Cell, Vol. 86, 391–399, August 9, 1996, Copyright ©1996 by Cell Press

XTcf-3 Transcription Factor Mediates β-Catenin-Induced Axis Formation in Xenopus Embryos

Miranda Molenaar,†‡ Marc van de Wetering,*‡ Mariette Oosterwegel,*§ Josi Peterson-Maduro,† Susan Godsave,† Vladimir Korinek,* Jeroen Roose,* Olivier Destrée,† and Hans Clevers*
*Department of Immunology
University Hospital
P. O. Box 85500
3508 GA, Utrecht
The Netherlands
†Hubrecht Laboratory
Netherlands Institute for Developmental Biology
Uppsalalaan 8
3584 CT, Utrecht
The Netherlands

Summary

XTcf-3 is a maternally expressed Xenopus homolog of the mammalian HMG box factors Tcf-1 and Lef-1. The N-terminus of XTcf-3 binds to β-catenin. Microinjection of *XTcf-3* mRNA in embryos results in nuclear translocation of β-catenin. The β-catenin–XTcf-3 complex activates transcription in a transient reporter gene assay, while XTcf-3 by itself is silent. N-terminal deletion of XTcf-3 (ΔN) abrogates the interaction with β-catenin, as well as the consequent transcription activation. This dominant-negative ΔN mutant suppresses the induction of axis duplication by microinjected β-catenin. It also suppresses endogenous axis specification upon injection into the dorsal blastomeres of a 4-cell-stage embryo. We propose that signaling by β-catenin involves complex formation with XTcf-3, followed by nuclear translocation and activation of specific XTcf-3 target genes.

Introduction

Tcf-1 and *Lef-1* define a small subfamily of vertebrate high mobility group (HMG) box transcription factor genes (van de Wetering et al., 1991, 1996; Oosterwegel et al., 1991; Castrop et al., 1992a; Travis et al., 1991; Waterman et al., 1991). This family also includes the less well characterized, embryonally expressed genes *Tcf-3* and *Tcf-4* (Castrop et al., 1992b; V. K. and H. C., unpublished data). Tcf-1 and Lef-1 were originally defined as lymphoid-specific transcription factors, but were later found to be expressed in a largely overlapping, complex pattern during murine embryogenesis (Oosterwegel et al., 1993). *Tcf-1* and *Lef-1* perform differential functions, as evidenced in gene disruption experiments. *Tcf-1*$^{-/-}$ mice have a severe defect in T lymphopoiesis, but are otherwise normal (Verbeek et al., 1995). *Lef-1*$^{-/-}$ mice lack hair follicles and other skin appendages, teeth, and the trigeminal nucleus and die perinatally (van Genderen et al., 1994). Lef-1 can play an architectural role in the activity of the T cell receptor α enhancer (Giese and Grosschedl, 1993; Carlsson et al., 1993). Lef-1 affects spatial enhancer structure, enabling contacts between factors that are bound elsewhere in the enhancer. Tcf-1 and Lef-1 do not behave as "classical" transcription factors, in that they do not activate transcription from reporter gene constructs that contain multimerized Tcf/Lef-binding sites (van de Wetering et al., 1993).

β-Catenin was originally identified as a 92–94 kDa protein associated with the cytoplasmic tail of cadherin cell adhesion molecules (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989). β-Catenin is the vertebrate homolog of the Drosophila segment polarity gene product, Armadillo (McCrea et al., 1991; Peifer et al., 1992). The central portion of these two proteins is made up of 13 Armadillo repeats, putative protein–protein interaction domains found in a range of proteins of diverse functions (Peifer et al., 1994).

The biological activities of β-catenin are not yet fully understood. In addition to a role in adhesion, β-catenin is also involved in a signaling pathway during Xenopus development. Antibody-mediated perturbation (McCrea et al., 1993) or injection of β-catenin mRNA (Funayama et al., 1995) induces axis duplication, while depletion of β-catenin inhibits dorsal mesoderm induction, thus abrogating axis specification (Heasman et al., 1994). These signaling properties of β-catenin in the vertebrate embryo are not unexpected, given the firmly established participation of *armadillo* in the *wingless* cascade in Drosophila (Peifer et al., 1991; Noordermeer et al., 1994).

Thus, the manipulation of β-catenin levels in the Xenopus embryo affects axis specification. This likely occurs as a result of the perturbation of a signaling cascade initiated by products of *Wnt* genes, vertebrate homologs of *wingless*. Wnt factors can induce axis duplication in Xenopus (McMahon and Moon, 1989). Vertebrate homologs of other components of the Wingless cascade are also implied in the Wnt dorsal induction pathway in the early Xenopus embryo. This has specifically been demonstrated for glycogen-synthase-kinase-3 (GSK3), the vertebrate homolog of Zeste-white-3 kinase (He et al., 1995), and for vertebrate Dishevelled (Sokol et al., 1995). Thus, a Wingless/Wnt cascade appears to be conserved between Drosophila and vertebrates. It is currently unknown, however, how signaling through β-catenin or Armadillo would affect the execution of downstream genetic programs.

Here we describe the in vitro and in vivo interactions between β-catenin and a Xenopus member of the Tcf/Lef family of transcription factors, XTcf-3. Based on our data, we propose that the β-catenin–XTcf-3 complex is responsible for activation of target genes in response to upstream (e.g., Wnt) signals that allow cytoplasmic β-catenin to interact with XTcf-3.

