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A Novel POZ/Zinc Finger Protein, *champignon*, Interferes With Gastrulation Movements in *Xenopus*

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ABSTRACT **We have cloned a novel *krüppel*-like transcription factor of *Xenopus* that encodes POZ/zinc finger protein by expression cloning. Overexpression of mRNA resulted in interference with gastrulation. Because the injected embryo looks like a mushroom in appearance at the neurula stage, we have named this gene *champignon* (*cpg*). In *cpg*-injected embryos, the blastopore appeared normally, but regressed thereafter. The injected embryos then elongated along the primary dorsoventral axis during the tailbud stage. Histologic sections and reverse transcription-polymerase chain reaction analysis showed that *cpg* had no effect on the cell differentiation. The animal pole region of *cpg*-injected embryos was thick during the gastrula stage, and mesodermal cells remained in the marginal zone. Furthermore, neither Keller-sandwich explants nor activin-treated animal cap explants excised from *cpg*-injected embryos elongated. These results suggest that *cpg* acts as a potent inhibitor of cell migration and cell intercalation during gastrulation.**

Key words: *Xenopus;* gastrulation; convergent extension; *champignon;* transcription factor; POZ domain; zinc finger domain

INTRODUCTION

During the early development of *Xenopus,* gastrulation involves very complicated changes in cell morphology. The morphogenetic movements during gastrulation involve formation of bottle cells, epiboly of the animal cap, migration of the deep mesoderm along the blastocoel roof, and convergent extension of the noninvoluting and involuting marginal cells (Keller, 1980; Keller et al., 1985, 1992; Keller and Danilchik, 1988; Hardin and Keller, 1988; Keller and Tibbetts, 1989; Wilson and Keller, 1991; Keller and Jansa, 1992; Shih and Keller, 1992a,b). Although bottle cells may play a role in orienting the direction of involution, they are not essential for gastrulation to continue, because the removal of bottle cells after their formation did not interfere with the involution of the marginal zone and closure of the blastopore (Keller, 1981). Similarly, neither epiboly of the animal cap nor migration of mesoderm along the inner surface of the blastopore is essential for involution of the marginal zones and closure of the blastopore, because the removal of the blastocoel roof, including the animal cap and the noninvoluting marginal zone, did not inhibit the involution of the marginal zone and closure of the blastopore (Keller and Jansa, 1992). When the dorsal sectors of the early gastrula embryos were excised and cultured in sandwich, they showed convergent and extension movements autonomously and mimicked the normal process of gastrulation (Keller, 1991). Therefore, convergent extension movements of the marginal cells is thought to play a major role in gastrulation. Moreover, it is recently reported that an active distortion of the vegetal cell mass, vegetal rotation, is required for the internalization of the mesendoderm during the first half of gastrulation (Winklbauer and Schurfeld, 1999).

Although the mechanism of gastrulation has been investigated extensively at the cellular level, as described above, information on the molecular mechanism of gastrulation is limited. When deep mesodermal cells migrate along the inner surface of the blastocoel roof, they attach to the extracellular matrix on the inner surface of the blastocoel roof. The adhesive glycoprotein fibronectin is one of the major components of the network of the extracellular matrix (Lee et al., 1984). Inhibition of cellular attachment to fibronectin by using a synthetic peptide for the cell-binding fragment of fibronectin led to the arrest of gastrulation (Boucaut et al., 1984; Winklbauer, 1990; Ramos and DeSimone, 1996; Ramos et al., 1996; Winklbauer and Keller, 1996). Some molecules related to convergent extension movements have also been isolated. C-cadherin–mediated cell-cell adhesion is associated with convergent extension movements during gastrulation (Lee and Gumbiner, 1995; Zhong et al., 1999). Overexpression of *Xcadherin-11* leads to posteriorised phenotypes due to the inhibition of convergent extension movements (Hadeball et al., 1998). Paraxial protocadherin plays an important role in convergent extension

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movements (Kim et al., 1998). The elimination of some sulfates also inhibits convergent extension movements (Wallingford et al., 1997). Recent studies showed that Wnt signalling other than the canonical Wnt pathway plays a role in convergent extension movements during gastrulation (Djiane et al., 2000; Tada and Smith, 2000) and that a pathway similar to that involved in planar polarity signalling in *Drosophila* is required for normal gastrulation (Sokol, 2000; Wallingford et al., 2000).

In the present study, we cloned a novel *krüppel*-like transcription factor that encodes an N-terminal POZ domain and three C-terminal zinc fingers of the C_2H_2 type by expression cloning. We suggest that this transcription factor acts as a potent inhibitor of gastrulation movements.

RESULTS

Isolation and Characterization of *cpg*

champignon (*cpg*) was isolated by expression screening of a cDNA library from *Xenopus* stage 7 embryos as a gene that caused gross change in morphogenetic movements when microinjected into both cells of two-cell stage *Xenopus* embryos. *cpg* encodes a putative protein of 470 amino acids with a predicted molecular mass of 52.6 kDa. The length of the *cpg* transcript is about 2.4 kb pairs as shown by Northern hybridization (data not shown). cpg contains an N-terminal POZ domain and three C-terminal *krüppel*-like zinc finger domains of the C_2H_2 type (GenBank accession no. AB029074), suggesting that *cpg* is a transcription factor. According to a search using PSORT, cpg has three putative nuclear localization signals (Fig. 1A). A homology search of a protein database using BLAST Search revealed that cpg is partially homologous to the transcription factors human c-krox (hc-krox; Widom et al., 1997) and mouse c-krox (mc-krox; Galera et al., 1994). The hc-krox contains a complete POZ domain and three zinc finger domains, whereas the mc-krox lacks most of the POZ domain. High identity of the amino acid sequences was found especially in the POZ domain and three zinc finger domains: the POZ domain of cpg was 63.5% identical to that of hc-krox, and the zinc finger domains of cpg were 93.5% identical to those of hc-krox and mc-krox (Fig. 1A). The region between the POZ domain and zinc finger domains of cpg was shorter than those of human and mouse c-krox and had low amino acid sequence identity with them. Therefore, we define that *cpg* is not the *c-krox* homologue gene, but a gene of *c-krox* family genes. The other *krüppel*-like transcription factors, such as human AMP-1 and mouse LRF, had some homology with cpg in the zinc finger domains (Fig. 1B), but the homologies in N-terminal region, including the POZ domain, were low.

The transcripts of *cpg* were present maternally and decreased gradually until the mid-blastula stage. Zygotic transcripts of *cpg* were increased gradually after the early gastrula stage (Fig. 2A). Because we could not detect regional differences in *cpg* expression by whole-mount in situ hybridization (data not shown) because of very low expression until the late gastrula stage, we examined the regional differences by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in dissected embryos at blastula (st. 8) and gastrula (st. 10) stages. The quantity of *cpg* transcripts did not differ in different regions in blastula and gastrula embryos (Fig. 2B). Whole-mount in situ hybridization of neurula embryos showed that *cpg* was expressed in the anterior region, especially in the cement gland precursor region (Fig. 2C,D). The strong expression was restricted to the cement gland after tailbud stages (Fig. 2E,F).

Overexpression of *cpg* Leads to Defective Gastrulation

One nanogram of synthetic *cpg* mRNA was injected into the lateral equatorial regions of the two blastomeres of the two-cell embryo, and the vegetal regions of the injected embryos were observed by time-lapse videomicrography. The blastopore of the injected embryos appeared precisely on the dorsal side at the initial gastrula stage (st. 10), spread laterally, and formed a large yolk plug (data not shown). In most cases, however, the blastopore failed to close thereafter (Fig. 4B), and conversely, the lateral regions of the blastopore disappeared (Fig. 3E). The injected embryos became mushroom-like in shape in the most severely defective cases at the neurula stage (Figs. 3E, 4C). After the neurula stage, the injected embryos elongated along the initial dorsoventral axis gradually until tailbud stages. The injected embryos looked like double layers of ectodermal and exposed endodermal cells externally at the tailbud stage (Fig. 3F). Most of the embryos injected with *cpg* mRNA at low doses invaginated slightly (Fig. 3C) and formed partial axial structures, including the head region (Fig. 3D; Table 1), but the blastopores remained open (Fig. 3D). The effects of *cpg* on gastrulation were, thus, dose dependent.

