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Loss of cell adhesion in *Xenopus laevis* embryos mediated by the cytoplasmic domain of XLerk, an erythropoietin-producing hepatocellular ligand

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ABSTRACT **The erythropoietin-producing hepatocellular (Eph) family of ligands and receptors has been implicated in the control of axon guidance and the segmental restriction of cells during embryonic development. In this report, we show that ectopic expression of XLerk, a *Xenopus* homologue of the murine Lerk-2 (ephrin-B1) transmembrane ligand, causes dissociation of *Xenopus* embryonic blastomeres by the mid-blastula transition. Moreover, a mutant that lacks the extracellular receptor binding domain can induce this phenotype. The carboxyl-terminal 19 amino acids of the cytoplasmic domain of XLerk are necessary but not sufficient to induce cellular dissociation. Basic fibroblast growth factor, but not activin, can rescue both the loss of cell adhesion and mesoderm induction in ectodermal explants expressing XLerk. Collectively, these results show that the cytoplasmic domain of XLerk has a signaling function that is important for cell adhesion, and fibroblast growth factor signaling modulates this function.**

The erythropoietin-producing hepatocellular (Eph) family of receptor tyrosine kinases is among the newest and largest families to emerge (1). The original Eph receptor tyrosine kinase cDNA was isolated from a human hepatoma library (2). Overexpression of Eph family transcripts has been observed in some melanomas and carcinomas of the colon, lung, and liver (3, 4). Most of the family members are expressed primarily, but not exclusively, in the nervous system. These receptors have been implicated to control axon guidance in retinotectal and commissural projections (5–9). Roles in axonal bundling (9, 10) and segmental integrity have been reported (11, 12). The large size of this family may provide the diversity of signals that are necessary to mediate specific events affecting neuronal guidance and cell movement during embryonic development.

Recent findings suggest that AL-1 (ephrin-A5) and its cognate receptor participate in axon bundling (5, 6, 10), whereas Elf-1 (ephrin-A2) has been demonstrated to cause repulsion of axons (8, 13). Additionally, Cek5 and Qek5 (EphB2) have been found to display complementary gradients with their proposed ligands in the retinotectal system, suggesting a role in retinal neuron guidance (14, 15).

The Eph ligands are membrane bound, and those ligands anchored by a glycosyl phosphatidylinositol tail belong to a subgroup having greater affinity for Eck-related receptors (EphA), whereas transmembrane ligands preferentially bind the Elk-related receptors (EphB) (16, 17). Genetic evidence suggests that one of the transmembrane Eph ligands, Lerk-2 (ephrin-B1), regulates axon guidance in the commissural neurons of the forebrain (7). Strikingly, proper axon guidance occurs in mice expressing a catalytically inactive cognate receptor (EphB2), suggesting that the ligand may transduce a signal upon association with the ectodomain of EphB2 (18, 19). In tissue culture cells, coexpression of ephrin-B1 and its cognate receptor (EphB1,B2) or coculturing cells expressing these proteins results in tyrosine phosphorylation of ephrin-B1 (18, 19). Therefore, it has been proposed that transmembrane Eph ligands are also receptor-like signaling molecules, but the nature of the cellular functions affected by ligand signaling is unknown.

We have isolated XLerk (20), an amphibian transmembrane ligand that has 72% overall identity with the closest mammalian family member, ephrin-B1 (Lerk-2) (21). However, the cytoplasmic domain of this molecule is the most conserved region with 95% identity, suggesting an important function for this domain. XLerk is most likely x-ephrin-B1, but this has not been determined with certainty at this time. XLerk is expressed at the highest levels in the olfactory epithelium and bulb, forebrain, hindbrain, retina, branchial arches, and myenteric plexus. XLerk transcripts are induced during gastrulation, a time of massive cell movement. XLerk transcripts are also substantially elevated during mesoderm induction in ectodermal explants and during neurulation stages of embryos (20).

To determine a possible cellular function for this Eph ligand, we ectopically expressed XLerk in the developing *Xenopus* embryo. Ectopic expression of XLerk causes a profound loss of cell adhesion by the blastula stage of development, and this phenotype can be induced in the absence of receptor binding. The intracellular signaling that results in cell dissociation can be rescued by activation of the fibroblast growth factor (FGF) receptor pathway in ectodermal explants expressing XLerk.

METHODS

Construction of XLerk Plasmids. A cDNA clone encoding full-length XLerk was tagged with a Flag octapeptide (IBI, Eastman Kodak) at either the C terminus or a nonconserved region of the ectodomain and inserted into a pSP64A vector. The partial cDNAs of XLerk encoding the transmembrane and cytoplasmic regions or cytoplasmic region alone were modified by adding a start codon ATG immediately before residue 215 or residue 251, respectively. The deletion mutants of XLerk were constructed by deleting the last C-terminal 19 amino acids or 39 amino acids from the full-length XLerk using PCR. All of these constructs were tagged with the Flag octapeptide and inserted into a pSP64A vector.

