

A maternal factor, OZ-1, activates embryonic transcription of the *Xenopus laevis* GS17 gene

NICK OVSENEK, AARON M. ZORN and PAUL A. KRIEG

Centre for Developmental Biology, Department of Zoology, University of Texas at Austin, Austin TX 78712, USA

Summary

We describe the identification of an enhancer sequence and a sequence-specific DNA-binding protein required for developmental expression of the *Xenopus laevis* GS17 gene. Using microinjection of recombinant plasmids into fertilized frog eggs, we have shown that a 14 base pair CT-rich sequence element, normally located about 700 bases upstream of the GS17 promoter, is sufficient to activate transcription of a heterologous reporter gene in gastrula stage embryos. This regulatory element has been called the OZ sequence. Sequences closely related to OZ are located in the promoter regions of several other genes expressed during *Xenopus* development.

Extracts prepared from *Xenopus* embryos show the presence of a DNA-binding factor, OZ-1, that specifically recognizes the OZ sequence. Mutations within the OZ element that abolish OZ-1 binding also abolish enhancer activity. The OZ-1 factor contains at least two proteins of approximate M_r 76×10^3 and 100×10^3 . The sequence-specific binding activity accumulates during oogenesis and remains present at approximately constant levels throughout early development.

Key words: *Xenopus laevis*, maternal transcription factor, OZ-1, NCAM expression.

Introduction

Development of a multicellular organism requires the ordered expression of genes in the correct places at the correct times. At the earliest stages of development the molecules regulating transcription in the embryo must be of maternal origin. Later in development factors encoded by the embryonic genome contribute to gene regulation until eventually the embryo becomes completely independent of maternal regulatory factors. In the amphibian, *Xenopus laevis*, fertilization of the egg is followed by a period of about 6-7 hours during which cell divisions are rapid and synchronous. Effectively no zygotic transcription is detectable during the cleavage stage of development (Newport and Kirschner, 1982). This early pattern of development changes abruptly at the midblastula transition (MBT) when the embryo contains about 4000 cells. The MBT is characterized by an increase in the duration of the cell cycle, a loss of synchrony of divisions, a reduction in the rate of DNA synthesis, and a sudden activation of transcription from the embryonic genome (Gerhart, 1980; Newport and Kirschner, 1982).

Transcription initiation at the MBT is quite specific, with only a limited number of embryonic genes being expressed precisely at this time. Sequences transcribed at the MBT include structural RNAs (snRNAs, tRNAs and 5S RNA) and also selected messenger RNAs. These mRNAs may encode embryo-specific products such as DG42 (Sargent and Dawid, 1983) and GS17

(Krieg and Melton, 1985), or proteins essential for basic cellular functions, for example EF-1 α (Krieg et al., 1989). Expression of many other essential sequences however, such as those encoding ribosomal RNAs (Busby and Reeder, 1983), ribosomal proteins (Baum and Wormington, 1985) and cytoskeletal actins (Mohun et al., 1984), does not occur until later in embryogenesis.

It is probable that the first genes expressed at the MBT are regulated by factors present in the unfertilized egg. Transcription factors synthesized during oogenesis may be stored in the egg, either as proteins, or as mRNAs to be translated later during development. In an effort to identify maternal factors and to understand how they regulate embryonic gene transcription, we investigated regulation of the embryo-specific gene, GS17. Transcription of this gene starts precisely at the MBT and stops during late gastrula. Previous experiments have shown that a 74 base fragment of DNA located about 700 bases upstream of the promoter contains sequences essential for MBT expression of GS17. This fragment is sufficient to promote high level transcription of reporter gene constructions in microinjected frog embryos (Krieg and Melton, 1987a). In this paper we describe the identification of a 14 base sequence, the OZ element, that appears to be the core of the GS17 enhancer. When incorporated into reporter gene constructions, this element alone is sufficient to promote high levels of transcription in gastrula embryos. Extracts prepared from *Xenopus* eggs and

embryos contain a DNA-binding activity, OZ-1, which interacts specifically with the OZ sequence. Large amounts of OZ-1 are present in the egg and levels of the factor remain constant throughout embryonic development.

