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Constitutive expression of a somatic heat-inducible *hsp70* gene during amphibian oogenesis

Bernard Billoud[1], Maria-Luz Rodriguez-Martin[1], Line Berard[1], Nicole Moreau[1,2] and Nicole Angelier[1,*] with the technical assistance of Marie-Christine Laine[1]

[1]Centre de Biologie Cellulaire, CNRS, ER47, 67, rue Maurice Günsbourg, 94205, Ivry-sur-Seine CEDEX, France
[2]Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France

*Author for correspondence

SUMMARY

We isolated and characterized a sequence coding for heat-shock protein 70 (HSP70) of the amphibian *Pleurodeles waltl*. Results from S1 nuclease protection assays led us to conclude that an *hsp70* gene, strictly inducible in somatic cells during heat shock, is constitutively active during oogenesis. By quantitative northern and western blot analysis, we showed that both hsp70 mRNA and HSP70-related protein levels increased in oocytes from stage II to stage VI under physiological conditions. Furthermore, by in situ hybridization to the nascent transcripts of lampbrush chromosome loops, we provided evidence for a clear-cut relationship between this increase in hsp70 mRNA and transcriptional activity during the lampbrush stage of oogenesis. These results strongly suggest that *hsp70* genes are actively transcribed throughout oogenesis. HSP70-related proteins localized in the cytoplasm of young oocytes are progressively transferred to the nucleus in the course of oogenesis and preferentially accumulated in the nuclei of some stage VI oocytes.

Key words: amphibian, oogenesis, lampbrush chromosomes, *hsp70* gene, transcription, protein nuclear transfer

INTRODUCTION

Heat-shock proteins, which represent one of the most evolutionarily conserved sets of proteins, are present in every cell type examined thus (for review, see Lindquist and Craig, 1988). In higher organisms, *hsp70* is a multigenic family that includes the transiently stress-inducible form(s) of HSP70 proteins involved in the cellular response to various types of stress, and one or more cognate protein(s) (HSC70) constitutively expressed under physiological conditions. Such constitutive gene expression has been reported in a wide variety of eukaryotic non-stressed cells from yeast, *Drosophila*, amphibian, mouse and human (Craig et al., 1983; Palter et al., 1986; Zimmerman et al., 1983; Bienz, 1984; Heikkila et al., 1987; Kurtz et al., 1986; Wu and Morimoto, 1986; Bensaude et al., 1983; Rosario et al., 1992). In *Xenopus*, *hsp70* genes are constitutively expressed during oogenesis (Heikkila et al., 1987; Horrel et al., 1987) and hsp70 mRNAs appear to be accumulated by stage III oocytes (Horrel et al., 1987). It is unclear, however, whether this indicates that a steady-state rate of transcription and decay is reached at stage III or that the *hsp70* genes are no longer transcribed after stage III. The HSP70 protein may be constitutively synthesized in *Xenopus* oocytes, since synthesis of two polypeptides that co-migrate with a HSP70-68 complex would occur in stage VI oocytes (Bienz and Gurdon, 1982). Many authors, however, failed to detect any HSP70 protein synthesis in defolliculated oocytes either before or after heat shock (King and Davis, 1987; Horrell et al., 1989). It seems that, in oocytes as well as in follicle cells which surround every oocyte, constitutive synthesis of HSP70 is below the level of detectability. Induction of HSP70 protein synthesis upon heat shock would be due to the follicular cells (Horrell et al., 1989).

In the present study, we addressed these questions in the amphibian urodele *Pleurodeles waltl*. During the middle and late stages of oogenesis, this amphibian exhibits, in its oocytes, particularly well-developed lampbrush chromosomes (for revue, see Callan, 1986; Angelier et al., 1990) enabling vizualization of transcriptionally active chromatin at the level of their lateral loops. By in situ hybridization of a homologous hsp70 gene-specific cRNA probe to lampbrush chromosomes, we were able to vizualize the precise site of *hsp70* gene transcription. Moreover, we provide evidence for such transcription at every stage of oogenesis. We also demonstrate that the amount of hsp70 mRNA regularly increased during oogenesis, suggesting that, following stage III, vitellogenic oocytes not only transcribed *hsp70* genes but also accumulated corresponding newly synthesized mRNAs. At the same time, we provide evidence for synthesis of HSP70-related proteins and their concomitant accumulation during oogenesis. Immunocytochemical localizations enabled us to observe nuclear transfer of these proteins in late oogenesis. This result is discussed

in relation to the possible function(s) of HSP70-related proteins in oocytes.

MATERIALS AND METHODS

Collection of oocytes

P. waltl females were raised in our laboratory at 20°C. Oocytes were collected and then defolliculated by two different techniques. Oocytes previously incubated for 15 minutes in modified Steinberg-EDTA (Masui, 1967) were manually defolliculated under the microscope with fine forceps as already described (Moreau et al., 1991). Alternatively, pieces of ovary were treated by collagenase as previously described (Moreau and Boucher, 1981). These defolliculated oocytes were then transferred to modified Barth's medium (MBS, Gurdon, 1976) and sized according to the six stages described by Bonnanfant-Jaïs and Mentré (1983). The absence of follicular cells was checked by microscopic examination of Hoechst 33258 (Sigma)-stained oocytes. The same results were obtained for protein and RNA analysis whatever the defolliculation procedure used.

Protein extraction and SDS-polyacrylamide gel electrophoresis

Batches of 20 defolliculated oocytes were incubated in 100 µl of MBS medium supplemented with 50 µCi of [^{35}S]methionine (specific activity 800 Ci/mmol, Amersham). Incubation was performed for $2\frac{1}{2}$ hours at 19°C. After incubation, oocytes were washed with three baths of MBS and then homogeneized in Tris-EDTA buffer (Chen and Stumm-Zollinger, 1986). The homogenate was centrifuged for 10 minutes at 10000 ***g***. Proteins of the supernatant were precipitated overnight at −20°C by 9 volumes of ethanol. The precipitate was recovered by 15 minutes centrifugation at 5000 ***g*** and treated for electrophoresis.

One-dimensional SDS-10% polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Two-dimensional electrophoresis was performed with equilibrium pH gradient electrophoresis (O'Farrell, 1975) using ampholines pH 3-11 in the first dimension, followed, in the second dimension, by electrophoresis on an SDS-10% polyacrylamide slab gel (Laemmli, 1970), and silver stained according to Morrissey (1981).

Antibody and immunoblotting

We used a mouse monoclonal antibody, mAbH3F18 which was raised against a *Plasmodium falciparum* 72×10^3 M_r HSP70-like protein (Mattei et al., 1989). The H3F18 epitope is a 16 aa sequence lying about 160 residues upstream of the protein C terminus.

Total oocyte proteins separated by SDS gel electrophoresis were transferred to an immobilon-PVDF membrane (Millipore) according to Towbin et al. (1979). Blots were incubated for 1 hour in 3% BSA in PBS at 40°C. They were washed with PBS, 1% Tween 20 and then incubated for 45 minutes with mAbH3F18 at room temperature. After washing, they were incubated with a ^{125}I-labelled (specific activity 3000 Ci/mmol, Amersham) anti-mouse antibody.

Molecular cloning, library immunoscreening, PCR amplification and sequence analysis

Antibody mAbH3F18 was used to probe a *Pleurodeles* cDNA library prepared from ovary poly(A)$^+$ RNA and constructed in λgt11 (Young and Davis, 1983a,b) This library was carried out using the strategy described by Huyn et al. (1985); cDNA was synthesized according to Okayama and Berg (1982), modified by Gubler and Hoffman (1983). The cDNA was inserted into λgt11 at the *Eco*RI site, and encapsided in vitro. Approximately 10^6 recombinant bacteriophages were amplified to produce the working library (Davis et al., 1980). Plating and preparation of nitrocellulosis filters (Schleicher and Schuell BA85) for screening were carried out essentially as described by Young and Davis (1983a). Filters were then treated with mAbH3F18 as described above. Three screening rounds were performed to obtain single phage clones. Pw70, one of the nine positive cloned sequences, was transferred into pGEM3Zf(+). From Pw70, two *Cla*I digestion products, respectively referred to as Pw70/M (Middle part, 707bp) and Pw70/3′ (534bp) were subcloned in pGEM7Zf(+). The nucleotide sequence of Pw70/M and Pw70/3′ was determined using the dideoxy chain-termination method (Sanger et al., 1977).