Embryo Injections. *Xenopus laevis* were obtained from Xenopus I (Ann Arbor, MI). Embryos were obtained by *in vitro* fertilization (22) and staged accordingly (23). Capped

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Abbreviations: Eph, erythropoietin-producing hepatocellular; FGF, fibroblast growth factor; bFGF, basic fibroblast growth factor; RT, reverse transcriptase; SEM, scanning electron microscopy.

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FIG. 1. XLerk-induced blastomere dissociation in *Xenopus* embryos. Each blastomere of the 2-cell stage embryo was injected with 2.5 ng of either capped XLerk mRNA or XLerk$^{1-215}$ mRNA, and development proceeded to the mid-blastula stage.

mRNA was synthesized according to the manufacturer (Ambion, Austin, TX). At the 2-cell stage, each blastomere was injected with 1–4 ng of the appropriate capped mRNA.

Scanning Electron Microscopy (SEM). Embryos were prepared for SEM according to the manufacturer (Ted Pella, Reading, CA). Briefly, the embryos were fixed in PBS containing 4% formaldehyde and 1.25% glutaraldehyde overnight at 4°C with gentle agitation. Following dissection, embryos were fixed again and stored at 4°C. Embryos were then washed three times with 0.1 M cacodylate buffer (pH 7.2) followed by postfixation in the same buffer containing 1% osmium tetroxide for 1 h. The embryos were dehydrated in graded ethanol (e.g., 35, 50, 70, 95, and 100%), infiltrated in a 1:1 mixture of absolute ethanol and Peldri, followed by pure Peldri for 1 h at 37°C. Following a change of pure Peldri, the solution was allowed to solidify at room temperature, and sublimation was completed in a vacuum chamber overnight. The embryos were attached on an SEM aluminum stub with double-sided adhesive tape and coated with gold palladium in a vacuum evaporator. Embryos were photographed with a Hitachi S-570 scanning electron microscope operated at 8 kV.

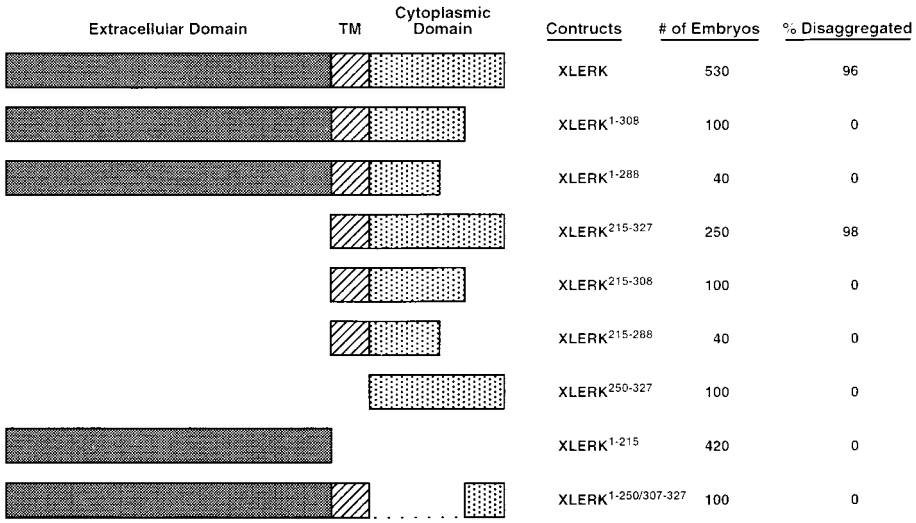
Animal Cap Assay. Animal caps were dissected at stage 8.5 and cultured at 23°C in 67% Leibovitz's L-15 medium (GIBCO/BRL), 7 mM Tris·HCl (pH 7.5) and 1 mg/ml gentamycin, with or without 50 ng/ml Activin (National Hormone Pituitary Program, NIH, Bethesda MD) or 100 ng/ml basic FGF (bFGF) (Pepro-Tech, Rocky Hill, NJ). The animal caps were harvested at stage 11 as determined by uninjected embryos.

