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Xenopus hsp 70 genes are constitutively expressed in injected oocytes

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***Xenopus* heat-shock genes are transiently heat-inducible in somatic cells, but they are also subject to a long-term developmental control in oogenesis and early embryogenesis. In order to understand whether different genes or different promoter elements are involved in the two types of control, several genomic clones coding for *Xenopus* heat-shock proteins, *hsp* 70 and *hsp* 30, were isolated, characterised and tested for expression in oocytes and COS cells. Three isolated *hsp* 70 genes are nearly identical in their promoter and mRNA leader sequences, indicating that there is only one type of *hsp* 70 gene. These promoters contain a consensus sequence element (CT-GAA--TTC-AG) upstream of the TATA-box, which is presumably required for their transient heat-inducibility. The two isolated *hsp* 30 genes show 5'-flanking sequences similar to each other, except that one of them shows a homology disruption precisely around the consensus sequence element. The same gene contains a frameshift mutation in the protein coding part and, since it cannot be expressed after introduction into oocytes or COS cells, it is probably a pseudogene. The other *hsp* 30 gene is strongly heat-inducible in injected oocytes or transfected COS cells. In contrast, the *hsp* 70 genes are strongly heat-inducible in COS cells, but their expression is highly efficient in injected oocytes at the normal temperature and is not increased during heat shock. This represents correct cell type-specific regulation of a cloned reintroduced gene, since the endogenous *hsp* 70 genes are constitutively activated during oogenesis, leading to the accumulation of stored *hsp* 70 mRNA in oocytes.**

Key words: genomic clones/*hsp* sequences/expression/COS cells/developmental control

Introduction

Heat-shock proteins are found in most eucaryotic and procaryotic cells: their synthesis is induced if the cell is exposed to high temperature or various other stresses. These proteins are closely related in different species; they have similar mol.wts., their gene sequences share homologies and antibodies have been reported to cross-react with heat-shock proteins from several organisms (see various articles in Schlesinger *et al.*, 1982). Furthermore, the regulation of their genes is conserved; *Drosophila hsp* 70 genes are heat-inducible after introduction into monkey, mouse and frog cells and the promoter element required for this heat-inducibility has been mapped (Pelham, 1982; Mirault *et al.*, 1982; Corces *et al.*, 1981; Rungger and Voellmy, 1982; Bienz and Pelham, 1982). This element consists of a short oligonucleotide which can be found in front of most of the *Drosophila* heat-shock genes and which, if synthesised and fused to a non-inducible promoter, confers heat-inducibility on this promoter (Pelham and Bienz, 1982).

Heat-shock genes are not only transiently heat-inducible, but also, in some cases, they are subject to a developmental regulation. Expression of a subset of heat-shock genes under normal physiological conditions has been reported for various developmental stages of *Drosophila* (Sirotkin and Davidson, 1982; Zimmerman *et al.*, 1983; Mason *et al.*, 1984) and for *in vitro* cultivated early mouse embryos (Bensaude *et al.*, 1983). Frog *hsp* 70 genes appear to be constitutively active during oogenesis and become heat-inducible in early embryos; frog *hsp* 30 genes are not expressed in oocytes or early embryos and become heat-inducible for the first time in tadpoles (Bienz, 1984). The question arises as to whether the developmental regulation is dependent on the same genes or promoter elements and on the same regulatory factors as the transient heat-induction.

In order to approach this question, I have isolated and characterised several *Xenopus* heat-shock genes, complementary to either *hsp* 70 or *hsp* 30 cDNA clones (Bienz, 1984). Sequence comparison between the *Xenopus* and the *Drosophila* genes and RNA mapping data allowed identification of the promoter and coding regions. The *Xenopus* promoters look very similar to *Drosophila* promoters: they all contain the consensus sequence element CT-GAA--TTC-AG (Pelham, 1982; Pelham and Bienz, 1982). As expected, the *Xenopus* genes are heat-inducible after introduction into somatic cells, with the exception of a potential *hsp*30 pseudogene. However, the *Xenopus hsp* 70 genes are highly and constitutively active in injected oocytes, which may reflect the developmental regulation of these genes in oogenesis.

Results

A genomic library was constructed by partially digesting *X.laevis* blood cell DNA with *Sau*3a I and by ligating size-selected fragments of 12-20 kb into a lamda vector (λ2001; Karn, 1983). Phages carrying a foreign DNA insert can be obtained by plating the ligation mixture onto selective host bacteria. An important advantage of λ2001 as a vector is that the library stays representative; thus the problem of selective loss of individual recombinant phages during amplification, apparently especially acute in the case of *Xenopus* genomic DNA, is avoided. A million plaques were screened by using single-stranded radioactive probes complementary to two cDNA clones (in M13) which have been reported previously (X4 and X16; Bienz, 1984). Eight phages carrying *hsp* 70 sequences (complementary to X16) and three phages carrying *hsp* 30 sequences (complementary to X4) were isolated.