Genomic DNA of the 5′ part of the *hsp*70 gene was obtained by PCR amplification (Mullis and Faloona, 1987) with AmpliTaq DNA polymerase (Perkin-Elmer Cetus) using 1 µg of *P. waltl* genomic DNA as a template. The 3′ primer (TCTTGTTCTTGGTGATGTCC) was derived from Pw70/M. The 5′ degenerated primer was designed from the consensus HSE sequence and its upstream nucleotides in *Xenopus*: AAGCCTCKSGAAIVTTCSIG (I=inosine, K=G or T, S=C or G, V=A or C or G) ; 33 thermocycles were performed for 30 seconds at 93°C, 30 seconds at 52°C, 60 seconds at 72°C. To deal with primer degeneracy, in the first 3 cycles the temperature increase from 52°C to 72°C was at 15°C/minute All other temperature shifts were at 60°C/minute (Compton, 1990). The sequence of amplified DNA was determined using a method derived from that of Sanger (DSCS kit, BRL).

Sequence analysis was performed using the BISANCE (Base Informatique Sur les Acides Nucleiques pour les Chercheurs Européens) software package (Dessen et al., 1990), provided by CITI2 (Centre Interuniversitaire de Traitement de l"Information).

Northern blotting and nuclease S1 protection assay

Ovaries and livers were surgically removed from *P. waltl* females and cut into several pieces. Half of these fragments were heat shocked for $2\frac{1}{2}$ hours at 34°C in Barth's medium (Rodriguez et al., 1991) before RNA extraction. In all batches, RNA was extracted using the LiCl-urea method (Auffray and Rougeon, 1980). RNA from defolliculated oocytes at different stages (see above) was extracted by the same method.

Poly(A)$^+$ RNA was purified according to Aviv and Leder (1972) by two successive chromatography rounds on oligo(dT)-cellulose. Northern blotting was performed according to Sambrook et al. (1988). RNAs were fractionated through an agarose/formaldehyde gel and blotted onto a nitrocellulosis membrane (Schleicher and Schuell BA85). The filter was prehybridized in 0.2 ml/cm^2 of 50% formamide, 6× SSC, 2× Denhardt's, 1 µg/ml sonicated salmon sperm DNA for 4 hours at 42°C. Hybridization was performed in 0.1 ml/cm^2 of the same buffer plus a denatured double-stranded [^{32}P]DNA Pw70 probe whose specific activity was typically 0.5 to 2×10^9 cpm/µg (Amersham Random Priming kit). Washing was for 2× 30 minutes at 65°C in 1× SSC, 0.1% SDS, and for 2× 30 minutes in 0.1× SSC, 0.1% SDS.

For the S1 protection assay (Pelham, 1982), an 850 bp single-stranded antisense DNA probe was generated from a *Pvu*II digest of the plasmid containing Pw70/3′. Hybridization of 10^5 cpm of this probe with 20 µg of total cellular RNA (assays), 20 µg of yeast tRNA (negative control) or 20 µg of tRNA + sense Pw70/3′ RNA (positive control) was performed overnight at 42°C. DNA was digested for 1 hour at 40°C with 600 units of nuclease S1, phenol/chloroform-extracted, ethanol-precipitated and analyzed on a 5% denaturing polyacrylamide gel.

Quantitative RNA slot blot hybridization

RNA quantitation experiments were derived from the technique described by Taylor et al. (1986). The exact amount of hsp70 mRNA in oocytes was determined by comparing the signal obtained by hybridization of a Pw70 probe to endogenous oocyte RNA and to a synthetic Pw70 RNA used as a standard.

(1) In order to deposit on the blot a total RNA amount corre-

sponding to a fixed number of oocytes whatever the extraction yield, we added an hsp70-unrelated [^{35}S]RNA (10^4 cpm for 10 oocytes) to the homogenization buffer in the first step of the extraction protocol. Assuming that the extraction yield of the cellular RNA is equal to that of the synthetic one, the RNA from a fixed number of oocytes can be associated with a known amount of radioactivity.

(2) In order to obtain a standard for hybridization with Pw70, a [^{35}S]-labelled hsp70 sense RNA was synthesized. The number of RNA copies was then determined by radioactivity measurement. Successive dilutions were made, allowing us to deposit on the filter known and increasing numbers of transcript copies, typically ranging between 10^7 and 5×10^8 copies per slot.

(3) RNA from (1) and (2) were deposited on the same blot. After hybridization with a [^{32}P]Pw70 probe and autoradiography, the signal was measured on the autoradiogram. A calibration curve for autoradiographic signal versus transcript number was plotted using the standard described in (2). Intrapolation in this curve of the measured values for oocytes at each oogenesis stage enabled us to calculate their hsp70 RNA content.

In practice, both synthetic RNAs used in (1) and (2) were transcribed in vitro as described below for RNA probes, with low specific activity [^{35}S]rUTP to prevent adding a background signal. RNA solutions (oocyte-extracted RNAs or hsp70 synthetic sense RNA dilutions) were blotted onto a nitrocellulosis membrane (Schleicher and Schuell BA85). Prehybridization, hybridization and washing were performed as described above for northern blots.

Autoradiography and scanning densitometry measurements

Northern blots were autoradiographed for 1 to 6 days on Kodak XAR5 films at −80°C with two reinforcing screens. Different exposures were used to improve the reliability of the standardization procedure. The autoradiograms were optically scanned either on a Shimadzu CS930 linear scanner or on a Biocom image processing device.

Immunoblots were autoradiographed on Amersham β-max films. The signal was quantified by both densitometry scanning (as above) and γ counting of corresponding cut-out filter pieces.

In situ localizations

In situ hybridizations on *P. waltl* lampbrush chromosome spreads were carried out as previously described (Penrad-Mobayed et al., 1991). Lampbrush chromosomes were prepared according to Gall (1954). Germinal vesicles of oocytes were manually isolated in 75 mM KCl, 25 mM NaCl, 10 mM Tris-Cl pH 7.2, 10 mM $MgCl_2$ and 10 mM $CaCl_2$ (Angelier et al., 1986). The nuclear envelope was removed, and the nuclear content was centrifuged (1500 ***g*** for 30 minutes at 8°C) onto the coverslip. Chromosome preparations were fixed in 70% ethanol, dehydrated through an ethanol series, washed in xylene to remove paraffin and air-dried from acetone. [^{35}S]UTP RNA probes (specific activity 3×10^8 cpm/μg) were synthesized in vitro from either strand of different subclones (Pw70/M or Pw70/3′) according to the manufacturer's instructions (Promega Biotech). The probe was reduced to an average size of 100 base pairs by alkaline hydrolysis and used at a final concentration of 4×10^5 cpm per slide. Hybridization was carried out as described by Penrad-Mobayed et al. (1991) in 40% formamide, 10 mM DTT, 4× SSC, 1× Denhardt's, 0.1% SDS, 1 μg/ml yeast tRNA and 1 μg/ml salmon sperm DNA. After washing in 0.1× SSC, 10 mM DTT for 3× 20 minutes at 65°C, the slides were air-dried, dipped in liquid emulsion (Kodak NTB2 twofold diluted in H_2O) and exposed at 4°C for 4 to 10 days. After development, lampbrush chromosomes were stained in Coomassie blue (Gall et al., 1981).

For immunolocalizations, 7.5 μm thick sections of ovary fixed with Romeis fixative (25 ml saturated $HgCl_2$, 20 ml TCA, 15 ml 37% formaldehyde) and embedded in polyester-wax (Hausen et al., 1985) were prepared as already described (Moreau et al., 1986). They were incubated for 1 hour with mAbH3F18 antibody (1/100 dilution) in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). After three washes in PBS, slides were incubated with a fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Miles scientific, Paris), washed and mounted in Mowiol.

