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A vegetally localized T-box transcription factor in *Xenopus* eggs specifies mesoderm and endoderm and is essential for embryonic mesoderm formation

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SUMMARY

Pattern formation in early embryogenesis is guided by maternal, localized determinants and by inductive interactions between cells. In *Xenopus* eggs, localized molecules have been identified and some, such as *Vg1* and *Xwnt-11*, can specify cell fates by functioning as inducers or patterning agents. We have used differential screening to identify new *Xenopus* genes that regulate mesodermal patterning, and we have isolated a new member of the T-box family of transcription factors. This gene, named *Brat*, is expressed maternally and its transcripts are localized to the vegetal hemisphere of the egg. During early embryonic cleavage, *Brat* mRNA becomes partitioned primarily within vegetal cells that are fated to form the endoderm. Zygotic expression of *Brat* begins at the onset of gastrulation within the presumptive mesoderm of the marginal zone. Consistent with its zygotic expression pattern, *Brat* induces, in a dose-dependent manner, a full spectrum of mesodermal genes that mark tissues across the dorsal-ventral axis, from the blood through the Spemann organizer. *Brat* also induces endoderm, consistent with its vegetal localization, making *Brat* a good candidate for a maternal determinant of the endoderm. We tested whether endogenous *Brat* is required for mesoderm formation by expressing a dominant-negative, transcriptional repressor form of *Brat* in embryos. This treatment inhibited mesoderm formation and severely disrupted normal development, thereby establishing that *Brat* plays a critical role in embryonic mesoderm formation and body patterning.

Key words: *Xenopus*, T-box, mesoderm, endoderm, transcription factor, dominant-negative protein

INTRODUCTION

Pattern formation in the embryos of most animals is guided by localized factors in eggs and inductive interactions that occur between cells of the developing embryo. Evidence has been gathered for decades supporting the existence of localized factors, or cytoplasmic determinants, in the eggs of a variety of phyla, and inductive interactions have been described in embryos throughout the animal kingdom (Slack, 1991a). For example, in *Drosophila* the localization of *bicoid* and *nanos* mRNAs to the anterior and posterior poles of the egg, respectively, is essential for normal anteroposterior axial patterning (Struhl, 1989). Furthermore, after cellularization of the *Drosophila* blastula, the TGFβ-related growth factor DECAPENTAPLEGIC (DPP) mediates inductive interactions that pattern the dorsal-ventral axis, and DPP also performs other important patterning functions in later development (Gelbart, 1989). In another example, the localization of *glp-1* mRNA to the AB blastomere in the *C. elegans* embryo is required for normal development of cells derived from that blastomere, but inductive interactions are required to establish the normal fates of the daughter cells (Moskowitz et al., 1994).

The existence of localized determinants in the eggs of chordates such as tunicates and amphibians has been inferred by classical embryonic manipulations such as the culture of tissue explants (Gilbert, 1994; Slack, 1993), or sometimes direct visualization (e.g. the yellow crescent of tunicates; Whitaker, 1977). More directly, in the vertebrate *Xenopus laevis*, mRNAs localized to the animal or vegetal pole of the egg have been isolated. Two of these, *Vg1* and *Xwnt-11*, are vegetally localized and encode secreted growth factors with mesoderm-inducing and/or -patterning activities (Ku and Melton, 1993; Melton, 1987a). Much of vertebrate development is guided by inductive interactions, and the first of these to occur in the *Xenopus* embryo establishes the mesoderm. Mesoderm induction can be triggered by growth factors in the FGF family and by TGFβ-related factors, such as BMPs, Activin, Vg1, and nodal (reviewed by Kessler and Melton, 1994; Jones et al., 1995). Nonetheless, cells isolated from different regions of the blastula marginal zone autonomously express early mesodermal marker genes, in accord with their spatial position, which provides evidence that maternal determinants may also influence formation of the mesoderm (Lemaire and Gurdon, 1994).

We sought to identify new mesoderm-specific genes in

Xenopus and, in the process, uncovered a gene that is expressed throughout the mesodermal germ layer. The gene is a new member of a family of transcription factors, called T-box genes, that function in embryonic pattern formation in a wide spectrum of animals. Mouse *Brachyury*, or *T*, was the first member isolated (Herrmann et al., 1990) and, henceforth, a variety of related genes have been isolated in metazoans from nematodes through mammals (Herrmann, 1995a), including *Xenopus Brachyury (Xbra)* (Smith et al., 1991) and *eomesodermin* (Ryan et al., 1996). All contain a DNA-binding domain of about 200 amino acids and about 40-50% identity, referred to as the T domain (Kispert, 1995). Outside of the T domain, there is no significant sequence identity among the various T domain proteins and little information regarding how they regulate transcription.

T-box genes function in a variety of developmental processes and the founding member of the family, *Brachyury* (*T*), has been extensively studied in mice, zebrafish and frogs (reviewed in Herrmann, 1995a). In all of these organisms, the *Brachyury* gene is expressed throughout the nascent mesodermal germ layer at blastula and early gastrula stages, and later it is specifically expressed in the notochord (Schulte-Merker et al., 1992; Smith et al., 1991; Wilkinson et al., 1990). Consistent with its embryonic expression, *Brachyury* functions in mesodermal patterning. Mice that are heterozygous for *Brachyury* display defects in trunk and tail development, and homozygous mice are severely defective in mesoderm formation and die early in utero (Herrmann, 1995b). In zebrafish, the *no tail (ntl)* gene encodes *Brachyury*, and heterozygous *ntl* mutations result in phenotypes that resemble heterozygous mutations of mouse T (Schulte-Merker, 1995). In *Xenopus*, *Brachyury (Xbra)* is capable of triggering ventral mesoderm differentiation when ectopically expressed in animal caps (Cunliffe and Smith, 1992) and the inhibition of *Xbra* function in vivo by a transcriptional repressor form of the protein blocks posterior mesoderm formation in a manner quite similar to the mouse and zebrafish T mutations (Conlon et al., 1996). A second *Xenopus* T-box gene, *eomesodermin*, has also been isolated recently (Ryan et al., 1996), and its expression pattern is similar to that of *Xbra*, but *eomesodermin* is not expressed in the notochord. *Eomesodermin* can induce mesoderm and a dominant-interfering version blocks normal *Xenopus* mesodermal patterning.

We have isolated a third example of a T-box gene from *Xenopus*, which we have named *Brat*. The gene is expressed maternally and its transcripts are localized to the vegetal pole of the egg and cleavage-stage blastula, making *Brat* perhaps the first example of a localized maternal transcription factor. The *Brat* gene is also expressed zygotically throughout the early mesoderm beginning at gastrulation. Consistent with its expression patterns, we demonstrate that ectopic expression of *Brat* induces a wide array of mesoderm, from ventral blood through dorsal mesoderm of the Spemann organizer. Mesoderm induction by *Brat* occurs in a graded fashion, with the character of the mesoderm shifting from ventral to dorsal as the dose of *Brat* is increased. *Brat* also induces endoderm, consistent with its maternal localization to the vegetal pole. We demonstrate that *Brat* is essential for the formation of mesoderm and for proper axial patterning by inhibiting its activity with a dominant-inhibitory form of the protein.

MATERIALS AND METHODS

Library screening

A stage 10^+ cDNA library was constructed using the Stratagene ZAP express kit and screened with a subtracted ventral stage 10^+ cDNA probe. Subtraction of common cellular sequences was performed using the Clontech photoactivatable biotin labeling kit. Maternal mRNA served as the driver in the subtraction, and first strand stage 10^+ ventral cDNA was the tracer. The non-subtracted first strand ventral cDNA (0.95 μg) was labeled with ^{32}P-dATP by random priming with a kit (Boehringer). Approximately 3.5×10^7 cts/minute of probe was used to screen 1.4×10^5 plaques on nylon filters. Hybridization conditions were 5× SSC, 5× Denhardt's, 0.5% SDS, 0.1 mg/ml salmon sperm DNA at 65°C. Filters were washed in 0.1× SSC, 0.1% SDS at 65° C for 30 minutes. Positive plaques were picked and pooled into 12 groups and screened a second time at a lower density (2,000 pfu per plate) with the same probe to isolate individual candidates. 100 positive plaques, selected at random, were converted to plasmids and used to produce digoxigenin-labeled probes for whole-mount in situ hybridization analysis on stage 10.5 embryos to determine whether they were expressed in the mesoderm. One of these was a partial-length cDNA of *Brat*. To isolate a full-length *Brat* cDNA a *Bam*H1 200 base pair (b.p.) fragment of the original isolate was used to rescreen the ventral cDNA library, as above.

