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Microdissection and cloning of DNA from landmark loops of amphibian lampbrush chromosomes

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Abstract. Microdissection of the "globular" and "granular" landmark loops of *Pleurodeles* lampbrush chromosomes and subsequent cloning of their DNA yielded several recombinant clones. The 6.6-kb insert of one of them was subcloned and the 600 bp of one subclone was characterized by Southern and slot hybridizations as well as by sequencing. This sequence, designated p130B, was shown to belong to a class of moderately repetitive DNA. RNA expression of this sequence was investigated by in situ hybridization of p130B to the nascent transcripts of lateral loops. Results showed that: (1) the same transcripts were not always found in matrices of landmarks exhibiting the same morphological features; (2) the same transcripts were expressed in loops of different morphological types. Based on these results we suggest that even if there is a morphological similarity of landmark loops, this does not reflect total similarity of their transcripts.

Introduction

Lampbrush bivalents from amphibian oocytes can be identified and mapped due to the presence of obvious landmark loops such as granular, globular and giant fusing loops, which show distinctive morphology and are observed at constant and reproducible sites along the chromosome axis (for a general review, see Callan 1986). Ultrastructural data about RNP matrices of landmark loops have shown that each matrix type exhibits the same organization for its transcripts (Angelier et al. 1990; Bonnanfant-Jais et al. 1986). It has been suggested that a similarity in landmark loop matrix texture might imply a similarity in DNA sequence organization that would be transferred to RNA transcripts (see Callan 1986).

Therefore, in the present study, we tried to determine whether or not a similarity in landmark loop matrix morphology implies a similarity in their transcripts. We analyzed the expression of nucleic acid sequences in *Pleurodeles waltl* landmark loops, which occur in different sites of the genome. Due to the large size of the *Pleurodeles* genome (19 pg) (Olmo 1973), it was difficult to recover landmark-loop-associated DNA sequences from a complete genome library. We therefore microdissected these loops and then microcloned their DNA (Scalenghe et al. 1981; Hennig et al. 1983; Huijser and Hennig 1987; Röhme et al. 1984; Fisher et al. 1985; Weith et al. 1987; Lüdecke et al. 1989).

This technique was successfully applied to amphibian oocyte lampbrush loops. The expression of one cloned sequence was analyzed by in situ hybridization. We show that: (1) the same transcripts are not always found in matrices exhibiting the same morphological features; (2) the same transcripts can be expressed in different loop types. A morphological similarity of landmark loops consequently does not reflect a total similarity of their transcripts.

Materials and methods

Lampbrush chromosome preparation. Lampbrush chromosomes were prepared as previously described (Gall 1954). Germinal vesicles of large oocytes were manually isolated in 75 mM KCl, 25 mM NaCl, pH 7.2, 0.01 M $MgCl_2$ and 0.01 M $CaCl_2$ (Angelier et al. 1986). The nuclear enveloppe was removed and the nuclear content was centrifuged (30 min, 1,500 *g*) onto the coverslip. Chromosome preparations were fixed in 70% ethanol for 30 min, dehydrated through an ethanol series, washed in xylene to remove paraffin wax and air-dried from acetone. Mitotic chromosomes were done as described by Macgregor and Andrews (1977) from intestinal epithelial cells of *Pleurodeles* females with the squash technique.

Microdissection and microcloning. Lampbrush chromosomes for microdissection were prepared as described above from oocytes that were incubated with 50 µg/ml actinomycin D for 60 min at 20° C. Micromanipulations were carried out in an oil chamber under a phase-contrast microscope (Scalenghe et al. 1981). The dissecting needle and cloning micropipettes were prepared in a de Fonbrune microforge, as earlier described (Pirotta et al. 1983). Landmark loops of bivalent VII were scratched using a glass needle directed by the de Fonbrune micromanipulator. They were then

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transferred to a nanoliter of GP buffer (4 vol 87% glycerol, 1 vol 0.05 M Na-K phosphate buffer, pH 6.8). After dissection of 500 loop pairs, the DNA was first digested with pancreatic RNase (Hennig et al. 1983), extracted with proteinase-K-SDS and phenol, and then digested with *Eco*RI as previously described (Scalenghe et al. 1981) with the following modification: because of the large amount of protein associated with landmark loops, 10% SDS instead of 0.1% (Scalenghe et al. 1981) or 0.5% (Hennig et al. 1983) was added from a supply drop until the material dissolved. The digested DNA was ligated to *Eco*RI-cut λ 641 (Murray et al. 1977) and packaged in vitro under conditions giving efficiencies of 10^8 plaques per pg vector. Recombinant clones were selected by plating on an *E. coli* pop 13b rk-mk+ host constructed by N. Murray (Pirotta et al. 1983). Individual plaques were picked and plate stocks were prepared by propagation on *E. coli* Q358, rk-mk+ using standard procedures (Maniatis et al. 1982).

Newt DNA preparation. Genomic DNA was purified from isolated erythrocyte nuclei using the same procedure as described by Moreau et al. (1981).

Newt RNA preparation. Total ovary RNA was extracted by the urea-LiCl method (Auffray and Rougeon 1980). For the purification of germinal vesicle RNA, we used the procedure already described by Epstein et al. (1986). The poly(A+) RNA population was purified by chromatography on oligo(dT)-cellulose successively performed twice.