RESULTS

Specificity of the antigen-antibody reaction

The specificity of antibody mAbH3F18 raised against *Plasmodium falciparum* HSP72 (Mattei et al., 1988) was checked by its binding to two-dimensional electrophoretically separated and blotted *P. waltl* oocyte polypeptides. We examined binding to a 10000 ***g*** fraction of whole oocytes. The reaction was positive with only one 74×10^3 M_r, pI=5.1 polypeptide (Fig. 1).

Isolation and characterization of the P. waltl hsp70 sequence

We constructed an ovary cDNA library in expression vector λgt11 (Young and Davis, 1983a,b). Immunoscreening of 5×10^5 clones with the mAbH3F18 antibody, whose specificity was previously checked, enabled us to isolate 9 positive transformed λgt11 clones. Partial sequence analysis revealed that all these positive clones were derived from identical cDNA encoding for polypeptides closely related to the carboxy-terminal part of the HSP70 protein family. One of the inserted sequences, called Pw70 (1241 bp), was therefore subcloned in pGEM3Zf(+). Two *Cla*I restriction fragments, 707 and 534 bp, were obtained from Pw70, and we will refer to them as Pw70/M (middle) and Pw70/3′, respectively. These sequences were analyzed by the dideoxy-chain-termination method (Sanger et al., 1977). Pw70 presented an open reading frame (the first 1194 bases) which encoded a 398 amino acid polypeptide (Figs 2 and 3). The nucleotide sequence and its deduced polypeptide were found to be highly homologous, respectively, to the 60% 3′-terminal part and the C-terminal part of *hsp70* genes and proteins known in other species.

In order to determine whether the Pw70 sequence was a partial sequence of a heat-inducible gene (*hsp*) or a cognate gene (*hsc*), test experiments for heat inducibility in somatic cells were carried out. Using the least conserved part of the sequence that is likely to be gene specific (Chappell et al., 1987), i.e. Pw70/3′, as a probe, we performed northern blot and S1 nuclease protection analysis on somatic cells before and after heat shock. Pw70/3′ was hybridized to northern blots of RNA from liver cells before and after heat shock. In normal liver cells, no related RNA was ever detected. In contrast, a very strong signal corresponding to a transcript size of about 3 Kb was observed in RNA from liver cells submitted to heat shock (Fig. 4). This result was similar to that reported for *Xenopus* hsp70 mRNA (Browder et al., 1987). S1 nuclease protection analysis confirmed these initial results. A 850-base single-stranded antisense DNA probe was generated from a *Pvu*II digest from the plasmid containing Pw70/3′ (534 bp). Fig. 5 shows that hybridization with RNA from liver cells submitted to heat shock led to the

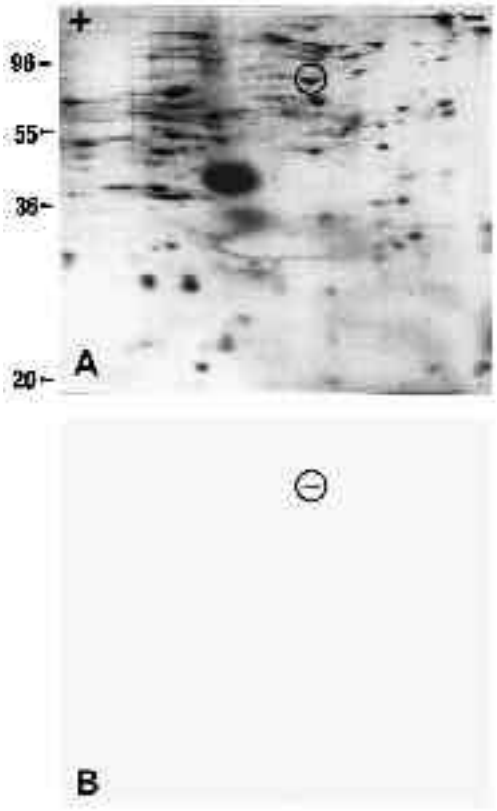

Fig. 1. (A) Two-dimensional electrophoresis of a 10000 ***g*** supernatant of 10 stage VI oocytes of *P. waltl* (silver staining according to Morrissey, 1981). Position of the HSP70 protein is denoted by a circle. Molecular mass designations on the left are in 10^3 M_r. (B) Corresponding immunoblot after incubation with an anti-*Plasmodium falciparum* HSP70 antibody (mAbH3F18). The antibody recognizes one single polypeptide denoted by a circle (74×10^3 M_r, pI=5.1).

protection of about 500 bases of the probe, i.e. almost the same size as Pw70/3′, whereas this fragment was absent from normal liver cells and controls with yeast tRNA alone. These results led us to conclude that Pw70/3′ was strictly heat-inducible in somatic cells, and therefore strongly suggested that Pw70 was part of the *Pleurodeles hsp70* gene.

In order to obtain the complete 5′ sequence of the *Pleurodeles hsp70* gene, we used the PCR procedure to amplify this segment from *P. waltl* genomic DNA using a 20-mer oligonucleotide of Pw70/M as the 3′ primer and a degenerate 5′ primer designed from the *Xenopus laevis* hsp70-regulating sequence, comprising the heat-shock responsive element (HSE) consensus (Pelham,1982; Pelham and Bienz, 1982). A 1.2 kb PCR product was obtained and sequenced. This approach enabled us to reconstitute all of the hsp70 coding sequence. Furthermore, this PCR assay and several others on genomic DNA using various primer pairs covering all of the transcribed sequence, showed that it contained no intron, again suggesting that it was actually an inducible hsp70 sequence (Yost and Lindquist, 1986).

Expression of hsp70 mRNA during oogenesis

Northern blot and S1 nuclease protection assays were performed to analyze the expression of Pw70 mRNA in oocytes under physiological conditions. In the first phase, total, poly(A)$^+$ and poly(A)$^-$ RNA were extracted from *Pleurodeles* ovaries and then probed under high-stringency conditions with Pw70. Both Pw70 probes, namely Pw70/M and Pw70/3′, hybridized to a single transcript of about 3 kb in total and poly(A)$^+$ RNA, but not in poly(A)$^-$ (Fig. 6). As contribution of small oocytes to total ovary RNA greatly exceeded that from the large oocytes, we could not totally exclude the possibility that poly(A)$^-$ hsp70 RNAs were present in late oocytes but could not be detected by northern blot assay. Nevertheless, this was an improbable hypothesis, as it would have implied that (i) the *hsp70* gene was expressed both as poly(A)$^+$ and poly(A)$^-$ RNAs, (ii) these two forms were undistinguishable by northern hybridization (see below, northern blots at different oocyte stages) and (iii) the poly(A)$^-$ RNA amount was undetectable in ovary but provided a significant signal in isolated large oocytes (see below, quantification). Such conditions had little chance to occur simultaneously, which allowed us to assume that oocytes contained poly(A)$^+$ hsp70 RNAs, but no poly(A)$^-$ hsp70 RNAs, whatever their stage.

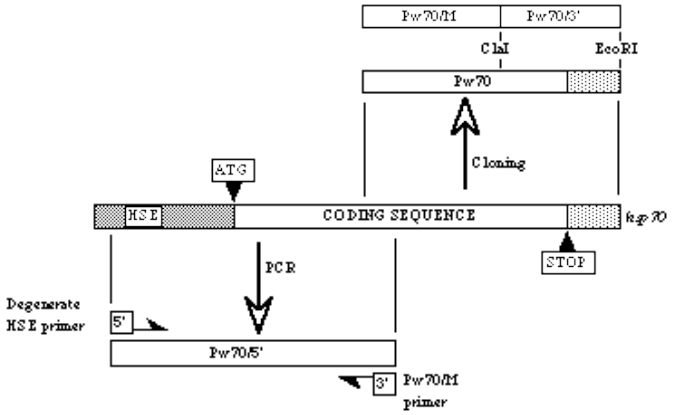

Fig. 2. Strategy for obtaining *P. waltl* hsp70 sequence. The first step is the cloning of the 3′ moiety of cDNA by immunoscreening of a cDNA library. The second step (PCR) uses sequence information from the cloned sequence, together with sequence homologies in regulation sequences of known hsp70 genes (HSE, i.e. heat-shock responsive element) to amplify the 5′ part from genomic DNA.

