Mutual antagonism of SoxB1 and canonical Wnt signaling in sea urchin embryos

Review

by

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Abstract

Early development of animal embryos involves establishing axial polarities that specify the anlage of major tissues in a 3-dimensional pattern. Cell fates are specified on this coordinate system through a combination of differential inheritance of maternal regulatory molecules and signaling interactions among cells. Correct patterning of cell fates along the primary axis of the sea urchin embryo depends on tightly regulating the ratio of activities of two nuclear regulatory proteins, SoxB1 and nuclear β–catenin. The latter acts at the top of the gene regulatory network that specifies mesoderm and endoderm and activates, directly or indirectly, signaling by Delta, Wnt8 and Nodal1. In contrast, SoxB1 initially accumulates in all nuclei but is progressively eliminated from presumptive mesoderm and endoderm by β-catenin-dependent transcriptional repression and by localized protein turnover, a novel pathway acting downstream of canonical Wnt signaling. A precise temporal program for SoxB1 down regulation is crucial for endomesoderm development because SoxB1 interferes with β–catenin's transcriptional regulatory function. The mechanisms we are beginning to understand that govern the β–catenin-SoxB1 antagonism in sea urchin embryos are likely to have broad significance, since Sox factors are involved in regulating many developmental processes in many deuterostome embryos.

Introduction

Early development of animal embryos involves establishing polarities that define the primary embryonic axes. Some of these are initially established through prelocalization of maternal molecules. For example, the eggs of many vertebrate and invertebrate embryos have morphologically visible Animal–Vegetal (A–V) axes that reflect underlying asymmetric distributions of both molecules and developmental potential. Classically, the animal pole is defined by the position of polar body extrusion during meiosis. In other cases, polarities are imposed after fertilization by epigenetic mechanisms; e.g., the sperm entry point determines the posterior end of the *Caenorhabditis* embryo [1] and approximately positions the ventral/posterior side of the *Xenopus* embryo [2]. Fates of different regions are then patterned on this axial coordinate system through a combination of differential inheritance of maternal regulatory molecules and signaling interactions among cells.

In the sea urchin embryo, the A-V axis is established during oogenesis, as animal and vegetal halves of eggs have different developmental capacities [3, 4]. Fertilized vegetal halves can develop into normal embryos, but fertilized animal halves become *dauer* blastulae consisting of poorly differentiated ectoderm. Recent studies provide compelling evidence that the unique feature of vegetal developmental potential is the ability of this region of the egg to activate the canonical Wnt pathway, which is diagrammed in Fig. 1. Activation is first evident when β–catenin accumulates in the nuclei of the vegetal-most blastomeres, the micromeres, at the 16-cell stage [5]. As discussed below, the specification of all mesenchyme and endoderm, as well as differentiation of oral and aboral ectoderm, depend upon that event. Therefore, at least

some of the pre-localized maternal regulatory molecules that establish the A-V axis must be proteins required for activating canonical Wnt signaling specifically in the vegetal hemisphere of the egg. Emerging evidence suggests that one such protein is disheveled (Dsh), the protein that couples activation of the Wnt receptor, Frizzled, to stablization of β–catenin. Both exogenously provided [6] and endogenous (Wikramanayake, personal communication) Dsh concentrate in the vegetal cortex of the egg. Thus, if Dsh is the only protein upstream of the β–catenin degradation machinery that is localized, it would be the key maternal vegetal determinant along the A-V axis.

Canonical Wnt signals are the earliest known communication between cells in the sea urchin embryo and lead to the nuclearization of β–catenin. This activates a regulatory cascade of other signals (Delta [7], nodal and BMP [8], and several others still undefined), as well as a cohort of genes encoding transcriptional regulatory proteins (reviewed in [9]). In this embryo, both new zygotic transcription and cell-cell signaling are activated within the first few cleavage divisions. β–catenin enters the nuclei of micromeres after the fourth cleavage and during the subsequent several cleavages a wave of β–catenin nuclearization spreads upward through the progeny of the macromeres (Fig. 2B; [5]). In the micromeres, this event initiates the gene regulatory program for their differentiation as the skeleton-producing primary mesenchyme and for their sending several signals required for development of endoderm and other secondary mesenchymal cells (Fig. 2A). In the macromeres, β–catenin also is necessary for receiving the micromere signals [5], for activating the many regulatory genes required for endomesoderm specification [9] and for sending additional signals to overlying ectoderm [10]. Therefore, the specification of all the major tissue territories of the embryo,

diagrammed in Fig. 2A, depends on canonical Wnt signaling. Consequently factors that regulate its activity in different blastomere tiers are also of crucial importance for normal development. Here we review the evidence that one of these proteins is the initially ubiquitous transcription factor, SoxB1, which inhibits the transcriptional activating function of β–catenin. Consequently, removal of SoxB1, which is mediated by several β–catenin-dependent processes, is required to allow embryo patterning to begin. Here we review our current understanding of the mechanisms underlying the remarkable antagonism between SoxB1 and nuclear β–catenin.

