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The finger motif defines a multigene family represented in the maternal mRNA of *Xenopus laevis* oocytes

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We have screened *Xenopus laevis* cDNA and genomic libraries for finger motif encoding sequences by use of a synthetic oligonucleotide probe coding for a stretch of conserved amino acids, the H/C-link, which joins individual finger loops in several multi-fingered proteins. Our studies reveal that a large number of different cDNA clones encode amino acid sequences predicting multiple units of the metal-coordinating finger structure. Derived proteins are different from each other as well as from the two examples of *Xenopus* finger proteins reported to date, TFIIIA and X.fin. The 109 finger repeats characterized are derived from 14 different cDNA clones and have been analysed for the presence of conserved and highly variable amino acids, revealing a close structural relatedness among each other as well as with a few selected finger domains from *Drosophila* and mouse proteins. The results from this comparative sequence analysis are also discussed in terms of the existing models for DNA binding. All sequences are identified in an ovary cDNA library but the patterns of mRNA level for individual finger clones vary greatly during early development. The prevalence of these structures in the oocyte suggests that part of the maternal information for the realization of the developmental program utilized in *Xenopus* embryogenesis might be transmitted in the form of regulatory, nucleic-acid-binding proteins.

Key words: Xenopus laevis oocytes/finger protein/cDNA and genomic clones/transcription in embryos

Introduction

Those genes which generate the set of maternal mRNAs stored in the cytoplasm of the *Xenopus* oocyte provide the entire protein encoding genetic information utilized in the earliest stages of embryogenesis. Even with the onset of transcription activity at the mid blastula transition (MBT), the quantitative transcriptional dominance of the maternally active genes among the zygotically expressed genes actively maintains the prevalence of maternal mRNAs. However, the function for the majority of these transcripts is unknown (Davidson, 1986, and references therein).

The high transcriptional competence of the *Xenopus* oocyte implies that it contains an excess of transcription factors and RNA polymerase (Gurdon and Melton, 1981) which are not sequestered in transcription complexes. One of those factors, the 5S gene-specific transcription factor TFIIIA, has been cloned (Ginsberg *et al.*, 1984) and the analysis of its amino acid sequence has led to the definition of a new class of nucleic-acid binding proteins, characterized by multiple entities of metal-coordinating loops or fingers (Miller *et al.*, 1985).

Classical genetic studies combined with the techniques of modern molecular biology have led to the structural description of several genes involved in the formation of spatial patterns in the *Drosophila* embryo. Some of these genes have been found to encode proteins which, similar to TFIIIA, contain several copies of the finger motif (Rosenberg *et al.*, 1986; Boulay *et al.*, 1987; Tautz *et al.*, 1987), and one of them (Tautz *et al.*, 1987) produces a maternal transcript. Earlier studies in *Drosophila* had already defined another class of regulatory genes in development, characterized by a different DNA binding structure, the helix-turn-helix motif of the homeo domain (Gehring, 1985). Both of these nuleic-acid-binding motifs define evolutionary conserved, eukaryotic multigene families (McGinnis *et al.*, 1984; Schuh *et al.*, 1986; Chowdhury *et al.*, 1987). The idea that structurally related proteins have related functions would suggest that at least some of these genes operate as transcription regulators or, in more general terms, as nucleic-acid-binding proteins. Furthermore, the hypothesis that maternal factors responsible for the spatial and temporal pattern of gene expression in *Xenopus* embryogenesis are partially defined by mRNA translated into nucleic-acid-binding finger proteins, predicts that multiple cDNA clones distinct from the one coding for TFIIIA, or the most recently described 37 finger protein X.fin (Ruiz i Altaba *et al.*, 1987), should be represented in an oocyte-derived cDNA library.

Initially using a mixed oligonucleotide probe against a conserved amino acid sequence joining individual repeat elements in many of the finger proteins, the H/C-link (Schuh *et al.*, 1986), we have isolated one cDNA clone encoding multiple copies of the finger repeat, which was then used for the identification of a large number of *Xenopus* ovary cDNA and genomic clones. About 20 of these cDNA clones have been sequenced and all of them were found to encode multiple copies of the finger motif. Comparative sequence analysis of the 109 repeats described in this study suggests that these clones define a subgroup of finger proteins sharing extended, conserved arrays of amino acids. Individual genes exhibit distinct patterns of expression, as judged from the levels of specific mRNAs present in different stages of early *Xenopus* development.

