Embryonic pattern formation without morphogens

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Summary

One of the earliest and most fundamental pattern-formation events in embryonic development is endoderm and mesoderm specification. In sea urchin embryos, this process begins with *blimp1* and *wnt8* gene expression at the vegetal pole as soon as embryonic transcription begins. Shortly afterwards, *wnt8*/*blimp1* expression spreads to the adjacent ring of mesoderm progenitor cells and is extinguished in the vegetal-most cells. A little later, the ring of *wnt8*/*blimp1* activity moves out of the mesoderm progenitors and into the neighboring endoderm cells. Remarkably, this moving ring of gene expression has now been shown to be controlled entirely by transcriptional *cis*-regulatory logic [(1)].

Keywords

Gene regulatory networks, pattern formation, embryonic development, systems biology, sea urchin endoderm and mesoderm specification

Background

How does the linear sequence of inherited-DNA control the complex spatio-temporal processes that underlie embryonic development? Ever since the publication of an ingenious proposal by Alan Turing in 1952 [(2)], morphogen-mediated 'reaction-diffusion' has been assumed to be responsible for many embryonic pattern formation processes (see for example [(3-5)]). In this view, the genome's role is essentially one of setting up the appropriate initial conditions by expressing the right set of proteins in the right nuclei.

Complex patterns emerge from complex chemical interactions among the diffusing proteins.

Now in a remarkable paper [1] published in Science, Joel Smith, Christina Theodoris and Eric Davidson offer a radically different paradigm: complex and dynamic pattern formation encoded directly in the genome.

In cleavage-stage embryos of the sea urchin species Strongylocentrotus purpuratus (Sp), two genes – *wnt8* and *blimp1* – are expressed first at the vegetal pole, and subsequently in a ring of cells that travels out towards the midline of the embryo, as illustrated schematically in Fig. 1A. The pattern of expression of these genes resembles the traveling wave-front generated when a stone is dropped into water.

Turing's theory of reaction-diffusion provides an enticingly elegant and simple explanation for such patterns: local (short-range) excitation combined with distal (long-range) inhibition. In the absence of diffusion, positive feedback can lead to stable, stationary islands of activity. Diffusion can act to trigger activity in neighboring territories, causing the active region to expand over time. The addition of negative feedback can cause recently-active regions to turn-off and become refractory. Combined together, these mechanisms can generate moving wave-front patterns of the type illustrated in Fig. 1A.

Reaction-diffusion models usually assume graded spatial distribution of the activating and inhibitory morphogens. Indeed, morphogens are defined as diffusible, secreted molecules that regulate the states of surrounding cells *in a graded, concentration-dependent manner* [6,7]. Competition between the activities of the two morphogens determines the size of the activity islands, and the distance between them. However, in spite of much research, no definitive example of a Turing-type reaction-diffusion pattern has been reported to date [8].

Dynamic pattern formation without gradients

Importantly, the model of Smith et al [1] does not require a graded morphogen concentration distribution. All excitation and inhibition activities are essentially binary (on or off), and diffusion is limited to the adjacent cells.

The cartoons in Fig. 1B depict cross-sections of Sp embryos during 4th to 7th cleavage. In these drawings (based on [9] and drawings by Dr. Andrew Ransick, Caltech), the vegetal pole is at the bottom and the animal pole at the top. The micromeres are the vegetal-most cells shown in shades of blue. The cells shown in gray are the progenitors of the endoderm. Mesoderm cells (green) arise from and separate these two groups of cells starting at 7th cleavage. The model of Smith et al describes a ring of *wnt8*/*blimp1* gene expression starting in the 4th cleavage micromeres, then moving into the (green) mesoderm cells at about 7th cleavage, and finally moving into the lower (Veg2) band of endoderm cells (daughters of the yellow cells in Fig. 1B).

Zygotic transcription of *wnt8* starts in the micromeres at around 4th cleavage. Smith et al show that the embryo contains a small amount of maternal Blimp1 mRNA at 4th cleavage. From fertilization until fifth cleavage, maternally-inherited Disheveled proteins are localized to the vegetal cortex [10] and maternal β-catenin is nuclearized in the micromeres [11,12]. Sp *wnt8* is a target of canonical Wnt signaling [13]. Synergistic action by maternal nuclear β-catenin and maternal *blimp1* triggers *wnt8* transcription in 4th cleavage micromeres and sets up a positive feedback loop, as shown schematically in Fig. 2A.

At 4th cleavage, maternal Otx(α) mRNA is restricted to the micromeres [14,15]. Later, zygotically Otx(α) is expressed in all vegetal cells [16]. *blimp1* is positively regulated by Otx(α) and nuclear β-catenin [17,18]. By 5th cleavage Otx(α) and nuclear β-catenin have cooperatively activated *blimp1* transcription in the micromeres (Fig. 2B). We now have two inter-cellular positive feedback loops in operation: one due to the fact that *wnt8* is itself a target of Wnt signaling, and a 2nd loop in which Blimp1 transcriptionally activates *wnt8* and Wnt8 activates *blimp1* via Wnt signaling.

The above pattern of *wnt8*/*blimp1* activity is initially restricted to the micromeres. Diffusion of Wnt8 to the adjacent mesoderm cells eventually initiates *wnt8* and *blimp1* activity in these cells.