Reverse Transcriptase (RT)-PCR Assay. RNA extraction from the animal cap explants was performed as described by the manufacturer (Triazol, GIBCO/BRL). To generate first strand cDNA, RNA from two animal cap equivalents was incubated with reverse transcriptase using oligo(dT) as primer. One-tenth of the reverse transcription product was used in each PCR reaction with [α-^{32}P]dCTP (3000 Ci mmol^{-1}; Amersham) added. The primers for EF-1α and Xbra were designed for PCR as previously described (24). The conditions for PCR of EF-1α were as follows: 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min for 19 cycles. The final step was 72°C for 10 min. Deviations from these conditions were as follows: gsc and Xbra were done for a total of 28 cycles using 55°C annealing temperature. The PCR products were electrophoresed on a 6% polyacrylamide gel and autoradiographed on Kodak XAR-5 film at −70°C for various exposure times.

DNA Ladder Fragmentation Assay. Stage 9 embryos were lysed, and the DNA was isolated according to the manufacturer (Gentra Systems, Research Triangle Park, NC). As a positive apoptotic control, an src-transfected murine myeloid cell line, 32D-23, was interleukin-3 starved for 48 h, and DNA was isolated from 1×10^6 cells. DNA was analyzed on a 1.8% agarose gel containing ethidium bromide and visualized by UV light.

Immunoprecipitation and Western Blot. Extracts were prepared from RNA-injected embryos at stage 9 in lysis buffer [137 mM NaCl/20 mM Tris (pH 8.0)/2 mM EDTA/1% Nonidet P-40] containing 2 mM phenylmethylsulfonyl fluoride, aprotinin (10 μg/ml), 0.5 mM sodium vanadate, and 20 μM leupeptin and extracted with an equal volume of 1,1,2 trichlorotrifluoroethane; insoluble material was removed by centrifugation at 14,000 $\times$ *g* for 10 min at 4°C. A polyclonal β-catenin antibody (gift of Barry Gumbiner, Sloan Kettering) was added for 3 h and then collected by incubation with protein A-Sepharose and centrifugation. Loading buffer was added, samples were centrifuged, and supernatants were fractionated on 8.5% SDS/PAGE. For Western analysis of extracts, a total of three embryo equivalents of lysate were resolved on an SDS/PAGE gel and transferred to Immobilon-P membrane (Millipore). The membrane was blocked in 5% powdered milk

Table 1. XLerk constructs and percentage of disaggregated embryos at the mid-blastula transition



Contructs	# of Embryos	% Disaggregated
XLERK	530	96
XLERK$^{1-308}$	100	0
XLERK$^{1-288}$	40	0
XLERK$^{215-327}$	250	98
XLERK$^{215-308}$	100	0
XLERK$^{215-288}$	40	0
XLERK$^{250-327}$	100	0
XLERK$^{1-215}$	420	0
XLERK$^{1-250/307-327}$	100	0

Each blastomere of the 2-cell stage embryo was injected with 2.5 ng of mRNA encoding the various XLerk constructs (20). Cell disaggregations were determined at stage 9 of development, and percentages were calculated from total number of embryos injected per sample.

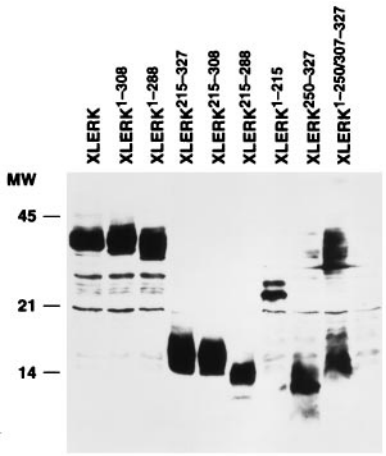


FIG. 2. Western blot analysis of embryos injected with RNA encoding normal and mutant forms of XLerk. 2-Cell embryos were injected with 2.5 ng of RNA encoding all XLerk forms, except in the cases of XLerk$^{215–327,215–307,215–287}$, where 0.5 ng of RNA were used. Extracts were prepared from stage 9 embryos, and three embryo equivalents were fractionated on 12% SDS-PAGE and then immunoblotted for the Flag epitope.

in 1× TBS containing 0.2% Tween-20 (TBST) for 2 h, washed three times in TBST, and incubated overnight at 4°C with primary antibody (Flag M2, Kodak; β-catenin, C-cadherin, Gumbiner lab; phosphotyrosine, UBI) at a dilution of 1:1000. The membrane was washed three times with TBST, incubated 30 min with horseradish peroxidase-coupled secondary antibody (Boehringer Mannheim) at a 1:5000 dilution, washed for 40 min with four changes of TBST, and developed using enhanced chemiluminescence (Amersham). In the case where phosphotyrosine was used as a primary antibody, 2% BSA and 1% fish gelatin in 1× TBST was used as a blocking reagent.