Results

Isolation of Xenopus ovary cDNA clones encoding finger-motif proteins

In order to determine whether multiple genes encoding finger proteins are expressed in the *Xenopus* embryo, we have screened a neurula stage cDNA library using a mixed oligonucleotide probe prepared against the H/C-link consensus

sequence HTGEKPY (Schuh *et al.*, 1986). Several positive clones were isolated and the 600-bp insert of one of them (XLcNF1: Xenopus Laevis cDNA Neurula Finger) has been sequenced. Six finger repeats were identified. This cDNA clone was used to screen a *Xenopus* ovary cDNA library under higher stringency conditions. About 50 strong, positive signals were detected; the inserts from 22 of these clones (designated as XLcOF: Xenopus Laevis cDNA Ovary Finger) were isolated (Figure 1) and 20 of them were sequenced. Each of these DNA fragments was found to encode multiple copies of the finger motif, connected by the H/C-link consensus sequence. Comparative sequence analysis reveals that 13 of these clones are distinct from each other (see Figure 1) as well as from the two published examples of *Xenopus* finger proteins, TFIIIA (Ginsberg *et al.*, 1984) and X.fin (Ruiz i Altaba *et al.*, 1987). Furthermore, it was established by Northern analysis that XLcNF1 is also already expressed in the oocyte (not shown), although it was not identified amongst the oocyte-derived clones. Out of the 14 different cDNA clones characterized in this study (see Table I), seven contain a poly(A) tail and a stop codon in frame with the finger repeats. Another one (XLcOF22) is lacking the poly(A) tail but shows the translation stop codon. Despite the fact that the majority of sequences obtained represent partial cDNA elements, we are confident that at least these seven or eight terminating fragments are derived from distinct genes. Further support for this notion comes from a genomic Southern blot analysis, using the inserts from several finger cDNA clones (Figure 2); with one exception (XLcOF9, as discussed below) a distinct pattern of only few fragments is visualized. The actual number of different finger transcripts present in the oocyte might be even higher than detected in this study.

In conclusion, these findings demonstrate that the finger motif defines a multigene family represented in polyadenylated RNA of *Xenopus* oocytes.

Structural analysis of finger motif encoding Xenopus ovary cDNA clones

DNA and protein sequence results obtained for two examples of *Xenopus* finger clones, XLcOF22 and XLcOF10, are shown in Figure 3. XLcOF22 has a stop codon at position 1345 and two potential initiation codons in frame with 12 finger repeats. The first ATG is in position 40 and, on the basis of initiation at this site, the derived protein comprises 435 amino acids (including the initiator methionine) and has a mol. wt of 57 173 daltons. No consensus polyadenylation signal was detected. Taking an additional portion of the 3′ untranslated region and a poly(A) tail into consideration, the length of this cDNA segment roughly corresponds to the XLcOF22 mRNA size observed in Northern blot analysis using poly(A)$^+$ RNA from *Xenopus* oocytes (as discussed below). These findings indicate that the coding sequence for the XLcOF22 protein is entirely defined by the structure shown in Figure 3. The seven different finger repeats in XLcOF10 are in frame with a termination codon at position 648 followed by a 3-fold repeated 95-bp sequence element, a modified polyadenylation signal in position 991 and the poly(A) tail. The presence of repetitive elements in the 3′ untranslated region of this cDNA clone explains the observed effects in the genomic Southern blot experiment (Figure 2; sequence of XLcOF9 is identical to XLcOF10); even under high-stringency washing conditions this probe produced a

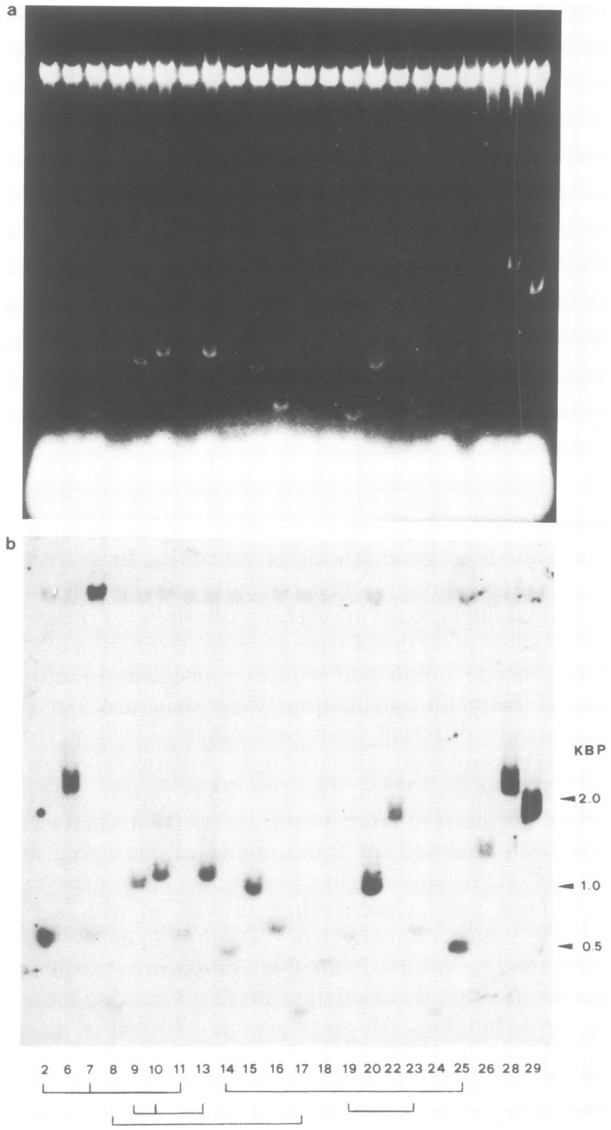

Fig. 1. Southern blot analysis of ovary cDNAs (XLcOF) encoding the finger motif. Recombinant cDNA clones from a *X.laevis* ovary λ gt11 phage library were identified by screening with XlcNF1 as labelled probe. *Eco*RI digests of phage DNAs were run on an agarose gel (**a**) and transferred to nitrocellular membrane. The autoradiograph (**b**) shows the cDNA inserts hybridizing to XLcNF1 after washing in 0.2 × SSC at room temperature. Brackets indicate partially homologous or identical clones identified by nucleotide sequence analysis.