Animal or vegetal injections of *cpg* mRNA at the two-cell stage gave less defective phenotypes than equatorial injection (Table 2). Embryos injected with *cpg* mRNA into the two dorsal blastomeres at the four-cell stage gave rise to severely defective embryos (Fig. 3G,H). However, embryos injected into the two ventral blastomeres of the four-cell stage embryos developed rather normally. In these ventrally injected embryos, gastrulation of the dorsal half was not affected at all and the anterior half of the resulting embryo was quite normal at the tailbud stage (Fig. 3I,J). These results indicate that the effects of *cpg* on gastrulation movements were restricted to the area near the injected site and that overexpression at the dorsal marginal zone was most effective.

Morphogenesis of *cpg*-Injected Embryos

The injected embryos developed normally until the formation of the large yolk plug (st. 10.5), but the

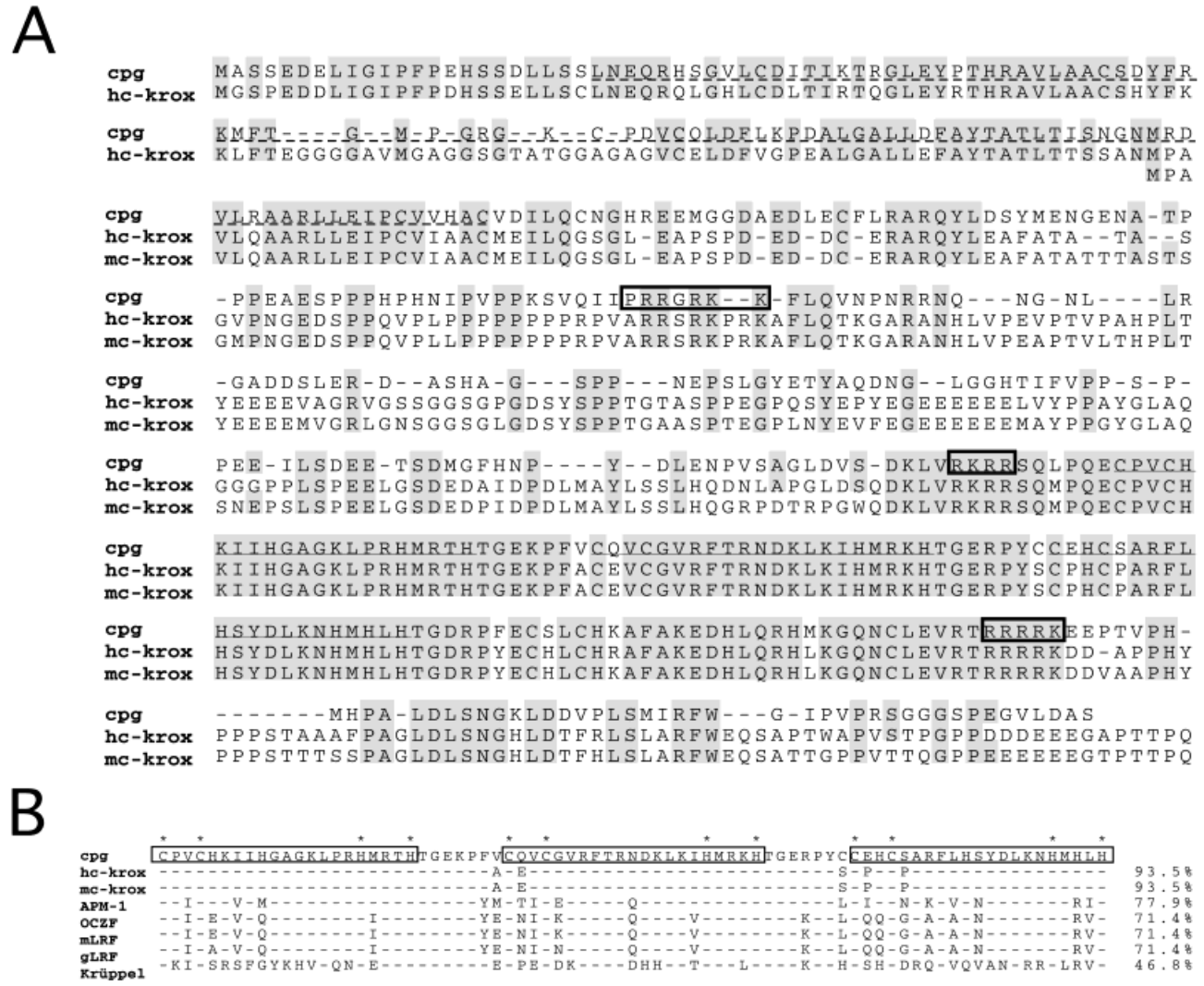

Fig. 1. **A:** Alignment of the cpg, human c-krox, and mouse c-krox amino acid sequences. The amino acid residues that are identical between the proteins are shaded gray. The putative nuclear localization signals identified by using PSORT are boxed. The POZ domain at the N-terminus is indicated by an interrupted underline. The zinc finger domains at the C-terminus are underlined. **B:** Homology of the zinc finger domains of cpg to those of other *krüppel*-like zinc finger proteins. The zinc finger domains are boxed. Asterisks indicate the cysteines and histidines of the zinc fingers.

blastopore did not invaginate thereafter (Fig. 4B). Although the invagiantion of blastopore was inhibited, large yolky cells were displaced toward the animal pole region at the mid- to late gastrula stages. A space between the blastocoel roof and the yolky cells was observed only near the leading edge of the yolky cells (Figs. 4B, 5B). A boundary between the ectodermal cells and mesodermal cells, which was grown by vegetal rotation movements, was not clear during gastrula stages; however, we could define three distinct layers at the neurula stage (Fig. 4C). The blastocoelic cavity was reduced gradually at the neurula stage, and the blastocoelic fluid was absorbed into the intercellular spaces in the endodermal mass (Figs. 4C, 5C). *cpg*-injected embryos differentiated neural tubes, notochords, and somites at the site where the blastopore first appeared (Fig. 4D). These axial organs did not elongate but made a lump in the dorsal marginal zone. This finding suggests that migration of the mesodermal cells toward the animal pole along the inner surface of the blastocoel was inhibited and mesodermal cells remained near the dorsal blastopore in *cpg*-injected embryos. A cement gland was frequently observed even in the most severely inhibited embryos. To confirm tissue differentiation in *cpg*-injected embryos, we assayed the expression of various molecular marker genes by RT-PCR. The expression of early mesodermal (*Xbra, goosecoid, noggin,* and *Xwnt-8*) and mesendodermal (*siamois*) marker genes was not changed at the gastrula stage by overexpression of *cpg* (Fig. 4E). At