In order to determine whether the detected mRNA was inducible in defolliculated oocytes and/or in follicular cells, the hsp70 gene-specific probe (see above) was hybridized to northern blots of total RNA isolated from either defolliculated or non-defolliculated, normal or heat-shocked oocytes. After heat shock, a very strongly induced RNA of about 3 kb was detected in undefolliculated oocytes, whereas no signal was obtained in RNA from oocytes alone without their follicular cells (Fig. 4). These results therefore showed that hsp70 mRNA was heat-inducible in follicular cells. Thus, they corroborated our previous results obtained from somatic cells, suggesting that Pw70 was a part of the *Pleurodeles hsp70* gene. Such a gene would not be heat-inducible in oocytes without follicular cells. In contrast, when Pw70/3′ was hybridized to RNA from normal oocytes

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acgctgcgtcacttagcatcgctgtgacaagagatacagagaggtaagcatcatcaacggctatactcggcatcactgcagaag

 M  S  A  P  K  G  V  A  F  G  I  D  L  G  T  T  Y  S  C  V  G  V  F  Q  H  G  K  V    28
ATGTCTGCACCTAAAGGAGTCGCATTTGGCATCGACCTGGGCACCACCTACTCCTGTGTGGGAGTCTTCCAGCATGGCAAGGTG   84

 E  I  I  A  N  N  Q  G  N  R  T  T  P  S  Y  V  A  F  T  D  T  E  R  L  I  G  A  P    56
GAGATCATCGCCAACAACCAAGGCAACCGAACCACCCCCAGCTATGTGGCCTTCACAGACACTGAGAGGCTCATTGGAGCGCCT   168

 A  K  N  Q  V  S  L  N  P  Q  N  T  V  F  D  A  K  R  L  I  G  R  K  F  N  D  T  V    84
GCCAAGAACCAGGTGTCCCTGAACCCCCAGAACACCGTCTTTGATGCCAAGAGACTGATTGGCAGGAAGTTTAATGACACAGTG   252

 V  Q  A  D  M  K  H  W  P  F  K  V  V  S  D  E  W  K  P  K  V  Q  V  E  Y  K  G  D    112
GTGCAGGCTGACATGAAGCACTGGCCCTTCAAGGTGGTGAGCGATGAGTGGAAGCCTAAGGTGCAGGTGGAGTACAAAGGGGAC   336

 N  K  T  F  F  P  D  E  V  S  S  M  V  L  I  K  M  K  E  I  A  E  A  Y  L  G  H  L    140
AACAAGACTTTCTTCCCTGACGAGGTGTCTTCCATGGTGCTGATCAAGATGAAGGAGATCGCTGAAGCATACCTGGGCCACCTA   420

 V  S  N  A  V  I  T  V  P  S  Y  L  T  A  S  Q  R  Q  A  T  K  D  A  G  V  L  A  G    168
GTCTCCAATGCTGTCATCACTGTGCCTTCTTATCTCACTGCTTCCCAGAGGCAGGCTACTAAGGATGCTGGGGTACTCGCTGGA   504

 L  N  V  L  S  I  V  N  E  P  T  A  A  A  I  A  Y  D  L  D  K  A  G  R  G  E  L  N    196
CTGAATGTCTTGAGCATCGTCAATGAGCCCACAGCAGCAGCGATCGCCTATGACTTAGACAAAGCAGGCAGAGGAGAGCTAAAT   588

 V  L  I  F  D  L  G  G  G  T  F  D  V  S  I  L  T  I  D  D  G  I  F  E  V  K  A  T    224
GTCCTGATCTTTGACCTTGGTGGAGGTACCTTTGATGTCTCCATTCTAACAATTGATGATGGCATCTTTGAAGTCAAGGCTACA   672

 A  G  D  T  H  L  G  G  E  D  F  D  N  R  M  V  N  H  F  H  E  P  F  K  R  K  H  K    252
GCTGGAGACACCCACCTGGGTGGAGAAGACTTTGACAATCGAATGGTCAATCACTTTCATGAGCCCTTCAAGAGAAAGCACAAG   756
                                                                  Δ 5′ of clone Pw70
 K  D  I  T  K  N  K  R  A  V  R  R  L  R  T  A  C  E  R  A  K  R  T  L  S  S  S  T    280
A GGACATCACCAAGAACAAGAGGGCAGTTCGGAGGCTGAGAACTGCCTGTGAGCGAGCCAAGAGAACCCTGTCTTCCAGCACT   840
  3′ PCR Priming site
 Q  A  S  I  E  I  D  S  L  F  E  G  I  D  F  Y  T  S  I  T  R  A  R  F  E  E  L  C    308
CAGGCCAGTATCGAGATTGACTCCCTTTTCGAAGGCATTGATTTCTACACTTCCATCACCAGAGCACGCTTTGAAGAGTTGTGT   924

 A  D  L  F  R  G  P  L  E  P  V  E  K  A  L  R  D  A  K  L  D  K  A  Q  I  H  E  I    336
GCTGACCTCTTCAGAGGACCCTTGGAACCTGTGGAGAAGGCTTTGAGGGATGCCAAATTGGACAAAGCTCAGATCCATGAAATT  1008

 V  L  V  G  G  S  T  R  I  P  K  I  Q  K  L  L  Q  D  F  F  N  G  R  E  L  N  K  S    364
GTGCTAGTTGGAGGATCCACCCGCATCCCTAAAATCCAGAAACTGCTGCAGGACTTTTTCAATGGCAGAGAACTGAACAAAAGC  1092

 I  N  P  D  E  A  V  A  Y  G  A  A  V  Q  A  A  I  L  M  G  D  K  S  E  N  L  Q  D    392
ATCAACCCAGACGAAGCTGTTGCATACGGTGCAGCTGTGCAAGCTGCCATCCTGATGGGCGACAAGTCTGAGAATTTGCAGGAC  1176

 L  L  L  L  D  V  A  P  L  S  L  G  L  E  T  A  G  G  V  M  T  V  L  I  K  R  N  S    420
CTGCTTCTCCTAGATGTAGCACCTCTCTCACTGGGTCTCGAGACAGCAGGTGGTGTGATGACTGTCCTCATAAAACGCAACTCC  1260

 T  I  P  T  K  Q  T  Q  I  F  T  T  Y  S  D  N  Q  P  G  V  L  I  Q  V  Y  E  G  E    448
ACCATCCCAACTAAGCAAACCCAGATCTTCACCACCTACTCTGATAACCAGCCTGGTGTCCTGATCCAAGTTTATGAGGGTGAA  1344

 R  A  M  T  K  D  N  S  L  L  G  K  F  E  L  S  G  I  P  P  A  P  R  G  V  P  Q  I    476
AGAGCAATGACAAAGGACAACAGTCTTCTGGGGAAATTTGAGCTGAGTGGAATCCCACCTGCCCCTCGTGGTGTGCCACAGATC  1428
                  <———————— Antibody epitope ————————>
 E  V  T  F  D  I  D  A  N  G  I  L  N  V  S  A  V  D  K  S  S  G  K  Q  N  K  I  T    504
GAGGTGACTTTTGATATCGATGCCAACGGTATCTTGAATGTCTCTGCTGTGGACAAGAGTTCAGGCAAACAGAACAAGATAACT  1512
          Cla I
 I  T  N  D  K  G  R  L  S  K  E  E  I  E  R  M  V  Q  E  A  E  R  Y  K  A  D  D  E    532
ATCACCAATGACAAAGGTCGGCTGAGCAAAGAGGAGATTGAGAGGATGGTGCAGGAGGCTGAGAGGTACAAAGCAGATGATGAA  1596