Subcloning and DNA sequencing. Phage DNA extraction was carried out following standard procedures (Maniatis et al. 1982). The restriction map of the clone λPw130 was constructed from double and partial enzymatic digests. A 600 bp *Bam*HI genomic fragment from λPw130 was subcloned in pGEM 3Z(f+) vector (Promega Biotech. COGER, France). Dideoxy DNA sequencing (Sanger et al. 1977) of both strands of the subclone p130B was performed using T7 DNA polymerase and (^{35}S) dATP. The subcloning and DNA sequencing were done according to the manufacturer's instructions (Promega Biotech. COGER, France). The sequence will appear in the EMBL nucleotide sequences databases under the accession number, X57350.

Transfer of nucleic acids to nitrocellulose filters. DNA blots were prepared from agarose gels according to Southern (1975). Slot-blot experiments were performed by spotting serial dilutions of alkali-denatured DNA onto nitrocellulose filters. Total RNA, poly(A−) (20 µg), poly(A+) (8 µg) and nuclear RNA (8 µg) were fractioned by formaldehyde agarose gel electrophoresis and blotted onto nitrocellulose (Maniatis et al. 1982).

Filter hybridizations. For Southern and slot-blots, insert DNA bands were purified by preparative electrophoresis as described by Stephenson et al. (1981) and labelled by nick translation (Maniatis et al. 1982) with (α^{32}P) dCTP (3000 Ci/mmol) at a specific activity of 10^8 cpm/µg. Hybridization was carried out at 42° C overnight in 40% formamide, 4× SSC, 0.1 M Na3PO4, 5× Denhardt's mix. (Denhardt 1966), 0.1% SDS, pH 7.2. Filters were washed in several changes of 40% formamide, 4× SSC at 42° C followed by two changes of 0.1× SSC at 65° C for 1 h and exposed on Kodak X-OMAT AR film with intensification for 1–7 days.

For Northern blots, ^{32}P-labelled cRNA probes (SA. 5×10^8 cpm/µg) were synthesized in vitro from either strand with T7 or SP6 RNA polymerase according to the manufacturer's instructions (Promega Biotech. COGER, France) Hybridization conditions were the same as for Southern blots. However, they were washed at 65° C twice in 4× SSPE, one in 1× SSPE, 0.1% SDS (30 min) and once in 0.4× SSPE, 0.1% SDS (30 min).

In situ hybridization. For mitotic chromosomes, ^{3}H-labelled complementary RNA (cRNA) (SA: 10^7 cpm/µg) synthesized from cloned DNAs with *E. coli* RNA polymerase (Pardue 1985) were used as probes. For lampbrush chromosomes, ^{35}S-labelled RNA probes (SA. 3×10^8 cpm/µg) were synthesized in vitro from either strand of p130B DNA with T7 or SP6 RNA polymerase according to the manufacturer's instructions (Promega Biotech. COGER, France).

The ^{35}S-radiolabelled probes were reduced by limited alkaline hydrolysis to an average size of 100–150 bp and used at a final concentration of 5×10^5 cts min^{-1} per slide. Hybridization was carried out as described by Gall et al. (1981) in 40% formamide, 4× SSC, 0.1 M Na_3PO_4, pH 7, and 300 µg/ml of *E. coli* and yeast tRNA at 42° C overnight. After washing for 1 h in 0.1× SSC, 10 mM dithiothreitol at 65° C, the slides were dehydrated through an ethanol series, dipped in NTB2 emulsion (Kodak) (diluted 1:1 with H_2O), and exposed at 4° C for 4 to 40 days. After development, mitotic and lampbrush chromosomes were stained with Giemsa and Coomassie blue R, respectively. For control, some preparations were pretreated before hybridization with 100 µg/ml pancreatic RNase at 37° C for 30 min (Pardue 1985).

Results

Microdissection and microcloning

Landmark loops of a granular or globular type were observed in 10 out of 12 bivalents in the *Pleurodeles* oocyte karyotype (Lacroix 1968). From unstained lampbrush spreads, these typical loops were easily identified (Fig. 1A). To reduce the chances of contamination by adjacent loops during microdissection, we inhibited RNA synthesis using actinomycin D in defined conditions (see Materials and methods), previously shown to cause the retraction of all lateral loops. The landmark loops (Fig. 1B) were always the last to disappear and could therefore be easily microdissected (Scheer et al. 1984; Penrad-Mobayed et al. 1986). Granular and globular loops from bivalent VII are particularly suitable because of their morphology. They were isolated by microdissection (Fig. 2A, B) and pooled in a 0.01 nl drop of glycerol/phosphate (Fig. 2C). Since the length of these landmarks was estimated at varying from 250 kb to 2,500 kb (N'Da and Angelier 1990), 500 loop pairs had to be microdissected to obtain 0.2 to 2 pg of DNA, the amount required for microcloning (Pirotta et al. 1983). After microdissection was completed, the DNA was extracted, digested with *Eco*RI, and ligated to the *Eco*RI-cut λ-641 vector in the oil chamber. After in vitro packaging, phages were plated on a *Lyc*7 host (pop13b) to recover the recombinant clones selectively.

