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A functional test for maternally inherited cadherin in *Xenopus* shows its importance in cell adhesion at the blastula stage

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SUMMARY

We report here on the consequences of reducing the expression of EP-cadherin at the earliest stages of *Xenopus* development. Injection of oligodeoxynucleotides antisense to maternal EP-cadherin mRNA into full-grown oocytes reduced the mRNA level in oocytes, and the protein level in blastulae. Adhesion between blastomeres was significantly reduced, as seen in whole embryos, and in assays of the ability of blastomeres to reaggregate in culture. This effect was especially conspicuous in the inner cells of the blastula and included the disruption of the blastocoel. The severity of the EP-cadherin mRNA depletion and of the disaggregation phenotype was dose dependent. This phenotype was rescued by the injection into EP-cadherin mRNA-depleted oocytes of the mRNA coding for a related cadherin, E-cadherin, that is normally expressed at the gastrula stage in the embryonic ectoderm.

Key words: cadherin, cell adhesion, *Xenopus*, blastula

INTRODUCTION

The construction of the *Xenopus* embryo from the fertilised egg relies on adhesion molecules. These are clearly required to hold cells together, but may also be involved in regionalization. The cell surface changes that underlie tissue segregation develop during the blastula stage, as shown by cell transplantation (Heasman et al., 1984) and cell sorting experiments (Turner et al., 1989). The first step towards understanding the mechanism of regionalisation in the embryo is the description and functional characterisation of the adhesion molecules present in the blastula.

Several classes of adhesion molecules are present at this stage of development, including members of the cadherin superfamily. At least one cadherin (EP-cadherin) is expressed both as mRNA and as protein in the *Xenopus* oocyte and fertilised egg, and in all embryonic cells at the blastula and gastrula stage (Choi et al., 1990; Ginsberg et al., 1991). A second cadherin (U-cadherin) is also ubiquitously expressed throughout early development up to the late neurula stage (Angres et al., 1991). Its relationship to EP-cadherin is uncertain, as only a short stretch of the extracellular domain of U-cadherin is sequenced, and this is not identical to the corresponding part of EP-cadherin. A third cadherin (XB-cadherin) may be an isoform of EP-cadherin (Herzberg et al., 1991).

After the mid-blastula transition, E-cadherin is synthesized in the presumptive ectoderm (Choi and Gumbiner, 1989; Angres et al., 1991). As the neural plate forms in the ectoderm, E-cadherin is down-regulated there and N-cadherin takes its place (Detrick et al., 1990; Fujimori et al., 1990; Ginsberg et al., 1991).

More recently, two other calcium-dependent adhesion systems have been described in the *Xenopus* blastula. One of these, M4B antigen, is a carbohydrate epitope associated with a membrane glycolipid and is differentially distributed in the blastula (Turner et al., 1992). The second group of adhesion receptors are members of the integrin family. Two α-subunits, α_3 and α_5, and the β_1 subunit are expressed as maternal mRNAs, while zygotic transcripts of α_{2-6} begin to accumulate during gastrulation (J. C. Smith et al., 1990; Whittaker and DeSimone, 1993; Gawantka et al., 1992).

The relative roles of these adhesion molecules in the blastula is unknown. The evidence that they are adhesive rests upon three types of data. Firstly, when cadherin cDNA is transfected into L-cells, they become adhesive, and furthermore transfected cells expressing different cadherin family members on their surfaces sort out from each other (reviewed in Takeichi, 1988). Secondly, antibody blocking experiments show that *Xenopus* blastomeres can be partially inhibited from aggregating in culture in the presence of either U-cadherin or M4B antibodies (Angres et al., 1991; Turner et al., 1992). Thirdly, mRNA expression experiments indicate that either a full-length or mutated form of N-cadherin mRNA, injected into *Xenopus* eggs causes disruption of ectoderm cells after gastrulation (Fujimori et al., 1990; Detrick et al., 1990; Kintner, 1992).

In this paper, we address the question of the role of EP-cadherin in the *Xenopus* blastula, using the direct approach of depleting the oocyte of the maternal EP-cadherin mRNA, using antisense deoxyoligonucleotides (oligos) (Shuttleworth and Colman, 1988; Dash et al., 1987), followed by fertilisation of the oocytes to study the effect on the blastula. This method was

shown to be effective in studying the role of maternally derived cytokeratin in the blastula (Torpey et al., 1992a; Heasman et al., 1992), as well as the roles of several other maternally inherited mRNAs (Weeks et al., 1991; Dash et al., 1987; El-Baradi et al., 1991; Kloc et al., 1989; Smith et al., 1991). We report here that depleting the EP-cadherin mRNA results in a lack of adhesion and severe disruption of the morphology of the blastula. EP-cadherin-depleted blastomeres show reduced ability to aggregate in culture. All the features of this phenotype are rescued by co-injection of the sense oligo, or subsequent injection into oocytes of excess E-cadherin mRNA prior to fertilization. These results show first that cadherin-mediated adhesion is important in the *Xenopus* blastula, and second that at least one other member of this family can fulfill the function of EP-cadherin in the blastula.

