J. Embryol. exp. Morph. **97 Supplement**, 211–221 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

Anti-sense RNA injections in fertilized eggs as a test for the function of localized mRNAs

D. A. MELTON AND M. R. REBAGLIATI
Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA

INTRODUCTION

In general, it is difficult to identify genes that play critical roles in developmental decisions in vertebrates because it is not possible to perform exhaustive screens for mutations that affect developmental processes. While some genes have been identified and analysed by conventional genetic methods, for example the mouse T locus (Bennett, 1975) or the axolotl *o* mutation (Brothers, 1976), it is likely that many genes that control vertebrate development will have to be identified by other means. In recent years, two methods for identifying such genes have been utilized. First, genes have been selected for study because they are expressed at a particular time or place during embryogenesis. For example, genes that are expressed only during gastrulation (Sargent & Dawid, 1983; Krieg & Melton, 1985) or at the cellular blastoderm stage (Roark, Mahoney, Graham & Lengyel, 1985) have been cloned and there are numerous examples of genes being studied because they are expressed in some, but not all tissues (e.g. Lynn *et al.* 1983) or are spatially localized within single cells (Jeffery, Tomlinson & Brodeurer, 1983; King & Barklis, 1985; Rebagliati, Weeks, Harvey & Melton, 1985). Second, genes that are potentially important for development have been cloned by virtue of their homology to *Drosophila* segmentation and homeotic genes. Several homeobox-containing genes have been isolated from frogs (Carrasco, McGinnis, Gehring & DeRobertis, 1984; Harvey, Tabin & Melton, 1986), mice and humans (reviewed by Gehring, 1985) and there is considerable interest in the possibility that these genes have a function in vertebrates homologous to their known functions in *Drosophila*. In all these cases it is necessary to have some test for the function of the cloned genes. We have explored the possibility of studying gene function by preventing the expression of the gene product *in vivo* by injecting anti-sense RNA. This report summarizes our recent efforts to prevent mRNA translation *in vivo* by injecting RNA that is complementary to messenger RNA, so called anti-sense RNA, into developing frog embryos.

We are interested in using anti-sense RNAs to study the function of localized maternal mRNAs in *Xenopus*. cDNA clones that correspond to maternal mRNAs

Key words: anti-sense RNA, frog egg, oocyte, microinjection, mRNA.

that are localized to either the animal (An1, 2 and 3) or vegetal (Vg1) pole of unfertilized eggs have been cloned and characterized (Rebagliati *et al.* 1985). Since the animal and vegetal poles of the egg give rise to the ectoderm and endoderm, respectively, it is possible that these RNAs play a role in specifying cell fates in different germ layers. Our studies have shown that as a consequence of their initial localization the An and Vg RNAs are distributed to different blastomeres during cleavage. Moreover, the An and Vg RNAs are found on polysomes during the cleavage period (Weeks, Rebagliati, Harvey & Melton, 1985). At present, we do not have any direct information about the distribution of the proteins translated from these localized mRNAs.

During the first 6–8 h of frog development, from fertilization until the mid-blastula stage, the cleaving embryo is transcriptionally inactive (Brown & Littna, 1964; Newport & Kirschner, 1982). Therefore it is not possible to provide anti-sense RNAs by injecting a DNA construction that directs transcription of an anti-sense RNA. To prevent translation of the An or Vg mRNAs with anti-sense RNAs it is necessary first to synthesize the anti-sense RNA *in vitro* and then directly inject these RNAs into the cytoplasm. The *in vitro* synthesis of anti-sense RNAs can be done simply and efficiently using SP6 vectors and SP6 RNA polymerase (Melton *et al.* 1984; Krieg & Melton, 1984).

The use of anti-sense RNAs to inhibit gene function *in vivo* has been pursued in several systems, most of which make use of DNA vectors that direct anti-sense RNA synthesis *in vivo* (Coleman, Green & Inouye, 1984; Izant & Weintraub, 1984; Kim & Wold, 1985; Petska *et al.* 1985; Crowley, Mellen, Gomer & Firtel, 1985). The direct injection of *in vitro* synthesized anti-sense RNAs has been reported in two systems: frog oocytes (Melton, 1985; Harland & Weintraub, 1985) and *Drosophila* embryos (Rosenberg *et al.* 1985). Rosenberg *et al.* (1985) were able to phenocopy the Krüppel mutant phenotype by injection of anti-sense RNAs complementary to Krüppel mRNA. In this case the RNA was injected into embryos at the syncytial blastoderm stage and a region of the developing embryo displayed an intermediate Krüppel phenotype. Experiments in frog oocytes showed that translation of injected globin mRNA could be specifically prevented by the injection of anti-sense globin RNA (Melton, 1985). RNAse digestion experiments showed that the block to translation was a result of RNA:RNA duplex formation *in vivo*. Moreover, this study showed that only a portion of the globin mRNA has to be hybridized in order to effect a block. As little as 50 bases of anti-sense RNA complementary to the 5′ end of the globin mRNA prevented globin protein synthesis in oocytes. Similar results were obtained by Harland & Weintraub (1985) in which they were able to prevent translation of thymidine kinase and chloramphenicol acetyl transferase (CAT) mRNAs in oocytes.