Microclone analysis

A total of 180 CI-phage clones was recovered. The insert size was estimated in restriction gels after *Eco*RI digestion of DNA recovered from minipreparations. Eighty clones had no detectable inserts. The insert size of the remaining 100 clones varied from 4 to 7.8 kb with an average of 7 kb. All these clones were tested for the presence of highly repetitive DNA sequences by hybridizing them with total ^{32}P-labelled *Pleurodeles* DNA used as probe. No detectable signals were obtained. In contrast, 39 cloned sequences showed positive signals when they were ^{32}P-labelled and hybridized to genomic *Pleurodeles* DNA, digested with *Eco*RI. Only these 39 cloned sequences were taken into consideration for the subse-

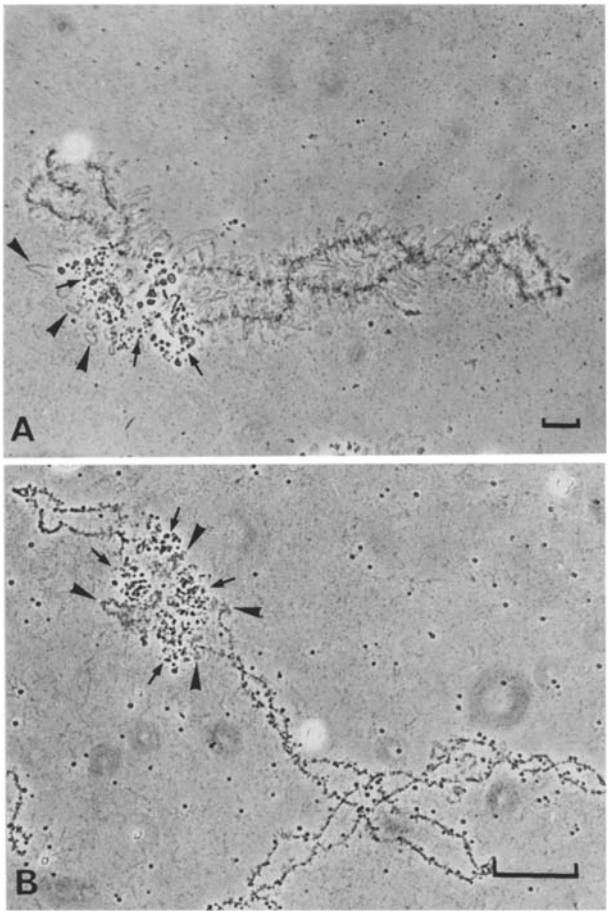


Fig. 1A, B. Inhibition of RNA synthesis in lampbrush chromosomes. **A** Bivalent VII observed in phase contrast. It is characterized by a large bundle of globular (*arrows*) and granular loops (*arrowheads*). **B** Bivalent VII from oocyte incubated with 50 μg/ml actinomycin D for 1 h. Chromosomes are foreshortened; normal loops are retracted; globular loops (*arrows*) and granular loops (*arrowheads*) are still developed. Bars represent 20 μm in **A** and 40 μm in **B**

