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Cold-inducible RNA binding protein is required for the expression of adhesion molecules and embryonic cell movement in *Xenopus laevis* ☆

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Abstract

We have previously shown that the *Xenopus* homologue of cold-inducible RNA binding protein, XCIRP-1, is required for the morphogenetic migration of the pronephros during embryonic development. However, the underlying molecular mechanisms remain elusive. Here, we report that XCIRP is essential for embryonic cell movement, as suppression of XCIRP by microinjection of anti-sense mRNA and morpholino antisense oligonucleotides (MOs) significantly reduced protein expression, inhibited the cell migration rate, and inhibited eFGF and activin-induced animal cap elongation. By immunoprecipitation and RT-PCR, we further showed that the mRNA of a panel of adhesion molecules, including αE- and β-catenin, C- and E-cadherin, and paraxial proto-cadherin, are the targets of XCIRP. Consistently, in animal cap explant studies, suppression of XCIRP by MOs inhibited the expression of these adhesion molecules, while over-expression of sense XCIRP-1 mRNA fully rescued this inhibition. Taken together, these results suggest for the first time that XCIRP is required to maintain the expression of adhesion molecules and cell movement during embryonic development.

Keywords: Cold-inducible RNA binding protein; *Xenopus*; Cell movement; Morphogenetic lineage migration; Neural development

The cold-inducible RNA binding proteins (CIRP) are a family of RNA binding proteins that possess one consensus-sequence RNA-binding domain, one major RNA binding motif, and one carboxyl-terminal glycine-rich domain [1]. CIRP from humans [2], rats [3], mouse [4], Mexican axolotls [5], bull frogs [6], *Xenopus laevis* [7–9], and *Anabaena variabilis* [10] have been identified. In *Xenopus*, three isoforms of CIRP have been reported: XCIRP, XCIRP-1, and XCIRP2. The XCIRP mRNA is present in the oocyte and expression increases rapidly after fertilization. During later stages of embryonic development, the mRNA is localized mainly in neural tissues and the presumptive pronephros, suggesting that it plays a role in kidney and neural development. Previously, using the capped antisense mRNA approach, we showed that XCIRP-1 is required for embryonic development as suppression of XCIRP-1 produced multiple defects in the brain and internal organs. We further demonstrated that XCIRP-1 is required in the specification and morphogenetic lineage migration of the embryonic kidney, the pronephros [8]. However, the underlying molecular mechanisms remain elusive.

In this study, we designed two morpholino antisense oligonucleotides (MOs) which cover conserved sequences to

☆ *Abbreviations:* CIRP, cold-inducible RNA binding protein; RT, reverse transcription; nt, nucleotide; PCR, polymerase chain reaction.

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suppress the expression of all three isoforms. These MOs enabled loss-of-function studies designed to specifically target the *XCIRP* mRNA of the CIRP family of genes and block their translation. We demonstrated that XCIRP has a function in early embryonic cell movement as suppression of XCIRP in early embryo development by either anti-sense mRNA or MOs reduced the rate of cell movement and inhibited eFGF and activin-induced animal cap elongation. In terms of molecular mechanisms, we further demonstrated that a panel of adhesion molecules are XCIRP targets: αE- and β-catenin, C- and E-cadherin, and paraxial proto-cadherin (PAPC). Suppression of XCIRP reduced the mRNA level of αE- and β-catenin, C- and E-cadherin, and PAPC. The co-expression of sense XCIRP-1 can fully rescue all of the aforementioned phenotypes. Taken altogether, results from these studies demonstrated that XCIRP is essential for embryonic development because it is required for the expression of a set of adhesion molecules, essential for embryonic cell movement and morphogenetic lineage migration.

Materials and methods

Embryo microinjection and animal cap explant culture. Adult pigmented *Xenopus laevis* specimens were obtained from *Xenopus* I, Inc. (Dexter, MI). *Xenopus laevis* embryos were obtained by artificial insemination after females were injected with 500 U of human chorionic gonadotropin. The embryos were chemically dejellied using 2% cysteine and then washed and transferred to Petri dishes containing 0.3 × MMR solution (0.1 M NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM Hepes, and 0.1 mM EDTA) and 3% Ficoll (Amersham Biosciences). Embryos were staged according to Nieuwkoop and Faber's table [11].

Microinjections were performed as previously described [8]. Briefly, embryos at the 2-cell stage were injected in the animal poles with various mRNAs and morpholino antisense oligonucleotides. The embryos were cultured up to 45 stages after injection. Animal caps were dissected from the injected embryos at a point between stages 8.5 and 9. They were cultured at 22 °C in 67% Leibovitz's L-15 medium (Invitrogen) with 7 mM Tris–HCI (pH 7.5) and gentamicin (50 μg/ml) with or without growth factors until various stages as indicated and harvested for RT-PCR analysis.

