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Involvement of the small GTPases XRhoA and XRnd1 in cell adhesion and head formation in early *Xenopus* development

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SUMMARY

The Rho family of small GTPases regulates a variety of cellular functions, including the dynamics of the actin cytoskeleton, cell adhesion, transcription, cell growth and membrane trafficking. We have isolated the first *Xenopus* homologs of the Rho-like GTPases RhoA and Rnd1 and examined their potential roles in early *Xenopus* development. We found that *Xenopus* Rnd1 (XRnd1) is expressed in tissues undergoing extensive morphogenetic changes, such as marginal zone cells involuting through the blastopore, somitogenic mesoderm during somite formation and neural crest cells. XRnd1 also causes a severe loss of cell adhesion in overexpression experiments. These data and the expression pattern suggest that XRnd1 regulates morphogenetic movements by modulating cell adhesion in early embryos. *Xenopus* RhoA (XRhoA) is a potential XRnd1 antagonist, since overexpression of XRhoA increases cell adhesion in the embryo and reverses the disruption of cell adhesion caused by XRnd1. In addition to the potential roles of XRnd1 and XRhoA in the regulation of cell adhesion, we find a role for XRhoA in axis formation. When coinjected with dominant-negative BMP receptor (tBR) in the ventral side of the embryo, XRhoA causes the formation of head structures resembling the phenotype seen after coinjection of wnt inhibitors with dominant-negative BMP receptor. Since dominant-negative XRhoA is able to reduce the formation of head structures, we propose that XRhoA activity is essential for head formation. Thus, XRhoA may have a dual role in the embryo by regulating cell adhesion properties and pattern formation.

Key words: Rho, Cell adhesion, Gastrulation, Pattern formation, *Xenopus laevis*

INTRODUCTION

Small Rho-like GTPases are involved in a variety of cellular processes (Van Aelst and D'Souza-Schorey, 1997). Like other small GTPases, they cycle between active (GTP-bound) and inactive (GDP-bound) states that are regulated by various guanine nucleotide exchange factors. Rho-like GTPases are able to induce major rearrangements of the actin cytoskeleton resulting in the formation of stress fibers and focal adhesions, lamellipodia and filopodia (Hall, 1998). Rho-like GTPases also regulate transcription via the JNK and p38 MAP kinase pathways (Boutros et al., 1998; Coso et al., 1995; Minden et al., 1995; Teramoto et al., 1996a). In addition, they can regulate cell adhesion (Braga et al., 1997; Nobes et al., 1998). This suggests that small Rho-like GTPases could play an important role in mediating morphogenetic changes during embryogenesis, where cell movements mediated through changes in the actin cytoskeleton and adhesion properties must be coordinated with patterning events via growth factor signaling.

Examples for the involvement of Rho GTPases in morphogenesis have been found in *Drosophila*. *Drosophila* DCdc42, DRhoA and DRac1 are involved in dorsal closure, a process during which the lateral ectoderm stretches over the dorsal amnioserosa (Harden et al., 1995, 1999; Strutt et al., 1997). DRhoA and its putative exchange factor, DRhoGEF, also play a role in gastrulation, presumably by mediating the apical constriction of involuting cells in the ventral furrow (Barrett et al., 1997). Genetic analysis of *DrhoA* has revealed its involvement in the establishment of cell polarity in eye and wing epithelia, acting in a cell polarity signaling cascade downstream of *frizzled* and *dishevelled* (Eaton et al., 1995; Strutt et al., 1997). Recent evidence suggests that Rho proteins are important for morphogenesis in vertebrates as well, since RhoB is involved in the delamination of neural crest cells from the neural tube in chick embryos (Liu and Jessell, 1998).

During *Xenopus* gastrulation, the modulation of adhesive properties within and between the germ layers provides a connection between cell identity and morphogenesis (Gurdon, 1992). Cell adhesion molecules, including cadherins (Kuhl et

al., 1996; Lee and Gumbiner, 1995; Takeichi, 1991; Zhong et al., 1999), protocadherins (Bradley et al., 1998; Kim et al., 1998) and integrins (Ransom et al., 1993; Smith et al., 1990; Whittaker and DeSimone, 1993) have been implicated in the differential regulation of cell adhesion and cell movements. Growth factors known to be involved in mesoderm patterning, such as activin and Xnr-3, also influence mesodermal morphogenesis and cell shape (Ramos et al., 1996; Smith et al., 1995). We show in this study that Rho-like GTPases may play an important role in early *Xenopus* morphogenesis through their ability to modulate cell adhesive properties.

Additionally, we provide evidence that *Xenopus* RhoA is involved in axial patterning events. The Spemann organizer, which is located at the dorsal marginal zone where gastrulation movements are initiated, plays a critical role during gastrulation as its transplantation into the ventral side of a host embryo leads to the formation of a second body axis (Spemann and Mangold, 1924). Since distinct regions of the organizer have different inducing potential, it can be further divided into head organizer and trunk organizer (Spemann, 1931). Several secreted molecules are expressed in the trunk organizer, including the dorsalizing factors noggin, chordin and follistatin. Each of them can bind to and inhibit the ventralizing Bone Morphogenetic Proteins (BMPs) (Iemura et al., 1998; Piccolo et al., 1996; Zimmerman et al., 1996). Mimicking trunk organizer activity on the future ventral side by inhibiting BMP signaling results in embryos with a secondary body axis lacking heads (Graff et al., 1994; Hawley et al., 1995; Suzuki et al., 1994), implying that the formation of head structures requires additional signals.

The head organizer is located in yolky endomesodermal cells in the deep layer of the organizer, including the leading edge of the most anterior cells that underlie the future head neuroectoderm after gastrulation. Cerberus is a secreted multivalent antagonist that is expressed in the head organizer and that has the ability to induce ectopic heads when misexpressed in the ventral vegetal side of the embryo (Bouwmeester et al., 1996; Piccolo et al., 1999). Cerberus binds to and inhibits the ventralizing factors BMP4 and Xwnt-8 and the dorsalizing nodal-related factor Xnr-1 (Jones et al., 1995) through distinct domains (Hsu et al., 1998; Piccolo et al., 1999). In addition to cerberus, the two secreted wnt-antagonists frzb and dickkopf-1 (dkk-1) (Glinka et al., 1998; Leyns et al., 1997; Wang et al., 1997) are expressed in the head organizer. Frzb contains structural motifs also found in the wnt-receptor frizzled but lacks a transmembrane domain, and binds directly to Xwnt-8 (Leyns et al., 1997; Wang et al., 1997). Xwnt-8 is a growth factor of the wingless family and plays a role in ventrolateral mesoderm formation during *Xenopus* gastrulation (Christian and Moon, 1993). In the current model of axis formation, inhibition of ventralizing BMP and wnt signals in the organizer is essential for both dorsal mesoderm and head formation. In the head organizer, nodal-related signals must be additionally blocked in order to prevent trunk mesoderm formation in the head region (Piccolo et al., 1999).

In order to analyze the function of Rho-like GTPases during *Xenopus* development, we have isolated RhoA and Rnd1 from *Xenopus laevis*. We examined their potential roles in cell adhesion and axis formation in *Xenopus* embryos. We show that XRnd1 has a strong disruptive effect on cell adhesion in overexpression assays and is expressed in tissues that are undergoing extensive morphogenetic movements. Therefore, it may be an important factor in mediating cell rearrangements in these tissues. Overexpression of XRhoA antagonizes XRnd1 function by increasing cell adhesion, possibly by acting as a XRnd1 antagonist. Furthermore, we provide evidence for a role of XRhoA in head formation. Overexpression of XRhoA in the ventral side with a BMP inhibitor leads to the formation of head structures, much like the phenotype seen when cerberus is overexpressed or when wnt inhibitors are coexpressed with BMP inhibitors. Consistent with this finding, dominant-negative XRhoA blocks head formation induced by cerberus and by dominant-negative Xwnt-8 together with dominant-negative BMP receptor (tBR). Thus, RhoA is the first intracellular signaling molecule implicated in head formation.

MATERIALS AND METHODS

Embryo manipulations and RNA injections

Eggs were fertilized in vitro, dejellied and resultant embryos cultivated as described previously (Cho et al., 1991). Staging was according to Nieuwkoop and Faber (1967). Dorsoventral polarity was determined as described by Klein (1987) and indicated amounts of synthetic mRNAs were injected into the desired blastomeres at 2 or 4 nl each unless indicated otherwise. For the synthesis of synthetic mRNA, linearized plasmids were transcribed using the T3 or SP6 Message Machine Kit (Ambion).

Construction of a cDNA library enriched for ventrally expressed messages

RNA was isolated from UV-hyperventralized embryos at stage 10.25 equivalent using the guanidine-acid-phenol method (Chomczynski and Sacchi, 1987). Poly(A^+) RNAs were purified by oligotex-dT30. *Not*I-oligo(dT) primed cDNA was synthesized using Superscript reverse transcriptase (GIBCO-BRL) according to the Gubler and Hoffman method (Gubler and Hoffman, 1983). An *Eco*RI adapter (New England Biolabs) was ligated to the resultant ds-cDNA followed by a *Not*I restriction digest. A Sephacryl S-400 column was used to remove DNA fragments less than 400 bp in length. The cDNA was cloned directionally into *Not*I-*Eco*RI digested pBluescript II KS+ (Stratagene). The average size of the cDNA inserts was approximately 1.2 kb.

Expression cloning of *Xenopus* Rnd1 and construction of XRnd1 plasmids

Pooled plasmid DNAs from 150-200 independent colonies of the UV-ventralized library were linearized with *Not*I and transcribed with T3 Megascript Kit (Ambion). 8 ng of these RNAs plus 2 ng of dominant-negative BMP receptor (tBR) RNA were injected into the ventral marginal zone of 4-cell stage embryos. Late neurula stages were scored for secondary axis formation. RNA pools that reduced the induced secondary axis phenotype were subdivided further until a single species of cDNA was isolated. Overall, 20 pools of RNA containing about 150 RNAs each were tested and of these, three fractions showed diminished formation of secondary axes.

pXβm-XRnd1

The coding and 3′ untranslated region of XRnd1 was subcloned into the *Nco*I/*Bam*HI sites of pSP64-Xβm, a pSP64-based vector containing a β-globin 5′ leader sequence immediately upstream of the initiator methionine (Krieg and Melton, 1984; Melton et al., 1984). The PCR primers 5′-CATGCCATGGGAAAGGAACGAAGGAA-CCCACA-3′, containing a *Nco*I site at the start codon and the T7 primer were used to generate the subcloned fragment. pXβm-XRnd1 was tagged at the N terminus with the hemagglutinin epitope (HA) in

the *Nco*I site using annealed HA-oligos with *Nco*I-compatible overhangs.

