What role might lampbrush chromosomes play in maternal gene expression?

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ABSTRACT The biological significance of lampbrush chromosomes from urodelan amphibians is far from being elucidated. Their particularly well developed lateral loops are the site of intense transcriptional activity, which can be visualized in electron microscopy using the Miller spreading procedure. All transcription units functioning in lampbrush loops synthesize RNA at a maximum rate. *In situ* hybridization has provided evidence for transcription of both unique coding sequences and highly repetitive sequences. The role of lampbrush transcripts in the production of maternal information remains unclear. RNAs transcribed from unique coding sequences are exported to the cytoplasm; there, they contribute either to maintaining the required level of maternal messenger RNA in a basal state during late oogenesis, or to increasing the store of these maternal RNAs throughout oocyte growth, i.e., until stage VI. For repetitive sequences, their intense transcription appears to be non-productive, in that RNAs are not translatable and might be useless products of readthrough transcription. The non-productive transcription of repetitive sequences, the expression of which is directly related to hyperdevelopment of lateral loops, raises the issue of the role of lampbrush chromosome transcription.

KEY WORDS: *Urodele amphibians, lampbrush chromosomes, maternal mRNA*

Introduction

Because of their lateral loops, the presence of which is directly related to transcriptional activity, lampbrush chromosomes of amphibian oocytes provide unique opportunities for analyzing DNA sequences expressed during oogenesis. However, despite numerous molecular and cytological studies of these chromosomes, their biological significance is far from being elucidated. In particular, the nature and purpose of transcripts synthesized at the level of lampbrush loops are unclear, and their role in developmental events remains subject to discussion.

In amphibians, as in most animal species, development is already programmed in oocytes even before fertilization. Indeed, it is well known that the growing oocyte accumulates maternal RNA; thus, the mature oocyte is a very large cell with much of the informational program for early embryogenesis stored within it (for review, see Davidson, 1986).

The classical view was that the store of maternal mRNA was synthesized on lampbrush chromosomes. Since these chromosomes were found to be a site of active RNA synthesis in amphibian oocytes, they were also assumed to be the site of maternal mRNA, with this maternal information being used during early embryogenesis, before the blastula, i.e., at a time when the young embryo is unable to synthesize its own mRNA (for review, see Davidson, 1976 and Sommerville, 1977). However, more recent data based on molecular analyses do not support such a hypothesis and have cast doubt upon this explanation.

First, the amount of maternal mRNA necessary for young embryo development is far less than the amount of RNA produced by lampbrush chromosomes (Sommerville, 1977). This has led to the assertion that intense RNA synthesis in the loops may be totally non-productive, i.e., none of the newly synthesized RNA would be exported to the cytoplasm, and therefore it would not be stored in oocytes (for review, see Davidson, 1986).

Secondly, up until now, no transcription of any single copy sequence has ever been detected on lampbrush loops. In contrast, *in situ* hybridizations provided early evidence for transcription of highly repetitive sequences at the loop level. Most, if not all, of the lampbrush loops were thus assumed to transcribe only repetitive sequences, and heterogenous transcripts exported from the lampbrush stage oocyte nucleus were thus believed to be non-translatable interspersed RNAs of unknown function (for review, see Sommerville, 1977; Callan, 1986).

Finally, the absolute amount of pA^+RNA per oocyte before the maximal lampbrush chromosome stage is approximately the same as after this stage; no further increase in pA^+RNA content occurs during the maximal lampbrush stage (Rosbach and Ford, 1974; Ford *et al.*, 1977). These results suggested that pA^+RNAs

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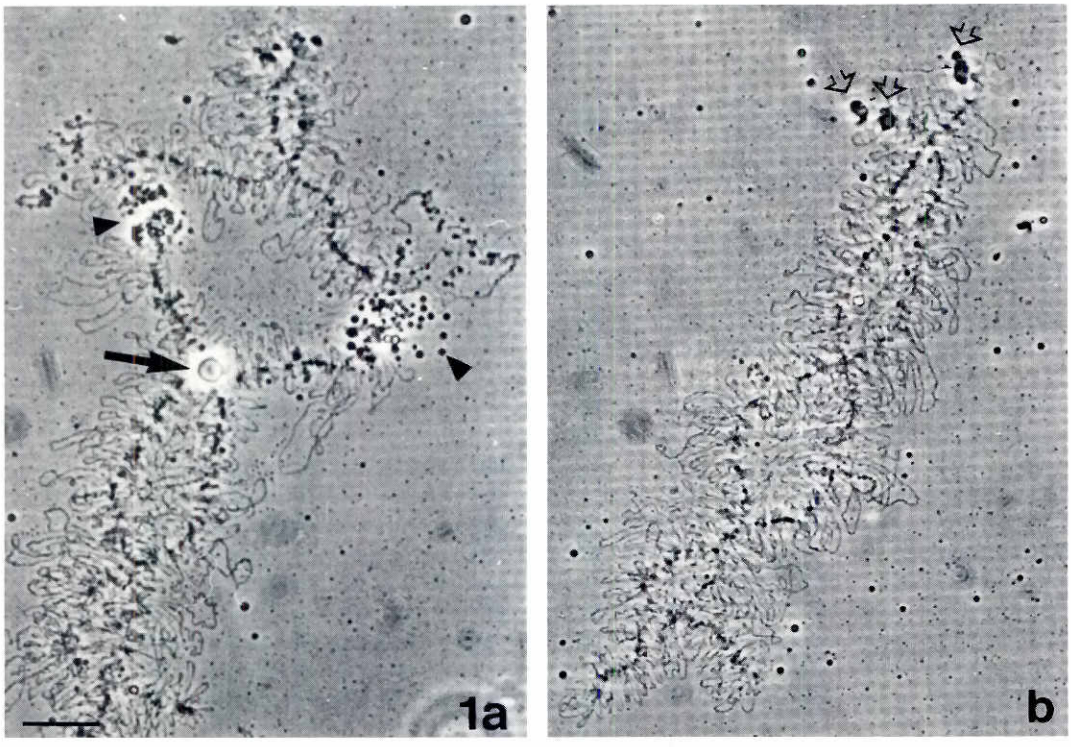

