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The maternal store of the xlgv7 mRNA in full-grown oocytes is not required for normal development in *Xenopus*

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Summary

We have attempted to analyze the function of a maternal mRNA xlgv7 which is distributed as an animal – vegetal gradient in stage 6 oocytes using a combination of antisense oligodeoxynucleotide injection into oocytes followed by *in vitro* maturation and fertilization. Injection of 20 ng of the antisense oligodeoxynucleotide resulted in the destruction of the xlgv7 mRNA to undetectable levels. Upon maturation and fertilization the resulting embryos develop with no specific defects suggesting that the maternal store of xlgv7 in stage 6 oocytes is not required and that the embryo can develop solely with the maternal store of the xlgv7 protein. Also, these results demonstrate the feasibility of this approach in destroying a specific maternal RNA and assaying its effect on development.

Key words: antisense oligo, maternal messenger RNA, destruction of mRNA, oocyte, *Xenopus laevis*.

Introduction

A central question in developmental biology concerns the role of maternal information in the differentiation of cell types during embryogenesis. The existence of regionally localized mRNAs for growth-factor-like molecules in *Xenopus* oocytes (Weeks and Melton, 1987; Kimelman and Kirschner, 1987) and the existence of numerous maternal effect mutations in vertebrates and in *Drosophila* (Driever and Nüsslein-Volhard, 1988) provide evidence for the involvement of molecules of maternal origin in regulating processes during embryogenesis.

Recently, we have reported the cloning of a cDNA encoding a maternal mRNA that exhibits a unique temporal and spatial distribution during development and in the adult tissues of the frog *Xenopus laevis* (Miller *et al.* 1989). This 2.4 kb mRNA is referred to as xlgv7 and is distributed in an animal-to-vegetal pole gradient in stage 6 (Dumont, 1972) oocytes (this paper). Following fertilization, the abundance of xlgv7 mRNA decreases through the late gastrula stage of development. The mRNA reaccumulates following the onset of neural induction. In the adult frog, the xlgv7 transcripts are enriched in the brain (Miller *et al.* 1989) and kidney (Cornish, Reddy, Etkin, unpublished observations). These data suggest that the xlgv7 gene product may play an important role in the development of several organ systems in *Xenopus*.

One possible approach to assess the function of the maternal xlgv7 mRNA during development would be the generation of embryos lacking, or containing a reduced amount of, this mRNA, even though there is a background of the maternal store of the xlgv7 protein (Reddy and Etkin, unpublished observations). Recently the introduction of antisense RNA and DNA into *Xenopus* oocytes and embryos has produced limited success in decreasing the expression of target genes (Harland and Weintraub, 1985; Wormington, 1986; Melton, 1985; Giebelhaus *et al.* 1988). An alternative strategy for creating minus mutant phenotypes employs the injection of antisense oligodeoxynucleotides into *Xenopus* oocytes (Kawasaki, 1985; Cazenave *et al.* 1986; Dash *et al.* 1987; Jessus *et al.* 1988; Shuttleworth and Colman, 1988; Smith *et al.* 1988). Antisense oligodeoxynucleotides hybridize to a specific transcript resulting in the degradation of the DNA–RNA duplex by RNase H cleavage of the target mRNA (Dash *et al.* 1987). This approach has been used to eliminate up to 96 % of the heat shock, histone H4, vegetally localized Vg1, and maternal D7 mRNAs in *Xenopus* oocytes (Shuttleworth and Colman, 1988; Smith *et al.* 1988). Smith *et al.* (1988) were able to show that destruction of a maternal RNA (D7) by injection of oligodeoxynucleotides affected progesterone-induced maturation. In this paper, we describe a novel combination of strategies to analyze the function of the maternal store of xlgv7 mRNA during development. This involves the

injection of antisense oligodeoxynucleotides into oocytes, followed by *in vitro* maturation and fertilization of the matured oocytes according to the method of Roberts and Gerhart (in preparation). We used this strategy instead of injection of oligodeoxynucleotides into fertilized eggs since injection of large amounts of oligodeoxynucleotides into fertilized eggs may be toxic (Etkin *et al.* 1984) and the xlgv7 maternal mRNA may be translated at maturation prior to its destruction. Surprisingly, we found that the destruction of the maternally stored xlgv7 mRNA does not affect the development through the larval stages. This result shows that the xlgv7 mRNA stored in full-grown oocytes is not required for normal development and that the embryo can develop with only the maternal store of the protein.

Materials and methods

Chemicals and media

Purified oligodeoxynucleotides were purchased from Genetic Designs Inc. L-Cysteine hydrochloride, Pepsin A from stomach mucosa, gentamicin sulfate and tetracyclin hydrochloride were all purchased from Sigma. Modified Ringer's (1×MR) is 100 mM-NaCl, 1.8 mM-KCl, 1.0 mM-$MgCl_2$, 2.0 mM-$CaCl_2$, 5.0 mM-sodium–Hepes pH to 6.5, 7.8 and 8.0 with HCl or NaOH. Modified Ringer pH 4.0 is 1×MR with 2.5 mM-sodium citrate as the buffer at pH 4.0 (Roberts and Gerhart, in preparation). ^{32}P-CTP and ^{32}P-γATP were from Amersham International.

