

Chromosomes of amphibian oocytes as a model for gene expression: significance of lampbrush loops¹

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ABSTRACT Amphibian lampbrush chromosome loops exhibit morphological variability in their RNP matrix. The biological significance of such variability remains unknown. In order to approach this problem, the structural organization of each RNP matrix type was analyzed in relation to transcriptional and post-transcriptional processes. First, autoradiographic and transcription inhibition studies in conjunction with macromolecular spread analysis revealed a particular transcription pattern in the most typical loops, i.e. the globular loops. This pattern was characterized by asynchronous variations in RNA synthesis in the different transcription units present in a given loop. Second, morphological and experimental studies provided evidence that the typical morphologies of different RNP matrices were interconvertible and that the differences between the different RNP matrices resulted from various degrees of tightness in packaging of transcription products. In particular, analysis of thermic-shock-induced changes in the structure of lampbrush chromosomes enabled us to visualize the progressive disorganization of dense RNP matrices into globular, granular and normal matrices. Furthermore, these studies suggested that changes in post-transcriptional processes might play a determining role in the specific morphology of the loops. In particular, the kinetics of each of these different processes, related to one another and/or proteins specific to one or another of these processes, might determine the morphological appearance of the loops. The immunological approach revealed that specific nuclear proteins might therefore interfere with each of these processes. Third, the problem of a possible relationship between the specific morphologies of lateral loops and the expression of particular DNA sequences was approached at the molecular level.

KEY WORDS: *amphibian oocytes, gene expression, lampbrush chromosomes, landmark loops*

Introduction

Lampbrush chromosomes of oocytes provide a system of considerable value in visualizing genomic DNA sequences expressed during oogenesis. Their characteristic features, the lateral loops, are directly related to the transcription process. They represent regions of intense RNA synthesis, and nascent RNA transcripts associate with proteins to form a ribonucleoprotein (RNP) matrix. In all Urodele amphibians, the majority of lateral loops conform to a normal type in which RNP matrices show the same organization. Some loops differ from normal loops in the size and organization of their RNP matrices, and display a distinct morphology. These loops are always observed at the same sites and thus constitute obvious landmarks which allow identification of bivalents in the oocyte

genome of many urodele species (reviewed in Callan, 1986).

Lampbrush chromosome function during oogenesis and embryonic development is far from being elucidated; in particular, the biological significance of morphological variability in lampbrush loops is unknown to date.

Our interest is focused on the functional significance of such characteristic loops. Functional interpretation of such structures requires a better understanding of their structural organization in relation to different events occurring at the level of each lampbrush loop. Each loop of each type serves as a template for RNA

Abbreviations used in this paper: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; RNP, ribonucleic protein; kD, kilodalton

¹This article is dedicated to the memory of Professor Daniel Sandoz, deceased on March 6, 1990. Professor D. Sandoz was the Director of the Centre de Biologie Cellulaire.

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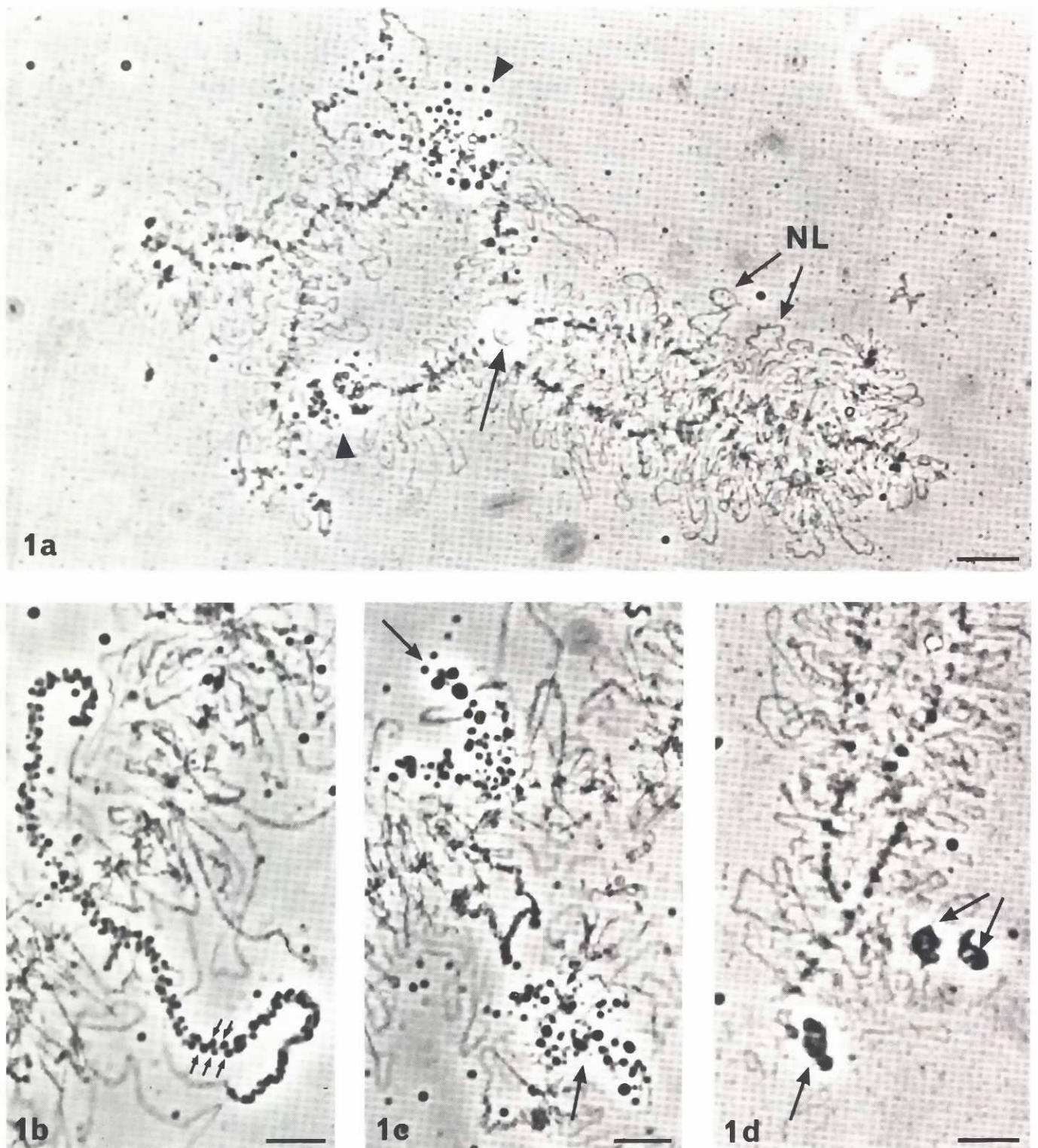


Fig. 1. *Pleurodeles waltl* lampbrush chromosomes as seen by phase contrast. In (a) bivalent XI characterized by two kinds of landmarks: a sphere at the midpoint (arrow) and two homologous globular loops (arrowheads). In (b, c, d) details of landmarks: (b) pair of granular loops showing an evident polarity of their RNP matrix. Note the zig-zag arrangement of the granules (arrows); in (c) homologous pairs of globular loops (arrows); in (d) homologous pairs of dense matrix loops (arrows); (NL) normal loop; (from N'Da et al., 1986). Bars represent 10 μ in (1a) and 4 μ in (1b, c, d).