Results

Identification of Four *Tcf/Lef* Homologs in Xenopus

Screening of a library of stage-17 Xenopus embryos with a *Tcf-1/Lef-1* probe yielded 12 cDNA clones, which

‡These authors contributed equally to this study.
§Present address: Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

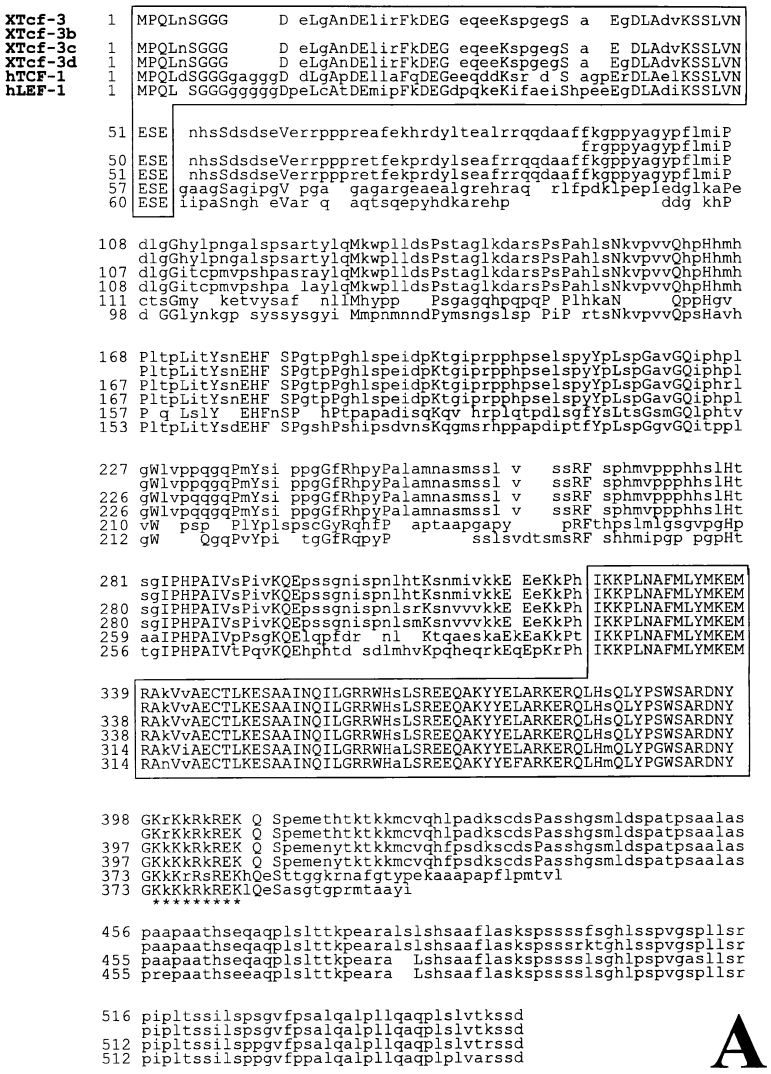

Figure 1. Four Novel *Tcf/Lef*-like Genes Expressed in Xenopus Embryos

(A) Four highly homologous gene products (XTcf-3, XTcf-3b, XTcf-3c, and XTcf-3d) were identified in a stage-17 embryonic cDNA library. These are compared with human Tcf-1 and Lef-1. The HMG domains are boxed, as is the conserved N-terminal region. The sequences diverge in the region C-terminal to the HMG box. A putative nuclear localization signal (NLS) overlaps with the C-terminus of the HMG box (asterisks; Prieve et al., 1996). (B) XTcf-3 and β-catenin constructs used in this study.

defined four virtually identical genes (Figure 1A). These genes belonged to the *Tcf/Lef* family, but diverged from *Tcf-1* and *Lef-1* in the region C-terminal to the HMG box. Based on high similarity to mammalian *Tcf-3* (V. K. and H. C., unpublished data), the Xenopus genes were termed *XTcf-3*, *XTcf-3b*, *XTcf-3c*, and *XTcf-3d*. Independently, 19 cDNA clones were obtained from 2-cell-stage embryos by reverse transcription–polymerase chain re-

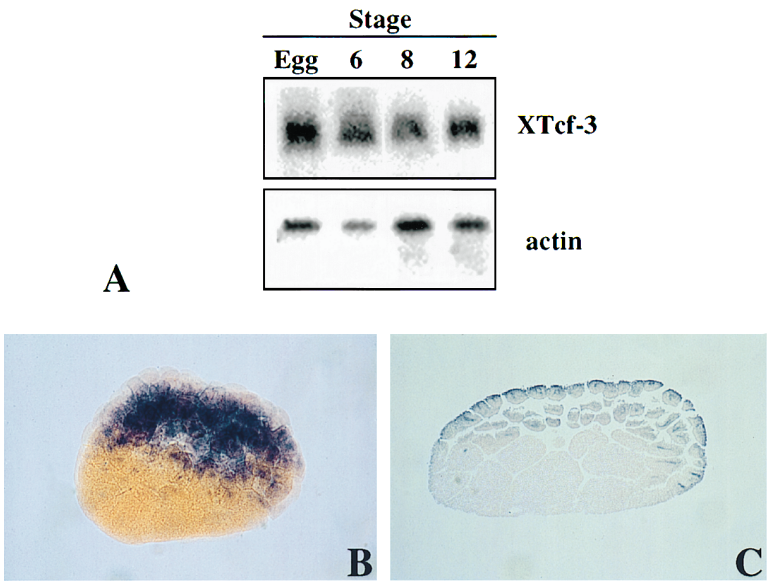

Figure 2. Expression Pattern of *XTcf-3* in Early Embryonic Stages

(A) Northern blot analysis of RNA extracted from unfertilized eggs (lane 1) and embryos of the stages 6, 8, and 12. The blot was probed for the 3.5 kb *XTcf-3* mRNA (top) and subsequently for actin (bottom).

(B and C) In situ hybridization for *XTcf-3* expression in early embryonic stages. (B) shows a stage-7 cleared embryo, viewed laterally. (C) shows a section of a stage-7 embryo.

action (RT–PCR) using degenerate primers for the conserved *Tcf/Lef* HMG box region. All clones were derived from the *XTcf-3* genes, which are identical in this region. This implied that the *XTcf-3* genes represent the predominant maternally expressed *Tcf/Lef* family members in the early Xenopus embryo. *XTcf-3* was arbitrarily chosen for further analysis.

The expression of *XTcf-3* during Xenopus embryogenesis was first documented by Northern blot analysis (Figure 2A). Given their virtual identity, expression of all four *XTcf-3* genes was visualized simultaneously with the *XTcf-3*-derived probe. A prominent band of approximately 3.5 kb was observed in RNA from unfertilized eggs and early cleavage stages, confirming the maternal expression of XTcf-3. The mRNA remained present during later cleavage stages and was also found after the midblastula transition (MBT). By in situ hybridization (Figures 2B and 2C), we observed *XTcf-3* mRNA in the blastocoel roof in stage-7 embryos and in the marginal zone. Much lower levels of expression were observed in vegetal cells of the embryo, which might relate to a general difficulty in visualizing mRNAs in this region by whole-mount in situ hybridization (Smith and Harland, 1992).