RT-PCR. Total RNA was extracted from embryos with a Trizol reagent (GIBCO Bethesda Research Laboratory) in accordance with the manufacturer's instructions. RT-PCR was performed using the Superscript Preamplification System (GIBCO Bethesda Research Laboratory). Primer sets and PCR conditions for EF-1α [12] have already been described. Primer sets and PCR conditions for αE-catenin and β-catenin, C- and E-cadherin, as well as paraxial proto-cadherin (PAPC), XCIRP, Cyclin B1, Nek2B, ARF6, SOX2, and BMP4 are as follows: αE-catenin (5′-GCAGATGGATTCGGCTGAATCCTG-3′ and 5′-GGTCATTAT CAGAGGGCTTCTCTTTG-3′, 31 cycles); β-catenin (5′-GCAGTTGC TGTATTCGCCTATTGAA-3′ and 5′-TACAAGTCAGTGTCAAAC CAGGCCAGT-3′, 31 cycles); C-cadherin (5′-TGATGTCCTCTCTCA GGTCAAG-3′ and 5′-TTATGGCTGTTCAAGTCCAGTG-3′, 29 cycles); E-cadherin (5′-CTACCTCCAGAAGATGAGACTC-3′ and 5′-GGAGCCACTGCCTTCGTAATCG-3′, 30 cycles); paraxial proto-cadherin (PAPC) (5′-CCGCAGATATCAAGACAGCTTCAT-3′ and 5′-CACGTCCAAAGTAGGTGAGATGCATA-3′, 33 cycles); XCIRP (5′-G ACGAAGGAAAACTCTTTATTG-3′ and 5′-CAGAAGAGCCACC TCTGTAA-3′, 32 cycles); Cyclin B1 (5′-CTAAAATTGGAGAGGTA ACTGC-3′ and 5′-TAACAGTCAGATGCTTGGTTAG-3′, 32 cycles); Nek2B (5′-AAGGATGGCAGAAGATAAAAT-3′ and 5′-AACAAA TAAAAGTGCATCATTG-3′, 33 cycles); ARF6 (5′-TAACAAACA AGATCTCCCTGAC-3′ and 5′-AGTAAAAGTATTCCAGCCCATA-3′, 33 cycles); SOX2 (5′-GAGGATGGACACTTATGCCCAC-3′ and 5′-GGACATGCTGTAGGTAGGCGA-3′, 30 cycles); BMP4 (5′-GCA TGTACGGATAAGTCGATC and 5′-GATCTCAGACTCAAC GGCA C-3′, 30 cycles). PCR conditions were as follows: 15 min at 95 °C, 1 min at 55 °C, and 1 min at 72 °C for the indicated number of cycles. Although data were retrieved from individual experiments, multiple PCR cycles were conducted before every experiment to determine the proper cycle settings. The results were confirmed in three independent experiments.

Design of morpholino antisense oligonucleotides. We designed two separate, non-overlapping, morpholino antisense oligonucleotides (24-mer) in the conserved region (Gene Tools LLC, OR) based on the cDNA sequence of the three XCIRP isoforms. The first one (MOa: 5′-ACGTTTGCTGCTCGTCAAAGAATT-3′) spans from the final base-pairs of the 5′UTR, the translation start site, to 21 base-pairs into the coding region of the XCIRP mRNA. The second one (MOb:5′-ACGTTTGCTGCTCGTCAAAGAATT-3′) spans from 30 to 7 bp upstream of the ATG translation start site. For control experiments, the standard control morpholino from Gene Tools LLC was used (sMO:5′-CATGTCTGACGAAGGAAAACTCTTT-3′). These sequences were screened against the GenBank nucleotide sequence data bank to confirm that the specific XCIRP morpholinos matched with all isoforms of the XCIRP genes while the standard control morpholino sequence did not match any genes in GenBank. The sense and anti-sense capped mRNAs were synthesized from linearized plasmid constructs by in vitro synthesis as described previously [8].

Assay of cell migration rate and whole-mount X-gal staining and histology. We injected a tracer mRNA encoding β-gal either alone as a control, or with sense XCIRP-1, or with morpholino antisense oligonucleotides (MO), or with the combination of MO and sense XCIRP-1 to the Dorsal Marginal Zone (DMZ) of the 4-cell stage embryo. These embryos were collected at appropriate stages and fixed. The rate of cell migration was determined by staining the embryos for the presence of β-gal with X-gal by standard staining techniques. For histological analysis, embryos were fixed in 2% paraformaldehyde, dehydrated, embedded in paraffin, sectioned, and stained as indicated.

Antibodies and immunoblotting. XCIRP-1 was amplified by PCR using 5′-GGCAC*GAATTC*TCTGACGAAGGAAAACTC-3′ forward primer (italics indicating *Eco*RI site) and 5′-GCGAT*CTCGAG* TTACTCGTGTGTAGCATAG-3′ reverse primer (italics indicating *Xho*I site) using pCR2.1-XCIRP-1 as a template [8]. The amplified fragment was gel-purified and ligated into pGEX 4T-1 (GE Healthcare). GST-XCIRP1 was expressed in *Escherichia coli* strain BL21-DE3 and purified with glutathione–Sepharose using the GST tag under standard conditions (GE Healthcare, GST Purification) to a purified yield of 4.8 mg/L culture. The GST tag was removed by proteolytic cleavage with thrombin under standard conditions (GE Healthcare). Untagged, purified XCIRP-1 was used to generate polyclonal antibodies in rabbits. For Western blotting, embryos were homogenized in a solution of 10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. Protein extracts were then subjected to SDS–PAGE and transferred to a polyvinylidene difluoride membrane. Immunoblotting analysis was conducted as described [9,13].

Identification of XCIRP target RNA by immunoprecipitation followed by RT-PCR. Xenopus embryos at stage 11 were homogenized in buffer A (20 mM Hepes, pH 7.5, 2 mM $MgCl_2$, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) containing 100 mM KCl as described previously [9]. Following centrifugation at 12,000*g* for 10 min, the lysates were incubated with rabbit polyclonal antibody against the recombinant XCIRP-1 bound to protein A-agarose in buffer A containing 100 mM KCl at 4 °C for 30 min. The co-precipitated RNA was purified three times by centrifugation. RNA was then recovered from the immunoprecipitates and reverse transcribed with oligo(dT) primer, and the presence of genes was determined by PCR using specific primer sets.

Results

Suppression of XCIRP expression by morpholino antisense oligonucleotides or anti-sense mRNA produced defects in Xenopus embryonic development

We suppressed XCIRP expression by microinjection of different concentrations of MOa and MOb into both blastomeres of 2-cell stage embryos and observed the resultant phenotypes. While over-expression of XCIRP-1 mRNA by microinjection of 1 ng of sense XCIRP-1 mRNA did not produce any effect on embryonic development, suppression of XCIRP by MOs dose dependently produced defective embryos. As shown in Table 1, at 1 nM of MOa or MOb, 13% and 10% of the embryos had defective phenotypes, respectively, and 82% were normal. At 5 nM of MOa or MOb, 70% and 76% of the embryos had defective phenotypes, respectively, 21% and 17% were normal, respectively. We also showed that co-injection of 1 ng of sense XCIRP-1 mRNA with 5 nM of MOs, significantly rescued the embryos from deformation (Table 1). Similar results were also obtained when anti-sense mRNA of XCIRP-1 were used instead of MOs.