Materials and methods

Plasmid constructions

The plasmid 64X β G contains a 2.4 kb *EcoRI*-*HindIII* fragment of the *Xenopus* adult β -globin gene inserted into pSP64 (Krieg and Melton, 1987a). This fragment includes 477 bp of 5' flanking sequence, the entire coding region and a polyadenylation signal. To test for enhancer activity, putative enhancer sequences are inserted into the unique *EcoRI* site at -477. For the experiments presented in Fig. 2, a single copy of the double-stranded OZ oligonucleotide (5'-GTTTTGTG-TCCCCCTCTCAGGTGT-3') or single copies of two mutant OZ oligonucleotides, M1 (5'-GTTTTGTGTCCTCCGGTCT-CAGGTGT-3') or M2 (5'-GTTTTGTGTCCTCCCTAACAGGTGT-3') were inserted into the blunt-ended *EcoRI* site.

Microinjection of fertilized eggs

Xenopus laevis eggs were fertilized and injected as previously described (Krieg and Melton, 1985). Two-cell stage embryos in 20% Steinberg's solution/5% Ficoll were injected with 75 pg of supercoiled plasmid DNA dissolved in water. Following microinjection, the Ficoll concentration of the media was gradually lowered to 1.5% by gastrula stage, in order to accommodate normal development. Only normally developing embryos were used for RNA analysis. Embryos were staged according to Nieuwkoop and Faber (1967).

RNA and DNA analysis

Nucleic acids were extracted from pooled samples of at least 25 embryos in each experiment. Total RNA was isolated as described by Melton and Cortese (1979) and purified further by precipitation with 4 M LiCl. Initiation of β -globin mRNA transcription from injected constructions was assayed by 5' end mapping, using RNase protection (Krieg and Melton, 1987b). A 500 base, 32 P-labeled antisense probe was prepared from the plasmid, pX β Gprobe, which contains a fusion of *Xenopus* β -globin genomic and cDNA sequences. Transcripts originating at the β -globin cap site produce a 354 base protected fragment. Two embryo equivalents of total RNA were used for each protection assay. In all expression experiments, the presence of equal amounts of template DNA in different groups of injected embryos was confirmed by Southern blotting of total nucleic acids.

Preparation of whole-cell extracts and analysis of DNA-binding proteins

Whole-cell oocyte and embryo extracts were obtained essentially by the method of Mohun et al. (1989a; Method 3) using 10 μ l of Buffer II (Wu, 1984) per embryo. Homogenates were incubated on ice for 30 minutes and spun in a microcentrifuge at 4°C for 5 minutes at 14,000 rev/minute. Supernatants were dialyzed overnight at 4°C against buffer III (Wu, 1984), centrifuged as above, frozen on dry ice and stored at -80°C. The binding reactions for DNA mobility shift assays contained one embryo equivalent of whole-cell extract mixed with 0.1 ng of 32 P-labeled, double-stranded OZ oligonucleotide (see above), 200 ng poly (dI-dC) and 200 ng of *Bst*UI/*Hae*III-digested pUC19 DNA in 10 mM Tris (pH 7.8), 50 mM NaCl, 1 mM EDTA, 0.5 mM DTT and 5% glycerol.

Binding reactions were incubated for 20 minutes at 22°C and immediately loaded onto 5% non-denaturing polyacrylamide gels containing 6.7 mM Tris-HCl pH 7.5, 1 mM EDTA, 3.3 mM sodium acetate. Following electrophoresis, gels were exposed to X-ray film overnight at -80°C. Photo-affinity crosslinking of OZ-1 was performed using the method of Chodosh et al. (1986) with modifications as described by Ovsenek and Heikkila, (1990).