MATERIALS AND METHODS

Oocytes and embryos

Female *Xenopus laevis* were anaesthetized in 0.1% 3-amino-benzoic acid ethyl ester (Sigma) and lobes of the ovaries were surgically removed. Full-grown oocytes were manually defolliculated, injected using a Medical System Corp. microinjection apparatus and cultured in oocyte culture medium (OCM) as previously described (Heasman et al., 1991). They were labelled using vital dyes, matured using progesterone (final concentration 2 μM) and fertilized using the host transfer technique (Holwill et al., 1987; Heasman et al., 1991). Sterile technique was essential throughout. After a period of 3 hours, the eggs were stripped every 30 minutes, fertilized in vitro using a sperm suspension and maintained in 0.1× MBSH (Heasman et al., 1991).

Oligonucleotides

A panel of eleven oligos (18- and 21mers) complementary to different regions of EP-cadherin RNA were chosen at random and tested for their ability to deplete EP-cadherin in oocytes by injection of 3-6 ng into the equatorial region. The most efficient oligos (61 and 62) were selected using northern analysis, and they, together with the oligo complementary to 61, sense oligo 84, were synthesized in a partially modified form (Integrated DNA Technologies Inc. USA) (Dagle et al., 1990). The * represents phosphoramidate linkages.

oligo 61 5′ C*C*T*C*TCCAGCTCCC*T*A*C*G 3′ (antisense to an 18mer in the precursor sequence of the EP-cadherin mRNA)

oligo 84 5′ C*G*T*A*GGGAGCTGGA*G*A*G*G 3′(sense of 61)

oligo 62 5′ C*C*A*C*GTTCATTCTCAGA*A*A*C*C 3′(antisense to a 21mer in the first extracellular domain EC1 of the mRNA)

The oligos were diluted in sterile distilled water before use and injected in dilutions of 0.1-0.5 ng/nl and volumes of 5-15 nl. Oligos were centrifuged for 10 minutes in a microfuge at 4°C before use to prevent needle blockage.

E-cadherin plasmid and in vitro transcription

The E-cadherin cDNA subcloned into SP64T vector was the gift of Dr C Kintner and has been described previously (Kintner, 1992). The template was linearized with *Sma*I and transcribed in vitro using SP6 RNA polymerase. It was purified on a spin column, ethanol precipitated and resuspended in sterile distilled water for injection. Injections were carried out in the equatorial zone of the oocytes using concentrations of 50-500 pg/nl and volumes of 2-10 nl. RNA was centrifuged for 10 minutes in a microfuge at 4°C before injection.

Northern analysis

Oocyte RNA was extracted as described by Gurdon et al. (1985). Electrophoresis and northern blotting were performed as described by Hopwood et al. (1989) using 2.5 oocyte equivalents per lane. The probe was synthesized by random priming of the excised insert (*Bam*HI, *Eco*R1) of EP-cadherin cDNA (Ginsberg et al., 1991) and of E-cadherin (*Eco*R1, *Eco*R5) (Kintner 1992).

Immunohistochemistry

Embryos were fixed in 100% ethanol at −20°C and embedded in polyethyleneglycol distearate. Sections were dewaxed in acetone, rehydrated and stained using a rabbit pan-cadherin antibody at 1:100 dilution (Geiger et al., 1990)) and fluorescein-labelled secondary goat anti-rabbit antibodies. To decrease the level of autofluorescence contributed by the yolk platelets, the sections were treated with 0.01% eriochrome black (Sigma) which shifts the autofluorescence signal of the yolk platelets towards the red.

Aggregation assays

These were carried out on mid-blastula cells as described previously (Turner et al., 1992), except that aggregation was carried out in OCM, over a time course of 1 and 2 hours.

Electron microscopy

Embryos were fixed in 3% glutaraldehyde, 3.5% paraformaldehyde and 2% DMSO pH7.2 for 2 hours at room temperature, followed by overnight at 4°C. Embryos were rinsed and cut in half and postfixed with 1% osmium tetroxide. After dehydration, embryos were critical point dried, sputter-coated with gold and examined in a JEOL 6400 electron microscope.

RESULTS

Antisense depletion of EP-cadherin mRNA in the oocyte results in a lack of adhesion between blastomeres

In these experiments, three antisense oligos were used, which were complementary to different parts of the EP-cadherin mRNA sequence, near the N terminus of the molecule (see Materials and Methods), and which depleted the amount of EP-cadherin mRNA in a dose-dependent manner (Fig. 1). 1 ng of oligos 61 and 62 was found to reduce the mRNA level to less than 10% of the control level. We have shown previously that oocytes depleted of particular mRNAs do not resynthesize them over culture periods of several days (Heasman et al., 1992). Furthermore, when EP-cadherin mRNA-depleted (EP-depleted) oocytes were fertilized by the host transfer technique (Holwill et al., 1987; Heasman et al., 1991) the mRNA remained depleted at the midblastula stage (Fig. 2). The number of oocytes that fertilize successfully using this method varies from experiment to experiment. For each experiment, all arrested or abnormal 1- to 2-cell-stage embryos in all batches were removed from the analysis, and experiments in which uninjected control embryos developed abnormally were not scored.