Fig. 1. *Pleurodeles waltl* lampbrush chromosomes as seen by phase contrast. (a) *Bivalent XI characterized by two kinds of landmarks: a sphere at the midpoint (arrow) and two homologous globular loops (arrowheads).* **(b)** *Bivalent X characterized by dense matrix loops in a subterminal position on the two homologous chromosomes. Bar, 20 μm. From Angelier et al. (1990).*

reach their final level of accumulation at an oocyte stage at which lampbrush chromosomes are not yet active. Furthermore, Golden *et al.* (1980) showed that the pA⁺RNA population in mature oocytes is not different from that present in previtellogenic oocytes.

Therefore, doubts have been raised as to whether unique coding sequences for structural genes are transcribed during the lampbrush stage of oogenesis. In line with this hypothesis, lampbrush chromosomes would not participate in production of maternal mRNA stored in oocytes.

In light of these recent studies, it is clear that the nature and purpose of the RNA transcripts remain open to question. What role, then, might lampbrush chromosomes play in maternal gene expression?

Lampbrush chromosomes of urodele amphibians constitute a unique model which is particularly suitable for addressing this question. Indeed, due to their extremely well-developed loops which are very active in transcription, *in situ* hybridization of labeled DNA or RNA probes to the nascent transcripts of loops can enable visualization and identification of sequences which are synthesized during lampbrush stage oogenesis.

The present article examines the nature and purpose of RNA transcripts synthesized on lampbrush loops. It reports two possible strategies for addressing these aspects. Both are based upon identification of RNA lampbrush transcripts by *in situ* hybridization of specific probes to the nascent transcripts of lateral loops. In the first strategy, the nature and fate of transcription products are visualized from their synthesis site (the loops) to the site of their use in oocyte, egg and embryo. The second strategy consists of determining whether or not unique sequences coding for proteins known to be expressed in oocytes or young embryos are transcribed at the level of lampbrush loops.

General features of the lampbrush chromosomes of urodele amphibians

Outstanding characteristic

Oocytes of all urodele amphibians contain spectacular diplotene prophase chromosomes in which DNA is decondensed into several thousand lateral loop pairs displayed along the chromosome axis. Lateral loops, which are responsible for the typical aspect of lampbrush chromosomes, represent regions of intense RNA synthesis, and nascent RNA transcripts associate with proteins to form a ribonucleoprotein (RNA) 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. Thus, in addition to lateral loops termed "normal," giant, granular, globular and dense matrix loops can be observed in lampbrush chromosomes of *Pleurodeles waltl* and *P. poireti* (Lacroix, 1968; N'Da *et al.*, 1986; Angelier *et al.*, 1990). Peculiar analogous loops with similar morphologies have also been identified in lampbrush chromosomes of other Urodeles. In *Triturus cristatus*, *Triturus marmoratus* and other amphibians, the more conspicuous ones are termed "giant fusing" loops (Callan and Lloyd, 1960; Mancino *et al.*, 1969; Nardi *et al.*, 1972). In *Notophthalmus viridescens*, they are referred to as "sequential labelling" loops, and in *Triturus cristatus*, *T. marmoratus* and *T. alpestrus apuanus*, they are called "lumpy" loops (Gall, 1954; Callan and Lloyd, 1960; Mancino and Barsacchi, 1965; Nardi *et al.*, 1972; Ragghianti *et al.*, 1972). All these typical loops are observed in constant, reproducible sites along the chromosome axis, and thus constitute obvious landmarks enabling identification and mapping of the different bivalents of the oocyte karyotypes of all urodele species studied

thus far (Fig. 1a-b; for review, see Callan, 1986).

Intense transcriptional activity in loops

Electron microscopy of lampbrush chromosomes carried out by the Miller spreading procedure (Miller and Beatty, 1969) provided a convincing molecular interpretation of intense transcriptional activity in all lampbrush loops. All transcription units functioning in lampbrush loops synthesize RNA at a maximum rate (Fig. 2). This rate may be very high, since initiation of transcription occurs as frequently as permitted by the RNA polymerase translocation rate. One RNA molecule is initiated even before elongation of the previously initiated molecule is terminated (Fig. 2). When the transcription rate is at its highest level, lengths of single transcription units in urodele lampbrush chromosomes are enormous (Angelier and Lacroix, 1975; Scheer *et al.*, 1976; Angelier *et al.*, 1986). The units transcribed by RNA polymerase II range in length from a few microns to over 100 μm, and may release transcripts that, when fully extended, are of comparable lengths (Fig. 3).