As a prerequisite to using anti-sense techniques to study the developmental function of maternal messages, like the An and Vg mRNAs, we have compared the properties of anti-sense RNAs in developing eggs as opposed to oocytes. Two issues are considered in this report: (1) the stability of anti-sense RNAs injected

into fertilized eggs and (2) the formation of RNA:RNA hybrids *in vivo* between injected anti-sense RNA and endogenous or injected mRNAs.

STABILITY OF ANTI-SENSE RNAS INJECTED INTO DEVELOPING EGGS

Previous studies have shown that mRNAs isolated from intact cells are unusually stable when injected into oocytes or fertilized eggs. Indeed, the half-life of globin mRNA injected into oocytes was estimated to be more than several days (Gurdon, Lingrel & Marbaix, 1973). Injection studies with synthetic RNAs, prepared *in vitro* by transcription with SP6 RNA polymerase, have shown that a 5′ cap is essential for RNA stability in oocytes. Uncapped RNAs are rapidly degraded (Green, Maniatis & Melton, 1983; Krieg & Melton, 1985). Drummond, Armstrong & Colman (1985) have shown that a poly A tail can increase the stability of synthetic RNAs in injected oocytes. In all these studies, the injected RNAs were sense RNAs, i.e. protein-coding RNAs that could be translated.

Our experiments show, as expected from studies with injected oocytes, that a 5′ cap is required for anti-sense RNA stability when the synthetic RNAs are injected into fertilized eggs. An anti-sense RNA complementary to ~680 bases of the An1 mRNA was synthesized *in vitro* with SP6 RNA polymerase, injected into fertilized eggs at the time of first cleavage and extracted at various times thereafter. Uncapped RNA is quickly degraded and we find no trace of the injected RNA after 1 h (Fig. 1). In contrast, significant amounts of capped RNA persist for at least 8 h. In neither case does the injection of the RNA have any noticeable effect on development. The injected embryos continue to divide and form normal blastula, gastrula and neurula. In these experiments the 5′ caps were added by synthesizing the RNA in the presence of a molar excess of diguanosine triphosphate. We find no significant difference between unmethylated (GpppG), monomethyl (m^7GpppG) or dimethyl (m^7Gpppm^{7}G) caps in terms of their effect on RNA stability.

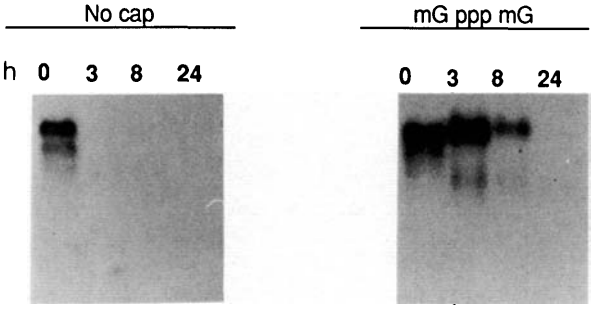

Fig. 1. A 5′ cap is required for the stability of anti-sense RNAs in developing embryos. Uncapped or capped 680 base anti-sense An1 RNA was synthesized *in vitro* with SP6 polymerase in the presence of ^{32}P-α-UTP to label the transcripts (Melton *et al.* 1984). RNAs were capped at the 5′ end by including m^7Gpppm^{7}G in the transcription reaction. Uncapped and capped anti-sense An1 RNAs were injected into eggs during first cleavage, extracted 0, 3, 8 or 24 h later and fractionated on denaturing agarose gels.