'smear' of signals without any detectable specific bands.

A comparative structural analysis of the 109 different finger repeats described in this study and the consensus sequences from various other vertebrate finger proteins are shown in Table I. A number of conserved structural features defining a subgroup of eukaryotic finger proteins emerges: (i) the spacing between the first and second cysteine of each repeat elements is strictly two amino acids; (ii) the length of the loop between the second cysteine and the first histidine is strictly 12 amino acids; (iii) only two positions in the 12-membered loop are fully conserved (F in position 4 and L in position 10); the spacing of the two histidines is three amino acids, with the exception of XLcOF22 (Figure 3), which has one amino acid each inserted in finger 1, 9 and 11;

Table I. Comparative structural analysis of finger repeats

Type	Sequence length (bp)	Poly(A)	Finger number	Consensus sequence[b]
XLcNF1	586	+	6	C S E C G K – F S L – S – L H – H Q – – H T G E K P F –
XLcOF2	561	–	6	C T E C G K – F S – K – – L Q – H Q R – H T G E K P F T
XLcOF6	1959	+	16	C T E C G K S F T – K S I L – – H – – T H T G E K P F T
XLcOF8	174	–	2	C T – C G K – F – – K D – L – K H – R – H T G E K P F –
XLcOF10	1036	+	7	C S E C G K – F K – – S – L – – H Q – – H T G E K P F –
XLcOF14	485	–	6	C – E C G K – F – – K – – L – – H – L I H T G E K P F –
XLcOF15	935	+	6	C T E C G K – F S – K S – L – – H Q K – H T G E K P F T
XLcOF18	425	+	3	C T E C G – C F T – – Y – L T – H – R – H T G E K P F T
XLcOF19	594	–	7	C S E C G K – F – – R S – L T – H – R T H T G E K P F S
XLcOF20	952	–	10	C T E C G K – F S – N – – L – – H – R V H T G E K P F –
XLcOF22	1637	(–)[a]	12	C S E C G K C F S – – S – L – – H Q R T H T G E K P F S
XLcOF26	1236	+	7	C T E C G K – F – – K – – L – – H Q – – H T G E K P F T
XLcOF28	2053	+	14	C T E C – K – F – – – S – L – – H – R T H T G E K P F –
XLcOF29	1725	–	7	C S E C G K C F T – – – – L – – H – – – H T G E K P F –
	Consensus sequence[c]			C $\frac{T}{S}$ E C G K – F $\underline{S}$ – $\underline{K}$ S – L – – H $\underline{Q}$ R – H T G E K P F $\underline{T}$
X.fin			37	C – – C – K – F – Q – S – L – K H – R T H T G E K P Y –
mkr 1			7	C – E C G K T F – – – S N L I – H Q R I H T G E K P Y –
mkr 2			9	C – E C G K A F – – – S S L T – H Q R I H T G E K P Y –
Krüppel			4	C – E C D R – F – – – H – L K – H M R V H T G E K P $\frac{Y}{F}$ –

[a]XLcOF22 does not contain a poly(A) tail but has a translation stop codon in frame with the finger repeats.
[b]Amino acids which are found at fixed positions in at least 50% of all fingers of individual clones.
[c]Amino acids which are found at least in seven out of the 14 consensus sequences shown. Underlined amino acids are only present in five or six cases.
For comparison we have included the consensus sequences of X.fin (Ruiz i Altaba *et al.*, 1987), mouse *mkr 1, mkr 2* (Chowdhury *et al.*, 1987) and *Drosophila Krüppel* (Rosenberg *et al.*, 1986).