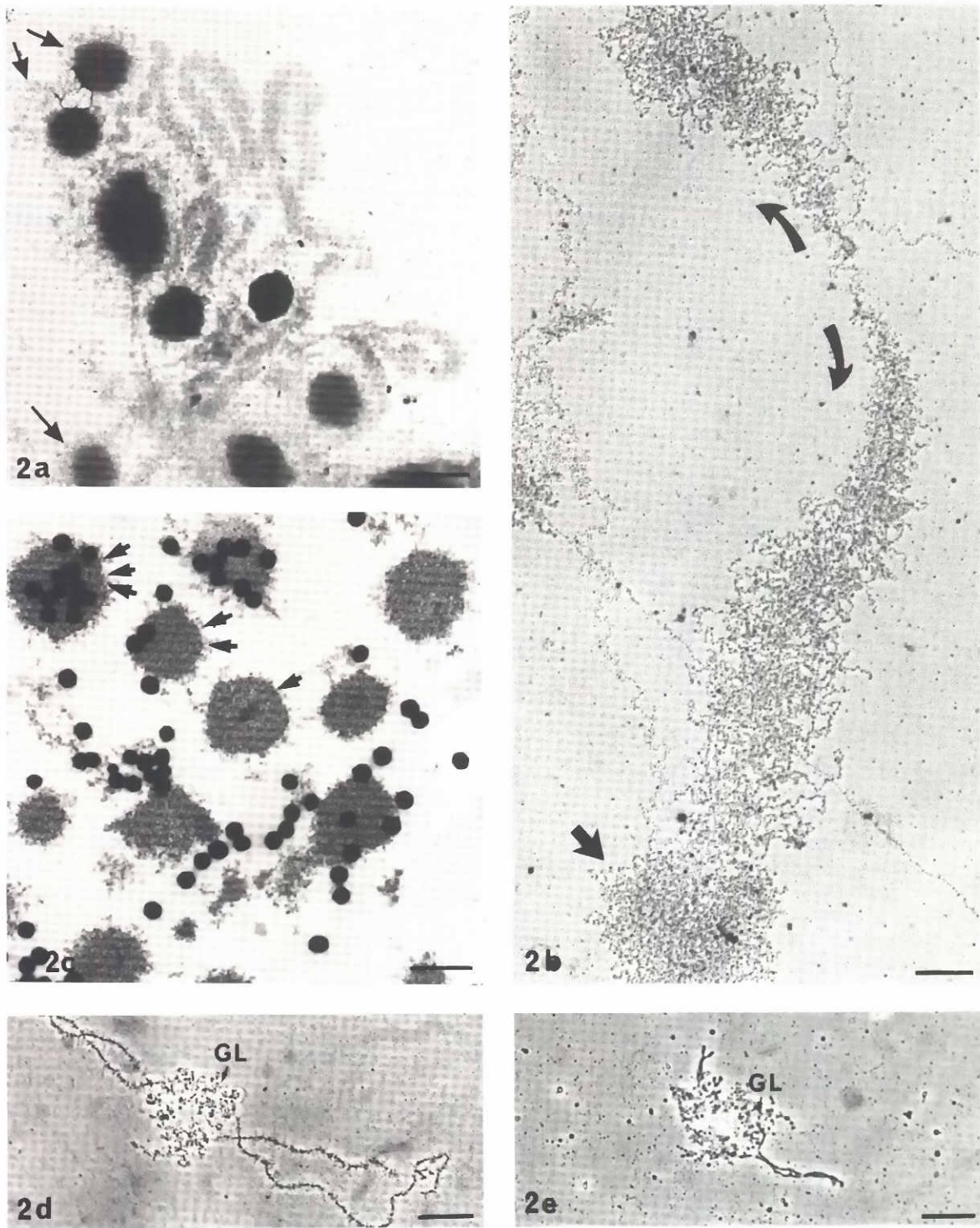


Fig. 2. Visualization of transcription in globular loops. (a) Region of one chromosome isolated in 0.05M KCl at pH 9 and exhibiting one globular loop (electron microscopy); globular components are more or less disorganized and are due to a dense aggregation of RNP fibrils (arrows). Chromatin spreads after gradual disorganization of lampbrush chromosomes using Angelier and Lavaud's method (1982). (b) Globular loop isolated in 0.01M borate buffer at pH 9.22 showing several transcription units with opposite polarities (curve arrows). When transcription units are unfolded they are packed into globules (arrow). Chromatin spreads using Miller's procedure. (c) Electron microscope autoradiograph of one globular loop after 24h of oocyte incubation with tritiated uridine and cytidine, thin section showing that globules are strongly labeled (3 arrows), slightly labeled (2 arrows) or unlabeled (1 arrow). (d, e) Inhibition of RNA synthesis in lampbrush chromosomes. Actinomycin D at 50 $\mu\text{g}/\text{ml}$. (d) Bivalent VII from oocyte control. (e) The same bivalent after 2h incubation; chromosomes are foreshortened and all loops have disappeared except globular loops (GL), which only disappear 6h later. Bars represent 5 μ in (a), 0.5 μ in (b), 2 μ in (c) and 40 μ in (d) and (e).