Results

Identification and characterization of the OZ element

We have identified a conserved sequence element that acts as a powerful enhancer in the frog embryo. We have named this approximately 14 base long element the OZ sequence. The OZ sequence was originally identified by comparing sequences within the *GS17* enhancer fragment (Krieg and Melton, 1987a) to the regulatory regions of other genes that are expressed during *Xenopus* early development. First, analysis of the promoter of a *Xenopus* homeobox gene, *Xhox4*, defined a regulatory region which included sequences similar to those within the *GS17* fragment (Peter Vize and Doug Melton, personal communication). We then surveyed other *Xenopus* genes and found the same conserved element (the OZ sequence) in the promoter region of the *Xenopus NCAM* gene (unpublished data). Fig. 1 shows the sequence of the OZ element within the relevant portions of the *NCAM* and *GS17* genes. The OZ sequence in the *Xenopus NCAM* promoter is identical to the *GS17* sequence in 13 out of 14 positions and is present in the same orientation with respect to the direction of transcription. The OZ sequence shows no evidence of dyad symmetry and does not appear to be related to previously described transcription regulating elements. The existence of this sequence in the promoter regions of several developmentally expressed genes suggests that it could play a role in regulation of gene expression in the frog embryo.

We have tested the transcription regulating activity of the OZ sequence, in vivo, by injecting reporter gene constructions into fertilized frog eggs. Plasmids were prepared in which one copy of the wild-type OZ oligonucleotide (plasmid WT) or one copy of mutant oligonucleotides (plasmids M1 and M2) was inserted into the *EcoRI* site of the reporter construction, p64X β G (see Fig. 2A). Plasmid 64X β G contains the entire coding region of a *Xenopus* adult β -globin gene



Fig. 1. Alignment of sequences in the 5' flanking regions of the *Xenopus GS17* and *NCAM* genes, to show the presence of a conserved sequence element. Identical residues are indicated by asterisks. The 14 bp region of the *GS17* gene that we have named the OZ element is indicated.