Immunofluorescence. Embryos injected with 2.5 ng of capped mRNA at the 2-cell stage were harvested at stage 9 and fixed in Carnoys buffer (60% ethanol/30% chloroform/10% glacial acetic acid) for 15 min at room temperature. The embryos were dehydrated to 70% ethanol and placed at 4°C overnight. Upon embedding in paraffin, blocks were serially sectioned at 6 μm. Slides were incubated in xylene followed by ethanol dehydration and PBS.

Flag M2 antibody at 1:500 was overlayed on the slides and placed at 4°C overnight. After washing in PBS for 1 h, fluorescent anti-mouse antibody (1:200) was added, and slides were incubated for 1 h at room temperature. After subsequent washing in PBS for 1 h, slides were coverslipped with Gel mount and examined under a fluorescent microscope.

RESULTS

To assay for functional effects of ligand signaling, *in vitro* synthesized RNA encoding XLerk was injected into the cleaving *X. laevis* embryo. During the early blastula stage, a profound effect on cell adhesion was observed in which blastomeres of the animal hemisphere dissociate from each other and slough off the embryo (Fig. 1). This phenotype of cell disaggregation was not observed in embryos injected with RNA encoding XLerk$^{1–215,}$ a mutant protein consisting of only the extracellular domain (Fig. 1).

To determine which regions of XLerk are required for inducing the loss of cell adhesion, various deletion mutants were tested in the *Xenopus* embryo system (Table 1). Expression of XLerk$^{215–327}$, which lacks the extracellular domain, also induced cell dissociation, suggesting that extracellular interaction between XLerk and its cognate receptor tyrosine kinase or other protein(s) was unnecessary at this high level of expression. These results indicate that the intracellular domain

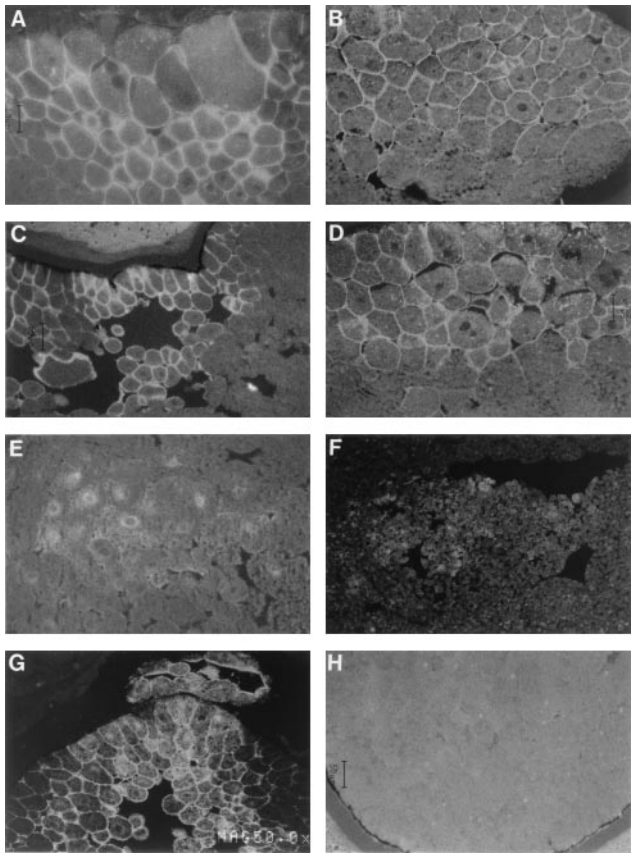


FIG. 3. Localization of normal and mutant forms of XLerk by immunofluorescence. Embryos were injected with 2.5 ng of RNA encoding normal and mutant XLerk constructs. At stage 9, the embryos were fixed in Carnoy's solution, embedded in parafin, and sectioned; then, immunofluorescence was performed using Flag M2 antibody and fluorescein-conjugated anti-mouse-Ig. These immunofluorescent images show that XLerk mutants retaining the transmembrane and cytoplasmic domains are localized to the membrane. (*A*) XLerk; (*B*) XLerk$^{215–327}$; (*C*) XLerk$^{1–308}$; (*D*) XLerk$^{215–308}$. Note that an intact blastocoel is revealed in embryos expressing a C-terminal deletion of the last 19 amino acids in *C*. (*E*) Diffuse staining of XLerk$^{250–327}$ that retains only the cytoplasmic domain. (*F*) Very low levels of diffuse staining of XLerk$^{1–215}$, which just retains the ectodomain. (*G*) Membrane localization of XLerk$^{1–250/307–327}$ and also an intact blastocoel. (*H*) Flag peptide competition control showing the specificity of the staining.

of this Eph ligand is promoting loss of cell adhesion. Removal of the last 19 or 39 carboxyl-terminal amino acids in the context of either the full-length XLerk or XLerk$^{215–327}$ resulted in a loss of the cellular dissociation phenotype (Table 1). Disaggregation was also not detected in embryos expressing only the cytoplasmic region (XLerk$^{251–327}$). Thus, the transmembrane domain and the last carboxyl-terminal 19 amino acids are necessary for XLerk to induce cell dissociation.

