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The Xenopus cerberus gene is able to induce ectopic heads in Xenopus embryos. At the time of its identification, cerberus shared significant homology with only one other protein, the putative rat tumor suppressor protein Dan. Sequence analysis has revealed that cerberus and Dan are members of a family of predicted secreted proteins, here called the can family. The identification of a can-family member in the nematode Caenorhabditis elegans, CeCan1, suggests that this family is of ancient origin. In the mouse, there are at least five family members: Cer1, Drm, PRDC, Dan, and Dte. These genes are expressed in patterns that suggest that they may play important roles in patterning the developing embryo. Cer1 marks the anterior visceral endoderm at E6.5. Dte is expressed asymmetrically in the developing node. Dan is first seen in the head mesoderm of early head fold stage embryos and Drm is expressed in the lateral paraxial mesoderm at E8.5. The region of homology shared by these genes, here called the can domain, closely resembles the cysteine knot motif found in a number of signaling molecules, such as members of the TGFβ superfamily. Epitope-tagged versions of Cer1 show that, unlike in TGFβ superfamily members, the cysteine knot motif is not processed away from a proprotein. Recent experiments in Xenopus have suggested that cerberus may act as an inhibitor of BMP signaling. To examine this further, the ability of Dan, Cer1, and human DRM to attenuate Bmp4 signaling has been assessed in P19 cells using pTlx-Lux, a BMP-responsive reporter. All three genes are able to inhibit Bmp4 signaling. These data suggest that the different family members may act to modulate the action of TGFβ family members during development.

Key Words: mouse; embry; cerberus; Dan; Cer1; Drm; Dte; Bmp4.

INTRODUCTION

The Xenopus cerberus gene was identified as a Spemann organizer-associated transcript that encodes a secreted protein able to induce ectopic heads when injected into Xenopus eggs (Bouwmeester et al., 1996). At the time of its isolation, cerberus shared homology with only one other protein. This protein, Dan, identified by virtue of the repression of the Dan gene in rat fibroblasts transformed with Rous sarcoma virus, had been predicted, on the basis of sequence analysis, to act as a DNA binding protein (Ozaki and Sakiyama, 1993). The apparent discrepancy in the predicted character of the cerberus and Dan proteins leads to the initial conclusion that the identified homology was artifactual. However, recent biochemical analysis has shown that Dan, like cerberus, is a secreted glycoprotein (Biben et al., 1998; Nakamura et al., 1997), suggesting that the shared domain may reflect a shared function.

The domain of homology identified in cerberus and Dan, here called the can domain, is related to domains in the Norrie disease protein (NDP) and the mucins (Biben et al., 1998). Modeling studies have suggested that the NDP domain is structurally related to the cysteine knot motif of the transforming growth factor β (TGFβ) superfamily (Meitinger et al., 1993). This motif occurs in a number of growth factors, including nerve growth factor, TGFβ2, and platelet-derived growth-factor (McDonald and Hendrickson, 1993).

The can domain is present in at least five mouse genes and one Caenorhabditis elegans gene, which together make up the can family. The mouse genes include the mouse Dan gene (Ozaki et al., 1996), the mouse cerberus-related gene, Cer1 (Belo et al., 1997; Biben et al., 1998; Thomas et al., 1997), and the PRDC gene (Minabe-Saegusa et al., 1998). Additionally, the motif is found in the mouse homolog of the recently described rat Drm gene (Hsu et al., 1998; Topol et al., 1997) and in a novel mouse gene that we have called dante (Dte).

To better understand the likely functions of the murine
gene family, the expression patterns of Cer1, Dte, Dan, and Drm were examined from embryonic day (E) 6.0 through E10.5. Cer1 is the earliest family member to be expressed in the embryo, being present at E6.5 in the anterior visceral endoderm (AVE). Dte is expressed from the early bud stage in the definitive node. Dan and Drm are initially expressed in the paraxial mesoderm.

Structural analyses have shown that the cysteine knots of TGFβ are cleaved away from a proprotein precursor during secretion (Gentry et al., 1988). Using epitope-tagged forms of Cer1, we show that this protein is not processed in a similar fashion.