In a series of experiments where 1 ng of oligo 61 was injected into oocytes, the embryos derived from these oocytes were studied. Cleavage occurred at the same rate in these embryos as in the control batches. EP-depleted embryos were normal in external appearance (Fig. 3A) at the blastula stage. In these experiments, a number of embryos were taken at random at the blastula stage and their animal caps dissected off

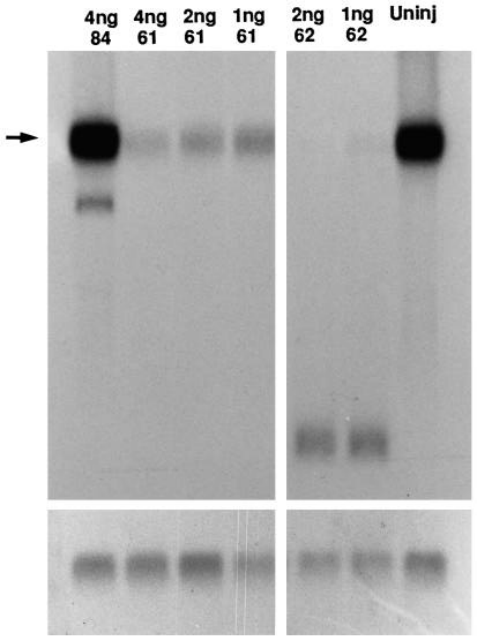

Fig. 1. Northern blot of RNA from 2.5 oocyte equivalents per lane after injection of 4 ng sense oligo; and 4 ng, 2 ng and 1 ng antisense 61 oligo; 2 ng and 1 ng of antisense oligo 62 and uninjected control, respectively. The antisense oligos deplete EP-cadherin mRNA (arrow) in a dose-dependent fashion. This blot was stripped and reprobed for EF1α RNA, to indicate RNA loading.

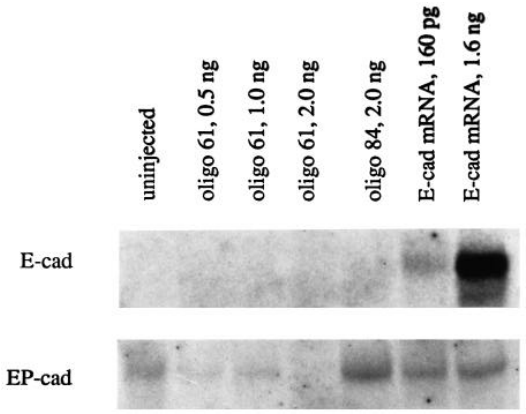

Fig. 2. Northern blot of RNA from 2.5 blastula equivalents per lane after injection of 0.5 ng, 1.0 ng, 2.0 ng of antisense oligo, and 160 pg and 1.6 ng E-cadherin mRNA, respectively, into the oocytes. The blot was probed first for EP-cadherin mRNA, then stripped and reprobed for E-cadherin mRNA. No resynthesis of EP-cadherin mRNA is seen by the blastula stage after injection of antisense oligo into the oocyte. E-cadherin mRNA injected into the oocyte remains stable at least until the blastula stage.

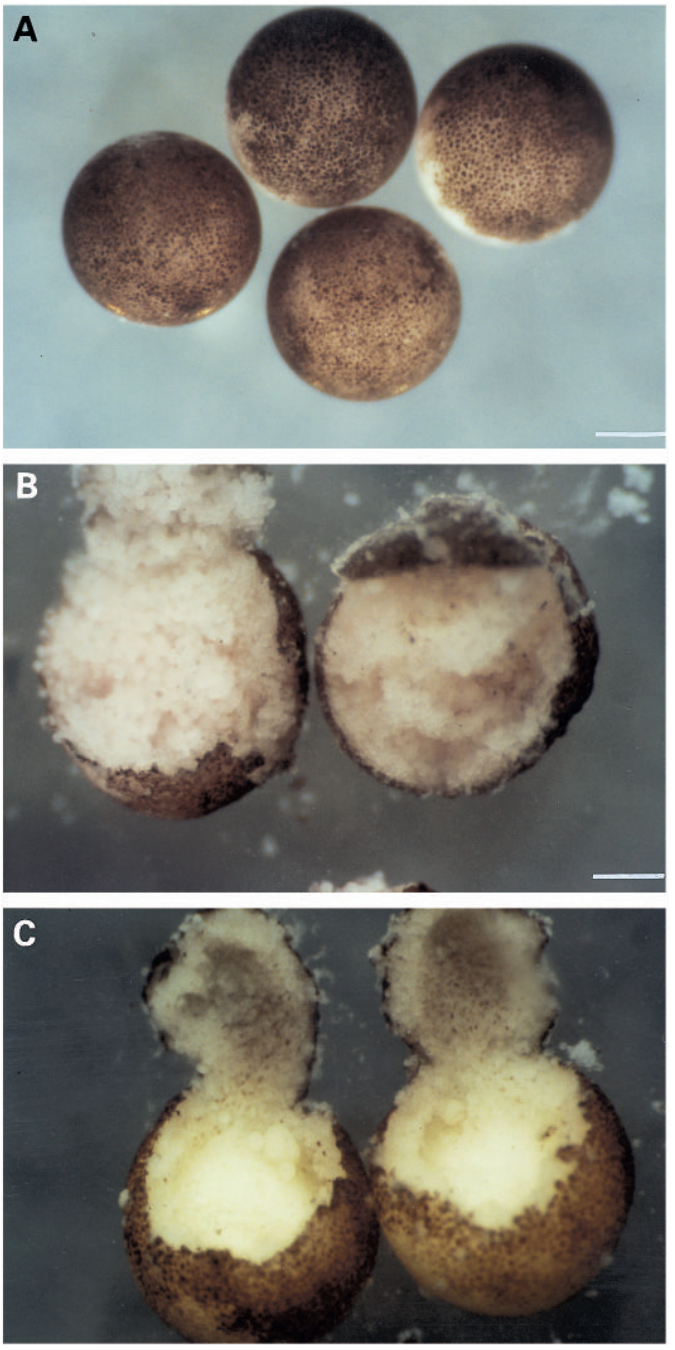

Fig. 3. (A) The external surface of blastulae injected as oocytes with 1 ng antisense oligo. Despite the apparently normal external surface, the inner cells are highly disaggregated (B) compared to controls (C). The control used here was injected with an oligo that depletes maternal MyoD mRNA, and is indistinguishable from those injected with 1 ng antisense + 2 ng sense oligo, or 2 ng sense oligo only. (A) Bar, 0.43 mm; (B,C) bar, 0.35 mm