Animals

Adult *Xenopus laevis* frogs were reared in charcoal-filtered water (Gurdon, 1967). Embryos were staged according to Nieuwkoop and Faber (1967).

Fertilization of in vitro *matured oocytes*

In vitro maturation and fertilization were done according to the method of Roberts and Gerhart (in preparation) with slight modifications.

Xenopus laevis females were injected with 50 i.u. of pregnant mare serum gonadotropin (Sigma) 15 h before use. Surgically removed oocytes were defolliculated manually in modified Barth's solution (MBS, Etkin and Maxson, 1980) and 5 nl of oligonucleotides or water, was injected into the oocyte cytoplasm as described by Etkin and Maxson (1980). After 2 h of healing at 18°C in MBS, oocytes were matured with 1 μM-progesterone (Sigma). At the first signs of germinal vesicle breakdown (usually 2 h after addition of progesterone), oocytes were washed four times in 50 % Liebowitz L-15 medium (Gibco) containing 1 mM-L-glutamine, 15 mM-Hepes–NaOH and 0.4 mg ml^{-1} BSA, pH 7.8 (Wallace and Misulovin, 1978), and cultured in the above medium for the next 5 h prior to fertilization.

Prior to fertilization, eggs were transferred for 45 s to modified Ringer's solution (1×MR) pH 4.0 and then treated for 5 s with pepsin (1.5 μg ml^{-1}) in 1×MR pH 4.0. After washing 3 times with 1×MR pH 7.8 eggs were transferred for 5 s to 1 % cysteine in MR/3 pH 8, washed in 1×MR pH 6.5, MR/3 pH 7.8 and subsequently placed in agarose wells containing MR/3 pH 7.8. Coelomic envelopes loosened by pepsin/cysteine treatment were removed manually under the dissecting microscope. Matured eggs lacking the coelomic envelopes were fertilized by overlaying with sperm suspension in jelly water in MR/3 pH 7.8. After 30 min the medium was changed to MR/10 pH 6.5 with antibiotics (50 μg ml^{-1} gentamycin and tetracycline) and dividing eggs and resulting embryos were cultured at 18 °C.

The oligonucleotides used in these experiments were designated, no. 1, which is the sense oligo; 01, the antisense complementary to nucleotides 39–55; and 2.2, which is complementary to nucleotides 1642–1658 of the xlgv7 mRNA (Miller *et al.* 1989).

RNA preparation and Northern blotting

Total RNA was prepared from injected and control oocytes, eggs and embryos by phenol extraction method (Etkin and Maxson, 1980). RNA was separated on formaldehyde 1 % agarose gels and blotted to Zeta probe blotting membranes (BioRad) according to Davis *et al.* (1986). Filters were hybridized overnight at 65 °C (Amasino, 1986) to 2×10^6 cts min^{-1} ml^{-1} of randomly primed ^{32}P probes (specific activity 10^9 cts min^{-1} μg^{-1}, synthesized from the *Eco*RI insert of xlgv7 clone (Miller *et al.* 1989) and from the *Pst*I/*Sac*I insert of *Xenopus* histone H4 clone (gift from Michael Perry) according to the method of Feinberg and Vogelstein (1984). RNA from different oocyte regions was a gift of Dr M. L. King.

Oligonucleotide hybridization

Northern blot filters containing oocyte poly(A^+) RNA, which was prepared as described in Davis *et al.* (1986), were hybridized to oligonucleotides 01, 1 and 2.2 end-labeled with T4 polynucleotide kinase (Pharamacia) to a specific activity of ~2000 Ci mmol^{-1} under the conditions described in Smith *et al.* (1988).

In situ *hybridization*

Xenopus ovaries were fixed in 100 % methanol. *In situ* hybridization was done according to Carrasco and Malacinski (1987). RNA probes complementary to coding (antisense) and noncoding (sense) strands were prepared from xlgv7 cDNA cloned into pGem 7Zf(+) using SP6 and T7 polymerase activity and ^{32}P-labeled UTP (Amersham International). The specific activity of probes was 1×10^9 cts min^{-1} μg^{-1}.

Results

Xlgv7 RNA is found in a gradient from animal to vegetal pole in oocytes

The distribution of the xlgv7 mRNA in oocytes was determined by Northern blot analysis of the RNA isolated from the animal, middle and vegetal regions of stage 6 oocytes and by *in situ* hybridization. Northern blots showed an enrichment of the xlgv7 mRNA in the RNA isolated from animal pole sections of stage 6 oocytes. There was a small amount of xlgv7 mRNA detected in the middle region, but little if any detected in equivalent amounts of RNA isolated from the vegetal third (Fig. 1). Rehybridization of the blots with a *Xenopus* histone H4 probe showed a shallower gradient of the H4 mRNA along the animal – vegetal axis (Fig. 1).