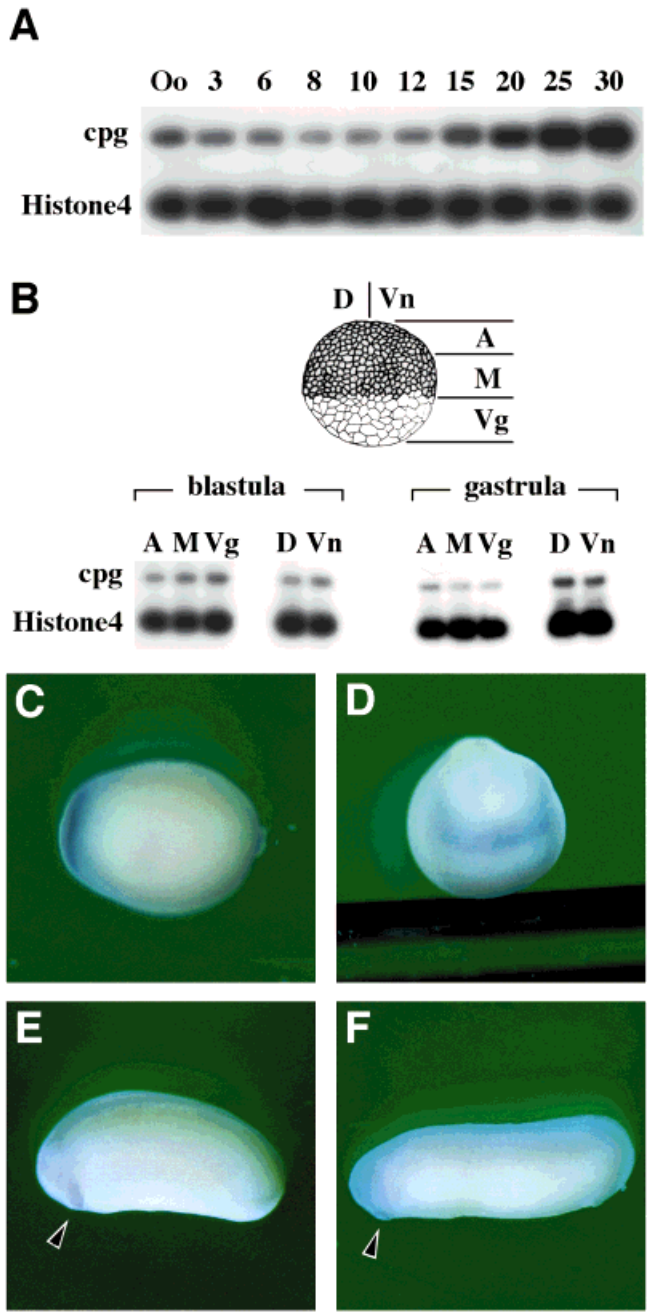

Fig. 2. Temporal and spatial expression of *cpg* during *Xenopus* development. **A:** Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed by using 1 μg of total RNA extracted from *Xenopus* embryos at different stages. Oo indicates the oocyte stage and numbers indicate the developmental stages. Maternal transcripts decreased gradually until the mid-blastula stage (st. 8), and zygotic expression increased after the initial gastrula stage (st. 10). **B:** Quantitative RT-PCR to determine spatial expression was performed by using total RNA extracted from different dissections of embryos at the mid-blastula stage (st. 8) and the initial gastrula stage (st. 10). *cpg* was expressed ubiquitously during these stages. **C–F:** Localization of *cpg* transcripts in *Xenopus* embryos after the neurula stage by whole-mount in situ hybridization. At the neurula stage (st. 16), *cpg* was expressed in the cement gland precursor region in C (ventral view) and D (anterior view). After the tailbud stage, *cpg* was expressed in the anterior region especially in the cement gland (arrowheads) in E (st. 25) and F (st. 30).

the late neurula stage, the expression of late neural (*N-CAM* and *Otx-2*) and late mesodermal (*MyoD* and *GATA-2*) marker genes was also unchanged (Fig. 4F). These results show that the defect in gastrulation movements is not due to the lack of mesodermal differentiation.

To investigate the morphogenesis of *cpg*-injected embryos in detail, we have observed the fine structure of *cpg*-injected embryos by scanning electron microscopy. The animal pole region of *cpg*-injected embryos was 6 to 7 cells thick at the mid-gastrula stage (Fig. 5A,B). However, the ectodermal region seemed to become thin abruptly after being lined with the endodermal cells (Fig. 5B). Thus, normal process of epibolic expansion was inhibited at the early and mid-gastrula stages. The endodermal cells were scattered, and the blastocoelic fluid filled the opening in the endodermal mass (Fig. 5C). We sometimes observed by time-lapse videomicrography that the blastocoelic fluid leaked from the tear at the vegetal surface. Normal-shaped bottle cells were observed at the blastopore of *cpg*-injected embryos (Fig. 5D). These observations suggest that formation of bottle cells was not inhibited, but the epibolic expansion of the animal hemisphere, migration of the mesodermal cells along the inner surface of the ectoderm, and vegetal rotation movements were inhibited by overexpression of *cpg*. To investigate the morphogenetic movements further, we next examined convergent extension movements in *cpg*-injected embryos.

cpg Interferes With Convergent Extension Movements

As Keller sandwich explants are known to show convergent and extension movements autonomously (Keller et al., 1985; Keller and Danilchik, 1988), we observed the movements of Keller sandwich explants taken from *cpg*-injected embryos. The Keller sandwich explants from normal embryos (four independent experiments, n = 31) elongated and mimicked convergent extension movements (Fig. 6A). However, elongation of the sandwich explants from *cpg*-injected embryos (n = 28) was considerably inhibited (Fig. 6B). To further test the effects of *cpg* expression on convergent extension, we also analyzed the morphology of the animal cap explants. When the animal cap explants (n = 68) from normal embryos were treated with high doses of activin, they elongated and differentiated various tissues in a dose-dependent manner (Fig. 6C) (Symes and Smith, 1987; Symes et al., 1994). Overexpression of *cpg* inhibited the elongation of the activin-treated animal cap explants (n = 73) almost completely (Fig. 6D). This inhibition was not due to the failure of mesoderm differentiation, because the activin-treated animal cap explants from *cpg*-injected embryos expressed mesodermal marker genes (*Xbra, noggin,* and *MyoD*) at the same level as those from normal embryos (Fig. 6G: lanes 2,4). Without activin treatment, the animal cap explants (n = 25) from *cpg*-injected embryos neither elongated nor differentiated any mesodermal tissues

but did form spherical masses similar to those (n = 63) from control embryos (Fig. 6E,F). These results indicate that overexpression of *cpg* inhibits convergent extension movements. Molecular analysis of the animal cap explants showed that the expression of the mesodermal marker genes was not induced by overexpression of *cpg* alone (Fig. 6G; lane 5). We also used RT-PCR to assay the expression of molecular marker genes that are known to be related to convergent extension movements during gastrulation: the expression of *C-cadherin* (Lee and Gumbiner, 1995; Zhong et al., 1999) and *PAPC* (Kim et al., 1998) was not affected by overexpression of *cpg* (Fig. 6H).

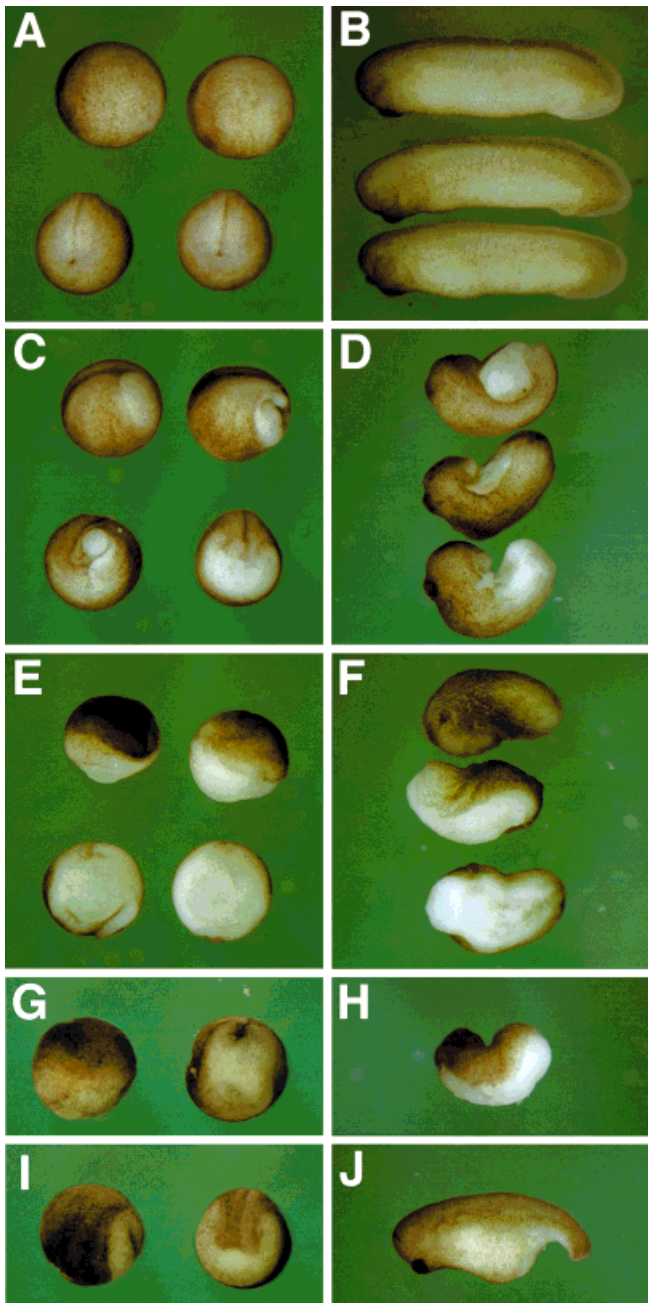

Fig. 3. Overexpression of *cpg* interfered with gastrulation. **A,C,E,G,I:** Neurula stage embryos (st. 20). **B,D,F,H,J:** Tailbud stage embryos (st. 30). A,C,E: Upper, lateral view; anterior is to the left. Lower, blastopore view. G,I: Left, lateral view, anterior is to the left. Right, blastopore view. *cpg* mRNA (1 ng) was injected into the lateral equatorial regions of the two-cell embryo (C–F). *cpg* mRNA (1 ng) was injected into two dorsal (G,H) or two ventral (I,J) equatorial regions of the four-cell embryo. A,B: Control embryos. C,D: Embryos showing slightly defective phenotypes. Some of the injected embryos had a slightly opened blastopore in C and formed partial axial structures, including the head region in D. E,F: Embryos showing severely defective phenotypes. The blastopore of most of the injected embryos did not close but regressed during the neurula stage in E. The injected embryos elongated along the initial dorsoventral axis gradually during the tailbud stage and looked like flat layers of double sheets of the ectodermal and endodermal tissues in F. G,H: The morphology of the embryos that were injected into two dorsal regions at the four-cell stage was similar to that seen in E and F. I,J: Only ventral gastrulation was inhibited by overexpression of *cpg* in the ventral region.