In these experiments about 4 ng of ^{32}P-labelled anti-sense RNA at a specific activity of about 3×10^7 cts min^{-1} μg^{-1} was injected into each fertilized egg. The injected anti-sense RNA is about 680 bases long and does not contain a poly A tail. We have not carefully measured the half-life of the injected RNA, but our results show that much of it (about 50 %) remains intact until the midblastula stage (about 8 h after fertilization). However, we find that nearly all (>90 %) of the injected RNA is degraded by 24 h or the neurula stage. To test whether it is possible to further stabilize the injected anti-sense RNAs so that they would persist through neurulation and later in development, we modified the 3′ end of the injected RNA in several ways.

In the light of previous studies (for example, Drummond *et al.* 1985) which suggested that a poly A tail can effect mRNA stability we have added a poly A tail to the synthetic RNAs. In the experiment shown in Fig. 2, Vg1 RNA was synthesized *in vitro* and extended at its 3′ end with poly A polymerase (Sippel, 1973). The number of adenosine nucleosides added to the 3′ end was estimated by gel electrophoresis to be 100–300 A residues. Fig. 2 shows that this long poly A tail has no significant effect on the stability of injected Vg1 RNA. While much of the injected RNA is present 8 h after injection, none is detectable at 24 h.

We reasoned that an artificial 3′ end may increase the stability of injected anti-sense RNAs. We therefore modified our SP6 transcription vectors so that tracts of C, G or A-C tails could be synthesized at the 3′ end of the anti-sense An1 RNA. The vector modifications were done by adding dG, for example, with deoxynucleotide terminal transferase (Roychoudhury & Wu, 1980). In this way we were able to prepare anti-sense An1 RNAs with various 3′ tails and test their stability in injected embryos. The results presented in Fig. 3 show that none of these artificial

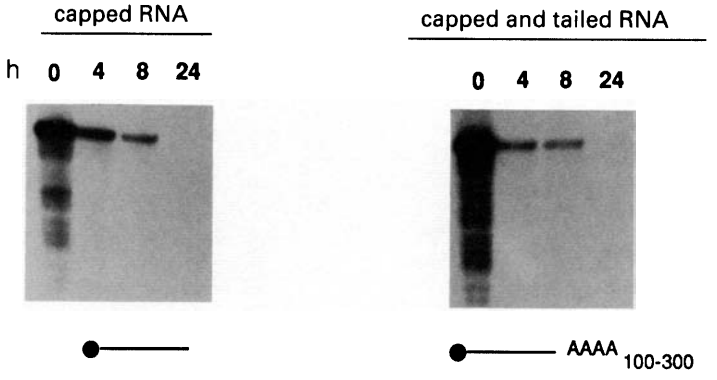

Fig. 2. A 3′ poly A tail does not significantly increase the stability of synthetic RNAs in injected eggs. m^7GpppG capped Vg1 RNA was synthesized and tailed with poly A polymerase. Tailed or untailed Vg1 RNA was injected during first cleavage, extracted at 0, 4, 8 or 24 h and analysed by RNAse mapping with a ^{32}P-RNA probe complementary to 300 bases of Vg1 RNA sequence immediately upstream of the poly A tail. RNAse digestion products were fractionated on 4 % polyacrylamide urea gels. Black dot, m^7GpppG cap; black line, 2·3 kb of Vg1 RNA containing protein coding sequence plus 3′ untranslated trailer. $AAAA_{100-300}$ represents the 100–300 A residues added by poly A polymerase.

3′ ends significantly increases the stability of the RNA *in vivo* as compared to untailed anti-sense An1 RNA.

There is much we do not know about the mechanism by which the injected anti-sense RNAs are degraded. The RNA may be digested by an exonuclease, an endonuclease or both. We do not know whether the injected anti-sense RNA is complexed with cellular proteins or interacts with ribosomes. It is possible that sense RNAs (mRNAs) are more stable in injected embryos because they are translated and protected from degradation by ribosomes. Presumably the anti-sense RNAs are not translated because they have no ribosome-binding sites and/or long open reading frames.

We conclude that synthetic anti-sense An1 RNA is not stable after about 8 h of development. It is possible that other RNAs will show different stability profiles, particularly synthetic messenger RNAs, and this issue is presently under investigation. Nonetheless, we note that enough of the injected anti-sense An1 RNA persists to allow us to assay for RNA hybrid formation with the endogenous An1 mRNA. Indeed, studies in injected oocytes have shown that RNA hybrids between sense and anti-sense globin RNAs form within 5 h *in vivo*. Moreover, the endogenous An1 mRNA is normally degraded by midgastrulation, about 10 h after fertilization (Rebagliati *et al.* 1985). It is therefore possible that the An1 protein serves its function early in development and that preventing translation of the An1 mRNA, by injected anti-sense RNA, could show an altered phenotype.