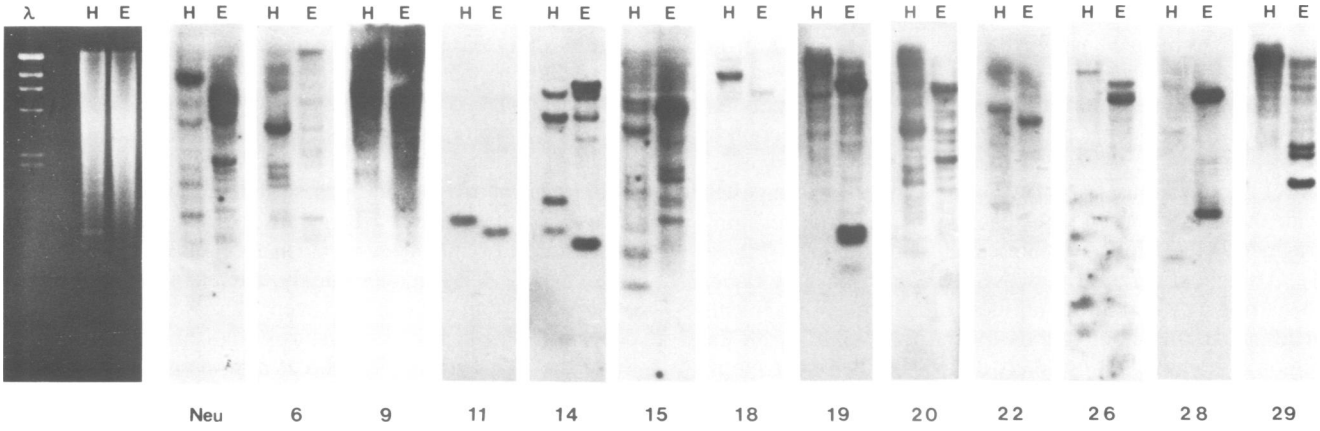

Fig. 2. Southern blot analysis of *X.laveis* genomic DNA. Genomic DNA isolated from *X.laevis* red blood cells was digested with *Eco*RI (E) or *Hin*dIII (H) and electrophoretically separated (50 μg/lane; see left side). A *Hin*dIII digest of λ DNA was run as size marker. DNA fragments were transferred to nitrocellulose membranes and hybridized with labelled XLcNF1 (Neu) and XLcOF sequences as indicated below the blots. Autoradiographs after washing in 0.05 × SSC at 65°C are shown.

(iv) finally, the sequence (TGEKPF–) and length (seven amino acids) of the element joining the second histidine of one finger with the first cysteine of the next one are conserved. Inspection of the data shown in Table I reveals that the finger consensus sequence of the XLcNF and XLcOF proteins is most closely related to the mouse *mkr 1* and *mkr 2* sequences (Chowdhury *et al.*, 1987). The observed high degree of sequence conservation in the finger domain of all of the XLcOF clones explains why XLcNF1 was such an efficient probe for the isolation of many different finger cDNA clones in *Xenopus*.

Other members of the finger protein family, such as *Xenopus* TFIIIA (Ginsberg *et al.*, 1984), the *Drosophila* hunchback gene product (Tautz *et al.*, 1987), the most recently described nerve-growth-factor-induced rat NGF1-A gene product (Milbrandt, 1987) or the yeast transcriptional regulator protein ADR 1 (Hartshorne *et al.*, 1986; Blumberg *et al.*, 1987), which all belong to the C/C–H/H class, share some, but not all, of the structural features listed above. It appears as if separate subfamilies of finger repeat proteins can be classified on the basis of such narrowly defined structural criteria. It is tempting to speculate that the