using tungsten needles. The internal animal and vegetal cells were strikingly disaggregated (Fig. 3B). The normal smooth surface of the blastocoel was disrupted, and in most cases the blastocoel was partially or totally obliterated by dissociated cells (Fig. 4A). Animal and vegetal cells were equally affected, although the most superficial layer of cells surrounding the embryo was not visibly affected. Dissected embryos were scored for disaggregation and the phenotype was found to be highly consistent (Table 1).

When dissected pieces of control and EP-depleted blastulae were cultured for 4 hours (sibling embryos at the early gastrula stage), the disaggregated cells continued to divide normally,

but did not adhere (Fig. 5B). However, after 12 hours of culture, the same fragments regained their adhesiveness, and were identical to control fragments (Fig. 5C). EP-depleted embryos that were not dissected at the blastula stage continued to develop, undergoing gastrulation and neurulation (Fig. 5D,E) At the neurula stage, the EP-depleted embryos were abnormal compared to the controls, but distinct axial structures had formed (Fig. 5E compared to Fig. 5F).

The severity of the phenotype was dose dependent, 0.5 ng of the same oligo causing partial disaggregation, while 2-3 ng of oligo 61 arrested development at the blastula stage (Table 1).

The experiment was repeated with a second oligo (62) that depletes the EP-cadherin mRNA more effectively than oligo 61 (Fig. 1). 1 ng of this oligo also caused the disaggregated phenotype at the blastula stage (Table 1). When dissected blastulae were cultured overnight, however, their cells continued to divide but did not reaggregate. Furthermore, sibling oligo 62 EP-depleted embryos that were not dissected at the blastula stage, failed to complete gastrulation (data not shown).

A third unmodified oligo (45) depletes EP-cadherin less effectively than oligos 61 and 62, and embryos derived from oligo 45-injected oocytes developed a partially disaggregated phenotype (data not shown).

In these experiments, controls included no injection, 2 ng of sense (84) or irrelevant oligo (AMD7 specifically depletes MyoD mRNA) or 1 ng of antisense + 2 ng of sense oligo. In no cases did these embryos develop with a disaggregated phenotype (Figs 3C, 4B; Table 1).

Table 1. The effect of depletion of maternal EP-cadherin mRNA on blastula adhesion

Experiment	Number of normal 4- to 64-cell embryos	No. dissected at midblastula	No. with disaggregated phenotype
Expt1			
3 ng 61	0	0	–
1 ng 61	27	10	10++
1 ng AMD7	43	10	0
Expt 2			
0.5 ng 61	13	5	5+
1 ng 61	9	6	6++
2 ng 61	0	0	–
2 ng 84	5	5	0
1 ng 61+2 ng 84	4	4	0
Expt3			
1 ng 61	29	12	12++
2 ng 61	0	0	–
1 ng 84	21	10	0
1 ng 61+2 ng 84	15	6	0
Expt4			
0.5 ng 61	35	4	4+
1 ng 61	30	12	12++
2 ng 61	0	0	–
2 ng 84	23	4	0
1 ng 61+E cad	18	12	7+
Expt5			
0.5 ng	45	5	5+
1 ng 62	38	10	10++
1 ng 84	35	10	0
1 ng 62+E cad	41	10	10+

In 5 seperate experiments, oocytes were injected with antisense (61,62,) or control oligos (AMD7,84,84+61) or antisense+ E-cadherin mRNA 1.6 ng. In each experiment, a small number were dissected and scored for disaggregated(++) or partially disaggregated(+) phenotype. Other embryos in these experiments were used for northern analysis, histology at light and electron microscopic levels, immunocytochemistry, aggregation experiments or allowed to develop further.

The disaggregated phenotype can be rescued by injection of mRNA coding for a related cadherin type, E-cadherin, into EP-depleted oocytes

It is important to prove that the disaggregated phenotype seen in EP-depleted embryos is specifically the result of the depletion of this mRNA. The obvious control would be to inject EP-cadherin mRNA into EP-depleted oocytes. However, we have shown in other experiments (Raats et al., unpublished observations) that phosphoramidate-modified oligos are extremely stable in oocytes and will continue to deplete their target mRNA when it is subsequently injected. To circumvent this, we injected mRNA coding for the related E-cadherin into EP-depleted oocytes. In this experiment, we injected oocytes first with 1 ng antisense oligo, followed 24 hours later, in half the oocytes, by 1.6 ng E-cadherin mRNA. Additional controls included injection of E-cadherin mRNA only, and 2 ng sense oligo only. All oocytes were cultured for the same length of time (48 hours) before fertilisation. At the blastula stage, 12 embryos from each batch were dissected. All of the EP-depleted embryos had a severely disaggregated phenotype, whilst none of the sense-injected embryos were affected (Fig. 6A,B). Of the embryos that were EP-depleted and subsequently injected with E-cadherin mRNA, 5 were normal and 7 of them were partially rescued (Fig. 6C). Embryos derived from oocytes injected with E-cadherin RNA alone were normal at the blastula stage. This experiment was repeated using oligo 62 instead of oligo 61 to deplete the mRNA with similar results. Again,injection of E-cadherin mRNA into EP-depleted oocytes partially rescued the embryos from disaggregation (Table 1).