To confirm the effects of MOs on XCIRP expression, immunoblotting analysis of the embryo lysates from stage 11 embryos was performed. As shown in Fig. 1A, XCIRP protein expression was suppressed by MOa/MOb injection while increased by XCIRP-1 mRNA injection.

Morphologically, over-expression of the sense XCIRP-1 mRNA (1 ng) did not produce any effect on embryonic development as observed up to stage 45. However, suppression of XCIRP mRNA by MOa or MOb produced defects which could be recognized starting from gastrulation stages as shown in Figs. 1B and C (stage 11, midgastrula stage). Severe defects in neural development were observed as shown: failure of anterior neural tube closure (stage 18, neural groove stage) and deformed neural development in tailbuds and tadpoles (stages 32 and 45).

Histological analysis of the sections of the stage 11 embryos showed aberrant cell movement and decreased advancing of the dorsal blastopore lip (D) and ventral blastopore groove (V) which resulted in a large yolk plug (Fig. 1D). Parasagittal section of the stage 18 embryos (Fig. 1E) showed that XCIRP-MOa inhibited the formation of the sensorial layer of neurectoderm (N), caused the failure of the somitogenic mesoderm (M) to segment, and showed marked defects in the formations of brain and eye anlages (BE). The anterior transversal section of the stage 18 embryos (Fig. 1F) showed that XCIRP MOa caused marked defect in the formation of the neural crest (NC), neural tube (NT), and notochord (Noto).

Suppression of XCIRP reduced cell movement and inhibited eFGF- and activin-induced animal cap elongation

To test whether aberrant cell movement is one of the possible mechanisms, we injected a tracer mRNA encoding β-gal either alone as a control, or with 1 ng of sense XCIRP-1, or with 5 nM of MOa, or with both sense XCIRP-1 and MOa to the Dorsal Marginal Zone (DMZ) of the 4-cell stage embryos. The embryos were allowed to develop until the control group reached stage 11, then, embryos were fixed and cell movement was visualized by β-gal whole mount staining with X-gal. As shown in Fig. 2A, no difference was observed in the X-gal staining between control β-gal injected and the sense XCIRP-1 injected embryos. However, MOa injection caused a marked decrease of cell movement as indicated by a significant reduction in both the area and the intensity of the β-gal staining in these embryos. This inhibitory effect could be fully restored by co-injection of 1 ng sense XCIRP-1. Similar results were also observed when the anti-sense mRNA of XCIRP-1 was used instead of MOa.

Activin and eFGF have both been shown to be essential for coordinating cell movements associated with gastrulation [14–16], and these processes can be studied ex vivo in *Xenopus* animal caps (ACs) explants. Therefore, an AC elongation assay was used to further evaluate the activity of MO in cell movement. We injected 1 ng of either control β-gal mRNA, or sense XCIRP-1 mRNA, or MOa, or combined sense XCIRP-1 mRNA with MOa, into the two blastomeres of the 2-cell embryos. ACs were dissected out and incubated as indicated in Materials and methods. As

Table 1
Abnormal phenotype induced by XCIRP transcripts[a]

Injection samples	No. of embryos	Normal	Defect. phenotype[b]	Dead
1 ng β-gal RNA	75	94% (70)	0% (0)	6% (5)
1 ng S RNA	75	90% (68)	5% (4)	5% (4)
5 nM MOa	75	21% (16)	70% (52)	9% (7)
5 nM MOb	75	17% (13)	76% (57)	7% (5)
1 nM MOa	75	82% (62)	13% (10)	5% (3)
1 nM MOb	75	82% (61)	10% (8)	8% (6)
5 nM MOa + 1 ng S RNA	75	62% (47)	21% (16)	17% (12)
5 nM MOb + 1 ng S RNA	75	56% (42)	27% (20)	17% (13)

[a] Values are the combined data of three independent experiments with each experiment consisting of 25 embryos. Bracketed figures indicate the number of embryos with the specified phenotype.
[b] Defective phenotypes include malformations of the brain and the internal organs.

shown in Fig. 2B, activin and eFGF induced AC elongation in both control and XCIRP-1 injected embryos. However, the injection of MOa completely inhibited eFGF and activin-induced AC elongation. Furthermore, co-injection of sense XCIRP-1 with MOa fully reversed the inhibition caused by MOa. In AC without growth factor incubation, no AC elongation was observed (Fig. 2B, bottom panel).