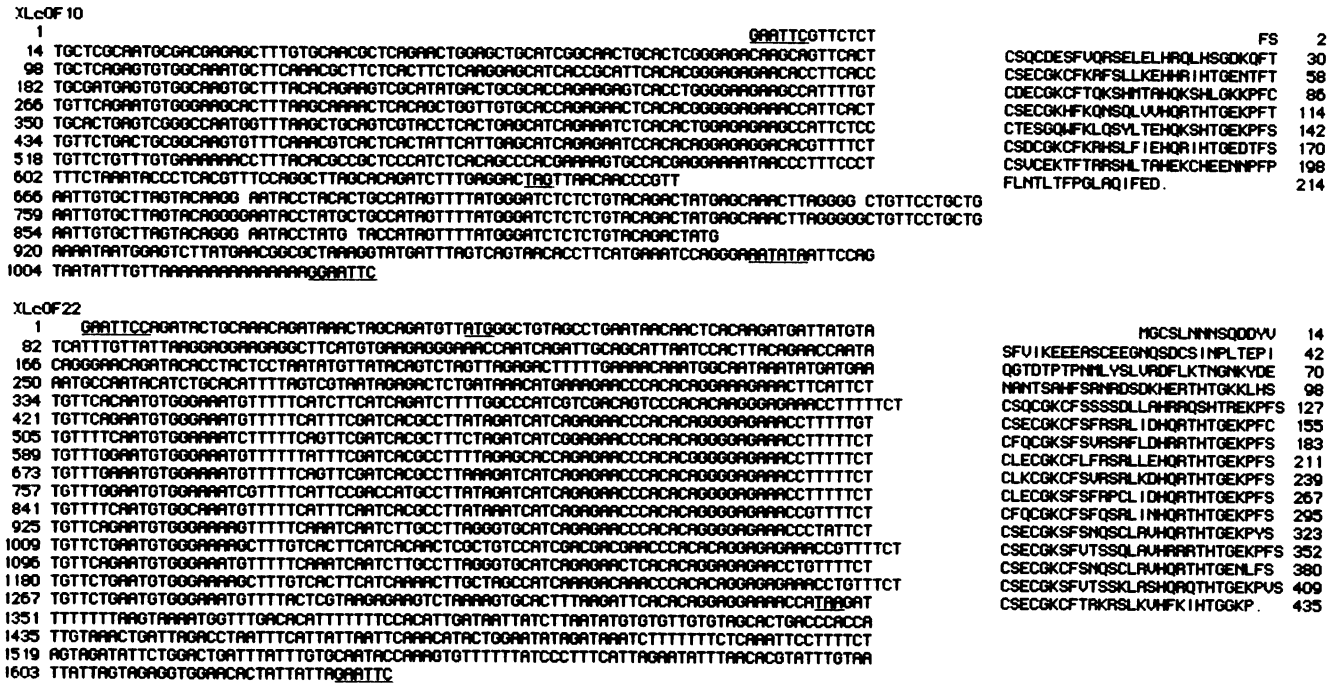

Fig. 3. Sequences of XLcOF10 and XLcOF22. Nucleotide sequences are written in 5′ to 3′ direction and aligned for the finger coding repeats. The deduced amino acid sequences are shown on the right side. XLcOF10 contains two complete copies and one incomplete copy of a repetitive element within the 3′ untranslated region as indicated. *Eco*RI sites, initiation and stop codons, as well as a modified polyadenylation site, are underlined.

members of such subfamilies share related functions in different organisms.

Isolation of Xenopus finger genes

A *Xenopus laevis* genomic DNA library in Charon 24A has been screened using the ^{32}P-labelled insert from XLcNF 1 described in the previous section as probe. About 100 000 p.f.u. representing $<50\%$ of one genome equivalent have been screened, resulting in the detection of 35 strong signals. Plaque purified phage DNA (designated as XLgF: Xenopus Laevis genomic Finger) was digested with the restriction endonuclease *Eco*RI and the resulting fragments were blotted on nitrocellulose membranes (Figure 4). After hybridization with XLcNF1 and washing under low-stringency conditions all these clones reveal fragments at least partially homologous to the probe used, with the region of homology most likely defined by the finger-motif-encoding domain. Only two of these clones (XLgF32 and XLgF33) are identical in their restriction and hybridization patterns, indicating that the rest represent distinct genomic fragments (part of which may, of course, be overlapping). The high-stringency wash identifies two strongly hybridizing fragments (XLgF3 and XLgF13), most closely related to XLcNF1. Partial sequence analysis of one of them (XLgF13) reveals sequence identity with the XLcNF1 clone over a region of at least 200 bp. Hybridization with other cDNA clones under high-stringency conditions combined with nucleotide sequence analysis of subcloned fragments led to the identification of two additional genomic clones (XLgF10 corresponding to XLcOF29, XLgF19 corresponding to XLcOF15) (data not shown). Thus, at least three of the genomic fragments isolated are homologous to the cloned cDNA sequences. We conclude that the *X.laevis* genome contains numerous genes encoding proteins of the H/C-link family with multiple copies of the finger motif.

Levels of Xenopus finger protein encoding mRNA during development

The Northern blot analysis shown in Figure 5 reveals that, as expected, poly(A)$^+$ RNA transcripts derived from any of the *Xenopus* finger protein cDNA clones assayed are readily detected in the oocyte. As judged from their electrophoretic mobility, the length of RNA molecules shown is in the range of 1.8 to ~4 kb. With the exception of XLcOF6, specific transcripts can also be detected in later stages of development. Some of the RNAs disappear in the latest stages of early embryogenesis tested (such as XLcOF15 and XLcOF20), whereas other transcripts are maintained up to the somite or tailbud stages (such as XLcOF10, XLcOF22 and XLcOF29). Most interestingly, the relative level for one of these RNAs (XLcOF28) even increases in the latest developmental stages tested (neurula and somite; Figure 5). Since the signal using XLcOF6 as a probe is only detected in ovary RNA, it was imperative to exclude the possibility that it results from follicle cell RNA. Using collagenase-treated, staged oocytes free of follicle cells, high levels of XLcOF6 RNA were only detected in early stages of oogenesis (not shown).

These distinct temporal patterns of RNA distribution most likely indicate stage-specific, differential activation on the level of transcription for some of these genes, although the possibility of differential polyadenylation cannot be excluded on the basis of the experimental results obtained. Similar effects have been described for the expression of X.fin (Ruiz i Altaba *et al.*, 1987).

The size of the transcript detected by use of XLcOF22 as a probe is ~2 kb. As already mentioned above, this estimate is in good agreement with the size predicted from the cDNA sequence reported (Figure 3). The presence of a repetitive element in the 3′-untranslated region of XLcOF10 (Figure 3) provides an explanation for the 'smear'