To determine whether the C-terminal residues were sufficient to cause this phenotype, we expressed a mutant consisting of the extracellular and transmembrane regions juxtaposed to the carboxyl-terminal 21 amino acids (XLerk$^{1–250/307–327}$). Cell dissociation was not observed in these embryos, indicating that the last 19 amino acids are necessary but not sufficient to cause loss of cell adhesion (Table 1). Western blot analysis confirmed that the various XLerk proteins were expressed in the embryos at similar levels, with the exception of XLerk^{215} series mutants. RNA encoding these mutants must be injected at levels 5-fold lower than other XLerk construct RNAs to maintain equivalent protein expression (Fig. 2).

To determine if changes in protein localization were responsible for the observed differences in the XLerk mutant-

induced cell dissociation, immunocytochemistry was performed on embryos injected with RNA encoding the different XLerk constructs. The results show that those mutants retaining the extracellular domain or transmembrane domain localized predominantly to the plasma membrane, whereas XLerk$^{251–327}$ and XLerk$^{1–215}$ were found in the cytoplasm or were secreted (Fig. 3).

To assess whether the cell dissociation phenotype resulted in any changes within the interior of the embryo, SEM was performed. Embryos expressing full-length XLerk displayed extensive loss of cell adhesion, resulting in a loss of blastocoel formation at stage 8 of embryonic development (Fig. 4). Higher magnification revealed filiform protrusions projecting from individual blastomeres (data not shown). These projections are often observed in nonadherent and motile cells (25). In embryos injected with RNA encoding mutants lacking the C-terminal 19 or 39 amino acids (XLerk$^{1–308}$ RNA and XLerk$^{1–288}$ RNA, respectively), the blastocoel was present and generally appeared normal when compared with uninjected stage 8 embryos (Fig. 3*C*, Fig. 4). Some embryos expressing XLerk$^{1–308}$ formed a blastocoel roof lined with cells that were more rounded than in normal or XLerk$^{1–288}$-expressing embryos (Fig. 4), suggesting that XLerk$^{1–308}$ retains some influence on events in these embryonic cells.

Because C-cadherin is a maternally expressed protein that is essential for the maintenance of cell adhesion in the *Xenopus* blastula (26), we examined whether the observed dissociation could be inhibited by enhanced cadherin function. Injection of 1 ng of C-cadherin RNA along with 4 ng of XLerk RNA inhibited the dramatic cell dissociation observed at the surface of the blastula stage embryo (data not shown). These embryos appeared normal when compared with the XLerk RNA-injected embryos, displaying adherent intact cell surfaces through the blastula stage. Whereas increased cadherin expression rescues cell surface adhesion, it does not rescue the ability of an embryo to progress through gastrulation. These embryos fail to gastrulate properly, resulting in embryonic death at a time when apoptosis is developmentally regulated (27, 28). Western analysis demonstrated that the levels of C-cadherin in embryos injected with this RNA were several-fold above normal, and the expression of XLerk was not substantially diminished in these coinjected embryos (Fig. 5*A*).

Because cadherin-mediated adhesion requires that its cytoplasmic tail be linked with the cytoskeleton through the catenins (29, 30), we examined whether ectopic XLerk expression affected C-cadherin expression or its binding to β-catenin. Extracts were prepared from embryos injected with XLerk RNA and cultured until the midblastula stage (when cell dissociation is strongly evident). Western analysis of the embryonic lysates and β-catenin immunocomplexes using probes for C-cadherin, β-catenin, or phosphotyrosine show no de-