The insert DNA of these phages was subsequently characterised by restriction mapping and Southern blot analysis. It turned out that among the 11 isolated phages, only six phages carried different DNA inserts (one phage was isolated twice and two phages three times each, probably because the library was amplified). These contain altogether four different *hsp* 70 genes, two of them in tandem array, and two *hsp* 30 genes in tandam array (Figure 1a). One *hsp* 70 gene (*hsp* 70B) was isolated three times in phages carrying different inserts, sug-

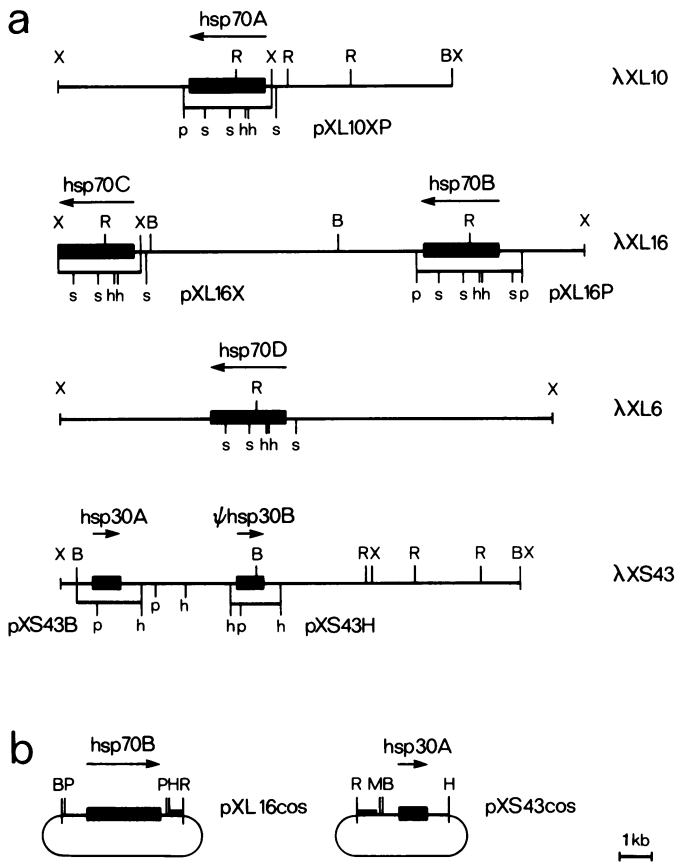

Fig. 1. Isolation and subcloning of *Xenopus* heat-shock genes.
(a) Restriction maps of four different λ clones (λXL10, λXL16, λXL6, carrying four different *hsp* 70 genes; λXS43, carrying two different *hsp* 30 genes). The inserted DNA was mapped with *Bam*HI (B), *Eco*RI (R) and *Xho*I (X); it is inserted into the *Bam*HI site (which is normally not restored) and is flanked on either side by an *Xho*I, an *Xba*I and a *Sac*I site (contained in the cloning site of λ2001; the latter two sites are not marked in the figure). Restriction sites given in small letters (h = *Hind*III, p = *Pst*I, s = *Sac*I) were mapped in the gene areas, but not in the remaining spacer DNA. With one exception, all the different gene regions were subcloned into pUC12 as fragments indicated in the figure (pXL10XP, pXL16X, pXL16P, pXS43B, pXS43H). Black boxes indicate the length of the transcripts, arrows indicate the transcriptional direction. **(b)** Construction of plasmids suitable for expression in COS cells. For pXL16cos, an *hsp* 70 gene fragment from pXL16P (P = *Pst*I), flanked by a *Bam*HI (B) and a *Hind*III (H) site, was inserted into a plasmid (pML) containing an SV40 origin of replication (pXTK1). For pXS43cos, an *Eco*RI/*Sma*I (R, M) fragment containing an SV40 origin of replication (from pTKS10) was inserted into the cloning site of pXS43B (see Materials and methods). Black boxes and arrows, as above; thin black boxes indicate the fragments containing the SV40 origins of replication. Vector sequences are not to scale.

gesting that the total number of four different *hsp* 70 genes may represent the complete genomic set; Southern blot analysis of genomic DNA indicates however that there is at least one extra gene (not shown). In all *hsp* 70 phages, the restriction site pattern in the cDNA complementary region was nearly identical, pointing to a high sequence conservation between them. No attempt was made to clone a representative number of *hsp* 30 genes (5 – 10 copies per haploid genome; not shown).

For sequence analysis and expression studies, suitable restriction fragments containing a complete gene were isolated from the genomic clones named λXL10, λXL16 and λXS43 and subcloned into pUC (Figure 1a). One *hsp* 70 and one *hsp* 30 gene were subcloned into a vector which contains an SV40 replication origin and which is therefore suitable for expression in COS cells (Figure 1b).

The hsp 70 protein sequence is highly conserved between Xenopus and Drosophila

The nucleotide sequence of the *hsp* 70A gene was determined by the dideoxy method, using two *Sac*I fragments and a *Sac*I/*Pst*I fragment from XL10 (Figure 1a). A single open reading frame coding for 647 amino acids was found (Figure 2). Within this region, a *Sau*3a I fragment of 116 bp exactly matches the sequence of a previously isolated cDNA clone (Bienz, 1984). A striking homology can be found if the predicted 647 amino acids are aligned with the *Drosophila hsp* 70 amino acid sequence (Karch *et al.*, 1981): 74% of all amino acid residues are identical and about half of the substitutions are conservative changes (Figure 3). The protein seems to be divisible into two domains, whereby the amino-terminal three quarters are strongly conserved (82% identical residues) and the carboxy-terminal quarter is much more diverse (51% identical residues). This might suggest that there is less selective pressure on the function of the carboxy-terminal domain or that the latter is adapted to species-specific requirements. Although only one *Xenopus hsp* 70 coding sequence has been determined, the differences between the various *hsp* 70 genes are likely to be minor, since many restriction sites in the gene and flanking regions are conserved (nine out of 10) and since there is only one major protein spot on a two-dimensional gel (Bienz and Gurdon, 1982).