XRnd1 N27

The single amino acid change from T to N at position 27 was achieved by generating two PCR products containing the mutation from XRnd1 in pBS-KS+. For the first PCR reaction, T3 primer and the 5′-ACTGTGGGAAGAACGCAATGCTGCAA-3′ primer were used. The second PCR reaction used T7 primer and the 5′-TTGCAGCA-TTGCGTTCTTCCCACAGT-3′ primer. These gel-purified PCR products containing an overlapping region were used in a third PCR reaction, with T3 and T7 outside primers. The *Hin*dIII fragment of this PCR product was then used to replace the *Hin*dIII fragment in the wild-type construct.

XRnd1G22/N27

The G22/N17 double mutant was created from the pBS-KS XRnd1 N27 mutant plasmid using the same strategy. In this case, the primers for the first PCR reaction were T3 and 5′-TCCCACAGTGACCAT-CTCCCACC-3′ and for the second PCR reaction T7 and 5′-GGTGGGAGATGGTCACTGTGGGA-3′. The PCR product from the third PCR reaction was cloned between the *Eco*RI/*Not*I sites of pBS-KS+.

pXβm-XRnd1 HA/CAAX–

The entire coding region except the amino acids of the CAAX-box was PCR-amplified with turbo-Pfu (Stratagene) from pXβm-XRnd1 HA using SP6 primer and the 5′-CCGCTCGAGGCTTTTGGCCT-TCTCTTT-3′ primer, containing a *Xho*I site (underlined). The PCR product was then cloned between the *Eco*RV/*Xho*I sites of pBS-KS+.

Cloning of XRhoA and construction of XRhoA plasmids

Low stringency hybridization was performed with a random-primed full-length human RhoA cDNA (gift from A. Hall) on a *Xenopus* oocyte library as described (Blitz and Cho, 1995). Five full-length *Xenopus* RhoA clones were isolated.

The point mutation to convert XRhoA into the constitutively active form XRhoA V14 (Garrett et al., 1989) was generated with upstream PCR primer 5′-ACATGCATGCAACATCTC-3′, containing an *Sph*I site, and T3 primer. The resulting PCR product was digested with *Bam*HI and *Sph*I and used to replace the corresponding fragment in the wild-type construct. XRhoA N19, a dominant-negative RhoA (Qiu et al., 1995), was generated using the downstream primer 5′-ACATGCATGCGGGAAAAACTGCCTTCTG-3′ and T7 primer. The PCR product was digested with *Sph*I and *Hin*dIII and used to replace the same fragment in the wild-type clone. CSKA XRhoA N19, in which XRhoA N19 is under the control of the cytoskeletal actin promoter (CSKA), was cloned by inserting an *Eco*RI fragment of XRhoA N19, containing the entire coding region, into the *Eco*RI site of the pCSKA vector (Christian and Moon, 1993).

Preparation of RNA and northern blot analysis

Total embryonic RNA was isolated from staged *Xenopus* eggs and embryos according to Chomczynski and Sacchi (1987). The stages were egg, 4-cell, 64-cell, stage 8 (mid-blastula), stage 9 (late blastula), stage 10.25 (early gastrula), stage 11 (mid-gastrula), stage 12.5 (late gastrula), stage 15 (mid-neurula), stage 20 (late neurula), stage 25 (mid-tailbud), stage 28 (late tailbud) and stage 41 (tadpole). Northern blot analysis was performed as described (Blitz and Cho, 1995). For the XRnd1 probe, a random-primed *Hin*dIII/*Not*1 fragment and for the XRhoA probe, a *Sac*I/*Xho*I fragment, were used. Both fragments contained mostly 3′ noncoding region in order to avoid potential cross-hybridization with related Rho messages. C-src (Collett and Steele, 1993) was used as an internal control for RNA loading.

Whole-mount in situ hybridization analysis

In situ hybridization was performed essentially as described previously (Harland, 1991), except that BM purple (Boehringer Mannheim) was used as the chromogenic substrate. For XRnd1 probe synthesis, pBS XRnd1 was digested with *Hin*dIII and transcribed using the T7 message machine Kit (Ambion) with digoxigenin-UTP (Boehringer Mannheim), generating an antisense probe consisting mainly of 3′-untranslated region. For the XRhoA in situ probe, pBS XRhoA was digested with *Sac*I and transcribed using the T7 message machine kit (Ambion), also generating a probe consisting mainly of 3′-untranslated region.

Histology and immunofluorescence

For lineage tracing, embryos were injected with 50-100 pg of β-galactosidase RNA and stained with X-gal. For histological sectioning, embryos were dehydrated in ethanol followed by incubation in Histosol (National Diagnostics) and embedding in paraplast (Oxford Labware). 12 μm sections stained with X-gal were counterstained with EosinY (J. T. Baker Chemical Company). Other sections were stained with Hematoxylin (Fisher), counterstained with EosinY and mounted with Permount (Fisher Scientific).

For immunofluorescence, embryos were fixed in MEMFA (Harland, 1991) for 1.5 hours and stored in methanol. Embedding, sectioning and immunofluorescence were carried out as previously described (Fagotto and Gumbiner, 1994). The monoclonal anti-HA antibody 12CA5 (Berkeley Antibody Company) was used at 2 μg/ml overnight at 4°C and secondary antibody (Cy3-coupled donkey anti-mouse, Jackson Immunoresearch) was diluted 1:500 and incubated for 2 hours at room temperature. Cy3 fluorescence was photographed on a Zeiss Axiophot microscope under a Rhodamine filter.

Subcellular fractionation and western blotting

Subcellular fractionation of injected and control embryos was carried out as previously described (Fagotto and Gumbiner, 1994) with the following modifications: 10 embryos per batch were homogenized in 100 μl homogenization buffer. For isolation of the membrane fraction, the supernatant from the first 750 ***g*** centrifugation was spun at 100,000 ***g*** for 45 minutes. The resulting supernatant was collected as the cytosolic fraction and the pellet, containing the membranes, was directly resuspended in 1× SDS/PAGE sample buffer. 10% of each resulting fraction, the equivalent of one embryo, was analyzed by 12% SDS-PAGE. After transfer to nitrocellulose, HA-tagged proteins were detected using monoclonal anti-HA antibody 12CA5 followed by peroxidase-conjugated goat anti-mouse secondary antibody (Jackson Immunoresearch). The secondary antibody was visualized with ECL reagents (Amersham).

RESULTS

Isolation of the *Xenopus* Rho-like GTPases XRnd1 and XRhoA

We performed an expression screen anticipating the identification of molecules that affect BMP signaling or morphogenesis by isolating inhibitors of dominant-negative BMP receptor (tBR)-induced secondary axes from a ventralized cDNA library (see Materials and Methods). The first such isolated cDNA is XRnd1, a *Xenopus* homolog of the recently described human Rnd1, a member of a novel group of Rho-like GTPases (Nobes et al., 1998). *Xenopus* and human Rnd1 are quite similar with 86% amino acid identity (Fig. 1A). Human and *Xenopus* Rnd1 share unique characteristics that distinguish them from other Rho-like GTPases. Their C termini are considerably longer than in other small GTPases and they contain substitutions in amino acids (highlighted in black) that have been shown to confer oncogenicity to c-ras by impairing GTP hydrolysis (Foster et al., 1996; Nobes et al., 1998). In fact,

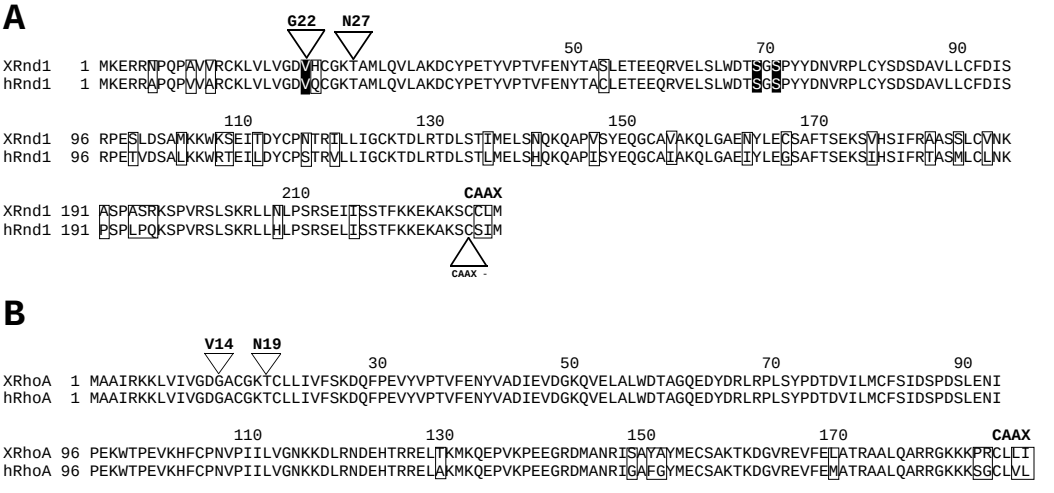

Fig. 1. Alignment of *Xenopus* Rnd1 and RhoA with their human homologs. Boxed amino acids indicate differences between the *Xenopus* and human proteins. (A) Alignment of *Xenopus* and human Rnd1. Amino acids that are likely to cause constitutive activity of Rnd1 are highlighted in black. Arrowheads indicate mutations that were generated in XRnd1 and that led to a loss of activity in overexpression assays. The C-terminal CAAX-box, site of lipid modification, is indicated. (B) Alignment of *Xenopus* and human RhoA. Arrowheads indicate the amino acids that were mutated to generate the constitutively active (V14) and dominant-negative (N19) versions of the protein. The GenBank accession numbers for the XRnd1 and XRhoA cDNAs are AF151015 and AF151014, respectively.

it has been shown that hRnd1 has very little intrinsic GTPase activity and is likely to be permanently in a GTP-bound state, making it constitutively active (Nobes et al., 1998). Also, the C-terminal CAAX-box motif for prenylation is a consensus site for farnesylation in *Xenopus* and human Rnd1, whereas most other Rho-like GTPases are geranyl-geranylated (Adamson et al., 1992).