We also attempted to analyze the distribution of the xlgv7 transcripts by *in situ* hybridization to sections of different-staged oocytes (Fig. 2 A–D). Interestingly, we observed that the xlgv7 RNA was found distributed

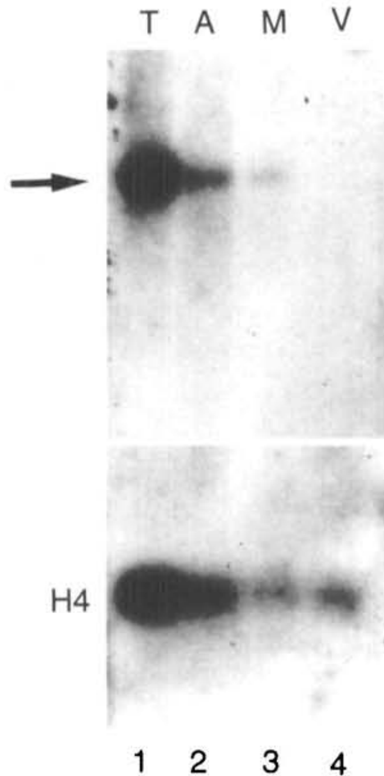

Fig. 1. Gradient of xlgv7 mRNA distribution in stage 6 oocytes. Poly(A)$^+$ RNA from total oocytes (lane 1), animal pole (lane 2), middle region (lane 3), and vegetal pole (lane 4) was separated on formaldehyde–agarose gels, blotted, and hybridized to random prime labeled xlgv7 cDNA and *Xenopus* histone H4 probe. The arrow points to the xlgv7 mRNA and the H4 represents the histone H4 transcript. Approximately 2 μg of RNA was loaded per lane except in lane 1 in which a larger quantity (approximately 4 μg) was loaded.

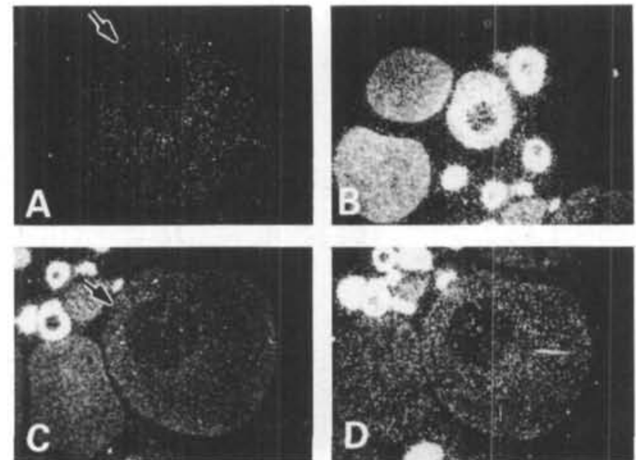

Fig. 2. *In situ* hybridization with xlgv7 probe. Sagittal sections of stage 6 oocytes hybridized to control (sense) xlgv7 transcript (A); B and C show stage 2, 3 and 6 oocytes hybridized with the antisense strand of xlgv7; D is a longer exposure of C. Arrowheads mark the position of animal pole in stage 6 oocytes. Magnification ×15 (darkfield).

evenly in stage 2 oocytes and showed a slight perinuclear accumulation in stage 6 oocytes (Fig. 2 B,C,D). It also appears that the hybridization signal is reduced in stage 6 oocytes (Fig. 2 B,C). This suggests that there was a decrease in the abundance of the xlgv7 mRNA during oogenesis. The decrease in abundance of xlgv7 mRNA during oogenesis is consistent with data from Northern blots of RNA of different-staged oocytes (data not shown). These data indicate that the xlgv7 mRNA is distributed in a gradient along the animal–vegetal axis in stage 6 oocytes with the highest levels at the animal hemisphere region in a perinuclear location.

Selective, oligodeoxynucleotide-mediated degradation of xlgv7 mRNA in oocytes

We were interested in assessing the function of the localized xlgv7 mRNA during development. Our approach was to attempt to destroy or at least decrease the abundance of this mRNA by selective degradation with injected oligodeoxynucleotides. The injected oocytes were matured and fertilized *in vitro*. We used two different antisense 17-mer oligonucleotides (referred to as 01 and 2.2) complementary to 5′ and 3′ region of xlgv7 mRNA, respectively, and one sense control 17-mer (1) that has no complementarity to this transcript were injected into stage 6 oocytes.