The simplest interpretation of the above data was that E-cadherin expression was able to rescue the adhesiveness of blastomeres. To test this directly, we analysed embryos from the above experiment in two ways.

First we analysed the expression of total cadherin protein by immunocytochemistry in these blastulae. Immunocytochemistry was carried out using an antibody that reacts with all cadherin types (Geiger et al., 1990). This showed a clear reduction in staining in EP-depleted blastulae, compared with sense-injected controls (Fig. 6D,E), and reappearance of cadherin by subsequent E-cadherin mRNA injection into the EP-depleted batch of oocytes(Fig. 6F).

Second, we tested adhesiveness of individual blastomeres from these embryos in simple reaggregation assays (Turner et al., 1992). Dissociated blastomeres were compared for their ability to reaggregate after disaggregation in Ca^{2+}- and Mg^{2+}-free saline. The results are shown in Fig. 7. Sense-injected, and EP-depleted+E-cadherin blastomeres reaggregated rapidly into spherical masses, whereas EP-depleted blastomeres aggregated to less than 50% of these control levels. The aggregation of all three classes was further reduced by the inclusion of an antibody that recognises the M4B glycolipid (Fig. 7).

In similar experiments, oligo 45 EP-depleted blastomeres were tested in their ability to reaggregate. EP(45)-depleted

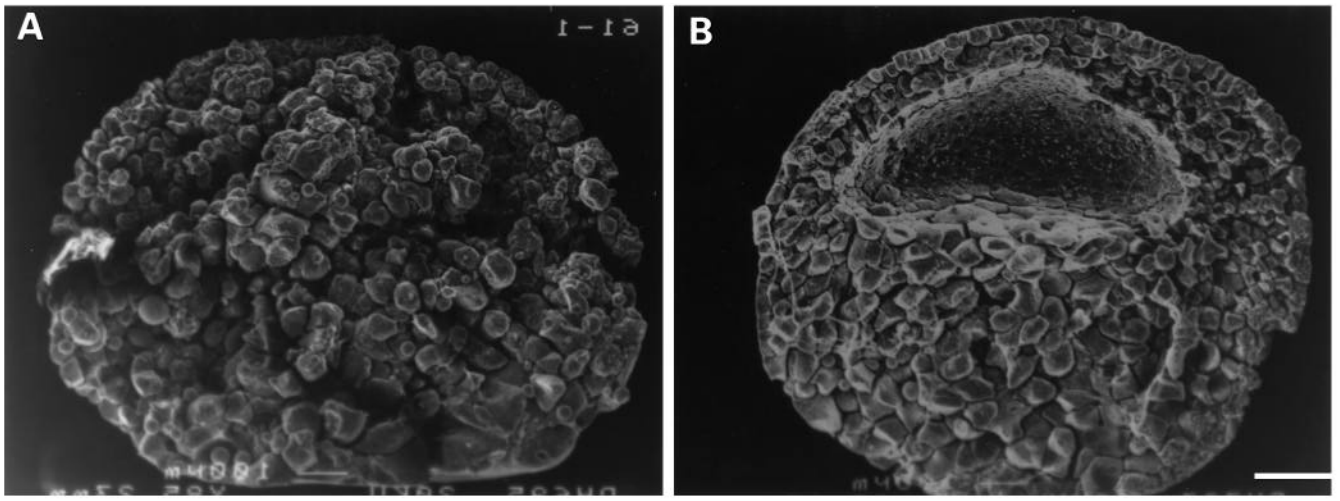

Fig. 4. (A,B) Scanning electron micrographs of blastulae derived from antisense (A) and control (B) oocytes, cut open to reveal the disrupted blastocoel after EP-cadherin RNA depletion. Bar, 0.17 mm

blastomeres showed an intermediate degree of reaggregation compared EP(61)-depleted cells and sense-injected controls (data not shown).

DISCUSSION

Antisense oligos as tools to deplete maternal mRNAs

One of the most powerful techniques to study the role of a protein in development is to prevent its synthesis in the embryo and study the effect. The most successful approach to this in vertebrate development has been the use of homologous recombination in ES cells, followed by the production of transgenic mice derived from these (Thomas and Capecchi, 1987). Unfortunately, if genes that are important early in development are knocked out in this way, the embryos die in utero, making the analysis of the primary phenotype difficult. In *Xenopus*, the transgenic approach is not possible due to the long life cycle and lack of pluripotential cell lines. However, it is possible to target maternally inherited gene products using the antisense technique described here and previously (Torpey et al., 1992a). Some of these proteins are clearly important in development, because zygotic transcription does not occur until the mid-blastula transition, by which time the embryo has already specified its dorsoventral axis, and shows considerable region specification. In the experiments described here, oligos complementary to the extracellular coding region of EP-cadherin mRNA were used. These were the only two oligos, out of eleven, that efficiently depleted the target mRNA. In our experience, less than 30% of randomly selected oligo sequences are effective in removing their target mRNAs.The reasons for this are unclear. The most likely explanation is that long stretches of the target mRNAs are not accessible to the oligo, due perhaps to secondary structure, or binding to other molecules at these sites.