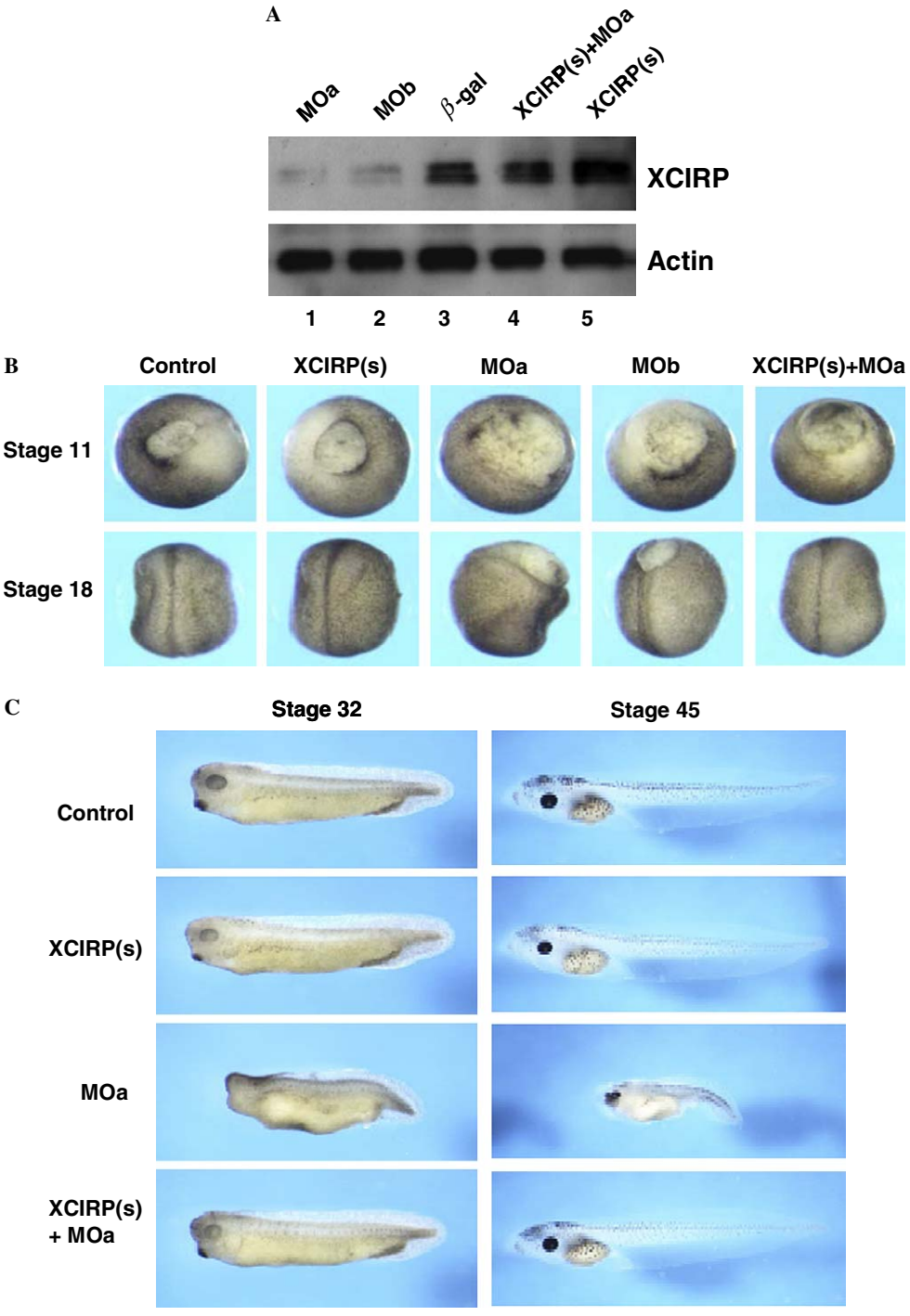

Fig. 1. Suppression of XCIRP by MOs induces gastrulation defect. The two blastomeres of the 2-cell stage embryos were injected with 2 ng of sense XCIRP, 5 nM MOa XCIRP, 5 nM MOb XCIRP, and (s + MOa) XCIRP transcripts or equal amount of β-gal mRNA (control, c). (A) The embryos were allowed to develop to stage 11, and the embryos were then homogenized and subjected to Western blotting analysis. Lane 1, MOa; lane 2, MOb; lane 3, β-gal; lane 4, sXCIRP + MOa; and lane 5, sXCIRP. (B,C) Embryos were allowed to develope to stages 11, 18, 32, and 45, as indicated. (D–F) Histological analyses of the control and defected embryos stained with H and E. (D) At stage 11, sagittal sections showed defects in the movement of cells. (E) At stage 18, parasagittal section showed malformations of neurectoderm (N), mesoderm (M), brain, and eye anlages (BE). (F) At stage 18, anterior transversal section showed defect in neural crest (NC), neural tube (NT), and notochord (Noto).