Another small Rho-like GTPase, *Xenopus* RhoA (XRhoA), was isolated through low stringency hybridization screening of a *Xenopus* oocyte library. It has 94% amino acid identity with human RhoA (Fig. 1B). Both constitutively active (RhoA V14) and dominant-negative (RhoA N19) versions have been described (Garrett et al., 1989; Qiu et al., 1995). Analogous mutations were generated in *Xenopus* RhoA in order to study XRhoA's role during *Xenopus* development (Fig. 1B, arrowheads, see Figs 4 and 7 for functional analysis).

Spatio-temporal expression of XRnd1 and XRhoA in the early *Xenopus* embryo

In order to understand the potential roles of XRnd1 and XRhoA during early *Xenopus* development, their spatiotemporal expression patterns were examined. Developmental northern blots of XRnd1 and XRhoA reveal that RNAs encoding these small GTPases are expressed both maternally and zygotically at relatively consistent levels (Fig. 2A,B, respectively). Whole-mount in situ hybridization was used to examine the spatial expression patterns of XRnd1 and XRhoA. In early gastrula stage embryos, XRnd1 is expressed in the ectoderm (Fig. 3A, left). A high level of expression is seen in the forming dorsal blastopore lip as the tissue of this region begins to migrate inward (Fig. 3A, arrowhead, right). This high level of mesodermal expression spreads around the entire blastopore during gastrulation, coinciding with formation of the blastopore as it spreads ventrally (Fig. 3B). XRnd1 expression then persists around the circumference of the blastopore until it is completely closed at late neurula stage (data not shown). Punctate expression of XRnd1 in the endoderm is also apparent at gastrula stages (Fig. 3B). XRnd1 expression persists in the involuted mesoderm as seen in stage 10.5 embryos that were cut along the midline before hybridization (Fig. 3C). In the early neurula, high levels of XRnd1 expression are seen in somitogenic mesoderm but expression is absent from the notochord (Fig. 3D,E). At mid-neurula stage, XRnd1 expression is detected in forming somites (Fig. 3F-H) and

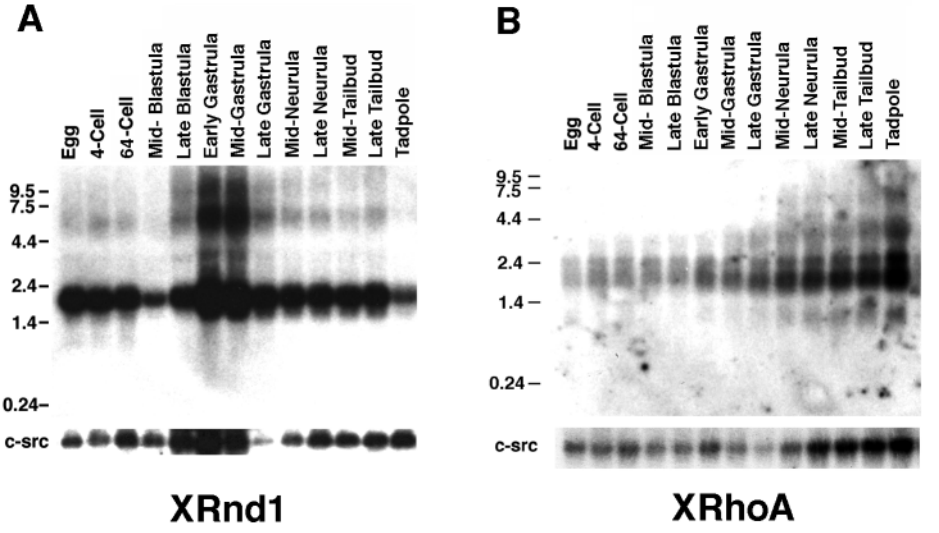

Fig. 2. Developmental northern blot hybridization analysis of XRnd1 and XRhoA. C-src expression is shown as a loading control in both panels. (A) XRnd1 is expressed in early *Xenopus* embryos both maternally and zygotically. The size of the major band corresponds to the size of the isolated XRnd1 cDNA (1.9 kb). (B) XRhoA RNA is present both maternally and zygotically. The positions of marker RNAs (kb) are shown.

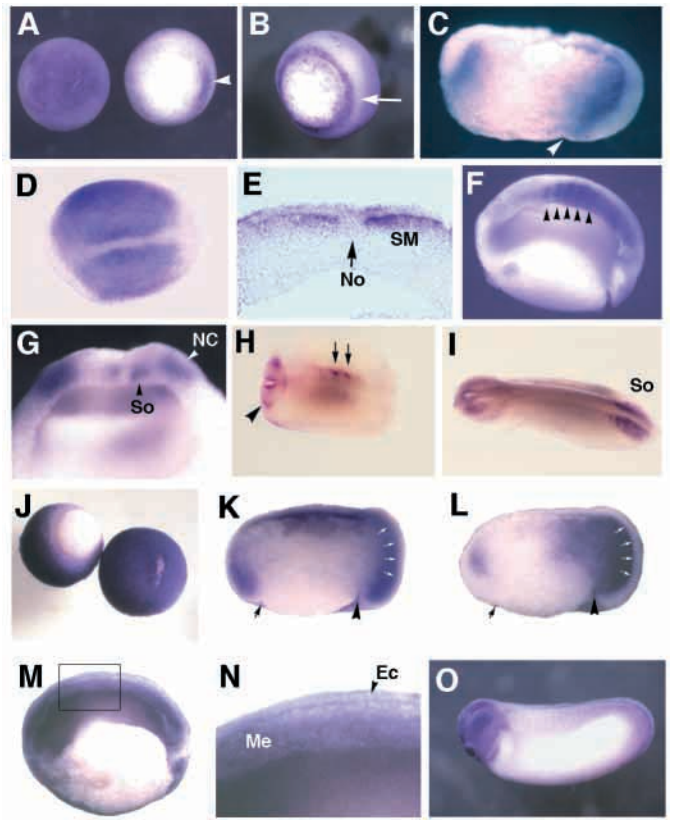

Fig. 3. Whole-mount in situ hybridization analysis of XRnd1 and XRhoA transcripts. (A-I) XRnd1, (J-O) XRhoA. (A,C,K,L) Dorsal is right. (D,F,H,I,M,N,O) Anterior is left. (A) Animal (left) and vegetal (right) view of early gastrula (stage 10) embryos. XRnd1is expressed in the ectoderm and dorsal blastopore lip. The arrowhead indicates the location of the dorsal blastopore lip. (B) Vegetal view of a midgastrula (stage 11) embryo. Note the high level of XRnd1 expression around the blastopore (arrow). (C) Mid-gastrula (stage 10.5) embryo that was cut along the midline. The arrowhead indicates the location of the dorsal blastopore. XRnd1 expression persists in the involuted mesoderm. (D) Dorsal view of an early neurula (stage 13) embryo. XRnd1 expression is absent from the midline. (E) Histological cross section of the same embryo as in D, which reveals high level of XRnd1 expression in the somitogenic mesoderm (SM), but not in the notochord (No). (F) Parasagittally cut midneurula (stage 20) embryo showing the high level of XRnd1 expression in unsegmented somitogenic mesoderm and expression in newly formed somites (arrowheads). (G) Anterior transverse section of a mid-neurula stage embryo (stage 20) revealing XRnd1 expression in cranial neural crest cells (NC) and somites (So). (H) Dorsal view of an early tailbud stage (stage 22) embryo. Note XRnd1 expression in newly formed somites (arrows) and cranial neural crest cells (arrowhead). (I) Dorsolateral view of a tailbud stage (stage 27) embryo. XRnd1 expression now remains only in the posterior region of the somitogenic mesoderm (So). (J) XRhoA expression: vegetal (left) and animal (right) view of gastrula (stage 11) embryos. XRhoA expression is present in the ectoderm and marginal zone. (K,L) two halves of a gastrula (stage 10.5) embryo probed for XRhoA (K) and cerberus (L) to demonstrate the presence of XRhoA expression in the organizer region. Note that embryos were cut along the midline prior to in situ hybridization analysis. The image of the embryo in K was horizontally flipped to allow for easier comparison with L. Small white arrows indicate the boundary between involuted mesoderm and overlying ectoderm. The black arrowhead shows the position of the dorsal blastopore lip and the small arrow indicates the ventral blastopore lip. Note that both cerberus and RhoA are expressed in the ventral marginal region. XRhoA is also expressed in the ectoderm, although the surface staining appears weaker in K due to lighting conditions used for photography. (M) Sagittally sectioned neurula embryo (stage 13) showing the expression of XRhoA in the ectoderm and mesoderm. (N) Enlargement of the boxed area in M; Me, mesoderm; Ec, ectoderm. (O) Tailbud stage (stage 23) embryo. XRhoA expression is enriched in the head and to some extent in the tail region.

cranial neural crest cells (Fig. 3G,H). Interestingly, the expression of XRnd1 in the somitogenic mesoderm is downregulated after somite formation (compare anterior and posterior somitogenic mesoderm in Fig. 3F). The neural crest staining appears to be restricted to the dorsal portion of the neural crest, where it delaminates from the neural tube (Fig. 3G,H). In tailbud stages, XRnd1 expression is restricted to newly formed somites and the remaining posterior unsegmented somitogenic mesoderm (Fig. 3I). Overall, the expression of XRnd1 appears to coincide with the movement and rearrangement of cells during embryogenesis, particularly in the involuting marginal zone, somitogenic mesoderm and neural crest.

XRhoA is expressed somewhat differently than XRnd1. At gastrula stage, XRhoA is expressed in the ectoderm and marginal zone, and appears to be absent from the endoderm (Fig. 3J). However, RT-PCR analysis reveals that XRhoA is also expressed in the endoderm (data not shown). Embryos that were cut along the midline at midgastrula stage, followed by wholemount in situ hybridization analysis show that RhoA is present in the involuted mesoderm (Fig. 3K). This region corresponds to organizer tissue and XRhoA expression overlaps with the expression of the head-inducing molecule cerberus at this stage (Fig. 3L). Note that both XRhoA and cerberus are also expressed in the ventral involuting marginal zone. In mid-neurula stages, XRhoA is expressed both in the ectoderm and mesoderm (Fig. 3M,N). In tailbud stages, expression is enriched in head and tail ectoderm and mesoderm (Fig. 3O). These results demonstrate that RNA expression of the small GTPases XRnd1 and XRhoA is differentially regulated in the *Xenopus* embryo, especially XRnd1 expression, which coincides with sites of changing cell adhesion. This raises the possibility that XRnd1 may participate in morphogenesis during early embryogenesis.