It is well-documented that oligos may themselves be toxic in animal experiments (R. C. Smith et al., 1990; Woolf et al., 1990), and, in addition, may deplete non-target mRNAs (Woolf et al., 1992). Fertilised eggs are particularly prone to non-specific effects of oligo injection (Heasman et al., 1992). In the experiments described here, neither degradation of non-target mRNAs, nor other non-specific effects are problems, since 2 ng of the sense oligo has no effect itself (and will prevent the effect of the antisense oligo), and the phenotype seen after injection of two antisense oligos complementary to different parts of the EP-cadherin mRNA is rescued by the subsequent injection of E-cadherin mRNA.

The role of EP-cadherin in the blastula

Embryos developing from oocytes depleted of EP-cadherin mRNA have a striking and reproducible disaggregated phenotype. The most superficial layer of cells was least affected. This ‘epithelial’ layer is known to have a well-developed network of cytokeratin filaments and associated junctions (Klymkowsky et al., 1987; Torpey et al., 1992b; Heasman et al., 1992), which may explain the absence of an effect. While animal and vegetal cells appear to be equally affected, neither of them is completely disaggregated, nor are they totally devoid of cadherin as visualised by immunocytochemistry. The remaining cadherin may be EP-cadherin protein produced on residual mRNA resistant to oligo-mediated breakdown, or it could be other classes of cadherin, which are not distinguishable using the pan-cadherin antibody. Since U-cadherin has not yet been sequenced, it is not known whether its mRNA will be depleted by the oligo used here. A second adhesion system that may be providing the residual adhesiveness shown here is the carbohydrate antigen M4B. The fact that M4B antibody further reduces the adhesiveness of EP-depleted blastomeres supports this possibility and suggests the two systems act in an additive fashion.

A consistent observation here was that a higher dose of oligo 61 (2 ng), which reduces the amount of EP-cadherin mRNA more than 1 ng of 61, arrested the embryos at the blastula stage. We have not distinguished in these experiments whether this is a specific effect or the result of non-specific toxicity. The latter is unlikely because the oocytes are cultured for 48 hours after injection of oligo, which is sufficient time for the oligo

to breakdown. Also, embryos injected with 2 ng of sense oligo develop normally. This suggests that embryos severely depleted of maternal EP-cadherin cannot develop to the gastrula stage.

Considering the degree of disruption observed in all blastula stage EP-depleted embryos, it was surprising that in some experiments they were able subsequently to gastrulate and form embryonic axes. It was clear from studying fragments of these embryos in culture that adhesiveness was reestablished between 4 and 12 hours after their dissection at the mid-blastula stage. The most likely explanation is that zygotic transcripts provide the adhesion molecules necessary for this process. The results further suggest that mesoderm induction and axis specification can occur in the EP-depleted embryos,