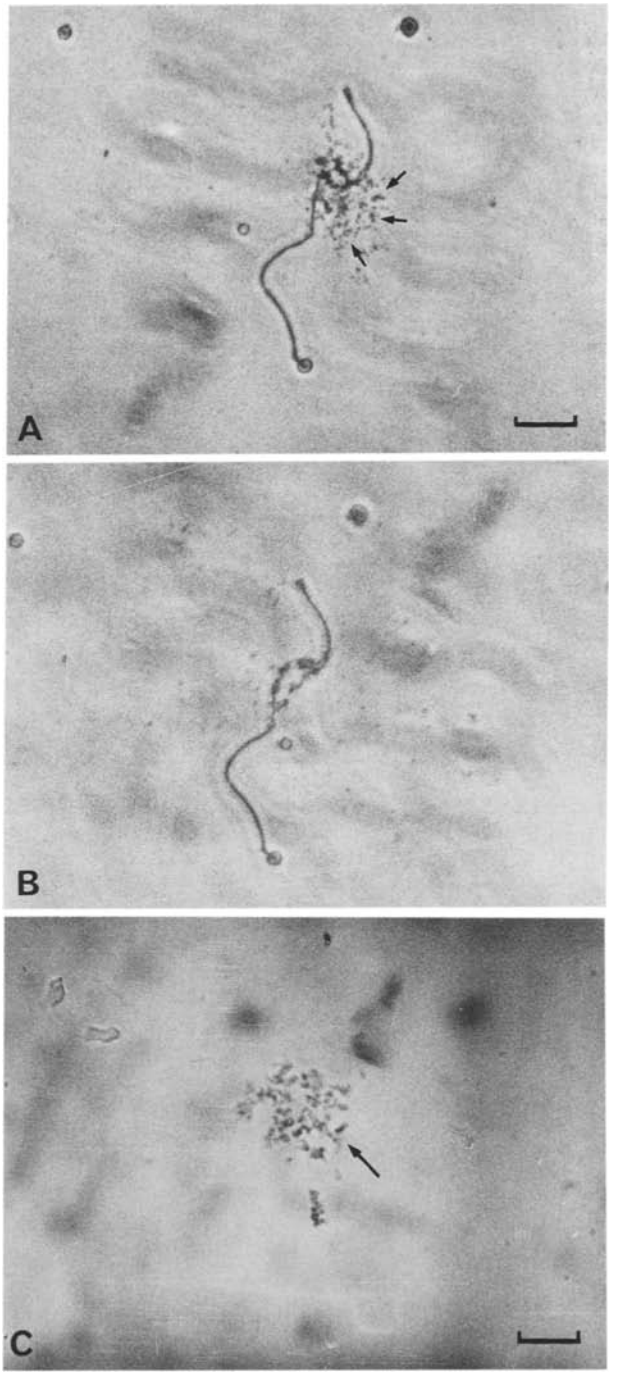


Fig. 2A–C. Microdissection of landmark loops of bivalent VII as seen in phase contrast in the oil chamber. **A** Before microdissection, only landmark loops are visible along the chomosome axis (*arrows*). **B** After microdissection, the same bivalent devoid of landmark loops. **C** Dissected material in a nanoliter drop of buffer. Bars represent 20 μm in **A** and **B** and 10 μm in **C**

quent molecular studies. The remaining clones displayed strong sequence homology with *Escherichia coli* DNA. Such contamination, though less important, had already been reported in other microcloning experiments (Scalenghe et al. 1981). However, the efficiency of our microcloning could be considered as satisfactory. Indeed, taking into account the amount of microcloned DNA (from 0.2 to 2 pg), the yield of positive clones can be estimated at ranging from 18 to 180 clones per DNA picogram.

Among the 39 positive clones, 30 had inserts of about 7.2 kb in length (group 1), 6 of about 6.8 kb (group 2) and 3 of about 6.6 kb (group 3). Sequence homology between these clones was investigated by cross-hybridization. A strong hybridization signal was obtained between cloned sequences of the same group. We can therefore assume that clones of each of the three groups have identical inserts. Inserts of groups 1 and 2 cross-hybridized. Moreover, they showed the same hybridization pattern, which is typical of repeated sequences. They hybridized with a large number of genomic DNA restriction fragments, as shown for one of them, λPW111 (Fig. 3A). On the other hand, clones of group 3 did not cross-hybridize with those of groups 1 and 2. The typical hybridization pattern is shown for λPw130 (Fig. 3B). It hybridized to a single fragment in *Bgl*II digest, to two fragments in *Eco*RI and to three in *Bam*HI digests. These very few hybridizing fragments represent either single copies or a clustering of repeats.

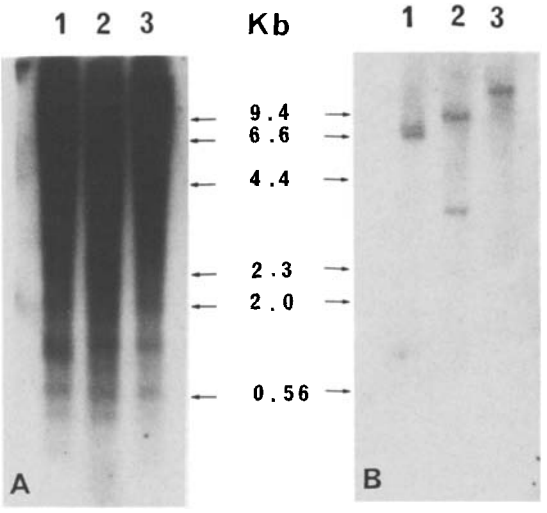


Fig. 3A, B. Southern hybridization of λPw111 and λPw130 to *Pleurodeles* genomic DNA. DNA was cut with *Eco*RI (*1*), *Bam*HI (*2*), *Bgl*II (*3*), electrophoresed on 1% agarose gel, transferred to nitrocellulose filter and hybridized with ^{32}p-labelled inserts of λPw111 (**A**) and λPw130 (**B**). In *lane* (*2*) *Bam*HI, the smallest band (600 bp) is not visible in this autoradiogram. All *lanes* were loaded with 20 μg DNA. Autoradiography was carried out at −70° C for 72 h. *Arrows* point to the position of selected *Hin*dIII-restricted fragments of λ DNA

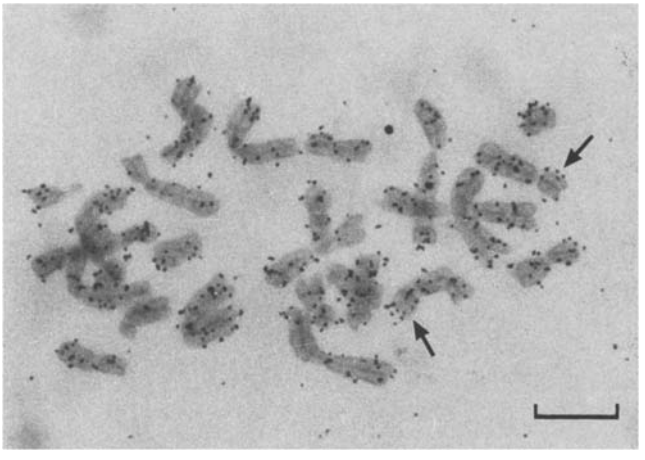
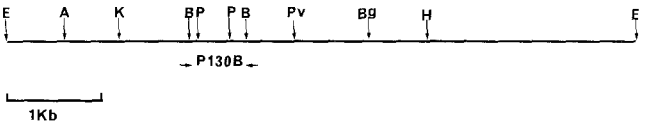