Overexpression of XRnd1 disrupts cell adhesion

Overexpression studies by RNA injections were performed to determine the function of XRnd1 during early development. Overexpression of XRnd1 mRNA in the animal pole induced lesions resulting from detached cells around the injection site within 1-2 hours after injection (Fig. 4A). These lesions healed externally by the blastula stage so that, although the injected embryos appeared normal externally, the animal cap tissue was actually very thin. At mid-blastula stage, the animal cap tissue is normally 4-5 cell layers thick (Fig. 4B). Histological sections of XRnd1-injected embryos together with the lineage tracer β-galactosidase RNA revealed that the injected cells (blue) have lost adhesion to neighboring cells (Fig. 4C). Almost all injected cells, except for those in the outermost layer, which are interconnected by tight junctions, have fallen into the blastocoel. When similar amounts of XRnd1 mRNA were

injected into the future dorsal side of 4-cell embryos, various degrees of head truncations and blastopore defects were observed (data not shown).

In an effort to generate a potential dominant-negative version of XRnd1 with the purpose of generating loss-of-function phenotypes, we introduced several mutations into XRnd1 (see Fig. 1A). A threonine-to-asparagine substitution at position 27 was introduced since the equivalent substitutions in RhoA and Rac result in dominant-negative forms of these proteins. In addition, we substituted valine at position 22 with glycine, generating the XRnd1 G22/N27 double mutant. These mutations should interfere with GTP binding (Nobes et al., 1998). In a third mutant, we deleted the CAAX-box motif. None of these mutants act as the dominant-negative form since they were not able to reverse the disruption of cell adhesion caused by wild-type XRnd1 overexpression (data not shown). They also were unable to disrupt cell adhesion when overexpressed. These results suggest that proper GTP binding and the CAAX-box motif are necessary to disrupt cell adhesion.

XRhoA overexpression antagonizes loss of cell adhesion by XRnd1

Since the small GTPases RhoA and Rac reportedly have a positive influence on cell adhesion (Bobak et al., 1997; Braga et al., 1997; Takaishi et al., 1997), we tested whether they could rescue the loss of cell adhesion caused by XRnd1 overexpression. XRnd1 mRNA was coinjected with equal amounts of constitutively active XRhoA (XRhoA V14) or human Rac1 (hRac1 V12) mRNA. Overrexpression of XRhoA mRNA (200 pg) shows a two- to threefold increase in XRhoA protein level over the endogenous level of XRhoA (data not shown). In embryos coinjected with XRnd1 and XRhoA V14, adhesion between the animal pole cells was fully restored

Table 1. XRhoA induces heads in the absence of BMP signaling and is necessary for head formation

	RNAs injected	*n*	Secondary head (%)*	
Experiment 1				
A	tBR (2 ng)	93	0	
B	tBR (2 ng) + XRhoA (200 pg)	19	73	
C	tBR (2 ng) + XRhoA (300 pg)	59	18	
D	tBR (2 ng) + XRhoA (400 pg)	18	50	
E	tBR (2 ng) + hRhoA V14 (100 pg)	56	57	
F	tBR (2 ng) + hRhoA V14 (200 pg)	57	40	
G	tBR (2 ng) + hRhoA V14 (300 pg)	30	30	
H	tBR (2 ng) + XRhoA V14 (100 pg)	29	34	
I	tBR (2 ng) + XRhoA V14 (200 pg)	63	38	
J	tBR (2 ng) + XRhoA V14 (300 pg)	25	24	
K	XRhoA (200-400 pg)	36	0	
L	tBR (2 ng) + XRhoA N19 (200 pg)	76	2	
M	tBR (2 ng) + hRac1 V12 (200 pg)	13	0	
Experiment 2				
A	tBR (1 ng)	76	0	
B	tBR (1 ng) + dnXwnt-8 (50 pg)	108	57	
C	tBR (1 ng) + dnXwnt-8 (50 pg) + CSKA XRhoA N19 (200 pg)	69	7	
D	tBR (1 ng) + dnXwnt-8 (50 pg) + CSKA XRhoA N19 (300 pg)	72	6	
E	tBR (1 ng) + dnXwnt-8 (50 pg) + CSKA XRhoA N19 (400 pg)	29	14	
F	tBR (1 ng) + dnXwnt-8 (100 pg)	164	76	
G	tBR (1 ng) + dnXwnt-8 (100 pg) + CSKA XRhoA N19 (200 pg)	55	51	
H	tBR (1 ng) + dnXwnt-8 (100 pg) + CSKA XRhoA N19 (300 pg)	42	7	
Experiment 3				
A	Cerberus (100 pg)	98	35	
B	Cerberus (100 pg) + XRhoA N19 (25 pg)	48	4	
C	Cerberus (100 pg) + XRhoA N19 (50 pg)	52	6	
D	Cerberus (100 pg) + CSKA XRhoA N19 (100 pg)	51	8	
Experiment 4			% reduced eyes/heads	% open blastopores
A	β-galactosidase (50 pg)	26	4	0
B	CSKA XRhoA N19 (100-200 pg)	48	37	18
C	XRhoA N19 (50 pg)	60	45	10
D	XRhoA N19 (50 pg) + XRhoA (150 pg)	66	13	8

*Secondary heads were scored when either cement glands or eyes were present.

n, total number of embryos analyzed. At least two independent experiments were typically carried out for every experiment.

Experiment 1. Coinjection of tBR and XRhoA results in the formation of ectopic head structures. 4-cell stage embryos were injected in two ventral blastomeres with the indicated amounts of mRNAs. The variability in the number of ectopic head structures in experiment 1B-D is due to the variation of head-inducing potential with tBR and XRhoA overexpression, depending on the batch of embryos used.

Experiment 2. dnXRhoA significantly reduces head formation induced by tBR together with dnXwnt-8. The indicated mRNAs and DNA (CSKA XRhoA N19) were injected into two ventral blastomeres at the 4-cell stage.

Experiment 3. dnXRhoA reduces ectopic heads induced by cerberus. One ventral-vegetal blastomere (D4) was injected at the 32-cell stage.

Experiment 4. dnXRhoA reduces head size when injected dorsally. DnXRhoA RNA or DNA was injected into the two dorso-animal blastomeres at the 8-cell stage.

Table 2. XRhoA does not behave as an inhibitor of wnt- or Xnr-1-signaling in injection assays

	RNA/ DNA injected*	*n*	
Experiment 1			% complete secondary axes
A	Xwnt-8 (5 pg)	47	66
B	Xwnt-8 (5 pg) + XRhoA (400 pg)	29	89
Experiment 2			% secondary axes
A	Xnr-1 (30 pg)	31	58
B	Xnr-1 (30 pg) + XRhoA (300 pg)	21	85
Experiment 3			% a- and microcephalic
A	CSKA Xwnt-8 (80 pg)	27	55
B	CSKA Xwnt-8 (80 pg) + XRhoA (200 pg)	21	38
C	CSKA Xwnt-8 (80 pg) + XRhoA (400 pg)	23	39

*The indicated amounts of mRNA or DNA (CSKA Xwnt-8) were injected into one ventral vegetal blastomere at the 8-cell stage (Experiments 1 and 2) or into two dorsoanimal blastomeres at the 8-cell stage (Experiment 3).
n, total number of embryos analyzed. At least two independent experiments were performed for every indicated experiment.

(compare Fig. 4C,D), as indicated by well-adhered, blue-stained cells. The animal cap tissue is as thick and sometimes thicker than control animal cap tissue in these embryos, suggesting that XRhoA V14 can antagonize XRnd1 by restoring cell adhesion. Injecting equal amounts of dominant-negative or wild-type XRhoA failed to restore cell adhesion (data not shown), indicating that an active, GTP-bound XRhoA is required for the rescue of cell adhesion. Interestingly, XRnd1-induced loss of cell adhesion could not be rescued by constitutively active hRac (Fig. 4E). These results suggest either that XRnd1 specifically disrupts XRhoA-dependent cell adhesion or that XRhoA is an antagonist of XRnd1.

Subcellular localization of XRnd1

Since the loss of cell adhesion caused by XRnd1 overexpression is rapid and severe, we examined XRnd1's subcellular localization to determine whether it acts directly at the cell membrane. Overexpressed HA-tagged XRnd1 is localized predominantly at the cell membrane, as observed by immunofluorescence on sections of injected embryos (Fig. 4F). Subcellular fractionation of embryonic extracts of HA-XRnd1-injected embryos also shows that most of the protein is in the membrane fraction and only very little XRnd1 is located in the cytoplasmic fraction (Fig. 4G, arrow). This finding is consistent with the membrane localization of hRnd1 in fibroblasts (Nobes et al., 1998), suggesting that Rnd-type GTPases are predominantly membrane-localized. The CAAX-box deficient mutant, which presumably can not be farnesylated, and which has no influence on cell adhesion in overexpression studies, is found only in the cytoplasmic fraction and not in the membrane fraction (Fig. 4H). This suggests that XRnd1 acts at the cell membrane and that this membrane association is essential for the disruption of cell adhesion in *Xenopus* embryos.

XRhoA is a head inducer

BMP signaling in the mesoderm is important for specifying its ventral fate. Inhibition of BMP signaling on the ventral side of the embryo leads to the formation of incomplete secondary axes by dorsalizing the mesoderm (Graff et al., 1994; Hawley et al., 1995; Suzuki et al., 1994) (Fig. 5B). The induced secondary axes possess notochord and hindbrain structures, but lack forebrain, eyes and cement glands. In an expression screen designed to identify molecules that would inhibit the formation of secondary axes induced by tBR, we identified the small GTPase XRnd1 (Fig. 5C). We examined whether other small GTPases could also affect the formation of secondary axes. XRhoA and hRac1 were examined in this overexpression assay. Coinjection of XRhoA with tBR mRNA resulted in the induction of well-defined head structures, including cement glands and eyes (Fig. 5D and Table 1, Exp. 1A-D). The variability in the number of ectopic head structures in Table 1, Exp. 1B-D is due to batch variation of head-inducing potential with tBR and XRhoA overexpression. Histological sections of these embryos reveal the presence of cement glands, neural tubes and one or two eyes (Fig. 5E). Interestingly, the sectioned embryos did not contain any histologically visible notochord, indicating that head organizer function was mimicked in embryos injected with tBR and XRhoA (Fig. 5F) (Piccolo et al., 1999). Coexpression of constitutively active human RhoA (hRhoA V14) or constitutively active *Xenopus* RhoA (XRhoA V14) with tBR mRNA also resulted in the formation of head structures (Table 1, Exp. 1E-J). The induction of head structures appears to be specific for active RhoA, as coinjection of constitutively active hRac1 (hRac1 V12) or dominant-negative XRhoA (XRhoA N19) together with tBR failed to induce this phenotype (Table 1, Exp. 1L,M). Importantly, expression of XRhoA alone in the ventral side had no overt phenotypic effects and was unable to induce head structures or secondary axes (Table 1, Exp. 1K). These results suggests that RhoA is able to mimick head-inducing signals in the absence of BMP signaling, similar to the phenotypes resulting from overexpression of wnt or nodal inhibitors together with tBR (Glinka et al., 1997; Piccolo et al., 1999).