The situation for the *hsp* 30 genes is different. Most of the nucleotide sequence of the *hsp* 30A gene (in pXS43B) and of the *hsp* 30B gene (in pXS43H) has been determined (Figure 4). Sequences similar to the previously isolated cDNA clones (Bienz, 1984) could be found, but in both cases the open reading frame stopped before the 3′ end of the cDNA clones. In the *hsp* 30A gene, there is an insertion of 21 bp (generated probably by some duplication event during evolution: a 10-bp stretch is repeated three times) which leads to two terminator codons in the middle of the predicted 30-K protein. Upstream from this insertion, the nucleotide divergence from the cDNA clone is $<4\%$. It is conceivable that this gene is expressed *in vivo* and gives rise to a half-sized *hsp* 30-like protein. It should be mentioned that the *hsp* 30 cDNA clones can hybrid-select a group of mRNAs from heat-shocked cells, which are translatable into several proteins *in vitro* of which the predominant one co-migrates with *hsp* 30 (unpublished).

In the *hsp* 30B gene, an A residue close to the carboxy-terminal end is deleted. The resulting frameshift leads to an additional 19 amino acids, that is, the carboxy-terminal tenth of the protein is completely changed. Noticeably, the nucleotide sequence divergence between this gene and the cDNA clones is higher than 11%. It is likely that this gene is not expressed *in vivo*, because its protein structure would be substantially altered. The amino acid sequence homology between the *Xenopus hsp* 30 cDNA clones and the *Drosophila* small heat-shock genes has been established previously (Bienz, 1984). It is highest (up to 45%) in the middle part of the protein over a stretch of ~80 amino acids, but is hardly detectable in the preceding 80 or in the following 40 amino acids.

Xenopus heat-shock gene promotors contain the consensus sequence element CT-GAA--TTC-AG