Fig. 4. Hybridization of λPw130 to metaphasic chromosomes. Autoradiograph of mitotic chromosomes after in situ hybridization with ^{3}H-labelled cRNA complementary to λPw130 cloned sequence. The label is observed at many sites in the 24 chromosomes of the karyotype. *Arrows* indicate the chromosome VII. Exposure: 120 days. Bar represents 15 μm

Chromosomal localization of the cloned sequences

The distribution of these cloned sequences in the genome was investigated by in situ hybridization to mitotic metaphase chromosome using radioactive cRNA probes. For both clones, λPw111 and λPw130, significant hybridization signals were revealed at many sites in different chromosomes, as shown for one of them (Fig. 4). We can therefore conclude that both clones λPw111 and λPw130 contain repetitive sequences interspersed over the chromosome set.

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CCGGCGTATCAGGGTACGACGGATATCGTGGGGGGGGAAATTGCTTTCGG

51
TTCCGACTCTGCCATTAATATGGCAAGTCAACACATTAATATCCATAACAG

101
CGGTGTGATGTCGGGAAATGTCACCACTGCAGGTGATATGAACGTTATGC

151
CTGGGGGGGGCACTGCGTGTCGCTAAAACCACTATCGGCGGCAACCTGGA

201
GAATGGCGGCACGGTTCAAATGAATAGCGAAGGGGGGAAACCGGGGAAT

251
GTACTGACCGTTAACGGGCAACTATACCGGAAACAATGGCCTGATGACGT

301
TCAACGCGACGCTGGGCGGCGATAATTCGCCCACCGATAAGATGAACGTG

351
AAAGGCGATACCCAAGGGAACACTCGCGTTCGGGTTGATAACATTGGCGG

401
CGTCGGTGCACAAACGGTCAACGGTATTGAACTCATTGAGGTTGGCGGTA

451
ATTCTGCAGGTAACTTCGCGCTGACCACCGGAACTGTCGAAGCTGGGGCT

501
TACGTCTACACGCTGGCTAAAGGGAAGGGGAATGACGAGAAAAACTGG

551
TATCTGACCAGTAAATGGGACGGTAACGCCAGCGGATACACCCGATCCCA

601      605
TCAATAA
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Fig. 5. Nucleotide sequence of the subclone p130B. We refer to this sequence as the strand of p130B DNA, which is transcribed in lampbrush chromosomes. At the *top* is shown a restriction map of the cloned sequence λPw130 (origin of p130B). *A*, *Ava*I; *B*, *Bam*HI; *Bg*, *Bgl*II; *E*, *Eco*RI; *K*, *Kpn*I; *P*, *Pst*I; *Pv*, *Pvu*II

Cloned sequences of λP130:p130B

Restriction analysis of λPw130 was carried out (Fig. 5). Fragments of *Eco*RI *Bam*HI (1.9 kb), *Bam*HI *Bam*HI (0.6 kb), and *Bam*HI *Eco*RI (4.1 kb) showed the same Southern hybridization pattern as the whole insert (Fig. 3 B). One of them, the 0.6 kb *Bam*HI, called p130B, was characterized. It cross-hybridized with all restricted fragments of λPw130 and all other clones of group 3. Such results therefore suggest that P130B sequences are common, at least in part, to clones of group 3. They demonstrate, moreover, that λPw130 is internally repeated. Analysis of p130B nucleotide sequence gave no evidence for an internal or inverted repeats (Fig. 5). A search within the Genbank data did not yield any homology with either known repetitive DNA sequences or a known protein sequence. In order to estimate the number of genomic copies of p130B, slot-blot hybridizations to genomic *Pleurodeles* DNA were carried out. Assuming complete homology between hybridizing geno-

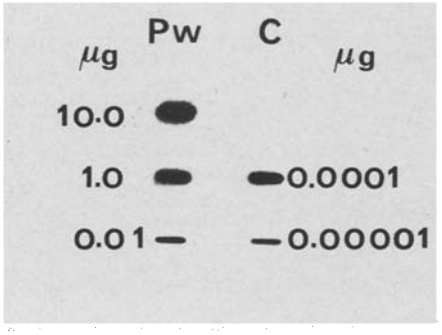

Fig. 6. Slot blot hybridizations of p130B to *10* μg, *1* μg, 0.01 μg of whole alkali-denatured DNA from *Pleurodeles waltl* (*Pw*); 10^{-4} μg and 10^{-5} μg slots of p130B DNA as controls (*C*)

mic DNA and p130B DNA, we estimated that these sequences represented 0.01% of *Pleurodeles* genome (Fig. 6). On the basis of genomic DNA content of 19 pg, we calculated that there are 3000 p130B copies per haploid genome.