XRhoA acts downstream of, or in parallel with, the head inducer cerberus

The inhibition of BMP4, wnt-8 and Xnr-1 signals in the head organizer region is essential for head formation. Cerberus is a multifunctional antagonist expressed in the head organizer region that physically binds all three growth factors and inhibits their signaling (Piccolo et al., 1999). Thus, one possible mechanism through which RhoA could induce heads in the absence of BMP signaling is by inhibiting wnt-8 or Xnr-1

signaling. We tested this possibility by coinjecting threshold amounts of Xwnt-8 (that induce fully duplicated axes in most, but not all injected embryos), together with XRhoA RNA to test whether axis formation could be blocked. We found that XRhoA did not block wnt-signaling in this assay (Table 2, Exp. 1A,B). Nor did XRhoA block late (zygotic) wnt-signaling. Expression of Xwnt-8 in the dorsal side at late blastula stage by injecting Xwnt-8 DNA transcribed from a cytoskeletal actin promoter (CSKA Xwnt-8) results in acephalic or microcephalic embryos (Christian and Moon, 1993). Coinjecting CSKA-Xwnt8 together with XRhoA results only in a slight reduction in the number of micro-and acephalic embryos (Table 2, Exp. 3A-C), suggesting that XRhoA does not act as a zygotic wnt-signal inhibitor. XRhoA also does not behave as an inhibitor of Xnr-1 signaling, since coinjection of XRhoA together with threshold amounts of Xnr-1 mRNA into the ventral side of embryos did not reduce secondary axes induced by Xnr-1 (Table 2, Exp. 2A,B). These results suggest that head induction by XRhoA is not a result of an inhibition of Xnr-1 or wnt-8 signals.

XRhoA is required for the formation of ectopic head structures

The above experiments suggest a role for XRhoA in head formation. To test whether XRhoA function is necessary for head formation, we injected dominant-negative XRhoA (XRhoA N19) mRNA or a DNA expression construct in the two dorsal-animal blastomeres, which later contribute to ectodermal head tissue. We used the DNA construct, in addition to the RNA injections, to rule out the possibility that any observed effect was due to the delay in cytokinesis sometimes seen with injections of dominant-negative RhoA in early cleavage stages (data not shown; Drechsel et al., 1997; Kishi et al., 1993). At low levels, dnXRhoA overexpression resulted in the formation of reduced eyes and smaller neural tubes (Fig. 6B,E and Table 1, Exp. 4B,C). Injection of higher amounts of dnRhoA result in the formation of open blastopores (data not shown). The head-reducing effect was specific for the inhibition of XRhoA, since the microcephalic phenotype was rescued by coinjection of wild-type XRhoA (Fig. 6C and Table 1, Exp. 4D).

Coexpression of tBR and dnXwnt-8 in the ventral side leads to the formation of ectopic head structures, often containing two eyes (Glinka et al., 1997) (Fig. 6F). To determine

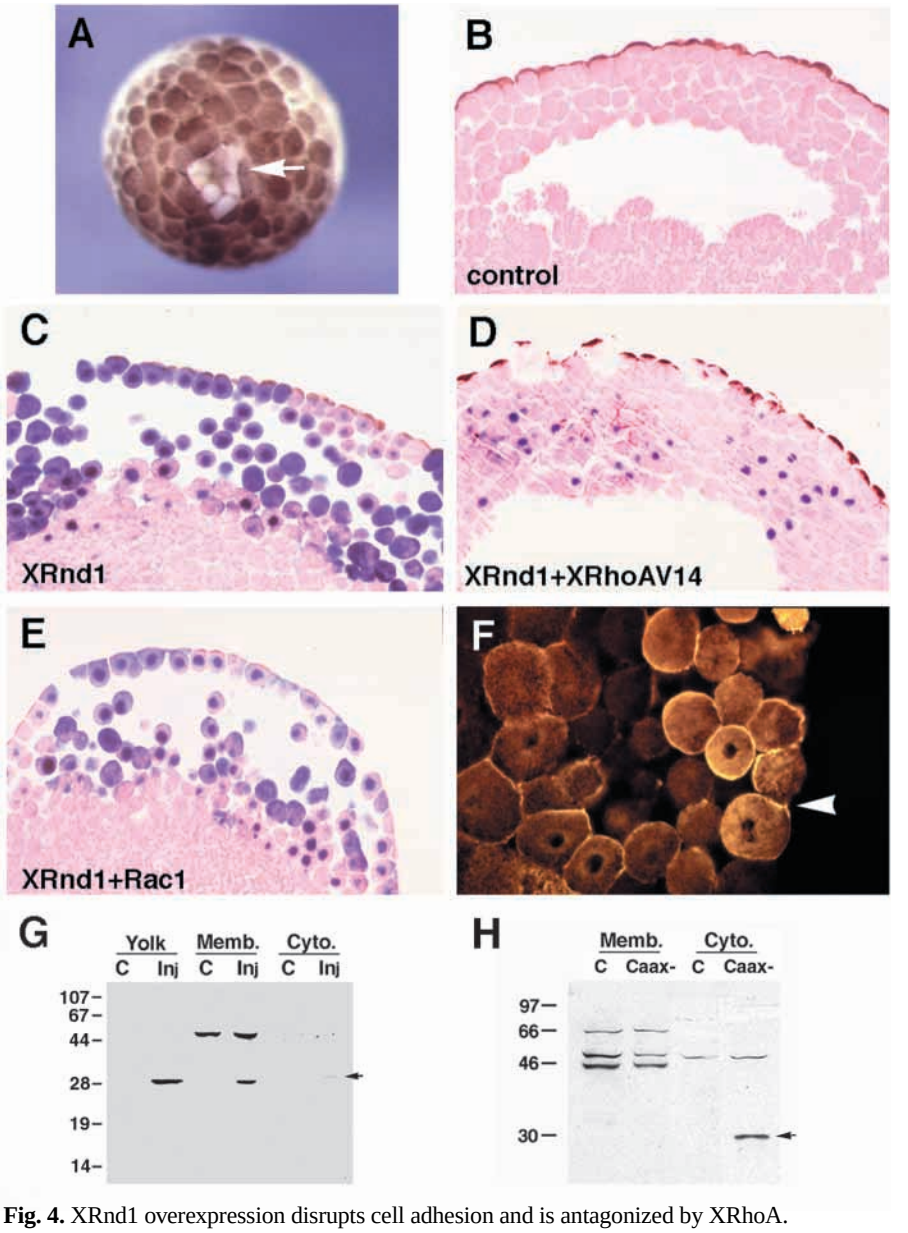

Fig. 4. XRnd1 overexpression disrupts cell adhesion and is antagonized by XRhoA. Overexpression of XRnd1 in the animal pole causes lesions (arrow) within 1-2 hours of injection. (B) Histological section through the animal pole of a blastula (stage 9) control embryo. (C) Section through the animal pole of a stage 9 embryo injected at the 4-cell stage with 250 pg of XRnd1 mRNA and β-gal mRNA as a lineage tracer. The injected blue cells have lost contact with their neighbors and fallen into the blastocoel cavity. LacZ staining appears to be throughout the cells, but in other samples nuclear staining of the XRnd1 expressing cells is detected. (D) Rescue of the XRnd1 cell adhesion defect by coinjection of 250 pg constitutively active XRhoA mRNA (XRhoA V14) with 250 pg of XRnd1 mRNA. Injected (blue) cells adhere well to their neighbors. (E) Cross section through an embryo injected with 250 pg of XRnd1 RNA and 1 ng of constitutively active human Rac1 (Rac1 V12) mRNA. Overexpression of hRac1 V12 is not able to rescue the loss of cell adhesion caused by XRnd1. (F) Immunofluorescence analysis for HA-tagged XRnd1 in the animal pole region of an injected blastula (stage 9) embryo. Immunostaining is mainly localized at the cell membranes (arrowhead). (G) Subcellular fractionation and western blot analysis of HA-XRnd1-injected (Inj) and uninjected control (C) embryos. The protein (lower 30 kD band) is predominantly found in the membrane (Memb.) and not in the cytoplasm (Cyto.). Its presence in the yolk fraction could be due to contamination with unlysed cells. The higher 44 kD band is due to background staining in the membrane fraction. (H) Subcellular fractionation and western blot analysis of HA-XRnd1-CAAX-injected embryos. The CAAX-form of XRnd1 (arrowhead) is found in the cytoplasmic fraction (Cyto.), but not in the membrane fraction (Memb.). C, uninjected control. The positions of molecular mass (kD) markers are shown.