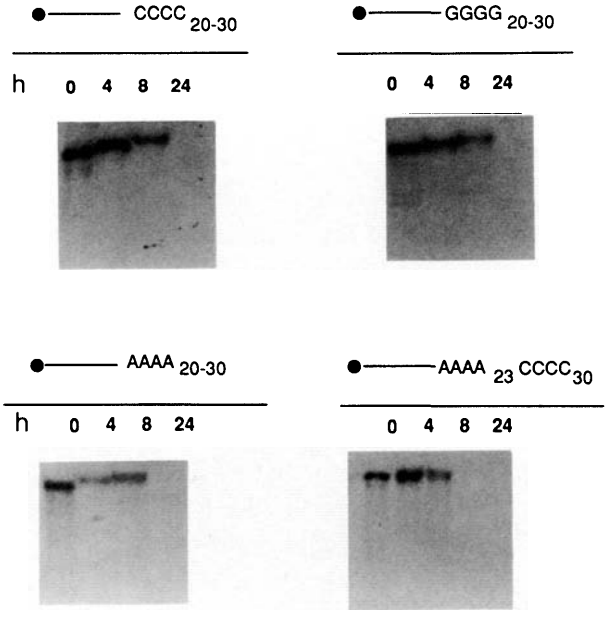

Fig. 3. Artificial 3′ tails do not increase the stability of anti-sense RNAs in embryos. Anti-sense An1 RNAs with homopolymeric tracts or A-C tracts were synthesized *in vitro* and the RNAs were injected and analysed on 4 % polyacrylamide urea gels. Black dot, m^7GpppG cap; black line, anti-sense An1 RNA of 680 bases. Sequences of the tails and estimates of their lengths are given above each gel.

DEVELOPMENTAL EFFECTS OF ANTI-SENSE RNA INJECTIONS INTO FERTILIZED EGGS

Our initial experiments involved injecting anti-sense An1 RNA and anti-sense globin RNA as a control. A 200-fold excess of anti-sense An1 to sense An1 RNA was injected (10 ng *versus* 50 pg per egg) and the embryos were allowed to develop. In some experiments as many as 80 % of the injected embryos failed to gastrulate normally. Moreover, some of the injected embryos exhibited other defects, for example the failure to form normal heads, eyes or posterior structures. However, all of these altered phenotypes were also observed in the controls, though at a lower frequency. Uninjected embryos and those injected with anti-sense globin RNA gave rise to the same defects we observed in embryos injected with anti-sense An1 RNA. Moreover, injection of higher concentrations of anti-sense globin RNA ($\sim$50 ng embryo^{-1}) increases the frequency with which these defects appear. These results suggest that we are not observing any specific defects due to the injection of anti-sense An1 RNA and that apparently specific developmental defects, such as the absence of an eye, can be produced by nonspecific, perhaps toxic, effects.

We have extended these studies by examining the fate of the injected anti-sense An1 RNA with respect to hybrid formation *in vivo*. Our previous studies had shown that anti-sense globin RNA and sense globin RNA form an RNA:RNA duplex *in vivo* that is resistant to digestion with RNAses. In those experiments both the sense and the anti-sense RNAs were injected into oocytes. We have now applied this same RNAse test for RNA hybrid formation using anti-sense An1 RNA and have investigated both oocytes and developing embryos.

RNA:RNA hybrid formation in injected fertilized eggs

^{32}P-labelled anti-sense An1 RNA (about 5 ng) was injected into embryos and 4 h later total RNA was extracted and digested with RNAse. If injected anti-sense RNA forms a hybrid with endogenous An1 mRNA then a protected duplex would be observed. The results in Fig. 4 show that we find no evidence for an RNA:RNA hybrid between injected anti-sense An1 RNA and endogenous sense An1 mRNA. The autoradiograms in Fig. 4 are purposely overexposed to show the complete absence of any duplex in both injected oocytes and developing eggs. Note that a vast excess of anti-sense RNA was injected in these experiments, an amount that should have been sufficient to drive the hybridization reaction. These results were obtained irrespective of the site at which the RNA was injected, though in most cases the anti-sense RNA was deposited in the animal pole.

As noted above, our previous work had demonstrated that hybrids form between injected globin mRNA and injected anti-sense globin RNA in oocytes (Melton, 1985). We therefore tested whether An1 RNAs, sense and anti-sense, could form hybrids when both RNAs are injected. The results presented in Fig. 5 show that RNA hybrids do indeed form in injected oocytes. This is entirely consistent with the previous studies using globin RNAs. However, in eggs, the

injected anti-sense An1 and injected An1 sense RNAs do not form a stable RNA:RNA duplex. This result shows a marked difference between the oocytes and developing eggs.