Correlation between size of loops, C-value and percentage of repetitive sequences

Within the amphibians, in general, species with low C-values have shorter lampbrush loops and shorter transcription units on these loops than species with high C-values, when the transcription rate is at its maximum. Sommerville and Scheer (1981, 1982) and Scheer and Sommerville (1982) quantified the correlation between C-value and loop length for an anuran amphibian, *Xenopus laevis* (C-value 3-1 pg), and for urodele amphibians which have a fairly high C-value (Table 1). The results clearly show that a correlation exists between the C-value and the size of lampbrush loops. Thus, in *Xenopus laevis*, most of the loops fall within the size range 5-10 μm, whereas in *Pleurodeles waltl* (C-value 19 pg) and *Necturus maculosus* (C-value 78 pg), the mean size of loops is, respectively, 50 μm and 100 μm. Furthermore, it has been shown that urodeles which have high C-values also have a very high percentage of repetitive DNA sequences, so high that single copy sequences are difficult and even impossible to detect in studies of reassociation kinetics. Taken together, these two features of urodele genomes have suggested that most lateral loops must be transcribing repetitive sequences (reviewed by Sommerville, 1977)

What are the nature and purpose of lampbrush RNA transcripts?

The first step in addressing this question has been to recover lampbrush loop DNA sequences. Due to the large size of urodele genomes (see Table 1 and Olmo, 1983), it has been difficult to recover loop-associated DNA sequences from a complete genomic library. Indeed, in these large genomes, only 5% of total DNA is transcribed (for review, see Callan, 1986). Thus, only those which were easily accessible could be cloned and then

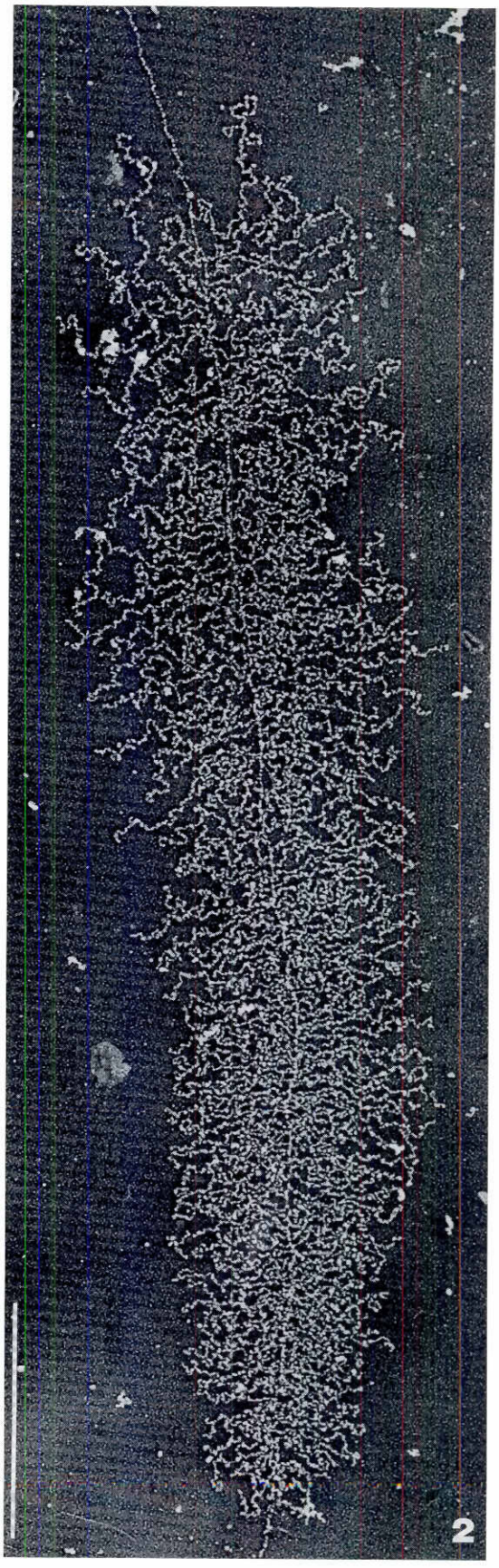

Fig. 2. Electron microscopy of *Pleurodeles waltl* lampbrush chromosomes spread according to the Miller procedure. *Transcription unit observed at the level of a lateral loop. RNA is transcribed at a high rate, as visualized by the numerous RNP fibrils along the DNP axial fiber. Bar, 5 μm. From Angelier and Lacroix (1975).*