Injection studies in Xenopus have suggested that cerberus and Cer1 act, in part, as BMP antagonists (Belo et al., 1997; Biben et al., 1998; Bouwmeester et al., 1996). This is supported by the recent demonstration of an interaction between BMP2 and the can-family members human CER1, Dan, and the Xenopus Drm homolog Gremlin (Hsu et al., 1998). To assess the ability of BMP inhibition by the mammalian proteins in a mammalian system we have used a previously described BMP-responsive Tlx2 promoter construct in embryonal carcinoma P19 cells (Tang et al., 1998). This study found that DRM, Dan, and Cer1 can all attenuate BMP signaling. Together these data suggest that the can-family members play important roles in patterning the early mouse embryo by inhibiting the action of specific TGFβ superfamily members.

MATERIALS AND METHODS

Whole-Mount in Situ Hybridization

Expression patterns were examined by whole-mount in situ hybridization. Antisense digoxigenin riboprobes were prepared from linearized plasmids using digoxigenin-dUTP (Boehringer Mannheim). E7.5 and E8.5 embryos were probed using a previously described protocol (Henrique et al., 1995). To examine E9.5 to E11.5 embryos, an alternative protocol was used (Conlon and Rossant, 1992). Staining times varied from a few hours to overnight, depending on signal strength. The Cer1 probe (1.6 kb) was made from expressed sequence tag (EST) 538769 using SalI and SP6 RNA polymerase. The Dte probe (1.2 kb) was made from EST 790229 using EcoRI and T3 RNA polymerase. The Dan probe (1.5 kb) was made from EST 464142 using EcoRI and T3 RNA polymerase. The Drm probe was made using a 1.2-kb genomic fragment of the mouse Drm locus containing the open reading frame (ORF) and 3' untranslated sequence. Double labeling of RNA and protein distributions were performed by the repeated application of the Henrique et al. (1995) protocol. The complete RNA protocol was followed by two repeated rounds of the antibody binding and washing steps: first a rabbit polyclonal anti-T antibody (Kispert and Herrmann, 1994), a kind gift from Dr. Bernard Herrmann, was applied and then a sheep anti-rabbit peroxidase antibody (Boehringer Mannheim). The distribution of antibody binding was visualized using DAB (Boehringer Mannheim).

Epitope Tagging

A full-length Cer1 clone was isolated using a modified 5'-RACE protocol and a plasmid-based E6.25 embryonic cDNA library. Briefly, two gene-specific primers, mcer(1), CTT-TTG-ATG-GGC-AGG-ATG-ACT, and mcer(2), ACT-GAA-CAT-GAA-CCG-ATG-CCA, were used in conjunction with a plasmid primer, T3, to amplify the 5' end of the Cer1 gene. Several isolated clones were sequenced and one, containing a consensus sequence, was sub-cloned into the Cer1 EST. PCR was used to add restriction sites to the Cer1 ORF sequence and to disrupt the stop codon. A modified Cer1 cDNA was cloned into pCDNA3.1-MYC (Invitrogen) to add a C-terminal Myc tag and into pCDNA3-HA to introduce a hemagglutinin (HA) tag at residue 42, downstream of the predicted signal sequence cleavage site. The epitope-tagged Cer1 constructs were transfected into mink lung cells (Mv1Lu) using Lipofectamine (Gibco BRL). Conditioned medium was collected after 48 h of culture and immunoprecipitated with either anti-MYC (9E10; Santa Cruz) or anti-HA (12CA5; Boehringer Mannheim) monoclonal antibodies. Bound conjugates were purified using protein A-Sepharose CL4B (Pharmacia), resolved by SDS-PAGE electrophoresis (Laemmli, 1970) and transferred to Westram membrane (Schleicher and Schuell). The precipitates were visualized by ECL (Pierce) in conjunction with goat anti-mouse peroxidase (Bio-Rad).