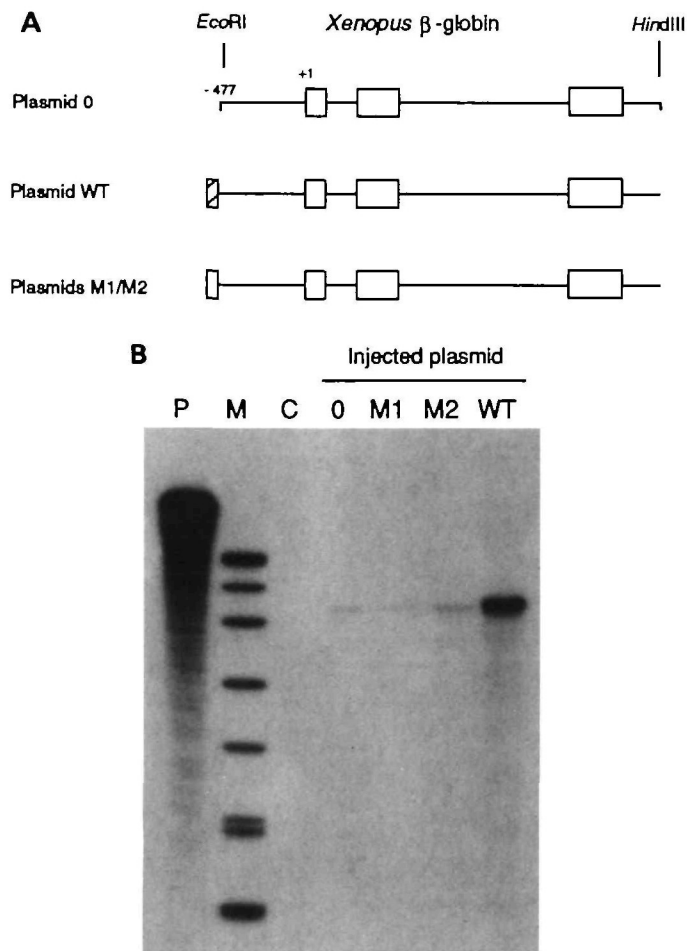


Fig. 2. Expression of enhancer test constructions injected into *Xenopus* embryos. (A) Diagrammatic representation of the reporter gene construction 64XβG (plasmid 0) and enhancer test constructions derived from it. The positions of the unique *EcoRI* (-477) and *HindIII* (approximately +1900) sites are indicated. Transcription is to the right and commences at the position shown as +1. Test plasmids contain one copy of either the wild-type OZ element (WT), or mutant OZ elements (M1, M2) inserted into the *EcoRI* site. (B) 5' end mapping analysis of β-globin mRNA transcription from injected plasmid constructions. RNA was isolated from injected embryos (and uninjected control embryos) at the gastrula stage of development and β-globin sequences detected by RNase protection analysis. Protected fragments were fractionated on a 5% acrylamide gel containing 8.3 M urea. Lanes are labeled to correspond to the test plasmids illustrated in Fig. 2A. Lane P contains undigested probe and lane C contains RNA from uninjected embryos. The size markers, M, are *MspI* fragments of pUC19. The protected fragment is 354 bases long.

and 477 bases of 5' flanking sequence, including the basal promoter elements (Krieg and Melton, 1987a). In the absence of additional enhancer sequences, only very low levels of β-globin transcription are observed when this plasmid is introduced into frog embryos. 75 pg of each plasmid DNA was microinjected into fertilized eggs and, after allowing the embryos to

develop to the gastrula stage, the amount of β-globin mRNA synthesized was measured by 5' end mapping. The use of 5' end analysis ensures that the assayed transcripts are derived from correct initiation at the β-globin promoter and are not the result of read-through transcription from cryptic promoters in the plasmid DNA. The results of the expression studies are presented in Fig. 2B. As expected, microinjection of p64XβG resulted in the transcription of very low levels of β-globin mRNA in gastrula stage embryos. Addition of the OZ element to the globin promoter resulted in a large increase (>10-fold) in globin transcription. In contrast, microinjection of the mutant constructions M1 and M2, resulted in no increase in transcription above background levels. Southern blot controls confirmed that equal amounts of template DNA were present in gastrula stage embryos injected with each of the different constructions (data not shown). Additional experiments indicate that β-globin transcription driven by the OZ sequence commences precisely at the MBT and that levels of transcription are independent of the orientation of the sequence element (data not shown). Overall, these results demonstrate that the OZ sequence is a strong transcriptional activator in the frog embryo and we conclude that it functions as the core region of the previously characterized *GS17* enhancer fragment (Krieg and Melton, 1987a).

Identification of a DNA-binding activity specific for the OZ sequence

DNA mobility shift assays (Fried and Crothers, 1981) were performed to detect the formation of a DNA-protein complex between the OZ sequence and protein(s) present in extracts prepared from frog embryos. Gastrula stage extracts were used in the initial experiments because the *GS17* gene is active at this stage of development. Fig. 3A shows the presence of a retarded complex formed between ³²P-labeled OZ element oligonucleotide and a factor present in the extracts. The specificity of this binding activity was tested by addition of increasing amounts of unlabeled OZ oligonucleotide competitor DNA to the binding reaction. As expected, this resulted in decreasing levels of the retarded band (Fig. 3A). No competition was observed after addition of a 200-fold excess of unlabeled mutant oligonucleotide. Furthermore, gel mobility shift analysis using radiolabeled mutant oligonucleotides did not reveal any detectable binding complex (data not shown). These results demonstrate that a factor with OZ element specific DNA binding activity is present in gastrula stage embryos. We have named this activity OZ-1. Together with the expression studies (Fig. 2B) these results strongly suggest that OZ-1 binding is required for transcriptional activation via the OZ sequence.

Additional competition experiments indicate that the OZ sequence in the NCAM promoter is also recognized by OZ-1. Fig. 3B shows that specific binding to the OZ oligonucleotide is competed by plasmid DNA containing the NCAM OZ element but not by plasmid vector sequences alone.