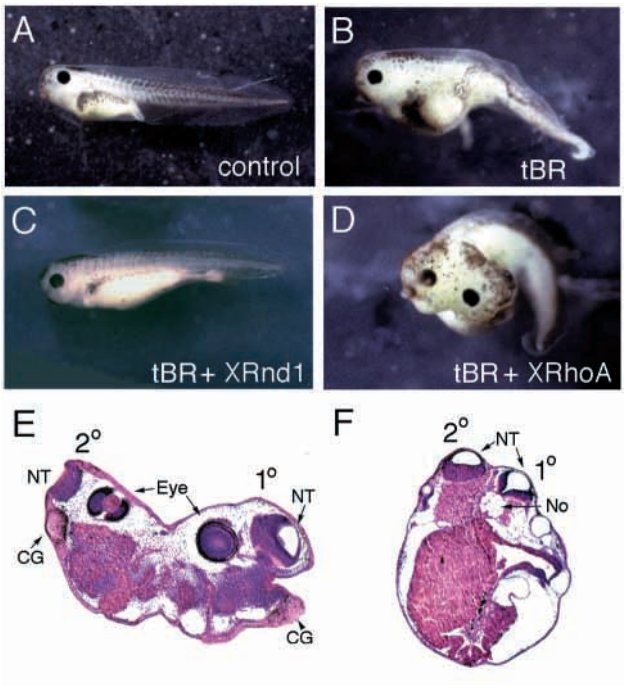

Fig. 5. XRhoA is involved in the formation of head structures. (A) Uninjected control embryo at stage 42. (B) Incomplete secondary axis of an embryo injected with 1 ng of dominant-negative BMP receptor (tBR) mRNA in the ventral side at 4-cell stage. (C) Formation of tBR-induced secondary axes is inhibited in embryos coinjected with tBR and 250 pg of XRnd1 mRNA. (D) Head structures (eyes and cement gland) are induced when 300 pg XRhoA mRNA are coinjected with 1ng tBR in the ventral side. (E) Histological section of the head region of the embryo shown in (D). The head of the secondary axis contains eyes, neural tube (NT) and cement gland (CG). (F) More posterior histological section of the same embryo as in (D) and (E) showing that the secondary axis does not contain notochord (No), indicating that head organizer activity is mimicked. 1°, primary axis; 2°, secondary axis.

if dnXRhoA could affect the formation of head structures induced by tBR together with dnXwnt-8, we coinjected dnRhoA DNA together with tBR and dnXwnt-8. In these coinjected embryos, the formation of head structures was significantly reduced (Fig. 6G, Table 1, Exp. 2A-H). This effect is not the result of a general inhibition of cell movements or cell death since, at the injected concentrations of dnXRhoA, the formation of other ectopically induced structures such as secondary axes is not reduced (Fig. 6G). The head inducer cerberus causes formation of ectopic heads when injected in the ventral-vegetal side (Bouwmeester et al., 1996). DnXRhoA overexpression also reduces the number of ectopic heads induced by cerberus in coinjection experiments (Fig. 6H,I, Table 1, Exp. 3A-D). We conclude that RhoA plays a key role in head formation in *Xenopus*, since blocking its activity leads to a significant reduction in the number of ectopically induced heads and also can reduce the size of head structures in the primary axis.

DISCUSSION

XRnd1 modulates cell adhesion during *Xenopus* gastrulation

We initially isolated XRnd1 during an expression screen designed to identify molecules that could inhibit secondary axis formation induced by tBR. With this approach, we expected to isolate molecules that are either involved in the regulation of BMP signaling or that could affect the formation of the secondary axis by their influence on morphogenesis. XRnd1 is apparently not involved in BMP signaling since it does not ventralize embryos when injected into the dorsal side

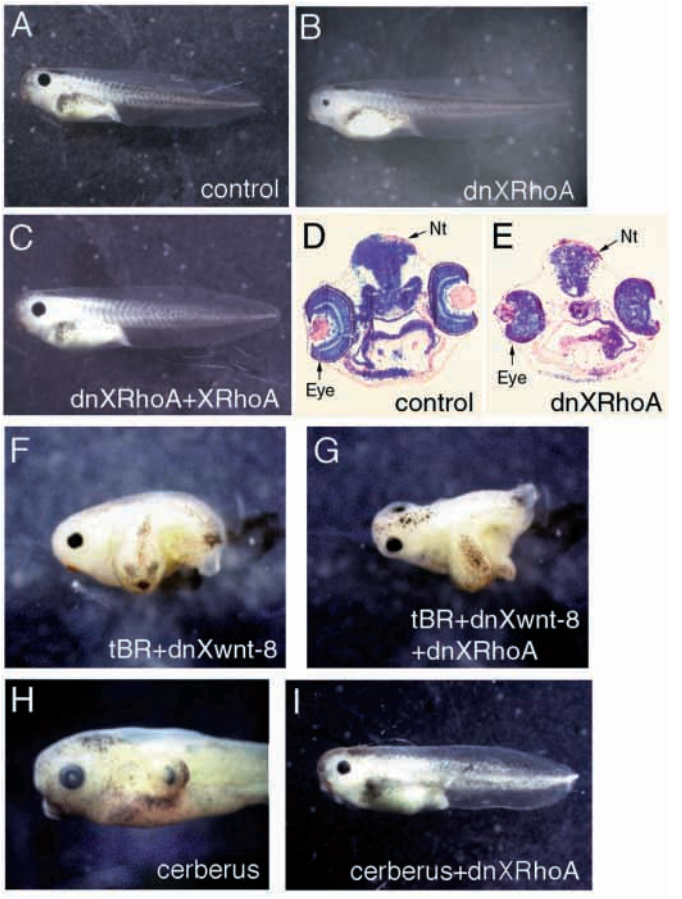

Fig. 6. Dominant-negative RhoA reduces eye size when injected dorsally and inhibits the formation of ectopic heads by cerberus and dnXwnt-8 in combination with tBR. (A) Control embryo. (B) Embryo injected with 50 pg of dnRhoA RNA in the dorsal-animal region (the future head region) at the 8-cell stage, which causes a reduction in anterior tissue as seen here by a reduction of eye size. (C) Embryo coinjected with dnRhoA (50 pg) and XRhoA (100 pg) in the dorsal-animal side at 8-cell stage. The eyes have normal size. (D) Histological section of control embryo injected with β-galactosidase mRNA in two dorsal-animal blastomeres at 8-cell stage at the eye level. (E) Histological section of the embryo shown in B injected with 50 pg of dnXRhoA and β-gal lineage tracer mRNA. Note the small size of eyes and neural tube (arrows). (F) Head structures (two eyes and a cement gland) are induced in embryos coinjected with 1 ng tBR and 50 pg dnXwnt-8 RNA in the ventral side at 4-cell stage. (G) The formation of these head structures is blocked in embryos coinjected with 1 ng tBR, 50 pg of dnXwnt-8 RNA and 200 pg of CSKA-dnXRhoA DNA, expressing dnXRhoA from a cytoskeletal actin promoter at midblastula transition. Note that the formation of secondary axis is not inhibited. (H) A rudimentary ectopic head is induced in embryos injected with 100 pg of cerberus RNA in the D4 blastomere at 32-cell stage. (I) The formation of ectopic heads by cerberus is inhibited when 50 pg dnRhoA mRNA are coinjected with 100 pg cerberus RNA into the D4 blastomere.

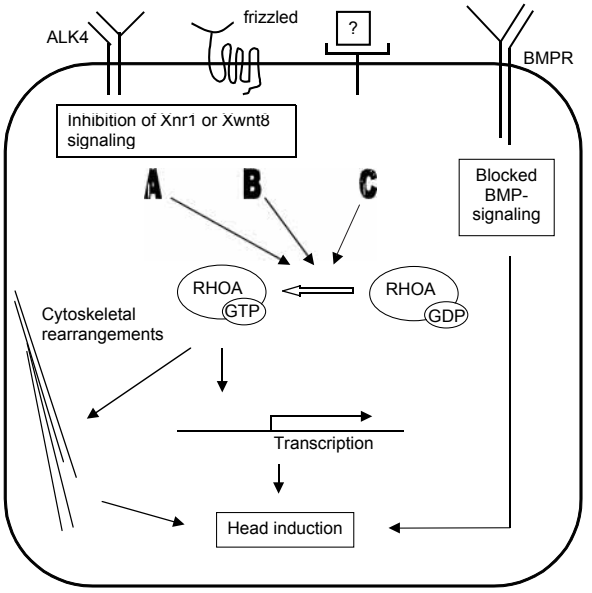

Fig. 7. Model of XRhoA's possible function in head induction. Cerberus binds and inhibits BMP4, Xwnt-8 and Xnr-1. The inhibition of either Xnr-1 (A) or Xwnt-8 (B) signaling may lead to activation of RhoA (indicated by GDP to GTP exchange). Alternatively, RhoA may be activated upon signaling of unknown factors involved in head induction (C). RhoA activation could then result in a variety of events involved in head formation, including transcriptional regulation of head-specific genes and morphogenesis via influences on the actin cytoskeleton. Head induction by XRhoA overexpression only occurs in synergy with BMP signal inhibition.

and also does not stimulate the BMP-responsive Xvent2 promoter (Candia et al., 1997; our unpublished observations). Several points of evidence suggest that XRnd1 may be involved in regulating morphogenesis in early *Xenopus* embryos. First, XRnd1 is expressed predominantly in tissues undergoing extensive morphogenetic movements, such as the involuting mesoderm during gastrulation and cranial neural crest cells. In the somitogenic mesoderm, XRnd1 expression is high in unsegmented areas and regions in which somites have just formed, after which its expression ceases. Several molecules for which roles in morphogenesis have been suggested in *Xenopus* are expressed in similar locations, such as paraxial protocadherin (PAPC) (Kim et al., 1998), the cell surface disintegrin metalloprotease ADAM 13 (Alfandari et al., 1997) and the receptor tyrosine kinase pagliaccio (Pag) (Winning and Sargent, 1994). Secondly, XRnd1 overexpression in animal cap tissue causes a dramatic loss of cell adhesion. This is also consistent with a potential role in modulation of cellular morphogenesis, since the involution of the marginal zone cells in the blastopore, rotation of the somites during somite formation and delamination and migration of neural crest cells require the loosening and rearrangements of cell-cell contacts (Duband et al., 1995; Wilson and Keller, 1991; Youn and Malacinski, 1981). In fact, most molecules identified so far that are implicated in embryonic morphogenesis are either cell adhesion molecules themselves, including cadherins (Costa et al., 1998; Kuhl et al., 1996; Lee and Gumbiner, 1995), integrins (Ramos et al., 1996) and protocadherins (Bradley et al., 1998; Kim et al., 1998), or modulate cell adhesion in some way via PDGF (Ataliotis et al., 1995), HGF (Ruiz i Altaba and Thery, 1996), or Xwnt5A (Moon et al., 1993). Interestingly, like XRnd1, Xwnt5A is able to block secondary axis formation induced by a variety of factors (Torres et al., 1996), suggesting that XRnd1 and Xwnt5A might function in a similar fashion, perhaps through a decrease in cell adhesion.