Inhibition of Bmp4 Signaling

pCDNA3 (Invitrogen) vectors containing the ORFs of murine Bmp4, Dan, and Cer1 and human DRM were constructed using PCR and subcloning. All constructs use endogenous Kozak and initiation sites and, except for the Dan construct, which contains an additional 270 bp of 3'UTR, contain only the ORF. These vectors were transfected into Cos cells using Lipofectamine (Gibco BRL) or into P19 cells using calcium phosphate precipitation (Macias-Silva et al., 1996). The pTLux-Luc vector was used in P19 cells to assess the level of BMP signaling (Tang et al., 1998) using a luciferase assay system (Promega) and a Berthold Lumat LB9501 luminometer. A pCMV-βgal vector and ONPG assay were used to control for transfection efficiencies. For all P19 assays, 2 μg of pTLux-Luc was cotransfected with 0.2 μg of pCMV-βgal. In the 1:1 assay, 1 μg of pCDNA3-BMP4 and 1 μg of pCDNA3-CAN were also added, while in the 1:4 assays, 0.4 μg of pCDNA3-BMP4 and 1.6 μg of pCDNA3-CAN were used. Controls were balanced using empty vector. For the conditioned medium assay, P19 cells were transfected with only the pTLux-Luc and pCMV-βgal vectors and grown in the presence of transfected Cos cell conditioned media.

RESULTS

The Can-Domain Family

The domain of homology shared by the Xenopus cerberus and rat Dan proteins, the can domain (Fig. 1A), was used to search genomic and EST databases by BLAST (Altschul et al., 1997). This screen identified three mammalian ESTs and a C. elegans cosmid (F35B12). The ESTs comprised a complete EST (272074) for the human homolog of rat Drm (Topol et al., 1997) and incomplete ESTs for Cer1 (538769) (Belo et al., 1997; Biben et al., 1998) and for a gene we have called dante (Dte) (790229). To enable functional studies of the family to be under-
taken, full-length versions of the family members have been sought. The complete Cer1 gene, encoding a predicted 272-aa protein, was isolated using 5′-RACE on a plasmid-based E6.25 embryonic library. The Dan gene was obtained as an EST (464412). Genomic clones of Drm were isolated using human DRM as a probe. Characterization of these identified the Drm ORF, 184 aa, in a single exon. A CeCan1 cDNA was isolated using a 3′ gene-specific primer and the common 5′ splice leader sequence SL1 (Krause and Hirsh, 1987) on mixed-stage C. elegans cDNA.

Screening for full-length Dte has thus far been unsuccessful, identifying only fusions between Dte and other tran-

FIG. 1. A comparison of identified can domains. (A) Cerberus/Dan homology. The can domains of the defining members of the family. (B) Can domains. Within-family lineup highlighting the cysteine knot fingerprints, underlined, and conserved amino acids. (C) Can/cysteine knots. Comparison of the can domains of cerberus and Dan with known cysteine knot motifs; can-family-specific residues are shown in blue.

FIG. 2. The can family, with can domains in orange and predicted signal sequences in blue. There is no significant homology outside of the can domains.
scripts. The absence of a conserved amino-terminal cysteine in the Dte can domain (Fig. 1B) may be due to the identified EST also being inappropriately processed. Although the EST has a poly(A) tail, the N-terminal 7 aa of the Dte can domain have poor codon usage and there is a putative 3' intron/exon junction at the boundary between these residues and a downstream region of good codon usage. Notwithstanding these concerns, the specific sequence and expression pattern of Dte, described below, suggest to us that this is likely a true gene and that cloning difficulties may simply reflect observed tight spatial and temporal controls of expression.

The predicted can domains (Fig. 1B) contain the core cysteine knot fingerprints C-X-G-X-C and C-X-C, identified using the PRINTS database (Attwood et al., 1998). In addition, these domains contain conserved residues that distinguish them from previously described cysteine knots, such as those of mouse Bmp4 (Kurihara et al., 1993), nodal (Zhou et al., 1993), human mucin2 (MUC2), Von Willebrand factor (VWF), and NDP (Berger et al., 1992; Bonthron et al., 1986; Chen et al., 1992; Gum et al., 1994) (Fig. 1C). Of these other cysteine knots, the can domain is most similar to those of MUC2, VWF, and NDP.

The extent of homology between the can domains varies from a high of 58% identity, between Cer1 and cerberus, to a low of 28%, between Cer1 and Dan. The predicted C. elegans CeCan1 can domain is most similar to that of PRDC, sharing 44% identity and 55% similarity. Outside of the can domain, family members share no obvious homology.