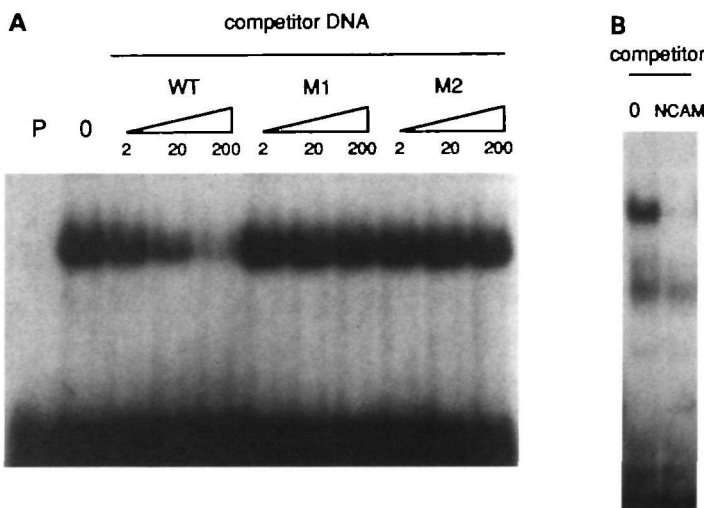


Fig. 3. A DNA-binding protein present in gastrula stage embryos interacts specifically with the OZ element. (A) ^{32}P -labeled OZ element oligonucleotide probe, (approximately 0.1 ng) was incubated with one embryo equivalent of crude whole-cell extract prepared from gastrula stage embryos and formation of a protein-DNA complex determined using the DNA mobility shift assay. Lane P contains probe alone and lane 0 contains extract in the absence of competitor oligonucleotides. The results show the effect of incubation with increasing amounts (2-200-fold excess) of either specific OZ oligonucleotide competitor (WT) or mutated oligonucleotide competitor DNA (M1 or M2). (B) The OZ element sequence in the *NCAM* promoter competes for factor binding. Probe, extracts and reaction conditions are identical to Fig. 3A. The lanes labeled 0 and NCAM show the results of a competition experiment in which the binding reaction contained either 200 ng of *AluI/Sau3A* digested Bluescript plasmid vector DNA (lane 0), or 200 ng of *AluI/Sau3A* digested Bluescript vector containing the *NCAM* promoter OZ sequence (lane NCAM). The *NCAM* DNA competes for binding of the OZ factor to the oligonucleotide probe.

OZ-1 binds as a heteromeric protein complex

Photo-affinity crosslinking was used to estimate the size of the protein(s) constituting the OZ-element binding activity. An OZ oligonucleotide probe was labeled with BrdU and ^{32}P and, following incubation with embryonic protein extracts, binding complexes were fractionated on a non-denaturing gel (see Methods). After UV irradiation of the gel the specific complex was excised and fractionated on an SDS-polyacrylamide gel. The results of the experiment are presented in Fig. 4. Autoradiography detected two labeled proteins of approximate M_r 76×10^3 and 100×10^3 . Precisely the same labeled bands were detected when crosslinking was performed with crude embryo extracts or with partially purified fractions obtained by heparin-agarose chromatography. Labeling could be eliminated by performing binding reactions in the presence of competing OZ-oligonucleotide, but not with oligonucleotides M1 or M2 (data not shown). These results suggest that the OZ-1 binding activity is composed of at least two proteins of $76 \times 10^3 M_r$ and $100 \times 10^3 M_r$. These

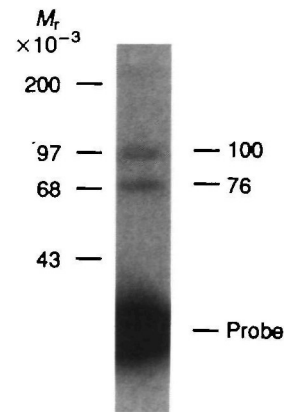


Fig. 4. UV photolabeling of proteins associated with the OZ oligonucleotide. Proteins in the OZ-1 binding complex were photo-crosslinked to BrdU/ ^{32}P -labeled OZ oligonucleotide as described in Methods. The labeled proteins were fractionated on a 10% SDS-polyacrylamide gel and visualized by autoradiography. Migration of ^{14}C -labeled protein markers ($M_r \times 10^{-3}$) is shown at the left of the figure. The positions of the labeled proteins and free probe are indicated to the right.

proteins may form a simple heterodimer or may be part of a more complex structure.