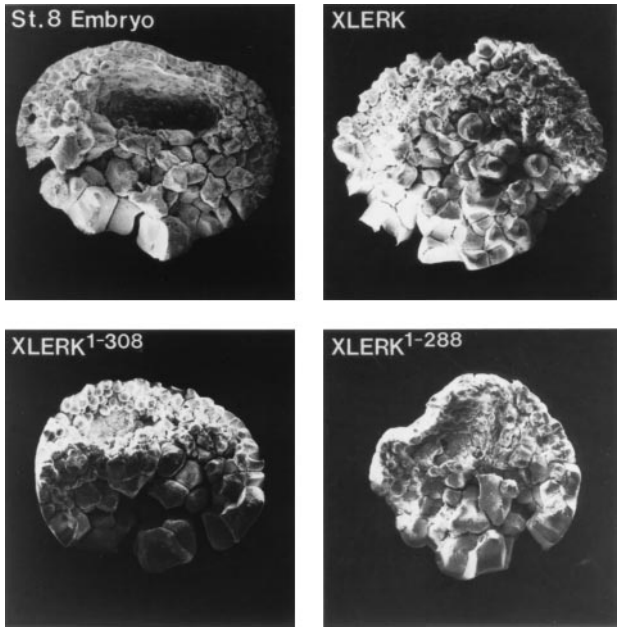


FIG. 4. Scanning electron microscopy of XLerk-expressing embryos during the early blastula stage of development. 2-Cell stage embryos were injected with 2.5 ng of capped XLerk, XLerk$^{1–308}$, or XLerk$^{1–288}$ mRNA in each blastomere. Embryos were then fixed at stage 8 in 4% formaldehyde/1.25% glutaraldehyde and prepared for SEM.

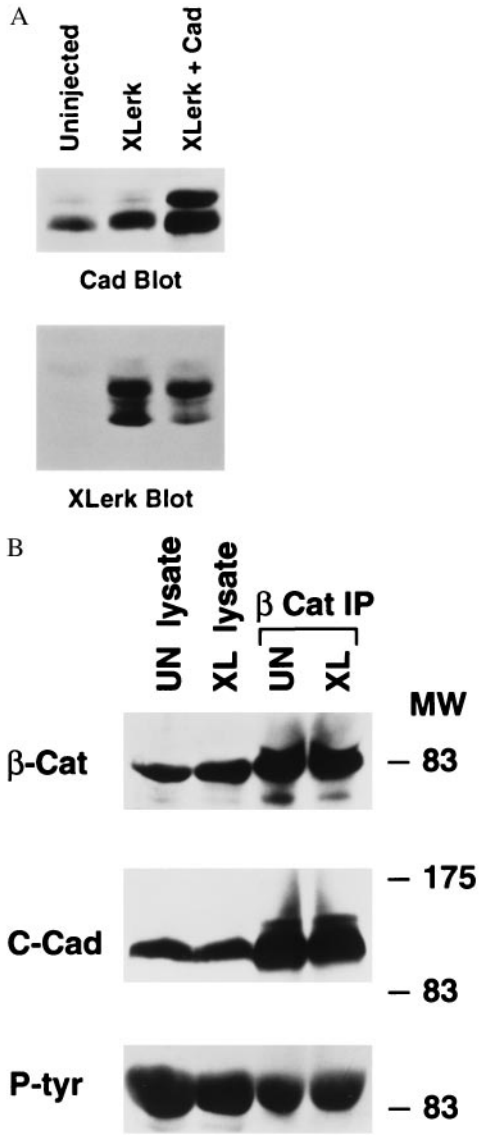


FIG. 5. (*A*) Western analysis of embryos overexpressing XLerk and C-cadherin. Embryos were either left alone or injected with 4 ng of XLerk RNA, or coinjected with 1 ng of C-cadherin RNA. At stage 9, extracts were prepared, and three embryo equivalents were fractionated by SDS-PAGE. Samples were immunoblotted with C-cadherin antibodies and a C-terminal XLerk antibody (cadherin appears as a doublet, perhaps representing the precursor and product forms). (*B*) C-Cadherin and β-catenin expression and association in XLerk-injected embryos. Embryos were either left alone or injected with 5 ng of XLerk RNA. Stage 9 embryos were harvested, and extracts were prepared. Three embryo equivalents were fractionated by SDS PAGE, and five embryo equivalents were subjected to immunoprecipitation analysis using β-catenin antibodies (β-Cat IP) and then fractionated by SDS-PAGE. Samples were immunoblotted with antibodies to β-catenin, C-cadherin, or phosphotyrosine.