Transcription of p130B sequences

Transcription of p130B was investigated by in situ hybridization to the nascent transcripts on lampbrush loops; 100 preparations from five different females were selected, in which the 12 bivalents of the karyotype were well identified. ^{35}S-labelled RNA probes were synthesized from either strands of the cloned DNA by using either SP6 or T7 polymerase (see Materials and methods). No labelling was observed when SP6-derived RNAs were used as probes. In contrast, with T7-derived RNAs, significant labelling was detected on a few loops in different bivalents. Since the number of hybridization sites on lampbrush chromosomes is much smaller than the one detected on mitotic chromosomes, we can assume that among the 3000 copies of p130B distributed in the genome, only few copies are expressed – those visualized by labelled lampbrush loops. These labelled loops conformed either to the normal or to the granular types. This in situ hybridization pattern was found to be exactly the same as that of the whole insert of λPw130.

Among the granular loops that are always present in the karyotype, i.e., those of bivalents VII, IX, X and XI, only one loop pair exhibited labelling. It was located in the microdissected region of the bivalent VII (Fig. 7A). This labelling was always slight, partial, and

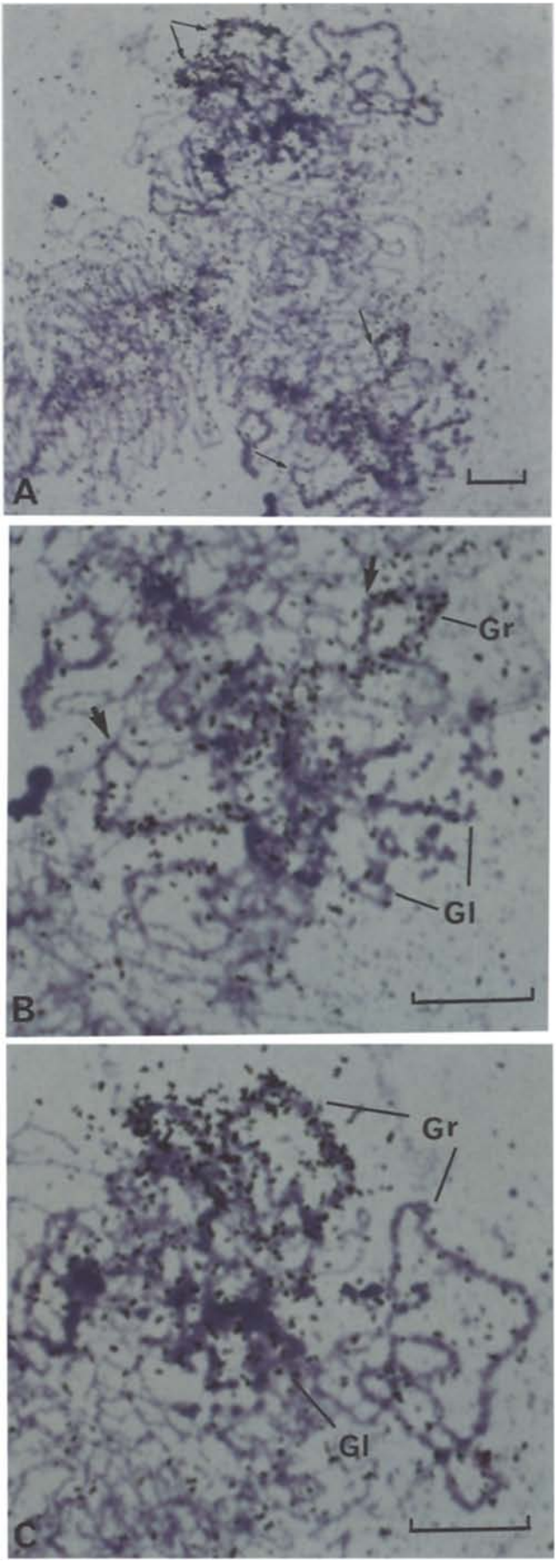

Fig. 7A–C. In situ hybridization to the nascent RNA transcripts on lampbrush chromosome from *Pleurodeles waltl*. The probe was ^{35}S-labelled cRNA synthesized from one of the two DNA strands of p130B using T7 polymerase. **A** Autoradiography of a part of the bivalent VII showing one pair of granular loops labelled (*arrows*). This loop was one of the landmark loops that were microdissected. **B, C** High magnification of the granular (*Gr*) and globular (*Gl*) loops of the two homologues shown in **A**. Only one of the granular loops is labelled, and this labelling is partial, as clearly shown in **B** (*arrows*). Exposure: 4 days. Bars represent 20 μm