XTcf-3 Binds to β-Catenin In Vitro

In a yeast two-hybrid screen for proteins interacting with an N-terminal fragment of human Tcf-1, we retrieved multiple cDNA clones encoding β-catenin. The shortest clone started at amino acid 252, while all clones encoded the complete C-terminus. Based on these findings, we wished to assess whether XTcf-3 also could physically interact with β-catenin. In vitro translated XTcf-3 bound to a gel retardation probe containing the *Tcf/Lef* consensus motif (Figure 3). In vitro translated β-catenin bound to XTcf-3, resulting in a supershift. A deletion clone of β-catenin that contained all 13 Armadillo repeats, but lacked the unique N- and C-terminus, still bound to XTcf-3. A small deletion removing the N-terminal 31 amino acids of XTcf-3 (ΔN) did not interfere with DNA binding, but abrogated the β-catenin interaction. The 60 N-terminal amino acids of Tcf/Lef proteins are conserved and likely constitute the interaction domain (Figure 1A).

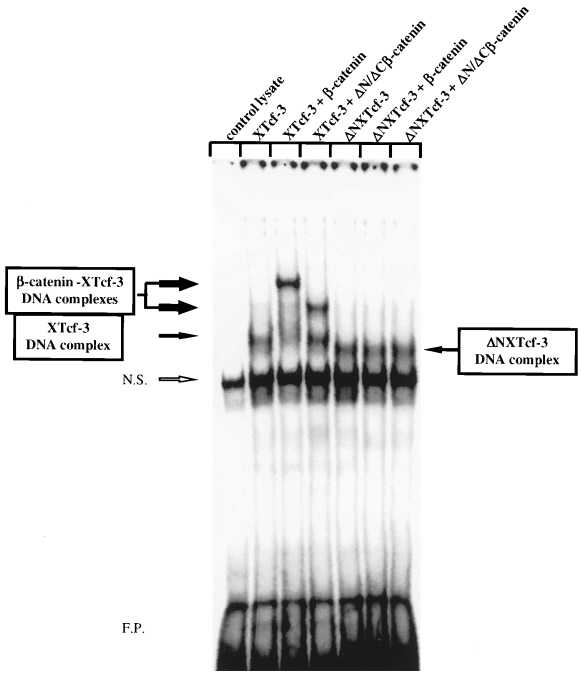

Figure 3. Physical Interaction of XTcf-3 with β-Catenin

In vitro translated XTcf-3 was subjected to gel retardation with an optimal Tcf/Lef probe (see Experimental Procedures). A nonspecific band (N.S.) resulted from an endogenous protein in the transcription/translation lysate (lane 1). F.P., free probe. Cotranslated full-length β-catenin and the deletion mutant ΔN/C β-catenin yielded supershifted bands (lanes 2–4). Deletion of the first 31 amino acids of XTcf-3 (ΔN) abrogated the interaction with β-catenin (lanes 5–7).

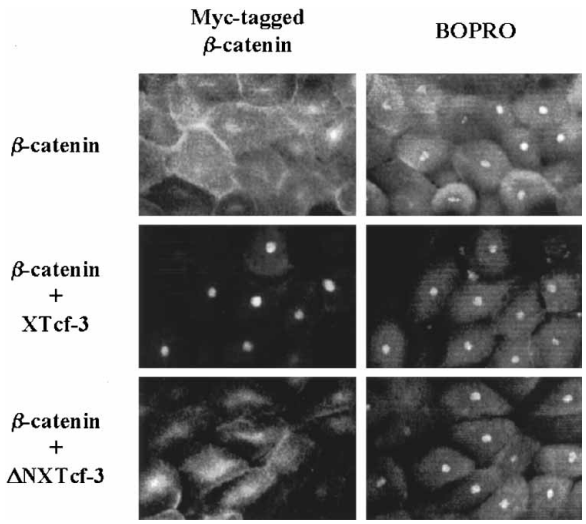

Figure 4. Nuclear Translocation of Injected β-Catenin by Coinjected *XTcf-3*

Stage-7 pre-MBT Xenopus embryos, analyzed by whole-mount immunohistochemistry. Three optical sections (Z-step, 5 μm) were projected onto each other for each picture. At left are the following: Myc-tagged β-catenin mRNA was injected alone (500 pg of RNA) (top) or in combination with 250 pg of RNA encoding wild-type XTcf-3 (middle) or ΔN (bottom). β-Catenin was visualized by staining for the Myc tag. Corresponding BO-PRO-3 nuclear staining is shown to the right.

XTcf-3 Translocates β-Catenin to the Nucleus

The interaction of XTcf-3 with β-catenin in vivo was then analyzed by microinjection of mRNAs encoding epitope-tagged versions of XTcf-3 and β-catenin into Xenopus embryos. Like Tcf-1 (Castrop et al., 1995) and Lef-1 (Travis et al., 1991), XTcf-3 and its deletion mutant ΔN accumulated in the nucleus (data not shown). Overexpressed, epitope-tagged human β-catenin accumulated in the cytoplasm before MBT (Figure 4), but was also occasionally seen in or near nuclei, as reported previously (Funayama et al., 1995). Coinjection with XTcf-3 resulted in translocation of the tagged β-catenin to the nucleus in pre-MBT blastomeres. As expected, coinjection with ΔN (incapable of interacting with β-catenin) did not result in nuclear translocation of β-catenin. By contrast, coinjection of β-catenin with an mRNA encoding amino acids 1–87 of XTcf-3 with a synthetic, C-terminal nuclear localization signal (PKKKRKV; N-NLS in Figure 1B) translocated β-catenin to the nucleus (data not shown). The latter experiment mapped the interaction domain to the N-terminus of XTcf-3.

The β-Catenin–XTcf-3 Complex Activates Transcription

In a transient reporter gene assay, we tested whether XTcf-3 was capable of activating transcription. To this end, we performed transfections with a CAT reporter plasmid containing a multimerized cognate motif for Tcf/Lef proteins (pTK(56)$_7$; van de Wetering et al., 1991). A murine B lymphoid cell line was chosen because it lacks endogenous *Tcf/Lef* mRNAs and contains low levels of endogenous β-catenin.