The extent of homology between the cerberus and Cer1 can domains, 58%, is much lower than that seen between the cysteine knots of individual BMPs; for instance, the cysteine knots of Bmp4 in the mouse and Xenopus are identical. It is, however, comparable with the level of homology between the cysteine knots of the mouse TGFβ family member nodal and its Xenopus relatives, XNr1-4, 53-64% (Jones et al., 1995; Joseph and Melton, 1997; Smith et al., 1995; Zhou et al., 1993). Whether there are other Xenopus cerberus-related genes or an as yet unidentified mouse cerberus gene is unknown.

All the fully characterized murine genes encode small proteins with predicted (Nielsen et al., 1997) amino-terminal signal sequences (Fig. 2). Within the predicted CeCan1 protein (339 aa) there is a putative transmembrane domain that starts with a methionine. It seems probable, given the nature of the other proteins, that this downstream methionine may be the start of a shorter, 241-aa, secreted form of CeCan1.

Expression Analysis

The expression patterns of the four murine genes, Cer1, Dte, Dan, and Drm, were examined using whole-mount in situ hybridization on E6.0–E10.5 embryos.

**Cer1.** Cer1 was the first family member expressed in the embryo. Expression was evident at the onset of gastrulation in the visceral endoderm in a stripe that extends from the embryonic/extraembryonic boundary down toward the distal tip of the embryo. Whether Cer1 is expressed before this time has not been investigated. The position of these cells relative to the developing primitive streak was established by double labeling for Cer1 transcripts and T protein (Fig. 3A). Cer1 expression was observed opposite the site of the primitive streak and therefore marks the anterior visceral endoderm in a domain similar to that of the recently described mouse Dickkopf-1 (Dck1) gene (Glinka et al., 1998) (Fig. 3B).

As the primitive streak extends, Cer1 expression was seen to spread within the endoderm, to reach a maximum extent at the late streak stage (Downs and Davies, 1993) (Fig. 3C). Fate maps (Quinlan et al., 1995) show that this domain of Cer1 expression underlies the future fore-, mid-, and hindbrain, and explant culture experiments (Ang et al., 1994) suggest that the late streak stage is the time at which anterior neural identity is specified. Cer1 is therefore present in the right place and at the right time to mediate anterior neural specification.

The lateral domain of expression of Cer1 begins to retract after the late streak stage so that by the early head fold stage (Fig. 3D), expression was observed solely in the anterior midline. At later stages, early somite stage through E10.5, Cer1 expression was seen only in the most posterior two or three somites, as reported by Biben et al. (1998)

**Dte.** Expression of Dte was first observed in the definitive node at the early bud stage (Fig. 4A), where it was expressed in the crown cells of the node (Bellomo et al., 1996). This pattern is similar to that of nodal (Zhou et al., 1993), a TGFβ family member required for primitive streak formation (Conlon et al., 1994) and implicated in the establishment of right–left asymmetries (Collignon et al., 1996; Levin et al., 1995, 1997; Lowe et al., 1996; Sampath et al., 1997). Interestingly, Dte expression became refined such that, by the early somite stage, expression was strongest on the right-hand side of the node (Figs. 4B and 4C). This is the first mouse gene seen to be expressed in such a pattern and contrasts with the later expression of nodal, which is expressed more strongly on the left-hand side of the node (Collignon et al., 1996; Lowe et al., 1996). After the node is engulfed in the developing tail bud, at E8.5, expression of Dte was no longer observed.

**Dan.** As Cer1 expression retracts, at the early head fold stage, Dan expression became apparent in the mesodermal wings that underlie the anterior neural plate (Figs. 5A and 5B). Prior to this stage expression could not be detected in the embryo proper. However, strong expression was seen in the ectoplacental cone (data not shown). By early somite stages, E8.0, Dan expression was seen in the paraxial mesoderm both within and rostral to the somitic column (Figs. 5C and 5D). At E8.5, expression was strongest throughout the two most caudal somites but appeared to be rapidly downregulated as these somites matured (Fig. 5E). More rostrally, a second wave of somitic expression was observed. At E8.5, this expres-
sion appeared to border the neural tube (data not shown), but by E9.5 expression was clearly restricted to the dermamyotome of somites along the entire body axis (Figs. 5F and 5H). This dermamyotome expression was maintained through E10.5 (Fig. 5G).

