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The maternal store of zinc finger protein encoding mRNAs in fully grown *Xenopus* oocytes is not required for early embryogenesis

Tarek El-Baradi, Tewis Bouwmeester, Richard Giltay and Tomas Pieler

Max-Planck-Institut für Molekulare Genetik, Otto-Warburg-Laboratorium, Ihnestrasse 73, 1000-Berlin 33, FRG

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A large family of C_2H_2 (*Krüppel*-like) zinc finger protein genes is maternally transcribed in *Xenopus* oocytes; many of the corresponding mRNAs are actively translated post-fertilization, before the onset of zygotic activation of transcription. With the aim of asking if any of these stored mRNAs have a function in *Xenopus* development, we made use of antisense oligonucleotide mediated, targeted RNA destruction. Injected oocytes lose the entire pool of C_2H_2 zinc finger protein encoding mRNAs. They are indistinguishable from control oocytes in their abilities to mature *in vitro* and to be fertilized *in vitro*. Embryos generated from such oocytes develop normally until tadpole stage. These findings do not rule out the possibility that C_2H_2 zinc finger protein genes are involved in developmental control in *Xenopus*. However, they do suggest that the biological function for at least some of the early expressed zinc finger proteins in *Xenopus* differs in important aspects from the way *Krüppel* or other DNA binding factors act as developmental regulators in *Drosophila*.

Key words: antisense oligonucleotides/embryogenesis/*Xenopus laevis*/zinc finger protein

Introduction

Many of the developmental control genes in *Drosophila* encode proteins which contain either a homeobox (Gehring, 1985) or zinc finger repeats (Rosenberg *et al.*, 1986). Using probes specific for these evolutionarily conserved domains, numerous candidate genes for the control of vertebrate development have been isolated (reviewed in Dressler and Gruss, 1988; Wright *et al.*, 1989b). While reverse and pseudogenetic analysis has provided evidence that at least some vertebrate homeobox encoding genes do indeed play a role in development (Ruiz i Altaba and Melton, 1989; Wright *et al.*, 1989a; Kessel *et al.*, 1990), a comparable analysis of vertebrate zinc finger protein encoding genes has not yet been reported.

We have previously shown that the *Xenopus* genome contains a large multigene family, comprising at least 100 different members, each of which encodes a *Krüppel*-like zinc finger protein (ZFP), (Köster *et al.*, 1988; Knöchel *et al.*, 1989; Nietfeld *et al.*, 1989). A similar analysis in other vertebrates has resulted in the identification of equally high or even higher numbers of ZFP genes. It is estimated that ~300 ZFP genes are present in the human genome (Bellefroid *et al.*, 1989) and numerous ZFP transcription units have also been isolated and characterized in mice (Chowdhury *et al.*, 1987; Chavrier *et al.*, 1988; Passananti *et al.*, 1989). Structural features inside and outside of the zinc finger repeats allow the definition of discrete subfamilies (Nietfeld *et al.*, 1989); conserved sequence elements outside of the finger domain were found to be associated with large numbers of *Xenopus* (Knöchel *et al.*, 1989) and human (E.J.Bellefroid, D.A.Poncelet, P.J.Lecoq, O.Revelant and J.A.Martial, submitted for publication) ZFPs.

Several ZFPs acting as developmental regulators in *Drosophila* embryogenesis have been identified. The earliest acting of these ZFPs are encoded by the gap class of segmentation genes, which organize the body pattern along the anterior–posterior axis under the influence of maternal genes (Nüsslein-Volhard *et al.*, 1987). Of the gap genes, only the *hunchback* gene product has a maternal component (Tautz *et al.*, 1987). However, although zygotic *hunchback* is essentially required for the formation of anterior segments, the maternal *hunchback* gene product is functionally redundant (Hülskamp *et al.*, 1990). The other, early acting gap ZFP gene is the zygotically activated *Krüppel* protein (Rosenberg *et al.*, 1986). These two, as well as other developmental regulatory ZFP encoding genes which were isolated on the basis of a developmental mutant phenotype, are expressed in a strictly localized fashion in the *Drosophila* embryo (Jäckle *et al.*, 1986; Boulay *et al.*, 1987; Coulter *et al.*, 1990; Orenic *et al.*, 1990).

In contrast, a family of *Drosophila* ZFPs clustered in a densely transcribed region, the *serendipity* locus, is transcribed during oogenesis and zygotically activated with a broad distribution in different embryonic tissues (Vincent *et al.*, 1985, 1988; Payre *et al.*, 1989). It has been proposed that the corresponding ZFPs are involved in the regulation of general cell functions, rather than directly in embryonic pattern formation (Vincent *et al.*, 1988).