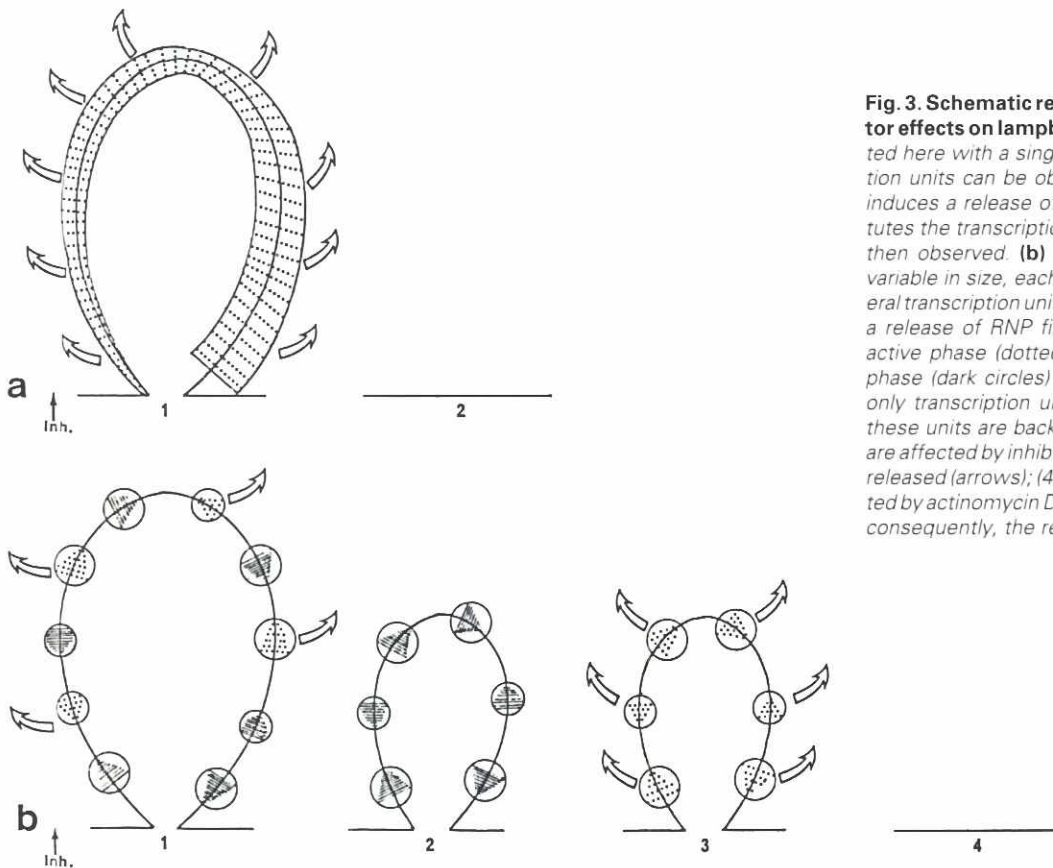


Fig. 3. Schematic representation of transcription inhibitor effects on lampbrush loops. (a) Normal loop represented here with a single transcription unit (several transcription units can be observed); (1) inhibition of transcription induces a release of all RNP fibrils (arrows) which constitutes the transcription unit(s); (2) a retraction of the loop is then observed. **(b)** Globular loops with several globules variable in size, each globule corresponding to one or several transcription units; (1) inhibition of transcription induces a release of RNP fibrils (arrows) of transcription units in active phase (dotted circles); transcription units in arrest phase (dark circles) are unaffected by actinomycin D; (2) only transcription units in arrest phase remain; (3) Once these units are back in activity phase (dotted circles) they are affected by inhibitors of transcription and RNP fibrils are released (arrows); (4) all transcription units have been affected by actinomycin D and all RNP fibrils have been released; consequently, the retraction of the loop occurs.

polymerase II dependent transcription, and consists of nascent heterogeneous nuclear RNA and proteins that must process, package or transport this RNA. The morphological features of each loop are genetically determined (reviewed in Callan, 1986).

Several questions concerning the significance of such morphological variability can therefore be posed. Is the morphology of the RNP matrix for each loop type: (i) related to a particular transcriptional activity? (ii) dependent on the post-transcriptional activity? (iii) inherent in a particular nucleic sequence?

This article concentrates on these questions and reports our own results concerning the structural and functional organization of lampbrush loops in a urodele, the newt *Pleurodeles waltl*.

Pleurodeles lampbrush chromosomes

As with all oocyte chromosomes of urodele amphibians, lampbrush chromosomes of the newt *Pleurodeles waltl* display several hundred lateral DNA loop pairs all along the chromosome axis. These loops vary in size and in the morphology of their matrices. In addition to lateral loops termed 'normal', giant, granular, globular, dense matrix loops and spheres are observed in constant, reproducible sites along the chromosome axis and thus constitute

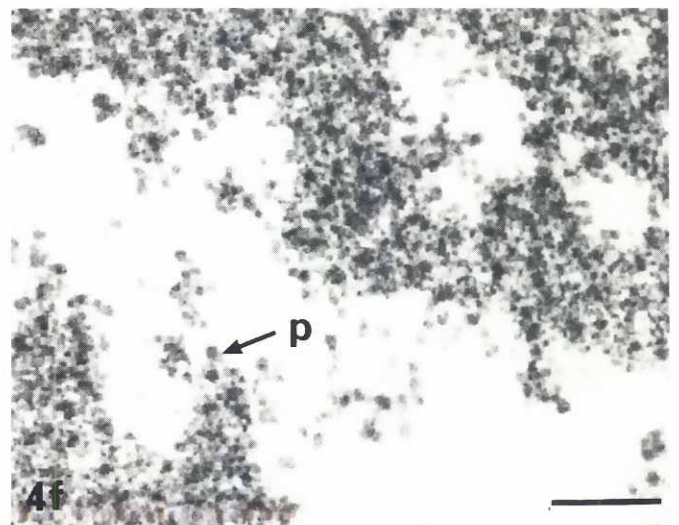
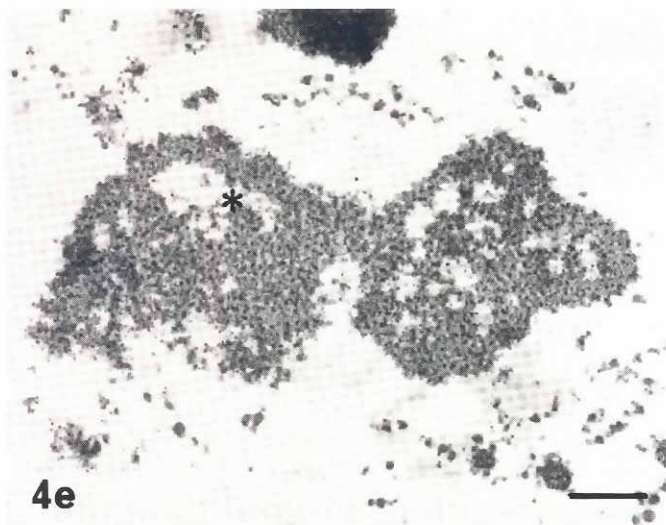
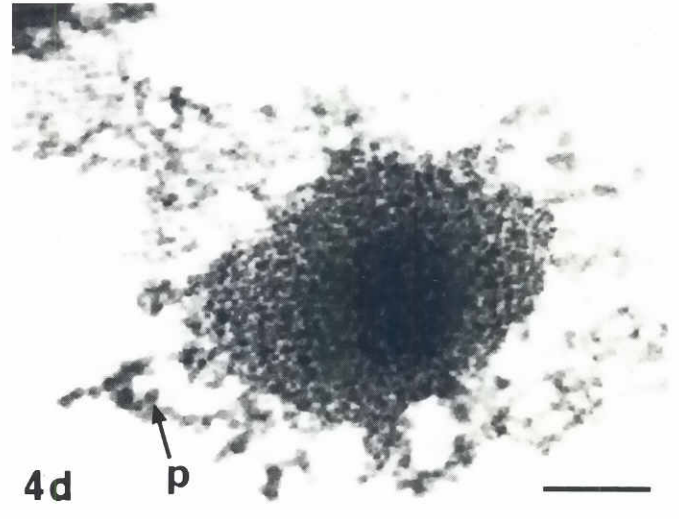
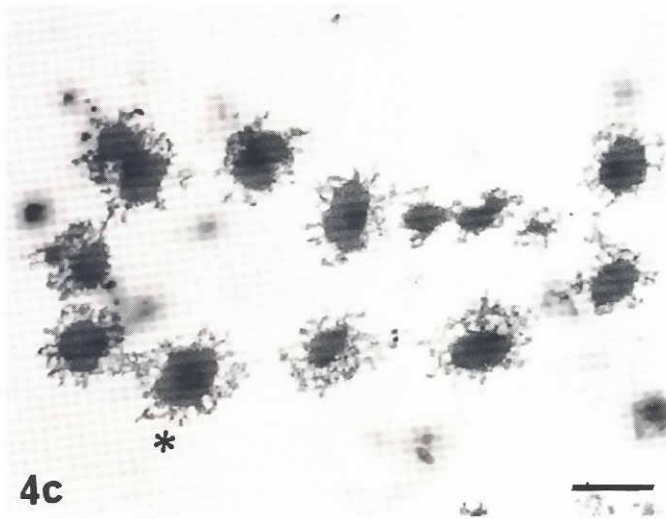
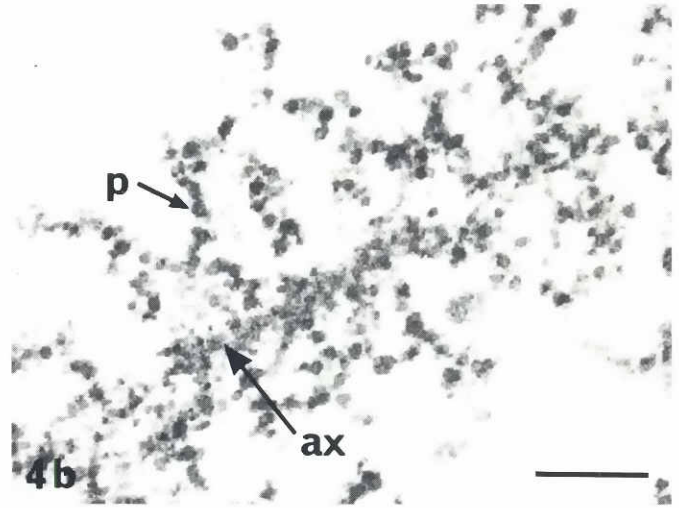
different kinds of typical 'landmarks' which allow identification and mapping of the twelve bivalents of the oocyte karyotype (Fig. 1, see also Lacroix, 1968). Examination in phase contrast of these different kinds of loops shows that (i) giant loops exhibit the same kind of RNP matrix as normal ones; (ii) the matrix of granular loops is composed of 1-3 μm granules presenting a zig-zag arrangement; (iii) the RNP matrix of globular loops is composed of very dense globules 3-4 μm in diameter; (iv) the matrix of dense matrix loops is reduced to a few dense lobules (Fig. 1a, b, c, d; see also N'Da *et al.*, 1986; N'Da, 1989).