Both POZ and Zinc Finger Domains Are Necessary for *cpg* Function

As shown in the previous section, *cpg* encodes an N-terminal POZ domain, three C-terminal *krüppel*-like zinc finger domains of the C_2H_2 type and three putative nuclear localization signals. To confirm that the phenotype of *cpg*-injected embryos is not due to artificial effects caused by overexpression of nonspecific genes and that *cpg* functions as a transcription factor, we first made *cpg* deletion constructs (Fig. 7) and a total of 1 ng of each mRNA was injected into the lateral equatorial regions of the two blastomeres of two-cell embryos. *cpg-* Δ *POZ* or *cpg-* Δ *Zn,* which lacks a part of the POZ domain or the zinc finger domains, respectively, did not interfere with gastrulation, and *cpg-* Δ *Zn-* or *cpg-* Δ *POZ*-injected embryos developed normally (Fig. 7). On the other hand, *cpg-* Δ *(POZ-Zn),* which lacks a part between the POZ domain and zinc finger domains, interfered with gastrulation movements and the phenotype of *cpg-* Δ *(POZ-Zn)*-injected embryos was similar to that of *cpg*-injected embryos (Fig. 7). These results suggest that *cpg* functions as a transcription factor, and both the POZ domain and zinc finger domains are required for its function.

The POZ domain is an evolutionarily conserved protein-protein interaction domain (Bardwell and Treisman, 1994) required for both transcriptional activation and repression (Kaplan and Calame, 1997). As shown in the previous section, *cpg* is highly homologous to *hc-krox,* especially in the POZ and zinc finger domains. Because *hc-krox* is a transcriptional repressor of the *type I collagen alpha 1 (COL1A1), fibronectin,* and *biglycan* genes (Widom et al., 1997; Heegaard et al., 1997), we expected that *cpg* would also control these target genes. Therefore, the expression of *Xenopus COL1A1, fibronectin,* and *biglycan* in *cpg*-injected embryos was examined by RT-PCR. However, the expression of *Xenopus COL1A1* (Goto et al., 2000) and *Xenopus biglycan* (data not shown) is very low until the late neurula stage. Preliminary experiments demonstrated that the embryos injected with *cpg-GFP* mRNA at the

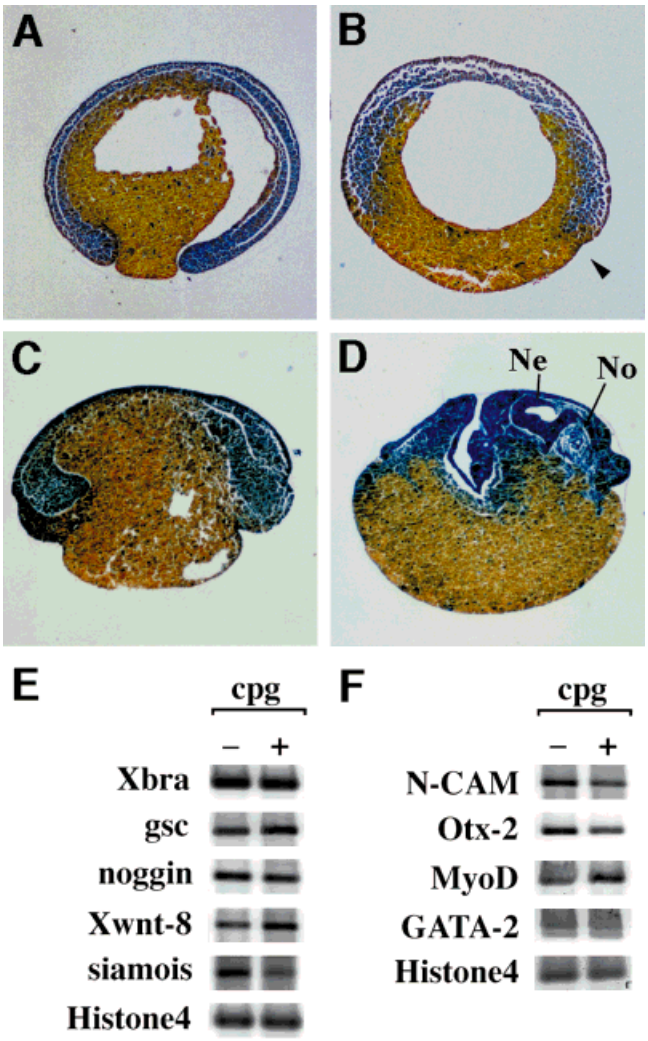

Fig. 4. Midsagittal sections (**A–D**) and reverse transcriptase polymerase chain reaction (RT-PCR) (**E,F**) of the control and *cpg*-injected embryos. *cpg* mRNA (1 ng) was injected into two lateral equatorial regions of the two-cell embryo. A: The control embryo (stage 11.5). B: A *cpg*-injected embryo at stage 11.5. Initial invagination occurred (arrowhead), but gastrulation did not proceed thereafter. Although *cpg* overexpression interfered with invagination, the yolky endodermal cells were displaced toward the animal pole region. The border between the ectodermal layer and inner cells was not clear. C: A *cpg*-injected embryo at stage 20 looks like a mushroom. The blastocoelic cavity has disappeared, and the three germ layers are clearly defined. D: A *cpg*-injected embryo at stage 30. Neural tube (Ne) and notochord (No) are shown apparently at the site where the blastopore first appeared. E,F: RT-PCR showed no significant effect of overexpression of *cpg* on the expression of the early mesodermal (*Xbra, goosecoid, noggin,* and *Xwnt-8*) and mesendodermal (*siamois*) marker genes at the gastrula stage (stage 11) in E, and the late neural (*N-CAM* and *Otx-2*) and mesodermal (*MyoD* and *GATA-2*) marker genes at the late neurula stage (stage 23) in F.

two-cell stage showed the same phenotype as those injected with *cpg* mRNA (data not shown). In these embryos, the fluorescent signals of cpg-GFP fusion protein were detected in nuclei even at stage 30 (data not shown). This finding indicates that the injected *cpg* is still functioning at the tailbud stage. Therefore, we assayed the effects of *cpg* on the expression of *Xenopus COL1A1* and *Xenopus biglycan* at the tailbud stage by injecting *cpg* mRNA at the two-cell stage. Overexpression of *cpg* strongly repressed the expression of both *Xenopus COL1A1* and *biglycan* (Fig. 8B). Expression of *fibronectin* was not changed by overexpression of *cpg* at the gastrula (Fig. 8A) and tailbud stages (Fig. 8B).

DISCUSSION

The Effects of *cpg* on Morphogenetic Movements

By expression cloning, we have cloned a novel POZ/zinc finger type transcription factor, *champignon* (*cpg*), which inhibits gastrulation if overexpressed in early embryos of *Xenopus*. The *cpg*-injected embryos developed normally until the early gastrula stage. At the mid-gastrula stage, however, the lateral regions of the blastopore disappeared and the blastopore failed to close thereafter. After the late neurula stage, the embryos gradually elongated along the primary dorsoventral axis. The phenotype of *cpg*-injected embryos is unique and different from that of exogastrulae or incomplete gastrulae caused by nonspecific genes or chemicals. Inhibition of gastrulation movements by *cpg* is more complete than that caused by overexpression of a dominant-negative form of *Xwnt11* (Tada and Smith, 2000) or *Xenopus frizzled 7* (Djiane et al., 2000) in which convergent extension movements were inhibited. Gastrulation movements involve the following five cellular processes: epiboly of the animal cap, formation of bottle cells, migration of the deep mesoderm, convergent extension of the marginal cells, and vegetal rotation movements (Keller, 1980; Keller et al., 1985, 1992; Keller and Danilchik, 1988; Hardin and Keller, 1988; Keller and Tibbetts, 1989; Wilson and Keller, 1991; Keller and Jansa, 1992; Shih and Keller, 1992a,b; Winklbauer and Schurfeld, 1999). In *cpg*-injected embryos, bottle cell formation occurred, but the other movements were inhibited. The expression of mesodermal marker genes was normal in *cpg*-injected embryos and in *cpg*-injected animal cap explants treated with activin, suggesting that *cpg* does not affect the differentiation of the mesoderm, but specifically affects the cellular movements. These results suggest that *cpg* may act on some basic molecules required for cellular movements, such as cytoskeletons, cell adhesion molecules, or extracellular matrices.