 A  Q  R  E  K  V  S  A  K  N  T  L  E  S  I  A  F  N  M  K  S  T  V  E  G  D  N  L    560
GCCCAGCGAGAGAAGGTCTCTGCAAAGAACACACTGGAGTCCATCGCTTTCAACATGAAGAGCACAGTGGAGGGTGACAATCTT  1680

 K  D  K  I  S  E  D  D  R  K  K  I  V  D  K  C  N  Q  T  I  S  W  M  E  N  N  Q  M    588
AAAGACAAGATCAGCGAAGATGACCGGAAAAAGATTGTGGACAAATGTAATCAGACCATTTCCTGGATGGAGAACAACCAGATG  1764

 A  E  K  E  E  Y  E  H  Q  Q  K  E  L  E  K  V  C  N  S  I  I  T  K  L  Y  Q  G  G    616
GCAGAGAAGGAGGAGTATGAGCACCAGCAGAAAGAACTGGAGAAAGTCTGTAATTCCATCATCACCAAACTTTACCAGGGCGGG  1848

 M  P  G  G  M  P  S  G  S  S  G  A  Q  A  R  Q  G  S  S  S  T  G  P  T  I  E  E  V    644
ATGCCCGGGGGAATGCCTAGTGGGAGCTCGGGAGCTCAGGCCCGCCAAGGGAGCTCCAGCACTGGACCTACTATTGAGGAGGTG  1932

 D  End                                                                                  645
GATtaatggcggacagtaatatattatttccatgtcccttccacagaagtgaattctgccgatatt                     1935
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Fig. 3. Nucleotide sequence of *P. waltl* hsp70, and its deduced amino acid sequence, featuring:
- 5′ end of the cloned part (Pw70): base 742.
- Sequence to which the 3′ PCR primer is complementary: bases 759 to 778.
- *Cla*I restriction site: bases 1444 to 1449. It enabled us to cut Pw70 into two parts, Pw70/M and Pw70/3′.
- Epitope of the antibody used for immunoscreening: aa 483 to 498.

not submitted to heat shock, a signal of about the same intensity was obtained in the presence or in the absence of follicular cells (Fig. 4). It is noteworthy that the intensity of this signal was much lower than that obtained from heat-shocked undefolliculated oocytes. Furthermore, S1 nuclease protection assays performed in the same conditions as those previously described (see above) revealed that a fragment of about 500 bp was protected from digestion when Pw70/3′ was probed to total RNA from defolliculated normal oocytes (Fig. 5). All these results suggest that the *Pleurodeles hsp70* gene, which is strictly inducible in follicular cells (i.e. somatic cells), is constitutively expressed in *P. waltl* oocytes in the absence of heat shock and is not heat-inducible in defolliculated oocytes.

Furthermore, since no signal was ever obtained using Pw70/3′ as a probe, whether by northern blot or by S1 nuclease protection assay in somatic cells (follicular and liver cells) under physiological conditions, we assumed that these probes were actually specific to the heat-inducible gene.

In order to analyze hsp70 mRNA expression during oogenesis, northern blots were carried out with total RNA from collagenase defolliculated oocytes at defined stages (from stage II to stage VI). One unique hsp70 RNA of about 3 kb was detected for each stage considered (not shown), i.e. the same size as that of the poly(A)$^+$ hsp70 RNA detected in total ovary RNA (see above). Quantification of hsp70 mRNA could therefore be efficiently undertaken by slot blot hybridization of total oocyte RNA using Pw70 as a probe

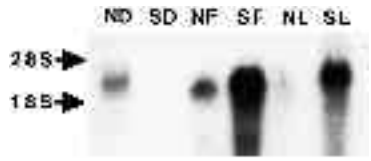

Fig. 4. High-stringency northern blot hybridization of 10 µg RNA with a [^{32}P]Pw70/3′ probe. ND, normal defolliculated oocytes; SD, heat-shocked defolliculated oocytes; NF, normal oocytes, with follicles; SF, heat-shocked oocytes, with follicle; NL, normal liver; SL, heat-shocked liver. “28S” and “18S” indicate the migration position of rRNAs. The same hybridization pattern is observed with Pw70/M or total Pw70 probes. Despite the high homology between hsp70 and hsc70, Pw70 probes do not detect the constitutive forms that are present in somatic cells.

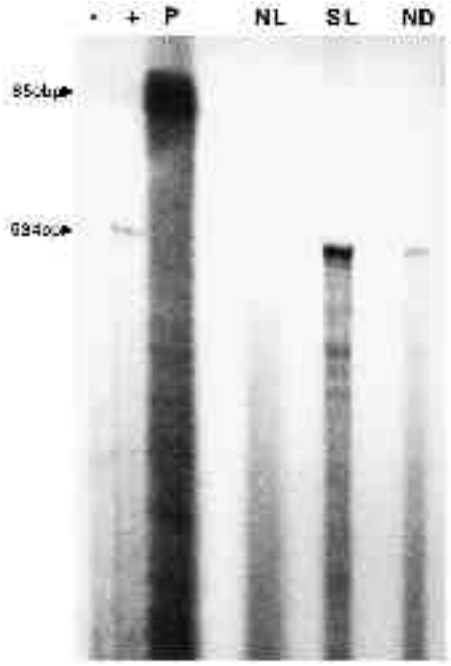

Fig. 5. Nuclease S1 digestion protection assay of an 850bp [^{32}P] probe consisting of Pw70/3′ (530 bp) + 320bp from the vector. −, negative control with yeast tRNA; +, positive control with sense Pw70/3′ cRNA; P, undigested probe; NL, normal liver; SL, heat-shocked liver; ND, normal defolliculated oocytes.
SL RNA strongly protects the probe from degradation, while NL RNA does not. Pw70/3′ can therefore be assumed to be a part of a gene strictly inducible in somatic cells. ND RNA protects the probe. Hsp70 RNA is therefore shown to be constitutively expressed in oocytes.

according to the procedure described above (see Materials and Methods). Scanning densitometry analysis of slot blot hybridization autoradiograms (Fig. 7A) provided evidence for a significant increase in hsp70 transcript amounts throughout oogenesis, including the final stages (stages III to VI), known as ‘lampbrush stages’.

In situ hybridization of probes Pw70/M and Pw70/3′ (shown to be a gene-specific probe, see above) antisense [^{35}S]cRNA to the nascent transcripts of lampbrush chromosome loops resulted in a strong signal on the same subterminal lateral loop pair of one of the 12 bivalents of *Pleurodeles* oocyte karyotype (Fig. 8). This bivalent was identified as number VIII. The labeled loop pair was of a “normal” type, and labeling was observed all along the loop. This site was the only one detected with Pw70 antisense probes. The same results were obtained with both Pw70/M and Pw70/3′ probes, whatever the lampbrush oocyte stage considered. Sense probes gave no detectable signal on any bivalent. These results strongly suggested that newly synthesized hsp70 RNA from lampbrush loops contributes to the hsp70 RNA increase observed during oogenesis.

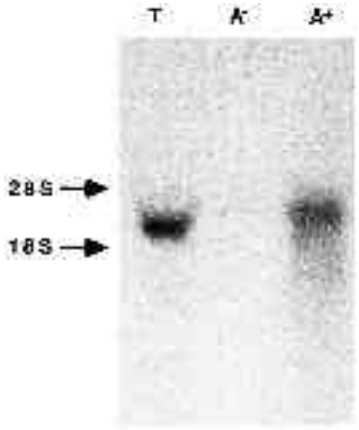

Fig. 6. Northern blot hybridization of ovary constitutive RNA with a [^{32}P]Pw70/3′ cDNA probe. T, 25 µg total RNA; A$^-$, 25 µg poly(A$^-$) RNA; A$^+$, 5 µg poly(A$^+$) RNA; ‘28S’ and ‘18S’ indicate the migration position of rRNAs. A signal is obtained with total and poly(A$^+$) RNA, but not with poly(A$^-$) RNA. The hsp70 mRNA is therefore present in the ovary under normal physiological conditions.