The results presented in Figs 4 and 5 show that stable RNA hybrids between injected anti-sense RNAs and injected or endogenous RNAs do not form in developing eggs. This may be because the RNA hybrids never form or because the hybrids are rapidly degraded. If the latter case were true, then it is possible that the endogenous An1 mRNA is being degraded following injection of a large excess of anti-sense RNA. To test this latter possibility, a large excess of anti-sense An1 RNA was injected into fertilized eggs. Immediately thereafter or 4 h later, the stability of the unlabelled endogenous An1 mRNA was assayed by Northern blots. The control was an injection of an equal amount of synthetic globin RNA. The results (Fig. 6) show that the injection of anti-sense An1 RNA has no significant effect on the levels of the endogenous An1 mRNA.

The experiments reported here are not sufficiently accurate to rule out the formation of a low level of RNA hybrids between injected and endogenous An1 RNAs, but it is clear that the majority of the endogenous An1 mRNA does not form a hybrid with the injected anti-sense RNA. It is therefore expected that assays for An1 protein synthesis would show no effect from the injection of

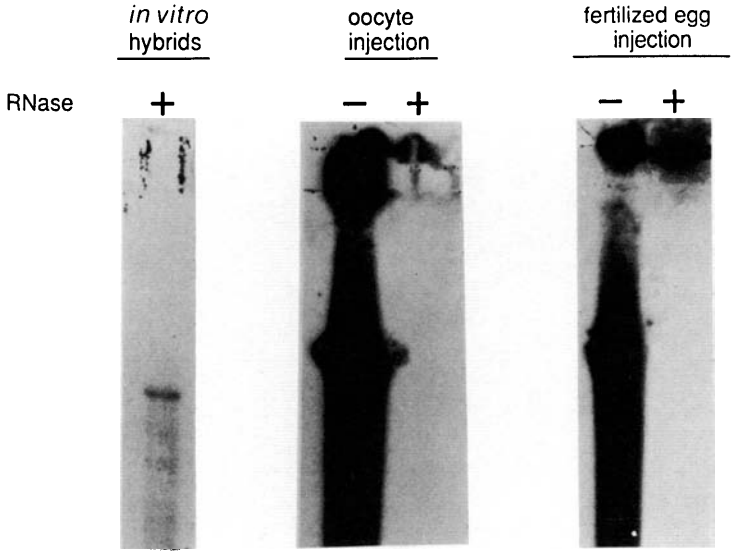

Fig. 4. RNA hybrids do not form between injected anti-sense RNA and endogenous sense mRNA in oocytes or embryos. ^{32}P-labelled anti-sense An1 RNA with an A-C tail (see Fig. 3) was injected into stage V/VI oocytes or into fertilized eggs during first cleavage. RNAs were extracted after 4 h and a portion of each sample was treated with RNAses A and T1 to assay hybrid formation. Untreated and RNAse treated samples were analysed on 4 % polyacrylamide urea gels. Lane marked '*in vitro* hybrids' shows the amount of hybrid formed when ^{32}P-labelled anti-sense An1 RNA is hybridized *in vitro* to 1 egg equivalent of An1 mRNA and then treated with RNAse. − and + denote untreated and RNAse-treated samples, respectively.