Nucleic acids

DNA sequencing was performed on 5′ and 3′ deletions of *Brat* with a Sequenase kit (U.S. Biochemical). Deletion clones of *Brat* were produced with the Promega Erase-a-Base kit. To construct *CS2-Brat*, full-length *Brat* cDNA was excised from pBK-CMV by *Sal*I/*Xho*I digestion, polished with Klenow and subcloned into the *Stu*I site of $CS2^+$ (Rupp et al., 1994). The dominant-negative *Brat-En*^{R} clone was constructed by inserting an 860 b.p. *Cla*I-*Bsu*36I N-terminal fragment of *Brat* cDNA (excised from the CS2+ vector) into the *Cla*I-*Eco*RI site of pCS2-*ENG-N* (unpublished gift of D. Kessler), which contains the first 298 amino acids of the *Drosophila* engrailed protein, nucleotides 169-1064. Capped synthetic mRNAs for microinjection experiments were synthesized with SP6 polymerase using the Ambion mMessage Machine kit. Templates for synthesis of synthetic mRNA were cut as follows: *CS2-Brat* with *Not*I, *CS2-Brat-En*^{R} with *Sac*II, *BUT13* (*Xbra*) (Rao, 1994) with *Eco*RI, *LacZ pSP64T* with *Xba*I. β-gal staining was performed according to Vize et al. (1991).

In situ methods

Whole-mount in situ hybridization was performed as described (Harland, 1991) using BM Purple colorimetric substrate. Antisense digoxigenin probes were synthesized from the following templates with appropriate RNA polymerases: *Brat* in pBK-CMV was cut with *Eco*RI and transcribed with T3; *Goosecoid*, pΔ*gsc* (Cho et al., 1991) was cut with *Xba*I and transcribed with T3; *Xbra*, *BU-K345* (gift of P. Wilson and A. Hemmatti-Brivanlou), was cut with *Xho*I and transcribed with SP6. For histological purposes, *Brat*-injected animal caps were fixed in MEMFA for 1 hour and embedded in paraplast. 10 μm sections were cut and stained with Giemsa. Whole-mount immunohistochemistry was performed as described (Hemmati-Brivanlou and Harland, 1989) using an alkaline phosphatase-conjugated secondary antibody and detected with BM Purple substrate (Boehringer). Antibodies used were 12/101 for muscle (Kintner and Brockes, 1984).

Embryological assays

Embryos in 3% Ficoll, 0.5× MMR were injected in the animal pole at the 2-cell stage with synthetic mRNA. Animal caps were cut at blastula stages 8-9 and cultured in 0.5× MMR until harvesting. RNA preparation, RT-PCR assays and primer pairs were as described (Henry et al., 1996; Thomsen, 1996). *Brat* and *Chordin* primers were as follows. *Brat*: upstream 5′-CAG TGC CGG ATT CCG TAT C

(1005-1023) and downstream 5′-GAG CTA CTG CTC CTT GTG (1290-1270); *Chordin* : upstream 5′-TTT CGC AAC AGG AGC ACA GAC (3439-3459) and downstream 5′-TAC CGC ACC CAC TCA AAA TAC (3722-3702). In mesoderm induction assays with proteins, animal caps cut at blastula stage 8-9 were treated with FGF or activin protein in 0.5× MMR, 0.1% BSA, cultured until sibling embryos reached stage 11 and RNA was prepared as above for northern blots. Primer sequences are available on the *Xenopus* XMMR home page (http://vize222.zo.utexas.edu).

RESULTS

Isolation of a new T-box gene, *Brat*

To gain a better understanding of the early events of mesoderm induction and patterning, we sought to isolate new mesoderm-specific genes by differential screening. We screened a stage 10^+ ventrolateral cDNA library with a gastrula stage ventral cDNA pool (details in Materials and Methods), and the expression patterns of 100 positive clones were analyzed by whole-mount in situ hybridization. Several were mesoderm-specific and among these one coded for a new member of the T-box family of transcription factors. We named this gene *Brat* because it is most closely related to the other vertebrate T-box genes, ***Bra****chyury* and ***T****bx-2* (Campbell et al., 1995) The *Brat* cDNA is 2.7 kb in length, and the Brat open reading frame is 456 amino acids (Fig. 1A). Within the T domain, Brat is about 50% identical at the amino acid level to other T domain proteins (Fig. 1B) but, outside of the T domain, Brat is not significantly similar to other members of the T-box family nor to any other proteins. In general, T-box genes do not share significant sequence identity outside of the DNA-binding domain.

Expression of the *Brat* gene in development

A developmental northern blot revealed that *Brat* transcripts are maternal and present throughout early development until neurula stage 18 (Fig. 2A). The presence of *Brat* transcripts in *Xenopus* eggs prompted us to examine their in situ spatial distribution during oogenesis. We found that *Brat* transcripts are uniformly distributed in stage I oocytes but become vegetally localized by stage II and remain so thereafter in oogenesis (Fig. 2B). The localization of *Brat* mRNA coincides precisely with the unpigmented, vegetal region of late stage oocytes (compare upper and lower panels of oocytes in Fig. 2B). This domain of localization approximately coincides with that of two other vegetally localized transcripts, *Vg1* and *Xwnt-11* (Ku and Melton, 1993; Melton, 1987b). *Brat* mRNA is tightly associated with the vegetal cortex since partial removal of the membrane from the vegetal pole of fixed oocytes abolishes the in situ hybridization signal (Fig. 2B).

Brat transcripts remain predominantly vegetal during the early cleavage stages of embryonic development. A northern blot on animal and vegetal halves of stage 6 embryos (approximately 32-64 cells) showed that *Brat* message is localized mainly within the vegetal blastomeres, although a low level of *Brat* mRNA is also present in cells of the animal hemisphere (Fig. 2C). Whole-mount in situ hybridization on 32-cell embryos confirmed that *Brat* mRNA is contained mostly within the C and D tiers of vegetal blastomeres (data not shown). Cells cleaved from the D tier form endoderm exclusively, while cells of the C tier contribute to both the endoderm and mesoderm (Dale and Slack, 1987). The presence of *Brat* transcripts in the D tier raises the possibility that it may act as a maternal determinant of endoderm formation. The localization of *Brat* transcripts within the C blastomeres also suggests that maternal *Brat* might function in mesoderm specification. Functional studies (below) support these notions.

Zygotic *Brat* transcripts first appear in the dorsal marginal zone just prior to the appearance of the dorsal blastopore lip (stage 10^-, Fig. 3A). Thereafter *Brat* expression extends ventrally so that, by stage 10^+, the *Brat* gene is transcribed

A.

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MRNCCRECGLSAGHLEPEASSNCASDVKSSPDMDSVSSQDSLYLPNTVGA
SLEDQDLWSQFHQEGTEMIITKSGRRMFPQCKIRLFGLHPYAKY
MLLVDFVPLDNFRYKWNKNQWEAAGKAEPHPPCRTYVHPDSPAP
GAHWMKDPICFQKLKLTNNTLDQQGHIILHSMHRYKPRFHVVQS
DDMYNSPWGLVQVFSFPETEFTSVTAYQNEKITKLKINHNPFAK
GFREQERSHKRDDVLKIHQQSPSKRQKRKKWEDSPEADISDFPKAICV
KEESIMDPAGVYQNWVSDHEANQGLTPHSPESEGANQEQQVPTSSSNFYN
KSHYRRSSQHLSSPFELGEPSSRRLTPDIATVPDSDPDSLAVFHVIPTQN
SAPERTCSMNFSMEAPMKQPLRGAMYSPYGADQWLVPAQGQYRPVGYTAY
PTDLSTQGAVAHPHSAMSDWSQYSLFPYSCW.
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B.