The spatial patterns of SoxB1 accumulation and β–catenin nuclearization are reciprocal.

SoxB1 was identified in our laboratory as an essential positive regulator of *SpAN* [11, 12], a gene that is transiently expressed between 4th and 8th cleavage stages in all but the vegetal-most blastomeres [12]. Detailed analysis of the *SpAN* promoter established that its activity was limited to non-vegetal cells to which SoxB1 became restricted after 4th cleavage. Between 4th and 8th cleavage, the size of the SoxB1-depleted (and the SpAN-negative) region progressively increases to include prospective secondary mesenchyme and endoderm, as illustrated in Fig. 2B. This dynamic pattern of SoxB1 expression is essentially reciprocal to the striking vegetal-to-animal wave of nuclearization of β–catenin within the vegetal hemisphere between the 4th and 9th cleavages described above (Fig. 2B). This observation raised the possibility that a negative regulatory relationship existed between these two proteins. This idea received support from the observation that embryos lacking nuclear β–catenin as a result of cadherin over-expression contain SoxB1 mRNA and protein at equivalent levels in all cells [13] and, in

accordance with the essential role of canonical Wnt signaling in endomesoderm specification, these embryos consist only of a ball of undifferentiated ectoderm [10]. Conversely, when stable β–catenin is expressed throughout the embryo, SoxB1 disappears in all but the apical ectoderm (Fig. 2A (black), 2C), which consists of special region of ectoderm at the extreme animal pole that is refractory to canonical Wnt-dependent signals [13, 14]. These embryos consist mostly of endodermal and mesenchymal cell types [10].

Mutual antagonism between SoxB1 and β–catenin.

The changes in SoxB1 levels as a function of gain and loss of nuclear β–catenin raised the possibility that SoxB1 is involved in the corresponding changes in cell fate specification. This idea was supported by the observation that the phenotype caused by mis/overexpression of SoxB1through injection of synthetic SocB1 mRNA is identical to that of a cadherin mRNA-injected embryo [15]. In both cases, all nuclei contain high levels of SoxB1 and all genes in the β–catenin-dependent endomesoderm gene regulatory cascade, including those operating near the top, are inactive (E.Davidson, L. Angerer, unpublished observations). This suggested that the inhibition of endomesoderm development by SoxB1 operates at or near the level of β–catenin's transcriptional regulatory function. That this indeed was the case was shown by the ability of SoxB1 to inhibit directly nuclear β–catenin function in the normal embryo. When SoxB1 was eliminated by morpholino-mediated translational interference, β-catenin's transcriptional activity, as monitored with Topflash, a β–catenin-dependent promoter driving luciferase expression, was dramatically elevated [15]. This proved that SoxB1 is a potent inhibitor

of the canonical Wnt signaling pathway in the normal embryo at the level of nuclear β–catenin function.

Antagonism between Sox factors and nuclear β–catenin during cell fate specification has been observed in several other model systems. For example, in mouse embryos containing developing chondrocytes that either lack Sox9 or express stable β–catenin, there is severe chondrodysplasia. Conversely, when these cells either express Sox9 or lack β–catenin, their proliferation and differentiation rates are decreased resulting in delays in endochondral bone formation[16]. Similarly in *Xenopus* embryos, Sox 17β prevents expression of genes involved in Wnt-dependent specification of dorsal fates and, along with Sox 7 and Sox3, can inhibit β–catenin's transcriptional activating function [17, 18].