Extensive analysis of RNA expression profiles for the multiple ZFP encoding genes during early *Xenopus* embryogenesis showed that these RNAs are all maternally transcribed, with the vast majority of ZFP transcripts uniformly distributed in eggs and embryos (Köster *et al.*, 1988, Knöchel *et al.*, 1989 and T.El-Baradi, T.Bouwmeester, R.Giltay and T.Pieler, manuscript in preparation). Similarly, many ZFP transcripts from other vertebrate organisms, including several human ZFPs are ubiquitously expressed (Chowdhury *et al.*, 1988a; Chowdhury *et al.*, 1989; Passananti *et al.*, 1989; E.J.Bellefroid, D.A.Poncelet, P.J.Lecoq, O.Revelant and J.A.Martial, submitted for publication); a different set of ZFP mRNAs is clearly localized in mouse embryos and/or adult tissue (Chowdhury *et al.*, 1988b; Mardon and Page, 1989; Wilkinson *et al.*, 1989; Cunliffe *et al.*, 1990).

Thus, on the basis of these characteristics, it appears likely, although at this point speculative, that two major groups of ZFP encoding genes can be distinguished in vertebrate and invertebrate systems: one with strictly localized spatial and

temporal expression characteristics and a function in embryonic pattern formation, and a second one with a rather broad expression profile and a so far unknown function. The molecular function of ZFPs is similarly diverse; many operate as positive and/or negative transcription regulators via their DNA binding activity (reviewed in Klug and Rhodes, 1987). Others, however, have a sequence specific RNA binding activity and function in RNA storage and/or transport (Pelham and Brown, 1980; Joho *et al.*, 1990; Guddat *et al.*, 1990).

These observations beg the question of whether it will be possible to identify vertebrate ZFPs which serve a biological function comparable to the various embryonic developmental regulator ZFPs identified in the model invertebrate system, *Drosophila*. Here, we describe the first *in vivo* functional analysis of vertebrate ZFPs. The entire pool of ZFP-encoding mRNAs in fully grown *Xenopus* oocytes was destroyed by antisense oligonucleotide injection. Embryos were generated from these manipulated oocytes using the surrogate mother technique (Holwill *et al.*, 1987). We find that ZFP mRNA-depleted oocytes go through normal early embryogenesis in *Xenopus*.

Results

Antisense oligonucleotides directed against the H/C link consensus sequence effectively destroy the maternal pool of C_2H_2 zinc finger protein-encoding mRNAs in Xenopus oocytes

Antisense techniques (reviewed in Weintraub, 1990) are the basis for experimental strategies aimed at studying the function of maternal genes in *Xenopus*. Injection of antisense oligonucleotides into *Xenopus* oocytes results in RNase H mediated cleavage of RNA–DNA hybrids (Dash *et al.*, 1987; Jessus *et al.*, 1988; Shuttleworth and Colman, 1988). Antisense oligonucleotide induced destruction of specific mRNAs in the oocyte should result in loss of function embryonic phenotypes for maternal effect genes. We designed a 14mer antisense oligonucleotide (termed H/C AS) directed against the H/C link region, a conserved, reiterated sequence which adjoins consecutive zinc finger elements and is one of the hallmarks of the *Krüppel*-type zinc finger (Schuh *et al.*, 1986), (Figure 1). The rationale for choosing this particular antisense oligonucleotide was that it could potentially destroy all ZFP transcripts simultaneously. This would increase the chance of determining if any of the ZFP transcripts are actually involved in developmental control.

Specificity and efficacy of H/C AS in destroying ZFP transcripts are illustrated in Figure 2, which shows a Northern blot analysis of total RNA from H/C AS injected, control injected and uninjected oocytes. The blot was first hybridized with a cDNA probe, which detects about half of all ZFP transcripts, because they share a large conserved non-finger region termed FAX (Knöchel *et al.*, 1989). Comparison of the FAX signal in RNA from control and experimental oocytes shows that ~95% of the FAX encoding transcripts has been destroyed by the H/C AS; a series of degradation products is visible, corresponding to 5′ segments of ZFP transcripts which presumably owe their stability to the fact that they are capped. Comparable levels of degradation were observed when the same RNA preparations were hybridized with six different probes for individual ZFP transcripts (three of which are shown: GF 5–1, GF 53–1 and GF 66–1), corresponding to unique finger regions of these specific ZFP transcripts. No degradation products are visible with these finger specific probes, showing that H/C AS effectively destroys the zinc finger encoding part of ZFP transcripts.