Our findings that XRnd1 causes a loss of cell adhesion are consistent with overexpression studies of human Rnd1 in tissue culture cells. HRnd1 causes loss of cell adhesion in Swiss 3T3 cells, and interferes with Rho- and Rac-mediated actin filament assembly (Nobes et al., 1998). Based on several unique amino acids in their effector domains, *Xenopus* and human Rnd1 are apparently constitutively active, raising the question of how their activity is regulated. One possibility is that inhibitory exchange factors similar to Rho-GAPs negatively regulate Rnd1. Alternatively, their activity might simply be regulated by differential expression. This form of regulation may play a role in the early *Xenopus* embryo as Rnd1 is dynamically expressed in the embryo at the RNA level. Rnd1 is also differentially expressed in humans, where it is found almost exclusively in liver and brain in adult tissues (Nobes et al., 1998). Chick RhoB is another example where a small GTPase is regulated at least partly by differential expression, in this case in response to BMP signaling in the neural tube (Liu and Jessell, 1998). It is interesting to note that both Rnd1 and RhoB share a farnesylation consensus site at their C terminus, whereas most other small GTPases are geranyl-geranylated. This raises the possibility that farnesylated Rhos form a subgroup of Rho-like GTPases that is regulated on the level of transcription. In contrast, RhoA is more widely expressed, both in chick and *Xenopus*, suggesting that exchange factors play a more important role in its regulation than RNA expression levels. The expression pattern of XRnd1 in the early *Xenopus* embryo, together with XRnd1's effect on cell adhesion, suggests that XRnd1's function in the embryo is to reduce cell adhesion in cells that are undergoing morphogenetic movements. XRnd1 possibly promotes cell intercalations through loosening of cell contacts in the involuting mesoderm. Convergence-extension movements in the involuting mesoderm are a result of such intercalation movements (Wilson and Keller, 1991). Further studies to identify which factors regulate the function and expression of XRnd1 could give more insights into the regulation of cell adhesion during early development.

XRhoA and XRnd1 play antagonistic roles in the regulation of cell adhesion

Activated XRhoA can antagonize the loss of cell adhesion caused by XRnd1 in the animal cap overexpression assay. This function seems to be specific for active RhoA, since wild-type XRhoA and activated hRac1 fail to do so. A positive role for RhoA in regulating cell adhesion has also been found in various cell culture systems. For example, inhibition of RhoA by *Clostridium botulinum* exoenzyme C3 transferase in cell culture, which specifically inactivates RhoA, interrupts cell adhesion (Bobak et al., 1997). In keratinocytes, blocking RhoA by microinjection of C3 transferase causes the depletion of E-cadherin from intercellular junctions (Braga et al., 1997), suggesting that the loss of cell adhesion could be due to RhoA's effect on cadherin-mediated cell adhesion.

RhoA also promotes the formation of stress fibers in response to certain growth factors such as PDGF (Ridley and Hall, 1992) and HGF (Takaishi et al., 1995). In *Xenopus*, PDGF plays a role in gastrulation where it apparently is involved in promoting adherence of the involuting mesoderm to the overlying ectoderm (Ataliotis et al., 1995). Since RhoA is a known downstream component of PDGF signaling (Zubiaur et al., 1995) it might be involved in promoting this adhesiveness. The formation of stress fibers by RhoA is disrupted by XRnd1 (Nobes et al., 1998). This suggests that XRhoA and XRnd1 have opposite effects on actin filament assembly as well as on cell adhesion, and possibly antagonize each other. In the early *Xenopus* embryo, the relative activities of XRhoA and XRnd1 could play an important role in regulating adhesiveness in developing tissues and be subject to regulation by various growth factors involved in regulating morphogenetic behavior.

XRhoA functions as a head inducer

For the proper formation of head structures, the head organizer releases several secreted inhibitors to prevent formation of trunk structures. These include cerberus, an antagonist of BMP4, wnt-8 and Xnr-1 signaling (Piccolo et al., 1999) and the wnt-signaling inhibitors frzb and dkk-1 (Glinka et al., 1998; Leyns et al., 1997; Wang et al., 1997). So far, all known head-inducing factors are extracellular inhibitory molecules and very little is known about intracellular factors that are positively involved in the head induction process. XRhoA is likely to be one of these factors. First, coinjection of XRhoA together with tBR in the ventral side leads to the formation of head structures. Secondly, overexpression of a dominant-negative form of XRhoA in the future head ectoderm region leads to a reduction in head size. Also, overexpression of dominant-negative RhoA with the head inducer cerberus or dnRhoA with tBR and dnXwnt-8 leads to a significant reduction in the number of induced heads. Lastly, XRhoA is expressed at the right time and place to act in the head induction pathway. These pieces of evidence support an essential role for XRhoA in head formation.

How can XRhoA function to induce head structures together with tBR? If XRhoA was an inhibitor of Xnr-1 or wnt-signaling, the head induction phenotype could be easily explained, since wnt- and Xnr-1 signaling inhibitors induce heads when overexpressd with BMP inhibitors (Glinka et al., 1997; Piccolo et al., 1999). However, in injection assays (Table 2), XRhoA does not behave as an inhibitor of wnt or Xnr-1 signaling, which suggests that is an unlikely possibility. Alternatively, XRhoA could be 'activated' in response to the inhibition of wnt-8 or Xnr-1 signals, i.e. via cerberus, or upon signaling in a parallel, yet unidentified pathway involved in head induction (see Fig. 7).

Some evidence supports the notion that XRhoA is linked to wnt-signaling. In *Drosophila*, *rhoA* has been shown to play a role in the establishment of cell polarity downstream of *frizzled (fz)* and *dishevelled (dsh)*, components of the wnt-signaling cascade (Strutt et al., 1997). Hypomorphic alleles of *Drosophila rhoA* show a cell polarity phenotype similar to dsh^1 and *fz* and overexpression phenotypes of *frizzled* and *dishevelled* can be rescued with the *rhoA* mutant, indicating that they act in the same pathway (Strutt et al., 1997).

Interestingly, the *Drosophila* JUN N-terminal kinase (JNK) *basket* is involved in cell polarity signaling (Strutt et al., 1997) and is activated by Dishevelled (Boutros et al., 1998). JNK can also be activated by RhoA in cell culture (Teramoto et al., 1996b). This raises the possibility for the involvement of JNK in head formation. This notion is especially intriguing since a similar signaling cascade, the ras-regulated MAPK pathway, is also involved during embryogenesis in mesoderm patterning (LaBonne et al., 1995). Alternatively, RhoA may influence gene regulation via NF-κb (Perona et al., 1997) or serum response factor (SRF) (Hill et al., 1995), factors known to be regulated by RhoA in other systems. Further identification of the factors involved in the regulation of RhoA activity and downstream RhoA targets will be necessary to elucidate RhoA's role during head formation.

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REFERENCES

Adamson, P., Marshall, C. J., Hall, A. and Tilbrook, P. A. (1992). Post-translational modifications of p21rho proteins. *J. Biol. Chem.* **267,** 20033-20038.

Alfandari, D., Wolfsberg, T. G., White, J. M. and DeSimone, D. W. (1997). ADAM 13: a novel ADAM expressed in somitic mesoderm and neural crest cells during *Xenopus laevis* development. *Dev. Biol.* **182,** 314-330.

Ataliotis, P., Symes, K., Chou, M. M., Ho, L. and Mercola, M. (1995). PDGF signalling is required for gastrulation of *Xenopus laevis*. *Development* **121,** 3099-3110.

Barrett, K., Leptin, M. and Settleman, J. (1997). The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in Drosophila gastrulation. *Cell* **91,** 905-915.

Blitz, I. L. and Cho, K. W. (1995). Anterior neurectoderm is progressively induced during gastrulation: the role of the *Xenopus* homeobox gene orthodenticle. *Development* **121,** 993-1004.

Bobak, D., Moorman, J., Guanzon, A., Gilmer, L. and Hahn, C. (1997). Inactivation of the small GTPase Rho disrupts cellular attachment and induces adhesion-dependent and adhesion-independent apoptosis. *Oncogene* **15,** 2179-2189.

Boutros, M., Paricio, N., Strutt, D. I. and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell* **94,** 109-118.

Bouwmeester, T., Kim, S., Sasai, Y., Lu, B. and De Robertis, E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382,** 595-601.

Bradley, R. S., Espeseth, A. and Kintner, C. (1998). NF-protocadherin, a novel member of the cadherin superfamily, is required for Xenopus ectodermal differentiation. *Curr. Biol.* **8,** 325-334.

Braga, V. M., Machesky, L. M., Hall, A. and Hotchin, N. A. (1997). The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *J. Cell Biol.* **137,** 1421-1431.

Candia, A. F., Watabe, T., Hawley, S. H., Onichtchouk, D., Zhang, Y., Derynck, R., Niehrs, C. and Cho, K. W. (1997). Cellular interpretation of multiple TGF-beta signals: intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* **124,** 4467-4480.

Cho, K. W., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the Xenopus homeobox gene goosecoid. *Cell* **67,** 1111-1120.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156-159.

Christian, J. L. and Moon, R. T. (1993). Interactions between Xwnt-8 and

Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of Xenopus. *Genes Dev.* **7,** 13-28.

Collett, J. W. and Steele, R. E. (1993). Alternative splicing of a neural-specific Src mRNA (Src+) is a rapid and protein synthesis-independent response to neural induction in *Xenopus laevis*. *Dev. Biol.* **158,** 487-495.

Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T. and Gutkind, J. S. (1995). The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* **81,** 1137-1146.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J. and Priess, J. R. (1998). A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell Biol.* **141,** 297-308.

Drechsel, D. N., Hyman, A. A., Hall, A. and Glotzer, M. (1997). A requirement for Rho and Cdc42 during cytokinesis in Xenopus embryos. *Curr. Biol.* **7,** 12-23.

Duband, J. L., Monier, F., Delannet, M. and Newgreen, D. (1995). Epithelium-mesenchyme transition during neural crest development. *Acta Anat.* **154,** 63-78.

Eaton, S., Auvinen, P., Luo, L., Jan, Y. N. and Simons, K. (1995). CDC42 and Rac1 control different actin-dependent processes in the Drosophila wing disc epithelium. *J. Cell Biol.* **131,** 151-164.

Fagotto, F. and Gumbiner, B. M. (1994). Beta-catenin localization during Xenopus embryogenesis: accumulation at tissue and somite boundaries. *Development* **120,** 3667-3679.

Foster, R., Hu, K. Q., Lu, Y., Nolan, K. M., Thissen, J. and Settleman, J. (1996). Identification of a novel human Rho protein with unusual properties: GTPase deficiency and in vivo farnesylation. *Mol. Cell. Biol.* **16,** 2689-2699.

Garrett, M. D., Self, A. J., van Oers, C. and Hall, A. (1989). Identification of distinct cytoplasmic targets for ras/R-ras and rho regulatory proteins. *J. Biol. Chem.* **264,** 10-13.