Mechanisms of SoxB1 antagonism of nuclear β–catenin transcriptional function. The molecular mechanism by which SoxB1 blocks nuclear β–catenin activity has not been established. The possibility that SoxB1 competes with the binding to DNA of Lef1, the factor through which β–catenin executes transcriptional activation (Fig. 1), was considered very likely since these two factors have similar HMG-box, DNA binding domains. However, mutations in the SoxB1 HMG box that eliminate its ability to bind DNA did not interfere with its ability to block endomesoderm development [15]. In agreement with this finding, the DNA binding domain of mouse Sox9 does not bind to well-characterized TCF-Lef sites [16]. A second possibility is that SoxB1 directly interferes with β–catenin's interaction with TCF-Lef. The carboxy terminal half of SoxB1 and sea urchin β–catenin can strongly interact in yeast two-hybrid assays (Z. Wei, unpublished observations). A physical interaction between *Xenopus* β–catenin and

mouse Sox9, also via sequences in the Sox9 C-terminal half, is thought to block the β–catenin/Lef1 interaction (Fig. 1), thereby preventing β–catenin-mediated transcriptional activation [16]. A similar molecular interaction model was derived for XSox17α-β and XSox3 and *Xenopus* β–catenin [17]. However, whether these interactions actually do occur in normal embryos has not been directly demonstrated, since the current evidence is based on mis/expression in which the concentration of the interacting proteins is artificially elevated or co-transfection assays in heterologous systems.

Mechanisms of β–catenin-dependent down regulation of SoxB1. The progressive clearance of SoxB1 from vegetal blastomeres between 4th and 9th cleavage stages is essential for normal development. The process begins with the asymmetric 4th cleavage of vegetal blastomeres (Fig. 2). Then two β–catenin-dependent processes, SoxB1 turnover and *SoxB1* transcriptional suppression, continue and complete this process.

Asymmetric cleavage. SoxB1 mRNA and protein accumulate to equal concentrations in all blastomeres of 2-, 4- and 8-cell embryos (Fig. 2B). During interphase, SoxB1 accumulates in nuclei, but at mitosis, it is released to the cytoplasm [19]. At the critical 16-cell stage, the micromeres contain 5-fold less cytoplasm than their sisters, the macromeres, and correspondingly less SoxB1 mRNA and protein. Furthermore, when equal amounts of protein from these purified blastomere types are used in an electorphoretic mobility shift assay, the micromeres contain about fourfold less SoxB1 binding activity per microgram of protein than extracts of the rest of the embryo [12]. The mechanism responsible for this additional reduction in SoxB1activity

is not understood, but it must be very rapid. Together, these two mechanisms produce an ~20-fold drop in SoxB1 that is sufficient to allow β–catenin to initiate its transcriptional function specifically in micromeres.

Selective β–catenin-dependent degradation of SoxB1 in vegetal cells. Although there is clear evidence for down regulation of *SoxB1* transcription during late cleavage (see below), this mechanism cannot account for the rapid decline of SoxB1 in micromeres soon after they form, given the large maternal stores of both SoxB1 mRNA and protein [12]. A significant loss of SoxB1 protein as a result of this mechanism is not expected until early blastula stages. Consequently there must be some post-transcriptional down regulation of SoxB1 protein in vegetal cells during cleavage stages. Direct evidence of this was obtained in embryos lacking endogenous SoxB1 as a result of morpholino injection, but containing SoxB1 synthesized from exogenous, morpholino-immune RNA [19]. In these embryos, all SoxB1 protein was derived from the injected, uniformly distributed RNA. Nevertheless, SoxB1 protein accumulated in the normal, non-vegetal pattern during early blastula stages. To test whether this was the result of differences along the A-V axis in control of SoxB1 translation or in the rate of turnover of SoxB1, mRNA encoding GFP-tagged SoxB1 but lacking untranslated sequences, in which translational control sequences most frequently are found, was injected into 1-cell zygotes. No GFP-SoxB1 accumulated in vegetal blastomeres of early blastulae, demonstrating that the embryo can degrade this protein differentially along the A-V axis [19]. Nuclear β–catenin is required for selective turnover of GFP-SoxB1 because it persists at similar levels in all cells of cadherin mRNA-injected embryos [19]. The

regulated turnover of SoxB1 constitutes a novel regulatory feature of the canonical Wnt signaling pathway.

The finding that a robust, nuclear β–catenin-dependent mechanism leads to the degradation of SoxB1 in vegetal blastomeres of early blastulae could explain the rapid loss of SoxB1 in micromeres. However, turnover of SoxB1-GFP was not observed in these cells. Perhaps the level of SoxB1-GFP exceeded the capacity of the turnover machinery, even though low concentrations of SoxB1-GFP were used in these experiments, in order to avoid inhibiting nuclear β–catenin function. It is also possible that the GFP moiety partially inhibit the turnover of the fusion protein. In this regard, it is interesting to note that, in an analogous experiment monitoring turnover of β–catenin-GFP along the A-V axis of sea urchin embryos, loss of signal in animal blastomeres was not observed until the 128-cell stage, three cleavages later than asymmetric nuclearization of β–catenin begins [6]. These observations raise the possibility that turnover of both SoxB1 and β–catenin occur much earlier than can be detected using GFP-tagged molecules**.**