H/C AS has perfect complementarity to at least one H/C link in about half of all ZFP transcripts, whose sequence we have determined. ZFP transcripts GF 5–1 and GF 53–1 belong to this class. In addition, H/C AS has partial complementarity with only one mismatch to at least one H/C link in the vast majority of the remaining ZFP transcripts, among them GF 66–1. Since H/C AS mediates equally well the destruction of GF 5–1, GF 53–1 and GF 66–1, it follows that this oligonucleotide is effective in the destruction of most if not all ZFP transcripts which we have isolated.

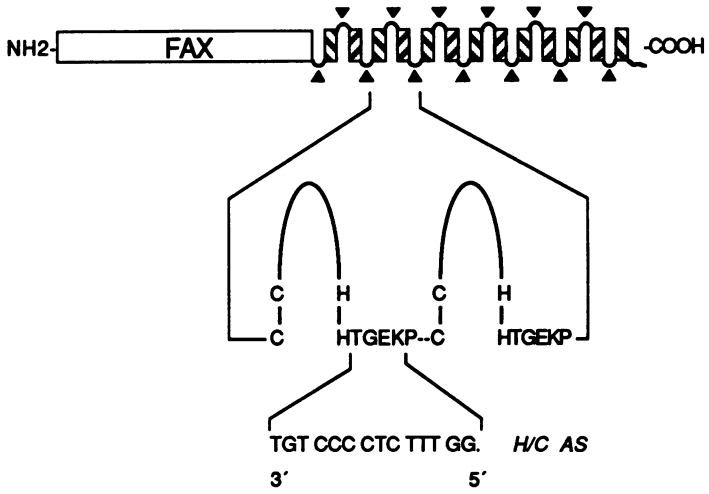

Fig. 1. Diagram of the generalized zinc finger protein structure. Tandem repeats of multiple zinc fingers are located at the C-terminus of a typical ZFP and they are characterized by structural features distinctive of zinc finger elements found in *Krüppel* and in other *Krüppel*-like proteins (Rosenberg *et al.*, 1986). One of these features is the strongly conserved H/C link, which adjoins consecutive fingers (Schuh *et al.*,1986) and it is indicated by the filled triangles. Also shown is the 14mer antisense oligonucleotide (H/C AS), directed against part of the consensus nucleotide sequence encoding the H/C link. FAX represents a highly conserved non-finger domain, comprising >200 N-terminally located amino acids and is present in about half of all ZFPs; a probe from the FAX domain detects the entire family of these transcripts (Knöchel *et al.*, 1989).

Levels of non-finger transcripts EF1a and histone H4 were not affected by oligonucleotide injection. From these results we conclude that the H/C AS specifically destroys the entire family of maternal ZFP transcripts to near completion in stage VI oocytes.

The minimal amount of H/C AS effective in destroying ZFP transcripts is 0.5−1.0 ng/oocyte (data not shown). This is 5- to 10-fold less than the reported minimal quantities of other antisense oligonucleotides effective in degrading specific mRNAs such as vg1 and histone H4 (Shuttleworth and Colman, 1988). It is also substantially less than the threshold of about 5 ng, above which oligonucleotides have been shown to cause non-specific toxic effects in cleaving embryos (Woolf *et al.*, 1990). The strong potency of the H/C AS predicts therefore that it is highly suitable for the production of ZFP transcript depleted embryos.

Fig. 2. Antisense oligonucleotide-mediated destruction of zinc finger protein mRNAs in stage VI *Xenopus* oocytes. Northern blot analysis was performed with total RNA from non-injected (N), injected with 1 ng H/C AS (AS) and injected with 1 ng control oligonucleotide (C) oocytes. Each lane contains 3 oocyte equivalents of total RNA; cDNA fragments utilized for probing are indicated; they detect either about half of all ZFP transcripts (FAX), individual ZFP transcripts (GF 66−1, GF 5−1 and GF 53−1) or mRNAs encoding histone H4 and EF1 alpha (EF-1A).

H/C AS mediated destruction of zinc finger protein transcripts does not affect Xenopus oocyte maturation or embryogenesis

We produced ZFP transcript depleted *Xenopus* embryos by *in vitro* maturing H/C AS injected oocytes and then fertilizing them by means of the surrogate mother procedure (Holwill *et al.*, 1987). In all the experiments performed, H/C AS injected oocytes were indistinguishable from controls in their ability to mature *in vitro* in the presence of progesterone. It has been reported that oligonucleotide mediated destruction of maternal mRNAs encoding the proto-oncogene products *c-mos* (Sagata *et al.*, 1988) and *ets-2* (Chen *et al.*, 1990) prevents hormone induced oocyte maturation of *Xenopus* oocytes. Our experimental data demonstrate that the maternal store of ZFP transcripts is not essentially required for oocyte maturation.