Glinka, A., Wu, W., Onichtchouk, D., Blumenstock, C. and Niehrs, C. (1997). Head induction by simultaneous repression of Bmp and Wnt signalling in Xenopus. *Nature* **389,** 517-519.

Glinka, A., Wu, W., Delius, H., Monaghan, A. P., Blumenstock, C. and Niehrs, C. (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391,** 357-362.

Graff, J. M., Thies, R. S., Song, J. J., Celeste, A. J. and Melton, D. A. (1994). Studies with a Xenopus BMP receptor suggest that ventral mesoderm-inducing signals override dorsal signals in vivo. *Cell* **79,** 169-179.

Gubler, U. and Hoffman, B. J. (1983). A simple and very efficient method for generating cDNA libraries. *Gene* **25,** 263-269.

Gurdon, J. B. (1992). The generation of diversity and pattern in animal development. *Cell* **68,** 185-199.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279,** 509-514.

Harden, N., Loh, H. Y., Chia, W. and Lim, L. (1995). A dominant inhibitory version of the small GTP-binding protein Rac disrupts cytoskeletal structures and inhibits developmental cell shape changes in *Drosophila*. *Development* **121,** 903-914.

Harden, N., Ricos, M., Ong, Y. M., Chia, W. and Lim, L. (1999). Participation of small GTPases in dorsal closure of the Drosophila embryo: distinct roles for *Rho* subfamily proteins in epithelial morphogenesis. *J. Cell Sci.* **112,** 273-284.

Harland, R. M. (1991). In situ hybridization: an improved whole-mount method for Xenopus embryos. *Meth. Cell Biol.* **36,** 685-695.

Hawley, S. H., Wunnenberg-Stapleton, K., Hashimoto, C., Laurent, M. N., Watabe, T., Blumberg, B. W. and Cho, K. W. (1995). Disruption of BMP signals in embryonic Xenopus ectoderm leads to direct neural induction. *Genes Dev.* **9,** 2923-2935.

Hill, C. S., Wynne, J. and Treisman, R. (1995). The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. *Cell* **81,** 1159-1170.

Hsu, D. R., Economides, A. N., Wang, X., Eimon, P. M. and Harland, R. M. (1998). The Xenopus dorsalizing factor Gremlin identifies a novel family of secreted proteins that antagonize BMP activities. *Mol. Cell* **1,** 673-683.

Iemura, S., Yamamoto, T. S., Takagi, C., Uchiyama, H., Natsume, T., Shimasaki, S., Sugino, H. and Ueno, N. (1998). Direct binding of follistatin to a complex of bone-morphogenetic protein and its receptor inhibits ventral and epidermal cell fates in early Xenopus embryo. *Proc. Natl. Acad. Sci. USA* **95,** 9337-9342.

Jones, C. M., Kuehn, M. R., Hogan, B. L., Smith, J. C. and Wright, C. V. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121,** 3651-3662.

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

Kishi, K., Sasaki, T., Kuroda, S., Itoh, T. and Takai, Y. (1993). Regulation of cytoplasmic division of Xenopus embryo by rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI). *J. Cell Biol.* **120,** 1187-1195.

Klein, S. L. (1987). The first cleavage furrow demarcates the dorsal-ventral axis in Xenopus embryos. *Dev. Biol.* **120,** 299-304

Krieg, P. A. and Melton, D. A. (1984). Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. *Nucleic Acids Res.* **12,** 7057-7070.

Kuhl, M., Finnemann, S., Binder, O. and Wedlich, D. (1996). Dominant negative expression of a cytoplasmically deleted mutant of XB/U-cadherin disturbs mesoderm migration during gastrulation in *Xenopus laevis*. *Mech. Dev.* **54,** 71-82.

LaBonne, C., Burke, B. and Whitman, M. (1995). Role of MAP kinase in mesoderm induction and axial patterning during *Xenopus* development. *Development* **121,** 1475-1486.

Lee, C. H. and Gumbiner, B. M. (1995). Disruption of gastrulation movements in Xenopus by a dominant-negative mutant for C-cadherin. *Dev. Biol.* **171,** 363-373.

Leyns, L., Bouwmeester, T., Kim, S. H., Piccolo, S. and De Robertis, E. M. (1997). Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* **88,** 747-756.

Liu, J. P. and Jessell, T. M. (1998). A role for rhoB in the delamination of neural crest cells from the dorsal neural tube. *Development* **125,** 5055-5067.

Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. and Green, M. R. (1984). Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12,** 7035-7056.

Minden, A., Lin, A., Claret, F. X., Abo, A. and Karin, M. (1995). Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* **81,** 1147-1157.

Moon, R. T., Campbell, R. M., Christian, J. L., McGrew, L. L., Shih, J. and Fraser, S. (1993). Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. *Development* **119,** 97-111.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of* Xenopus laevis. Garland Publishing, Amsterdam, North Holland.

Nobes, C. D., Lauritzen, I., Mattei, M. G., Paris, S., Hall, A. and Chardin, P. (1998). A new member of the Rho family, Rnd1, promotes disassembly of actin filament structures and loss of cell adhesion. *J. Cell Biol.* **141,** 187-197.

Perona, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R. and Lacal, J. C. (1997). Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. *Genes Dev.* **11,** 463-475.

Piccolo, S., Sasai, Y., Lu, B. and De Robertis, E. M. (1996). Dorsoventral patterning in Xenopus: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* **86,** 589-598.

Piccolo, S., Agius, E., Leyns, L., Bhattacharyya, S., Grunz, H., Bouwmeester, T. and De Robertis, E. M. (1999). The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature* **397,** 707-710.

Qiu, R. G., Chen, J., McCormick, F. and Symons, M. (1995). A role for Rho in Ras transformation. *Proc. Natl. Acad. Sci. USA* **92,** 11781-1175.

Ramos, J. W., Whittaker, C. A. and DeSimone, D. W. (1996). Integrin-dependent adhesive activity is spatially controlled by inductive signals at gastrulation. *Development* **122,** 2873-2883.

Ransom, D. G., Hens, M. D. and DeSimone, D. W. (1993). Integrin expression in early amphibian embryos: cDNA cloning and characterization of Xenopus beta 1, beta 2, beta 3, and beta 6 subunits. *Dev. Biol.* **160,** 265-275.

Ridley, A. J. and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70,** 389-399.

Ruiz i Altaba, A. and Thery, C. (1996). Involvement of Livertine, a hepatocyte growth factor family member, in neural morphogenesis. *Mech. Dev.* **60,** 207-220.

Smith, J. C., Symes, K., Hynes, R. O. and DeSimone, D. (1990). Mesoderm induction and the control of gastrulation in *Xenopus laevis*: the roles of fibronectin and integrins. *Development* **108,** 229-238.

Smith, W. C., McKendry, R., Ribisi, S., Jr. and Harland, R. M. (1995). A

nodal-related gene defines a physical and functional domain within the Spemann organizer. *Cell* **82,** 37-46.

Spemann, H. and Mangold, H. (1924). Über die Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Roux' Arch. Entw. Mech. Org.* **100,** 599-638.

Spemann, H. (1931). Über den Anteil von Implantat und Wirtskeim an der Orientierung und Beschaffenheit der induzierten Embryonalanlage. *Roux' Arch. Entw. Mech. Org.* **123,** 389-517.

Strutt, D. I., Weber, U. and Mlodzik, M. (1997). The role of RhoA in tissue polarity and Frizzled signalling. *Nature* **387,** 292-295.

Suzuki, A., Thies, R. S., Yamaji, N., Song, J. J., Wozney, J. M., Murakami, K. and Ueno, N. (1994). A truncated bone morphogenetic protein receptor affects dorsal-ventral patterning in the early Xenopus embryo. *Proc. Natl. Acad. Sci. USA* **91,** 10255-10259.

Takaishi, K., Sasaki, T., Kameyama, T., Tsukita, S. and Takai, Y. (1995). Translocation of activated Rho from the cytoplasm to membrane ruffling area, cell-cell adhesion sites and cleavage furrows. *Oncogene* **11,** 39-48.

Takaishi, K., Sasaki, T., Kotani, H., Nishioka, H. and Takai, Y. (1997). Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. *J. Cell Biol.* **139,** 1047-1059.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251,** 1451-1455.

Teramoto, H., Coso, O. A., Miyata, H., Igishi, T., Miki, T. and Gutkind, J. S. (1996a). Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. *J. Biol. Chem.* **271,** 27225-27228.

Teramoto, H., Crespo, P., Coso, O. A., Igishi, T., Xu, N. and Gutkind, J. S. (1996b). The small GTP-binding protein rho activates c-Jun N-terminal kinases/stress-activated protein kinases in human kidney 293T cells. Evidence for a Pak-independent signaling pathway. *J. Biol. Chem.* **271,** 25731-25734.

Torres, M. A., Yang-Snyder, J. A., Purcell, S. M., DeMarais, A. A., McGrew, L. L. and Moon, R. T. (1996). Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early Xenopus development. *J. Cell Biol.* **133,** 1123-1137.

Van Aelst, L. and D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. *Genes Dev.* **11,** 2295-2322.

Wang, S., Krinks, M., Lin, K., Luyten, F. P. and Moos, M., Jr. (1997). Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* **88,** 757-766.

Whittaker, C. A. and DeSimone, D. W. (1993). Integrin alpha subunit mRNAs are differentially expressed in early *Xenopus* embryos. *Development* **117,** 1239-1249.

Wilson, P. and Keller, R. (1991). Cell rearrangement during gastrulation of *Xenopus*: direct observation of cultured explants. *Development* **112,** 289-300.

Winning, R. S. and Sargent, T. D. (1994). Pagliaccio, a member of the Eph family of receptor tyrosine kinase genes, has localized expression in a subset of neural crest and neural tissues in *Xenopus laevis* embryos. *Mech. Dev.* **46,** 219-229.

Youn, B. W. and Malacinski, G. M. (1981). Somitogenesis in the amphibian *Xenopus laevis*: scanning electron microscopic analysis of intrasomitic cellular arrangements during somite rotation. *J. Embryol. Exp. Morph.* **64,** 23-43.

Zhong, Y., Brieher, W. M. and Gumbiner, B. M. (1999). Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J. Cell Biol.* **144,** 351-359.

Zimmerman, L. B., De Jesus-Escobar, J. M. and Harland, R. M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* **86,** 599-606.

Zubiaur, M., Sancho, J., Terhorst, C. and Faller, D. V. (1995). A small GTP-binding protein, Rho, associates with the platelet-derived growth factor type-beta receptor upon ligand binding. *J. Biol. Chem.* **270,** 17221-17228.