Sequences flanking the coding regions at their 5′ ends most

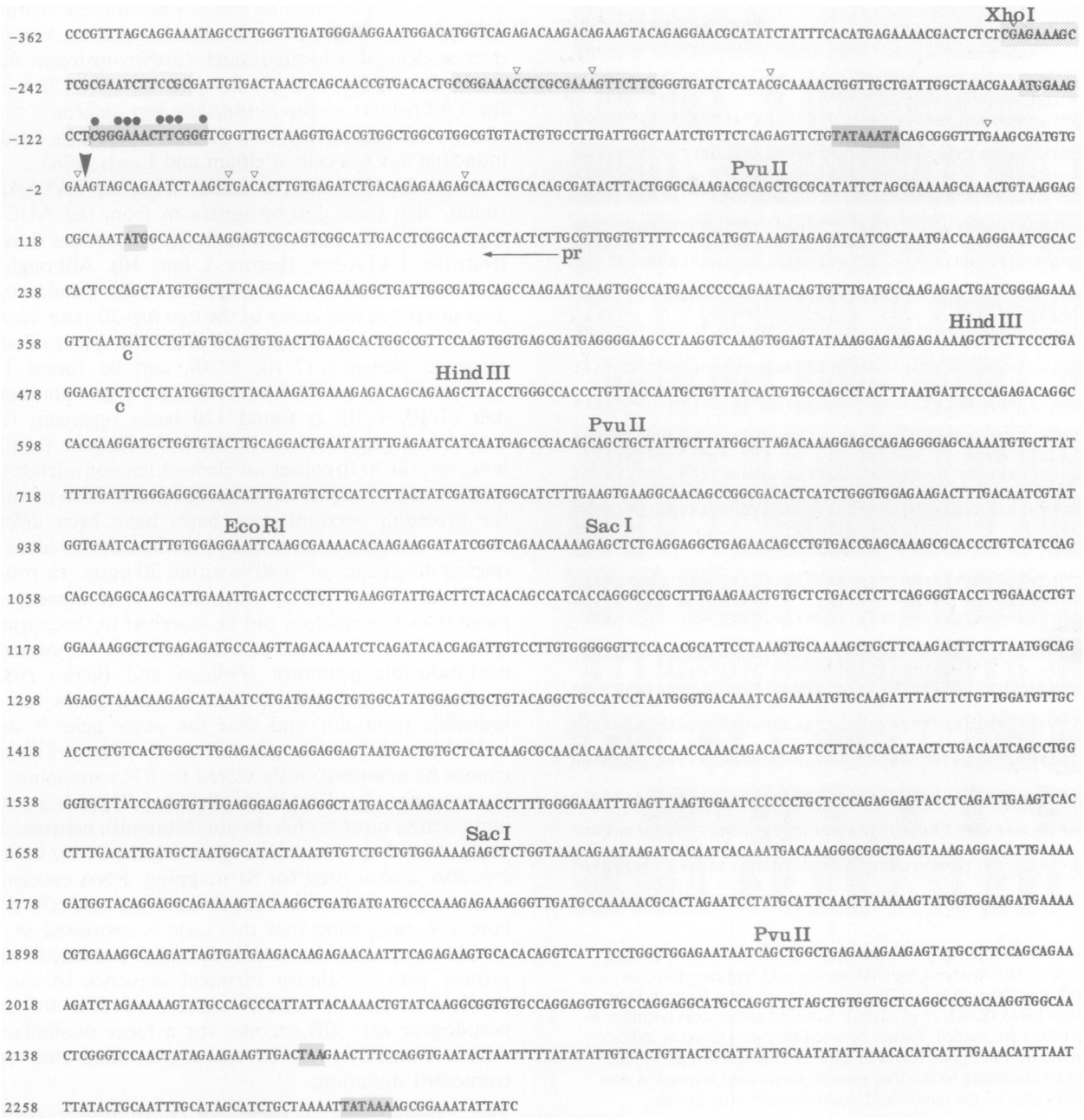

Fig. 2. Nucleotide sequence of a *Xenopus hsp* 70 gene. The most likely RNA start site (long arrow) was given position 1. Several restriction sites are marked. Triangles above nine different nucleotides in the 5′-flanking area indicate residues which are substituted in the *hsp* 70B gene (pXL16P). Various sequences are darkly shaded: the consensus sequence element (dots above the sequence indicate bases matching the consensus sequence CT-GAA--CTT-AG), the TATA-box, the translation start and stop codons and the likely polyadenylation site (S1 mapping data not shown). Lightly shaded sequences indicate 'weak' consensus sequence elements (7/10 matches). The *Sau*3a I fragment between the two c was previously cloned as cDNA (X16; Bienz, 1984). pr marks the 3′end of the M13 clone (from the sequence library) which was used for preparing S1 mapping probes.

likely contain the regulatory elements required for the transient and the developmental expression of the heat-shock genes. If there were two types of *hsp* 70 genes, one being constitutively expressed in oogenesis and one being transiently heat-inducible in somatic cells, these 5′-flanking sequences should differ between the two types of genes. In order to establish the sequence variability in this region, a *Pvu*II/*Xho*I fragment from pXL16X (*hsp* 70C) and a *Pvu*II/*Pst*I fragment from pXL16P (*hsp* 70B) were cloned into M13 and the sequence was determined. The sequence conservation between the three genes is striking: these 348 bases are identical between the *hsp* 70A and C genes; there are nine base substitutions within the same stretch in the *hsp* 70B gene (Figure 2). It is likely that all the *hsp* 70 genes have the same basic structure in their 5′-flanking region; there is no evidence for two types of *hsp* 70 genes. The situation for the *hsp* 30 genes is similar: a few sequence homology disruptions close to the coding region (probably in the mRNA leader) and one other disruption further upstream (which will be discussed later) can be observed between the two genes (Figure 4). Apart from these, >90% of the nucleotide residues are conserved.

The 5′-flanking sequences were searched for promotor elements. A sequence element which matches the consensus

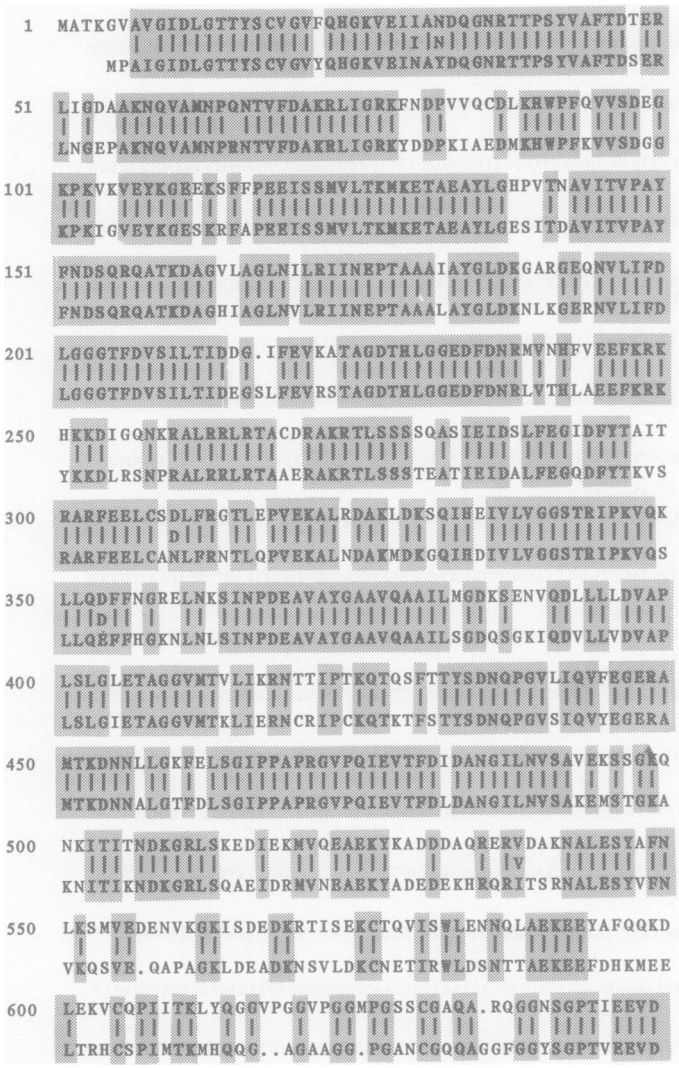

Fig. 3. Sequence comparison between the *Xenopus* and the *Drosophila hsp* 70 protein. The *Xenopus hsp* 70A amino acid sequence (top line) was aligned with the *Drosophila hsp* 70 amino acid sequence (bottom line) derived from 56H8 (Karch *et al.*, 1981). Identical amino acid positions in the two proteins are shaded. Letters between the two sequences indicate variant amino acid positions found in another *Drosophila* gene (132E3) which lead to homology to the frog protein. Gaps were inserted as dots. The numbers refer to the amino acid position in the frog protein.