As observed previously for Tcf-1 and Lef-1 (see Introduction), XTcf-3 or ΔN alone did not activate transcription above background levels. However, cotransfection of XTcf-3 with β-catenin potently activated transcription. No effect was seen when ΔN was cotransfected with β-catenin. As expected, a control reporter CAT plasmid carrying mutated versions of the Tcf/Lef-binding motif (pTK(56Sac)$_7$; van de Wetering et al., 1991) always scored negative. A typical experiment is depicted in Figure 5. Staining for XTcf-3 and β-catenin in the transfected lymphocytes recapitulated the observations made in microinjected embryos: in brief, transfected β-catenin was predominantly cytoplasmic, but was translocated to the nucleus upon cotransfection with intact XTcf-3. Again, the ΔN protein itself was nuclear,

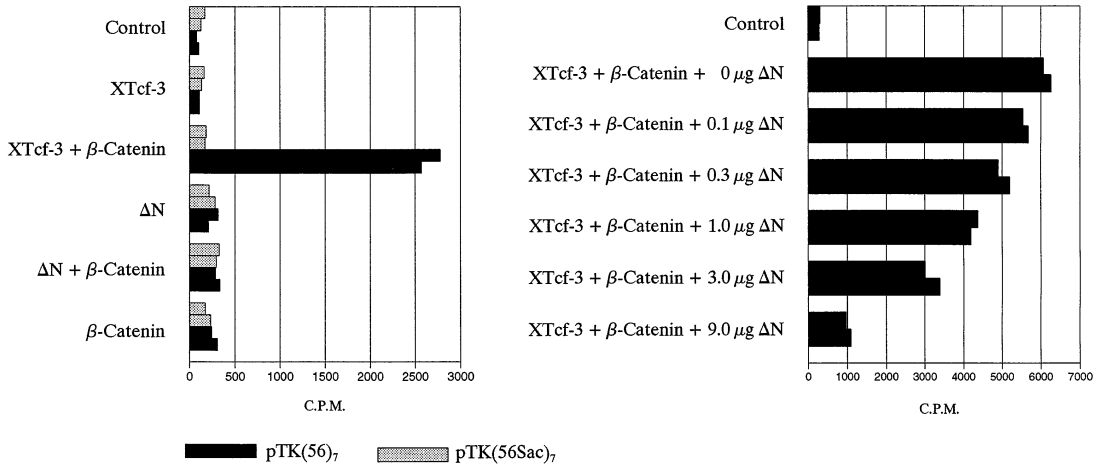

Figure 5. β-Catenin–XTcf-3 Complex Transactivates Transcription

Cells were transfected either with a CAT vector containing a minimal herpesvirus thymidine kinase promoter and an upstream concatamer of the Tcf/Lef cognate motif (pTK(56)$_7$) or with the negative control vector (pTK(56Sac)$_7$). Left, cotransfections were performed with the indicated plasmids: 1 μg of CAT reporter plasmid; 2 μg of XTcf-3 expression vectors; 4 μg of β-catenin expression vector; and empty pCDNA vector as stuffer. Right, cotransfection of 1 μg of XTcf-3 with 4 μg of β-catenin and the indicated amounts of ΔN expression plasmids; pCDNA was used as stuffer. Relative CAT activity is presented in counts per minute. Both values of duplicate transfections are given.

Table 1. ΔN Suppresses β-Catenin-Induced Axis Formation

RNA Injected	Amount (pg)	Second Axis (%)	n
β-Catenin	500	52	50
β-Gal	250		
β-Catenin	500	0	50
ΔN	250		
β-Catenin	500	2	50
ΔN	100		
β-Gal	100		
Β-Catenin	500	25	48
ΔN	100		
XTcf-3	100		
noggin	200	19	98
β-Gal	250		
noggin	200	35	100
ΔN	250		

Embryos were injected at the 4-cell stage in the equatorial region of one ventral blastomere and screened for secondary axis induction at stages 18–22. Percentages include complete and partial secondary axes. n, number of embryos.

but it failed to transport β-catenin to the nucleus (data not shown).

Thus, XTcf-3 and the ΔN protein in isolation accumulated in the nucleus, presumably bound to their cognate DNA motifs, but were transcriptionally inert. By contrast, the β-catenin–XTcf-3 complex potently activated transcription. The ΔN mutant failed to interact with β-catenin and consequently remained inert when cytoplasmic β-catenin was available. Cotransfection of increasing amounts of the ΔN mutant with activating amounts of β-catenin and wild-type XTcf-3 in the CAT assay resulted in a progressive suppression of the induced transcriptional activation (Figure 5). This indicated that the ΔN mutant could act as a dominant-negative mutant in the proposed pathway, providing a tool for probing the function of XTcf-3 in vivo.

The ΔN Mutant of XTcf-3 Suppresses Axis Formation

We analyzed the in vivo function of XTcf-3 in the well-established axis formation assay in Xenopus embryos. Injection of full-length *XTcf-3* RNA in Xenopus embryos did not lead to severe axial perturbations, nor did it facilitate axis duplication upon coinjection of suboptimal amounts of β-catenin RNA (data not shown). This was likely due to the relatively large pools of endogenous XTcf-3, and suggested that the amount of endogenous XTcf-3 is not a limiting factor in axis specification.