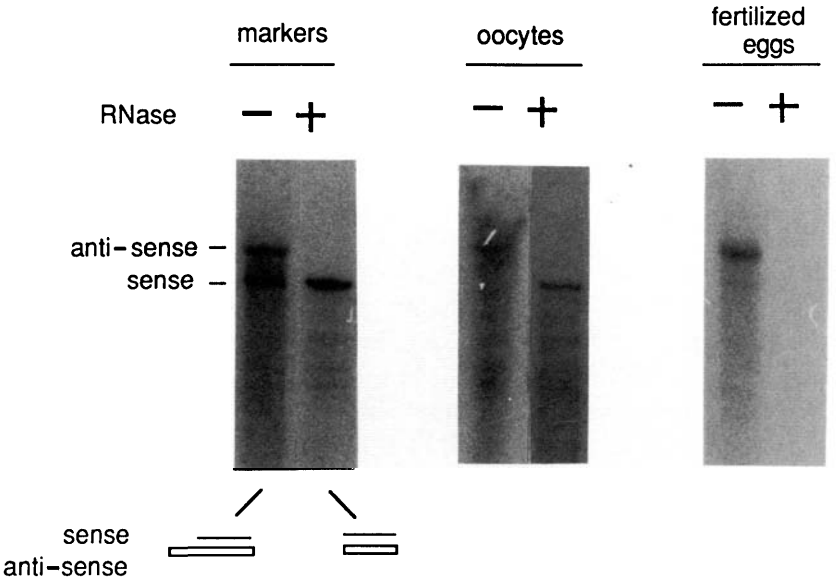

Fig. 5. RNA hybrid formation between injected sense RNA and injected anti-sense RNA in oocytes and eggs. ^{32}P-labelled An1 RNAs were synthesized and capped as described. The anti-sense RNA was identical to that used in Fig. 4; sense RNA was a 680 base transcript complementary to the anti-sense transcript. The two RNAs were injected sequentially into oocytes or fertilized eggs and analysed as described in Fig. 4. Lane labelled 'markers' shows the bands produced when the two RNAs are hybridized *in vitro* and fractionated on gels, either with or without an intervening RNAse treatment.

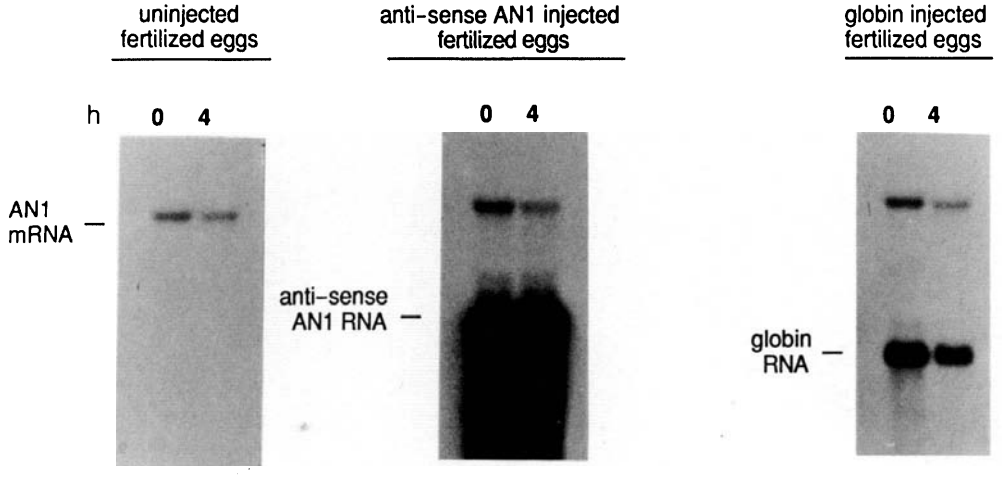

Fig. 6. The effect of injected anti-sense RNA on the levels of the endogenous mRNA in embryos. At 0 or 4 h RNA was extracted from uninjected fertilized eggs or eggs injected during first cleavage with either the A-C tailed anti-sense An1 RNA or synthetic *Xenopus* β-globin mRNA. Extracted RNAs were fractionated on a denaturing agarose gel, blotted onto Gene Screen and hybridized with a labelled RNA probe complementary to the endogenous An1 mRNA. Bands below An1 RNA in injected eggs are the injected ^{32}P-labelled anti-sense An1 and globin RNAs.

anti-sense An1 RNA. Experiments to assay the levels of An1 protein synthesis following the injection of anti-sense An1 RNA are in progress.

CONCLUSIONS AND FUTURE PROSPECTS

Our results show that injected anti-sense RNAs behave differently in oocytes and eggs. In oocytes, the RNAs are more stable and RNA:RNA hybrids do form between injected sense and anti-sense RNAs. In fertilized eggs, the injected anti-sense RNAs are less stable and seem to be degraded some time shortly after the MBT. Moreover, we find no evidence for hybrid formation in eggs. Hybrids with injected anti-sense An1 RNA do not form with either the endogenous An1 mRNA or injected An1 sense RNA. These results reinforce our conclusion that the defective development we sometimes observe following injection of anti-sense An1 RNA cannot be attributed to a specific block of An1 mRNA translation. Rather, the defective development is likely to be due to toxic or other effects of injected RNAs.