More importantly, H/C AS injected oocytes were also indistinguishable from controls in their abilities to be fertilized after *in vitro* maturation and passage through a surrogate mother in the five independent experiments listed in Table I; experimental embryos ranging from morula to stage 39 tadpoles were comparable to appropriate controls with respect to their morphology, rates of development and survival percentages (Table I and Figure 3). Tadpoles allowed to grow further up to stage 48 were also found to be indistinguishable from controls.

In each of the experiments listed in Table I, an aliquot of 5−10 injected and control oocytes was taken for Northern blot analysis of H/C AS induced ZFP transcript degradation (data not shown). Since survival rates of embryos generated from microinjected oocytes were slightly reduced in comparison to control embryos (Table I), we wanted to exclude the possibility that surviving H/C AS treated embryos represent a subpopulation derived from oocytes which received lower than average amounts of oligonucleotide. Therefore, ^{32}P-labelled H/C AS was utilized for microinjection. Radioactivity per oocyte did not deviate significantly from the average; moreover, the distribution of label in surviving embryos was similar to the distribution of label in injected oocytes.

Table I. Developmental fates of embryos reared from oligonucleotide-injected oocytes and non-injected oocytes.

Injected	Amount (ng/oocyte)	Implanted (No.)	Cleavage (%)	Tadpole (%)
H/C AS	0.6	110	56	21
Non-injected	−	150	60	35
H/C AS	0.8	90	32	11
Control	0.8	60	38	25
H/C AS	1.0	92	40	17
Non-injected	−	101	55	25
H/C AS	1.5	55	36	9
Non-injected	−	145	26	9
H/C AS	5.0	96	18	8
Control	5.0	60	8	0
Non-injected	−	150	43	24

Percentages were calculated as the fraction of implanted oocytes developing into morphologically normal embryos. Developmental aberrations did occur, but at comparable frequencies, both in embryos reared from H/C AS injected oocytes and in embryos derived from control oocytes. Frequently observed developmental aberrations included irregular cleavage at morula stages, exogastrulation and microcephaly.