As the anterior limb bud forms, Dan expression was seen in the outer mesenchymal layers of the dorsal and ventral buds and in an anterior proximal domain (Fig. 5F). Limb expression continued through E10.5, with expression remaining restricted to the outer dorsal and ventral mesenchyme (Figs. 5G and 5I). The apparent lack of expression in the ectoderm may be due to disruption of this tissue during in situ hybridization.

Starting from E9.5, strong expression was observed in the emerging branchial arches and the edge of the olfactory pit (Fig. 5J). This expression became very pronounced by E10.5, at which time the entire distal facial primordia stained strongly (Fig. 5K).

**Drm.** Expression of Drm was first seen at the somite stage (E8.5), when it was confined to a stripe of mesoderm at the lateral edge of the presomitic mesoderm and, more anteriorly, in the lateral-most aspects of the developing somites (Figs. 6A and 6F). The lateral expression was still apparent at E9.5 and an additional domain of expression was seen in the medial aspects of the dermamyotome of maturing anterior somites (Figs. 6C and 6G). At E10.5, expression of Drm in the somites was still restricted to the medial and lateral extremes of the dermamyotome, with prominent expression seen in the epithelial balls of the lateral myotome and overlying dermis of the interlimb region (Fig. 6D).

In the neural tube at E8.5, expression of Drm was seen in the anterior ventral forebrain (Fig. 6B). By E9.5, expression was restricted to the roof plate at three axial levels: the forebrain (Fig. 6E), the midbrain–hindbrain junction area (Fig. 6H), and the posterior neural tube (Fig. 6C). At E10.5, neural tube expression was no longer apparent. A new domain of head expression was seen at E11.5 in the developing outer ear (Fig. 6I).

Drm expression was observed in the dorsal and ventral mesenchyme of the emergent limb buds at E9.5 (Figs. 6C and 6G). Limb expression was evident through E10.5 (Fig. 6D) and E11.5 (Fig. 6I), becoming restricted first to a dorsal proximal domain and then to the proximal interdigital regions.
Cer1 Processing

The similarity between the can domain and the cysteine knots of TGFβ superfamily members suggests that, as in these proteins (Gentry et al., 1988), the can domain may be cleaved away from a proprotein during processing. To test whether Cer1 is cleaved, two epitope-tagged versions of Cer1 were constructed. In one, an HA tag (Field et al., 1988) was inserted downstream of the putative signal sequence cleavage site and, in the other, a Myc tag (Cravchik and Matus, 1993) was added to the C-terminal end of the protein (Fig. 7A).

Both tagged proteins were efficiently secreted into the medium of transiently transfected Mv1Lu cells (Fig. 7B, lanes 2 and 4). As with the native protein (Belo et al., 1997), the tagged versions run higher than predicted (predicted 30 HA and 32 Myc, actual 40 and 44). This is most likely due to the reported glycosylation of Cer1 (Biben et al., 1998). As both tagged versions are readily identified it appears that Cer1 is not processed beyond the probable removal of the secretion signal sequence. When Cer1-HA protein expressed in Mv1Lu cells was examined under nonreducing conditions a band of double the expected size was seen (Fig. 7A).

**FIG. 4.** Dte expression (A) in the crown cells of the definitive node at early bud (EB) stage and (B) at early somite (Esom) stage. (C) Ventral view of embryo shown in (B) showing more prominent expression on the right-hand side. L, left; R, right.
FIG. 5. Dan expression (A and B) in head mesoderm (hm) at early head fold (EHF) stage and (C and D) in nascent somite (som) and rostral paraxial mesoderm (rpm) at early somite (Esom) stage. (E) A second domain of somitic expression in anterior somites at E8.5. (F and H) Expression in the dermamyotome (dm) and forelimb (fl) bud at E9.5. (G and I) Expression in dm and limb at E10.5. (J) Expression in brachial arches (ba) and in a ring around the olfactory pit (op) of E9.5 head and (K) in the entire distal facial primordia at E10.5.
7B, lane 6), as observed previously using a C-terminal tagged form of Cer1 (Biden et al., 1998), suggesting that the observed lack of processing is not artifactual and that Cer1 may form homodimers.