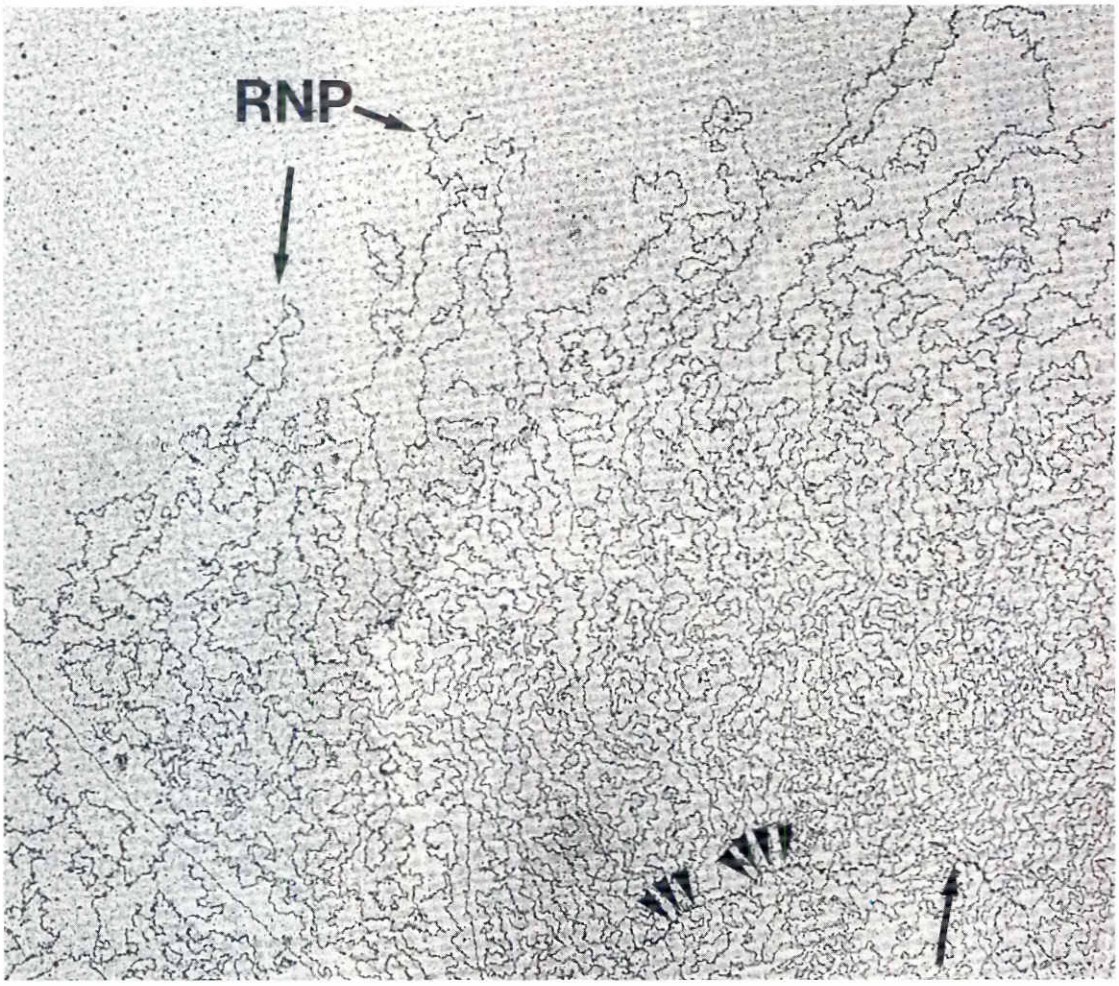

Fig. 3. Detail of a transcription unit showing the large length of RNP transcripts. *Arrowheads indicate the position of RNA polymerase II. Bar, 1 µm. RNP, ribonucleoprotein; DNP, deoxyribonucleoprotein.*

localized by *in situ* hybridization to the nascent transcripts of lampbrush loops. These were either middle and highly repetitive sequences of a satellite type (MacGregor and Andrews, 1977; MacGregor, 1979; Varley *et al.*, 1980a,b; Diaz *et al.*, 1981), or sequences corresponding to the histone genes contained in a 9 kb DNA repeat which is present at 600-800 copies per haploid genome (Diaz *et al.*, 1981; Stephenson *et al.*, 1981a,b; Diaz and Gall, 1985). Up until now, no transcribed unique coding sequence has ever been detected on lampbrush chromosome loops.

TABLE 1

CORRELATION BETWEEN THE C-VALUE AND THE LENGTH OF LOOPS

amphibian species	C value (pg)	length of loops (µm)
Necturus maculosus	78	>100
Amphiuma means	65	>100
Ambystoma mexicanum	38.4	>50
Triturus cristatus carnifex	23	30-50
Triturus cristatus cristatus	19	30
Pleurodeles waltl	19	30
Xenopus laevis	3.1	5

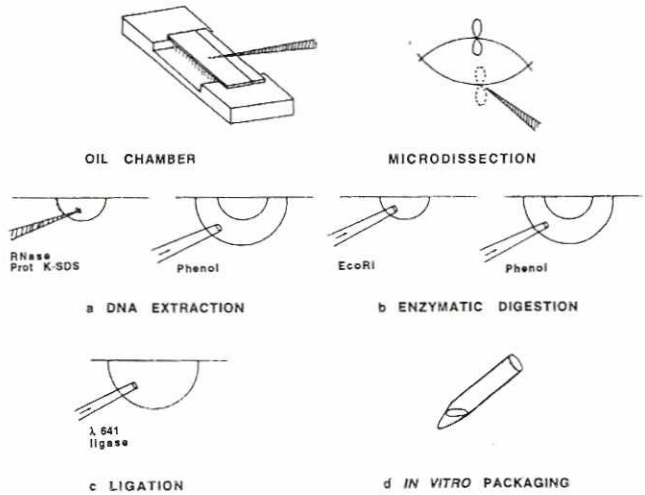

Fig. 4. *Schematic illustration of* **microcloning procedure** *applied to lateral loops of amphibian lampbrush chromosomes.*