It is interesting that spatially regulated turnover is the major mechanism that regulates the levels of both SoxB1 and nuclear β–catenin, which may reflect the fact that the concentration of these mutually antagonistic proteins must be rapidly adjusted and closely regulated during cleavage stages. Interestingly, there is emerging evidence in another system that a Sox factor and β–catenin can regulate each other's turnover. Co-transfection of mouse HA-tagged Sox9 and myc-tagged, stable, Xβ–catenin leads to reduction in the levels of both proteins compared to those in singly transfected cells [16]. In sea urchin embryos at the 16-cell stage, nuclear β–catenin concentrations are highest

in the micromeres while SoxB1 concentrations are the lowest as a result of asymmetric cleavage. If reciprocal degradation operates here, then SoxB1degradation might be dominant over that of β–catenin and would be begin first in the micromeres where the ratio of nuclear β–catenin to SoxB1 is the highest. The fact that endogenous SoxB1 disappears faster from these cells than in the progeny of the macromeres is consistent with this possibility.

Although regulation of turnover of transcription factors by β–catenin is a new finding, selective turnover of several other transcription factors involved in early cell fate specification has recently been reported. In both *C. elegans* and *Drosophila* embryos, this mechanism is important in establishing the germline [20, 21], which is the first lineage to be separated during the maternal-to-zygotic transition. Seydoux's laboratory has shown that several transcription factors are degraded during early cleavage stages specifically in somatic blastomeres by the cullin/ubiquitin system [22], thereby achieving rapid asymmetric partitioning of these proteins to the germline. Similarly, the germ cell determinant, *oskar*, is degraded throughout the *Drosophila* embryo, except at the posterior pole, where it is stabilized by PAR-1-dependent phosphorylation [21]. In *Xenopus,* regulated turnover of Xom, a transcriptional repressor of *goosecoid*, occurs rapidly during early gastrulation and is important for cell fate specification along the dorsal-ventral axis [23].

Transcriptional suppression. During cleavage and early blastula stages, the SoxB1 mRNA population includes both maternal and embryonic transcripts [12]. The large maternal component obscures exactly when and where *SoxB1* transcription begins in the embryo, but *SoxB1* transcript levels decrease in vegetal blastomeres by 7th to 8th

cleavage [12]. The evidence that down regulates *SoxB1*transcription, either directly or indirectly, in vegetal blastomeres is that all cells of cadherin-injected blastulae accumulate equivalently high levels of *SoxB1* mRNA, as shown by in situ hybridization [19].

The ability of a β–catenin-dependent mechansim to down regulate transcription of *SoxB1* might appear to be a redundant level of control, superimposed on control at the level of SoxB1 protein turnover. That this is not the case became evident with the observation that SoxB1, directly or indirectly, negatively regulates its own transcription. SoxB1 mRNA levels increase tenfold in embryos lacking SoxB1 protein as a result of morpholino-mediated translational interference [18]. Thus, canonical Wnt signals provide a two-pronged regulation of SoxB1 activity, diagrammed in Fig. 3, to ensure that both early and late functions of nuclear β-catenin are executed during the specification and differentiation of endomesoderm in the sea urchin embryo.

Concluding remarks

While negative regulation of nuclear β–catenin function is a critical feature of SoxB1's developmental role, it certainly is not its only function. SoxB1 is expressed in the ectoderm throughout early development and oral-aboral polarity is not established in its absence. The closest relative of SpSoxB1in the large Sox superfamily is the SoxB class factor, Sox2. Mammalian Sox2, the earliest known Sox factor to be expressed in pre- and peri-implantation mouse embryos [24] is crucial for the development of the first lineages, i.e., the epiblast, extraembryonic endoderm and trophectoderm [25]. However, its primary function is thought to be the maintenance of the epiblast in an undifferentiated state [24]; homozygous null Sox2 embryos lack an epiblast that can be rescued in

chimeras formed with wild type ES cells, which contribute only to the epiblast. SoxB1 expression continues in the early sea urchin embryo in pluripotent cells that are only weakly biased toward ectodermal fates. If the early function of SoxB1 is to maintain this state while animal cells await vegetal signals, then canonical Wnt signaling may eliminate SoxB1 not only to eliminate its inhibition of nuclear β–catenin function, but also to erase this "default" ectodermal bias from vegetal cells. The cross-regulatory interactions of SoxB1 and nuclear β–catenin are classic examples of how the egg-to-embryo transition is achieved through the interplay between maternal factors and early signaling pathways that generate the initial partitioning of developmental potential in the sea urchin embryo.