Inhibition of Bmp4 Signaling

When either cerberus or Cer1 mRNA are injected into Xenopus eggs and animal caps analyzed, the observed changes in gene activity (Belo et al., 1997; Biben et al., 1998) are similar to those caused by the known BMP inhibitors noggin and chordin (Lamb et al., 1993; Piccolo et al., 1996; Sasai et al., 1995; Zimmerman et al., 1996). In particular, anterior neural markers are activated in the absence of mesodermal markers. In addition, Hsu et al. (1998) have recently demonstrated a direct interaction between BMP2 and the can-family members Dan, human CER1, and Xenopus Gremlin. To assess the ability of the mammalian proteins to inhibit BMP signaling in a mammalian system, we have used pTlx-Lux, a BMP-responsive promoter element from the murine Tlx-2 gene driving a luciferase reporter gene (Tang et al., 1998), in embryonal carcinoma P19 cells (McBurney and Rogers, 1982). The addition of exogenous BMP2 causes a three- to fourfold induction of the pTlx-Lux construct in P19 cells. Cotransfection of P19 cells with pTlx-Lux and a murine Bmp4 expression vector, pCDNA3-BMP4, gave a level of induction, five- to sevenfold (Figs. 8A and B), similar to that

**FIG. 6.** Drm expression (A and F) in the intermediate mesoderm (im) at E8.5 and (C and G) in the lateral dermamyotome (ldm) of posterior somites and medial dermamyotome (mdm) of anterior somites, the roof plate (rp) of the posterior neural tube, and the fore- and hindlimb (fl and hl) buds at E9.5. (D) Expression in the limbs and the lateral dermamyotome of the interlimb region at E10.5. (E and H) Expression in fore- and midhindbrain junction rp at E9.5. (I) Expression in the developing outer ear (oe) and (J) in the proximal dorsal (pd) and interdigital (id) regions of the limbs at E11.5.
seen with purified BMP2 ligand, thereby demonstrating that P19 cells are competent to both transmit and receive a BMP signal. Whether Dan, Cer1, or human DRM is able to attenuate BMP signaling was assessed by cotransfecting pCDNA3-BMP4 with pCDNA3 carrying the relevant can-family member, pCDNA3-CAN. During characterization of the genomic Cer1 loci a coding sequence polymorphism was found between mouse strains. In 129Sv an R replaces the M220 found in the Cer1 domain of CD1 and C57BL6. The likely impact of this change, if any, is unknown. The functional studies of Cer1 were made using the CD1/C57BL6 allele.

When equal amounts of each vector, pCDNA3-BMP4 and pCDNA3-CAN, were transfected into P19 cells, only DRM clearly inhibited Bmp4 signaling, reducing induction from seven- to threefold (Fig. 8A). However, when the ratio of Bmp4 to can family was increased from 1:1 to 1:4, all three family members repressed Bmp4 signaling (Fig. 8B). The levels of induction seen with each family member alone do not differ significantly from background (Fig. 8B). That these effects are not due to an inhibition of Bmp4 processing or secretion is suggested by the finding that conditioned medium from Cos cells transiently transfected with pCDNA3-DRM, like conditioned medium containing noggin, also inhibits Bmp4 signaling (Fig. 8C). This is in accord with the interaction between the Xenopus Drm homolog Gremlin and BMP2, a close relative of Bmp4 (Hsu et al., 1998).

**DISCUSSION**

The identification of a region of homology in the Xenopus head-inducing protein cerberus (Bouwmeester et al., 1996) and the putative rat tumor suppressor protein Dan (Ozaki and Sakiyama, 1993) has enabled us to identify a family of
genes that contain this domain. We have called the domain the can domain and the family the can family in recognition of its defining members.

There are at least five mammalian can-family members: Cer1, Drm, PRDC, Dan, and Dte. In addition, a family member has been isolated from the nematode C. elegans, CeCan1, suggesting that the family is of ancient origin.

The can domain contains the core features of a cysteine knot, a tertiary structure found in a number of growth factors, such as members of the TGFβ superfamily (McDonald and Hendrickson, 1993). Additionally, the can domain shares extended homology with the predicted cysteine knots of NDP and human mucin2 (Fig. 1C). Like these other cysteine knot-containing proteins, all the fully characterized mammalian can-domain family members are predicted to be secreted, a prediction that has been confirmed for Dan and Cer1 (Belo et al., 1997; Biben et al., 1998; Nakamura et al., 1997) and by our results.