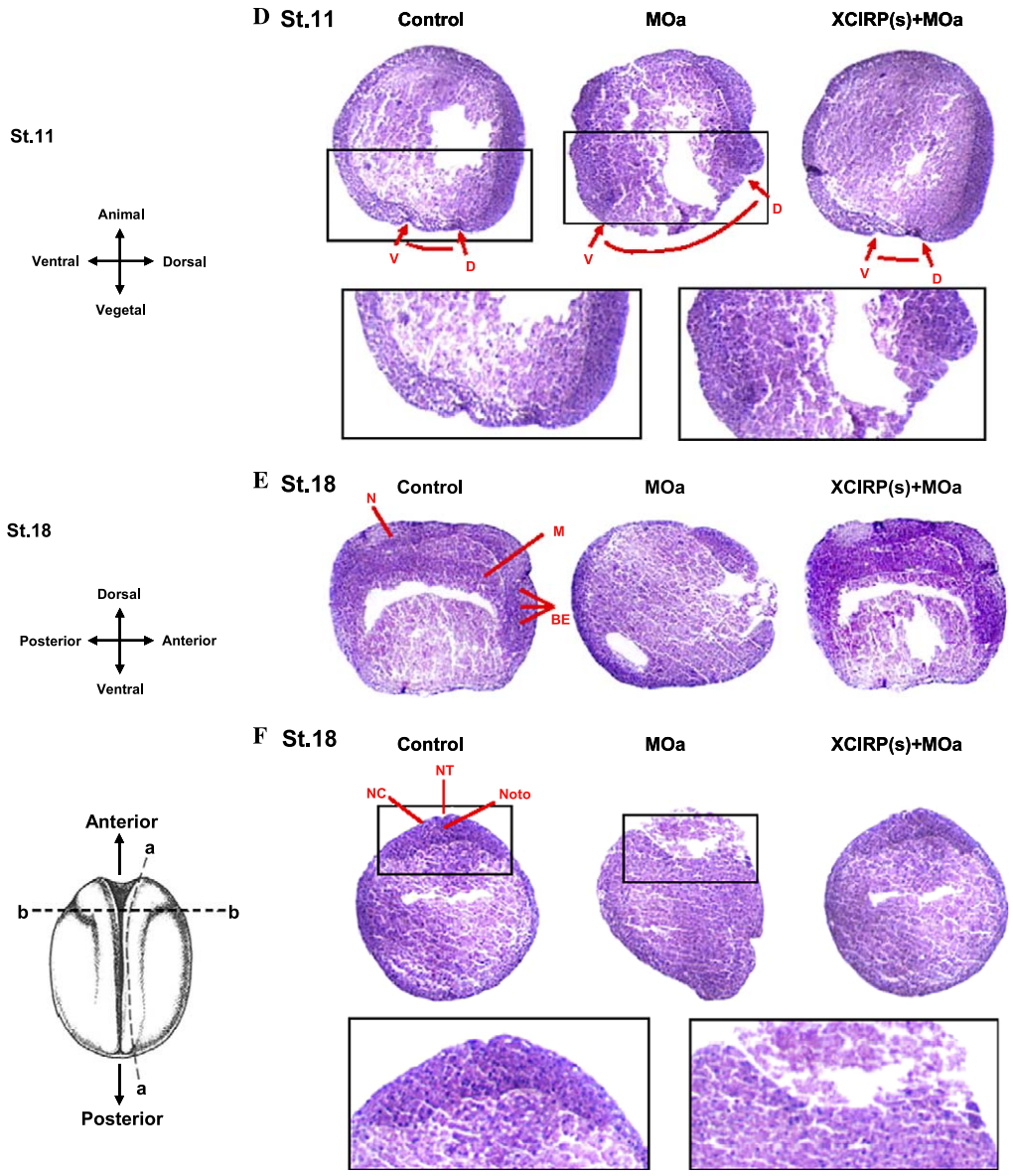

Fig 1. (*continued*)

Suppression of XCIRP disrupted the morphogenetic lineage migration of A1 blastomeres

Morphogenetic lineage movement plays an important role in the patterning and specification of cells during organogenesis. The A1 blastomere eventually forms most of the hindbrain, the spinal cord, a substantial portion of all the head mesenchyme, provides a minor contribution to the forebrain, the somites, and the notochord. We were thus inspired to study the effects of XCIRP inhibition specifically in terms of the migration and differentiation of the A1 blastomeres. We injected the morpholino antisense oligonucleotides (MOa) and/or tracer RNA encoding β-gal (0.05 ng) into an A1 blastomere of an embryo at the 32-cell stage. The migrations of the A1 blastomeres were determined at the tailbud stage (stage 30) by X-gal whole-mount staining of the tracer β-gal. As expected, the X-gal stain distributed proportionally in all of the corresponding tissues in control tailbud injected with β-gal alone (Fig. 3A). However, tailbuds injected with MOa had significantly reduced X-gal stain in the brain and spinal cord regions (Fig. 3B). Histological section of the brain revealed a reduced X-gal staining as well as defective brain structures (Fig. 3D) as compared to the control β-gal injected embryos (Fig. 3C).

Identification of a panel of adhesion molecules as the XCIRP target mRNA in Xenopus embryos

It has been shown in *Xenopus* oocyte that XCIRP-2 is required for the stabilization and the maintenance of expression of specific mRNAs including Cyclin B1and Nek2B [9]. It is proposed that XCIRP stabilizes these specific mRNAs through interaction with them. Therefore, we