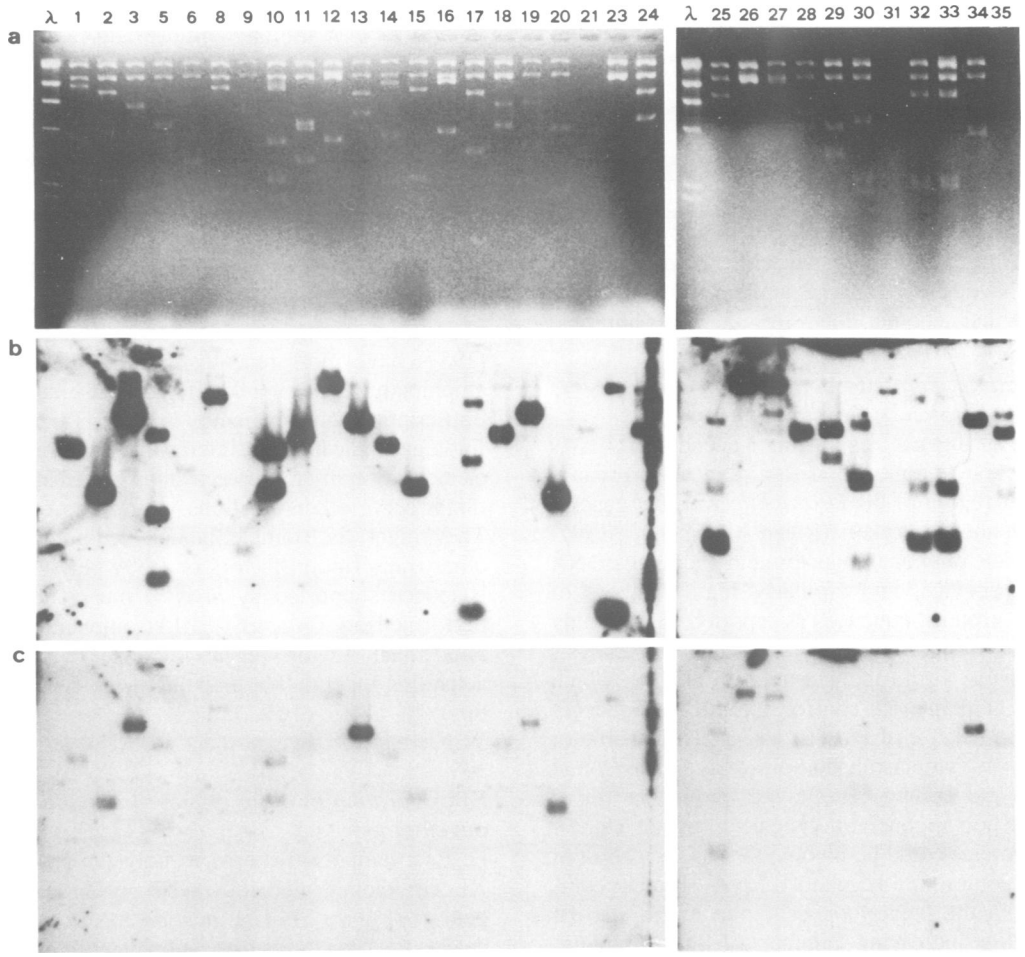

Fig. 4. *X.laevis* genomic clones (XLgF) encoding finger proteins. A *X.laevis* genomic DNA library in Charon 24A was screened with XLcNF1 as labelled probe. DNAs of 35 clones yielding a positive hybridization signal were isolated, digested with *Eco*RI and separated on agarose gels (**a**). Two *Hin*dIII digests of λ DNA were run as size markers. After blotting to nitrocellulose membranes the DNA was hybridized to ^{32}P-labelled XLcNF1. Autoradiographs after washing under non-stringent conditions (0.2 × SSC; room temperature) are shown in (**b**) and after washing under high stringency (0.05 × SSC, 65°C) in (**c**).

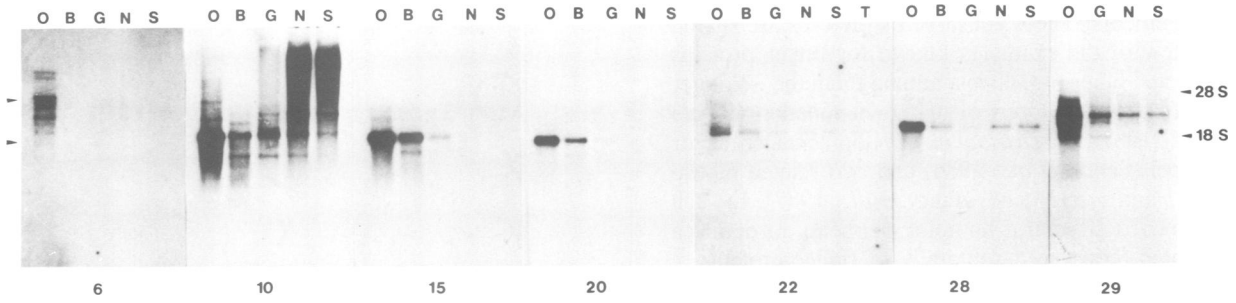

Fig. 5. Northern blotting of *X.laevis* RNAs from different developmental stages. Glyoxylated poly(A)$^+$ RNA (5 μg/lane) from total ovary (O) (oocytes of all maturation stages), blastula (B) (stages 7–9; Nieuwkoop and Faber, 1975), gastrula (G) (stages 10–12), early neurula (N) (stages 13–16), somites 1–15 segregation (S) (stages 17–25) and hatched tadpoles (T) (stages 38–40) were run on agarose gels and transferred to positively charged nylon membranes. Autoradiographs after hybridization with XLcOF sequences and washing in 40 mM phosphate buffer at 70°C are shown.

in the high mol. wt area of the Northern blot (Figure 5), caused by minor DNA contaminations in the RNA preparations of later developmental stages. In addition to several signals of minor intensity, which may be caused by hybridization to identical or similar repeat elements in otherwise unrelated messages, a major band of ~2 kb was observed.