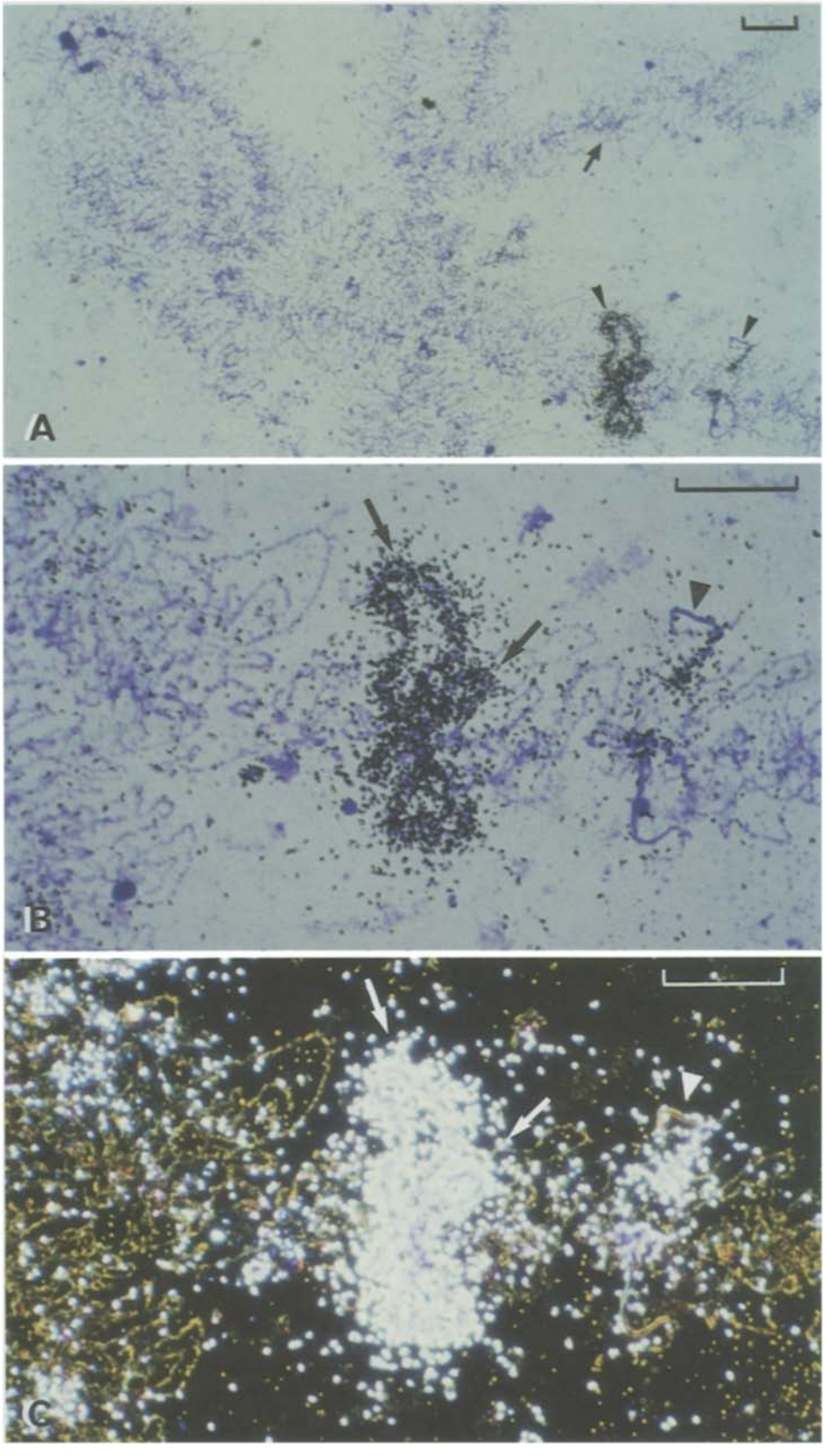

Fig. 8A–C. In situ hybridization to the nascent RNA transcripts on lampbrush chromosomes from *Pleurodeles waltl*. The probe was ^{35}S-labelled cRNA synthesized from one of these two DNA strands of p130B using T7 polymerase. **A** Autoradiograph of part of the bivalent IV with two hybridization sites on one homologue (*arrowheads*) and none on the other homologue (*arrow*). **B**, **C** High magnifications of **A** in a bright field (**B**) or with darkbackground (**C**) illumination. One hybridization site concerns two clustered normal loops of different size, uniformly and heavily labelled (*arrows*). The other site concerns one granular loop partially labelled (*arrowhead*). Exposure: 4 days. Bars represent 20 μm

limited to the granular part of the loop (Fig. 7B, C). Other granular loops, such as those mapped on bivalents II and IV, are only transiently formed during the oocyte development. Such loops always showed label (Fig. 8B, C).

Among the residual 10000 normal loops distributed along the chromosome axes of the 12 bivalents, only two sites displayed hybridization. One site formed two normal loops clustered in the middle region of one-half of the bivalent IV (Fig. 8A). These loops were strongly and uniformly labelled (Fig. 8B, C). However, this hybridization site involved only one of the two homologous chromosomes, while the other homologue remained unlabelled. The other site corresponded to one loop pair in both homologues in bivalent X (data not shown).

The weak labelling observed in granular loops, compared to that of normal loops, may be due to the transcription of a limited set of p130B copies or to poor accessibility of probes. In these loops, RNA transcripts are compacted in a dense matrix (Bonnanfant-Jaïs et al. 1986). The partial labelling observed in granular loops may point to the occurrence of several transcription units along these loops; p130B could hybridize to the transcripts of only one of them. The presence of several transcription units within a single loop has previously been described (Angelier and Lacroix 1975; Scheer et al. 1976; Diaz et al. 1981).

p130B expression was also investigated by Northern hybridization to ovary RNA. No positive signal was detected with either total RNA, poly(A+), poly(A−) RNA or nuclear RNA while the same RNA blots gave strong signals with other probes such as hsp70 or hsp90 (not shown).