The effect of the injection of oligodeoxynucleotides on the xlgv7 mRNA was determined by Northern blot analysis of total mRNA extracted from oligodeoxynucleotide injected and control oocytes. Injection of antisense (01 and 2.2) oligodeoxynucleotides (50–60 ng per oocyte) resulted in the degradation of full-length xlgv7 mRNA within 2–3 h, usually to undetectable levels (Fig. 3A,B,C). A small residual amount and breakdown products of the mRNA were detectable on long exposures of the same autoradiograph (Fig. 3A,B). On the other hand, the injection of control noncomplementary oligodeoxynucleotides did not have any detectable effect on xlgv7 mRNA integrity (Fig. 3A,B). Injection of 10 ng of the antisense oligodeoxynucleotides resulted in a considerable amount of residual xlgv7 mRNA (Fig. 3C). In a total of 5 experiments in which RNA from 62 oocytes and fertilized eggs injected with between 20–60 ng of antisense oligodeoxynucleotides was analyzed by Northern blots, 46 (74 %) showed no detectable xlgv7 mRNA, while 16 (26 %) showed degradation products and in approximately half of this latter group a faint signal (1–4 % of control xlgv7 levels) co-migrating with the authentic xlgv7 mRNA. This suggested a very low level of residual mRNA remained intact in some of the oocytes. We have not detected any differences in the ability of either the 2.2 or 01 antisense oligodeoxynucleotide to destroy the xlgv7 mRNA in injected oocytes. In 10 different experiments in which we have analyzed RNA from pools of 5–10 oocytes injected with 20 ng of antisense oligodeoxynucleotide, we have observed 96–100 % destruction of the xlgv7 mRNA (see Fig. 5 below for example). In the developmental studies, we routinely used 20 ng of oligodeoxynucleotide/oocyte (see below).

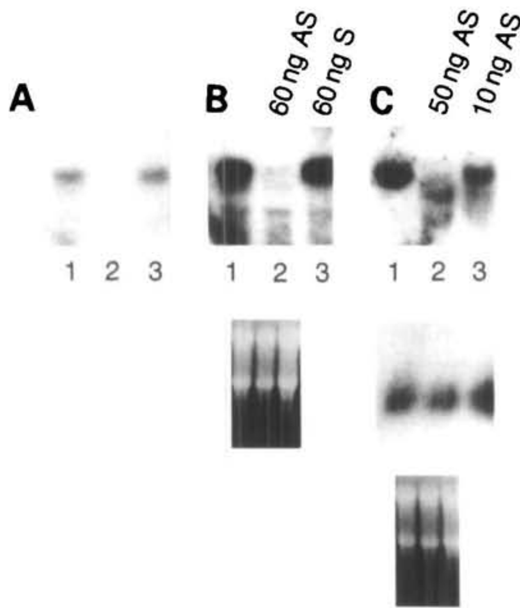

Fig. 3. Northern blots and ethidium bromide staining of total RNA (20 μg per lane) from stage 6 oocytes injected with different amounts of sense and antisense oligodeoxynucleotides. Panels A and B are different exposures of the same blot hybridized to random labeled xlgv7 probe. Lane 1, RNA from sham-injected control oocytes; Lane 2, RNA from oocytes injected with 60 ng of antisense no. 01; Lane 3, RNA from oocytes injected with sense no. 1 oligonucleotide. Oocytes were incubated for 2 h post-injection. Panel B also shows the ethidium bromide staining of the same gel before blotting. Panel C. Lane 1, RNA from control sham-injected oocytes; Lane 2, RNA from oocytes injected with 50 ng of antisense; Lane 3, RNA from oocytes injected with 10 ng of antisense oligodeoxynucleotides no. 2.2. Panel C also shows the ethidium bromide staining pattern of the gel before blotting and the same blot re-probed with *Xenopus* histone H4 random labeled probe. The 18s refers to the position of 18S rRNA on the gel.

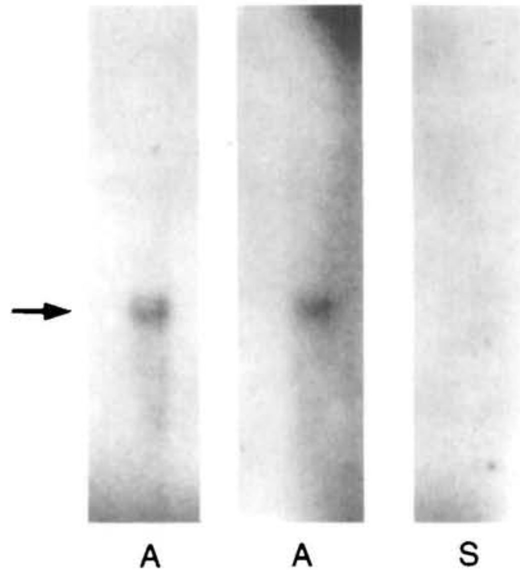

Fig. 4. Hybridization of oligodeoxynucleotides to Northern blot of poly $(A)^+$ RNA from noninjected stage 6 oocytes. Strips from two independent blots containing 20 μg and 5 μg of poly $(A)^+$ RNA probed with antisense oligonucleotide (01) showing specific hybridization to xlgv7 mRNA (arrow). (S) Under the same conditions control sense oligonucleotide (1) does not hybridize to oocyte poly $(A)^+$ RNA. Similar results were obtained with the 2.2 antisense probe.

The only case in which we have observed the non-specific degradation of xlgv7 mRNA by control sense oligodeoxynucleotides occurred following injection of unpurified oligodeoxynucleotides. Therefore routinely we have used sense and antisense oligodeoxynucleotides that were HPLC- and gel-purified for all experiments.

To assess the integrity of total mRNA from injected oocytes, we examined the 18S and 28S rRNA on the ethidium-bromide-stained gels prior to blotting and/or we rehybridized many of the Northern blots with random labeled probe prepared from *Xenopus* histone H4 cDNA. Fig. 3B,C shows that, even at the highest dose of injected oligodeoxynucleotides (60 ng/oocyte), the levels of rRNA and H4 mRNA in injected oocytes remain comparable to the noninjected controls.