Taken together, the data obtained from Northern blot experiments using poly(A)$^+$ RNA preparations from *Xenopus* embryos suggest that individual members of the H/C finger protein family are subject to distinct temporal patterns of expression in early embryogenesis.

Discussion

Two different structural elements detected in a number of developmental genes from *Drosophila*, the helix−turn−helix motif and the finger repeat, define distinct families of potentially nucleic-acid-binding proteins. Both of these structures are conserved in evolution and believed to define functionally related gene families (Gehring, 1985; Schuh *et al.*, 1986; Chowdhury *et al.*, 1987). Initially using the conserved H/C-link consensus sequence (Schuh *et al.*, 1986) as a probe, we have isolated a large number of finger-motif-encoding cDNA and genomic clones from *Xenopus* libraries.

Common structural elements define an evolutionary conserved finger protein gene subfamily

The abundance of distinct, finger-motif-encoding mRNAs in the *Xenopus* oocyte appears, at first glance, surprising. Using the finger region of the *Drosophila Krüppel* gene as a probe, Melton and co-workers (Ruiz i Altaba *et al.*, 1987) were able to isolate only one finger-protein-encoding cDNA from *Xenopus* oocytes, and similarly high numbers of different finger protein sequences as reported in this study have, to our knowledge, not been reported in the analysis of other organisms. From the example of TFIIIA, which binds to the 5S gene internal control region in a sequence-specific way (Engelke *et al.*, 1980) and is also capable of forming a specific, cytoplasmic complex with 5S ribosomal RNA (Pelham and Brown, 1980), we know that finger repeats may function as general nucleic-acid-binding entities. Thus, finger proteins could be involved in the formation of the multiple structural and regulatory RNA−protein complexes formed in the eukaryotic cell, and might also be examples for the increasing number of DNA-binding, transcriptional regulator proteins characterized.

A comparative analysis of the amino acid sequences which constitute the repeats of *Xenopus* finger genes reveals a high number of conserved positions and other structural features (Table I; as detailed in Results). The H/C-link sequence which was utilized as a probe for the screening of *Xenopus* libraries is, as expected, one of these common structural elements. These conserved structures are shared with only few of the examples known for finger proteins in *Xenopus* and other organisms, among them the 37 finger protein X.fin from *Xenopus* of unknown function (Ruiz i Altaba *et al.*, 1987), the *Drosophila* developmental regulator gene Krüppel (Schuh *et al.*, 1986) and two related mouse genes *mkr 1* and *mkr 2* (Chowdhury *et al.*, 1987). In such a way, extended structural homology appears to define a closely related, conserved subfamily of finger proteins.

Significance of highly variable positions in the finger loop

Brown and Argos (1986) have predicted that seven amino acids of the finger loop, including the inner histidine, form an α-helix. Subsequently, Ruiz i Altaba *et al.* (1987) suggested on the basis of the analysis of the 37 finger repeats in X.fin that, in analogy to the helix−turn−helix motif for other DNA-binding proteins, variable residues in this α-helix may help determine binding specificity. This idea is in good agreement with the recently proposed three-dimensional finger model (Berg, 1988), which suggests the formation of an antiparallel β-sheet followed by an α-helix. Upon DNA binding the α-helical region is postulated to contact the DNA in the major groove. Inspection of the 109 fingers identified in the XLcNF and XLcOF protein family reveals that the majority of the highly variable positions are located in or directly flanking to the potentially α-helical region (Figure 6). Thus, these highly variable amino acids may indeed provide the structural key for the sequence specificity observed in the binding of finger proteins to nucleic acids.

However, DNA or RNA binding may not be the only function of the finger repeats. On the basis of a comparative structural analysis Brown and Argos (1986) have reported another interesting observation. The potentially α-helical regions in the finger repeats show an obvious hydrophobic/hydrophilic sidedness. In addition, Giniger and Ptashne (1987) have most recently demonstrated that a putative amphipathic α-helix linked to a DNA-binding unit forms a transcription-activating entity in yeast. Taken together, these findings indicate the interesting possibility that finger elements of transcription-activating proteins, such as TFIIIA, might serve other functions in addition to DNA binding. They might, for example, be involved in the interaction with other transcription factors or RNA polymerase. This idea is indeed supported by *in vitro* mutagenesis experiments performed on *Xenopus* TFIIIA; amputation of one of the N-terminal finger repeats abolishes the transcription-activating function but maintains wild-type levels of DNA-binding activity (W.E.Jack, T.Pieler, B.Moorefield, W.Nietfeld, W.Swiggard and R.G.Roeder, in preparation).

Finger protein in gene expression in Xenopus development

Distinct temporal patterns of poly(A)$^+$ RNA for different XLcOF clones were observed during *Xenopus* embryogenesis (Figure 5). The increase in the amount of mRNA detected in later stages of development for one of these clones (XlcOF28) indicates a transcriptional reactivation.