The structural organization of these different kinds of RNP matrix was studied in relation with (i) transcriptional process, (ii) post-transcriptional processes, (iii) the nature of DNA sequences.

Evidence for a particular mode of transcription in 'landmark' loops

In order to ascertain whether special transcriptional activity could be observed in these landmark loops, RNA synthesis was analyzed in the most typical landmark, i.e. the globular loops. To approach this question, autoradiographic and transcription inhibition studies were carried out in conjunction with macromolecular

Fig. 4. Electron micrographs of thin sectioned landmark loops. (a) Normal, giant loops. **(b)** Detail of normal loop matrix showing RNP fibrils extending from the loop axis (ax); **(c)** globular loops; **(d)** portion of the globular loop shown in **(c)** (asterisk); **(e)** dense loop. **(f)** Portion of the dense loop shown in **(e)** (asterisk). The RNP matrices of all kind of loops show the same basic structure: a 30 nm particle 'p' in **(b, d, f)**. From N'Da *et al.* (1986). Bars represent 1 μm in 4a, c, e and 0.25 μm in (b, d, f).



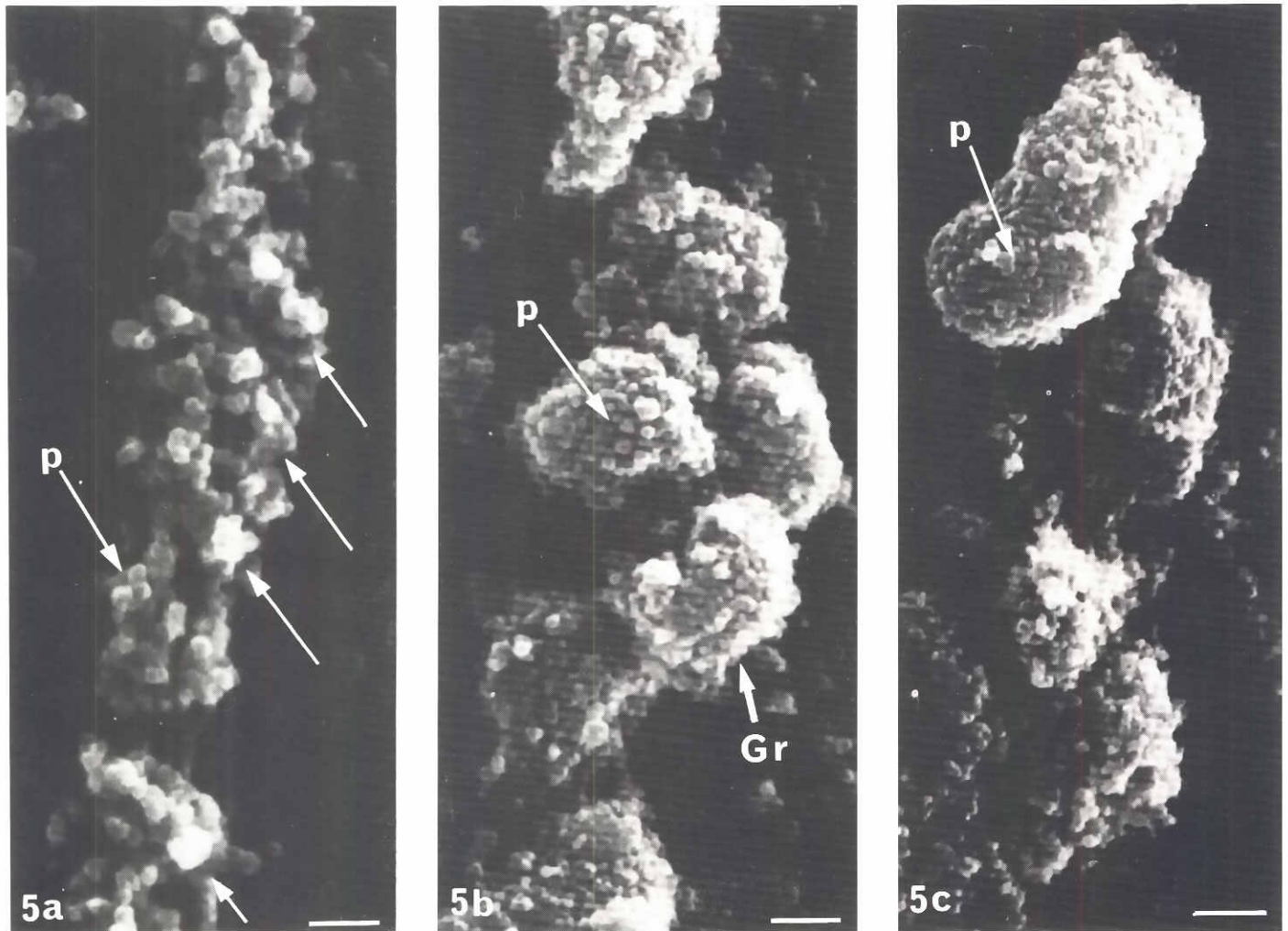


Fig. 5. SEM of lampbrush chromosome spreads. RNP matrix organization of normal (a), granular (b) and dense (c) loops. RNP particles (p) of the same size (50 nm including the 20 nm gold coat) are observed in all kinds of RNP matrices. In the normal loop (a) RNP particles display a helical arrangement around the loop axis (arrows). In the granular loop (b), each granule (Gr) is composed of RNP particles (p) and is formed by the packaging of adjacent transcripts; granules exhibit a helical arrangement around the loop axis. The dense loop (c) exhibits a twisted appearance; the RNP matrix forms a continuous sheath around the loop axis. Bars represent 0.2 μm in 5a and 0.5 μm in (b) and (c).