We note that only one RNA has been tested in these experiments, namely An1. Other mRNAs may be susceptible to anti-sense blocking. An1 mRNA is unusual in that it is a localized maternal RNA and it may therefore be packaged or sequestered in such a way that it is inaccessible to the anti-sense RNA. However, our results, especially those with injected anti-sense RNA and sense RNAs suggest that hybrids simply may not form in developing eggs. Interestingly, this does not seem to be true for *Drosophila*. The phenocopy of a Krüppel mutant phenotype was obtained by injecting anti-sense RNAs into developing *Drosophila* eggs. In those experiments the uncapped anti-sense RNA was digested to short pieces (about 150 bases long) prior to injection. While it is formally possible that the digestion of the anti-sense RNA to short pieces is an important difference, we think that unlikely because uncapped RNAs are rapidly degraded in frog cytoplasm.

It may be possible to block An1 mRNA translation by the injection of complementary oligonucleotides. However, our preliminary results and those of others suggest that standard DNA oligos are exceedingly unstable following injection into the cytoplasm. Nonetheless, it may be possible to increase the stability of injected oligos by modifying the ends (Toulme *et al.* 1986) or preparing oligos with chemical bonds that are not easily hydrolysed (see e.g. Smith *et al.* 1986). In addition, it may be possible to gain some insight to the function of the localized mRNAs by interfering with the activity of their protein products. The injection of antibodies directed against fibronectin (Boucaut, Darribére, Boulekbacke & Thiery, 1984) and gap junction proteins (Warner, Guthrie & Gilula, 1984) suggest that this may be an informative approach.

Finally, we note that experiments involving the injection of synthetic mRNAs may also provide hints about the function of localized maternal messages. We have previously shown that SP6 transcripts of full-length cDNA act as functional mRNAs in injected oocytes. Thus, for example, the injection of sense An1 RNA

(mRNA) into the vegetal pole blastomeres should direct the synthesis of An1 protein in those cells. Since these blastomeres never normally have the An1 protein this type of experiment may provide information on the function of the An1 protein or on the mechanisms responsible for the localization of the An1 mRNA.

REFERENCES

BENNETT, D. (1975). The T locus of the mouse. *Cell* **6**, 441–454.

BOUCAUT, J. C., DARRIBÉRE, T., BOULEKBACHE, H. & THIERY, J. P. (1984). Antibodies to fibronectin prevent gastrulation but do not perturb neurulation in gastrulated amphibian embryos. *Nature, Lond.* **307**, 364–367.

BROTHERS, A. J. (1976). Stable nuclear activation dependent on a protein synthesized during oogenesis. *Nature, Lond.* **260**, 112–115.

BROWN, D. D. & LITTNA, E. (1964). RNA synthesis during the development of *Xenopus laevis*, the South African clawed toad. *J. molec. Biol.* **8**, 669–687.

CARRASCO, A. E., MCGINNIS, W., GEHRING, W. J. & DE ROBERTIS, E. M. (1984). Cloning of an *X. laevis* gene expressed during early embryogenesis coding for a peptide region homologous to *Drosophila* homeotic genes. *Cell* **37**, 409–414.

COLEMAN, J., GREEN, P. J. & INOUYE, M. (1984). The use of RNAs complementary to specific mRNAs to regulate the expression of individual bacterial genes. *Cell* **37**, 429–436.

CROWLEY, T. E., NELLEN, W., GOMER, R. H. & FIRTEL, R. A. (1985). Phenocopy of discoidin 1-minus mutants by antisense transformation in *Dictyostelium*. *Cell* **43**, 633–641.

DRUMMOND, D. R., ARMSTRONG, J. & COLMAN, A. (1985). The effect of capping and polyadenylation on the stability, movement and translation of synthetic messenger RNAs in *Xenopus* oocytes. *Nucl. Acids Res.* **13**, 7375–7394.

GEHRING, W. J. (1985). The homeo box: a key to understanding development? *Cell* **40**, 3–5.

GREEN, M. R., MANIATIS, T. & MELTON, D. A. (1983). Human β-globin pre-mRNA synthesized *in vitro* is accurately spliced in *Xenopus* oocyte nuclei. *Cell* **32**, 681–694.

GURDON, J. B., LINGREL, J. B. & MARBAIX, G. (1973). Message stability in injected frog oocytes: long life of mammalian α and β globin messages. *J. molec. Biol.* **80**, 539–551.

HARLAND, R. & WEINTRAUB, H. (1985). Translation of mRNA injected into *Xenopus* oocytes is specifically inhibited by anti-sense RNA. *J. Cell Biol.* **101**, 1094–1099.

HARVEY, R. P., TABIN, C. J. & MELTON, D. A. (1986). Embryonic expression and nuclear localization of *Xenopus* homeobox (Xhox) gene products. *EMBO J.* **5**, 1237–1244.