The work reviewed here was greatly facilitated by a number of useful properties of the sea urchin embryo. It has become an ideal system for exploring the regulatory interactions at both cellular and molecular levels because of the combination of excellent methods for perturbing gene function and for manipulating combinations of blastomeres. The optical clarity and simplicity of the sea urchin embryo are particular advantages for monitoring the effects of these experimental challenges in all cell types in real time. As a consequence, rapid progress has been made from the work of just a few labs in understanding the functions and regulation of the canonical Wnt signaling pathway in the initial patterning of this embryo, which are among the best understood in any developing system.

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Figure legends

Fig. 1. Canonical Wnt signaling. For simplicity, this diagram illustrates only the components of the canonical Wnt signaling pathway that will be discussed in this review; for a complete listing of all components, the reader is referred to http://www.stanford.edu/~rnusse/wntwindow.html). Dsh (disheveled); gsk3β (glycogen synthase kinase 3β).

Fig. 2. A) Fate map of blastomeres along the animal-vegetal axis of the sea urchin embryo. Different developmental lineages are color-coded as described at right. B) The domains of SoxB1 accumulation (purple) and nuclear β–catenin (green) during cleavage stages (see text for details). C) The consequences of misexpressing cadherin or SoxB1 (purple) is that the embryo consists of poorly differentiated ectoderm, whereas misexpressing stable β–catenin (green) is that it consists mostly of endoderm and mesenchyme.

Fig. 3. Canonical Wnt signals down regulate SoxB1 through degradation of the protein to rapidly reduce its level in vegetal blastomeres and by transcriptional repression of the gene to block SoxB1's negative autoregulation.

Figure 1

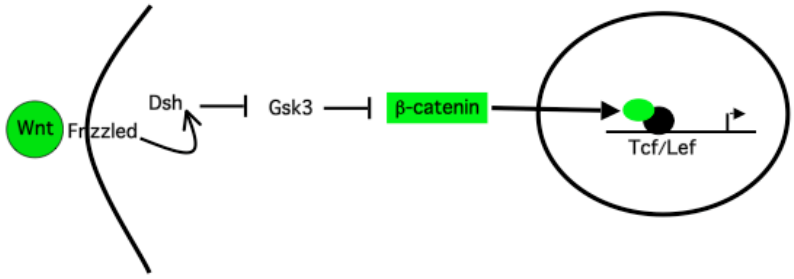
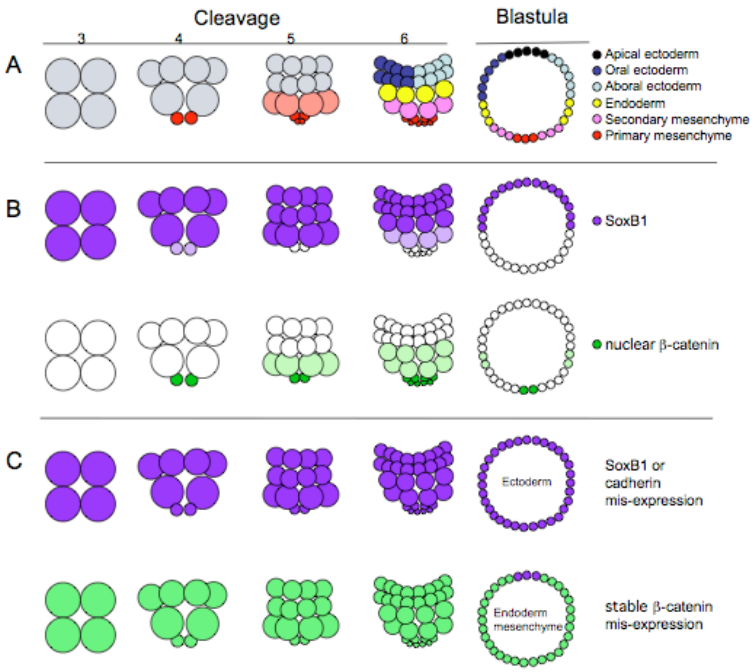


Figure 2


Cleavage
Blastula
3
4
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A
Apical ectoderm
Oral ectoderm
Aboral ectoderm
Endoderm
Secondary mesenchyme
Primary mesenchyme
B
SoxB1
nuclear β-catenin
C
Ectoderm
SoxB1 or
cadherin
mis-expression
Endoderm
mesenchyme
stable β-catenin
mis-expression

Figure 3