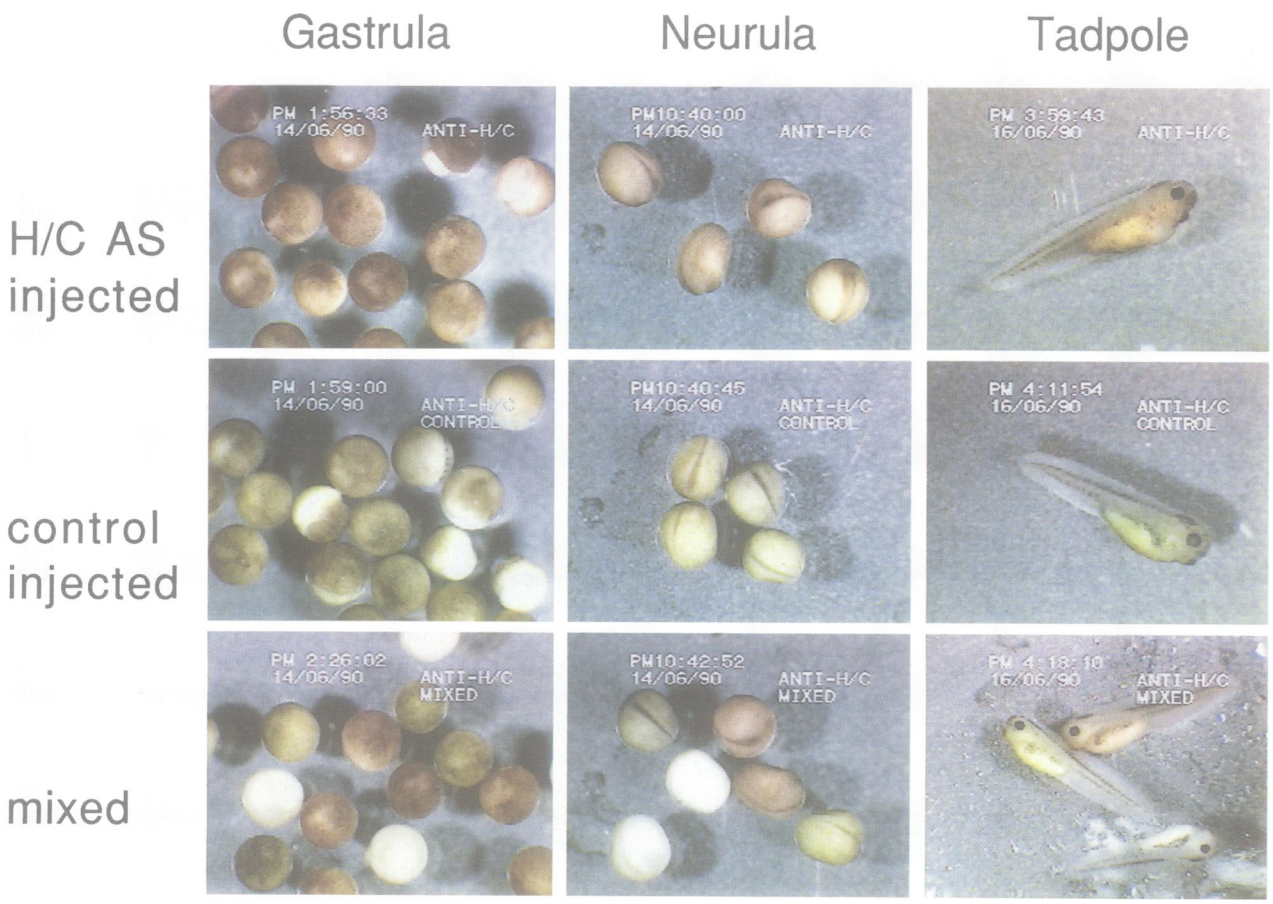

Fig. 3. Development of embryos reared from H/C AS injected oocytes. H/C AS injected (red) and control-injected (green) oocytes were colour coded by incubation in vital dyes to make them distinguishable from the endogenous oocytes laid by the surrogate mother frog (white). Embryos raised from manipulated and endogenous oocytes were allowed to develop to stage 39 tadpoles with documentation at gastrula and neurula stages. The number of embryos shown does not reflect the actual number of embryos obtained, which is given in Table I.

Thus, we conclude that the H/C AS induced elimination of almost the entire maternal pool of ZFP transcripts does not obviously affect oocyte maturation, egg fertilization or any of the subsequent developmental events that lead to a stage 48 tadpole.

Zinc finger protein genes are not retranscribed before the mid-blastula transition

Microinjected oligonucleotides have a very short half-life of only several minutes (Woolf *et al.*, 1990, and our own data, not shown). Since progesterone induced oocyte maturation takes 3–4 h, ZFP transcripts might be resynthesized before fertilization. We therefore determined levels of ZFP transcripts in antisense injected and control embryos by the developmental Northern analysis shown in Figure 4. The blot was sequentially hybridized with probes specific for the family of FAX encoding transcripts and with a probe specific for GF 5–1. The analysis revealed that the maternal pool of these transcripts in H/C AS injected oocytes remains depleted throughout maturation, fertilization and early blastula stages. These stages precede the mid blastula transition (MBT), the developmental timepoint at which zygotic transcription commences. After MBT, GF 5–1 and FAX encoding transcripts reappear in experimental gastrula stages (Figure 4). Levels of EF1a and histone H4 transcripts are comparable in control and experimental oocytes/embryos.

These experiments clearly demonstrate that the H/C AS induced disruption of ZFP transcripts is maintained in the timespan between oocyte maturation and MBT.

Zinc finger protein transcripts are actively translated post-fertilization in cleavage stage embryos

One possible explanation for the indifference of embryos to H/C AS injection would be that ZFP transcripts are not translated between egg maturation and MBT, i.e. the period during which H/C AS has its destructive effect. In fact, mRNAs coding for ribosomal proteins are translationally repressed during this same period, having been actively translated in oogenesis (Pierandrei-Amaldi *et al.*, 1982; Hyman and Wormington, 1988). Destruction of such translationally silent mRNAs would most probably not be deleterious to early development.

We therefore tested if ZFP transcripts are translated in early embryological stages preceding MBT. Polysomal and non-polysomal RNA preparations from cleavage stage embryos were probed for zinc finger protein encoding transcripts (Figure 5). The fact that ZFP transcripts, detected either as a family of transcripts (FAX) or as an individual transcript (GF 66–1) are found in EDTA-releasable polysome fractions, shows that at least a subset of ZFP transcripts are actively translated in early embryos and this excludes the possibility that maternal zinc finger protein-encoding mRNAs are not utilized post-fertilization.