spread analysis in electron microscopy using a modified version of Miller's procedure (Angelier and Lavaud, 1982; see also Angelier and Lacroix, 1975, Angelier *et al.*, 1986). The results allowed us to draw several important conclusions which can be summarized as follows:

Globular loops are the site of RNA synthesis, as previously demonstrated for other loops in other species (reviewed in Callan, 1986). This transcriptional activity was proven by autoradiography in light microscopy after incorporation of RNA precursors into the globular matrix (see Penrad-Mobayed *et al.*, 1986). These globular matrices appeared to be made up of several transcription units, as revealed by macromolecular spread analysis (Fig. 2a, b). RNA synthesis appears to occur in the different transcription units of the same globular loop with asynchronous variations. This important conclusion was provided by autoradiographic data combined with those obtained by macromolecular spread analysis. Indeed, glo-

bules do not simultaneously incorporate tritiated precursors (Fig. 2c; see also Penrad-Mobayed *et al.*, 1986) although they represent transcription units. Globular loops display a particular pattern of incorporation; they are poorly and only partially labeled after a 24 h period whereas the other kinds of loops appear uniformly and strongly labeled. This means that all globules are indeed the sites of RNA synthesis, but such synthesis occurs along these different transcription units at variable speeds. It might be slowed down or even arrested. Several hypotheses were advanced for interpreting this variation (see Penrad-Mobayed *et al.*, 1986). In particular, it was assumed that this variation might be caused by an arrest in RNA polymerase movement along these transcription units without release of RNP fibrils. This hypothesis, i.e. the transcription activity phase and the transcription arrest phase occurring successively in the same unit, seemed to be the most plausible, in particular for explaining the slow retraction of globular loops observed in our

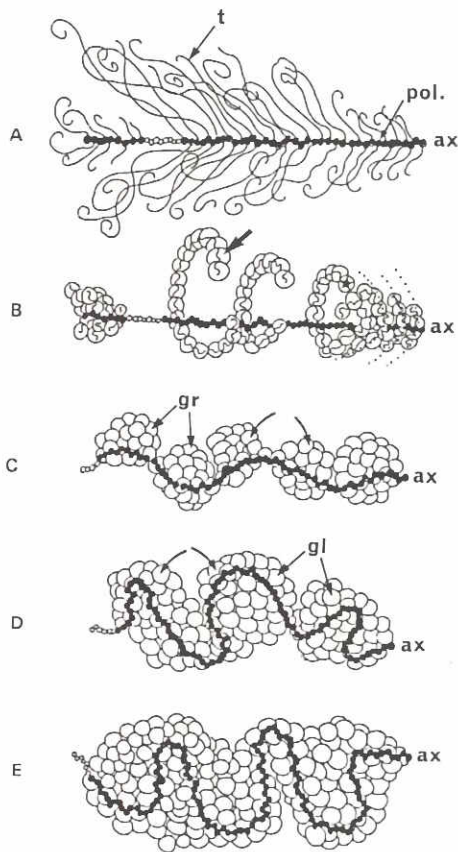


Fig. 6. Schematic representation of the progressive compaction of the RNP matrix and concomitant coiling of the loop axis. (A) Transcription figure visualized according to Miller's procedure. Thin arrow shows RNA polymerase (pol.) at the basis of each transcript (t). (B) The normal loop RNP matrix. RNP transcripts (thick arrow) coil around the loop axis (ax) in such a way that 30 nm RNP particles (star) of the transcripts show a helical arrangement (dotted lines). (C) The granular loop RNP matrix. Interactions between RNP particles of adjacent transcripts induce their aggregation into granules (gr). This transcript aggregation leads the loop axis (ax) to curve in at the level of each granule. (D) The globular loop RNP matrix. Aggregates of adjacent RNP transcripts grow progressively larger. The coiling of the loop axis (ax) becomes more pronounced as the process of compaction involves RNP transcripts situated further and further apart; (gl) globule. (E) The dense loop RNP matrix. The compaction of the RNP transcripts is maximum. The matrix forms a continuous sheath around the loop axis (ax) which exhibits a concomitant extreme coiling (from Bonnanfant-Jais et al., 1986).

transcription inhibition experiments using actinomycin D or α -amanitin (Fig. 2d, c; see also Penrad-Mobayed *et al.*, 1986). Indeed, in these loops, only transcription units 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 (Fig. 3).

In conclusion, landmarks such as globular loops exhibit a particular transcription pattern. However, the question of whether there is a direct relationship between matrix morphology and this particular transcription mode remains to be elucidated.

Evidence for a relationship between matrix morphology and post-transcriptional activities

Morphological evidence

Electron microscopy (EM) and scanning electron microscopy (SEM) studies of the different types of loops provided good evidence that the differences between the RNP matrices resulted from the various degrees of tightness in the packaging of the transcription products.

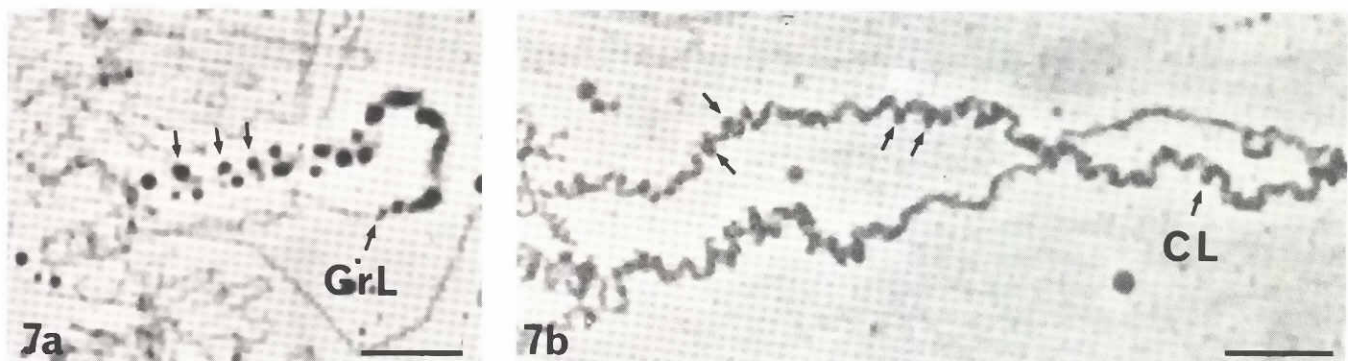


Fig. 7. Cold-induced changes in lampbrush loops. (a) Typical granular loops (GrL) from control lampbrush chromosomes at 20°C; note the zig-zag arrangement of granules (arrows). (b) After 8 days at 8°C, typical hyperdeveloped 'cold loops' (CL) are observed in place of granular loops; some residual granules (arrows) are recognizable; note the remaining spiraled organization of the RNP matrix. Bars represent 5 μ m.