Discussion

Similar morphology of landmark loops does not reflect total similarity of their transcripts

The main purpose of this study was to detect if similarity of landmark loop matrix morphologies implies total similarity of their transcripts. The sequence p130B and the whole insert of the lambda clone Pw130 were, however, not found expressed in all landmark loops showing the same morphology. The expression was restricted to only one of the ten granular loop pairs. The granular loop involved was one of the landmark loops that were microdissected. Since a hybridization signal was also detected in three loop pairs of normal type, two on bivalent IV and one on bivalent X, this sequence was, moreover, found expressed in loops exhibiting various morphologies. We can, therefore, conclude that similarity in landmark loop matrix morphologies does not reflect total similarity of their transcripts. Such results do not entirely exclude a possible relationship between the expression of specific sequence(s) and a specific morphology of the landmark loop matrices. However, none of the 39 recovered clones was found to be always expressed in all landmark loops of one specific type.

Distribution and transcription of cloned sequences in Pleurodeles genome

From microclone analysis, sequences cloned from landmark loop DNA of *Pleurodeles waltl* are mainly represented by two clones λPw111 and λPw130. These clones contain repetitive sequences. Since Southern hybridization experiments using *Pleurodeles* genomic DNA as a probe gave no signal, we can therefore hypothesize that these sequences are likely to belong to a class of moderately rather than highly repetitive sequences. For λPw111, its Southern and in situ hybridization patterns on mitotic chromosomes allow us to conclude that cloned sequences are repetitive and highly interspersed over the chromosome set. For λPw130, analysis of one subclone provides evidence that it is internally repeated. Moreover, from Southern (1975) and in situ hybridizations on mitotic chromosomes, we can conclude that λPw130 represents clusters of repeats, such clusters being distributed at many sites in different chromosomes. A similar distribution has already been reported for sequences of satellite type in various amphibian species (Epstein et al. 1986; Wu et al. 1986; Varley et al. 1991; Vignali et al. 1991).

Our results also suggest that the complexity of the clones we recovered in total is quite restricted. That might mean that the microdissected loops are either mainly composed of such sequences or that we have cloned a highly selective fraction, possibly because the other sequences might be restricted to turn up in our microcloning experiment. The first possibility seems hardly credible since the in situ hybridization pattern on mitotic chromosomes revealed many sites, none of them preferential. We therefore concluded that microdissected loops share these sequences with other sites. It is still difficult, however, to design a model of the DNA arrangement of this loop.

The present study also provides information concerning the transcription of p130B sequence in oocytes. In situ hybridization of cRNAs probes to the nascent transcripts of lampbrush loops using strand-specific probes demonstrates that only one of the two strands of Pw130 DNA is transcribed. This transcription cannot be explained according to the general model proposed by Gall et al. (1983) for lampbrush TUs, in which repetitive sequences are transcribed by read-through from upstream structural gene promoter. This model has been corroborated by data from most repetitive sequences already studied in amphibians (Diaz et al. 1981; Jamrich et al. 1983; Barsacchi-Pilone et al. 1986; Wu et al. 1986; Randsholt et al. 1989). It implies that the two strands of these repetitive sequences are transcribed because some genes are read in one direction and the others in the opposite one, which is not the case for p130B sequences. Since the p130B transcripts were not stored in oocyte (see Northern hybridization results), the significance of this expression must still be elucidated.

Microdissection and microcloning

Microcloning, easily applicable in several other systems such as polytene and metaphasic chromosomes, encounters some special problems here due to: (1) the very low DNA content in lampbrush loops; (2) the very large amount of binding proteins in such loops; (3) the possible contamination by adjacent loops during microdissection. In order to resolve these technical problems inherent to amphibian lampbrush chromosomes, we had to (1) microdissect numerous loop pairs, (2) increase the SDS concentration during the DNA extraction step, and (3) recover bivalents exhibiting only landmark loops by using selective inhibition of transcription as described above (Fig. 1; see also Scheer et al 1984; Penrad-Mobayed et al. 1986). Such a microcloning procedure had previously been applied to Y-lampbrush chromosomes in *Drosophila* spermatocytes (Hennig et al. 1983). Microdissection of one specific lampbrush loop was possible in *Drosophila*, since males exhibiting only one loop type in their spermatocyte nuclei would be constructed by genetic procedures (Hackstein and Hennig 1982). In the present paper, it is shown for the first time that it is possible to microclone DNA from specific amphibian lampbrush loops, opening the way for a molecular approach of amphibian lampbrush loops.

Concluding remarks

These results are the first data concerning amphibian landmark loop transcripts. They provide evidence that a morphological similarity of landmark loops is not inherent to a total similarity of their transcripts. However, it is obvious that such results cannot exclude the possibility of some relationship between specific morphologies of landmarks and specific transcripts. The present state of our research does not allow us to come to any conclusions on this point. Nevertheless, we think that our approach by microdissecting and microcloning landmark loops DNA remains one of the best ways to resolve this particular problem in amphibian lampbrush chromosomes.

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