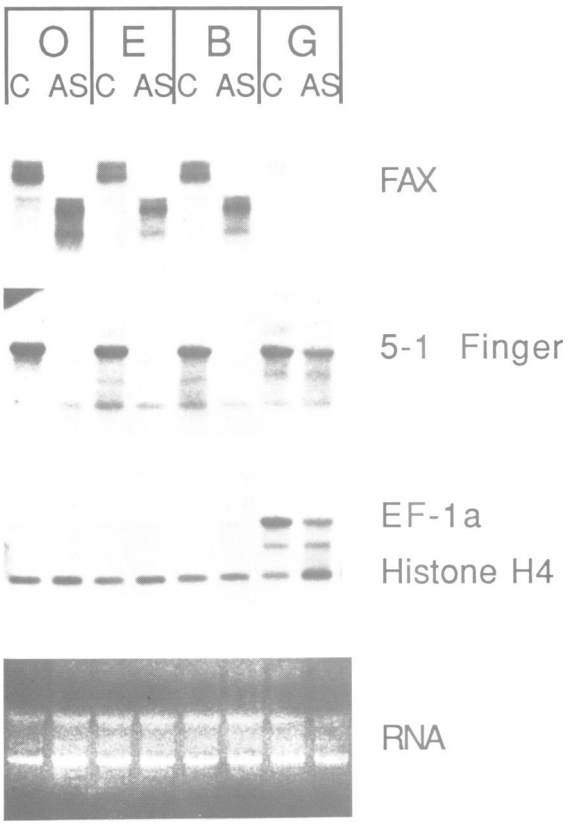

Fig. 4. Antisense oligonucleotide-mediated destruction of zinc finger protein mRNAs in *Xenopus* embryos. Injected and control stage VI oocytes were fertilized by means of the surrogate mother procedure (Holwill *et al.*, 1987). Total RNA from control (C) and experimental (H/C AS) oocytes and embryos was analysed for levels of ZFP transcripts using a subset of the probes described in (Figure 2). Each lane contains 3 oocyte or embryo equivalents of total RNA. Blots were also hybridized with histone H4 and EF1a. EF1a transcription is strongly activated at MBT, the developmental timepoint at which zygotic transcription starts. O: stage VI oocytes, E: unfertilized eggs, B: stage 6 blastula and G: stage 12 gastrula.

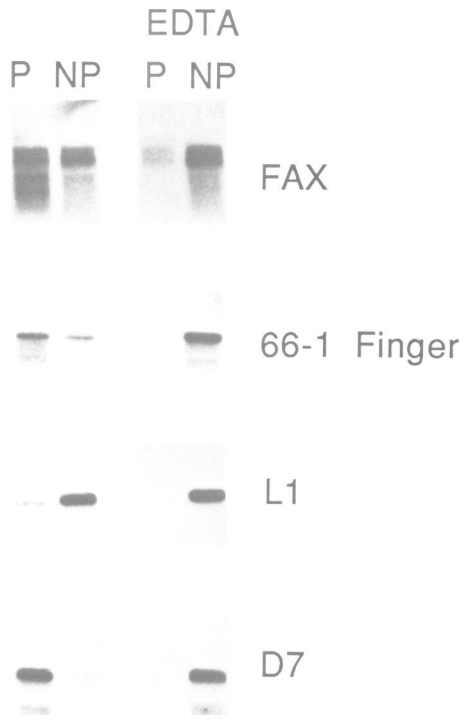

Fig. 5. Polysomal recruitment of ZFP transcripts in cleavage stage embryos. Northern blot analysis of total RNA from polysomal pellet (P) and non-polysomal supernatant (NP) fractions, isolated from cleavage stage embryos either in the presence or in the absence of EDTA. The blot was hybridized with the FAX probe and then with the finger probe specific for GF 66−1. Controls show the polysomal distributions of ribosomal protein L1 encoding mRNA, which is translationally repressed upon maturation (Pierandrei-Amaldi *et al.*, 1982; Hyman and Wormington, 1988) and of D7 mRNA, which is translationally activated upon maturation (Smith *et al.*, 1988).

Discussion

The rationale for isolating vertebrate genes with structural homology to conserved motifs in *Drosophila* developmental control genes is based on the assumption that these genes will exhibit some sort of functional relationship. While this assumption has been proven to be correct for at least some homeobox-encoding genes (Ruiz i Altaba and Melton, 1989; Wright *et al.*, 1989a; Kessel *et al.*, 1990) it still has not been substantiated for zinc finger protein encoding genes. Specific destruction of the entire pool of more than 100 different *Krüppel*-type zinc finger protein (ZFP)-encoding mRNAs has been achieved in this study by injecting an antisense oligonucleotide directed against the conserved H/C link consensus sequence, which is a reiterated motif in all of these RNA molecules. In embryos generated from such manipulated oocytes by use of the surrogate mother technique (Holwill *et al.*, 1987), disruption of ZFP transcripts is maintained up to the mid blastula transition (MBT). ZFP transcripts are actively translated in the earliest embryological stages preceding MBT. However, maturation, fertilization and early development of embryos generated from ZFP transcript depleted oocytes is indistinguishable from control oocytes/embryos.