As reported previously (Funayama et al., 1995), injection of β-catenin in the vegetal ventral region of early cleavage-stage embryos leads to axis duplication. We found that injection of 500 pg of β-catenin RNA (together with 250 pg of control β-galactosidase [β-gal] RNA) consistently induced axis duplication in >50% of the embryos (Table 1; Figure 6A). Coinjection with comparable amounts of XTcf-3-encoding RNA did not modify this β-catenin effect (data not shown). However, ΔN was found to be a potent suppressor of β-catenin-induced axis duplication (Table 1; Figure 6B). Coinjection of 500 pg of β-catenin RNA with 250 pg of ΔN RNA strongly suppressed axis duplication and in most cases led to

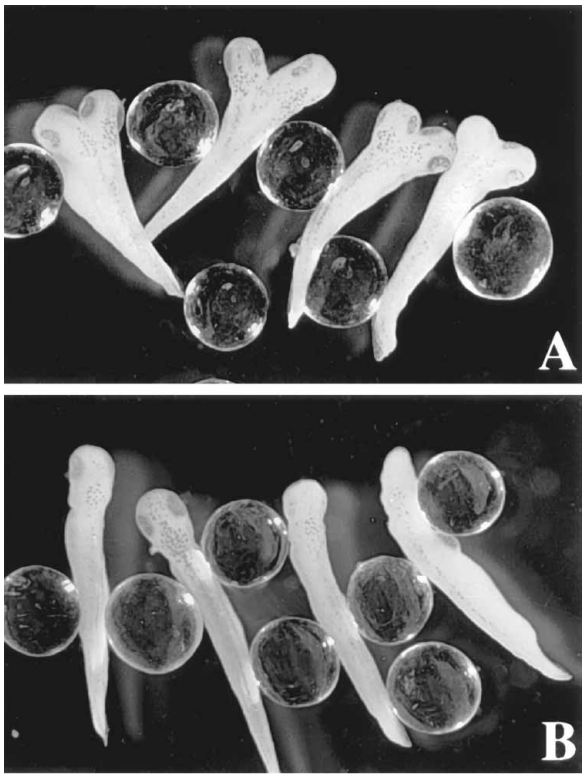

Figure 6. Suppression of β-Catenin-Induced Axis Formation in Xenopus Embryos

Stage-35 embryos injected at the 4-cell stage in a single ventral blastomere with 250 pg of β-catenin RNA alone (A) or in combination with 250 pg of ΔN RNA (B).

normal development of the embryos. The frequency of axis duplication was still reduced upon coinjection with 50 pg of ΔN RNA (data not shown). The ΔN-mediated suppression could partially be relieved by coinjection of wild-type *XTcf-3* (Table 1). Axis duplication induced by injection of *noggin* mRNA, which presumably acts in a parallel pathway, could not be suppressed by ΔN (Table 1). This demonstrated the specificity of ΔN-mediated suppression.

Depletion of β-catenin suppresses endogenous axis formation (Heasman et al., 1994). To test the effects of our dominant-negative XTcf-3 mutant on endogenous axis formation, we injected ΔN RNA in both dorsal blastomeres of 4-cell-stage embryos. This consistently led to failure of axis formation (Table 2; Figure 7). The effect was maximal when injections were performed in the equatorial region, much weaker when injected in the vegetal region, and virtually absent when injected in the animal region (Table 2). ΔN-injected embryos developed normally through the cleavage and blastula stages and completed gastrulation in most cases. Subsequently, the embryos failed to form neural folds and at a sibling stage 27 lacked visible head–tail and dorsal–ventral axes (Figure 7). A closed blastopore was present in these embryos, identifying an anteroposterior axis. In some cases, the presence of a vestigial dorsal fin (Figure 7C) indicated a rudimentary dorsal–ventral axis. Histological sectioning of the embryos revealed no obvious

Table 2. Equatorial Injection of ΔN Suppresses Endogenous Axis Formation

Position	n	DAI	Percent
Animal	86	4/5	90
		1/2/3	8
		0	2
Equatorial	99	4/5	25
		1/2/3	8
		0	67
Vegetal	80	4/5	63
		1/2/3	21
		0	16

Embryos were injected at the 4-cell stage in each dorsal blastomere with 250 pg of ΔN RNA at the position indicated. The embryos were scored at stages 26–30 according to the standard dorsoanterior index scale (DAI; Kao and Elinson, 1988). A normal embryo is assigned DAI5, while embryos lacking dorsoanterior structures are assigned DAI0.

notochord, somites, or neural tube, nor were any other clear structures (e.g., cement gland) present (Figure 7B). Embryos showed a three tissue-layer configuration. A very short archenteron was found just inside the blastopore. In most embryos, including those with a rudimentary fin, the anterior ectoderm was thickened and showed disordered folding. The phenotype resembled that generated by reduction of β-catenin levels reported by Heasman et al. (1994). The expression of the dorsal marker *goosecoid* (Cho et al., 1991) was very low or absent in stage-11 embryos (Figure 7E). Injection of equivalent amounts of β-gal RNA had no effect on axis specification (Figure 7D). Coinjection of β-gal RNA with ΔN RNA and subsequent staining with X-Gal revealed that the injected cells actively participated in early embryonic morphogenetic events, but that proper axis specification did not occur (Figure 7C).

Discussion

The present study supports the notion that the novel Xenopus transcription factor XTcf-3 acts directly downstream of β-catenin in embryonic axis specification. We originally found evidence for the interaction of Tcf/Lef transcription factors with β-catenin in a yeast two-hybrid screen using human Tcf-1 as bait. Since the Xenopus embryo provides the best-characterized model system for vertebrate β-catenin signaling, we studied the in vitro and in vivo interaction of β-catenin with the maternally expressed Xenopus *Tcf/Lef* homolog, *XTcf-3*. The XTcf-3 protein binds to the consensus Tcf/Lef DNA motif, while the N-terminus of XTcf-3 associates with the Armadillo repeat region of β-catenin, as evidenced in a gel retardation assay. Microinjection of *XTcf-3* mRNA reveals that the protein accumulates in the nucleus. β-Catenin, expressed in the absence of XTcf-3, is mostly cytoplasmic. Coexpression of β-catenin with XTcf-3 results in translocation of β-catenin to the nucleus. A small deletion of the N-terminus of XTcf-3 abrogates the in vitro association of β-catenin, as well as the nuclear translocation of β-catenin in injected Xenopus embryos.

XTcf-3 by itself binds DNA in vitro, but is inert in a transient reporter gene assay. By contrast, cotransfection of XTcf-3 with β-catenin potently activates transcription. Again, N-terminal deletion of XTcf-3 abrogates the β-catenin-dependent activation of transcription. Thus, the transcriptional activation by XTcf-3 is dependent on complex formation between XTcf-3 and β-catenin. The ΔN deletion mutant of XTcf-3 represents a dominant-negative mutant in the proposed pathway, in that it suppresses the activation of transcription mediated by the β-catenin–wild-type XTcf-3 complex.