IZANT, J. G. & WEINTRAUB, H. (1984). Inhibition of thymidine kinase gene expression by anti-sense RNA: a molecular approach to genetic analysis. *Cell* **36**, 1007–1015.

JEFFEREY, W. R., TOMLINSON, C. R. & BRODEUR, R. D. (1983). Localization of actin messenger RNA during early ascidian development. *Devl Biol.* **99**, 408–417.

KIM, S. K. & WOLD, B. J. (1985). Stable reduction of thymidine kinase activity in cells expressing high levels of anti-sense RNA. *Cell* **42**, 129–138.

KING, M. L. & BARKLIS, E. (1985). Regional distribution of maternal messenger RNA in the amphibian oocyte. *Devl Biol.* **112**, 203–212.

KRIEG, P. & MELTON, D. (1984). Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned DNAs. *Nucl. Acids Res.* **12**, 7057–7070.

KRIEG, P. A. & MELTON, D. A. (1985). Developmental regulation of a gastrula-specific gene injected into fertilized *Xenopus* eggs. *EMBO J.* **4**, 3463–3471.

LYNN, D. A., ANGERER, L. M., BRUSKIN, A. M., KLEIN, W. H. & ANGERER, R. C. (1983). Localization of a family of mRNAs in a single cell type and its precursors in sea urchin embryos. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2656–2660.

MELTON, D. A. (1985). Injected anti-sense RNAs specifically block messenger RNA translation *in vivo*. *Proc. natn. Acad. Sci. U.S.A.* **82**, 144–148.

MELTON, D. A., KRIEG, P. A., REBAGLIATI, M. R., MANIATIS, T., ZINN, K. & GREEN, M. R. (1984). Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucl. Acids Res.* **12**, 7035–7056.

NEWPORT, J. & KIRSCHNER, M. (1982). A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. *Cell* **30**, 687–696.

PETSKA, S., DAUGHERTY, B., JUNG, V., HOTTA, K. & PETSKA, R. (1985). Anti-mRNA specific inhibition of translation of single mRNA molecules. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7325–7328.

REBAGLIATI, M., R., WEEKS, D. L., HARVEY, R. P. & MELTON, D. A. (1985). Identification and cloning of localized maternal RNAs from *Xenopus* eggs. *Cell* **42**, 769–777.

ROARK, M., MAHONEY, P. A., GRAHAM, M. L. & LENGYEL, J. A. (1985). Blastoderm-differential and blastoderm-specific genes of *Drosophila melanogaster. Devl Biol.* **109**, 476–488.

ROSENBERG, U. B., PREISS, A., SEIFERT, E., JACKLE, H. & KNIPPLE, D. C. (1985). Production of phenocopies by Krüppel anti-sense RNA injection into *Drosophila* embryos. *Nature, Lond.* **313**, 703–706.

ROYCHOUDHURY, R. & WU, R. (1980). Terminal transferase-catalyzed addition of nucleotides to the 3′ termini of DNA. In *Methods in Enzymology*, vol. **65** (ed. L. Grossman & K. Moldave), pp. 43–62. New York: Academic Press, Inc.

SARGENT, T. D. & DAWID, I. B. (1983). Differential gene expression in the gastrula of *Xenopus laevis*. *Science* **222**, 135–139.

SIPPEL, A. E. (1973). Purification and characterization of adenosine triphosphate: ribonucleic acid adenyltransferase form *Escherichia coli. Eur. J. Biochem.* **37**, 31–40.

SMITH, C. C., AURELIAN, L., REDDY, M. P., MILLER, P. S. & TSO, P. O. P. (1986). Antiviral effects of an oligo(nucleoside methylphosphonate) complementary to the splice junction of herpes simplex virus type I immediate early pre-mRNAs 4 and 5. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2787–2791.

TOULME, J. J., KRISCH, H. M., LOREAU, N., THUONG, N. T. & HELENE, C. (1986). Specific inhibition of mRNA translation by complementary oligonucleotides covalently linked to intercalating agents. *Proc. natn. Acad. Sci. U.S.A* **83**, 1227–1231.

WARNER, A. E., GUTHRIE, S. C. & GILULA, N. B. (1984). Antibodies to gap junctional protein selectively disrupt junctional communication in the early amphibian embryo. *Nature, Lond.* **311**, 127–131.

WEEKS, D. L., REBAGLIATI, M. R., HARVEY, R. P. & MELTON, D. A. (1985). Localized maternal mRNAs in *Xenopus laevis* eggs. *Cold Spring Harb. Symp. quant. Biol.* **50**, 21–29.