cpg Is a Novel Transcription Factor

Comparison of the predicted amino acid sequence of cpg with a protein database revealed that *cpg* is most homologous to the transcription factors human *c-krox* (Widom et al., 1997) and mouse *c-krox* (Galera et al., 1994), which contain the POZ and zinc finger domains. The amino acid sequences of the POZ and zinc finger domains were highly conserved between cpg and these proteins. However, the region between the POZ domain and zinc finger domains of cpg was shorter than those

TABLE 1. The Dose-Dependent Effects of *cpg* on Gastrulation[a]

Injected RNA	Dose (/blastomeres)	% Severe defect of gastrulation (no head structure)	% Slight defect of gastrulation (posterior axis defect)	% Normal
cpg	1 ng n = 82	90	9	1
	500 pg n = 92	87	12	1
	250 pg n = 93	36	47	17
β-*gal*	1 ng	0	0	100

[a]Dose-dependent inhibition of gastrulation movements in *cpg* injected embryos. *cpg* mRNA (1 ng) or β-*gal* mRNA (1 ng) for control was injected into two lateral equatorial regions of the two-cell embryo. The phenotype was scored at the tailbud stage. Injection of a low dose of *cpg* mRNA (250 pg) resulted in slightly defective phenotype of gastrulation. These embryos developed to partially defective embryos, as shown in Fig. 3D, at the tailbud stage. The injection of higher doses of *cpg* mRNA (≧500 pg) strongly interfered with gastrulation. These embryos developed to severely defective embryos, as shown in Fig. 3F.

TABLE 2. The Injected Site-Dependent Effects of *cpg* on Gastrulation[a]

	Injected region	% Severe defect of gastrulation (no head structure)	% Slight defect of gastrulation (posterior axis defect)	% Normal
2-cell	Animal n = 124	23	35	42
	Marginal n = 96	83	17	0
	Vegetal n = 109	45	32	23

[a]Equatorial injections of *cpg* mRNA produced most severely defective embryos. *cpg* mRNA (1 ng) was injected into the animal, lateral equatorial, or vegetal pole regions of both blastomeres of the two-cell embryo, and the resulting phenotypes were scored at the tailbud stage. The injection into the lateral equatorial region most severely affected gastrulation movements. The phenotypes followed those of Table 1 and Figure 3.

of hc-krox and mc-krox and showed no obvious homology with them. Accordingly, we conclude that *cpg* is not a *Xenopus* homologue of *hc-krox* and *mc-krox* but a new member of the *c-krox* family genes. Nevertheless, remarkably high identities in the three zinc finger domains at the C-terminus with human and mouse c-krox suggest that the target genes of *cpg* may be similar to those of *hc-krox* and *mc-krox.* It has been reported that the target genes of *hc-krox* and *mc-krox* were fibrous proteins, which constitute the extracellular matrix. Although *hc-krox* represses the transcription of human *COL1A1* and *fibronectin* (Widom et al., 1997), *mc-krox* activates the transcription of mouse *COL1A1* and *COL1A2* (Galera et al., 1994, 1996). The main difference in amino acid sequence between hc-krox and mc-krox is the length of the POZ domain at the N-terminus. The POZ domain, also known as BTB, is an evolutionarily conserved protein-protein interaction domain and is found at the N terminus of 5–10% of the C_2H_2-type zinc finger transcription factors (Bardwell and Treisman, 1994). This domain is strongly implicated in the regulation of gene expression through the local control of chromatin conformation (Albagli et al., 1995). We showed that cpg contains a full-length N-terminus POZ domain that is highly homologous to that of hc-krox. Because *cpg- Δ POZ* and *cpg- Δ Zn* did not affect gastrulation and *cpg- Δ (POZ-Zn)* interfered with gastrulation, both full-length POZ domain and zinc finger domains are required for the function of *cpg.* These data suggest that *cpg* may be a novel transcriptional repressor whose functional domain is most similar to that of *hc-krox.* Actually, *cpg* repressed the expression of *Xenopus COL1A1* and *Xenopus biglycan* at the tailbud stage.

The Target Genes of *cpg*

As mentioned above, *cpg* is most similar to *hc-krox,* which is known to be a transcriptional repressor of *fibronectin* and *type I collagen* (Widom et al., 1997) and binds to the 5′-flanking region of the human *biglycan* (Heegaard et al., 1997). However, we cannot explain the phenotype of *cpg*-injected embryos by the repression of these genes. Fibronectin is known to play a role in migration of the mesoderm (Nakatsuji, 1986; Winklbauer, 1990). Although the mesoderm migration was inhibited in *cpg*-injected embryos, the phenotype of *cpg*-injected embryo was different from phenotypes caused by a synthetic peptide for the cell-binding fragment of fibronectin or an antibody against fibronectin (Ramos and DeSimone, 1996; Ramos et al., 1996; Winklbauer and Keller, 1996). Furthermore, *Xenopus* embryos contain high levels of maternal *fibronectin* transcripts, and zygotic transcription begins only after the late gastrula stage (st. 12) (Lee et al., 1984; DeSimone

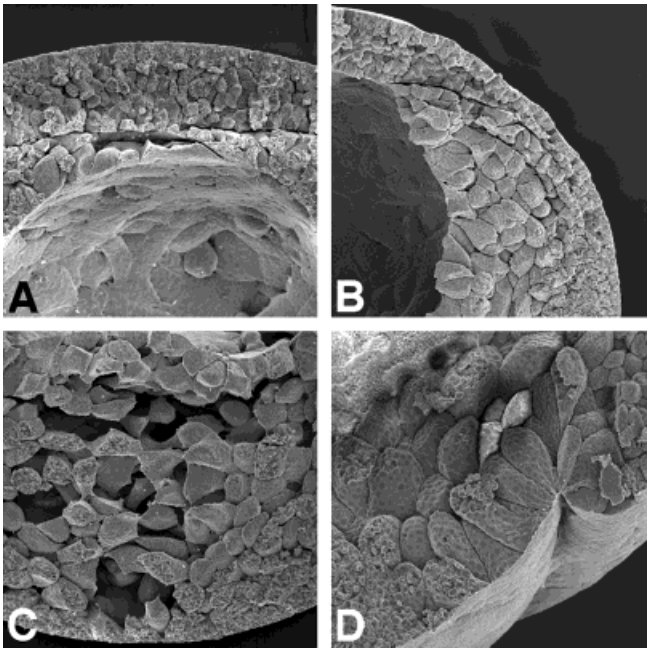

Fig. 5. Scanning electron microscopic images of *cpg*-injected embryos. *cpg* mRNA (1 ng) was injected into two lateral equatorial regions of the two-cell embryo, which was then fixed at the mid-gastrula stage (stage 11.5). **A:** Epiboly of the animal cap was inhibited, and the ectodermal layer of the animal pole was thick. **B:** The ectodermal layer of the lateral region became thin after lined with the yolky endodermal cells. **C:** Endodermal cells were scattered and did not closely adhere to each other. **D:** Normal-shaped bottle cells were formed.

et al., 1992). Actually, the level of *fibronectin* transcripts was unchanged in *cpg*-injected embryos at the gastrula and tailbud stages. Therefore, the defect in gastrulation caused by *cpg* may not be related to fibronectin function.