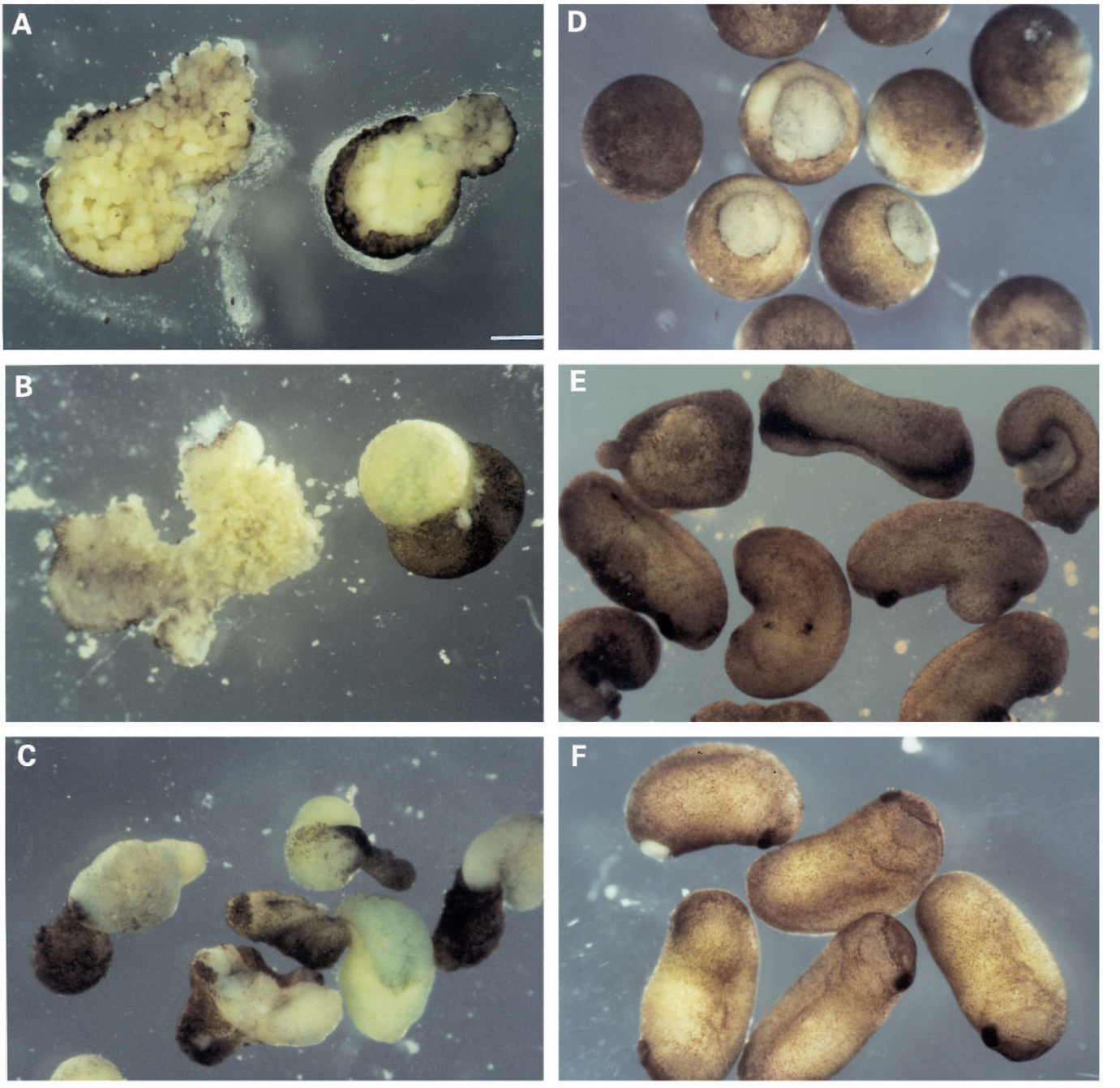

Fig. 5. (A) A newly dissected blastula after antisense oligo injection into the oocyte, together with a control embryo (1 ng antisense + 2 ng sense oligo). After 4 hours (B) in 1× MBSH the control embryo has reaggregated whereas the antisense-injected embryo remains a disaggregated mass. After 12 hours (C) both batches of embryos have reaggregated and are indistinguishable. Both have exogastrulated due to the high ionic strength of the saline (1× MBSH) used to allow reaggregation. Antisense-injected blastulae that are left undissected will gastrulate (D) and form abnormal tailbud embryos (E). These have a variety of abnormalities, but form dorsoventral and anteroposterior axes. Control embryos at the same stage are shown in F. Bar, 0.55 mm.