To test the specificity of their reaction with the xlgv7 mRNA, all three oligodeoxynucleotides were hybridized to the filters containing poly(A)+ RNA extracted from noninjected oocytes. Both antisense oligodeoxynucleotides hybridize to a single band which co-migrates with authentic xlgv7 mRNA (Fig. 4 and data not shown). Under the same conditions, the control sense oligodexoynucleotide does not show any hybridization to poly(A)+ mRNA (Fig. 4). Although the conditions of hybridization of the oligodeoxynucleotides to mRNA *in vitro* (Northern blots) are certainly different from those existing *in vivo* when the oligodeoxynucleotides are injected into oocyte cytoplasm, the result suggests that there is high sequence-specificity of the interaction of these oligodeoxynucleotides with xlgv7 mRNA.

Development of the embryos depleted of xlvg7 mRNA

Following fertilization, *Xenopus* embryos do not synthesize the xlgv7 mRNA until the neurula stage of development (stage 15, Miller *et al.* 1989). Between fertilization and the neurula stage, the level of xlgv7 transcripts decreases 5- to 10-fold. The lack of new synthesis during early embryogenesis suggests that the early embryo relies solely on the maternal xlgv7 transcripts and/or the maternal store of this protein. Therefore, we wanted to determine the effect of the destruction of this localized messenger RNA on development. To analyze the functional importance of the maternal xlgv7 mRNA in *Xenopus* development, we have injected the antisense oligodeoxynucleotide 2.2 into the cytoplasm of defolliculated stage 6 oocytes. The oocytes were allowed to heal for 2 h, then were matured and fertilized *in vitro*. We observed the development of the embryos until gastrula and post-neurula stages.

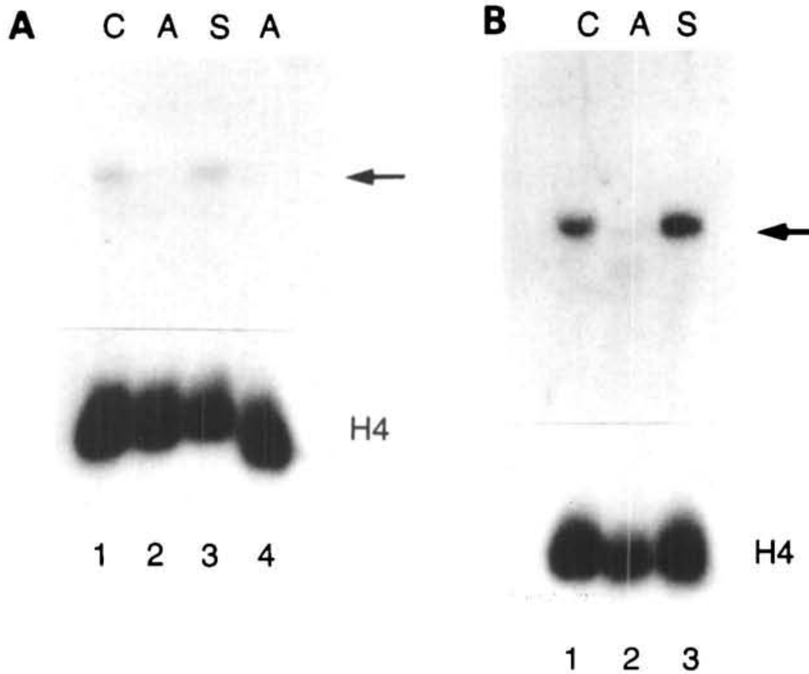

Fig. 5. A and B. Northern blot of total RNA (40 μg per lane) from matured oocytes (A), and 2-cell-stage embryos (B) originating from the oocytes injected with 20 ng of oligonucleotides. The blots were hybridized with random labeled xlgv7 and *Xenopus* histone H4 probes. Lanes A1 and B1, RNA from control oocytes that were sham injected; Lanes A2, 4 and B2, RNA from oocytes injected with antisense (2.2) oligonucleotide; Lanes A3 and B3, RNA from control oocytes injected with sense (no. 1) oligonucleotide. Arrow points the position of xlgv7 transcript.

In these experiments, we injected 20 ng of oligodeoxynucleotide per oocyte. We wanted to inject the smallest effective dose to keep the amount of oligodeoxynucleotides and free nucleotides to a minimum. Controls consisted of oocytes injected with the same amount of sense oligodeoxynucleotide and sham-injected oocytes. At different times following injection, oocytes, eggs or embryos at various stages of development were killed and assayed for the levels of the xlgv7 and histone H4 mRNAs by Northern blot analysis. The results show that RNA from matured oocytes and 2- to 4-cell-stage embryos injected with antisense oligodeoxynucleotides were deficient in the xlgv7 mRNA (Fig. 5A,B). Densitometry scans of these autoradiograms indicated that at least 96 % of the xlgv7 mRNA was destroyed in each sample that was injected with 20 ng of the antisense oligodeoxynucleotide. In contrast, this RNA species was always present in the RNA extracted from sham and sense oligodeoxynucleotide-injected controls (Fig. 5). Assaying these blots with a probe for *Xenopus* H4 showed no major differences in the levels of H4 mRNA further confirming that the destruction of the xlgv7 mRNA was specific (Fig. 5).