Structural homology to the finger domain of the *Drosophila* developmental regulator gene *Krüppel* (Schuh *et al.*, 1986) raises the possibility that members of the *Xenopus* XLcOF gene family contribute to the generation of maternal factors involved in the formation of the spatial

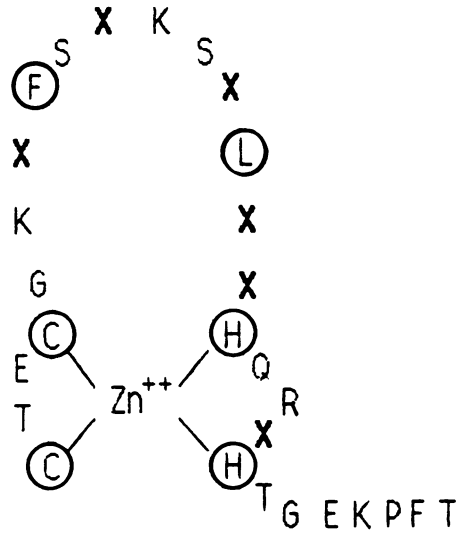

Fig. 6. Conserved amino acids in the finger motif. The conserved amino acids (as outlined in Table I) are shown at the corresponding positions of the proposed finger model (Miller *et al.*, 1985). Highly variable positions are denoted by ×.

and temporal pattern of gene expression in *Xenopus* development. On the basis of numerous classical studies, localized maternal factors in the *Xenopus* oocyte have been implicated to be important for the specification of cell fate (as reviewed in Davidson, 1986). It is tempting to speculate that part of the localized maternal information for the realization of the developmental programme is transmitted in the form of mRNA, which encodes regulatory, nucleic-acid-binding proteins. It will be interesting to test if any of the XLcOF clones presented in this study exhibits properties one would predict for these putative developmental regulators.

Materials and methods

Screening of cDNA and genomic libraries

In initial experiments we used a neurula stage cDNA library. Synthesis of cDNA and cloning in λ gt10 was performed using commercially available kits and following the instructions given by the supplier (Amersham). This library was screened with a [γ-^{32}P]ATP-labelled mixed oligonucleotide probe derived from the conserved H/C-link sequence HTGEKPY(5′-CA-YACNGGNGARAARCCNTAY-3′). After transfer of the phage DNA to nitrocellulose filters the hybridization reaction was done overnight in 10 × Denhardt's solution (Denhardt, 1966), 1% SDS and 6 × SSC at 57°C. Filters were washed in 2 × SSC, 0.1% SDS and 1 mM EDTA at 40°C and subjected to autoradiography. Having isolated and characterized a finger-protein-encoding clone (XLcNF1), this sequence served as an oligonucleotide-primed, [^{32}P]dCTP-labelled probe to screen a *X.laevis* ovary cDNA library in λ gt11 (2.5 × 10^5 primary recombinants; kindly provided by Dr J.Kleinschmidt, Heidelberg) and a *X.laevis* genomic DNA library in Charon 24A (4 × 10^5 primary recombinants; kindly provided by Dr G.Spohr, Geneva). After transfer to nitrocellulose filters the DNA was hybridized overnight at 60°C in 10 × Denhardt's solution, 1% SDS and 4 × SSC. After washing in 0.2 × SSC, 0.1% SDS and 1 mM EDTA at room temperature the filters were subjected to autoradiography at −70°C using Du Pont Cronex 4 films.

Southern blotting

After electrophoretic separation of DNA fragments on 1% agarose gels and transfer to nitrocellulose filter (Southern, 1975), hybridization with the appropriate probe was performed in 10 × Denhardt's solution, 4 × SSC, 1% SDS and 1 mM EDTA at 65°C. Filters were washed either in 0.2 × SSC, 0.5% SDS, 1 mM EDTA at room temperature (non-stringent conditions) or in 0.05 × SSC, 0.5% SDS, 1 mM EDTA at 65°C (high stringency) and subsequently exposed to Kodak X-AR films for autoradiography at −70°C.

Isolation of RNA and Northern blotting

Total cellular RNA from ovaries and embryos of different developmental stages (staged according to Nieuwkoop and Faber, 1975) was prepared by the guanidinium isothiocyanate/hot phenol method (Maniatis *et al.*, 1982). Poly(A)$^+$ RNA was selected by two consecutive runs on oligo(dT)-cellulose columns. Glyoxylated poly(A)$^+$ RNA was run on 1.2% agarose gels. After transfer to Gene Screen Plus membrane (NEN), hybridization with oligonucleotide-primed labelled cDNAs was performed in 0.5 M sodium phosphate (pH 7.2), 7% SDS, 1 mM EDTA and 1% BSA at 70°C for 14 h. After washing in 0.04 M sodium phosphate (pH 7.2), 1% SDS and 1 mM EDTA (twice at 65°C and once at 70°C) the membranes were subjected to autoradiography as described above.

DNA sequencing

Total cDNA inserts as well as suitable restriction fragments were subcloned in M 13 mp8/mp9 vectors (Messing and Vieira, 1982) and sequenced using the dideoxy chain termination technique (Sanger *et al.*, 1977).

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