The ΔN mutant provides a tool for probing the in vivo function of XTcf-3. ΔN suppresses the formation of an

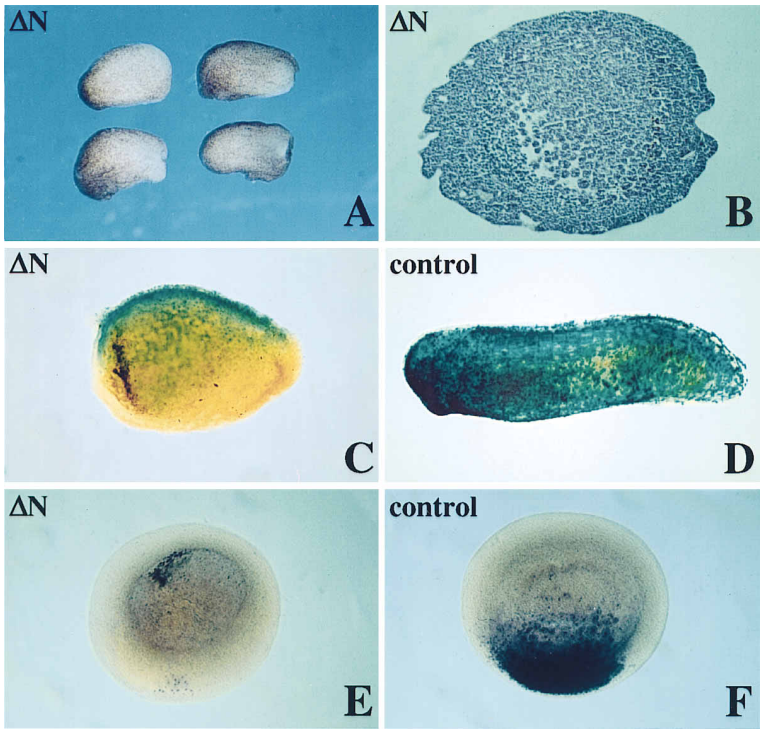

Figure 7. Suppression of Endogenous Axis Formation

(A–D) Embryos allowed to develop until sibling stage 27. Anterior is to the left. (A) shows phenotypes of embryos injected with 250 pg of ΔN RNA in both dorsal blastomeres at the 4-cell stage. (B) shows a horizontal section of an embryo as in (A), with blastopore to the right.

(C and D) Embryos injected with a combination of 250 pg of ΔN RNA and 250 pg of β-gal RNA (C) or β-gal RNA alone (D).

(E and F) In situ hybridization with *goosecoid* on embryos injected with 250 pg of ΔN (E) or 250 pg of β-gal (F) analyzed at stage 11. Dorsal view of cleared embryos with the dorsal blastopore lip to the bottom. Some nonspecific staining occurs in the blastocoel cavity in (E).

ectopic axis induced by microinjected β-catenin, indicative of an inhibitory effect downstream in the β-catenin pathway. In addition, injection of the XTcf-3 deletion mutant into the equatorial region of the two dorsal blastomeres of a 4-cell-stage embryo also suppresses endogenous axis specification. The phenotypes of the resulting embryos are very similar to those observed by Heasman et al. (1994), who depleted the endogenous β-catenin pool by an antisense strategy or by overexpression of cadherins.

Based on these data, we propose the following model. Signaling through the *wingless*/*Wnt* pathway results in the generation of cytoplasmic β-catenin in a form that allows the Armadillo repeat region to interact with the N-terminus of Tcf/Lef factors. XTcf-3 is the most likely candidate to mediate β-catenin signaling in the Xenopus embryo, although we cannot formally rule out the involvement of other, yet to be cloned, factors from this family. The nuclear localization signal in XTcf-3 mediates the translocation of the β-catenin–XTcf-3 complex to the nucleus. The HMG box of XTcf-3 binds in a sequence-specific fashion to the regulatory sequences of specific target genes. Other regions in the XTcf-3 protein might contribute to the specificity of target gene selection. In our model, activation of transcription of target genes only occurs when XTcf-3 is complexed to β-catenin. When no signals are transduced through the *wingless*/*Wnt* pathway, XTcf-3 (which is not complexed to β-catenin in that situation) may also occupy the regulatory sites of the target genes, but will fail to activate transcription.

Embryonic axis formation initiates before major activation of the zygotic genome. Likely target genes of XTcf-3 are some of the earliest zygotic genes activated after MBT, which themselves are capable of inducing a secondary axis, e.g., *siamois* (Lemaire et al., 1995) or *goosecoid* (Cho et al., 1991). It has been shown that both Xenopus (Watabe et al., 1995) and zebrafish (Joore et al., 1996) *goosecoid* promoters can be activated in vivo by Wnt-1.

In conclusion, the current data place the products of the *XTcf-3* genes directly downstream from β-catenin and indicate the mechanism by which the axis-specification signal of β-catenin is transduced to specific genes in the nucleus. The existence of multiple Tcf/Lef transcription factors and β-catenin homologs implies that the type of interaction described in this study occurs in multiple developmental processes.

Experimental Procedures

Cloning of *XTcf-3*

A stage-17 Xenopus embryo cDNA library in λGT10 (Kintner and Melton, 1987) was screened at low stringency with murine *Tcf-1* (Oosterwegel et al., 1991) and *Lef-1* (Travis et al., 1991) cDNA probes according to standard procedures; 12 positive clones were subcloned into pBluescript SK and sequenced. RT–PCR was performed on cDNA prepared from 2-cell-stage embryos with the primers ATG AAG/AGAG/AATGC/AGG/A/TGCG/A/T/CAAT/GG and TCC/TCGT/CGCG/ACTG/CG/CG/AG/ACCG/AGGATA based on the highest conserved HMG box sequences of human and mouse Tcf-1 and Lef-1. The approximately 120 bp PCR products were subcloned and sequenced.

Two-Hybrid Screen

A fragment encoding amino acids 4–359 of human Tcf-1 was inserted into the bait vector pMD4 (a gift of Dr. L. van 't Veer). A mouse brain cDNA library in pGADRx was obtained from Dr. van 't Veer. A thymus cDNA library in pGAD10 was purchased from Clontech. pMD4-Tcf and the pertinent cDNA libraries were transformed into the HF7c yeast strain (Clontech). Plasmids were recovered from $His^+/LacZ^+$ clones, tested for the absence of interaction with irrelevant baits, and sequenced. Of 363 recovered cDNA clones, 40 were found to encode β-catenin.