Similarly, although our data indicated that *Xenopus COL1A1* and *biglycan* seem to be target genes of *cpg,* and although the expression patterns of *cpg* and *COL1A1* are complementary (Goto et al., 2000) at the tailbud stage, the defect in gastrulation caused by *cpg* may not be related to *Xenopus COL1A1* and *biglycan* during gastrulation, because the quantity of *Xenopus COL1A1* (Goto et al., 2000) and *biglycan* (data not shown) transcripts is very small during gastrulation and coinjection of *cpg* mRNA with *Xenopus COL1A1* and *biglycan* mRNA did not rescue the defect in gastrulation movements caused by *cpg* (data not shown). Furthermore, although *C-cadherin* (Lee and Gumbiner, 1995; Zhong et al., 1999) and *PAPC* (Kim et al., 1998) are known to affect convergent extension movements during gastrulation, *cpg* did not change the level of expression of either gene. Andreazzoli et al. (1997) showed that the convergent extension was inhibited by overexpression of *Xotx1* or *Xotx2* genes. This finding suggests that the normal *otx* expression may inhibit convergent extension as part of the normal development of anterior ectodermal structures. As *cpg* is expressed predominantly in the anterior region especially in the cement gland precursor region and inhibits convergent extension, it is expected that *cpg* upregulated the expression of *Xotx* genes and thereby inhibited convergent extension movements. However, *Xotx2* was not up-regulated in *cpg*-injected embryos. This finding suggests that *Xotx2* is not involved in the inhibition of convergent extension by *cpg*. Recently *OCZF,* a new zinc finger gene, with the POZ domain has been isolated, and this gene was shown to bind to the *hc-krox*–binding consensus sequence (Kukita et al., 1999). Furthermore, it was reported that POZ/zinc finger proteins may function as homo- and heterodimeric complexes (Galera et al., 1996; Li et al., 1997; Ahmad et al., 1998; Davies et al., 1999). These findings suggest that there may be other zinc finger proteins that also control the target genes of *cpg*. Isolation of the other *cpg*-like POZ/zinc finger genes and the target genes of *cpg* will be necessary to clarify the mechanism of gastrulation movements.

Roles of *cpg* In Vivo

The transcripts of *cpg* were present maternally, but we could not detect regional differences in *cpg* expression until the neurula stage. After the neurula stage, the transcripts of *cpg* become more abundant and localized to the anterior region, especially in the cement gland. This suggests that *cpg* is related to the cement gland formation at later developmental stages. The transcripts of *cpg* were present ubiquitously at gastrula stages and the phenotype caused by *cpg* overexpression is general over the whole gastrula, i.e., ventral injection inhibits gastrulation of the ventral half, and dorsal injection inhibits dorsal gastrulation movements. Then, how does overexpression of *cpg* inhibit gastrulation movements? One possible explanation is that cpg represses a molecule that is necessary for normal gastrulation movements. The other is that overexpression has a dominant negative effect and blocks the normal function of endogenous cpg, because high levels of a transcription factor could well titrate out cofactors. This is supported by the reports that POZ family genes cooperate with cofactors to regulate the transcription of target genes (Kaplan and Calame, 1997; Dhordain et al., 1998; Ahmad et al., 1998). Isolation of the target genes and cofactors of cpg is necessary to give an accurate evaluation of these two possibilities.

So far, only a few genes that are related to cellular movements in gastrulation are isolated. We preliminarily compared constituent proteins between *cpg*-injected embryos and control embryos by two-dimensional gel electrophoresis at the mid-gastrula stage and found that isoelectric mobility of a 62-kDa protein was shifted by *cpg* (data not shown). Isolation and characterization of this protein may serve for analyzing the downstream of *cpg*. Although we are not able to find a gene that is directly related to the inhibition of gastrulation by *cpg,* the unique function of *cpg* is likely to be

a clue to analyze the molecular mechanisms of gastrulation.

EXPERIMENTAL PROCEDURES

Eggs and Embryos

Eggs were obtained by injecting *Xenopus laevis* females with 400 units of human chorionic gonadotropin; they were fertilized *in vitro*. Embryos were dejellied with sodium thioglycollate and cultured in modified Steinberg's solution (MSS). Developmental stages were determined according to that of Nieuwkoop and Faber (1967).

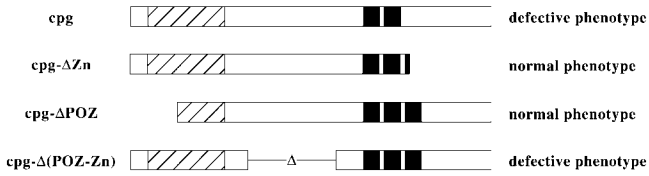

Fig. 7. Both the POZ and zinc finger domains of cpg were necessary to function as a transcription factor. *cpg- Δ POZ* (four independent experiments, n = 80) and *cpg- Δ Zn* (n = 80), which lack a part of the POZ domain and zinc finger domain, respectively, did not interfere with gastrulation. *cpg- Δ (POZ-Zn)* interfered with gastrulation and the phenotype of *cpg- Δ (POZ-Zn)*-injected embryos (n = 80) was similar to that of *cpg*-injected embryos (n = 80).

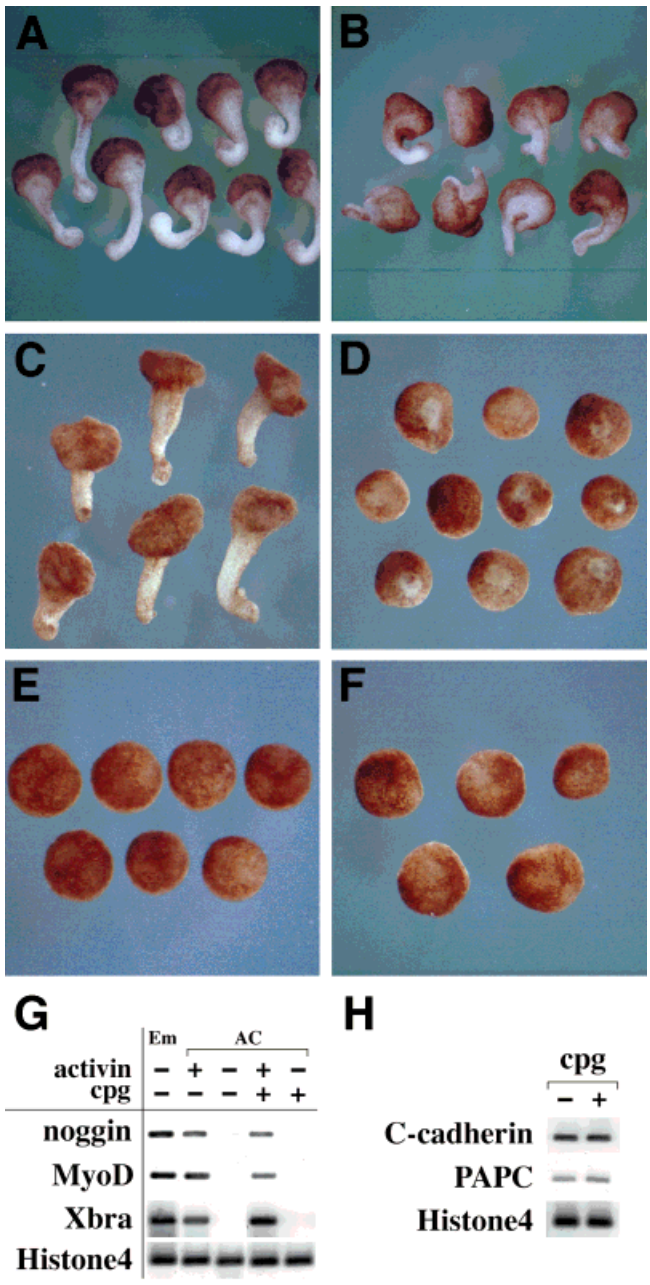

Fig. 6. *cpg* inhibited extension of Keller sandwich explants and the animal cap explants treated with activin. **A:** Keller sandwich explants from control embryos (four independent experiments, n = 31). **B:** Keller sandwich explants from *cpg*-injected embryos. *cpg* mRNA (1 ng) was injected into two lateral equatorial regions of the two-cell embryo. Dorsal sectors of *cpg*-injected embryos were excised at stage 10 and sandwiched. Overexpression of *cpg* caused a defect in the elongation (n = 28). **C:** Animal cap explants from control embryos. The animal caps were excised from stage 8 embryos and incubated for 6 hr with recombinant activin protein (10 ng/ml) and cultured in MSS until control embryos reached stage 20. A high dose of activin induced elongation of the animal cap (n = 68). **D:** Animal cap explants from *cpg*-injected embryos. *cpg* mRNA (1 ng) was injected into the animal pole regions of both blastomeres at the two-cell stage, then explanted at stage 8, and treated with a high dose of activin (10 ng/ml) for 6 hr. *cpg* completely interfered with the elongation of the explants treated with a high dose of activin (n = 73). **E:** Control animal cap explants without any treatment (n = 63). **F:** *cpg* alone did not induce any morphologic changes and resulted in formation of spherical masses similar to those in E (n = 25). **G:** RT-PCR of RNA extracted from the animal caps (stage 11) showed no significant effect of the overexpression of *cpg* on the expression of the mesodermal marker genes induced by activin (*noggin, MyoD,* and *Xbra*). **H:** *cpg* mRNA (1 ng) was injected into the lateral equatorial region of the two-cell embryo. RT-PCR showed no significant effect of overexpression of *cpg* on the expression of genes *C-cadherin* and *PAPC,* which are known to be related to the gastrulation movements.

