triturus (Anfibi, Urodeli) I. Triturus alpestris apuanus. Caryologia 18:637–665

Mancino G, Barsacchi G (1966) Le mappe dei cromosomi lampbrush di Triturus (Anfibi, Urodeli) II. Triturus helveticus helveticus. Riv Biol19:311–351

Mancino G, Nardi I, Corvaja N, Fiume L, Marinozzi (1971) Effects of α-amanitin on Triturus lampbrush chromosomes. Exp Cell Res 64:237–239

Miller OL, Bakken AH (1972) Morphological studies of transcription. Acta Endocrinol (Suppl) 68:155–177

Miller OL, Hamkalo BA (1972) Visualization of RNA synthesis on chromosomes. Int Rev Cytol 33:1–23

Nardi I, Ragghianti N, Mancino G (1972) Characterization of the lampbrush chromosomes of the marbled newt Triturus marmoratus. Chromosoma 37:1–22

N'Da E, Bonnanfant-Jaïs ML, Penrad-Mobayed M, Angelier N (1986) Size uniformity of ribonucleoprotein matrix particles in loops of Pleurodeles waltlii lampbrush chromosomes visualized by electron microscopy. J Cell Sci 81:17–27

Old RW, Callan HG, Gross KW (1977) Localization of histone gene transcripts in newt lampbrush chromosomes by in situ hybridization. J Cell Sci 27:57–79

Reich E, Cerami A, Ward DC (1976) Actinomycin. In: Gottlieb D, Shaw PD (eds) Antibiotics, mechanism of action, vol 1. Springer-Verlag, Berlin-Heidelberg-New York, pp 715–725

Scheer U, Franke WW, Trendelenburg MF, Spring H (1967) Classification of loops of lampbrush chromosomes according to the arrangement of transcriptional complexes. J Cell Sci 22:503–519

Scheer U, Sommerville J, Bustin M (1979) Injected histone antibodies interfere with transcription of lampbrush chromosomes loops in oocytes of Pleurodeles. J Cell Sci 40:1–20

Snow MHL, Callan HG (1969) Evidence for a polarized movement of the lateral loops of newt lampbrush chromosomes during oogenesis. J Cell Sci 5:1–25

Sommerville J (1973) Ribonucleoprotein particles derived from the lampbrush chromosomes of newt oocytes. J Mol Biol 78:487–503

Sommerville J (1981) Immunolocalization and structural organization in nascent RNP. In: Bush H (ed) Cell nucleus, vol VIII. Academic Press, New York, pp 1–52

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

Received February 2, 1986 / in revised form July 30, 1986
Accepted by M. Trendelenburg