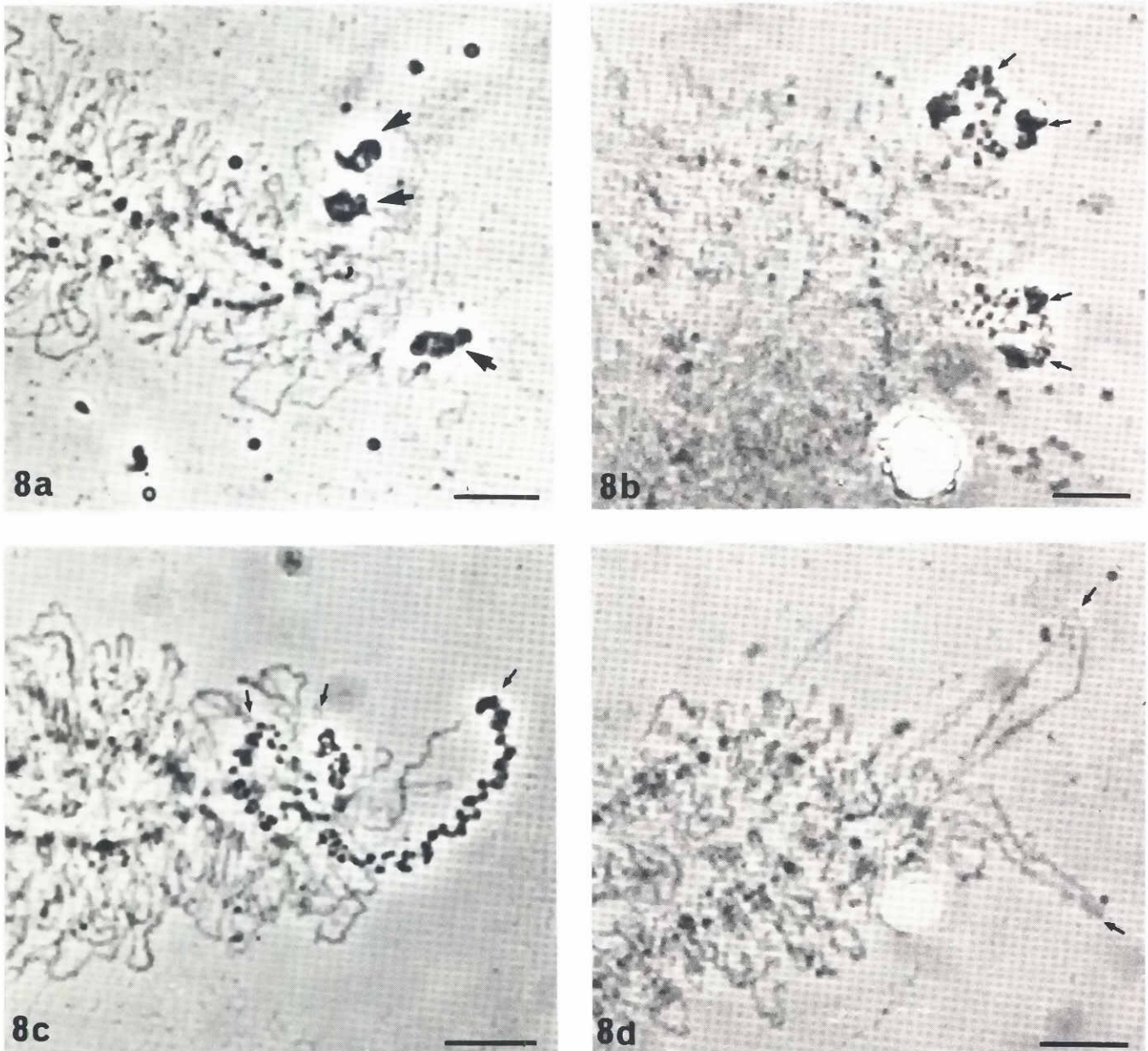


Fig. 8. Effects of heat treatment on lampbrush chromosome structure. Part of bivalent X from untreated female (20°C, **a**) and female subjected to 32°C for 2 days (**b**), 5 days (**c**) and 8 days (**d**). In control, matrices of dense loops (arrows) which characterize bivalent X are reduced to lobules (**a**). In (**b**, **c** and **d**), progressive disorganization of dense matrices is visualized. The matrices successively exhibit globular (**b**, arrows), granular (**c**, arrows) and giant aspects (**d**, arrows); phase contrast. Bars represent 5 μ .

Observations in EM on thin sections and in SEM have shown that the basic structure of RNP matrices of all kinds of loops is a 30 nm RNP particle (Figs. 4 and 5); however, the spatial arrangement of this particle in the RNP matrices was found to differ from one loop type to another. Indeed, SEM observations clarified this spatial arrangement in the matrices of all loop types examined. These observations have suggested that the specific morphology of normal, granular, globular and dense loop matrices depends on the degree of compaction of the transcription products (Fig. 5), with this

compaction resulting both from the progressive packaging of RNP transcripts and concomitant gradual coiling of the loop axis as schematically represented in Fig. 6 (Angelier *et al.*, 1984; Bonnanfant-Jaïs *et al.*, 1985, 1986; N'Da *et al.*, 1986).

Nevertheless, we could not be sure that this morphological evolution observed on landmark loops as a whole corresponded to the transformation of a particular loop from a normal to a granular loop and then from a globular to a dense loop, although there exist several arguments in favor of such a transformation (Bonnanfant-