Table 1 shows the results of 8 independent experiments analyzing the development of sense, antisense and control embryos from injection into oocytes through early cleavage stages. Of the nearly two hundred embryos analyzed in each group, there were no significant differences in the development of both control and sense-injected embryos from fertilization through early cleavage. However, the antisense-injected embryos showed a small difference (56 % normal development) compared to the control and sense-injected embryos (78 % and 76 %, respectively). We do not feel that this difference is related to the loss of the xlgv7 mRNA since it is based on poor development of the antisense embryos from 3 of the experiments, while development was comparable or better than the controls in the other 5 experiments. Table 1 also compares the survival of oocytes following injection until fertilization. The losses during this period were due to abnormal maturation, cytolysis, mechanical damage due to handling, and samples taken for RNA analysis.

Table 2 shows the development from first cleavage through the gastrula stage of development. There does not appear to be any significant difference in the development of the two groups. The discrepancy in the numbers of embryos at the cleavage stages between Table 1 and 2 was because many embryos were used for RNA analysis at the cleavage stages. Most of the embryos from all three groups appear to mature, cleave and gastrulate normally (Fig. 6).

The data in Tables 1 and 2 include experiments that were terminated during cleavage stages to analyze the abundance of the xlgv7 mRNA. In several experiments, we allowed the embryos to continue development through the post-neurula stages resulting in 21 sense, 28 antisense, and 29 control post-neurula embryos. Table 3 shows the results of these individual experiments from injection of the DNA into oocytes through post-neurula development. It is clear that a significant number of antisense-injected embryos survive and develop from cleavage to post-neurula stages (experiment 5 shows 46 %; experiment 8 shows 78 %). Of the total of 28 post-neurula antisense-injected embryos, 68 % (17) were completely normal while 32 % (11) showed abnormalities such as spina bifida, retained yolk plug, and microcephaly. Similar numbers of these same abnormalities were detected in the sense-injected embryos (Table 3 and Fig. 6). We conclude that these abnormalities are nonspecific and due to the experimental procedure and are not related to the depletion of the xlgv7 transcript.

We were also concerned with the possibility that the surviving embryos may be a subset of embryos contain-

Table 1. *Fertilization and cleavage of injected oocytes*

Experiment	DNA injected	Number injected	Number fertilized	Cleavage
1				
	sense	30	30	23
	antisense	15	13	5
	control	10	10	7
2				
	sense	25	17	12
	antisense	25	17	13
	control	25	20	10
3				
	sense	25	20	20
	antisense	25	13	13
	control	25	18	18
4				
	sense	25	14	11
	antisense	25	19	4
	control	25	20	20
5				
	sense	38	32	21
	antisense	40	24	13
	control	35	25	15
6				
	sense	35	35	18
	antisense	49	49	32
	control	32	12	12
7				
	sense	25	22	17
	antisense	25	23	20
	control	25	23	20
8				
	sense	25	22	8
	antisense	55	37	18
	control	20	15	9

Summary DNA injected	Number injected	Number fertilized	%	Cleavage	%†
sense	228*	192	84	140	73
antisense	259*	195	75	110	56
control	197*	143	73	111	78

* Losses between injection and fertilization were due to abnormal maturation, death of oocytes, and samples taken from RNA analysis.
† Represents percentage of fertilized eggs that cleaved.

Table 2. *Development of injected cleaving embryos to gastrula stage*,†*

Treatment	Cleaved	Stage 7–10	%
sense	120	62	52
antisense	88	38	43
control	95	43	45

* based on a summary of 8 experiments in Table 1.
† Discrepancy between numbers of cleaving embryos in Table 1 and Table 2 are due to death of some embryos and the use of others for RNA analysis.

ing residual xlgv7 mRNA. We feel that this is unlikely since the analysis of xlgv 7 mRNA levels in siblings from the same group of embryos in experiment 5 (Tables 1 and 3) showed the loss of at least 96 % of the xlgv7 mRNA at cleavage stages (Fig. 5B lane 2). Table 3 shows that 46 % of these embryos developed to post-

Table 3. *Development of injected embryos to post-neurula stages**

Exp.	DNA	No. injected	No. fertilized	No. stage 2–10	No. post-neurula	%†
5§						
	S	38	32	21	5	23
	AS	40	24	13	6	46
	C	35	25	15	8	53
8						
	S	25	22	8	8	100
	AS	55	37	18	14	78
	C	20	15	9	7	78
9§						
	S	–	–	13	8	62
	AS	–	–	9	8	89
	C	–	–	16	14	88