Expression of the HSP70 protein during oogenesis

HSP70 expression was investigated by immunoblots using the anti-hsp70 mAbH3F18 previously characterized by Mattei et al. (1989). The 16 amino acid epitope recognized by mAbH3F18 begins at about 160 residues upstream from the C-terminal aa of HSP70. As previously shown, this antibody specifically recognizes a protein of 74×10^3 M_r which is likely to be a HSP70-like protein constitutively present in *Pleurodeles* oocytes under normal conditions (see above and Fig. 1). Total extracts from collagenase-defolliculated oocytes at every stage of oogenesis (stage II to stage VI) were analyzed for their HSP70 content by immunoblotting (Fig. 7B). The sample of each lane corresponded to the same number of oocytes. Immunoblots were developed with ^{125}I-labelled anti-mouse antibody to increase the sensitivity of the assay and to enable direct quantification (see Materials and Methods). A weak signal was detected at stage II and increased to its maximum level in mature stage VI oocytes (Fig. 7B). This increase in the amount of HSP70 was correlated with the accumulation of hsp70 mRNA. These results suggest that such a dramatic increase depends on the biosynthetic activity of the oocytes throughout oogenesis. In order to determine whether HSP70 protein synthesis occurs at every stage of oogenesis, defolliculated oocytes separated into distinct developmental stages were incubated with [^{35}S]methionine. Extracts containing equal

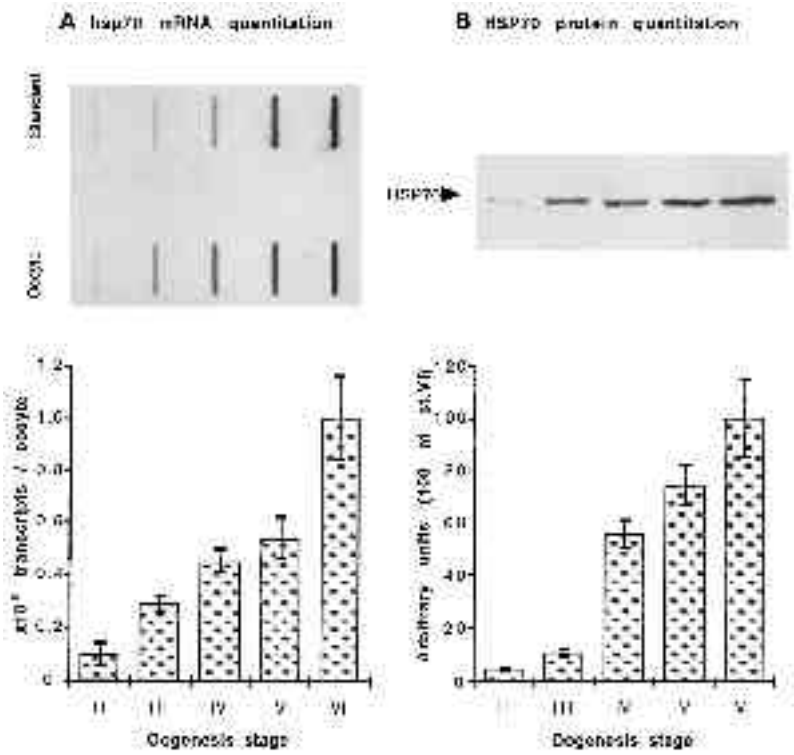

Fig. 7. (A) Autoradiography of an RNA slot-blot of different stages oocytes hybridized with the Pw70/3′ probe, and quantitation of hsp70 mRNA. Standard: synthetic sense Pw70/3′ RNA. Left to right, 5×10^7, 1×10^8, 2×10^8, 4×10^8, and 5×10^8 copies. Oocyte: total RNA of 5 oocytes per slot. Left to right, stages II,III, IV, V and VI. We determined by optical scanning of the autoradiogram that there are, respectively 1×10^7, 2.9×10^7, 4×10^7, 5×10^7, and 1×10^8 copies of the hsp70 mRNA per oocyte at stage II, III, IV, V and VI. (B) Western blot analysis of proteins from an equal number of different stage oocytes, incubated with the mAbH3F18 antibody, and then revealed with an ^{125}I-labelled IgG. Left to right, stages II, III, IV, V and VI. The amount of HSP70 was quantified in protein extracted from oocytes at each vitellogenic stage of oogenesis, i.e. stages II to VI of oogenesis. The signal of each track was quantified by both linear scanning and γ radioactive counting of cut-out filter pieces (these two methods give identical results). Taking 100 at stage VI (end of oogenesis) as a base, we estimate at respectively 4.5, 10.6, 26, 74.5, and 100 arbitrary units, the amount of HSP70 protein per oocyte at stages II, III, IV, V and VI.

amounts of incorporated radioactivity were separated by two-dimensional electrophoresis and autoradiographed. A radioactive spot corresponding to HSP70 was observed at every stage, including stage VI (Fig. 9).

Localization of HSP70 protein during oogenesis

The localization of HSP70 during oogenesis was immunohistochemically examined using the mAbH3F18 antibody in sections of isolated ovaries (Fig. 10). In previtellogenic oocytes, immunohistochemical staining was significant in the cytoplasm, while the nucleus was faintly fluorescent (Fig. 10A). In vitellogenic oocytes (stages III to V), HSP70 appeared to be localized between yolk platelets in the cytoplasm of both the animal and vegetal hemispheres. Mitochondrial aggregates showed particularly strong fluorescence. Immunoreactivity was also detectable in the nucleus (Fig. 10B). In all stage VI oocytes, HSP70 remained localized between yolk platelets in the cytoplasm. However, immunoreactivity was variable in the nuclear area (Fig. 10C-D). Furthermore, it is noteworthly that in some oocytes, the nucleus was moderately stained, with staining concentrated in the perinuclear region, whereas in others, nuclear fluorescence was strong with no preponderant labelling of the perinuclear area. During the time course of vitellogenesis, the level of nucleus immunoreactivity therefore progressively increased. Whatever the oogenesis stage considered, follicle cells were intensively labelled, particularly in their cytoplasmic region.

DISCUSSION

P. waltl hsp70 sequence

Immunoscreening and PCR amplification enabled us to recover a sequence that was closely related to *hsp70* genes from other species. In particular, the deduced polypeptide was strongly homologous to the known HSP70 and HSC70 proteins in other species. For example, we found an identity of 85.9% with *Xenopus lævis* HSP70 (Bienz, 1984), 83.3% with *Rattus norvegicus* HSC73 (Sorger and Pelham, 1987), 78.3% with *Drosophila melanogaster* HSC70 deduced from the hsc4 gene (Perkins et al., 1990), 70.1% with *Drosophila melanogaster* HSP70 deduced from the hsp2 gene (Ingolia et al., 1980) and 69.2% with *Plasmodium falciparum* HSP70 (Yang et al., 1987) to which mAb H3F18 was raised (Table 1). These results led us to conclude that this sequence belongs to the multigenic hsp70 family.

Table 1. Sequence comparison between hsp70 in *P. waltl* and other species

	Nucleotide (%)	Protein (%)
Xenopus hsp70	78.4	85.9
Rattus hsc73	76.5	83.3
Drosophila hsc70	73.6	78.3
Drosophila hsp70	68.8	70.1
Plasmodium hsp70	65.6	69.2

Whether a heat-shock gene is heat inducible (hsp gene) or a cognate gene (hsc gene) depends on the regulation of this gene in normal somatic cells: a heat-inducible gene is efficiently expressed only after heat induction, whereas a cognate gene has a high basal level of expression in most cells and is not, or only weakly, heat inducible. For example, the *Xenopus hsp70* gene has been shown to be strictly heat inducible in somatic cells (Bienz, 1984).