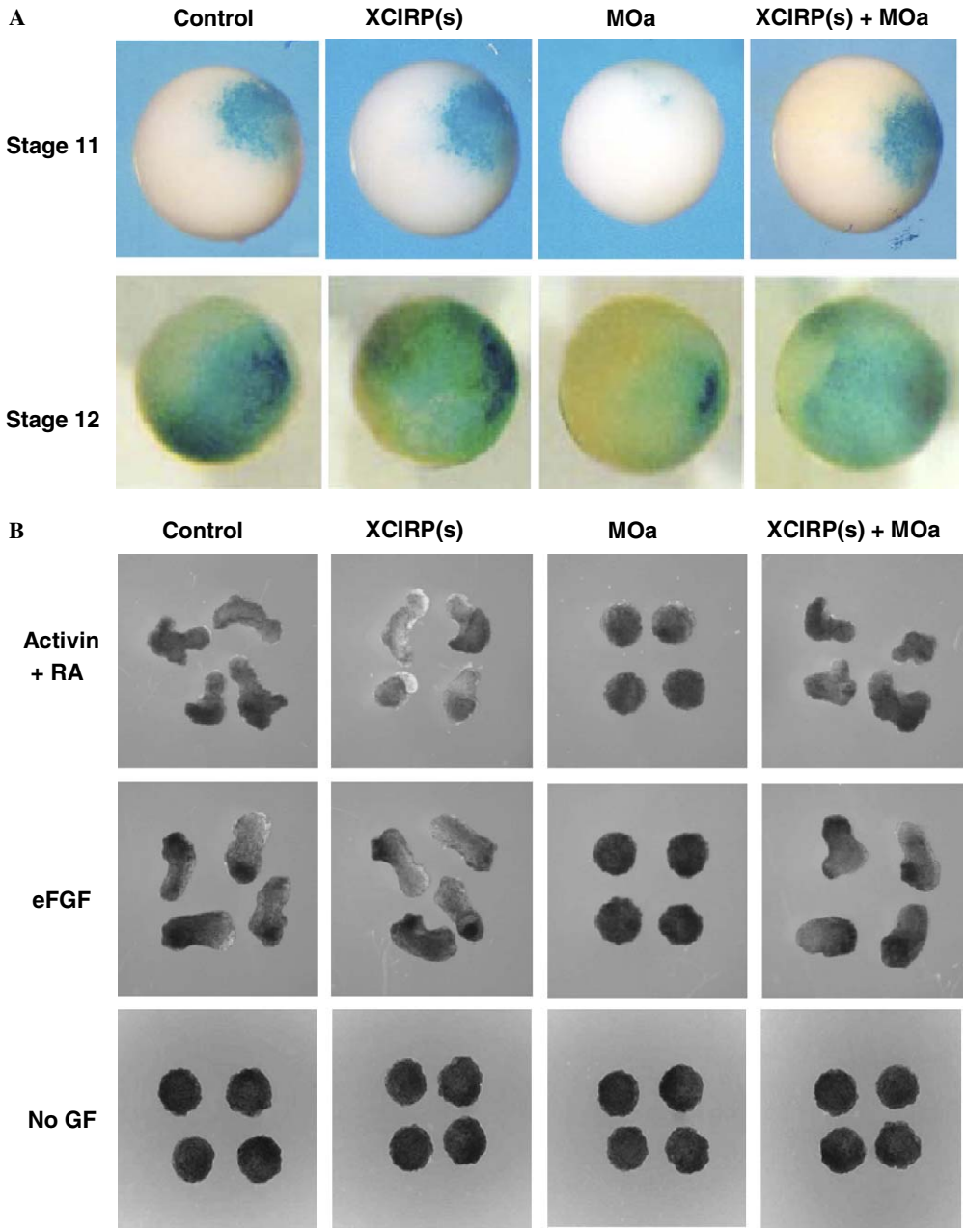

Fig. 2. Suppression of XCIRP inhibited cell movement and eFGF and activin-induced animal cap elongation. (A) Four-cell stage embryos were microinjected at the Dorsal Marginal Zone with a single injection of 2 ng of β-gal (control), 2 ng of sense XCIRP-1 (XCIRP(s)), 5 nM MOa, and combination of XCIRP(s) + MOa as indicated. The embryos were allowed to develop until the control group reached stage 11 and then fixed for X-gal whole-mount staining of the β-gal. (B) The two blastomeres of the 2-cell stage embryos were injected with 2 ng of β-gal mRNA (control), 2 ng of sense XCIRP(s), 5 nM MOa, or XCIRP(s) + MOa. Embryos were developed to stage 8.5, animal cap explants prepared, and an elongation assay was conducted as described in Materials and methods.

attempted to identify these mRNAs by immunoprecipitation with rabbit polyclonal antibody against the recombinant XCIRP-1 followed by RT-PCR. *Xenopus* embryos at stage 11 were homogenized, the lysates were incubated with rabbit polyclonal antibody against the recombinant XCIRP-1 bound to protein A-agarose. The co-precipitated RNA was reverse transcribed with oligo(dT) primer, and the presence of genes was determined by PCR using specific primer sets as described in Methods. We first showed that the previously identified oocyte XCIRP2-interacting mRNAs, *Xenopus* cyclin B1 and Nek2B mRNAs, were detected in the immunoprecipitates. In addition, a panel of adhesion molecules, the αE- and β-catenin, C- and E-cadherin, were also detected (Fig. 4A).

Suppression of XCIRP by MO inhibited the expressions of a panel of adhesion molecules

To test whether MOa and MOb could reduce the mRNA level of the interacting mRNA, animal cap (AC)

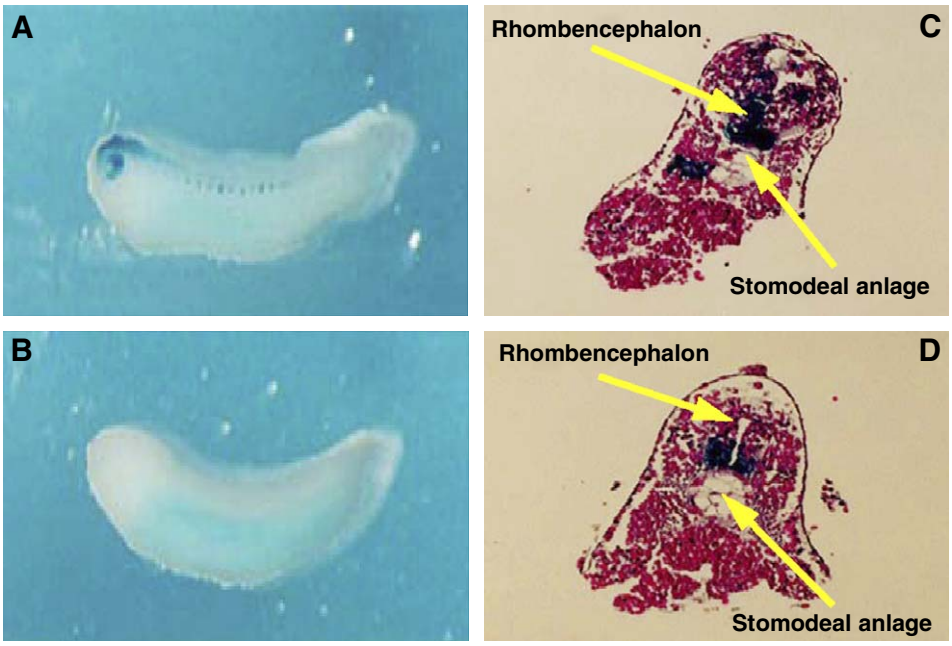

Fig. 3. Suppression of XCIRP disrupted the morphogenetic lineage migration of A1 blastomeres. The morphogenetic lineage migration of A1 blastomeres was examined by injecting to one of the A1 blastomeres (at 32-cell stage) with the following mRNAs. (A,C) 0.05 ng of β-galactosidase (β-gal); (B,D) β-gal with 5 nM of MOa. The morphogenetic migration pattern of the A1 blastomeres was visualized by X-gal whole-mount staining (A,B) and histological analysis of the dorsal section (C,D) of the stages 28–30 tailbuds.

explant studies were then conducted to confirm that CIRP is indeed required to stabilize these interacting mRNAs. ACs were incubated with eFGF at stage 8.5 and harvested at stage 22 for RNA analysis. We showed that suppression of XCIRP by MOa effectively decreased the expression of all isoforms of XCIRPs (Figs. 4B and C, lanes 3 and 5), and reduced the expression of cyclin B1, Nek2B, the pan neural marker *NCAM*, and a set of the cell movement related genes: αE- and β-catenin, C- and E-cadherin, as well as paraxial proto-cadherin (PAPC). However, they had no effect on the expressions of ARF6, another cell movement related gene, SOX2, or BMP4 (Fig. 4B, lanes 3 and 5). Each inhibition could be rescued by full length sense XCIRP-1 transcript (Figs. 4B and C, lanes 4 and 6). In contrast, microinjection of β-gal or sense XCIRP-1 alone had no effect on gene expression (Figs. 4B and C, lanes 1 and 2).