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Brat    VGASLEDQDLWSQFHQEGTEMIITKSGRRMFPQCKIRLFGLHPYAKYMLLVDFVPLDNFRYKWNK
Xbra    LKV...ER...TR.KELTN...V..N......VL.VSMS..D.N.M.TV.L...AA..H.W.YVN
T       LRVG..ESE..LR.KELTN...V..N......VL.VNVS..D.N.M.SF.L...TA..H.W.YVN
Zf-T    IKL....AE..TK.KELTN...V..T......VLRASVT..D.N.M.SV.L...AA..N.W.YVN
Trg     LRI..D.RE..LR.QNLTN...V..N......VV..SAS..D.A.M.TV.LE..QI.SH.W.YVN
Tbx2    PKVT..AKE..D...KL....V..........PF.V.VS..DKK...I..M.IVAA.DC...FHN
Omb     PKVT..GK...EK..KL....V......Q....M.F.VS..DAK...I..L.IVAA.DY...FHN

Brat    NQWEAAGKAEPHPPCRTYVHPDSPAPGAHWMKDPICFQKLKLTNNTLDQQGH---IILHSMHRYK
Xbra    GE.VPG..P..QA.SCV.I.....NF........VS.S.V....KMNGGGQ----.M.N.L.K.E
T       GE.VPG..P..QA.SCV.I.....NF......A.VS.S.V....KLNGGGQ----.M.N.L.K.E
Zf-T    GE.VPG..P..QS.SCV.I.....NF......A.VS.S.V..S.KLNGGGQ----.M.N.L.K.E
Trg     GE.VPG....VP.SNPI....E..NF......E..S.A.V..T.K.NGNGQ----MM.N.L.K.E
Tbx2    SR.MV....DPEM.K.M.I......T.EQ..AKPVA.H.......IS.KH.F---T..N...K.Q
Omb     SR.MV....DPEM.K.M.I.....TT.EQ..QKVVS.H.......IS.KH.FVSTT..N...K.Q

Brat    PRFHVVQSDDMYNSPWGLVQVFSFPETEFTSVTAYQNEKITKLKINHNPFAKGFREQERSHKRD
Xbra    ..I.I.RVGGTQRM----ITSH.....Q.IA.......E..A...K......A.LDAKERNDYK
T       ..I.I.RVGGPQRM----ITSHC....Q.IA.......E..A...KY.....A.LDAKERNDHK
Zf-T    ..I.I.KVGGIQKM----ISSQ.....Q.IA.......E..A...K......A.LDAKERSDHK
Trg     ..V.L.RVGSEQRH----.VTYP....Q.IA.......EV.S...KY.....A.LDAKERPDTL
Tbx2    ....I.RAN.ILKL.YSTFRTYV....D.IA......DK..Q...DN........DTGNGRREK
Omb     ....L.RAN.ILKL.YSTFRTYV.K....IA.......K..Q...DN......L.DTGAGKREK
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Fig. 1. The Brat Protein. (A) The predicted amino acid sequence of *Brat* encodes a protein of 456 amino acids. The DNA-binding domain, T domain, is located between the arrows and is highlighted in bold. (B) A comparison of the T domain of Brat with six other T domain containing proteins. Identical amino acids are indicated by dots, and spaces (dash) were introduced to maximize homology. The degree of identity between these domains and the T domain of Brat is: *Xenopus* Brachyury (Xbra, 47%), mouse Brachyury (T, 45%), zebrafish T (Zf-T, 46%), *Drosophila* T-related gene (Trg, 45%), mouse Tbx2 (54%), and *Drosophila* optomotor blind (Omb, 51%). The accession number for Brat is U89707.

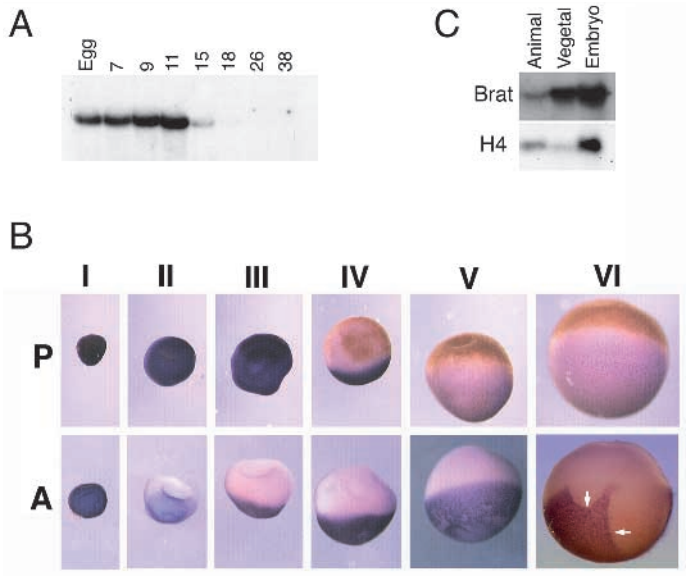

Fig. 2. Expression pattern of the *Brat* gene in *Xenopus* development. (A) A Developmental northern blot on total embryonic RNA revealed a single *Brat* transcript of approximately 3 kb that is expressed maternally and zygotically into mid-neurulation (Stage 15). Stages analyzed were egg, blastula (stages 7,9), gastrula (stage 11), neurula (stages 15,18) tailbud tadpole (stage 26) and swimming tadpole (stage 38). Five embryo equivalents were loaded per lane and RNA recovery and integrity was equal across the blot (not shown). (B) Localization of *Brat* mRNA in oocytes by whole-mount in situ hybridization. *Brat* mRNA is expressed throughout oogenesis and is localized to the vegetal pole by stage II. Pigmented oocytes (P) are shown in the upper panel, animal pole up, to orient the stain relative to the animal and vegetal pole. Albino oocytes (A) are shown in the lower panel to clearly display the domain occupied by *Brat* transcripts. During the in situ procedure, the cortex was partially removed from several stage VI oocytes to demonstrate that the bulk of *Brat* transcripts are sequestered in the cortex. Arrows indicate the edge where the cortex was torn off, revealing the underlying, unstained cytoplasm. (C) Vegetal localization of *Brat* mRNA in early cleavage stage embryos. A northern blot of RNA from animal and vegetal halves of stage 6 embryos. Seven explants or three embryos were analyzed and the blot was reprobed with *Histone H4* to contrast the localization of *Brat* mRNA with that of a cytoplasmic mRNA.

throughout the marginal zone (Fig. 3B). The timing and distribution of *Brat* transcripts appear nearly identical to those of *Xenopus Brachyury (Xbra)* at these early stages (compare Fig. 3A,B with D,E). However, by late gastrulation (stage 12), differences in their expression patterns are apparent (Fig. 3C,F). Both *Brat* and *Xbra* transcripts are present throughout the mesodermal ring around the closing blastopore. *Brat* transcripts, however, are not present in the involuting axial mesoderm that will form the notochord, whereas *Xbra* transcripts are present (Fig. 3C,F). Late in gastrulation and through neurula stage 15 *Brat* is expressed in the posterior mesoderm but, by stage 18, its transcripts are not detected (Fig, 2A and data not shown).

Functional analysis of *Brat*

Xbra and *Brat* encode putative DNA-binding factors, and *Xbra* has been shown to function as a transcriptional activator that can trigger mesoderm formation when ectopically expressed in

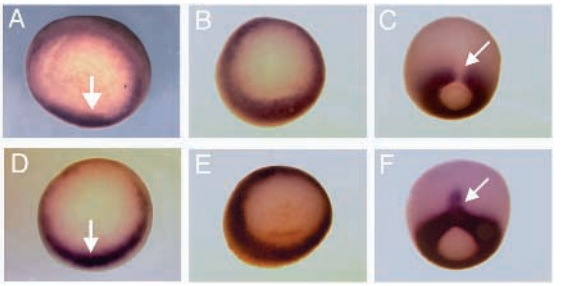

Fig. 3. Comparison of the spatial distribution of *Brat* and *Xbra* transcripts during gastrulation by whole-mount in situ hybridization. *Brat* expression is shown in the upper panel, *Xbra* expression is shown in the lower panel. (A,D) Zygotic expression of *Brat* and *Xbra* is first detected just prior to the appearance of the dorsal blastopore lip (arrow), stage 10^{-}. (B,E) By stage 10^{+} both *Brat* and *Xbra* are expressed throughout the marginal zone. (C,F) During late gastrulation, stage 12, *Brat* is expressed in the ventrolateral mesoderm surrounding the yolk plug, but not in the axial mesoderm of the future notochord (arrow). *Xbra* is also expressed in the ventrolateral mesoderm, but, in contrast to *Brat*, it is expressed in the notochord (arrow).

Xenopus animal caps (Cunliffe and Smith, 1992). We compared the mesoderm-inducing potential of *Brat* with *Xbra* and found that *Brat* indeed induces mesoderm in animal caps, but that the mesoderm-inducing activity of *Brat* differs from that of *Xbra*. *Brat* and *Xbra* both induced the ventrolateral mesodermal markers *XMyoD* and *Xwnt-8* (Fig. 4A), although *Xbra* induced *Xwnt-8* to a lesser degree than *Brat*. The two factors show a distinct difference in their activities, however, because *Brat* induces the *goosecoid* and *XFKH-1* genes that mark the Spemann organizer, the most dorsal mesodermal derivative, but *Xbra* does not.