sequence CT-GAA--TTC-AG in at least eight out of 10 positions (8/10) and which is located closely to a TATA-box, is usually sufficient to make a promotor heat-inducible (Pelham, 1982; Pelham and Bienz, 1982). In the *hsp* 70 genes, a sequence TATAAATA, identical to the one in the *Drosophila hsp* 70 genes, is found some 150 bp upstream from the initiator AUG codon, leaving space for a short mRNA leader of ~130 bases. Indeed, 5′ ends of RNA extracted from heat-shocked somatic cells map ~23 nucleotides downstream of this TATA-box (Figure 5, lane 1), implicating its function *in vivo*. Six consensus sequence elements can be found upstream of the TATA-box (Figure 2): the most related one (8/10) is located closest to the TATA-box (74 bases away), overlapped by a second one (7/10) 10 bases away. Another two pairs of two elements (both 7/10) at the same 10 base spacing from each other are located 141 and 206 bases upstream from the TATA-box. Heat-inducibility of the *Xenopus hsp* 70 gene in somatic cells would probably be dependent on the closest (8/10) consensus element, by analogy to the *Drosophila* genes. One unusual feature in the *Xenopus hsp* 70 promotor structure is that the consensus sequence element is located much further upstream than most of the *Drosophila* ones (74 as opposed to 14–28 bases from the TATA-box) – the *Drosophila hsp* 23 gene is the exception in this respect (spacing 99 bases) and it is hardly heat-inducible in COS cells (Pelham and Lewis, 1983).

In the *hsp* 30 genes, a similar sequence TATAAAA was found, also some 150 bp upstream from the AUG codon. Again, RNA 5′ ends map some 20 nucleotides downstream from the TATA-box (Figure 5, lane 10). Although this indicates the function of a TATA-box in this location *in vivo*, it does not prove that either of the two *hsp* 30 genes is expressed *in vivo*. In the *hsp* 30A gene (in pXS43B), a pair of consensus sequence elements (7/10, 8/10), can be found 16 bases upstream from the TATA-box (Figure 4) and another similar pair (7/10, 7/10) is found 120 bases upstream from the TATA-box (not shown). In the *hsp* 30B gene (in pXS43H) however, the 8/10 consensus element has considerably diverged (this gap of sequence conservation has been mentioned in the preceding section): two bases have been deleted and another eight bases have been substituted; this leads to a sequence divergence of >40% within 20 bases, surrounded by highly conserved (95%) sequences. As a consequence, no more than five residues can be matched to the consensus sequence, which is not in general sufficient for constituting a heat-inducible promotor (Pelham and Bienz, 1982). The prediction is that only one of the two genes has a heat-inducible promotor and that the other gene is not heat-inducible and therefore possibly a pseudogene. This question cannot be unambiguously solved by RNA mapping, because the sequence of at least three other *hsp* 30 genes is not known and because most probes do not distinguish between different genes. However, if a probe spanning the 21-bp insert in the *hsp* 30A gene is used for S1 mapping, RNA containing this insert can be detected in heat-shocked somatic cells (Figure 5, lane 17), suggesting that this gene is expressed *in vivo*. It should be recalled that it encodes for a shortened heat-shock protein with an almost identical sequence to the protein predicted from the cDNA clones, whereas the potential pseudogene *hsp* 30B encodes for a more dissimilar *hsp* 30 with a completely altered carboxy-terminal end, due to a frameshift mutation.

The Xenopus hsp 70 genes are constitutively expressed in oocytes

In order to test the predictions resulting from structural considerations, the various *Xenopus* heat-shock genes were assayed for expression and regulation after introduction into oocytes or somatic cells (monkey COS cells). The plasmids used for these expression studies are depicted in Figure 1 and the conditions used for introducing and expressing the genes were described earlier (Pelham, 1982; Bienz and Pelham, 1982). The results concerning the *Xenopus hsp* 70 genes are striking: whereas the *hsp* 70B gene is strongly heat-inducible after introduction into COS cells (Figure 5, lanes 6–7), all the *hsp* 70 genes are efficiently expressed in injected oocytes without a heat shock and this high level of expression is not increased after heat shock (Figure 5, lanes 2–5). A *Drosophila hsp* 70 gene injected at the same time is strongly heat-inducible (Figure 5, lanes 8–9), as previously found (Bienz and Pelham, 1982), which rules out the possibility that the oocytes have accidently been heat-shocked. A *Bam*HI/*Xho*I subclone derived from the right-hand side of