In *Pleurodeles*, our results from test experiments for heat inducibility in somatic cells, e.g. northern blot hybridizations and more specifically S_1 nuclease protection assays,

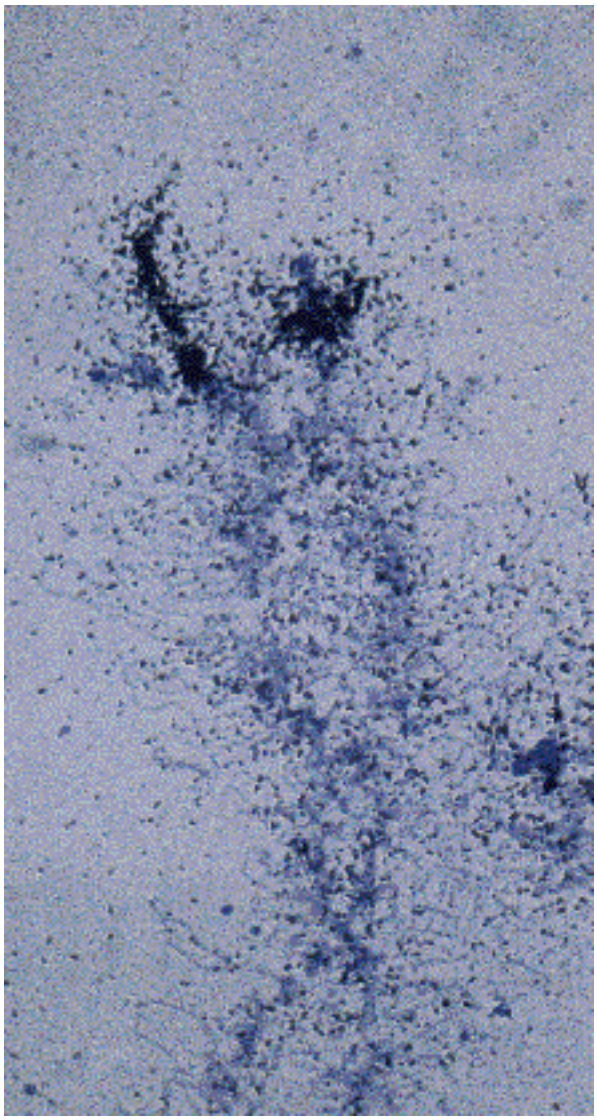

Fig. 8. In situ hybridization to the nascent RNA transcripts on lampbrush chromosomes from *P. waltl*. The probe is an antisense ^{35}S-labelled *P. waltl* Pw70/3′ cRNA. One hybridization site is detected on the subterminal part of the two homologues of the bivalent VIII. 1.5 cm represents 20 μm.

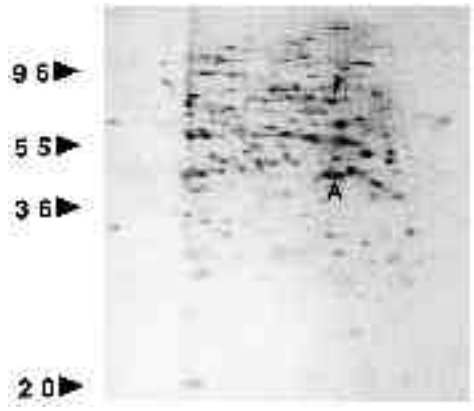

Fig. 9. Autoradiograph of a two-dimensional polyacrylamide gel showing that HSP70 is synthesized in stage VI oocytes. The molecular mass of polypeptides was determined according to electrophoretic migration of standards and is expressed (left) in 10^3 M_r. The separated proteins corresponded to a 10000 ***g*** supernatant of 10 defolliculated oocytes incubated for $2\frac{1}{2}$ hours at 19°C with [^{35}S]methionine. The positions of HSP70 (arrowhead) and actin (A) are indicated.

strongly suggest that Pw70 is part of the heat-inducible *hsp70* gene and does not correspond to the cognate gene (hsc70). Such a conclusion is also supported by our results from PCR analysis of the genomic sequence, which reveal the absence of introns all along the transcript. Such a characteristic was described as being specific to heat-inducible hsp70 sequences (Yost and Lindquist, 1986).

Constitutive expression of a heat-inducible *hsp70* gene in *Pleurodeles* stage VI oocytes

Our results from test experiments for heat inducibility in defolliculated oocytes, i.e. in the absence of somatic cells, revealed constitutive expression of hsp70 in the absence of heat shock in defolliculated oocytes. In contrast, in heat-shocked defolliculated oocytes, we observed the disappearance of hsp70 RNA. Since the same signal was obtained in RNA from both normal and heat-shocked defolliculated oocytes when the same blots were probed with rDNA (data not shown), we can assume that such hsp70 RNA disappearance during heat shock really exists and is not due to a physiological or experimental loss of RNA. Furthermore, since HSP70 protein synthesis was found to occur in defolliculated oocytes during heat shock, these results suggest that the hsp70 mRNA previously synthesized by oocytes in normal conditions is used for the translation process during heat shock, and that no hsp70 RNA neosynthesis simultaneously occurs. This hypothesis is supported by recent results obtained by Rodriguez et al. (1991), which showed that, in *Pleurodeles* oocytes during heat shock, all lampbrush loops disappeared and therefore all RNA synthesis, including hsp70 RNA synthesis, was arrested. These results can only be observed in the absence of follicular cells. Indeed, when follicular cells surrounding oocytes were present during heat-shock treatment, the hsp70 RNA strongly induced in these somatic cells did not enable us to detect the disappearance of hsp70 mRNA synthesized by the defolliculated oocyte before heat shock. In contrast, since hsp70 mRNA transcription in somatic cells is observed only under stress conditions, RNA detected in undefolliculated oocytes in the absence of heat shock can only be due to oocytes themselves. This RNA is likely to correspond to the constitutive expression of a heat-inducible *hsp70* gene. Overall, these results are particularly interesting because they imply that, as previously proposed for *Xenopus* oogenesis (Bienz and Gurdon, 1982; Bienz, 1984), a bona fide *hsp70* gene strictly inducible in somatic cells is constitutively active during oogenesis.

Expression of *hsp70* genes during *Pleurodeles* oogenesis

Using Pw70 probes, which were demonstrated to be hsp70 gene-specific (see above), we showed, by molecular hybrid-