Repetitive sequences are actively transcribed on lampbrush loops of all urodeles, but corresponding interspersed RNAs are not stored in oocytes

To gain access to the transcribed DNA sequences of lampbrush loops, it was hypothesized that the most direct procedure was to

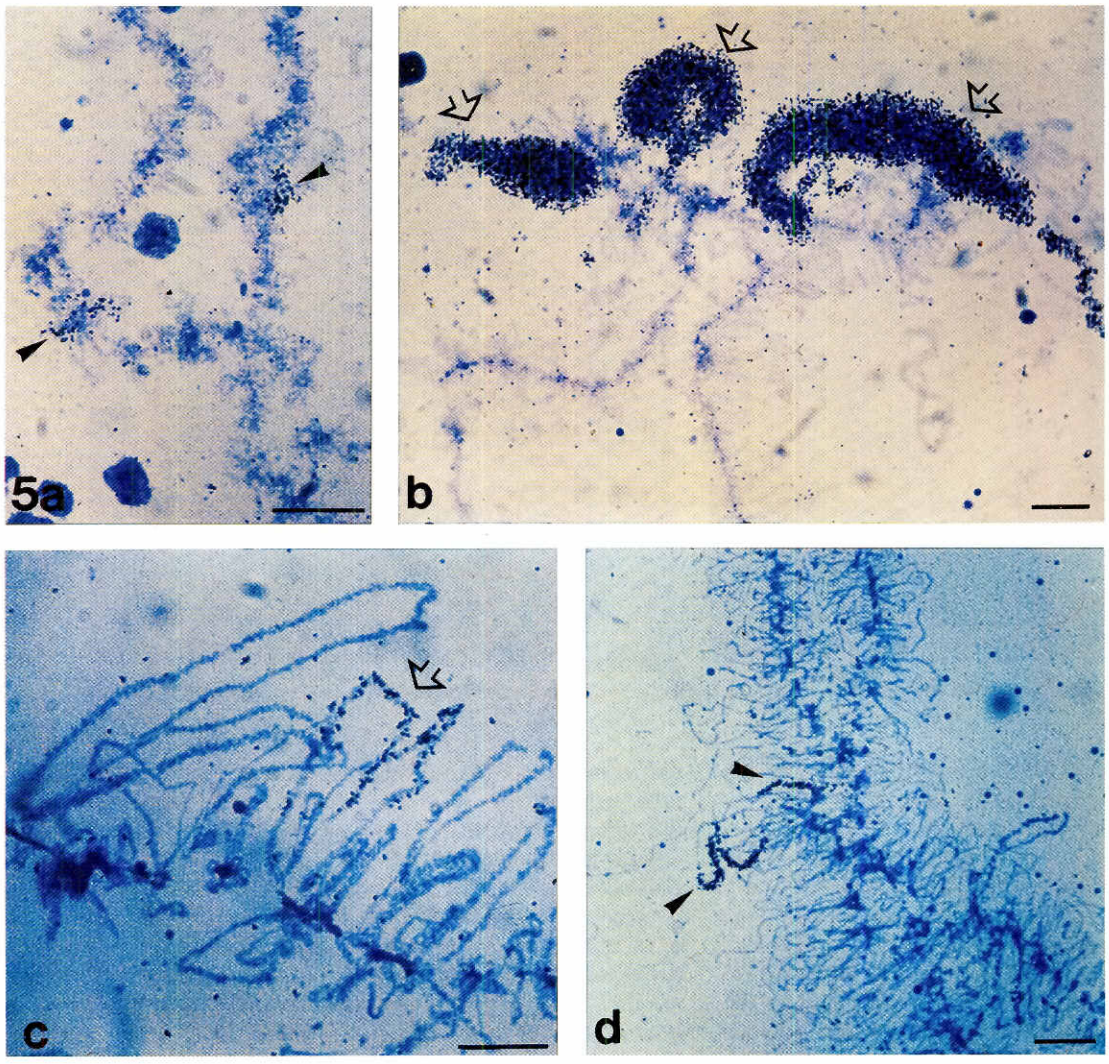

Fig. 5. *In situ* hybridization of *Pleurodeles* cRNA probes *to the nascent transcripts of lampbrush chromosomes of* **(a)** *Xenopus laevis;* **(b)** *Notophthalmus viridescens;* **(c)** *Euproctus;* **(d)** *Pleurodeles waltl. These cRNA probes were synthesized in vitro from repetitive sequences recovered by microcloning of Pleurodeles lampbrush loop DNA. Bars, 20 μm.*