* The data in this table are from two separate experiments from Table 1 (numbers 5 and 8) in which the embryos were allowed to develop to tailbud and larval stages and a third experiment which includes data from gastrulation to the larval stages.
† This represents the percentage of embryos developing from cleavage through tailbud (stages 25–27) or young larval stages.
‡ Normally developing tailbud and larvae consisted of 81 % for sense-injected 68 % for the antisense-injected, and 90 % for the controls. The defects consisted of spina bifida, microcephaly, and other non-specific defects.
§ RNA analysis was performed on siblings at cleavage stages. Embryos were either devoid of any detectable xlgv7 mRNA or contained trace amounts (no greater than 1–4 %).

neurula stages with no specific abnormal phenotype. Also in experiment 8 (Table 3) 78 % developed from cleavage to post-neurula stages, while in experiment 9 (Table 3) 89 % developed to post-neurula stages. Analysis of the xlgv7 RNA levels at cleavage stages showed less than a 1 % residual compared to sense-injected controls (data not shown). This strongly suggests that many of the embryos developing to post-neurula stages lack or contain greatly reduced amounts of the xlgv7 mRNA.

The xlgv7 mRNA is re-expressed during development in antisense-injected embryos

Resynthesis of the xlgv7 mRNA in *Xenopus* development begins around the time of neural induction and its accumulation reaches the highest level between stages 15 and 28 (Miller *et al.* 1989). It was of interest to determine if the embryos originating from oocytes injected with antisense oligodeoxynucleotide were able to resynthesize the xlgv7 transcripts to control levels found in noninjected embryos. Fig. 7 shows that the abundance of the mRNA hybridizing to xlgv7 cDNA probe in antisense-injected stage 25/27 embryos was similar to that in sham- and sense-oligodeoxy-nucleotide-injected controls.

Discussion

In the present study, we have attempted to analyze the function of a maternal mRNA, xlgv7. Our strategy was to inject antisense oligodeoxynucleotides into oocytes

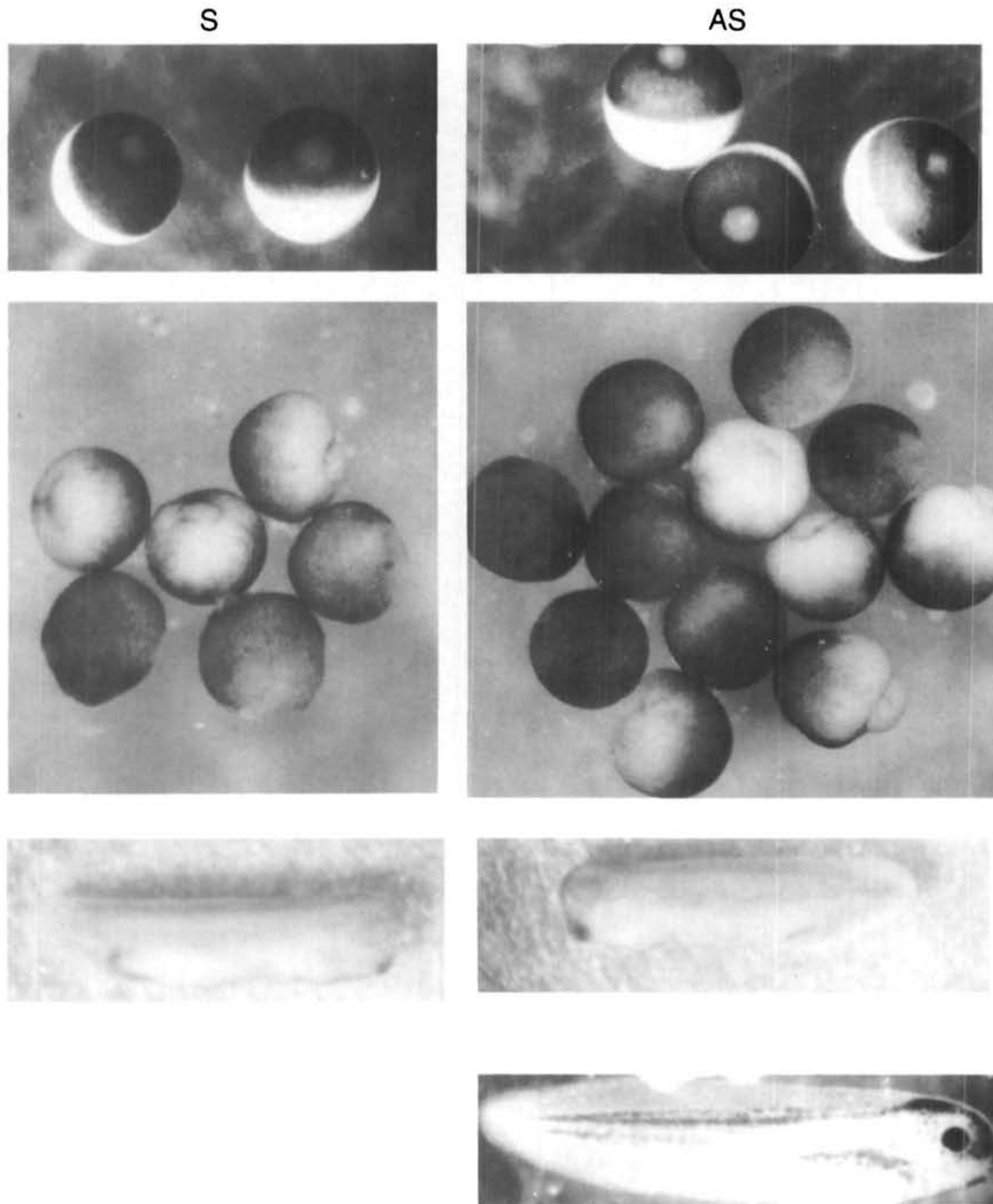

Fig. 6. Maturation and development of oocytes injected with sense (S) and antisense (AS) oligodeoxynucleotides.