We examined mesoderm induction by *Brat* in greater detail and found that *Brat* induces a full spectrum of early markers for ventral, lateral and dorsal mesoderm in a dose-dependent fashion (Fig. 4B). Low doses of *Brat* mRNA (14 picograms, pg) induce ventral and posterior mesodermal genes, such as *Xwnt-8*, *Xhox3*, *Xlhbox6* and *XMyoD*. Intermediate doses (42-140 pg) induce *XFKH-1*, a marker expressed in the organizer and adjacent dorsolateral tissue, and doses at or above 350 pg induce Spemann organizer-specific genes, such as *chordin* and *goosecoid*. Note that, as the dose of *Brat* is increased, the expression of ventral-posterior markers (*Xhox3*, *Xlhbox6* and *XMyoD*) declines as organizer gene expression increases, reflecting a shift in the proportion of dorsal or ventral mesoderm induced by *Brat*. All doses of *Brat* that induce mesoderm also induce the *Xbra* gene, consistent with the relatively uniform embryonic expression pattern of *Xbra* across the dorsal-ventral axis of the marginal zone. *Brat*-injected animal caps also express late stage markers of dorsal and ventral mesoderm (Fig. 4C) in agreement with the induction of early mesodermal markers. At the equivalent of tadpole stage 28, *Brat* induces the muscle-specific *actin* gene, a marker for somitic muscle, and *alpha globin*, a marker of red blood, the most ventral mesodermal tissue.

To confirm that *Brat* induces mesodermal tissues, as opposed to simply activating mesoderm-specific genes, we examined histological sections of animal caps injected with *Brat* mRNA. At low doses (50 pg) of *Brat*, animal caps form

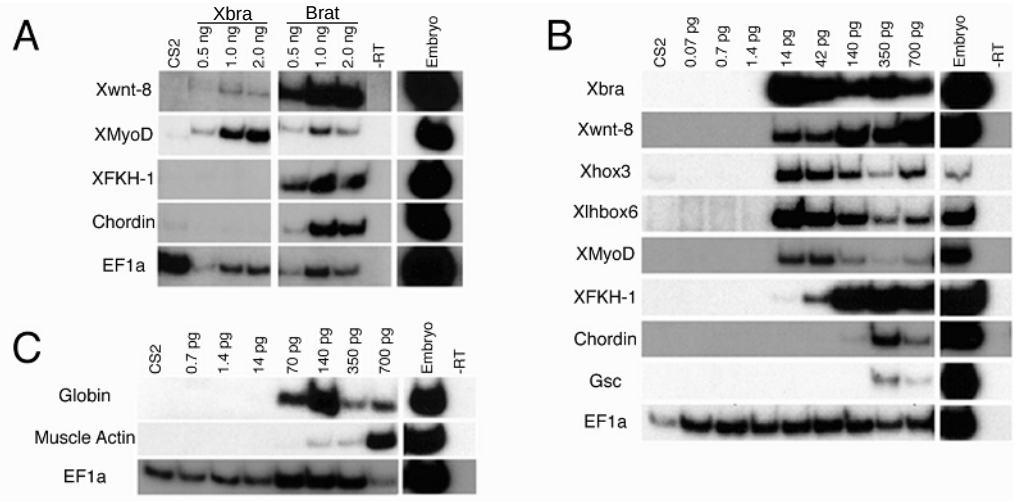

Fig. 4. Analysis of mesoderm induction by *Brat* and *Xbra* in animal caps. (A) Comparison of *Xbra* and *Brat* activities reveals that both induce the ventrolateral markers *Xwnt-8* (Christian et al., 1991) and *XMyoD* (Frank and Harland, 1991); only *Brat* however, induces the dorsal mesoderm markers *XFKH-1* (Dirkson and Jamrich, 1992) and *chordin* (Sasai et al., 1994). (B) A more detailed examination of mesoderm induction by *Brat*. At low doses of injected mRNA (14-42 pg), *Brat* induced ventral-posterior mesoderm, as scored by the expression of *Xwnt-8*, *Xhox3* (Ruiz i Altaba and Melton, 1989), *Xlhbox6* (Wright et al., 1990), *Xbra*, and *XMyoD*. At intermediate doses (42-140 pg) *XFKH-1* is induced, followed by organizer-specific genes *goosecoid* (*gsc*) (Cho et al., 1991) and *chordin* at higher doses (350-700 pg). (C) In tadpole stage 28 animal caps *Brat* induces *globin*, a marker for blood (the most ventral mesoderm derivative), and *muscle actin*, which marks dorsal mesoderm. Animal caps were injected with synthetic mRNA at the 2-cell stage and mesoderm induction was analyzed by RT-PCR at either stage 11 (A,B) or stage 28 (C).

vesicles, characteristic of ventral mesoderm (Fig. 5B). At high doses (500 pg) of *Brat*, the animal caps elongate and form muscle (Fig. 5E) a dorsal mesodermal tissue, as shown by immunohistochemical staining of *Brat*-injected animal caps with the muscle-specific antibody, 12/101. In *CS2*-injected animal caps, no muscle tissues form (Fig. 5D), while 33% of *Brat*-injected animal caps (n=27) form muscle (Fig. 5E). At low doses of *Brat*, 50-250 pg, little muscle tissue forms. As a positive control, all *BVg1*-injected animal caps (n=22) form muscle (Fig. 5F). Although *Brat* can induce organizer-specific markers, as well as muscle, we did not observe the formation of notochord tissue (n>30). These histological examinations confirm our findings with molecular markers that *Brat* induces ventral and dorsal mesoderm.

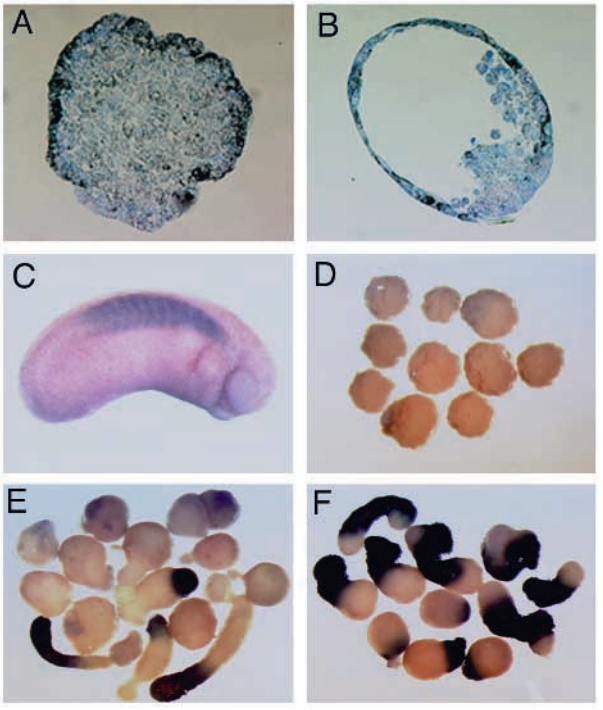

Fig. 5. *Brat* induces mesodermal tissues. (A) Control-injected animal caps form solid balls of atypical epidermis. (B) At low doses, 50 pg, *Brat*-injected animal caps form vesicles, characteristic of ventral mesoderm. Caps in (A) and (B) were scored at stage 28. (C-F) Whole-mount staining for muscle with the 12/101 antibody. (C) Sibling stage 24 whole embryo. Muscle tissue is stained in the segmented somites. (D) CS2-injected animal caps, 250 pg mRNA. No muscle tissue formed, n=11. (E) *Brat*-injected animal caps, 500 pg mRNA. Muscle formed in 33% of the injected caps, n=27. (F) *BVg1*-injected animal caps, 5 pg mRNA. Muscle staining is seen in all caps, n=22.

The localization of maternal *Brat* transcripts within vegetal cells led us to test whether *Brat* might also function in endoderm specification. *Brat*-injected animal caps were scored at the equivalent of tadpole stage 28 for induction of the *Xlhbox8* gene, a marker of anterior endoderm of the pancreas and liver (Wright et al., 1988), and the *Intestinal fatty acid binding protein* (*IFABP*) gene (Shi and Par Hayes, 1994), a general endoderm-specific marker (Fig. 6). *Brat* mRNA doses at or above 70 pg induce both markers, which prompts us to suggest that *Brat* might act as a maternally localized determinant for endoderm differentiation.