The indifference of embryos to the decimation of the large number of maternal transcripts represented by the ZFP family is surprising. It certainly contrasts with the idea that maternal mRNAs are important in guiding early embryogenesis (Davidson, 1986). An indication for the relevance of maternal mRNA for embryogenesis is the fact that the bulk of the maternal mRNAs is translationally repressed in the egg, but many become activated upon fertilization and they are almost completely recruited into polysomes at MBT (Dworkin and Dworkin-Rastl, 1990).

The astonishing tolerance of *Xenopus* embryos to ZFP transcript destruction might be explained by one or more of the following possibilities: (i) The destruction of ZFP transcripts might be incomplete in a quantitative sense. Thus, trace amounts of ZFP transcripts in H/C AS treated embryos might still produce sufficient protein for normal function. Especially if vital ZFP functions were enzymatic, then minute amounts of these proteins might suffice for wild-type activity. Alternatively, strong regulatory feed-back loops might ensure continued synthesis of normal ZFP amounts even at substantially diminished levels of ZFP transcripts; (ii) The destruction of ZFP transcripts might be incomplete in a qualitative sense. We have shown that the majority of

ZFP transcripts which we have characterized, even those with H/C links which are only partially complementary to H/C AS, are destroyed by the oligonucleotide. However, not all of the C_2H_2 ZFPs contain an H/C link. In fact, *Krüppel* is the only C_2H_2 ZFP member of the gap class of segmentation genes which contains H/C links (Schuh *et al.*, 1986); the other, *hunchback* (Tautz *et al.*, 1987), does not contain this sequence element. It therefore remains possible that putative, non-H/C link *Xenopus* ZFP genes are expressed at normal levels in H/C AS treated oocytes/embryos; (iii) Maternal ZFP transcripts might not be translated between egg maturation and MBT, i.e. the period during which H/C AS has its destructive effect. In fact, mRNAs coding for ribosomal proteins are translationally repressed during this same period, having been actively translated during oogenesis (Pierandrei-Amaldi *et al.*, 1982; Hyman and Wormington, 1988). However, our finding that ZFP transcripts either as a family of mRNAs or as a single transcript are found in the polysomal fractions from embryos limits this possible explanation to only a subset of ZFP transcripts; (iv) Some maternal transcripts might be dispensable because they become retranscribed after MBT, and these zygotic counterparts are sufficient for normal function. However, it should be kept in mind that a large number of *Xenopus* ZFP transcripts is strictly maternal in early embryonic development (unpublished data); (v) Finally, and probably most importantly, maternal ZFP transcripts might be translated in the course of oogenesis, thereby producing a stockpile of maternal ZFPs, sufficient for normal function, even after transcript ablation in fully grown oocytes.

Thus, the present study certainly does not preclude the possibility that some maternally expressed ZFP genes in *Xenopus* are actually involved in developmental control. However, we feel that the sheer magnitude of the *Xenopus* ZFP gene family makes it unlikely that the majority of these genes are enrolled in developmental control. Moreover, even if some of these maternally expressed ZFP genes do control aspects of embryogenesis in *Xenopus*, then their mode of action is not likely to be related to that of *Krüppel* or to other DNA binding transcription factors acting as developmental regulators in *Drosophila*. Important common features of the function for the latter are critical dependence on restricted regional expression and on precise local concentration of the proteins (Pankratz *et al.*, 1990). Neither feature seems to be of paramount importance for ZFP function in *Xenopus*. We have found localized expression of ZFP transcripts at any stage of *Xenopus* development to be the exception (El-Baradi *et al.*, manuscript in preparation). Moreover, the fact that destruction of translationally active ZFP transcripts goes by unnoticed to the embryo is not consistent with a general dependence of ZFP function on concentration.

That zinc finger proteins might have other activities than regulation of transcription is suggested by our unpublished observations that at least one ZFP interacts sequence specifically *in vitro* with RNA but not with DNA. It is relevant to note in this respect that other C_2H_2 type zinc finger proteins, namely TFIIIA (Pelham and Brown, 1980) and p43 (Joho *et al.*, 1990), bind sequence specifically to 5S RNAs and that the former protein has a proven role in storage (Pelham and Brown, 1980) and nuclear export (Guddat *et al.*, 1990) of this molecule.