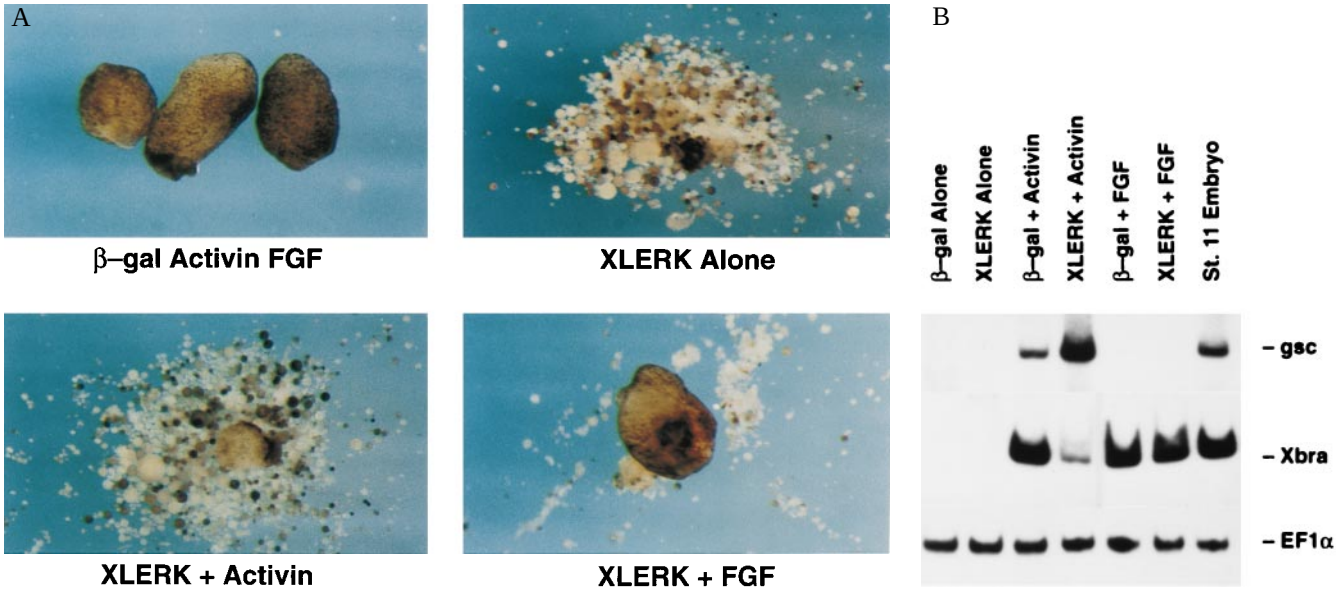


FIG. 6. (*A*) Morphological analysis of animal caps demonstrating the rescue of XLerk-injected embryos by bFGF. Animal caps were dissected at stage 8.5 and cultured at 23°C in 67% Leibovitz's L-15 medium, 7 mM Tris·HCl (pH 7.5), and 1 mg/ml gentamycin with or without 50 ng/ml Activin or 100 ng/ml bFGF. The animal caps were harvested at stage 26 as determined by uninjected embryos. These results are representative of four independent experiments. (*B*) Effect of XLerk on gene expression in the animal cap. Embryos were injected with either 5 ng of XLerk mRNA or β-galactosidase mRNA at the 2-cell stage. The animal caps were explanted at stage 8 and cultured in 50 ng/ml activin or 100 ng/ml bFGF until stage 11. Upon harvesting, RT-PCR analysis using primers specific for gsc, Xbra, and EF-1α was performed.

tectable effect on C-cadherin expression or its association with β-catenin (Fig. 5*B*). Additionally, there was no observable change in tyrosine phosphorylation of β-catenin that is associated with an interaction with cadherin (Fig. 5*B*).

We had shown previously that XLerk transcripts are elevated during mesoderm induction by both activin and bFGF in ectodermal explants (animal caps) (20). Therefore, it was of interest to determine how these mesoderm-inducing factors affect XLerk-induced dissociation within this system. Animal caps from XLerk-injected embryos were cultured in the presence of activin, bFGF, or alone, until mid-gastrulation (stage 11) and the early tailbud stage (stage 26). As shown in Fig. 6*A*, animal caps harvested from XLerk-expressing embryos and cultured alone displayed blastomere dissociation. This dissociation was not a result of cell death as evidenced by continual division of the blastomeres from the animal cap until stage 26. It has been shown that blastomeres of *Xenopus* embryos cultured in calcium- and magnesium-free media also lose their adhesive properties but continue to divide (31). Blastomere dissociation was also observed in XLerk-expressing animal caps cultured in the mesoderm inducer, activin (Fig. 6*A*). Most surprisingly, however, was the absence of cell dissociation in XLerk-expressing animal caps cultured with bFGF. These cells regained their adhesive properties and morphologically resembled the control β-galactosidase-expressing animal caps cultured in bFGF until stage 26 (Fig. 6*A*). These results suggest that activation of the FGF signal transduction pathway rescues XLerk-induced blastomere dissociation.