The *Brat* gene responds to mesoderm-inducing factors

Mesoderm is induced within the marginal zone of the *Xenopus* embryo by factors secreted from the vegetal cells at blastula stages. Mesoderm can be induced in blastula stage animal caps in vitro when placed in contact with vegetal cells or exposed to particular growth factors of the TGFβ and FGF families (Kessler and Melton, 1996). Like other mesoderm-specific genes, we anticipated that, in animal caps, *Brat* would respond to mesoderm-inducing factors, and this prediction holds true. *Brat* gene expression can be induced in animal caps exposed to basic FGF (bFGF) or activin B proteins, and the level of induction is concentration-dependent (Fig. 7A). The lowest concentration of factor that induced *Brat* was 6.4 ng/ml for

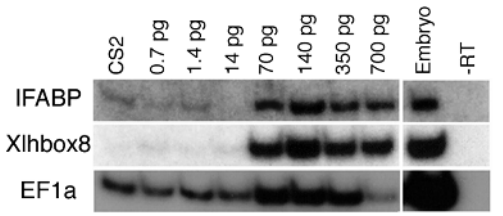

Fig. 6. RT-PCR analysis of endoderm induction by *Brat* in animal caps at stage 28. *Brat* induces the endodermal markers, *intestinal fatty acid binding protein* (*IFABP*) and *Xlhbox8*. *IFABP* is a general marker of endoderm while *Xlhbox8* is an anterior endoderm marker of pancreas and liver.

bFGF and 2 ng/ml for activin B and, as the concentration of either factor was increased, we observed a concomitant increase in the level of *Brat* gene expression (Fig. 7A). The *Brat* gene can also be induced by *BMP-4* or *Vg1* (in its functional form, *Bvg1*; Thomsen and Melton, 1993) when these factors are expressed in animal caps from microinjected synthetic mRNA (Fig. 7B). These results demonstrate that *Brat* can be induced by growth factors that specify either ventral mesoderm (FGF and BMP-4) or dorsal mesoderm (activin and BVg1), consistent with the expression of the *Brat* gene throughout the early mesoderm.

Brat is essential for mesoderm formation in the embryo

The expression of *Brat* in the marginal zone and its ability to induce mesoderm ectopically in animal caps predict that *Brat* plays an essential role in the formation of mesoderm in the embryo. To test this hypothesis, we sought to block the function of endogenous *Brat* with a dominant negative version of *Brat*. Natural mutations in the mouse *Brachyury* gene (Herrmann, 1995a) and studies with mutated forms of *Xbra* (Conlon et al., 1996; Rao, 1994) indicate that a transcriptional activation domain lies within the C terminus of both proteins, outside of the DNA-binding domain. Deletion of the C terminus of *Xbra* generates a dominant negative mutant protein that can antagonize wild-type *Xbra* in mesoderm induction assays (Rao, 1994). Furthermore, replacement of the C terminus of *Xbra* with the transcriptional repressor domain of the *Drosophila* engrailed protein creates a more potent dominant negative inhibitor of *Xbra* (referred to as *Xbra-En*^{R}), and its expression in *Xenopus* embryos blocks posterior mesoderm formation (Conlon et al., 1996). This domain of engrailed has been shown to function as a transcriptional repressor (Han and Manley, 1993). When this domain is fused to the DNA-binding portion of a transcriptional activator, it turns it into a transcriptional repressor that can act in a dominant-negative manner to block the function of the endogenous factor (Badiani et al., 1994). We sought to create a similar dominant negative form of *Brat*, so we replaced its C terminus with the *Drosophila* engrailed transcriptional repressor domain to form *Brat-En*^{R} (Fig. 8A).

The effects of *Brat-En*^{R} expression in *Xenopus* embryos are shown in Figs 8 and 9. By whole-mount in situ hybridization, we found that expression of *Brat-En*^{R} in the marginal zone inhibits the expression of general and organizer-specific mesodermal genes (Fig. 8). We injected *Brat-En*^{R} mRNA together with *LacZ* mRNA (to mark the injected cells) into the marginal

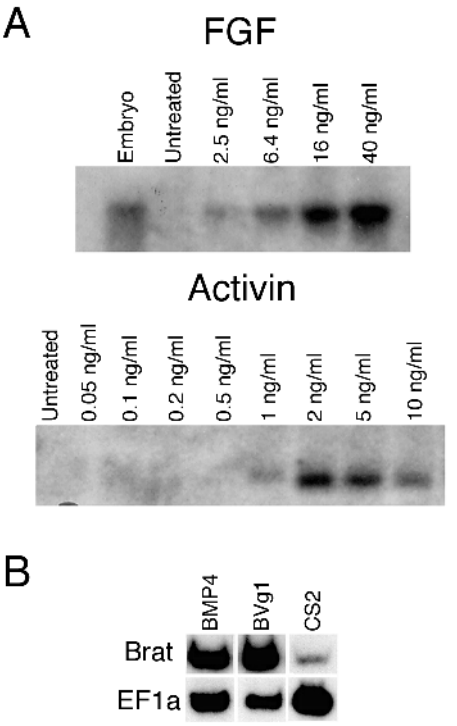

Fig. 7. *Brat* is induced by peptide growth factors in the FGF and TGF-β families. (A) *Brat* displays dose-dependent induction by FGF or activin protein. Animal caps were excised at stage 8-9 and exposed to the indicated concentrations of FGF or activin protein. Caps were harvested at stage 11, and *Brat* induction was analyzed on a northern blot. RNA recovery was equal across the blot (not shown). (B) *Brat* is induced by *BVg1* and *BMP-4*. Caps were injected with 30 pg of *BVg1* mRNA or 1.8 ng *BMP-4* mRNA, excised at stage 9, harvested at stage 11, and scored for *Brat* expression by RT-PCR. *EF1-alpha* expression is a control for RNA recovery and cDNA synthesis. Maternal *Brat* transcripts in the animal pole (see Fig 2c) account for the background signal in vector-injected animal caps.

zone at one end of the first cleavage furrow (Fig. 8B). Injected embryos were cultured to early gastrula, stage 10, and the expression of several mesoderm-specific genes was scored. Control-injected embryos showed a normal pattern of *Xbra* expression in the marginal zone (Fig. 8C), but in embryos injected with *Brat-En*^{R} *Xbra* expression was eliminated in the injected cells (Fig. 8D,E). Furthermore, *Brat-En*^{R} inhibited the expression of *goosecoid (gsc)* within the territory of the Spemann organizer (Fig. 8F-H). Similarly, *Brat-En*^{R} also inhibited *Xlim-1* expression in the organizer (data not shown). The inhibition of both pan-mesodermal and Spemann organizer markers by *Brat-En*^{R} demonstrates that *Brat* activity is essential for mesoderm formation across the entire dorsal-ventral axis of the *Xenopus* embryo.

At the phenotypic level, inhibition of *Brat* function disrupts body patterning. At the early gastrula stage, expression of *Brat-En*^{R} inhibited the formation of the dorsal blastopore lip of the organizer when expressed on the dorsal side (Fig. 9B). The inhibition of dorsal lip formation in these embryos is consistent with the inhibition of organizer specific genes by *Brat-En*^{R} (above, Fig. 8). Similarly, expression of *Brat-En*^{R} mRNA on the ventral side of the embryo inhibited ventral lip formation (Fig. 9C). At tadpole stages, embryonic patterning defects

caused by *Brat-En*^{R} are clearly evident. Embryos injected with *Brat-En*^{R} mRNA on the dorsal side lack a head (or have a very small head rudiment) and develop severe dorsal trunk defects (Fig. 9E) due to incomplete gastrulation and neural plate closure (arrow in Fig. 9E). Ventral expression of *Brat-En*^{R} caused the reduction or loss of tail, trunk and ventral structures (Fig. 9F). These results demonstrate that *Brat* activity is necessary for correct embryonic body patterning.

To demonstrate that *Brat-En*^{R} specifically antagonizes the function of *Brat*, we co-expressed both transcripts in the lateral marginal zone of developing embryos. Trunk defects resulting from the expression of *Brat-En*^{R} (Fig. 10B) in the lateral marginal zone were rescued by coinjection of twice as much wild-type *Brat* mRNA (Fig. 10C). Wild-type *Brat* also rescued defects produced by dorsal and ventral injection of *Brat-En*^{R} mRNA (data not shown). We also tested whether or not *Xbra* can substitute for *Brat* and rescue the patterning defects caused by *Brat-En*^{R}. We found that *Xbra* did not rescue *Brat-En*^{R} defects at any ratio of *Xbra* to *Brat-En*^{R}, ranging from 0.25:1 to 5:1 (Fig. 10D). The rescue of *Brat-En*^{R} phenotypes by wild-type *Brat* demonstrates that *Brat-En*^{R} is a specific dominant-negative inhibitor of *Brat* in the embryo. Furthermore, the failure of *Xbra* to rescue dominant-negative *Brat* phenotypes argues that the two genes do not function in a simply redundant manner in development.

DISCUSSION

By differential screening, we have isolated a cDNA encoding a T-box transcription factor, named *Brat*, that displays a unique set of properties. The *Brat* gene is expressed during oogenesis and its transcripts are localized to the oocyte vegetal pole where they later become incorporated into vegetal cells during embryonic cleavage stages. Zygotic expression of *Brat* commences just before gastrulation and *Brat* transcripts are present throughout the marginal zone. Consistent with its expression patterns, *Brat* can induce mesoderm and endoderm as a function of dose, and blocking *Brat* activity in the embryo with a dominant-negative version of the protein inhibits mesoderm formation and severely disrupts body patterning. Thus, *Brat* performs an essential role in *Xenopus* embryonic development.