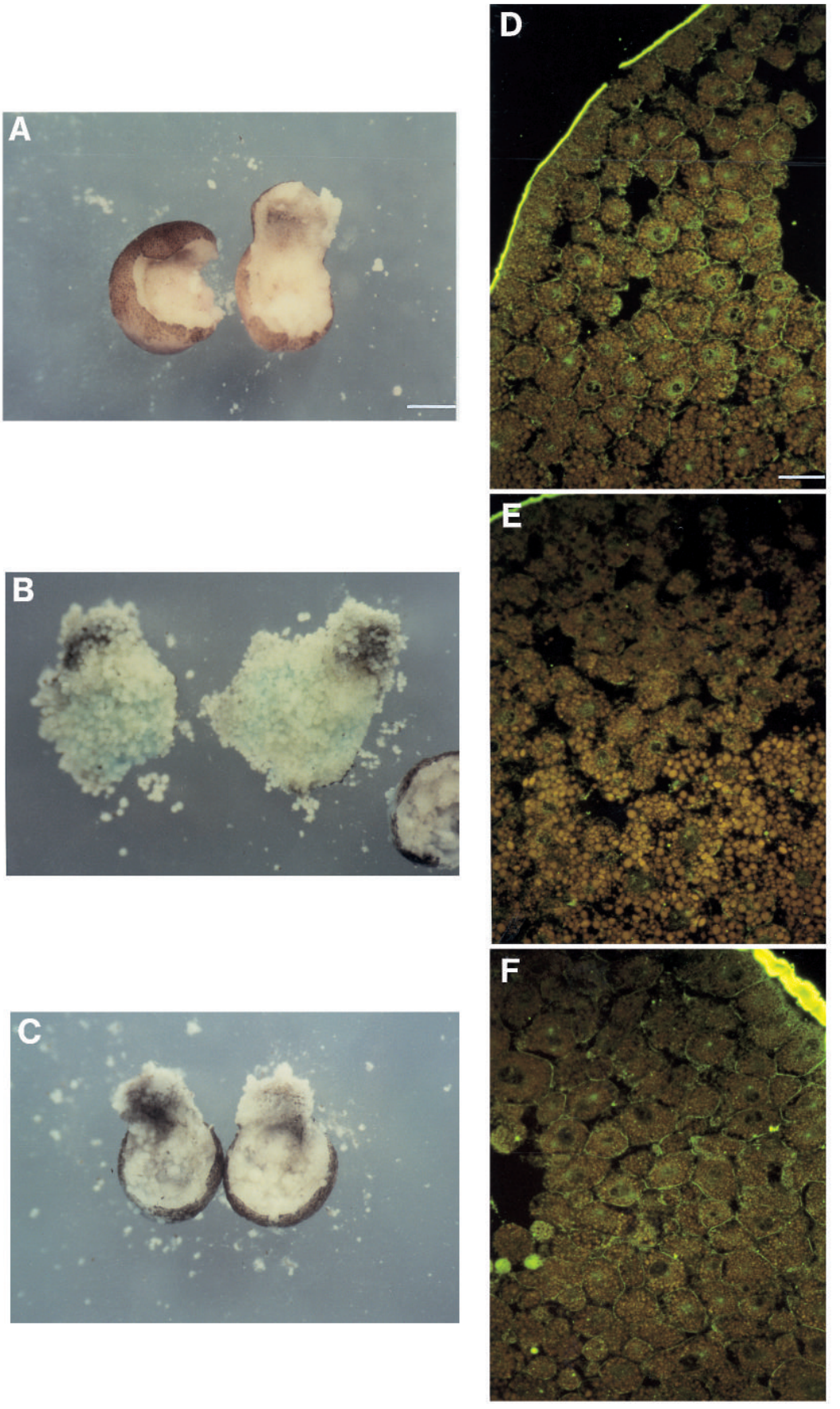

Fig. 6. (A) Blastulae derived from oocytes injected with 2 ng sense oligo, dissected open at the late blastula stage. (B) An embryo at the same stage after 1 ng antisense oligo injection to show the loss of interblastomere adhesion. The embryos shown in C were also injected with the antisense oligo, but 24 hours later were injected with 1.6 ng E-cadherin mRNA. Normal blastula morphology has largely been restored. (D-F) Equivalent embryos fixed, sectioned and stained with a pan-cadherin antibody. Cadherin can be seen around the blastomeres in the 2 ng sense oligo-injected embryos (D). It is missing from the surfaces of the blastomeres after 1 ng antisense oligo (E) and present again after 1 ng antisense oligo followed by E-cadherin mRNA (F). Notice that the blastocoel is obliterated in E, and restored in F. Each picture is from an equivalent region of the blastula, and shows animal and marginal cells. In all cases, the antibody stains nuclei and the vitelline membrane non-specifically. (A-C) Bar, 0.55 mm; (D-F) Bar, 0.05 mm.

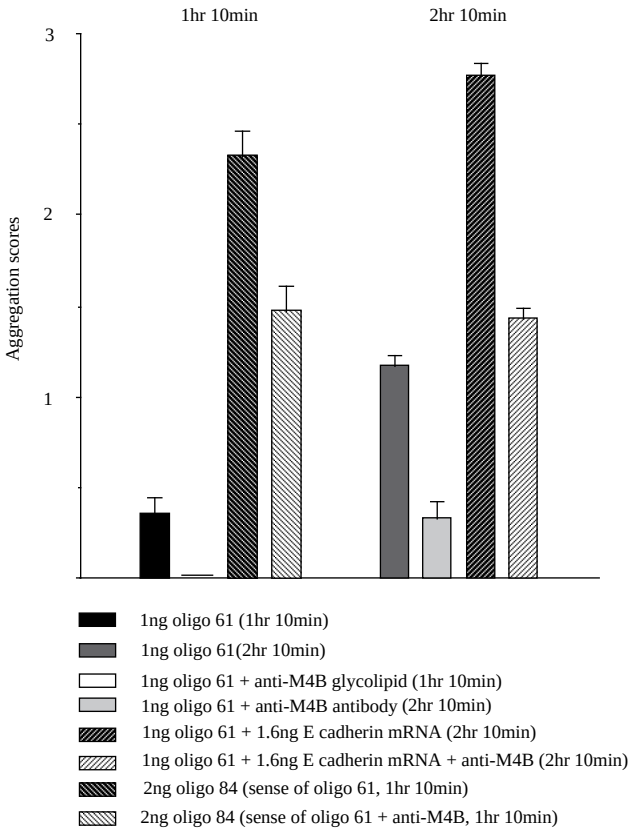

Fig. 7. Bar charts to show the rates of reaggregation of isolated groups of 50 animal blastomeres disaggregated in Ca^{2+}- and Mg^{2+}-free saline, and allowed to reaggregate in OCM for the two time periods shown. A score of 3 indicates that the aggregates had become smooth spheres, while 0 indicates no reaggregation. For further details of scoring system, see Turner et al. (1992). EP-cadherin mRNA depletion causes reduced blastomere aggregation, compared with sense-injected controls. This is rescued by the injection of E-cadherin mRNA. In all cases, addition of anti-M4B glycolipid further reduces aggregation.

even though the cellular structure, including the blastocoel, is disrupted in these embryos. However, a more efficient depletion of EP-cadherin mRNA, caused by 2 ng of 61 or 1 ng of 62 oligos arrests development before or during the gastrula stage. Furthermore, blastomeres from these batches disaggregated at the blastula stage do not reaggregate overnight. The cells continue to divide in isotonic saline, showing that they are still alive. This suggests that a basal level of maternal EP-cadherin expression is necessary for development to proceed through gastrulation.

The phenotype can be rescued by a related cadherin

In the rescue experiments, E-cadherin was used instead of EP-cadherin because phosphoramidate oligos are very stable, and will efficiently breakdown injected complementary RNA (J. Raats et al., unpublished observations). It is interesting that E-cadherin mRNA can allow the normal cellular structure of the blastula to form, even though it is not normally expressed until the gastrula stage, and then only in the ectoderm (Choi and Gumbiner, 1989; Angres et al., 1991). This suggests that E-cadherin can substitute for EP-cadherin as an adhesion molecule in the blastula cell membrane.

The observations reported here show that antisense depletion from oocytes of a maternally expressed cadherin causes the predictable phenotype of disaggregated blastulae. This establishes directly the role of cadherins as important adhesion molecules in the developing *Xenopus* embryo, and therefore provides an important supplement to studies in culture, and inferences from their spatial and temporal expression pattern.

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