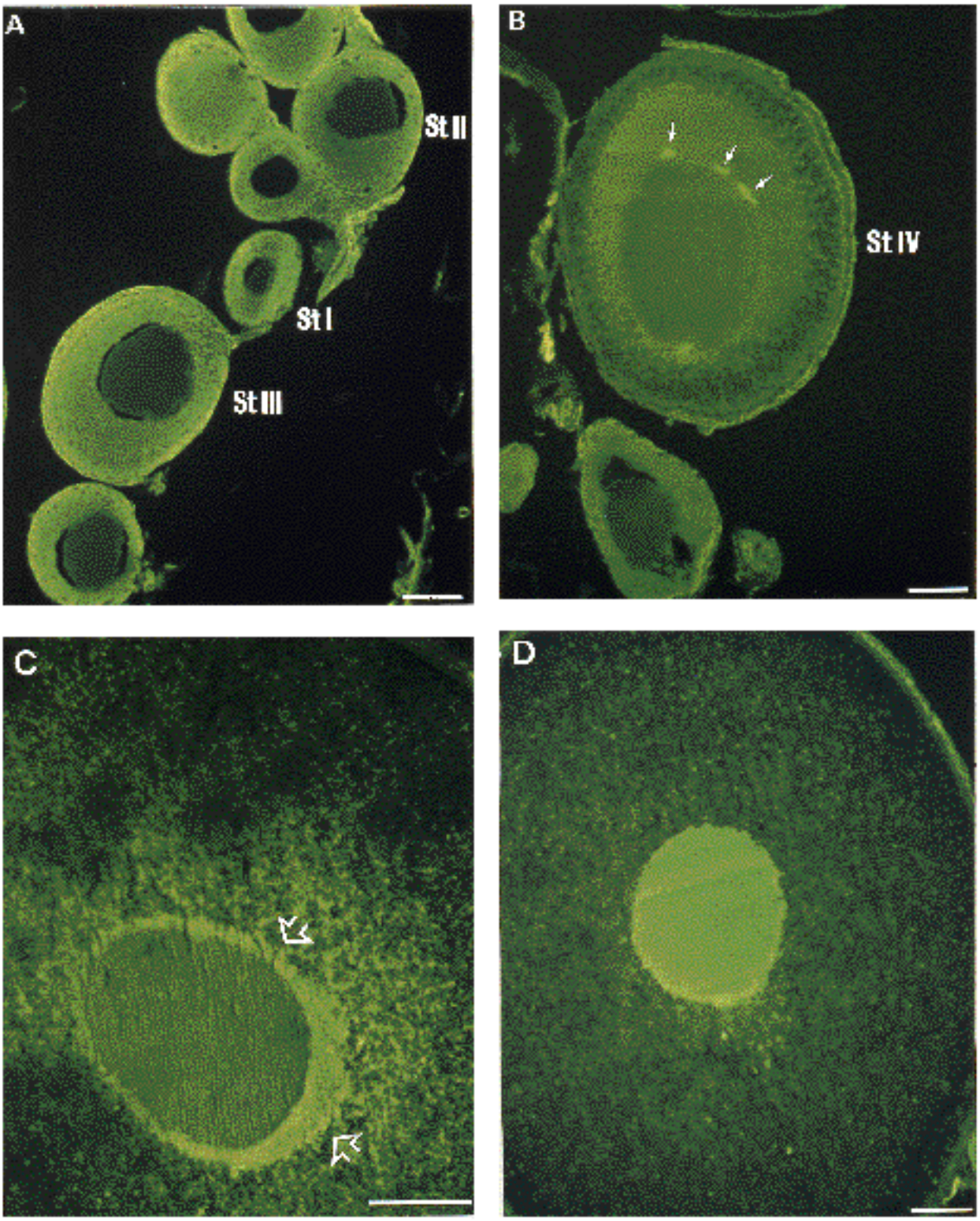

Fig. 10. Immunolocalization of HSP70 protein on ovary sections. (A) Previtellogenic (stage I and II) and stage III oocytes. Strong cytoplasmic staining is observed. The nucleus is only faintly stained. (B) Stage IV-V oocyte. HSP70 is localized between yolk platelets. Note that mitochondrial aggregates are particularly fluorescent (arrows). Nucleus immunoreactivity is higher than in (A). (C,D) In stage VI oocyte, staining is either preferentially perinuclear (C, arrows) or nuclear (D). Bar represents 200 μm.

ization, that hsp70 mRNA constitutive expression takes place throughout oogenesis. Indeed, results from quantification of such expression provided evidence for progressive accumulation of hsp70 mRNA during oogenesis. The amount of hsp70 mRNA constantly increases in oocytes from stage II to stage VI, and a mature oocyte thus contains 1×10^8 molecules of hsp70 mRNA (see results). Two hypotheses were envisaged. Such an increase may result from demasking of preexisting RNAs synthesized very early during oogenesis, or it may be due to a relative ratio of synthesis to degradation of hsp70 mRNA, which leads to a net accumulation of transcripts during vitellogenesis. The first hypothesis can be ruled out since the RNA extraction method presumably enables recovery of all RNA molecules, whether masked or not. Our results from in situ hybridization of hsp70 cRNA probes to nascent transcripts of lampbrush chromosome loops strongly support the second hypothesis, and unequivocally provide evidence for true transcription of hsp70 mRNA at every stage studied. The same hybridization site was always detected on the same bivalent whatever the stage of vitellogenesis. According to this hypothesis, the increasing amount of hsp70 mRNA is likely to be due to stabilization of newly synthesized transcripts. However, we cannot exclude an increase in the hsp70 transcription rate in late oogenesis. Such a hypothesis has already been advanced by Bienz (1984) to explain the accumulation of stored hsp70 mRNAs constitutively transcribed throughout oogenesis in *Xenopus*. This author suggested that mRNA stabilization may play a consequent role in such accumulation.

Our results concerning constitutive hsp70 expression analysis during oogenesis raise the issue of lampbrush chromosome transcription. These chromosomes have been found to be the site of active RNA synthesis in the amphibian oocyte and were initially assumed to be the site of synthesis of maternal mRNA (for review, see Davidson, 1976; Sommerville, 1977). However, based on the molecular studies of Golden et al. (1980), it is unlikely that such RNAs are directly related to the production of pA^+ RNAs stored in oocytes. Those authors showed that pA^+ RNAs reached their final level of accumulation at an oocyte stage at which lampbrush chromosomes were not yet active. In contrast, our data show an increase in hsp70 transcripts concomitant with hsp70 RNA synthesis, as visualized by in situ hybridization to newly synthesized lampbrush loop transcripts. Our results led us to conclude that lampbrush chromosome loops actively participate in production of at least one species of pA^+ RNAs stored throughout vitellogenesis, as we had shown for hsp70 mRNAs. However, those results cannot be extrapolated to all pA^+ RNAs.

Simultaneously with the accumulation of hsp70 mRNA, a correlated increase in the amount of HSP70-related protein has been reported during oogenesis. To detect the protein present unambiguously, we performed immunoblots of total proteins of stage II to stage VI oocytes rather than more classical immunoprecipitation of labelled proteins, which would have limited analysis to that of the proteins synthesized during the labelling period. However, since the antibody used recognized an epitope common to HSP70 and HSC70 in other species (Mattei et al., 1989), we were unable to identify *hsp70* or *hsc70* products specifically, and therefore could not conclude as to whether these proteins resulted from translation of hsc70 mRNAs or from that of constitutively expressed hsp70 mRNAs. Nor could we assume that these HSP70-related proteins resulted from translation of newly synthesized RNAs since, in growing oocytes, a large fraction of the synthesized mRNA is not used for immediate translation but is dormant and/or masked and stored for future translation (Richter et al., 1984). We cannot rule out the possibility, however, that such HSP70-related protein synthesis, during of oogenesis process is entirely encoded for by de novo translation, since we observed their biosynthesis throughout oogenesis.

Nuclear transfer of HSP70-related proteins in stage VI oocytes

On the basis of cytological analysis, we have shown that HSP70-related proteins are localized in the cytoplasm in early oogenesis and are then progressively transferred to the nucleus in the course of oogenesis so that the concentration of the protein becomes very high in some nuclei of stage VI oocytes. However, since this increase concerned only a limited number of oocytes with no particular features, it was impossible to identify them from total ovaries and to draw quantitative conclusions about such HSP70 nuclear transfer. Proteins that migrate between the cytoplasm and nucleus are of interest since they may be involved in control processes implicated in cell maintenance, growth, replication and differentiation. In *Xenopus*, several types of behavior have been reported for proteins involved in nuclear metabolism. PCNA protein localization in the nucleus has been observed to take place very early during oogenesis (Leibovici et al., 1990). In contrast, the *c-myc* nuclear proto-oncogene encodes for a protein that is localized only in the cytoplasm throughout oogenesis and which is translocated to the nucleus just after fertilization (Gusse et al., 1989). These two proteins have been shown to be involved in the early cleavage stage of embryonic development and probably during the replication process. As concerns HSP70 relocalization, it is interesting to point out that this protein is transferred to the nucleus in stage VI oocytes, i.e. at the time that the oocyte is able to mature. It could therefore be hypothesized that HSP70 is involved in the processes by which stage VI oocytes acquire competence to undergo hormonally stimulated maturation. HSP70 is well known to be a 'chaperone' protein. Beckman et al. (1992) have recently shown that its interaction in co-translational fashion with nascent polypeptides prevents their folding up until maturation. The cytoplasmic localization of HSP70 in all *Pleurodeles* oocytes active in protein synthesis is in good agreement with such interactions. HSP70 may also interact with certain proteins that undergo various post-translational modifications (Chirico et al., 1988; Deshaies et al., 1988). In this case, we can assume that HSP70 interacts with specific nuclear proteins involved in gene control of oocyte maturation. The high concentration of HSP70 observed in *Pleurodeles* stage VI oocytes supports this hypothesis.

The oocyte hsp70 message content would therefore be required to provide for protein synthesis needs during oogenesis, and particularly during oocyte maturation. It might also be required after fertilization, up until mid-

blastula transition, when transcription from the embryonic nucleus begins anew.

The sequence presented here appears in EMBL under the accession number X71951.

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