CHAPTER 1

Maternal VegT and β-Catenin: Patterning the *Xenopus* Blastula

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1.1 Introduction

Loss of the maternal T-box transcription factor VegT has a devastating effect on development. Embryos fail to gastrulate, lack the expression of all early zygotic genes characteristic of the endoderm germ layer and also fail to activate ventral, general and dorsal mesodermal gene expression (Zhang et al. 1998; Kofron et al. 1999; Xanthos et al. 2001, 2002). All activity in the activin receptor/Smad 2 signaling pathway is lost (Lee et al. 2001). Embryos depleted of maternal β-catenin (and therefore deprived of maternal Wnt signaling) also have severe defects. Gastrulation is delayed, embryos develop without heads, dorsal axes and tails and lack neural, dorsal mesodermal and dorsal endodermal gene expression (Heasman et al. 1994; Wylie et al. 1996; Xanthos et al. 2002). Many early zygotic genes have been shown to be targets of these two signaling pathways (see Xanthos et al. 2002 for the expression profiles of zygotic genes in VegT^{-} and β-catenin^{-} embryos). The challenge now is to understand the networks downstream of VegT and β-catenin that are responsible for embryonic patterning. In particular, two aspects of patterning will be considered here: cell fate specification in the animal-vegetal axis, and asymmetrical gene expression in the dorso-ventral axis of the embryo during the late blastula to early gastrula stages.

1.2 Cell Fate Specification in the Animal-Vegetal Axis

One simple hypothesis for the mechanism of VegT regulation of fate specification in the animal-vegetal axis involves dose-dependent thresholds. For the growth factor, activin, different doses applied to animal caps resulted in different cell fates (Green and Smith 1990). Similarly, high doses of VegT may be required to activate endodermal gene expression, while lower doses may be sufficient to activate mesodermal genes (Kimelman and Griffin 1998).

Several studies have shown that VegT mRNA is localized during oogenesis to the vegetal half of the oocyte, the area that, after fertilization, is inherited

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only by cells destined to form endoderm or mesoderm (Lustig et al. 1996; Stennard et al. 1996; Zhang and King 1996; Horb and Thomsen 1997). Although no evidence has been found of a gradient of either mRNA or protein in the vegetal hemisphere (Stennard et al. 1999), some suggestion that there may be gene specification according to dose comes from explant experiments (Zhang et al. 1998). While embryos injected with a high dose of VegT antisense oligo have no endodermal and little mesodermal gene expression, embryos injected with a lower dose of VegT oligo, that causes only a partial depletion of VegT mRNA, show fate transformations. Vegetal cells that should normally form endoderm now follow mesodermal and neural fates.

One test of the hypothesis that endodermal versus mesodermal fate specification depends on the amount of VegT is to add back different doses of *VegT* mRNA into VegT$^-$ embryos and analyze the rescued gene expression. In this experiment the mRNA is injected into VegT$^-$ embryos at the 8-cell stage, pla-