Potential Roles of the Mammalian Can-Domain Genes

To better understand the likely roles of the murine can-domain gene family, two initial studies have been undertaken. The effect of these proteins on Bmp4 signaling has been investigated and the gene expression patterns of several can-family members and their observed domains of expression suggest possible roles for these genes during development.

Cer1 and the AVE

The characterization of Cer1 and other genes, such as Hex, expressed in the AVE of the early mouse embryo led Beddington and colleagues to propose that this region might influence the site of primitive streak formation and be responsible for the establishment of anterior neural character (Thomas and Beddington, 1996; Thomas et al., 1998). This model is supported by the phenotype of Smad2-deficient mice, in which AVE-specific gene expression is not established and the entire epiblast turns on the primitive streak-specific markers brachyury and FGF8 ( WALDROP et al., 1998).

The finding that Cer1 inhibits TGFβ superfamily signaling and represses trunk formation in Xenopus (Belo et al., 1997; Hsu et al., 1998) suggests that the AVE may act, not as a positive inducer of anterior character, but rather as an inhibitor of posterior character. This proposal is supported by the presence in the AVE of lefty (OU lid-Abdelghani et al., 1998), another inhibitor of BMP signaling (MENO et al., 1997), and mouse Dck1, a recently described Wnt inhibitor (Gilika et al., 1998). If the AVE acts negatively, it might ensure the appropriate displacement of the anterior and posterior ends of the developing primary body axis. During growth, the region of the epiblast farthest from the AVE might escape repression and initiate primitive streak formation.

Dte and the Left-Right Axis

Experiments in the chick have established that a number of genes, including shh, ptc, Act-R1I, and Cnr-1, are expressed asymmetrically in Hensen’s node and that the node plays a critical role in specifying the left-right axis (LEVIN et al., 1995; Pagai-Westphal and T Abin, 1998). In the mouse, in contrast, although the node is likely required for left-right axis determination, as demonstrated by the failure of this event in HNF3β−/− mice ( Dufort et al., 1998), nodal is the only gene, until now, that has been found to be...
asymmetrically expressed in the node (Collignon et al., 1996; Lowe et al., 1996).

Dte expression, like that of nodal, is symmetric initially, but, by the early somite stage, expression of both genes becomes biased such that Dte is stronger on the right and nodal on the left. Given the ability of the can-family members to block BMP signaling it is tempting to speculate that Dte may block nodal signaling, thereby greatly increasing the effective nodal gradient. It should be noted, however, that it is unknown whether the differential expression of nodal in the node is important for right-left axis formation, although the disruption of this event in nodal HNF3β double heterozygotes suggests that it might (Collignon et al., 1996).

**Drm and Somitic Development**

The expression pattern of Drm is strikingly similar to that of chick noggin (Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998). Both genes are expressed, first, in the intermediate mesoderm and then in the medial dermamyotome of maturing somites. This similarity and the ability of both noggin and DRM to inhibit BMP signaling suggests that these genes may act together to pattern the developing somites.

Chick noggin promotes somitic MyoD expression by repressing BMP4 signals emanating from the lateral plate mesoderm (Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998). Drm is expressed in the medial and lateral aspects of the dermamyotome, both of which are sites of myogenesis. DRM may then act in a manner similar to that of chick noggin to enable MyoD expression in these sites. An alternative model is suggested by the observation that DRM is able to repress cell growth and is most highly expressed in terminally differentiated, nondividing cells (Topol et al., 1997). The cells that enter the myogenic pathway become postmitotic (Parker et al., 1995); DRM may then be acting to repress myogenic cell division.

The expression pattern of Drm is quite distinct from that of its Xenopus homolog gremlin. Gremlin is expressed at high levels in the pronephric duct and migrating neural crest cells (Hsu et al., 1998), sites of expression not seen in the mouse. These inconsistencies may be due to differences in the distribution of BMP ligands in these morphologically very distinct embryos.

These initial investigations of the can family suggest that the family members act as homodimers to inhibit BMP-like signals. Future work will investigate the specificity of the inhibition using different members of the TGFβ superfamily, establish whether homodimers are formed, and determine which regions of the proteins mediate the inhibition. In vivo, both gain- and loss-of-function studies are being undertaken to better understand the patterning roles of these genes.

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