followed by *in vitro* maturation and fertilization. We hoped to detect a specific phenotype during development in embryos that lacked or contained reduced amounts of the xlgv7 mRNA. We have shown that injection of 20 ng of the antisense oligodeoxynucleotide destroys at least 96 % of the xlgv7 mRNA in injected oocytes. When fertilized, these oocytes develop without any specific abnormalities.

The lack of any apparent phenotype was surprising since the xlgv7 mRNA is found in an A–V gradient suggesting a specialized function. We estimate that there are approximately 10^4–10^5 molecules of the xlgv7 mRNA/stage 6 oocyte based on comparison with the abundance of histone H4 mRNA. Also, we know that the xlgv7 gene is not re-expressed until the neurula stage (Miller *et al.* 1989). Western blot data using a polyclonal antibody produced against the bacterially expressed xlgv7 protein showed that the level of maternally stored protein remains relatively constant during early development in both sense- and antisense-injected embryos (Kloc and Etkin, unpublished observations). Therefore we interpret our results as demonstrating that the maternal store of the mRNA in stage 6 oocytes is not required for normal development

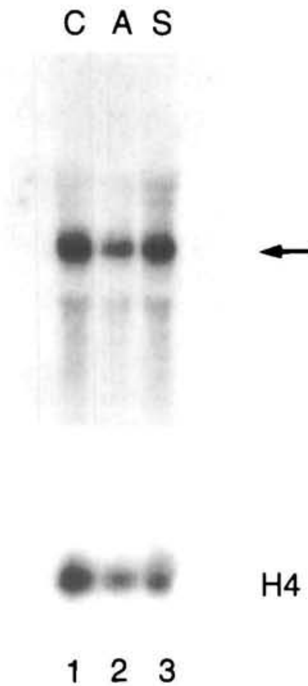

Fig. 7. Resynthesis of xlgv7 mRNA in embryos. Northern blot of total RNA (25 μg per lane) from stage 25 embryos originating from oocytes injected with oligodeoxynucleotides (20 ng per oocyte). The blot was probed with xlgv7 and reprobed with *Xenopus* histone H4 random-labeled probes. Lane 1, RNA from sham-injected embryos; Lane 2, RNA from oocytes injected with antisense DNA (no. 2.2); Lane 3, RNA from embryos injected with sense DNA (no. 1). Arrow marks the position of xlgv7 transcript.

through the neurula stage of development and that the embryo can survive on the maternal store of protein and/or the small amount of residual xlgv7 mRNA that may remain after antisense injection.

Another example in which depletion of a maternal mRNA did not affect development can be found in the sea urchin. Sea urchin histone mRNAs are sequestered in the GV in oocytes. Wells *et al.* (1986) produced merogones in which one half of the embryo contained the nucleus (thus the full complement of maternal histone mRNA) while the other half was anucleate and contained little if any histone mRNA. Fertilization of both halves resulted in two normally developing small embryos. The lack of maternal mRNA for the histone messengers had no obvious effect on early development. This situation is somewhat different than in *Xenopus* since in the sea urchin transcription begins immediately after fertilization while in *Xenopus* it does not begin until the mid-blastula stage. However, it does demonstrate the ability to develop of an embryo lacking the maternal store of a specific mRNA.

In *Dictyostelium* the expression of heavy chain myosin has been interfered with by insertional mutagenesis (De Lozanne and Spudich, 1987) and by transformation with antisense constructs (Knecht and Loomis, 1987). The phenotypes were relatively subtle such as effects on cytokinesis and aggregation behavior in some cells, otherwise, the myosin-deficient cells appear to develop and function normally. All of these results suggest that organisms may contain a redundancy of information required for survival and development and the depletion of a single component may not result in a specific phenotype.

It is also possible that the xlgv7 gene product functions during early oogenesis and/or at neurulation and the maternal product accumulated during oogenesis is not utilized. Regardless, it is clear that the mRNA component for xlgv7 in stage 6 oocytes is redundant and is not required for further development. It will be interesting to examine the situation for other maternally stored gene products.

While the depletion of the localized maternal store of the xlgv7 mRNA did not produce a distinct phenotype, the results have demonstrated the feasibility of using this experimental approach to deplete a specific maternal mRNA by injection of antisense oligodeoxynucleotides into oocytes and assessing its function by maturation and fertilization *in vitro*. Perhaps this strategy will be more successful in cases where the mRNA is not translated in oocytes or in conjunction with injections of antibodies to inactivate proteins. We feel that this approach will be generally applicable in the analysis of the function of maternal RNAs during embryogenesis.

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