microdissect the lampbrush loops and then clone their DNA. Indeed, the characteristic features of lampbrush chromosomes, namely the hyperdeveloped lateral loops, enabled the use of microtechniques originally applied to polytene chromosomes of *Drosophila* (Scalenghe *et al.*, 1981). Thus, these microtechniques were successfully applied to lampbrush loops of the urodele *Pleurodeles waltl*: precise loops were microdissected and their DNA cloned (Fig. 4; Penrad-Mobayed *et al.*, 1991). The molecular characterization of the recovered clones showed that all cloned sequences were of an intermediate or highly repetitive type. No single sequence has ever been obtained using this procedure. Transcription of such sequences was localized by *in situ* hybridization not only to the microdissected loops, but also to several other loops. Nevertheless, corresponding interspersed RNAs have never been found exported to the cytoplasm and, consequently, stored in the oocyte (Penrad-Mobayed *et al.*, 1991). Taken together, these results support the hypothesis previously advanced by Sommerville (1977). The percentage of repetitive sequences would be so high in urodeles such as *Pleurodeles*, which has a high C-value (19 pg), that single copy sequences would be very difficult to recover even by the direct procedure described above. Interestingly, these repetitive sequences cloned from *Pleurodeles* lampbrush loops have also been found to be transcribed on lampbrush loops of other urodeles, including the lateral loops of *Euproctus*, the sequential labelling loops of *Notophthalmus viridescens* and even the lampbrush loops of the anuran amphibian *Xenopus laevis* (C-value: 3.1 pg) (Fig. 5). Transcription of repetitive sequences such as satellite DNA has already been visualized in anurans including *Rana catesbeiana* (Wu *et al.*, 1986) and *Xenopus laevis* (Jamrich *et al.*, 1983; Wu *et al.*, 1986). *In situ* hybridization of a DNA sequence obtained by microcloning to lampbrush loops of different urodeles corroborates these results; furthermore, it provides evidence for a strong homology between repetitive sequences transcribed during the lampbrush stage of all urodeles, and even of many amphibian species. It can be presumed, however, that the degree of repetition of such homologous sequences in lampbrush loops varies from one amphibian species to another in direct relation to the C-value.

Unique coding sequences are actively transcribed on lateral loops and lampbrush chromosomes can participate in storage of maternal information

Another approach toward analyzing the nature of lampbrush RNA transcripts in urodeles is to provide evidence for transcription

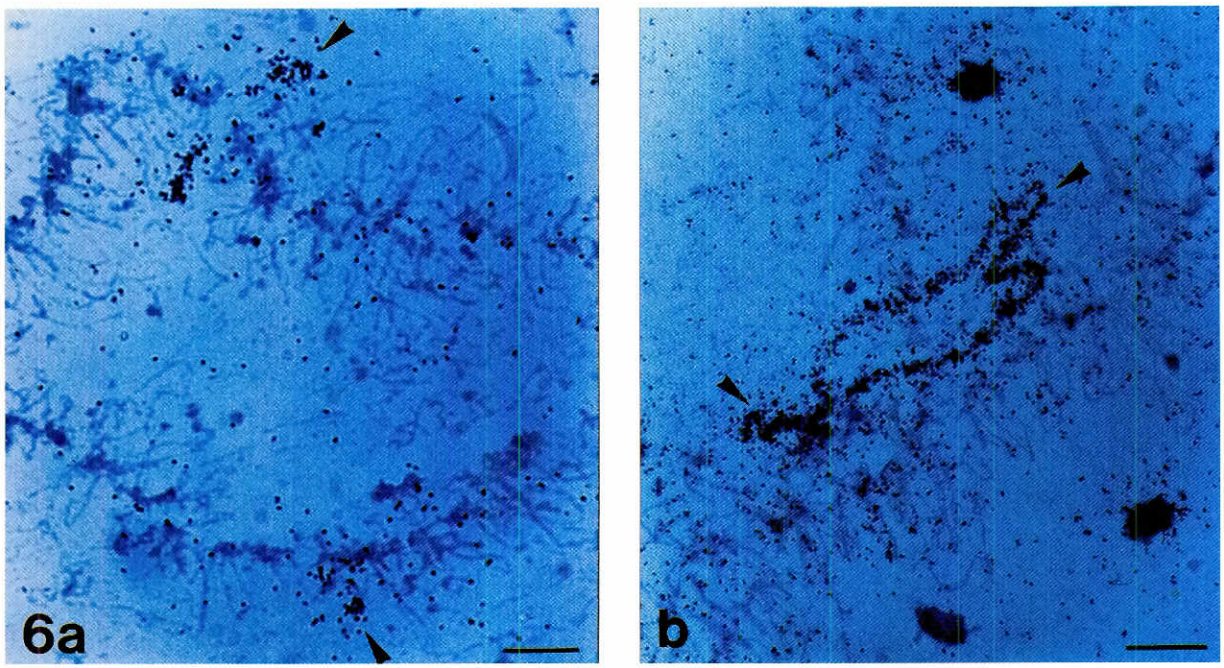

Fig. 6. *In situ* hybridization of antisense cRNA probes to the nascent transcripts of lampbrush loops of *P. waltl*. **(a)** *Xenopus c-myc cRNA probe;* **(b)** *Xenopus Eg1 cRNA probe. Bars, 20 μm.*

on lampbrush chromosomes of RNA transcribed from known coding sequences. However, in urodeles, up until recently, no study of developmental gene expression of a unique coding sequence was ever carried out, due to the fact that no probes were available. In contrast, in the anuran *Xenopus laevis*, many RNAs could be identified as members of the class of maternal RNA. Those RNAs are expressed before fertilization, have reached their final level of accumulation at the beginning of oogenesis (stage II), and then are progressively degraded during late oogenesis and early embryogenesis. Among these maternal RNAs, c-myc RNA and Eg_1 RNA (strongly homologous to cdc2) have been extensively studied (Taylor *et al.*, 1986; Paris *et al.*, 1991). Both are implicated in fundamental events linked to early embryogenesis, including proliferation and cell cycle control, and their sequences are highly conserved during evolution. Heterologous c-myc and Eg_1 probes originating from *Xenopus laevis* were therefore used to test whether or not corresponding homologous sequences were transcribed on lampbrush loops in urodeles. Results from *in situ* hybridization of these probes to the nascent transcripts of *Pleurodeles waltl* have strongly suggested that lateral loops can transcribe these unique coding sequences (Fig. 6; unpublished results).