Thus, the results reported here, together with the findings on ZFP structure, expression and function in vertebrate and invertebrate systems discussed, clearly indicate that we have to distinguish between functionally distinct subfamilies of ZFPs. Therefore, a broad structural relatedness is certainly not an absolute criterion for a similar general relatedness in biological function. This does not of course imply that there are no true functional homologues in *Xenopus* of the zinc finger-encoding genes involved in the control of *Drosophila* development. Recently, a *Xenopus* homologue of *Drosophila snail* has been isolated (Sargent and Bennett, 1990) and its localized expression is promising in the context of a possible role in regulating aspects of *Xenopus* embryogenesis. If the two postulated major families of ZFPs, those with specific, spatially and temporally highly restricted and those with rather broad expression characteristics, are indeed functionally diverse, this may explain why the remarkable functional correlation existing between certain homeobox protein homologues in vertebrates and *Drosophila* has not yet been established for zinc finger proteins.

Materials and methods

Oligonucleotide preparation and oocyte injections

Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and purified by high performance liquid chromatography (HPLC), followed by polyacrylamide gel electrophoresis, gel elution, repeated ethanol precipitations and lyophilization. For microinjection, oligonucleotides were dissolved in distilled water.

Ovaries were surgically removed from unprimed *Xenopus laevis* females, and stage VI oocytes (staging according to Dumont, 1972) were manually defolliculated with a set of extra-fine forceps (Dumont no. 4). During all subsequent manipulations, defolliculated oocytes were kept in modified 50% Liebovitz L-15 medium containing 1 mM L-glutamine, 15 mM HEPES-NaOH and 0.4 mg/ml BSA, pH 7.8 (Wallace and Misulovin, 1978) except when explicitly mentioned otherwise. Oocytes were microinjected with 10–15 nl of H/C AS solutions ranging in concentration between 0.1 and 0.5 mg/ml. Similar amounts of a 20mer oligonucleotide with no known complementarity to any of the sequences listed in the EMBL database were injected as controls. Injected and uninjected oocytes were incubated for 1 h at room temperature and subsequently processed, either for Northern blot analysis or for fertilization by means of the surrogate mother procedure (see below).

RNA preparation and Northern blot analysis

For Northern blot analysis, total RNA was isolated from samples of 5–10 oocytes or samples of 3–5 staged embryos as described in Krieg *et al.* (1989). RNA was glyoxylated, fractionated on a 1% agarose gel, transferred to a nylon membrane (Genescreen) and hybridized with randomly primed, ^{32}P-labelled cDNA fragments, exactly as described in Köster *et al.* (1989).

Polysome analysis

Ten stage 2 embryos (staging according to Nieuwkoop and Faber, 1956) were homogenized in 1 ml polysome buffer (Richter and Smith, 1981) supplemented with 5 mM DTT and 100 U/ml RNasin. Insoluble cell debris was removed by low speed centrifugation at 12 000 *g* for 15 min at 4°C in an Eppendorf centrifuge. The clear supernatant was split into two equal fractions, one of which was adjusted to 50 mM EDTA. Both fractions were incubated on ice for 10 min before layering on 0.250 ml 20% sucrose cushions in a Beckmann TL 100.1 tube and subsequent centrifugation at 278 000 *g* for 5 min at 4°C. Total RNA from the pellet and supernatant fractions was analysed by Northern blot analysis as described above.

Surrogate mother procedure

The surrogate mother procedure was performed essentially as described (Holwill *et al.*, 1987). In brief, 100–200 each of injected and control oocytes were matured *in vitro* in the presence of 2 μg/ml progesterone (Sigma). Donor oocytes had previously been selected on the basis of perfect morphology and of the ability to mature *in vitro* within a period of 4 h. After white spot formation at the animal pole had occurred, matured oocytes were immediately removed from the progesterone solution, washed and kept in 50% Liebovitz L-15. Immediately prior to transfer into a single surrogate mother frog, injected and control matured oocytes were colour coded with

solutions of vital dyes (Sigma) in normal-strength modified Barth saline (1× MBSH) (Gurdon, 1977) for 30 min.

Candidate surrogate mother frogs had been injected with 1000 I.U. of human gonadotropic hormone (Sigma), usually 8 h prior to oocyte implantation. Implantation was only initiated after frogs started egg laying. The actual surrogate mother was selected if eggs proved to be efficiently fertilized *in vitro*. The egg laying surrogate mother frog was anaesthesized, laid on ice and 200–400 oocytes in 1× MBSH were implanted into the abdominal cavity with as little buffer as possible. Finally, within an interval of usually 5 h, the surrogate mother frog was regularly squeezed for endogenous and colour coded eggs, which were then immediately fertilized *in vitro*.

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