To further characterize the effect of XLerk-induced cell dissociation on mesoderm induction in animal caps, RT-PCR analysis was performed in XLerk-expressing embryonic animal caps that were cultured until mid-gastrulation (stage 11). Animal caps expressing XLerk were cultured alone or in the presence of either activin or bFGF and harvested at mid-gastrulation for RT-PCR analysis. Expression of Xbrachyury (Xbra), a pan-mesodermal transcription factor (32), was dramatically reduced with activin treatment (Fig. 6*B*), yielding a similar result to that reported for animal caps dissociated in calcium and magnesium-free media (33, 34). However, Xbra expression was induced with bFGF treatment, consistent with the morphological rescue by bFGF (Fig. 6*B*). These results are in stark contrast to the control β-galactosidase-expressing animal caps, which show an increase in Xbra transcripts upon treatment with both mesoderm inducers (Fig. 6*B*) (32). Interestingly, in response to activin, animal caps expressing XLerk showed an increase in the transcripts for goosecoid (gsc), a dorsal-specific mesodermal mRNA that can be induced independent of cell–cell contact upon activin treatment (33–35).

Although the cells of embryos expressing high levels of XLerk undergo cell death during gastrulation, loss of cell adhesion occurs prior to this time. Further, induction of several marker genes was observed in XLerk-expressing animal caps cultured with bFGF or activin, demonstrating transcriptional activity (Fig. 6*B*; data not shown). DNA fragmentation, a characteristic feature of apoptosis (36), was not observed in stage 9 XLerk-expressing embryos in contrast to the control apoptotic T cells (data not shown) (37). These data indicate that the loss of cell adhesion observed in pregastrula stage embryos and in cultured animal caps is not due to cell death.

DISCUSSION

Our results suggest that XLerk transduces a signal that affects cell adhesion and that the carboxyl-terminal 19 amino acids of the cytoplasmic region constitute a domain that is critical for this event. Two laboratories have recently reported tyrosine phosphorylation of the Eph ligand, ephrin-B1, in cultured cells. This phosphorylation event was contingent upon ephrin-B1 association with the ectodomain of the EphB2 receptor tyrosine kinase in cultured cells (18, 19) or upon stimulation of the ephrin-B1 expressing cells with platelet-derived growth factor (19). The carboxyl-terminal 33 residues of the Eph transmembrane ligands is highly conserved, with three of five conserved tyrosine residues localized within the last 19 amino acids. We have not observed tyrosine phosphorylation of XLerk upon ectopic expression in embryos (L.D.C. and I.O.D., unpublished results), but its phosphorylation state in response to activation of the FGF receptor is presently being investigated. Although it has been reported that the extracellular interaction between ephrin-B1 and the EphB3 receptor tyrosine kinase induced cell–cell adhesion (38), XLerk mutants that lack the extracellular domain are also able to

modulate cell adhesion. An intriguing possibility is that XLerk, like many receptor tyrosine kinases, becomes active as a signal transducer when highly expressed and, perhaps like the Notch family of receptors, becomes constitutively active by truncation of the extracellular ligand interaction domain (39). Additional deletion of the transmembrane domain abolishes the cell dissociation phenotype, suggesting an important role for this region as well.

Recently, it has been reported that using truncated forms of x-EphA4 (X-sek1) and x-EphB1 (Xek, Xelk) (40, 41) leads to abnormal migration of the third arch neural crest cells in *Xenopus* (42). Additionally, overexpression of the *Xenopus* Eph ligand, x-ephrin-B2, causes scattering of the third arch neural crest cells (42). The morphological effects observed in our studies are similar to the loss of both cell adhesion and blastocoel formation that result from the expression of the activated Eph receptor tyrosine kinase, Pag (x-EphA4) in *Xenopus* embryos (43). These results suggest a possible similarity in function among the Eph receptors and ligands. Whereas overexpression of cadherin rescues surface adhesion in XLerk-injected embryos, the mechanism of cell disaggregation is unclear, as we also found no apparent disruption of the catenin–cadherin interaction that is important to cell adhesion in embryos (Fig. 5*B*) (26, 44, 45).

We have shown that dissociated blastomeres from embryonic animal caps that express XLerk exhibit elevated gsc transcripts in response to activin, whereas Xbra levels were greatly diminished. In contrast, culturing with bFGF rescues adhesion and mesoderm induction with similar changes in mRNA levels of either gene when compared with the control animal caps. One interpretation of our data is that FGF may modulate the activation of the XLerk signaling pathway. How FGF signaling rescues loss of cell adhesion mediated by the cytoplasmic domain in XLerk-expressing animal caps is presently unclear. The expression pattern of the FGF family during early development is very similar to XLerk expression, thus providing possible interactions during normal development (46). Moreover, it is most striking that the FGF receptor has been shown recently to possess a CAM homology domain and to be activated in response to cell adhesion molecules (L1, N-Cadherin, N-CAM) that play a role in axon guidance (47–49). Further, a functional FGF receptor is required for neurite growth stimulated by cell adhesion molecules (47). Because ephrin-B1 and its receptor (EphB2) have been shown to guide axonal growth (7, 50), it is possible that FGF may play an important regulatory role in this process.

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