Such preliminary results implied more extensive research in order to reach a conclusion on this important point. Recently, two analyses of genic expression during oogenesis in urodeles once again raised the issue of lampbrush chromosome transcription. These studies involved expression of two heat-shock genes, *hsp70* and *hsc90*, coding, respectively, for HSP70 and HSC90, two proteins implicated in the cellular response to stress (Billoud *et al.*, 1993; Coumailleau *et al.*, 1995). Sequences coding for these heat-shock proteins were obtained by screening a cDNA library of ovaries of *Pleurodeles waltl* using either an antibody against HSP70 or an *hsc90* cDNA probe from chicken. The recovered cDNA sequences, the specificity of which had been previously checked, were used as homologous probes to analyze the expression of *hsp70* and *hsc90* RNA during oogenesis. Results are summarized in Figure 7. *hsc70* RNA and *hsc90* RNA, previously identified as maternal messenger RNA, were found to be expressed according to two different modalities. Quantification of mRNA during oogenesis provided evidence for progressive accumulation of *hsp70* mRNA throughout oogenesis, with a maximum in stage VI oocytes, whereas the final level of accumulation of *hsc90* mRNA is reached earlier, around stage II, i.e., before the lampbrush stage; this early accumulation of *hsc90* mRNA is then followed by a progressive decrease up until stage IV. However, whatever the modality of RNA accumulation, transcription occurs throughout oogenesis, and even during the lampbrush phase, since *in situ* hybridization has provided evidence for *hsp70* and *hsc90* RNA on lampbrush loops (Fig. 7; Billoud *et al.*, 1993; Coumailleau *et al.*, 1995).

These results suggest that, in urodeles, maternal RNA expression either follows the classical rule according to which a peak level of mRNA accumulation is reached very early in oogenesis, or else there is progressive accumulation throughout oogenesis, with a maximum in stage VI oocytes. In the latter case, the fact that a progressive increase in *hsp70* transcripts is concomitant with *hsp70* RNA synthesis, as visualized by *in situ* hybridization to newly synthesized lampbrush loops, led us to conclude that lampbrush loops actively participate in production of at least one species of pA^+RNAs stored throughout oogenesis, as shown for *hsp70* mRNA (Billoud *et al.*, 1993). In the case of *hsc90* mRNA, which follows the classical rule, RNA transcripts synthesized on lampbrush loops might remain immature and therefore not stored in oocytes. According to this hypothesis, the RNA stored very early in oogenesis would not be replaced, or else the ratio of RNA synthesis to degradation would lead to a