Construction of cDNA Libraries and Expression Screening

Total RNA was isolated by the ultracentrifuge method using CsTFA (Pharmacia) (Okayama et al., 1987) or the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987) from embryos at various stages and mRNA was purified by using polyAtract mRNA Isolation System (Promega). Double-stranded cDNAs were obtained from the purified mRNA by using a ZAP-expression cDNA synthesis kit (Stratagene) and cloned into pCS2+ vector digested with *Eco*RI and *Xho*I. The plasmids were transformed into *E. coli* XL-1Blue-MRF′ and plated onto LB-ampicillin plates so that each plate contained approximately 100 individual colonies. The colonies of each plate were scraped and grown in liquid cultures for plasmid isolation by using the alkaline lysis method. Pools of the plasmids were linearized with *Not*I and used as DNA templates for capped mRNA

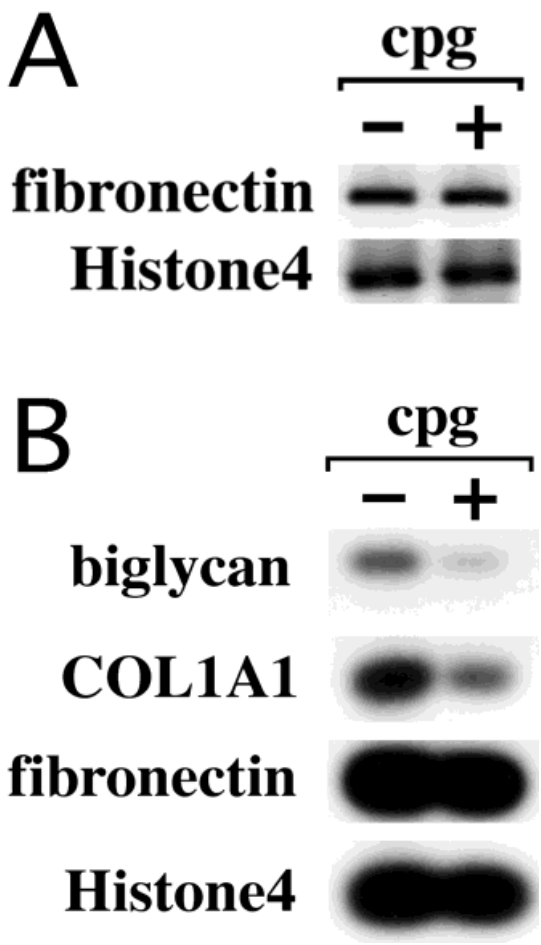

Fig. 8. Expression of *Xenopus fibronectin, biglycan,* and *COL1A1* in *cpg*-injected embryos. *cpg* mRNA was injected into two lateral equatorial region of the two-cell embryo. **A:** Reverse transcriptase polymerase chain reaction at the gastrula stage (stage 11) shows that *fibronectin* is not affected by overexpression of *cpg.* **B:** The expression of *Xenopus biglycan* and *COL1A1* was remarkably repressed, but fibronectin was not affected by overexpression of *cpg* at the tailbud stage (stage 30).

synthesis by using SP6 RNA polymerase. A total of 1 ng of the synthetic mRNA was microinjected into two blastomeres of a two-cell embryo or two dorsal or ventral blastomeres of a four-cell embryo and screened by morphogenesis after gastrulation. Two subsequent rounds of sib selection were performed to isolate a cDNA that caused gross changes in morphogenesis.

RT-PCR Analysis

Oligo(dT)-primed first-strand cDNA was prepared from 1 μg of total RNA by using Superscript II (Gibco). One-twentieth of the cDNA obtained was used for each PCR. As an internal loading control, the primers for the ubiquitously expressed *Histone4* were included in all PCRs. Aliquots containing one-tenth of the PCR products were loaded on 2% agarose gels, electrophoresed, and transferred to nylon membranes. The membranes were hybridized to the random-primed isotope-labeled fragment of each gene and autoradiographed. The sequences of gene primers used were as follows: *cpg* (GenBank accession no. AB029074) forward (nt 953-972), reverse (nt 1593-1574); *Histone4* (M21286) forward (nt 1498-1521), reverse (nt 1686-1663); *Xbra* (M77243) forward (nt 432-451), reverse (nt 733-714); *goosecoid* (M81481) forward (nt 430-447), reverse (nt 917-900); *noggin* (M98807) forward (nt 995-1012), reverse (nt 1275-1258); *Xwnt-8* (X57234) forward (nt 1126-1143), reverse (nt 1457-1440); *siamois* (Z48606) forward (nt 539-560), reverse (nt 848-827); *N-CAM* (M25696) forward (nt 2817-2834), reverse (nt 3159-3142); *Otx-2* (U19813) forward (nt 770-791), reverse (nt 1108-1087); *MyoD* (X16106) forward (nt 221-240), reverse (nt 512-493); *GATA-2* (M76564) forward (nt 982-1001), reverse (nt 1400-1381); *C-cadherin* (U04707) forward (nt 1372-1391), reverse (nt 1814-1795); *PAPC* (AF042192) forward (nt 2490-2509), reverse (nt 2955-2936); *Xenopus COL1A1* (AB034701) forward (nt 3396-3415), reverse (nt 3845-3826); *Xenopus biglycan* (AB037269) forward (nt 540-559), reverse (nt 1247-1228).

Whole-Mount In Situ Hybridization

In situ hybridization was performed by using digoxigenin-labeled RNA probe and alkaline phosphatase substrate (NBT) (Boehringer Mannheim) according to Harland (1991). The pCS2+ vector, including the cDNA of *cpg,* was linearized with *Eco*RI and transcribed with T7 RNA polymerase to produce the antisense probe.

Histology and Scanning Electron Microscopy

For histology, embryos were fixed overnight in a solution of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.5), dehydrated through a graded series of ethanol, cleared by xylene, and embedded in paraffin (Paraplast Plus, Sigma). Ten-micrometer-thick sections were deparaffinized in xylene, rehydrated in a graded series of ethanol, and stained with 0.1% azocarmine B for 20 sec and a mixture of 0.2% aniline blue and 0.4% orange G for 7 min.

For the SEM study, the embryos were fixed overnight in a solution of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.5). The fixed embryos were cut in half at the midsagittal plane by using a razor blade and then post-fixed with 1% OsO_4 in 0.1 M phosphate buffer. The samples were dehydrated through a graded series of acetone and critical point dried by using liquid CO_2.

Animal Cap Assay and Keller Sandwich Experiment

A total of 1 ng of *cpg* mRNA was microinjected into the equatorial region of two blastomeres of the two-cell embryo for Keller sandwich experiments or into the animal pole region of two blastomeres of the two-cell embryo for animal cap assays. Keller sandwich experiments were performed according to previously described methods (Keller, 1991). Animal caps were ex-

cised from stage 8 embryos and incubated either in the absence or presence of 10 ng/ml of human recombinant activin A in LCMR for 6 hr (Stewart and Gerhart, 1990). The explants were then cultured in MSS until the control embryos reached stage 20.

Construction of *cpg* Mutants

cpg- Δ Zn was constructed as follows: the *cpg* cDNA was digested with *Pst*I, and then only a large *Pst*I restriction fragment containing 713 bp (nt 633-1345) of the *cpg* cDNA was re-ligated into the residual *cpg* cDNA (nt 1-632). *cpg- Δ POZ* was constructed by ligating a *Sac*II-*Xho*I restriction fragment containing 2,047 bp (nt 375-2421) of the *cpg* cDNA into pCS2+ vector digested with *Stu*I and *Xho*I. *cpg- Δ (POZ-Zn)* was constructed by self-ligating after removing an *Aat*II-*Sca*I restriction fragment containing 348 bp (nt 709-1056) of the *cpg* cDNA.

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LITERATURE CITED

Ahmad KF, Engel CK, Prive GG. 1998. Crystal structure of the BTB domain from PLZF. Proc Natl Acad Sci USA 95:12123–12128.

Albagli O, Dhordain P, Deweindt C, Lecocq G, Leprince D. 1995. The BTB/POZ domain: A new protein-protein interaction motif common to DNA- and actin-binding proteins. Cell Growth Differ 6:1193–1198.

Andreazzoli M, Pannese M, Boncinelli E. 1997. Activating and repressing signals in head development: The role of *Xotx1* and *Xotx2*. Development 124:1733–1743.