OZ-1 is a maternal factor

Since transcription of *GS17* commences precisely at the MBT (Krieg and Melton, 1985) the DNA-binding factors that regulate *GS17* expression must already be present, in active form, in the mid-blastula embryo. The amount of *GS17* mRNA peaks at about mid-gastrula and then declines to undetectable levels by the mid-neurula stage of development. Because of this transient expression profile, it is of interest to determine the developmental expression of the transcription factors that regulate *GS17* expression. We assayed for the presence of OZ-1 during *Xenopus* embryogenesis using OZ oligonucleotide probe and whole-cell extracts from egg through to tailbud stage embryos. The results of this DNA mobility shift experiment (Fig. 5) reveal that OZ binding activity is present in all developmental stages examined. Indeed, the amount of OZ-1 complex, and the apparent migration of the complex, remain approximately constant throughout early development. Significantly, OZ-1 is present in large quantities in the *Xenopus* egg, providing clear evidence that at least one of the factors regulating embryonic expression of the *GS17* gene is a maternal product. This factor is stored in the egg in a form capable of binding DNA.

The finding that OZ-1 is present in eggs led us to examine its appearance during oogenesis. Whole-cell extracts were prepared from batches of stage I/II, stage III/IV, and stage V/VI oocytes and used in a DNA mobility shift assay with OZ element oligonucleotide probe. The results are presented in Fig. 6A. The amount of OZ-1 detected in stage V and VI oocytes is similar to that present in the mature egg. Stage III and IV oocytes contain reduced binding activity and levels are low in stage I and II oocytes. The final lane of Fig.

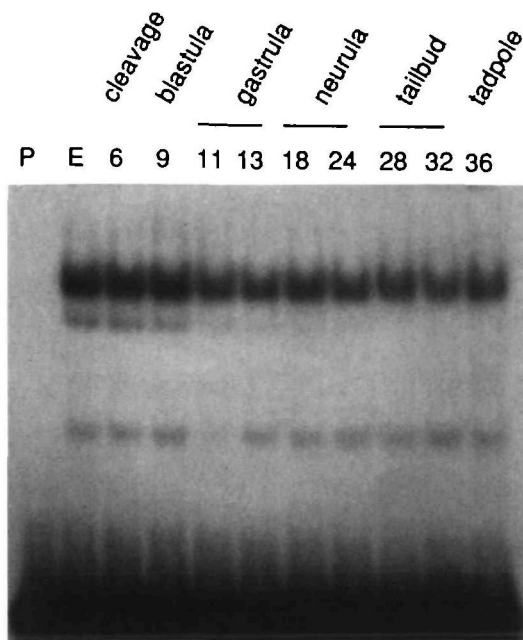


Fig. 5. Profile of OZ element binding activity during early *Xenopus* development detected by DNA mobility shift assay. Approximately 0.1 ng of ^{32}P -labeled OZ oligonucleotide was incubated with one embryo equivalent of whole-cell extract prepared from embryos at various stages of development. Lane P contains OZ probe in the absence of extract. The source of other samples is indicated by the *Xenopus* developmental stage (Nieuwkoop and Faber, 1967) at the top of each lane. Lane E is the result of an experiment using extracts prepared from unfertilized eggs. The additional faint bands present in the autoradiograph are non-specific complexes that are not competed by cold OZ oligonucleotides.

6A shows that extract from the equivalent of 10 stage I and II oocytes contains approximately the same amount of OZ-1 as one mature egg. These results show that synthesis of OZ-1 commences at the earliest stages of oogenesis. To determine the subcellular localization of OZ-1, stage 5 and 6 oocytes were manually enucleated and the isolated germinal vesicles and cytoplasmic fractions were assayed for OZ-specific binding. Fig. 6B shows that all detectable OZ binding activity in the oocyte is contained within the germinal vesicle.