Northern Blotting

RNA isolation and Northern blot hybridizations were performed as described previously (Destrée et al., 1992) with a full-length *XTcf-3* probe or a control actin probe.

In Situ Hybridization

Whole-mount in situ hybridization of albino Xenopus laevis embryos of different stages was performed as described elsewhere using antisense digoxigenin-labeled RNA (Harland, 1991). The *XTcf-3* cDNA in pBluescript SK was linearized with NcoI (base pair 1360 of cDNA sequence), resulting in a probe spanning the terminal 118 amino acids of the unique C-terminus, as well as 3′ untranslated sequences. *goosecoid* cDNA was prepared as described previously (Cho et al., 1991). Methods of egg collection, fertilization, microinjection, and culture of embryos have been described previously (Gao et al., 1994).

Whole-Mount Immunohistochemistry

Embryos were processed as described previously (Beumer et al., 1995; Veenstra et al., 1995). Myc-tagged β-catenin (Huelsken et al., 1994) was detected using a monoclonal antibody (9E10; Santa Cruz Biotechnology). HA-tagged XTcf-3 was detected using the monoclonal 12CA5. As secondary antibody, Cy5-conjugated donkey anti-mouse antibody (Jackson Laboratory) was used. Nuclear staining was visualized using BO-PRO-3 (Molecular Probes). Embryos were cleared and stored in 33% benzyl alcohol, 67% benzyl benzoate. Image analysis was performed on a Bio-Rad confocal laser scanning microscope.

Gel Retardation Analysis

XTcf-3, β-catenin, and their derivatives were individually transcribed and translated from the pT7TS vector using T7 RNA polymerase in the Promega T7 TnT rabbit reticulocyte lysate–coupled transcription/translation system according to the instructions of the manufacturer. Gel retardation analysis was performed as described elsewhere (van de Wetering et al., 1991). In brief, the double-stranded gel retardation probe derived from the T cell receptor α enhancer (Travis et al., 1991) (cccagagcTTCAAAGGgtgccctacttg, binding motif capitalized) was radiolabeled by T4 DNA kinase. Oligonucleotides were from Isogen (Amsterdam). For a typical binding reaction, the pertinent combinations of extracts (1 μl of each) and 1 μg of poly(dI–dC) were preincubated for 5 min at room temperature in 15 μl of 10 mM HEPES, 60 mM KCl, 1 mM EDTA, 1 mM DTT, and 12% glycerol. Probe (1 ng) was added and incubated for 20 min. The nondenaturing 4% polyacrylamide gel was run in 0.25× TBE at room temperature.

CAT Assays

Assay is described in detail elsewhere (van de Wetering et al., 1991). In short, 2×10^6 IIAI.6 B cells were transfected by electroporation with the various combinations of plasmids. CAT vectors were $(pTK(56)_7$ and $pTK(56Sac)_7$ (van de Wetering et al., 1991). cDNAs encoding tagged versions of β-catenin and XTcf-3 were inserted into the mammalian expression vector pCDNA (Invitrogen). After 48 hr, CAT values were determined as pristane/xylene-extractable, radiolabeled, butyrylated chloramphenicol.

Acknowledgments

We thank Dr. H. Bos for critically reading the manuscript, Drs. J. Huelsken and J. Behrens for β-catenin cDNAs, Dr. R. M. Harland for *noggin* cDNA, Dr. E. M. DeRobertis for *goosecoid* cDNA, Dr. D. A. Melton for the cDNA library, and Dr. P. Krieg for the pT7TS vector. H. C. is supported by a PIONIER and PROGRAM grant from NWO–GMW.

Received June 20, 1996; revised July 18, 1996.

References

Beumer, T.L., Veenstra, G.J.C., Hage, W.J., and Destrée, O.H.J. (1995). Whole-mount immunohistochemistry on *Xenopus* embryos using far-red fluorescent dyes. Trends Genet. *11*, 9.

Carlsson, P., Waterman, M.L., and Jones, K.A. (1993). The hLEF/TCF-1α HMG protein contains a context-dependent transcriptional activation domain that induces the TCRα enhancer in T cells. Genes Dev. *7*, 2418–2430.

Castrop, J., Hoevenagel, R., Young, J.R., and Clevers, H.C. (1992a). A common ancestor of the mammalian transcription factors TCF-1 and TCF-1α/LEF-1 expressed in chicken T cells. Eur. J. Immunol. *22*, 1327–1330.

Castrop, J., van Norren, K., and Clevers, H.C. (1992b). A gene family of HMG box factors with homology to TCF-1. Nucl. Acids Res. *20*, 611.

Castrop, J., van Wichen, D., Koomans-Bitter, M., van de Wetering, M., de Weger, R., van Dongen, J., and Clevers, H. (1995). The human Tcf-1 gene encodes a nuclear DNA-binding protein, uniquely expressed in normal and neoplastic T lineage lymphocytes. Blood *86*, 3050–3059.

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

Destrée, O.H.J., Lam, K.T., Peterson-Maduro, L.J., Eizema, K., Diller, L., Gryka, M.A., Frebourg, T., Shibuya, E., and Friend, S.H. (1992). Structure and expression of the *Xenopus* retinoblastoma gene. Dev. Biol. *153*, 141–149.

Funayama, N., Fagotto, F., McCrea, P., and Gumbiner, B.M. (1995). Embryonic axis induction by the armadillo repeat domain of β-catenin: evidence for intracellular signaling. J. Cell Biol. *128*, 959–968.

Gao, Z.M., Kuiken, G.A., Baarends, W.M., Koster, J.G., and Destrée, O.H.J. (1994). Characterization of a functional promoter for the *Xenopus wnt-1* gene *in vivo*. Oncogene *9*, 573–581.

Giese, K., and Grosschedl, R. (1993). LEF-1 contains an activation domain that stimulates transcription only in a specific context of factor-binding sites. EMBO J. *12*, 4667–4676.

Harland, R.M. (1991). *In situ* hybridization: an improved whole mount method for *Xenopus laevis*. Meth. Enzymol. *36*, 685–697.