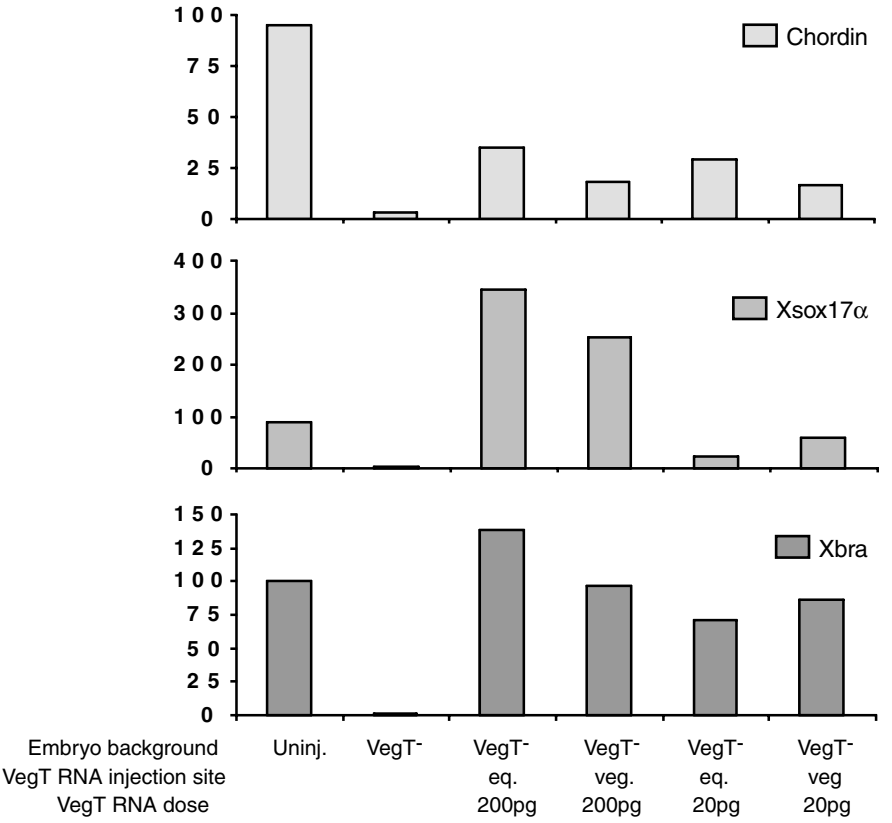

Fig. 1.1. VegT rescue experiments do not show dose-dependent thresholds for mesoderm and endoderm formation. VegT$^-$ embryos were injected with 20 or 200 pg of *VegT* mRNA at the 8-cell stage. RNA was injected into the four vegetal cells either equatorially (eq) or vegetally (veg). Embryos were frozen at the late gastrula stage and examined by real-time PCR for the expression of *chordin*, *Xsox 17* and *Xbra* mRNAs, as described in Xanthos et al. (2002)

cing the RNA into the four vegetal cells either at the vegetal pole or just below the horizontal cleavage plane. Figure 1.1 shows that, contrary to the idea of dose-dependent specification, a low dose (20 pg) of *VegT* mRNA rescues the expression of both endodermal markers (*Xsox17*) and mesodermal markers (*Xbra* and *chordin*). This dose also rescues gastrulation movements and normal development. In contrast, 200 pg of *VegT* mRNA causes a three-fold over-expression of endodermal markers and a slight over-expression of mesodermal genes. This dose is non-physiological, causes abnormal development and is unlikely to enlighten us about the mechanism of gene regulation in the normal embryo. Concentrating on the 20 pg dose, the injection site does influence the amount of *Xsox17* expression but not that of *Xbra* and *chordin* mRNA. Vegetal injection into the vegetal blastomeres produces more endodermal gene expression than equatorial injection into the same cell. This suggests that, for *Xsox17*, other localized factors are likely to act in concert with VegT to determine the extent of its expression. Thus the simple idea of VegT dosage determining cell fate specification is incomplete.

A similar conclusion comes from add-back experiments using VegT target genes of the TGFβ family. The nodal-related proteins Xnrs 1, 2, 4, 5 and 6 have all been shown to be either directly or indirectly regulated by VegT, and all have the ability to rescue the phenotype of VegT-depleted embryos (Kofron et al. 1999; Chang and Hemmati-Brivanlou 2000; Hyde and Old 2000). However, when *Xnr2* mRNA is injected into VegT⁻ embryos, the expression of both endodermal (*Xsox17*) and mesodermal markers (*Xbra*) is rescued at both high

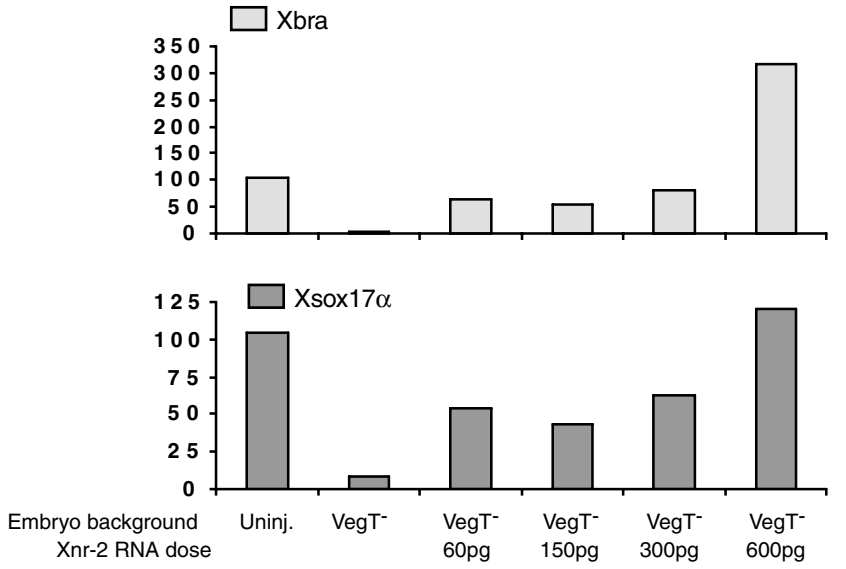

Fig. 1.2. Rescue of VegT⁻ embryos with *Xnr2* mRNA does not show dose-dependent thresholds for mesoderm and endoderm formation. VegT⁻ embryos were injected with different doses of Xnr2 mRNA at the 8-cell stage (vegetal injection). Embryos were frozen at the midgastrula stage and examined by real-time PCR for the expression of *Xbra* and *Xsox 17* mRNAs

and low doses (Fig. 1.2). It seems likely that VegT is not acting in isolation to regulate mesoderm and endodermal fate in the early embryo, but that fate specification depends on VegT along with other regulatory molecules.