Discussion

Our major objective in studying the regulation of genes expressed at the MBT is to understand how maternal factors control transcription in the early *Xenopus* embryo. Since no substantial transcription occurs during the cleavage stages of *Xenopus* development, activation of the first genes to be expressed in the embryo must be achieved by maternal factors. In order to identify such maternal transcription factors we have investigated the regulation of the *Xenopus* embryonic gene, *GS17*. In this report we demonstrate that the OZ element (a 14 base CT-rich sequence shared by a

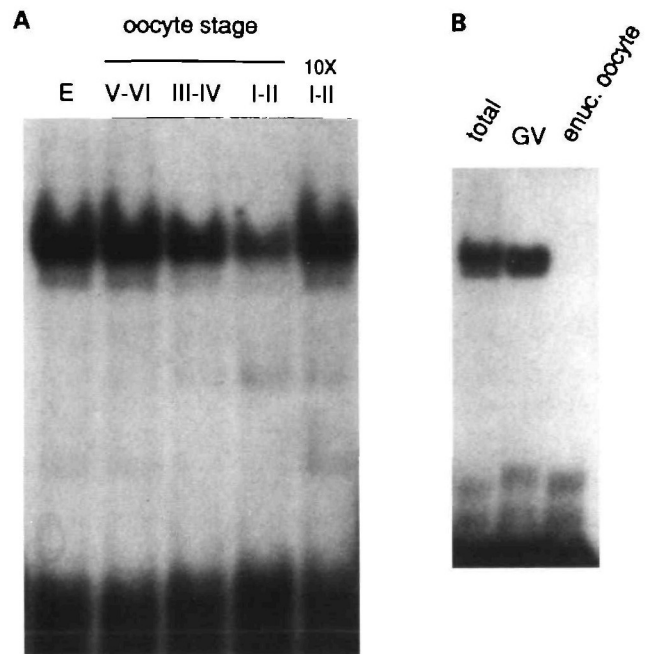


Fig. 6. DNA mobility shift analysis of OZ element binding activity during oogenesis. (A) Approximately 0.1 ng of ^{32}P -labeled OZ element oligonucleotide was incubated with one oocyte or egg equivalent of whole-cell extract. Extracts were prepared from pooled stage I and II, stage III and IV and stage V and VI oocytes as indicated. Oocyte stages were determined according to Dumont (1972). Lane E is the result of an experiment using extract from unfertilized eggs. The binding reaction assayed in the lane indicated as 10X I-II contained extract from the equivalent of 10 stage I and II oocytes. (B) OZ binding activity is located in the oocyte germinal vesicle. The subcellular location of OZ binding activity was determined by mobility shift analysis using extracts prepared from isolated germinal vesicles or from the remaining enucleated oocyte. The source of extract is indicated at the top of each lane.

number of *Xenopus* genes) is sufficient to activate transcription at the MBT in the *Xenopus* embryo. Thus the OZ element exhibits all the properties of the *GS17* enhancer. DNA mobility shift analysis showed that a binding activity specific for the OZ sequence, OZ-1, is present in protein extracts prepared from oocytes, eggs and embryos. Mutations within the OZ element that prevent OZ-1 binding also abolish enhancer activity. We conclude that OZ-1 is a transcriptional activator. OZ-1 accumulates in the oocyte and levels remain constant during early development.

Few enhancer sequences responsible for regulation of gene expression during *Xenopus* development have been identified. The OZ sequence appears to be a novel transcription element involved in the regulation of a number of *Xenopus* embryonic genes, including *GS17*, *NCAM* and *Xhox4*. In addition, OZ elements are present in the promoter regions of several mammalian genes and we have detected a binding activity equivalent to OZ-1 in extracts prepared from mammalian tissues (N. Ovsenek and A. Zorn, unpublished observations).