He, X., Saint-Jeannet, J.P., Woodgett, J.R., Varmus, H.E., Dawid, I.B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. Nature *374*, 617–622.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C.Y., and Wylie, C. (1994). Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. Cell *79*, 791–803.

Huelsken, J., Birchmeier, W., and Behrens, J. (1994). E-cadherin and APC compete for interaction with β-catenin and the cytoskeleton. J. Cell Biol. *127*, 2061–2069.

Joore, J., Fasciana, C., Speksnijder, J.E., Kruijer, W., Destrée, O.H.J., van den Eijnden-van Raaij, A.J.M., de Laat, S.W., and Zivkovic, D. (1996). Regulation of the zebrafish goosecoid promoter by mesoderm inducing factors and XWnt-1. Mech. Dev. *55*, 3–18.

Kao, K., and Elinson, R.P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. Dev. Biol. *127*, 64–77.

Kintner, C.R., and Melton, D.A. (1987). Expression of *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. Development *99*, 311–325.

Lemaire, P., Garrett, N., and Gurdon, J.B. (1995). Expression cloning of *siamois*, a Xenopus homeobox gene expressed in dorsal vegetal cells of blastulae and able to induce a complete secondary axis. Cell *81*, 85–94.

McCrea, P.D., Turck, C.W., and Gumbiner, B. (1991). A homolog of the *armadillo* protein in *Drosophila* (plakoglobin) associated with E-cadherin. Science *254*, 1359–1361.

McCrea, P.D., Brieher, W.M., and Gumbiner, B.M. (1993). Induction of a secondary body axis in *Xenopus* by antibodies to β-catenin. J. Cell Biol. *123*, 477–484.

McMahon, A.P., and Moon, R.T. (1989). Ectopic expression of the proto-oncogene *int-1* in Xenopus embryos leads to duplication of the embryonic axis. Cell *58*, 1075–1084.

Nagafuchi, A., and Takeichi, M. (1989). Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. Cell Regul. *1*, 37–44.

Noordermeer, J., Klingensmith, J., Perrimon, N., and Nusse, R. (1994). *Dishevelled* and *armadillo* act in the *wingless* signaling pathway in *Drosophila*. Nature *367*, 80–83.

Oosterwegel, M., van de Wetering, M., Dooijes, D., Klomp, L., Winoto, A., Georgopoulos, K., Meijlink, F., and Clevers, H. (1991). Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-ϵ and T cell receptor α enhancers. J. Exp. Med. *173*, 1133–1142.

Oosterwegel, M., van de Wetering, M., Timmerman, J., Kruisbeek, A., Destrée, O., Meijlink, F., and Clevers, H. (1993). Differential expression of the HMG box factors TCF-1 and LEF-1. Development *118*, 439–448.

Ozawa, M., Baribault, H., and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule ovumorulin associates with three independent proteins structurally related in different species. EMBO J. *8*, 1711–1717.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, B., and Wieschaus E. (1991). The segment polarity gene *armadillo* interacts with the *wingless* signaling pathway in both embryonic and adult pattern formation. Development *111*, 1029–1043.

Peifer, M., McCrea, P.D., Green, K.J., Wieschaus, E., and Gumbiner, B.M. (1992). The vertebrate adhesive junction proteins β-catenin and plakoglobin and the *Drosophila* segment polarity gene *armadillo* form a multigene family with similar properties J. Cell Biol. *118*, 681–691.

Peifer, M., Berg, S., and Reynolds, A.B. (1994). A repeating amino acid motif shared by proteins with diverse cellular roles. Cell *76*, 789–791.

Prieve, M.G., Guttridge, K.L., Munguia, J.E., and Waterman, M.L. (1996). The nuclear localization signal of lymphoid enhancer factor-1 is recognized by two differentially expressed srp1-nuclear localization sequence proteins. J. Biol. Chem. *271*, 7654–7658.

Smith, W.C., and Harland, R.M. (1992). Expression cloning of Noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos. Cell *70*, 829–840.

Sokol, S.Y., Klingensmith, J., Perrimon, N., and Itoh, K. (1995). Dorsalizing and neuralizing properties of *XDsh*, a maternally expressed homolog of *dishevelled*. Development *121*, 3487–3497.

Travis, A., Amsterdam, A., Belanger, C., and Grosschedl, R. (1991). Lef-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor α enhancer function. Genes Dev. *5*, 880–894.

van de Wetering, M., Oosterwegel, M., Dooijes, D., and Clevers, H. (1991). Identification and cloning of TCF-1, a T cell-specific transcription factor containing a sequence-specific HMG box. EMBO J. *10*, 123–132.

van de Wetering, M., Oosterwegel, M., van Norren, K., and Clevers, H.C. (1993). SOX-4, an SRY-like HMG box protein, is a transcriptional activator in lymphocytes. EMBO J. *12*, 3847–3854.

van de Wetering, M., Castrop, J., Korinek, V., and Clevers, H. (1996). Extensive alternative splicing and dual promoter usage generates TCF-1 protein isoforms with differential transcription control properties. Mol. Cell. Biol. *16*, 745–752.

van Genderen, C., Okamura, R.M., Farinas, I., Quo, R.G., Parslow, T.G., Bruhn, L., and Grosschedl, R. (1994). Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1 deficient mice. Genes Dev. *8*, 2691–2704.

Veenstra, G.J.C., Beumer, T.L., Peterson-Maduro, J., Stegeman, B.I., Karg, H.A., van der Vliet, P.C., and Destrée, O.H.J. (1995). Dynamic

and differential *oct-1* expression during early *Xenopus* embryogenesis: persistence of oct-1 protein following down-regulation of the RNA. Mech. Dev. *50*, 103–117.

Verbeek, S., Izon, D., Hofhuis, F., Robanus-Maandag, E., te Riele, H., van de Wetering, M., Oosterwegel, M., Wilson, A., MacDonald, H.R., and Clevers, H. (1995). An HMG-box-containing T-cell factor required for thymocyte differentiation. Nature *374*, 70–74.

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

Waterman, M.L., Fischer, W.H., and Jones, K.A. (1991). A thymus-specific member of the HMG protein family regulates the human T cell receptor Cα enhancer. Genes Dev. *5*, 656–669.

GenBank Accession Numbers

The accession numbers for the *XTcf-3* clones described in this paper are X99308, X99309, X99310, and X99311.