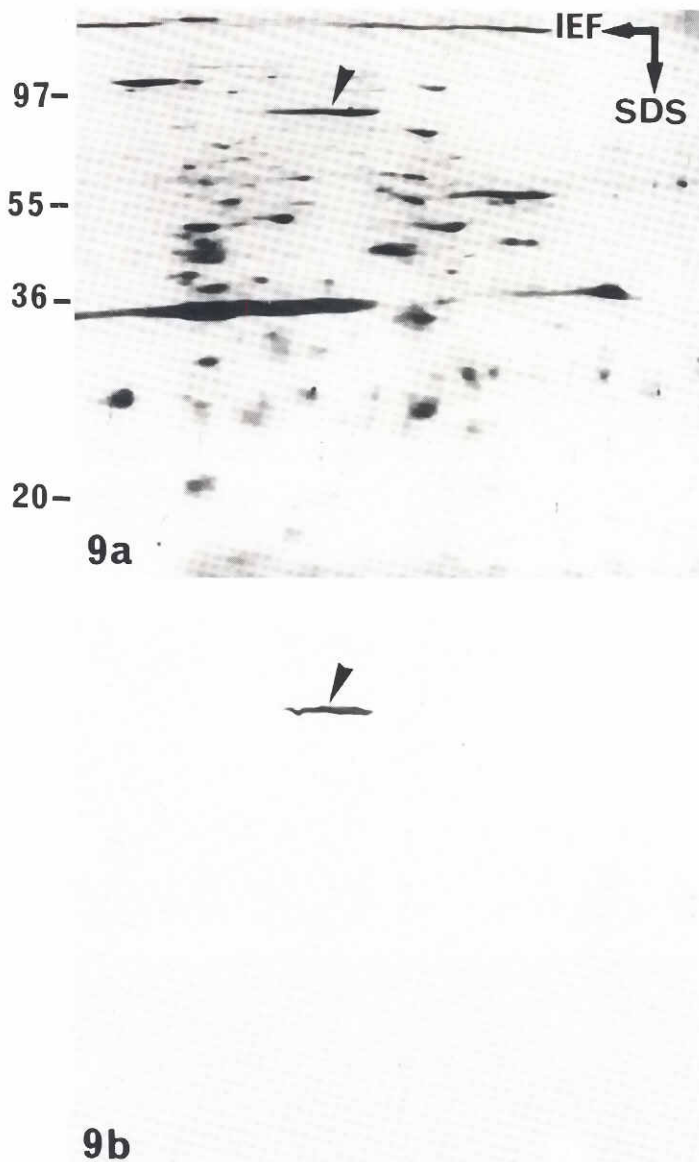


Fig. 9. Two-dimensional gel electrophoresis of nuclear proteins extracted from *Pleurodeles* oocytes silver staining. (a) The position of the 82 KD protein is noted by an arrowhead. Molecular weight designations at left are $\times 10^3$. **(b)** Corresponding immunoblot after incubation with purified 82 KD protein antibody. Arrowhead points to the positive spot.

Jaïs *et al.*, 1986). An experimental approach to this question allowed us to demonstrate that the different morphologies were interconvertible.

Experimental evidence: thermic shocks

The analysis of thermic-shock-induced changes in the structure of amphibian lampbrush chromosomes, enabled visualization of the morphological evolution of normal loops to landmark loops. It has thus become evident that the morphology of landmark loops resulted from the transformation of a particular normal loop into a granular, a globular, and then a dense matrix loop, as already suggested by our previous morphological studies (see above).

Indeed, when *in vivo* thermic shocks were applied to *Pleurodeles* females, striking modifications occurred in the structure of oocyte lampbrush chromosomes. Cold shocks induced the appearance of hyperdeveloped loops, the 'cold loops' which occur at constant, reproducible loci (Fig. 7; see also Angelier *et al.*, 1989a). These 'cold loops' were shown to result from the decompaction of specific landmarks, namely the granular loops, and a model for the structural evolution of granular loops to hyperdeveloped cold loops was proposed (N'Da, 1989; N'Da and Angelier, 1990). Heat shocks, in a similar way, induce changes which result in progressive disorganization of the RNP matrices of various loops. This was clearly demonstrated for dense matrix loops in which the degree of tightness in the packaging of transcription products is high. These dense matrices successively exhibit dense, globular, granular and finally normal aspects, with the degree of this disorganization being a function of the intensity and duration of the stress (Fig. 8; see also Rodríguez-Martín *et al.*, 1989).

We therefore assumed that thermic shocks induce a disturbance in post-transcriptional processes, particularly in packaging of RNP products in the landmark matrices. As a consequence of this disorganization, the DNP axis of the loops would be allowed to progressively relax and extend (N'Da, 1989; N'Da and Angelier, 1990; Rodríguez-Martín *et al.*, in preparation).

Such an interpretation can also be applied to all post-transcriptional processes, and it can be assumed that changes in pre-RNP processing (e.g. splicing), packaging and/or transport away from the loops might play a role in the specific morphology of the loops. The kinetics of each of these processes, related to one another and/or proteins specific to one or another of these processes, could determine the morphological appearance of the loops.

Immunological evidence

Morphological differences between RNP matrices might be due to intrinsic variations in the composition of these different types of RNP matrices, i.e. in the composition of RNP proteins associated with transcripts. Indeed, the important role of proteins in the structural and functional properties of RNP has already been suggested. Immunolocalization experiments using conventional or monoclonal antibodies directed against amphibian oocyte nuclear proteins have provided evidence that some proteins are common to all kinds of lateral loops, while others are specific to morphological or physiological states (Sommerville, 1981; Lacroix *et al.*, 1985; Moreau *et al.*, 1986, 1989; Roth and Gall, 1987).

In the *Pleurodeles* oocyte, we have identified an 82 KD nuclear protein (Fig. 9; pl 5.5) which might interfere with post-transcriptional processes such as packaging or transport of RNP away from the loops. Indeed, immunolocalizations, using a monospecific polyclonal antibody directed against the 82 KD protein provided evidence that this protein associates with RNP particles of all types of loops, but never simultaneously. When a loop type such as granular loops is involved, normal and globular loops remain unlabeled (Fig. 10e, f) and *vice-versa* (Fig. 10a, b, c, d, g, h). In a similar way when this 82 KDa protein associates with RNP particles present in the nucleoplasm, the association with RNP particles of loop matrices is no longer observed (Fig. 10i, j). We have suggested that this protein is specific of a defined physiological state: it might interfere with packaging and transport of RNP particles; the kinetics of these processes may vary from one loop to another (Moreau, 1987; Moreau *et al.*, in preparation).