Our experiments show that OZ-1 is a maternal product. Maternal factors could be stored in the egg in several ways. First, they could be stored as mRNAs that are translated into active DNA-binding proteins during the cleavage stages of development. Second, they could be stored in the egg as proteins that require posttranslational modification before they acquire the ability, either to bind to DNA or to act as transcription activators. Third, they could be stored as fully functional transcription activating proteins. Our experiments allow us to exclude the first of these possibilities for the OZ factor. Fig. 5 shows that the OZ factor is present as a DNA binding protein in the mature frog egg and Fig. 6A shows that this DNA binding activity is present at least as early as stage II of oogenesis. We therefore conclude that the OZ factor is not stored in the egg solely as a mRNA. In independent studies, binding activities associated with the CArG promoter element of cardiac actin (Mohun et al., 1989a) and the heat shock transcription element of HSP70 (Ovsenek and Heikkila, 1990) have also been shown to be present in the egg. It is becoming clear that numerous proteins regulating embryonic transcription are stored in the egg in a DNA-binding form. The high levels of these factors in the egg suggest that they are stockpiled for use during development rather than for regulation of gene expression during oogenesis.

A fundamental problem of developmental biology is to understand how maternal factors, present throughout development, can precisely regulate genes with different temporal and spatial patterns of expression. For example, while OZ-1 is present at constant levels throughout embryogenesis, the *GS17* gene is only transcribed for a brief period following the MBT. Therefore, levels of OZ-1 in the embryo do not correlate with the transcription activity of the *GS17* gene. A similar situation is encountered when examining the regulation of *Xenopus* genes, such as cardiac actin, cytoskeletal actin and *c-fos*, that contain the CArG box promoter element. Serum Responsive Factor, the protein that specifically binds the CArG box, is present at constant levels during early *Xenopus* development, and yet the two actin genes and the *c-fos* gene all show very different spatial and temporal patterns of expression in the frog embryo (Mohun et al., 1989a,b; Taylor et al., 1989). Several theories may be advanced to explain how sequence specific DNA-binding factors such as OZ-1 can be present throughout embryogenesis while the genes they regulate are only expressed transiently in the embryo. First, OZ-1 may require a posttranslational modification to acquire its transcription activating function. If this is true, the pattern of expression of the responsive gene need not correspond to the presence of the DNA-binding activity. However, the fact that embryonic transcription of *NCAM* starts at the mid-gastrula stage and continues throughout the lifetime of the organism suggests that the OZ factor persists in the embryo, at least in neural tissues, in a transcription activating form. Second, other factors in addition to OZ-1 may be required to regulate the expression of genes containing the OZ sequence.

Such factors could be repressors or activators and they could either interact directly with OZ-1 or bind to other sites in the promoter. Our results, however, provide no evidence to suggest that other factors interact with the OZ sequence. Migration of the retarded complex remains unchanged through development (Fig. 5) implying that the composition of the complex was constant. Third, the activation of transcription in the embryo could be regulated by the subcellular localization of transcription factors. Several examples are known where transcription regulating proteins translocate from the cytoplasm to the nucleus during embryonic development. These include the *Xenopus* c-myc protein (Gusse et al., 1989), the *Xenopus* *xnf7* protein (Miller et al., 1991) and the *Drosophila* dorsal gene product (Rushlow et al., 1989; Steward, 1989; Roth et al., 1989; Ip et al., 1991). Since we have demonstrated that all detectable OZ-1 protein is located in the oocyte nucleus (see Fig. 6B) such a mechanism cannot explain the lack of *GS17* expression during oogenesis. While we consider it unlikely, changes in subcellular localization of OZ-1 could regulate transcription at later stages in development. Finally, gene expression in the embryo could be modulated by the relative affinity of OZ-1 for variants of the OZ sequence in the promoters of different genes. While the amount of OZ-1 per embryo remains approximately constant during development from the egg to the late tailbud stage, the amount of DNA in the embryo increases more than 100,000-fold (Gerhart, 1980). As the amount of OZ-1 per nucleus decreases at later stages of development, it is possible that only the highest affinity binding sites will be occupied. This situation could conceivably lead to the different developmental expression patterns of genes containing variant OZ sequences.

Clearly, further experiments will be required to determine the precise mechanism by which OZ-1 regulates transcription in the frog embryo. Towards this end we are purifying the OZ-1 factor, so that its structure and properties may be examined in detail.

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