Brat is a localized maternal transcription factor

The *Brat* gene is a member of the T-box gene family of DNA-binding proteins, and it shares about 50% identity to other T-box genes within the DNA-binding domain. At least one member of the family, *Brachyury* functions as a transcriptional activator (Conlon et al., 1996; Kispert, 1995). We have not directly demonstrated that *Brat* also functions as a transcription factor, but that is almost certainly the case since we can create a dominant-negative version of *Brat* by fusing its DNA-binding domain to a transcriptional repressor domain of engrailed. Recent results have shown that the C terminus of a gene called *VegT*, which is nearly identical to *Brat*, activates transcription in a yeast one-hybrid assay (Zhang and King, 1996).

The developmental expression pattern of *Brat* suggests it has a dual role in the formation of endodermal and mesodermal tissues. The first phase of its expression is maternal when *Brat* transcripts become localized to the vegetal pole of oocytes early in oogenesis and, in full-grown oocytes, the transcripts reside mostly in the vegetal cortex. The timing of *Brat* transcript localization in oogenesis is similar to that of *Xwnt-11*, *Xcat-2* and *Xlsirts*, which are transported to the vegetal pole via the mitochondrial cloud, referred to as the METRO mechanism (Forristall et al., 1995; Kloc and Etkin, 1995). Unlike *Xcat-2* and *Xlsirts*, however, *Brat* transcripts are distributed more widely in the vegetal cortex, similar to *Vg1* mRNA (Melton, 1987b) which becomes localized via a microtubule-dependent mechanism (Yisraeli et al., 1990). It will be interesting to determine which transport system localizes *Brat* mRNA.

The vegetal localization of *Brat* mRNA in the egg results in its sequestration within cleavage stage cells that fate map to the endoderm and part of the mesoderm. At the 32-cell stage, the most vegetal tier of cells, the D tier, is fated to form endoderm, while the next layer of cells, the C tier, populate both endoderm and some of the mesoderm (Dale and Slack, 1987). The capacity of *Brat* to induce both endodermal and mesodermal marker genes in animal caps prompts us to suggest that *Brat* may function as a localized determinant for the specification of these germ layers. The capacity of vegetal pole explants of amphibian embryos to form rudimentary endodermal tissues (Holtfreter, 1938) and express endoderm-specific genes (Henry et al., 1996) provides indirect evidence for the existence of maternal determinants for the endoderm. Loss-of-function tests will be required to conclusively establish whether *Brat* is a bona fide endodermal determinant. The existence of maternal determinants for mesoderm is supported by the demonstration by Lemaire and Gurdon (1994) that presumptive mesodermal cells isolated from the blastula marginal zone express mesodermal genes (*Xwnt-8* and *goosecoid*) in a cell autonomous manner, and their expression properly reflects the ventral or dorsal origin of the explanted cells. Our demonstration that *Brat* induces mesoderm in animal caps would be consistent with the possibility that maternal *Brat* may act as a determinant for mesoderm formation. *Brat* may be the first example of a transcription factor that functions as a localized determinant in a vertebrate egg.

The function of *Brat* in mesodermal patterning

When *Brat* is expressed in animal caps, it can induce a full range of mesoderm, from the most ventral types through that of the Spemann organizer, and the type of mesoderm induced by *Brat* is dose-dependent. As the amount of *Brat* is increased, the character of the mesoderm shifts from ventral, through lateral, to dorsal. On the basis of these results, we hypothesize that a gradient of Brat protein (or activity) is generated across the dorsal-ventral axis of the marginal zone, with the low end of the gradient present in ventral cells and the high end present in dorsal cells. The level of Brat protein in cells along the dorsal-ventral axis would specify the particular character of the mesoderm. It is also possible that a gradient of Brat protein is set up along the animal-vegetal axis of the blastula, since a relatively high level of *Brat* mRNA is contained within vegetal pole cells compared to animal pole cells (Fig. 2C). The high level of Brat in vegetal cells might be sufficient to specify autonomous endodermal differentiation, but the lower level of Brat in animal cells is insufficient to specify mesoderm in isolated animal caps. Similarly, intact marginal zone tissue,

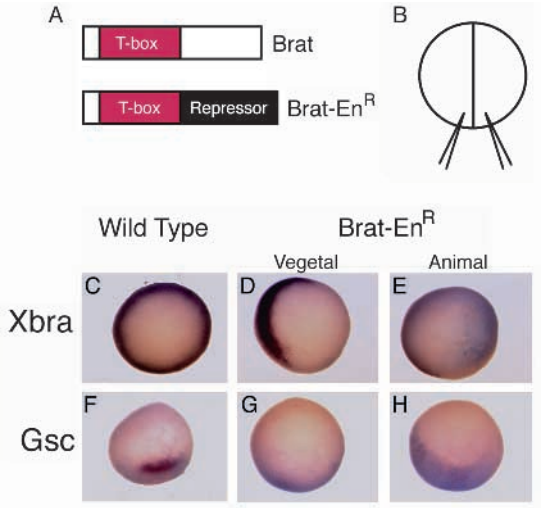

Fig. 8. A repressor form of *Brat*, *Brat-En*^{R}, inhibits expression of dorsal and ventral mesodermal markers. (A) Schematic diagram of wild-type *Brat* (upper) and a chimera of the N terminus of *Brat* and the *Drosophila* engrailed repressor domain, *Brat-En*^{R} (lower). The T-box is red, while the engrailed repressor domain is black. (B) Diagram illustrating the experimental design. 2 ng *Brat-En*^{R} and 0.5 ng *LacZ* mRNA was injected into the marginal zone of both blastomeres along the first cleavage plane at the 2-cell stage. Albino embryos were used in which dorsal-ventral differences are not apparent and therefore the site of injection is randomized with respect to the dorsal or ventral side. (C,F) Wild-type whole-mount expression patterns of *Xbra* and *gsc*. (D,G) Vegetal view of marker expression in embryos injected with *Brat-En*^{R}. (E,H) Animal view of the same embryos in D,G to show β-gal staining in the region coinjected with *Brat-En*^{R} and *Lac Z* mRNA. Note that the expression of *gsc* and *Xbra* is absent on the side expressing *Brat-En*^{R}. *Xlim* expression was also absent in these embryos (data not shown). Since the site of injection along the dorsal-ventral axis was randomized, *gsc* expression was present opposite the β-gal stain in 50% of the embryos (n=30), as expected. *Xbra* expression, on the contrary, was always eliminated in the injected cells, whether injected dorsally or ventrally.

isolated prior to the onset of inductive signaling by vegetal cells, do not form mesoderm, so maternal Brat trapped within these cells is also apparently insufficient to trigger mesoderm differentiation in intact tissue. Whether Brat is the agent that triggers mesodermal gene expression in dispersed marginal zone cells remains to be determined. We are pursuing experiments to examine Brat protein localization in development to begin addressing these issues.

Once mesoderm induction is underway in the *Xenopus* embryo, *Brat* may perform a pivotal role in the commitment of marginal zone cells to mesodermal fates in response to mesoderm-inducing signals. The vegetal cells are the source of mesoderm-inducing factors (Nieuwkoop, 1969; Slack, 1991b), and there is good evidence that some of these factors might be members of the TGF-β, FGF and Wnt growth factor families (Kessler and Melton, 1994). We have shown that the *Brat* gene can be induced by FGF, activin, BMP-4 and Vg1, and this ability to be activated by several types of mesoderm inducers may ensure that *Brat* is expressed throughout the marginal zone, even if the identity or concentration of mesoderm inducer changes along the dorsal-ventral axis. The expression of *Brat*, in turn, would trigger differentiation of the appropriate type of mesoderm. For instance, in the ventral marginal zone *Brat* induced by BMP signals would specify blood, whereas in the organizer domain *Brat* induced by Vg1 or an activin-like signal would specify organizer-specific tissues. Furthermore, since the *Brat* gene responds in a concentration-dependent manner to activin and FGF, cells positioned across a gradient of such factors might activate the *Brat* gene in proportion to the inducing signal. Activin in particular can act as a morphogen on animal cap cells and induce different types of mesoderm as a function of concentration (Green et al., 1992; Wilson and Melton, 1994). The level of *Brat* gene induction might provide the molecular mechanism by which cells convert the signal from a morphogen into a proportional genetic, and ultimately phenotypic, response. In the *Xenopus* embryo, however, the steady-state levels of *Brat* transcripts that we observe in situ are nearly equal in marginal zone cells during gastrulation, inconsistent with the presence of a morphogen gradient that directly affects *Brat* transcript levels. However, perhaps the dynamic appearance of *Brat* transcripts starting on the dorsal side of the marginal zone and moving ventrally reflects the presence of a transient gradient of mesoderm inducer(s).