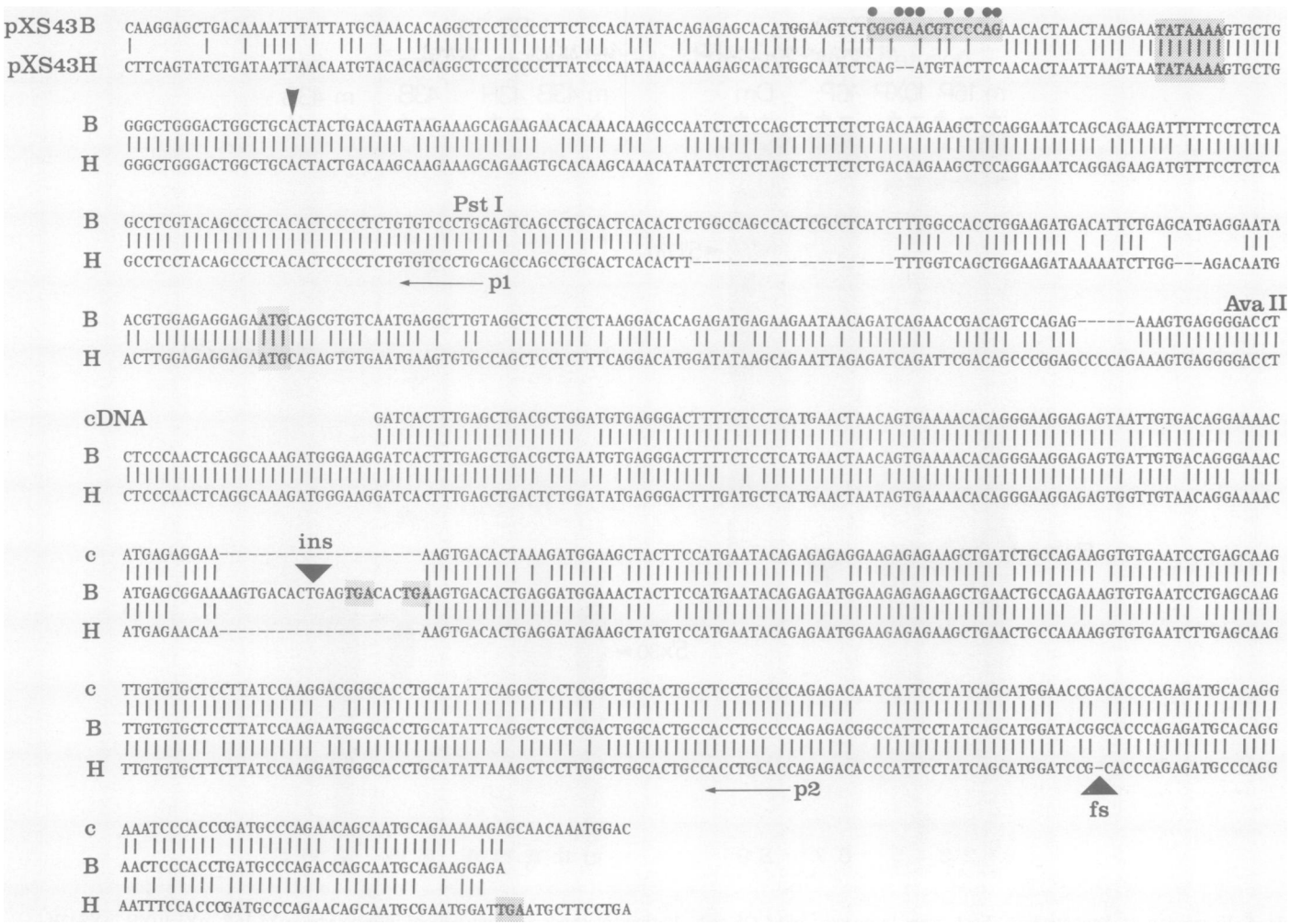

Fig. 4. Sequence comparison between two *Xenopus hsp* 30 genes and *hsp* 30 cDNA. The three lines represent the cDNA sequences (c) and the sequences of the *hsp* 30A gene (in pXS43B, B) and of the *hsp* 30B gene (in pXS43H, H), the putative pseudogene. Vertical lines indicate identical positions; gaps are marked by dashes. The long arrow points to the base where transcription initiation most likely starts. A 'weak' consensus sequence element (7/10 match) is lightly shaded (top line), whereas darkly shaded sequences mark the strong consensus sequence element (dots indicate bases matching the consensus sequence), the TATA-box and translation start and stop codons. The latter are created by the 21-bp insertion (ins) in B and by the one base deletion (fs) in H; the cDNA sequence probably finishes shortly after the given sequence. p1 and p2 indicate the 3′ ends of the M13 clones used for S1 mapping; the internal probe derived from pXS43B extends from p2 to the *Ava*II site.

λXL16, retaining ~2.5 kb 5′-flanking sequences, gives the same level of expression as pXL10XP, and so does the gene cloned into the COS cell vector (not shown), indicating that the observed expression at normal temperature is not fortuitously caused by adjacent procaryotic vector sequences.

The two *hsp* 30 genes behave differently in these functional assays. The putative pseudogene, *hsp* 30B (in pXS43H), is not expressed after introduction into oocytes (Figure 5, lanes 13 – 14) or COS cells (not shown). In some S1 mapping experiments, a low level of transcription at both temperatures can be observed, a result reminiscent of the expression of *Drosophila hsp* 70 genes from which the consensus sequence element has been deleted (Bienz and Pelham, 1982). In contrast, the *hsp* 30A gene (in pXS43B) is heat-inducible to similarly high levels of expression in injected oocytes (Figure 5, lanes 11 – 12) and transfected COS cells (Figure 5, lanes 15 – 16 and 18 – 19). After long exposures of the autoradiograms, some residual transcription can be observed at the lower temperature in oocytes, which could reflect rare initiation events directed by the TATA-box (see above). Thus, these results are as predicted: a promotor element with a good match to the consensus sequence CT-GAA--TTC-AG is required to make a gene heat-inducible. The results support the hypothesis that only one of the *hsp* 30 genes is a functional heat-shock gene *in vivo*.