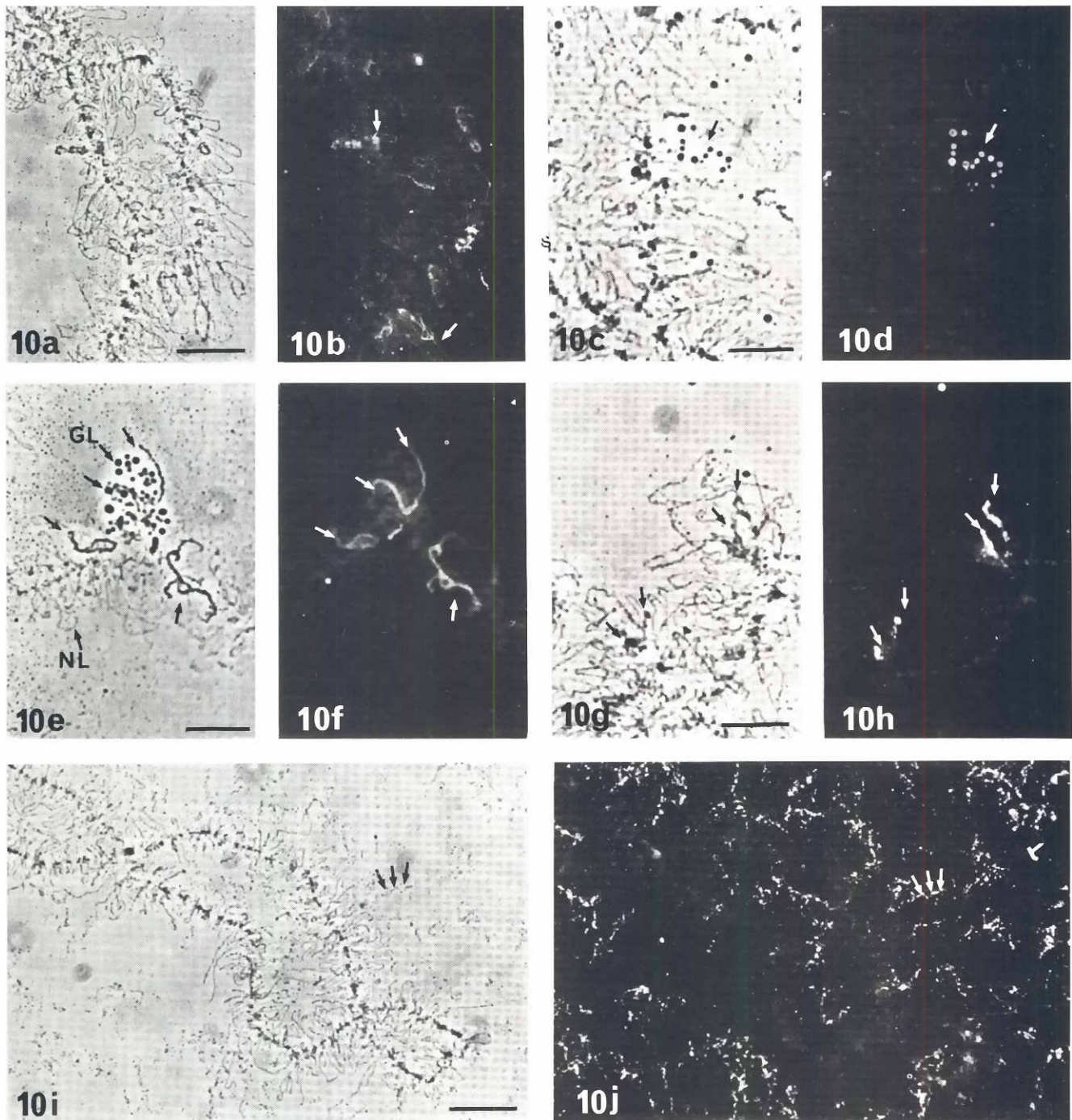


Fig. 10. Immunofluorescence of nuclear structures which specifically bind antibody against an 82 KD nuclear protein. Phase-contrast and fluorescence images of the same lampbrush chromosome regions after treatment with antibody are shown. (a, b) Some normal loops are labeled (arrows). (c, d) Fluorescence is only associated with globular loops (arrow); normal loops are unlabeled. (e, f) Granular loops are labeled (arrows) while globular (GL) and normal loops (NL) are unlabeled (arrows). (g, h) Note that only dense loops are labeled (arrows). (i, j) All lampbrush loops remain unlabeled; fluorescence is only associated with nucleoplasm particles (arrowheads). Bars represent 25 μ in (a, b, e, f, i, j) and 15 μ in (c, d, g, h).

Cold stress was found to induce drastic modifications in the composition of oocyte neosynthesized nuclear proteins (Angelier *et al.*, 1987, 1989a; N'Da, 1989; Moreau *et al.*, submitted). In particular, several proteins with high molecular weights which might well

have been RNPs were missing from the nucleus for as long as oocytes were maintained in the cold. This lack of specific neosynthesized proteins might disturb post-transcriptional processes and consequently be the main cause of the disorganization of granular

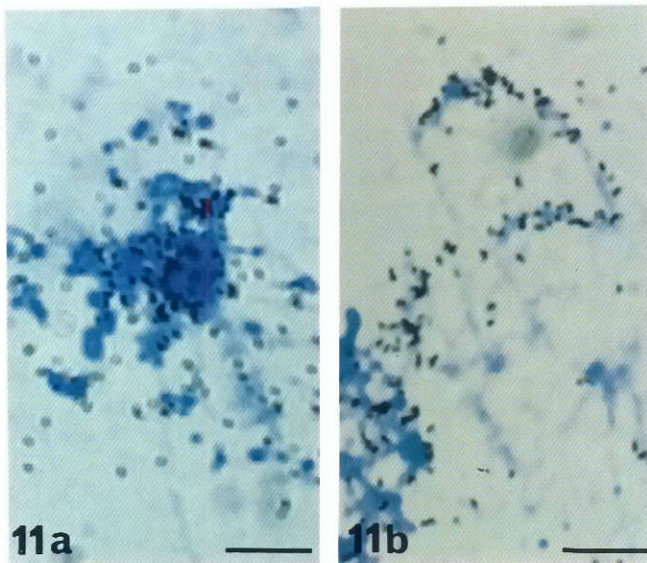


Fig. 11. *In situ* hybridization of microcloned DNA sequences to RNA transcripts on *Pleurodeles* lampbrush chromosomes. Globular loops (a); granular loops (b). Bars represent 5 μ .

and in general landmark RNP matrices.

Antibodies directed against such specific proteins as well as nucleic acid probes corresponding to DNA sequences coding for these proteins should enable us to determine which particular processes are responsible for the specific morphology of each landmark loop type.

Is the morphology of a specific RNP matrix inherent in a particular nucleic acid sequence?

Data reported above should lead us to rule out such a hypothesis. Indeed, they suggest that the morphologies of landmark loops are not related to a particular DNA sequence but rather are interconvertible. Why then, are landmark loops always observed at the same loci and why are their features stable and inherited? Taking all these characteristics into consideration, we hypothesized a possible relationship between the morphologies of landmark loops and the expression of particular DNA sequences. The first step in addressing this problem was to recover landmark loop-associated DNA sequences. To gain access to these sequences the most direct procedure was to microdissect these loops and then to microclone their DNA. To *Pleurodeles* oocyte lampbrush loops we successfully applied microcloning techniques (Penrad-Mobayed *et al.*, 1987) originally applied to polytene chromosomes by Scalenghe *et al.*, (1981). Clones recovered were characterized and their expression was then visualized by *in situ* hybridization on lampbrush chromosomes. DNA cloned sequences were found to be expressed in different kinds of loops (Fig. 11; Angelier *et al.*, 1988, 1989b). Analysis of the expression of subcloned DNA sequences should allow us to determine whether the same DNA sequences are expressed in the same kinds of landmarks (Penrad-Mobayed *et al.*, in preparation). The existence of such a relationship should enable us to follow the fate of such specific transcripts during oogenesis and embryogenesis. A relationship between a precise loop structure and a defined function during development might be envisaged.

Conclusion

The findings discussed here represent mere examples of variations in gene expression that might determine and control the specific morphology of the different lampbrush loop types. Numerous processes occur more or less simultaneously and consequently it is difficult to judge their causal relationships and to determine which process is predominantly responsible for the specific morphology of each RNP matrix. Immunological and molecular approaches combined with morphological and experimental studies should enable us to analyze in more detail such problems. In amphibian oocytes, any modification occurring in the activity of these processes is immediately reflected in the structure of lampbrush chromosomes by concomitant morphological variations. Such chromosomes provide therefore a model of considerable value for analyzing the different processes that control gene expression in oocytes and during development.

Acknowledgments

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