In order to establish whether or not *Brat* is essential for mesodermal patterning in the embryo, we blocked its activity with a dominant-negative version of *Brat*, called *Brat-En*^{R}, which is a chimera of the Brat DNA-binding domain and a transcriptional repressor domain of *Drosophila* engrailed. We assumed Brat is a transcriptional activator based on the fact that *Xbra* is an activator (Conlon et al., 1996). Expression of *Brat-En*^{R} in embryos blocked the induction of ventrolateral and Spemann organizer mesoderm and severely perturbed body patterning. Importantly, *Brat-En*^{R} specifically inhibits the functions of *Brat*, because coexpression of these proteins (at a 2:1 ratio of *Brat* to *Brat-En*^{R}) rescues the patterning defects

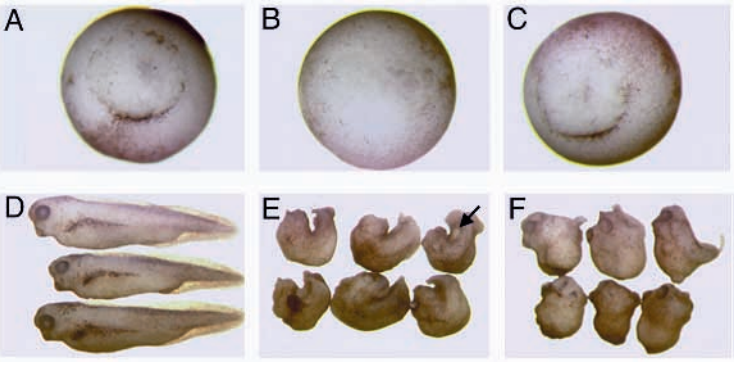

Fig. 9. *Brat-En*^{R} disrupts mesodermal patterning. (A) Vegetal view stage 11 wild-type embryo, positioned with the dorsal blastopore lip at the bottom. (B) Dorsal injection of *Brat-En*^{R} inhibits dorsal blastopore lip formation. (C) Ventrally injected embryo shows normal dorsal lip formation, but ventral lip formation is blocked. (D) Stage 35 wild-type embryo. (E) Embryos injected in the dorsal marginal zone with *Brat-En*^{R} mRNA do not develop anterior head structures (note the lack of eyes) and the neural plate does not close (arrow). (F) Ventral injection of *Brat-En*^{R} disrupts posterior mesoderm development, but anterior development is not affected. A total of 2 ng of *Brat-En*^{R} was injected into two dorsal or two ventral blastomeres at the 4-cell stage.

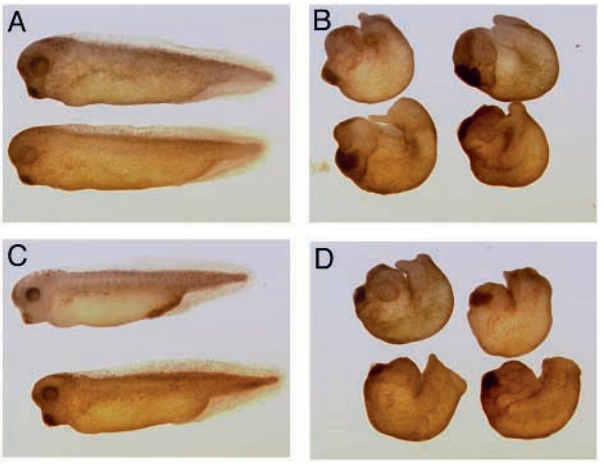

Fig. 10. Rescue of *Brat-En^R* phenotypes by *Brat* but not by *Xbra*. (A) Wild-type stage 34 embryo. (B) Embryos injected with 250 pg *Brat-En^R* into the lateral marginal zone. At this dose, the embryos have kinked backs and forked tails, and sometimes incomplete closure of the neural plate. (C) A 2:1 ratio of wild-type *Brat: Brat-En^R* rescues the embryonic defects caused by *Brat-En^R*. (D) A 2:1 ratio of *Xbra: Brat-En^R* does not rescue the *Brat-En^R* defects. In fact, no dose of *Xbra* tested (0.25:1 to 5:1) rescued the *Brat-En^R* phenotype.

caused by *Brat-En^R*. We therefore conclude that endogenous *Brat* is essential for the induction and patterning of the entire mesodermal germ layer.

How *Brat* functions together with other T-box genes in mesodermal patterning is important to establish, and some of our experiments point to functional interactions between *Brat* and *Brachyury*. For instance, we have shown that *Brat* induces the *Xbra* gene in animal caps, and in the embryo dominant-negative *Brat-En^R* blocks *Xbra* expression. Therefore *Brat* might directly activate transcription of the *Xbra* gene, but we have not established whether *Brat* functions in this manner or acts in some indirect way, such as by up-regulating FGF signaling (which maintains *Xbra* expression in embryos; Kroll and Amaya, 1996; Schulte-Merker and Smith, 1995). Other evidence indicates that *Xbra* and *Brat* perform some similar functions in the *Xenopus* embryo; however, it is unlikely that their roles are simply redundant. In animal cap assays, both genes can induce ventral and lateral mesoderm, but only *Brat* can induce organizer markers. Our loss-of-function experiments with dominant-negative *Brat*, and similar experiments with *Xbra* (Conlon et al., 1996), demonstrate that both proteins are required for ventral-posterior mesoderm formation and body patterning, but only *Brat* is essential for the formation of the organizer and head. Another important finding that illustrates a lack of functional redundancy between *Brat* and *Xbra* is that *Xbra* does not rescue embryonic phenotypes caused by dominant-negative *Brat*: Further work is required to fully understand the relationship between *Brat* and other T-box genes in mesodermal patterning.

Lastly, it is worth considering whether maternal Brat protein might control transcriptional responses to inductive signaling. That is, Brat protein sequestered in blastula cells during cleavage might act as a nuclear mediator of signals from growth factor receptors and activate transcription of mesoderm- or endoderm-specific genes. There is clear evidence that maternal transcription factors facilitate mesoderm-specific gene activation by growth factors. A maternal factor named FAST binds a *Mix.2* promoter element in response to activin signals (Chen et al., 1996) and the *goosecoid* gene is activated by unidentified maternal transcription factors that recognize an activin-responsive element (Watabe et al., 1995). Whether maternal Brat protein is a target of signal transduction pathways triggered by mesoderm or endoderm-inducing factors awaits the identification of Brat-binding sites in growth factor-inducible genes.

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Note: At the time our manuscript was submitted three reports on genes very similar, if not identical, to Brat were reported (Lustig et al., 1996; Stennard et al., 1996; Zhang and King, 1996).

REFERENCES

Badiani, P., Corbella, P., Kioussis, D., Marvel, J. and Weston, K. (1994). Dominant-interfering alles define arole for c-Myb in T-cell development. *Genes Dev.* **8,** 770-782.

Campbell, C., Goodrich, K., Casey, G. and Beatty, B. (1995). Cloning and mapping of a human gene (TBX2) sharing a highly conserved protein motif with the Drosophila omb gene. *Genomics* **28,** 255-260.

Chen, X., Rubock, M. J. and Whitman, M. (1996). A transcriptional partner for MAD proteins in TGFβ signaling. *Nature* **383,** 691-696.

Cho, K. W. Y., 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.

Christian, J. L., McMahon, J. A., McMahon, A. P. and Moon, R. T. (1991). *Xwnt-8*, a *Xenopus Wnt-1*/*int-1*-related gene responsive to mesoderm inducing factors may play a role in ventral mesodermal patterning during embryogenesis. *Development* **111,** 1045-1056.

Conlon, F. L., Sedgwick, S. G., Weston, K. M. and Smith, J. C. (1996). Inhibition of Xbra transcription activation causes defects in mesodermal patterning and reveals autoregulation of Xbra in dorsal mesoderm. *Development* **122,** 2427-2435.

Cunliffe, V. and Smith, J. C. (1992). Ectopic mesoderm formation in *Xenopus* embryos caused by widespread expression of a Brachyury homologue. *Nature* **358,** 427-430.

Dale, L. and Slack, J. M. W. (1987). Fate map of the 32 cell stage of *Xenopus laevis*. *Development* **99,** 527-551.

Dirkson, M. L. and Jamrich, M. (1992). A novel, activin-inducible, blastopore lip-specific gene of Xenopus laevis contains a fork head DNA-binding domain. *Genes Dev.* **6,** 599-608.

Forristall, C., Pondel, M., Chen, L. and King, M. L. (1995). Patterns of localization and cytoskeletal association of two vegetally localized RNAs Vg1 and Xcat2. *Development* **121,** 201-208.