Discussion

Xenopus heat-shock genes are structurally related to the *Drosophila* heat-shock genes with respect to promotor and coding region. They are tightly controlled by heat shock when functionally assayed in somatic cells. It is therefore striking that the *Xenopus hsp* 70 genes are very actively expressed without a heat shock after injection into *Xenopus* oocytes. The expression pattern of the injected genes apparently reflects the expression pattern of the endogenous oocyte genes: we estimated previously that the oocyte *hsp* 70 genes are actively transcribed during most or all of oogenesis, resulting in the accumulation of stored untranslated *hsp* 70 mRNA (Bienz and Gurdon, 1982). It also suggests that the underlying activating mechanism operates at the level of transcription initiation of these particular genes; *hsp* 70 transcription is not simply a consequence of general gene activity (and readthrough transcription) in the lampbrush chromosome stage. Messenger RNA stabilisation could play a consequent role in the accumulation of stored *hsp* 70 mRNA,

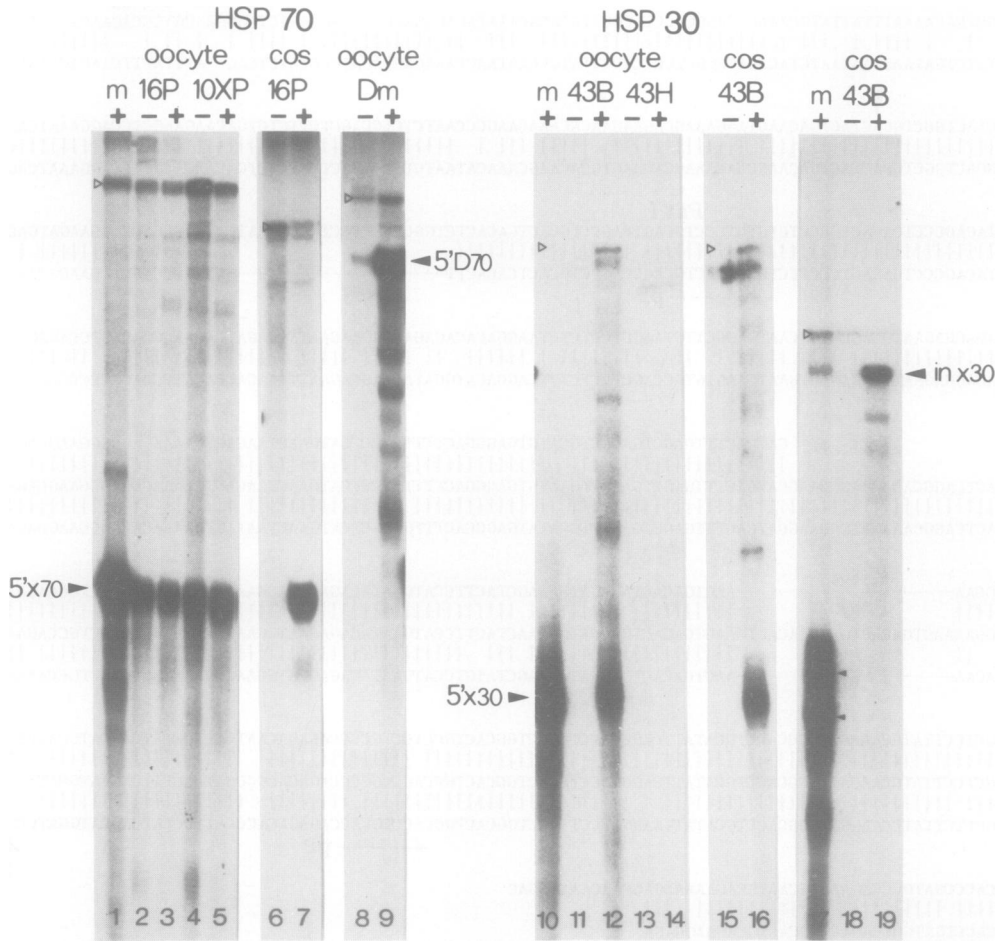

Fig. 5. Expression of *Xenopus* heat-shock genes in oocytes and COS cells. Various plasmids were introduced into oocytes (pXL16P, pXL10XP, pXS43B, pXS43H; as a control, Dm, 56H8 from *Drosophila*) or COS cells (pXL16cos, pXS43cos) and total RNA was extracted before (−) or after (+) a 2−4 h heat shock at 34°C in oocytes or 42.5°C in COS cells. The incubation time before the heat-shock was 2 h in oocytes and 30 h in COS cells. Total RNA from heat-shocked *Xenopus* tissue-cultured cells (m; 4 h at 37°C) was subject to the same S1 mapping for comparison. The probes used for S1 mapping are indicated in Figures 2 and 4: pr in **lane 1−7** gives rise to a protected band of ~180 bases (5'X70), p1 in **lane 10−16** to a band of ~120 bases (5'X30); both probes map 5' ends. The internal probe p2 in **lane 17−19** indicates *hsp* 30A gene-type transcripts (top band of ~190 bases: inX30) or cDNA-type transcripts (bottom bands of ~120 and 140 bases, small arrows; S1 nuclease cuts this probe at the site of the 21-bp insert, if it is hybridised to cDNA-type transcripts). The same RNA as in **lane 15** and **16** were mapped with this probe (**lane 18** and **19**) as a comparison with the mapping of authentic RNA from tissue-cultured cells (**lane 17**). The probe used to detect *Drosophila hsp* 70 transcripts (in **lane 8** and **9**, 5'D70) was described previously (Dudler and Travers, 1984). The top band (marked by triangle) in each lane represents reannealed probe. About 5−10 μg of total RNA was used in every lane.

but cannot account for the results obtained in transient oocyte assays.