Discussion

In this study, we demonstrated that XCIRP is required for embryonic gastrulation and neural development by facilitating proper cell movement. We showed that suppression of XCIRP inhibited cell movement and eFGF and activin-induced animal cap elongation, as well as inhibiting the expression of a specific set of adhesion molecules. As regulation of cell–cell adhesion is of particular importance for cell movement during gastrulation, we propose that XCIRP exerts its effect at least in part by maintaining the expressions of C- and E-cadherin, αE- and β-catenin, as well as paraxial proto-cadherin (PAPC), to facilitate proper cell movement.

Morpholino antisense oligonucleotide functions mainly as an inhibitor of translation of target mRNA. Therefore, it was surprising that MOa and MOb injections not only reduced XCIRP protein but also inhibited XCIRP mRNA expression. The possibility that MOa and MOb may have caused XCIRP mRNA degradation, or that XCIRP protein can also stabilize its own mRNA cannot be excluded and will require further investigation.

It has been well documented that C-cadherin-mediated cellular adhesion correlates with convergent extension movements of mesodermal cells [17]. E-cadherin has also been shown to modulate cell migration and maturation by regulating intracellular contacts [18]. The function and expression of the catenin family of proteins are closely related to those of the cadherins. It has been shown that embryonic cells respond to the over-expression of cadherin by raising levels of β-catenin, whereas depletion of maternal cadherin mRNA leads to a complete loss of β-catenin. Furthermore, αE-catenin is essential for connecting the cadherin/β-catenin complex to the sub-membrane cytoskeleton and thus, could be instrumental for the regulation of cell–cell adhesion [19]. The paraxial proto-cadherin (PAPC) encodes a transmembrane protein expressed initially in the Spemann's organizer and then in the paraxial mesoderm. Together with another member of the proto-cadherin family, axial proto-cadherin (AXPC), it subdivides the gastrulating mesoderm into paraxial and axial domains. PAPC has potent homotypic cell adhesion activity and plays an important role in the convergence and extension movements that drive *Xenopus* gastrulation. Therefore, the suppression of these adhesion molecules is likely to produce the observed phenotypes [20].

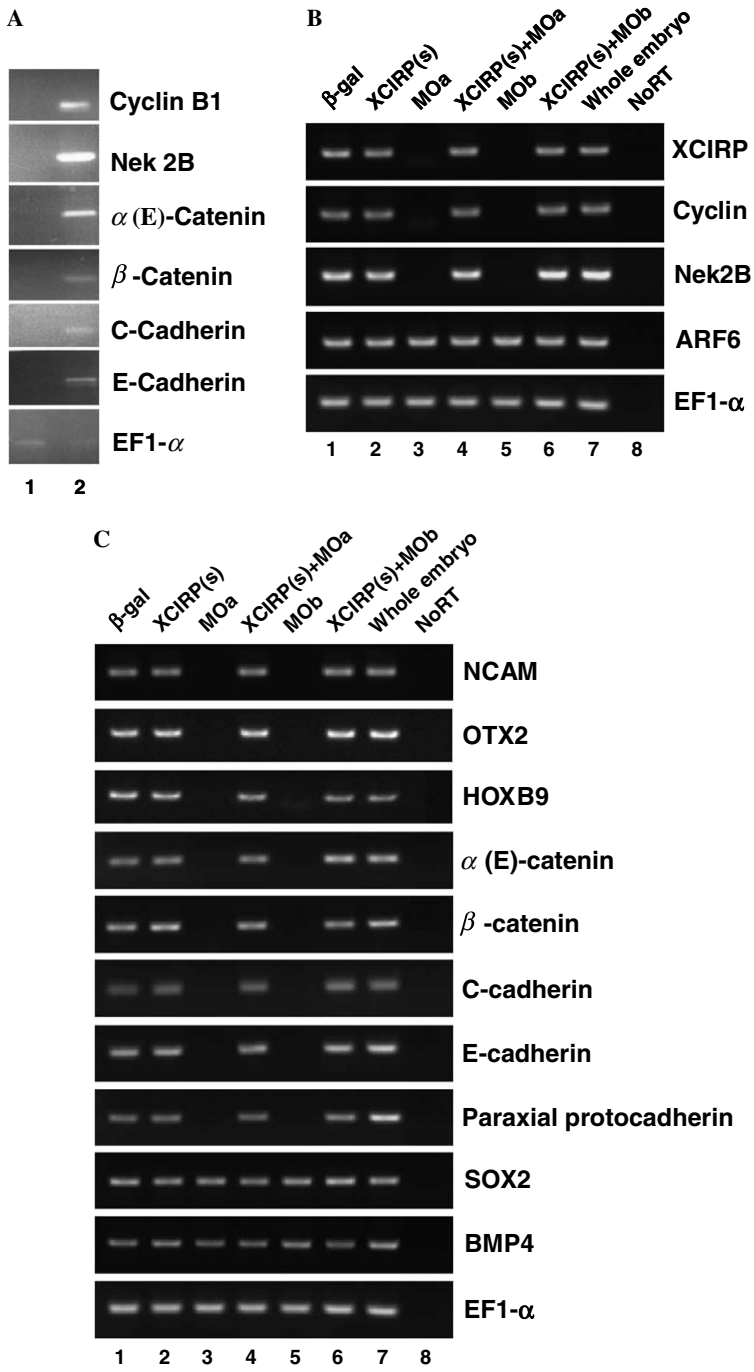

Fig. 4. Identification of a panel of adhesion molecules as XCIRP target mRNAs in the embryos. (A) *Xenopus* embryo lysates were immunoprecipitated with preimmune serum (lane 1) or the anti-XCIRP serum (lane 2), followed by RT-PCR with specific primer sets as indicated. (B,C) Embryos were injected with various mRNA and MOs, and animal caps were prepared as indicated. Total RNA was extracted from the animal caps when the control embryos were developed to stage 22. The expression of XCIRP, cyclin B1, Nek2B, ARF6, EF1α, NCAM, SOX2, BMP4, αE- and β-catenin, C- and E-cadherin, as well as paraxial proto-cadherin (PAPC) were determined by RT-PCR as described in Materials and methods.