1.2.1 Endodermal Transcription Factors Downstream of VegT Have General and Specific Roles in Fate Specification

The transcription factors Xsox17, GATA5 and Mixer are activated by VegT shortly after midblastula transition (MBT), and are expressed in the vegetal mass and excluded from the equatorial zone (Hudson et al. 1997; Henry and Melton 1998; Weber et al. 2000; Xanthos et al. 2001, 2002). One question is to what extent these genes direct separate or similar hierarchies of gene activation in the vegetal mass. In add-back experiments, we found that the genes were not redundant with each other. For example, Mixer rescues the expression of *Xsox 17* mRNA in VegT^{-} embryos, while GATA5 activates *Xlim1* expression and Xsox 17 does neither (Xanthos et al. 2001). A similar conclusion has been reached by genetic epistatis experiments using zebrafish mutants of the *Sox17*, *Mix-like* and *GATA5* genes (Alexander and Stainier 1999; Stainier 2002).

In contrast to their specific roles in endodermal fate specification, Mixer, Xsox17 and GATA 5 share the property of suppressing mesodermal gene expression when ectopically expressed (Hudson et al. 1997; Henry and Melton 1998; Weber et al. 2000). This suggests that these genes may share the common role of preventing mesodermal gene expression in the vegetal mass. What is not clear from these experiments is whether these endodermal transcription factors repress all mesodermal genes or have specific effects. Loss-of-function analysis will be required to clarify this.

1.2.2 The Importance of Inductive Interactions in Mesoderm and Endoderm Specification

The explant experiments of Nieuwkoop demonstrated that inductive signals released by vegetal cells can induce mesoderm formation in adjacent tissue. Recent studies have suggested that Xnrs are the best candidates for these inductive molecules released by vegetal cells. Smad 2 phosphorylation, indicative of signaling through the ALK4/7 receptor, is dependent on VegT activity (Lee et al. 2001). The expression of *Xnrs 1*, *2*, *4*, *5* and *6* is activated by VegT, and they all rescue mesoderm formation when re-introduced into VegT-depleted embryos (Kofron et al. 1999; Takahashi et al. 2000). It is also possible that some mesodermal gene expression occurs cell autonomously in the equatorial zone in cells which inherit *VegT* mRNA. Several laboratories have demonstrated that, once *Xnrs* are expressed, their level of expression may be amplified rapidly by positive feed-back loops (Lemaire et al. 1995; Osada et al. 2000; Rex et al. 2002; White et al. 2002).

In contrast to mesoderm induction, the evidence for endoderm induction is less pursuasive. When VegT$^-$ equatorial explants are co-cultured with wild-type vegetal cells, mesoderm is induced in the equators (Kofron et al. 1999). When VegT$^-$ vegetal explants are co-cultured with wild-type vegetal explants, the expectation would be that endoderm would be induced, since Xnrs rescue endoderm formation in VegT$^-$ embryos. However, only low levels of some early mes-endodermal markers such as *Xlim1* and *Bix4* are induced, and general endodermal markers such as *Xsox17* and *GATA 5* are not rescued at all (Xanthos et al. 2001). Paradoxically, cleavage mutant forms of both TGFβs derriere and Xnr2, cm derriere and cmXnr2 block the expression of endodermal genes in vegetal cells extremely efficiently, confirming that nodal signal/receptor interactions are important in endoderm specification (Xanthos et al. 2001). This apparent contradiction may be resolved either by hypothesizing that, within the vegetal mass, inductive activities happen over very few cell diameters or that they happen in an autocrine fashion. The activity of inducers may be limited by extracellular matrix components, by the presence or absence of co-receptors suuch as EGF/CFC proteins or by the availability of proprotein convertases required to cleave the nodal proproteins. Also, a purely mechanical obstacle, the limited contact area available between VegT$^-$ and wild-type explants, may limit the effectiveness of inductive interactions between vegetal explants in these co-culture experiments.