Bardwell VJ, Treisman R. 1994. The POZ domain: A conserved protein-protein interaction motif. Genes Dev 8:1664–1677.

Boucaut JC, Darribere T, Poole TJ, Aoyama H, Yamada KM, Thiery JP. 1984. Biologically active synthetic peptides as probes of embryonic development: A competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. J Cell Biol 99:1822–1830.

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

Davies JM, Hawe N, Kabarowski J, Huang QH, Zhu J, Brand NJ, Leprince D, Dhordain P, Cook M, Morriss-Kay G, Zelent A. 1999. Novel BTB/POZ domain zinc-finger protein, LRF, is a potential target of the *LAZ-3/BCL-6* oncogene. Oncogene 18:365–375.

DeSimone DW, Norton PA, Hynes RO. 1992. Identification and characterization of alternatively spliced *fibronectin* mRNAs expressed in early *Xenopus* embryos. Dev Biol 149:357–369.

Dhordain P, Lin RJ, Quief S, Lantoine D, Kerckaert JP, Evans RM, Albagli O. 1998. The LAZ3 (BCL-6) oncoprotein recruits a SMRT/mSIN3A/histone deacetylase containing complex to mediate transcriptional repression. Nucleic Acids Res 26:4645–4651.

Djiane A, Riou J, Umbhauer M, Boucaut J, Shi D. 2000. Role of *frizzled 7* in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*. Development 127:3091–3100.

Galera P, Musso M, Ducy P, Karsenty G. 1994. *c-Krox,* a transcriptional regulator of type I collagen gene expression, is preferentially expressed in skin. Proc Natl Acad Sci USA 91:9372–9376.

Galera P, Park RW, Ducy P, Mattei MG, Karsenty G. 1996. c-Krox binds to several sites in the promoter of both mouse type I collagen genes: Structure/function study and developmental expression analysis. J Biol Chem 271:21331–21339.

Goto T, Katada T, Kinoshita T, Kubota HY. 2000. Expression and characterization of *Xenopus type I collagen alpha 1* (*COL1A1*) during embryonic development. Dev Growth Differ 42:249–256.

Hadeball B, Borchers A, Wedlich D. 1998. *Xenopus cadherin-11* (*Xcadherin-11*) expression requires the Wg/Wnt signal. Mech Dev 72:101–113.

Hardin J, Keller R. 1988. The behaviour and function of bottle cells during gastrulation of *Xenopus laevis*. Development 103:211–230.

Harland R. 1991. In situ hybridization: An improved method for *Xenopus* embryos. In: Kay BK, Peng HB, editors. Methods in cell biology. Vol. 36. San Diago: Academic press Inc. p 685–695.

Heegaard AM, Gehron Robey P, Vogel W, Just W, Widom RL, Scholler J, Fisher LW, Young MF. 1997. Functional characterization of the human *biglycan* 5′-flanking DNA and binding of the transcription factor *c-Krox*. J Bone Miner Res 12:2050–2060.

Kaplan J, Calame K. 1997. The ZiN/POZ domain of ZF5 is required for both transcriptional activation and repression. Nucleic Acids Res 25:1108–1116.

Keller RE. 1980. The cellular basis of epiboly: An SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis*. J Embryol Exp Morphol 60:201–234.

Keller RE. 1981. An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation of *Xenopus laevis*. J Exp Zool 216:81–101.

Keller R. 1991. Early embryonic development of *Xenopus laevis*. In: Kay BK, Peng HB, editors. Methods in cell biology. Vol. 36. San Diago: Academic press Inc. p 61–113.

Keller R, Danilchik M. 1988. Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. Development 103:193–209.

Keller R, Jansa S. 1992. *Xenopus* gastrulation without a blastocoel roof. Dev Dyn 195:162–176.

Keller R, Tibbetts P. 1989. Mediolateral cell intercalation in the dorsal, axial mesoderm of *Xenopus laevis*. Dev Biol 131:539–549.

Keller RE, Danilchik M, Gimlich R, Shih J. 1985. The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. J Embryol Exp Morphol 89(Suppl):185–209.

Keller R, Shih J, Domingo C. 1992. The patterning and functioning of protrusive activity during convergence and extension of the *Xenopus* organiser. Development Suppl 81–91.

Kim SH, Yamamoto A, Bouwmeester T, Agius E, De Robertis EM. 1998. The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation. Development 125:4681–4690.

Kukita A, Kukita S, Ouchida M, Maeda H, Yatsuki H, Kohashi O. 1999. Osteoclast-derived zinc finger (OCZF) protein with POZ domain, a possible transcriptional repressor, is involved in osteoclastogenesis. Blood 94:1987–1997.

Lee CH, Gumbiner BM. 1995. Disruption of gastrulation movements in *Xenopus* by a dominant-negative mutant for *C-cadherin*. Dev Biol 171:363–373.

Lee G, Hynes R, Kirschner M. 1984. Temporal and spatial regulation of fibronectin in early *Xenopus* development. Cell 36:729–740.

Li X, Lopez-Guisa JM, Ninan N, Weiner EJ, Rauscher FJ III, Marmorstein R. 1997. Overexpression, purification, characterization, and crystallization of the BTB/POZ domain from the PLZF oncoprotein. J Biol Chem 272:27324–27329.

Nakatsuji N. 1986. Presumptive mesoderm cells from *Xenopus laevis* gastrulae attach to and migrate on substrata coated with fibronectin or laminin. J Cell Sci 86:109–118.

Nieuwkoop PD, Faber J. 1967. Normal table of *Xenopus laevis*. Amsterdam: North-Holland Publishing Company.

Okayama H, Kawaichi M, Brownstein M, Lee F, Yokota T, Arai K. 1987. High-efficiency cloning of full-length cDNA: Construction and

screening of cDNA expression libraries for mammalian cells. Methods Enzymol 154:3–28.

Ramos JW, DeSimone DW. 1996. *Xenopus* embryonic cell adhesion to fibronectin: Position-specific activation of RGD/synergy site-dependent migratory behavior at gastrulation. J Cell Biol 134:227–240.

Ramos JW, Whittaker CA, DeSimone DW. 1996. Integrin-dependent adhesive activity is spatially controlled by inductive signals at gastrulation. Development 122:2873–2883.

Shih J, Keller R. 1992a. Cell motility driving mediolateral intercalation in explants of *Xenopus laevis.* Development 116:901–914.

Shih J, Keller R. 1992b. Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus laevis.* Development 116:915–930.

Sokol S. 2000. A role for Wnts in morpho-genesis and tissue polarity. Nat Cell Biol 2:E124–E125.

Stewart RM, Gerhart JC. 1990. The anterior extent of dorsal development of the *Xenopus* embryonic axis depends on the quantity of organizer in the late blastula. Development 109:363–372.

Symes K, Smith JC. 1987. Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis.* Development 101:339–349.

Symes K, Yordan C, Mercola M. 1994. Morphological differences in *Xenopus* embryonic mesodermal cells are specified as an early response to distinct threshold concentrations of activin. Development 120:2339–2346.

Tada M, Smith JC. 2000. *Xwnt11* is a target of *Xenopus brachyury:* Regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. Development 127:2227–2238.

Wallingford JB, Sater AK, Uzman JA, Danilchik MV. 1997. Inhibition of morphogenetic movement during *Xenopus* gastrulation by injected sulfatase: Implications for anteroposterior and dorsoventral axis formation. Dev Biol 187:224–235.

Wallingford JB, Rowning BA, Vogeli KM, Rothbacher U, Fraser SE, Harland RM. 2000. Dishevelled controls cell polarity during *Xenopus* gastrulation. Nature 405:81–85.

Widom RL, Culic I, Lee JY, Korn JH. 1997. Cloning and characterization of *hcKrox,* a transcriptional regulator of extracellular matrix gene expression. Gene 198:407–420.

Wilson P, Keller R. 1991. Cell rearrangement during gastrulation of *Xenopus:* Direct observation of cultured explants. Development 112: 289–300.

Winklbauer R. 1990. Mesodermal cell migration during *Xenopus* gastrulation. Dev Biol 142:155–168.

Winklbauer R, Keller RE. 1996. Fibronectin, mesoderm migration, and gastrulation in *Xenopus.* Dev Biol 177:413–426.

Winklbauer R, Schurfeld M. 1999. Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus.* Development 126:3703–3713.

Zhong Y, Brieher WM, Gumbiner BM. 1999. Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. J Cell Biol 144:351–359.