The mechanisms by which XCIRP participates in the expression of these adhesion molecules remain elusive. It has been suggested that one of the physiological functions of XCIRP is to protect and restore native RNA conformations during stress (i.e., to serve as a chaperonin for RNA). Evidence for this role has been reported in human colorectal carcinoma (RKO) cells where CIRP, also termed A18 hnRNP, was found to move from the nucleus to the cytoplasm after UV irradiation. While there, it binds specifically to the 3′ untranslated region (UTR) of RNA molecules involved in the regulation of translation and in RNA stability [21]. The binding of proteins to the 3′-UTR of a transcript can in turn affect both the rate of translation initiation and the stability of a transcript. More recently, by studying the XCIRP2 interacting proteins and the binding mRNA substracts, it has been proposed that XCIRP2

interacts with these mRNAs and inhibits deadenylation of AU-rich element-containing mRNA, and can regulate the length of the poly(A) tail of mRNA in *Xenopus* oocytes [9].

In summary, results from our study suggest for the first time that a set of adhesion molecules are also the mRNA substrates of XCIRP and that XCIRP is essential for cell movement during *Xenopus* embryonic development.

Acknowledgments

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References

[1] C.G. Burd, G. Dreyfuss, Conserved structures and diversity of functions of RNA-binding proteins, Science 265 (1994) 615–621.

[2] H. Nishiyama, H. Higashitsuji, H. Yokoi, K. Itoh, S. Danno, T. Matsuda, J. Fujita, Cloning and characterization of human CIRP (cold-inducible RNA-binding protein) cDNA and chromosomal assignment of the gene, Gene 204 (1–2) (1997) 115–120.

[3] J.H. Xue, K. Nonoguchi, M. Fukumoto, T. Sato, H. Nishiyama, H. Higashitsuji, K. Itoh, J. Fujita, Effects of ischemia and H_2O_2 on the cold stress protein CIRP expression in rat neuronal cells, Free Radic. Biol. Med. 27 (1999) 1238–1244.

[4] H. Nishiyama, K. Itoh, Y. Kaneko, M. Kishishita, O. Yoshida, J. Fujita, A glycine-rich RNA-binding protein mediating cold-inducible suppression of mammalian cell growth, J. Cell Biol. 137 (1997) 899–908.

[5] R. Bhatia, D.K. Dube, A. Gaur, D.R. Robertson, S.L. Lemanski, M.D. McLean, L.F. Lemanski, Expression of axolotl RNA-binding protein during development of the Mexican axolotl, Cell Tissue Res. 297 (1999) 283–290.

[6] T. Saito, K. Sugimoto, Y. Adachi, Q. Wu, K.J. Mori, Cloning and characterization of amphibian cold inducible RNA-binding protein, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 125 (2000) 237–245.

[7] T. Uochi, M. Asashima, XCIRP (*Xenopus* homolog of cold-inducible RNA-binding protein) is expressed transiently in developing pronephros and neural tissue, Gene 211 (1998) 245–250.

[8] Y. Peng, K.H. Kok, R.H. Xu, K.H. Kwok, D. Tay, P.C. Fung, H.F. Kung, M.C. Lin, Maternal cold inducible RNA binding protein is required for embryonic kidney formation in *Xenopus laevis*, FEBS Lett. 482 (2000) 37–43.

[9] K. Aoki, K. Matsumoto, M. Tsujimoto, *Xenopus* cold-inducible RNA-binding protein 2 interacts with ElrA, the *Xenopus* homolog of HuR, and inhibits deadenylation of specific mRNAs, J. Biol. Chem. 278 (2003) 48491–48497.

[10] N. Sato, A family of cold-regulated RNA-binding protein genes in the cyanobacterium *Anabaena variabilis* M3, Nucleic Acids Res. 23 (12) (1995) 2161–2167.

[11] P.D. Nieuwkoop, J. Faber, Normal Table of *Xenopus laevis* (Daudin), North Holland, Amsterdam. The Netherlands, 1967.

[12] P.A. Krieg, S.M. Varnum, W.M. Wormington, D.A. Melton, The mRNA encoding elongation factor 1-alpha (EF-1 alpha) is a major transcript at the midblastula transition in *Xenopus*, Dev. Biol. 133 (1989) 93–100.

[13] R.H. Xu, J. Kim, M. Taira, D. Sredni, H.F. Kung, Studies on the role of fibroblast growth factor signaling in neurogenesis using conjugated/aged animal caps and dorsal ectoderm-grafted embryos, J. Neurosci. 17 (1997) 6892–6898.

[14] H. Isaacs, M. Pownall, J. Slack, eFGF regulates *Xbra* expression during *Xenopus* gastrulation, EMBO J. 13 (1994) 4469–4481.

[15] J.C. Smith, Mesoderm-inducing factors and mesodermal patterning, Curr. Opin. Cell Biol. 7 (1995) 856–861.

[16] J.C. Smith, J.E. Howard, Mesoderm-inducing factors and the control of gastrulation, Dev. Suppl. (1992) 127–136.

[17] W.M. Brieher, B.M. Gumbiner, Regulation of C-cadherin function during activin induced morphogenesis of *Xenopus* animal caps, J. Cell Biol. 126 (1994) 519–527.

[18] A.S. Yap, W.M. Brieher, M. Pruschy, B.M. Gumbiner, Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function, Curr. Biol. 7 (1997) 308–315.

[19] T. Kurth, I.V. Fesenko, S. Schneider, F.E. Munchberg, T.O. Joos, T.P. Spieker, P. Hausen, Immunocytochenical studies of the interactions of cadherins and catenins in the early *Xenopus* embryo, Dev. Dyn. 215 (2) (1999) 155–169.

[20] S.H. Kim, A. Yamamoto, T. Bouwmeester, E. Agius, E.M. Robertis, The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation, Development 125 (23) (1998) 4681–4690.

[21] C. Yang, F. Carrier, The UV-inducible RNA-binding protein A18 (A18 hnRNP) plays a protective role in the genotoxic stress response, J. Biol. Chem. 276 (2001) 47277–47284.