One clear gap in our knowledge of mesoderm and endoderm specification downstream of VegT is in understanding the sequence of events that leads to the different behaviors and gene expressions apparent in the vegetal mass and equatorial zone by the beginning of gastrulation. It seems entirely possible, given the diversity and complexity of patterns of expression, that many other maternal and early zygotic regulatory factors as well as VegT are involved, both at the transcriptional and post-transciptional level.

1.3 Patterning in the Dorso-Ventral Axis

Many early zygotic genes have been studied in detail because they are expressed asymmetrically across the dorso-ventral axis. Some genes, such as *goosecoid* and *cerberus*, are expressed almost exclusively on the dorsal side (Steinbeisser and de Robertis 1993; Bouwmeester et al. 1996), while others, including the *Xnrs 1*, *2* and *4*, *Xlim 1* and *Xhex*, have a temporal wave of expression, starting on the dorsal side before the ventral side (Jones et al. 1999; Osada et al. 2000; Kodjabachian et al. 2001; Xanthos et al. 2002). The cooperation of two maternal pathways, the VegT and the Wnt/β-catenin pathways, has been shown to be important for these asymmetric gene expression patterns (Steinbeisser et al. 1993; Watabe et al. 1995; Carnac et al. 1996; Crease et al. 1998; Agius et al. 2000; Nishita et al. 2000; Xanthos et al. 2002).

Since β-catenin is recognized to be the essential transactivator of Wnt signals, one means of identifying active areas of Wnt signaling in the early embryo has been to locate sites in the embryo where β-catenin is nuclearly lo-

calized. The first such site occurs in the dorsal area at the early to late blastula stage (Schneider et al. 1996; Larabell et al. 1997; Schohl and Fagotto 2002). This correlates loosely with the sites in which known target genes of Wnt signaling, including *goosecoid*, *siamois* and *Xnr3*, are expressed (Steinbeisser and de Robertis 1993; Lemaire et al. 1995; Smith et al. 1995). These genes are not expressed in maternal β-catenin⁻ embryos (Fig. 1.3). A recent additional site of β-catenin nuclear localization was shown to be in the ventral region at the late blastula stage (Schohl and Fagotto 2002). The role of this site of Wnt activity is unknown.

From many people's studies, two different modes of interaction of the maternal VegT and Wnt/β-catenin pathway can be recognized. Genes such as *goosecoid*, *siamois* and *Xnr3* are only expressed on the dorsal side of the embryo through the blastula and gastrula stages (Steinbeisser and de Robertis 1993; Lemaire et al. 1995; Smith et al. 1995; Xanthos et al. 2002). The second pattern, shown in Fig. 1.4, consists of a dorso-ventral temporal wave. An illus-