The mechanism of transient heat-shock gene activation in *Drosophila* somatic cells is understood in its basic features: the consensus sequence element (CT-GAA--TTC-AG) upstream of the TATA-box is required for heat-inducibility (Pelham, 1982; Pelham and Bienz, 1984) and a protein factor binds to this element upon heat shock *in vivo* and *in vitro* (Wu, 1984; Parker and Topol, 1984). This factor, called HSTF, can be found in unshocked cells in an inactive state (Parker and Topol, 1984). Since the *Xenopus* heat-shock genes contain a consensus sequence element in their promotors and since they are heat-inducible in somatic cells, it is likely that a very similar mechanism operates in *Xenopus*. Strong support for this was obtained with the putative *hsp* 30 pseudogene which cannot be activated by heat-shock: the consensus sequence element of this gene is heavily mutated, whereas the rest of the 5'-flanking sequences are highly conserved and the TATA-box and the RNA initiation site are identical in comparison with the heat-inducible *hsp* 30A gene. Apparently, the distance between the consensus sequence element and the TATA-box bears little relevance to the heat-inducibility in somatic cells; it is 74 bases in the *Xenopus hsp* 70 genes and 14−28 bases in most *Drosophila* heat-shock genes and in the *Xenopus hsp* 30A gene and the heat-inducibility of all these genes in transfected monkey COS cells is comparable.

What is the mechanism for the developmental regulation of the *Xenopus* heat-shock genes, particularly for the activation of the *hsp* 70 genes in oogenesis? Two alternative models can be envisaged. Either, oocytes contain a transcription factor, different from HSTF, which recognises and activates the *Xenopus hsp* 70 genes, but which is not present in somatic cells. The site of interaction could lie in the RNA-coding region or anywhere within the first 250 5'-flanking bases. It is tempting to speculate that it is located in the atypically long space between the consensus sequence element and the TATA-box. The *hsp* 30 genes would not be activated by this factor, because they do not contain the corresponding promotor element. Alternatively, unshocked oocytes might contain low levels of active HSTF. Due to its low concentration, it would only, under normal conditions, activate heat-shock

gene promotors with a high binding affinity, e.g., the *Xenopus hsp* 70 genes, but not promotors with a low affinity, e.g., the *Xenopus hsp* 30 or the *Drosophila hsp* 70 genes. Such a model could also explain the fact that *hsp* 30 genes are not heat-inducible till late in embryogenesis and heat-inducible to variable extents in adult somatic cells (Bienz, 1984): different levels of active HSTF under heat shock or normal conditions combined with different promotor affinities could indeed generally account for differential and developmental regulation of heat-shock genes. This model would predict that the exact sequence within and around the consensus sequence element is crucial for the distinction between heat-inducible and constitutive heat-shock genes. Evidence for the importance of these adjacent sequences has been reported: a *Drosophila hsp* 70 deletion mutant which retains only five bases 5′-adjacent to the consensus sequence is not reproducibly heat-inducible in *Xenopus* oocytes (Bienz and Pelham, 1982) and is heat-inducible only at a very low level in transformed flies (Dudler and Travers, 1984). Footprinting experiments *in vivo* and *in vitro* indicate that a region in the *Drosophila hsp* 70 gene spanning the consensus sequence element and 30−44 5′-flanking bases (including a second consensus sequence element) is protected by protein(s) during heat-shock (Wu, 1984; Parker and Topol; 1984). A deletion analysis of the *Xenopus hsp* 70 promotor will distinguish between the two models.

Materials and methods

Isolation and characterisation of genomic clones

A genomic library from *X.laevis* DNA was constructed in λ2001 using standard methods (Karn, 1983; Maniatis et al., 1982). Frog blood cell DNA, kindly provided by T.Mohun, was partially digested with *Sau*3a I, fractionated on a sucrose gradient and size-selected fragments (12−20 kb) were ligated into *Bam*HI/*Eco*RI-cut λ2001. The ligation mixture was packaged, using extracts from the strains NS428 and NS433 (Sternberg *et al.*, 1977), and $>10^5$ plaques/μg of DNA were obtained upon plating onto the selective strain Q359 (Karn, 1983). The library was then amplified with the non-selective strain Q358 and 10^6 plaques were screened with single-stranded radioactive probes derived from X4 and X16 (Bienz, 1984). Eleven positive phages (eight complementary to X16, three to X4) were plaque-purified and DNA was prepared from minilysates (Karn, 1983). Restriction mapping and Southern blot analysis identified six phages which carry different inserts (one was twice, two were three times independently isolated) and which altogether contain four different *hsp* 70 genes, complementary to X16, and two different *hsp* 30 genes, complementary to X4. Subclones in pUC12 (Vieira and Messing, 1982) were constructed for five different genes and the nucleotide sequence was determined for three of them, using the dideoxy method (Sanger *et al.*, 1980; Bankier and Barrell, 1983).

Expression assays

Plasmids containing an SV40 origin of replication were constructed for one *hsp* 70 and one *hsp* 30 in order to express them in monkey COS cells. For pXS43cos, an *Eco*RI/*Sma*I fragment containing the SV40 origin was purified from pXTK10 (Pelham and Bienz, 1982) and inserted into the cloning site of pXS43B. For pXL16cos, pXTK1 (based on pML; Pelham, 1982) was cut with *Bam*HI and *Hind*III and the vector fragment was ligated with a *Bam*HI/*Eco*RI fragment, containing the 5′ half, and with an *Eco*RI/*Hind*III fragment, containing the 3′ half of the *hsp* 70 gene, both isolated from pXL16P.

Expression of the various plasmids in oocytes or COS cells and S1 mapping analysis (using single-stranded M13 probes) of total RNA was performed as previously described (Bienz and Pelham, 1982; Pelham, 1982; Bienz, 1984).

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