Frank, D. and Harland, R. M. (1991). Transient expression of XMyoD in non-somitic mesoderm of *Xenopus* gastrulae. *Development* **113,** 1387-1394.

Gelbart, W. M. (1989). The *decapentaplegic* gene: a TGF-β homologue controlling pattern formation in *Drosophila*. *Development* **Supplement,** 65-74.

Gilbert, S. F. (1994). *Developmental Biology*. Sunderland, M.A.: Sinauer.

Green, J. B. A., New, H. V. and Smith, J. C. (1992). Responses of embryonic Xenopus cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* **71,** 731-739.

Han, K. and Manley, J. L. (1993). Functional domains of the Drosophila engrailed protein. *EMBO J.* **12,** 2723-2733.

Harland, R. M. (1991). *In situ* hybridization: an improved whole mount method for *Xenopus* embryos. *Methods in Cell Biology* **36,** 675-685.

Hemmati-Brivanlou, A. and Harland, R. M. (1989). Expression of an

engrailed-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* **106,** 611-617.

Henry, G. L., Brivanlou, I. H., Kessler, D. S., Hemmati-Brivanlou, A. and Melton, D. A. (1996). TGF-beta signals and a prepattern in *Xenopus laevis* endodermal development. *Development* **122,** 1007-1015.

Herrmann, B. G., ed. (1995a). The Brachyury gene. *Semin. Dev. Biol.* **6,** 381-435.

Herrmann, B. G. (1995b). The mouse Brachyury (T) gene. *Semin. Dev. Biol.* **6,** 385-394.

Herrmann, B. G., Labeit, S., Poustka, A., King, T. R. and Lehrach, H. (1990). Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343,** 617-622.

Holtfreter, J. (1938). Differenzierungspotenzen isolierter Teile der Anurengastrula. *Roux' Arch. EntwMech. Org.* **138,** 657-738.

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

Kessler, D. and Melton, D. (1994). Vertebrate embryonic induction: Mesodermal and neural patterning. *Science* **266,** 596-604.

Kintner, C. R. and Brockes, J. P. (1984). Monoclonal antibodies identify blastemal cells derived from dedifferentiating muscle in newt limb regeneration. *Nature, Lond.* **308,** 67-69.

Kispert, A. (1995). The Brachyury protein: a T-domain transcription factor. *Semin. Dev. Biol.* **6,** 395-404.

Kloc, M. and Etkin, L. (1995). Two distinct pathways for the localization of RNAs at the vegetal cortex in *Xenopus* oocytes. *Development* **121,** 287-297.

Kroll, K. L. and Amaya, E. (1996). Transgenic *Xenopus* embryos from sperm nuclear transplantation reveal FGF signaling requirements during gastrulation. *Development* **122,** 3173-3183.

Ku, M. and Melton, D. (1993). *Xwnt 11*: a maternally expressed *Xenopus* wnt gene. *Development* **119,** 1161-1173.

Lemaire, P. and Gurdon, J. B. (1994). A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the *goosecoid* and *Xwnt-8* genes along the dorsoventral axis of early *Xenopus* embryos. *Development* **120,** 1191-1199.

Lustig, K. D., Kroll, K. L., Sun, E. E. and Kirschner, M. W. (1996). Expression cloning of a *Xenopus* T-related gene (*Xombi*) involved in mesodermal patterning and blastopore lip formation. *Development* **122,** 4001-4012.

Melton, D. A. (1987a). Translation of messenger RNA in injected frog oocytes. *Methods in Enzymology* (S. Berger and A. Kimmel). 288-296.

Melton, D. A. (1987b). Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. *Nature* **328,** 80-82.

Moskowitz, I. P., Gendreau, S. B. and Rothman, J. H. (1994). Combinatorial specification of blastomere identity by glp-1-dependent cellular interactions in the nematode Caenorhabditis elegans. *Development* **120,** 3325-3338.

Nieuwkoop, P. D. (1969). The formation of mesoderm in urodelean amphibians. I. Induction by the endoderm. *Wilhelm Roux Arch. EntwMech. Org.* **162,** 341-373.

Rao, Y. (1994). Conversion of a mesodermalizing molecule, the Xenopus Brachyury gene, into a neuralizing factor. *Genes Dev.* **8,** 939-947.

Ruiz i Altaba, A. and Melton, D. A. (1989). Bimodal and graded expression of the *Xenopus* homeobox gene *Xhox3* during embryonic development. *Development* **106,** 173-183.

Rupp, R. A., Snider, L. and Weintraub, H. (1994). Xenopus embryos regulate the nuclear localization of XMyoD. *Genes Dev.* **8,** 1311-1323.

Ryan, K., Garrett, N., Mitchel, A. and Gurdon, J. B. (1996). Eomesodermin, a key early gene in Xenopus mesoderm differentiation. *Cell* **87,** 989-1000.

Sasai, B., Lu, H., Steinbeisser, H., Geissert, D., Gont, L. K. and DeRobertis, E. M. (1994). Xenopus chordin, a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* **79,** 779-790.

Schulte-Merker, S. (1995). The zebrafish no tail gene. *Sem. Dev. Biol.* **6,** 411-416.

Schulte-Merker, S., Ho, R. K., Herrmann, B. G. and Nusslein-Volhard, C. (1992). The protein product of the zebrafish homologue of the mouse T gene is expressed in nuclei of the germ ring and the notochord of the early embryo. *Development* **116,** 1021-1032.

Schulte-Merker, S. and Smith, J. C. (1995). Mesoderm formation in response to Brachyury requires FGF signalling. *Current Biology* **5,** 62-67.

Shi, Y. and Par Hayes, W. (1994). Thyroid hormone dependent regulation of the intestinal fatty acid binding protein gene during amphibian metamorphosis. *Dev. Biol.* **161,** 48-58.

Slack, J. M. W. (1991a). *From Egg to Embryo: Regional Specification in Early Development*. Cambridge: Cambridge University Press.

Slack, J. M. W. (1991b). The nature of the mesoderm-inducing signal in *Xenopus*: a transfilter induction study. *Development* **113,** 661-669.

Slack, J. M. W. (1993). Embryonic induction. *Mech. Develop.* **41,** 91-107.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a *Xenopus* Homolog of *Brachyury (T)* is an immediate-early response to mesoderm induction. *Cell* **67,** 79-87.

Stennard, F., Carnac, G. and Gurdon, J. B. (1996). A Xenopus T-box gene, antipodean, encodes a vegetally-localized maternal mRNA that can trigger mesoderm formation. *Development* **122,** 4179-4188.

Struhl, G. (1989). Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. *Nature* **338,** 741-744.

Thomsen, G. H. (1996). *Xenopus Mothers Against Dpp* is an embryonic ventralizing agent that acts downstream of the BMP-2/4 receptor. *Development* **122,** 2359-2366.

Thomsen, G. H. and Melton, D. A. (1993). Processed Vg1 protein is an axial mesoderm inducer in Xenopus. *Cell* **74,** 433-441.

Vize, P. D., Hemmati-Brivanlou, A., Harland, R. and Melton, D. A. (1991). Assays for gene function in developing *Xenopus* embryos. *Methods in Cell Biology* **36**, 367-387.

Watabe, T., Kim, S., Candia, A., Rothbacher, U., Hashimoto, C., Inoue, K. and Cho, K. W. (1995). Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between Xenopus and mouse. *Genes Dev.* **9,** 3038-3050.

Whitaker, J. R. (1977). Segregation during cleavage of a factor determining endodermal alkaline phosphatase development in ascidian embryos. *J. Exp. Zool.* **202,** 139-153.

Wilkinson, D. G., Bhatt, S. and Herrmann, B. G. (1990). Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature* **343,** 657-659.

Wilson, P. A. and Melton, D. A. (1994). Mesodermal patterning by an inducer gradient depends on secondary cell-cell communication. *Curr. Biol.* **4,** 676-686.

Wright, C. V. E., Morita, E. A., Wilkin, D. J. and DeRobertis, E. M. (1990). The Xenopus XlHbox 6 homeo protein, a marker of posterior neural induction, is expressed in proliferating neurons. *Development* **109,** 225-234.

Wright, C. V. E., Schnegelsberg, P. and DeRobertis, E. M. (1988). Xlhbox8: a novel Xenopus homeoprotein restricted to a narrow band of endoderm. *Development* **105,** 787-794.

Yisraeli, J., Sokol, S. and Melton, D. A. (1990). A two step model for the localization of maternal mRNA in Xenopus oocytes: Involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 RNA. *Development* **108,** 289-298.

Zhang, J. and King, M. L. (1996). *Xenopus* VegT RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesodermal patterning. *Development* **122,** 4119-4129.

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