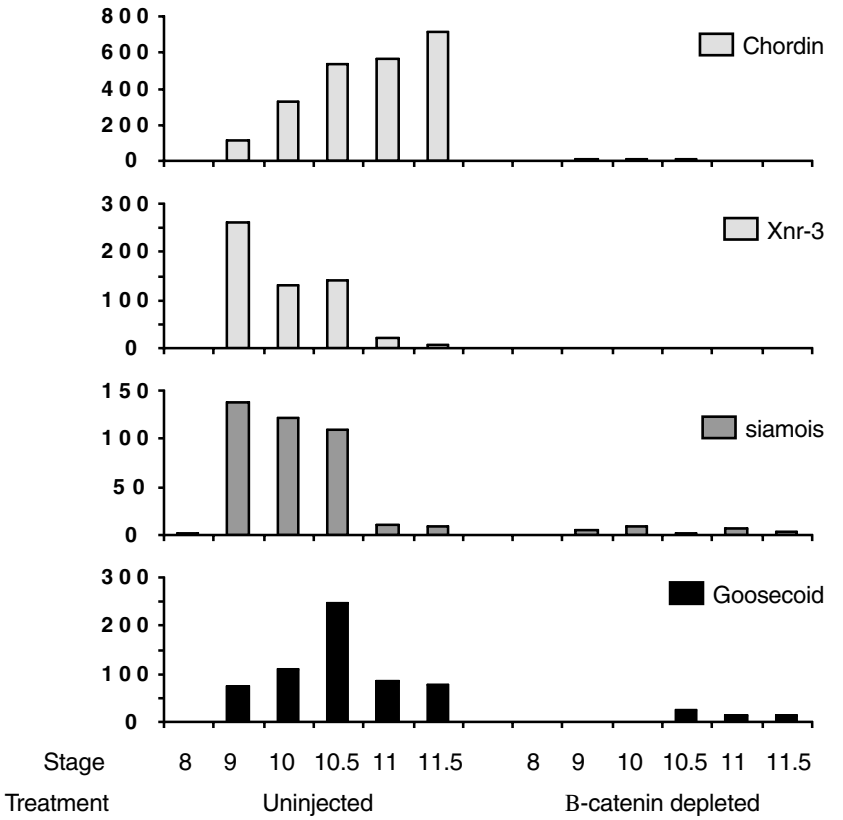

Fig. 1.3. Dorsal genes, *chordin*, *Xnr3*, *siamois* and *goosecoid*, are not expressed in β-catenin-depleted embryos. Wild-type and β-catenin-depleted embryos were frozen at 2-h intervals through the blastula and gastrula stages

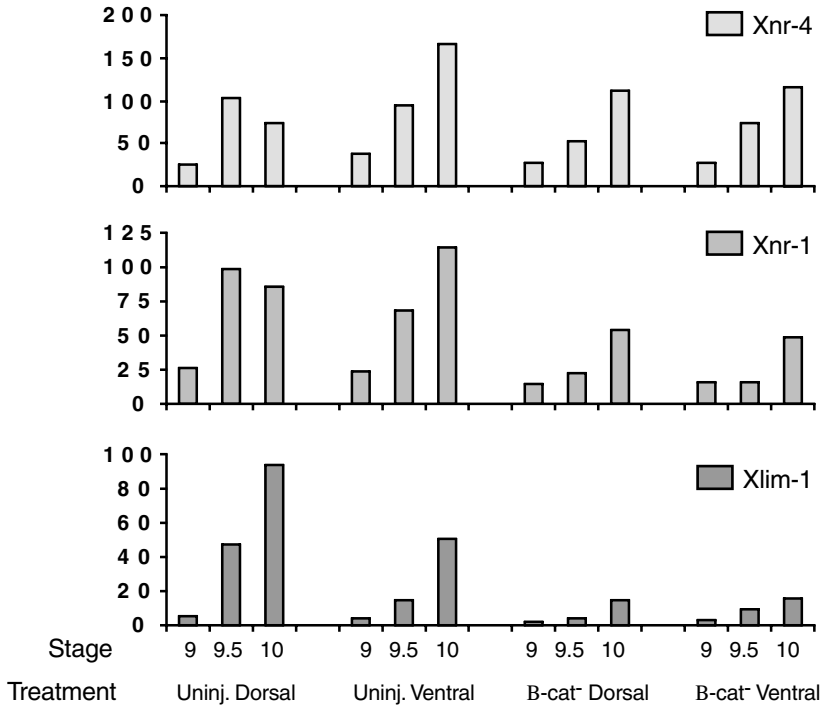

Fig. 1.4. *Xnr1*, *Xnr4* and *Xlim1* are regulated in their dorsal and ventral expression by β-catenin. Wild-type and β-catenin-depleted embryos were bisected into dorsal and ventral halves at the late blastula and early gastrula stages. Groups of four-half embryos were analyzed by real-time PCR for the expression of *Xnr1*, *Xnr4* and *Xlim1* mRNAs

tration of the two patterns can be made by comparing *goosecoid* and *Xnr1* expression in wild-type and β-catenin⁻ embryos. In wild-type embryos at the late blastula to early gastrula stage, *goosecoid* mRNA is expressed exclusively on the dorsal side, while *Xnr1* occurs in a dorso-ventral temporal wave, eventually being more highly expressed ventrally than dorsally. In β-catenin-depleted embryos, *goosecoid* is not expressed, while *Xnr1* is reduced but still activated. However, in β-catenin-depleted embryos, the *Xnr1* asymmetric temporal wave of expression is lost, and *Xnr1* mRNA is now expressed symmetrically across the embryo (Fig. 1.4). Both genes are off in VegT-depleted embryos. How can these patterns be explained in terms of gene regulation?

One scenario is suggested by studies with XTcf3-depleted embryos (Houston et al. 2002). β-Catenin binds to this maternal HMG box protein (Behrens et al. 1996; Molenaar et al. 1996). XTcf3 acts as a repressor of transcription of *goosecoid* (Houston et al. 2002). Thus, in XTcf3-depleted embryos, *goosecoid* is ectopically expressed in ventral cells. In wild-type embryos, nuclear β-catenin acts to relieve the repression of XTcf3 on *goosecoid* expression. VegT, or one of its target genes, cooperates to activate *goosecoid* expression concomitant with β-catenin de-repression of XTcf3-regulated inhibition.