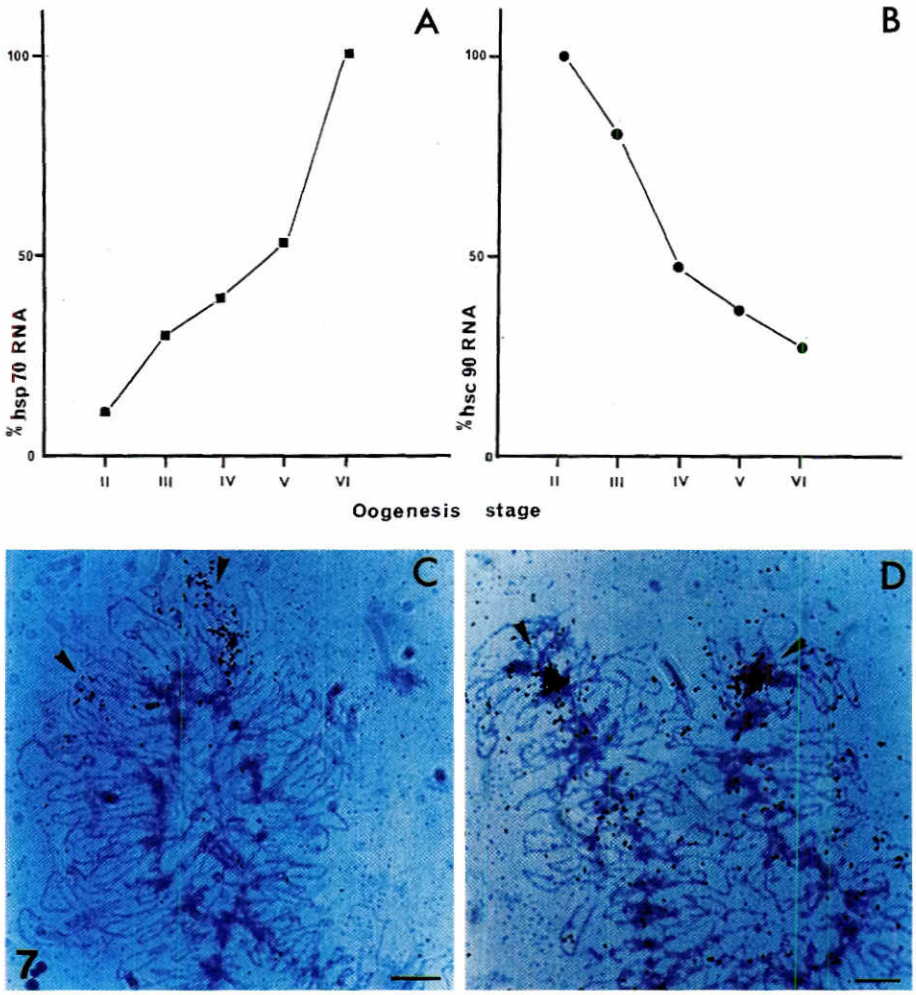

Fig. 7. Expression of *hsp70* and *hsc90* mRNA during oogenesis of *Pleurodeles waltl.* (A,B) *Quantification of hsp70 and hsc90 mRNA (stages II, III,IV, V and VI);* **(C,D)** *in situ hybridization of hsp70 and hsc90 cRNA probes to the nascent transcripts of Pleurodeles lampbrush loops; arrows indicate the single hybridization site for hsp70* **(C)** *and one of the four hybridization sites for hsc90* **(D)**. *Bars, 20 μm. From Billoud et al. (1993) and Coumailleau et al. (1995).*

decrease in the number of transcripts in late oogenesis; in the latter hypothesis, RNA transcripts from lampbrush loops would contribute only to maintaining a basal level of maternal RNAs in oocytes (Coumailleau *et al.*, 1995).

Discussion

Role of lampbrush transcripts in production of maternal information

The nature and purpose of lampbrush RNA raises the issue of the role of lampbrush chromosomes in the production of maternal information. This question has led to a remarkable series of interpretations. The most coherent, based on all available data, is as follows.

It is now clear that unique coding sequences are actually transcribed on lampbrush loops. Transcripts exported to the cytoplasm include translatable messenger RNAs of known function (for examples, see Billoud *et al.*, 1993; Coumailleau *et al.*, 1995). For most RNAs, the final level of accumulation is reached at the beginning of oogenesis (stages I-II), at a time when lampbrush chromosomes are not in their maximal phase, i.e., at a time when they are not yet active, or are just beginning to transcribe. In such an RNA accumulation pattern, the role of lampbrush RNA synthesized during late oogenesis (stage III to stage VI), i.e., during vitellogenesis, is simply to maintain, in a kinetic steady or basal state, the required level of maternal transcripts (for review, see Davidson, 1986). In contrast, for maternal RNA such as *hsp70* mRNA, this final level of accumulation is only achieved in late oogenesis in stage VI oocytes, and lampbrush chromosome transcription undoubtedly contributes to the production of maternal messenger RNAs, which are progressively stored during oogenesis. Interestingly, the only known example of such an accumulation pattern concerns hsp70 mRNA, the genome sequence of which does not exhibit any intronic sequence. Therefore, it is assumed that the regular increase in maternal RNA observed throughout oogenesis results from immediate export of lampbrush transcripts to the cytoplasm, without processing. Whatever the case, all these data strongly suggest that one of the basic functions of lampbrush chromosomes is to provide transcription products loaded in the cytoplasm, where they contribute either to maintaining, in a basal state, the required level of maternal messenger RNA in late oogenesis, i.e., during the growth phase of large oocytes, or to progressively increasing the supply of maternal messenger RNA, depending on the needs of stage VI oocytes. The latter interpretation is in good agreement with the *hsp70* gene regulation pattern in oocytes, where the response to stress is regulated only at the translational level, with messenger RNA *hsp70* being synthesized and stored in oocytes under normal conditions (Billoud *et al.*, 1993).

It is also clear that lampbrush loops actively transcribe repetitive sequences and produce heterogeneous transcripts, which either turn over or are exported to the cytoplasm as non-translatable, interspersed RNAs of unknown function. According to the model proposed by Gall over the last ten years, such RNA might represent useless products of readthrough transcription. Indeed, a series of investigations on histone gene transcription in oocytes of *Notophthalmus viridescens* has provided evidence that initiation from histone gene promoters can result in the transcription of downstream satellite sequences (Diaz *et al.*, 1981; Stephenson *et al.*, 1981a,b; Diaz and Gall, 1985). According to

these authors, readthrough transcription in histone genes would not be a peculiarity: "In our view read-through transcription is a general feature of the lampbrush chromosome stage and is not a peculiarity of the histone genes ..." (Diaz and Gall, 1985).

The fact that such interspersed RNAs are not translatable again raises the question of the actual significance of lampbrush loop structures. Indeed, as reported above, the extent of development of lateral loops is linked to the number of repeat sequences transcribed. What role can be attributed to these sequences and their transcription products? Are they, in fact, useless transcription products? With their hyperdeveloped loops, might lampbrush chromosomes be a vestige of an archaic type of organization maintained during evolution? Or should we be more conclusive, and maintain that such an intense transcriptional activity, if it is not made use of, represents an incredible waste?

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