While Wnt8 diffuses from the micromeres to the neighboring mesoderm progenitor cells, Blimp1 protein levels build up in the micromeres. Davidson and colleagues previously showed that *blimp1* has multiple *cis*-regulatory sites for auto-repression [(18)]. Since transcription of *wnt8* requires Blimp1, *blimp1* auto repression may be expected to shut down the transcription of both *wnt8* and *blimp1*. By mutating the *blimp1* auto-repression sites in a series of reporter constructs, Smith et al show that *blimp1* auto-repression is indeed responsible for the spatial clearance of *wnt8*/*blimp1* activity a few hours after *blimp1* is expressed (Fig. 2C). They hypothesize that *blimp1* auto-repression may be a multi-step process mediated through histone modifications.

Activation of *blimp1* and *wnt8* in the mesoderm progenitor cells essentially follows the same sequence of events, except that the initial activating input is provided by Wnt ligands diffusing from the micromeres, as shown in Fig. 2D-F. This entire process then repeats one more time to activate *wnt8*/*blimp1* transcription in the endoderm cells, and to shut down *wnt8*/*blimp1* transcription in the mesoderm.

Fig. 3A-C summarizes the spatial relationships between the cells, and the concomitant pattern of inter-cellular signaling. Note that the diffusion of Wnt8 ligand to adjacent cells plays a dual role in this system. Firstly, it creates a positive feedback loop within cells of the same type (micromeres in the case of Fig. 3A-C). Second, diffusing Wnt8 ligands act as an activating trigger to the adjacent layer of cells. The preceding descriptions emphasized the importance of these two roles to the pattern generation process. The inter-cellular positive feedback of *wnt8* has an additional property: it homogenizes the response of cells within the ring of *wnt8* expression.

Diffusion of Wnt8 ligands among adjacent cells means that each cell receives some of its Wnt8 input auto-catalytically, and some from each of its neighbors. In this way, *wnt8* activity is averaged spatially between each cell and its adjacent neighbors. Spatial averaging of this kind ensures that any cells lagging in *wnt8* expression (e.g. due to lower levels of maternally inherited factors) will be 'pulled up' to similar levels of expression as its neighbors. Thus, spatial averaging is another way in which the pattern formation process described by Smith et al is not morphogen gradient dependent. Fig. 3D illustrates this effect with example simulations of expression variability with and without spatial averaging.

Discussion

A key feature of the model of Smith et al is that the positive and negative feedback loops that control wave-front generation and decay are entirely controlled through *cis*--regulatory logic, not protein-protein interactions. Another remarkable feature is that none of the proteins involved in this system meet the definition of a morphogen. Maternal Otx(α) is restricted to the micromeres. At later stages, zygotic Otx(α) is expressed uniformly in all cells during the period of interest. *blimp1* zygotic expression is controlled by Wnt8, Otx(α) and itself, and is part of an all-or-nothing positive feedback loop. Thus, neither Otx nor Blimp1 meet the usual definition of a morphogen.

Wnt/Wingless proteins are known to act as morphogens in other species. In the imaginal wing disk of Drosophila embryos, Wingless proteins diffuse some 50 microns within 30 minutes, and create a distinct concentration gradient [(19)]. In mice, diffusing Wnt proteins have been proposed to lead to concentration gradients determining hair follicle spacing [(4)].

However, like *blimp1*, the expression of *wnt8* in Sp is all-or-nothing, not concentration-dependent. In the absence of Wnt signaling, Groucho-family proteins actively repress transcription of Wnt target genes. Competition between nuclear β-catenin and Groucho

proteins thus results in a push-pull switch action that leaves Wnt target genes either repressed or active, but not in-between (see [20] for a discussion).

Is the traveling ring pattern robust to small variations in factor concentrations and kinetic parameters? As long as the auto-repressive effect of *blimp1* on itself is long-lasting, then the system will always generate a wave-front that starts at the vegetal pole, expands towards the animal pole, and extinguishes *wnt8* and *blimp1* expression in cells behind the wave-front. So the overall characteristic behavior of the system is indeed very robust.

The model of Smith et al focuses on the core regulatory roles of Otx(α), *blimp1* and *wnt8*. At least three additional processes contribute to the robustness of the traveling wave of *wnt8*/β-catenin/*blimp1* activity. First, the localization of Disheveled to the vegetal cortex of the fertilized egg [10,12] implies an absence of maternal Dsh in the ectoderm progenitor cells. Thus, in the ectoderm, β-catenin is cytoplasmically degraded [12]. In the absence of nuclear β-catenin, the target genes of canonical Wnt signaling are actively repressed by Groucho, thus ensuring a different regulatory state for the ectoderm founder cells.

Second, the traveling ring of *wnt8*/*blimp1* activity stops after reaching the Veg1 layer of endoderm cells. Regulation of *blimp1* in these cells is mediated by additional factors, including Eve [17,18]. Further propagation of Wnt signaling into the adjacent ectoderm cells is thought to be inhibited by Animalizing Transcription Factors [21].

Third, in a process whose mechanism is not yet fully understood [21], maternal SoxB1 [22] – an inhibitor of β-catenin transcriptional activity – is excluded from the micromeres in 4th cleavage embryos. The exclusion of SoxB1 from the micromeres may provide an additional mechanism ensuring that *wnt8* and *blimp1* are initially only expressed in the micromeres.

Since repression of Blimp1 activity is transcriptional, the rate at which Blimp1 activation of *wnt8* transcription is extinguished will depend on the rate of Blimp1 protein clearance. This suggests that Blimp1 protein not-bound to the *blimp1* promoter may be short-lived.

In support of this hypothesis, Smith et al report that the half-life of Blimp1 mRNA is about half the average mRNA half-life in sea urchin embryos.

The model, and resulting insights provided by Smith et al also open up opportunities for further research. According to Smith et al, zygotic *wnt8* expression starts one cleavage before the start of *blimp1* transcription. Both *blimp1* and *wnt8* require nuclear β-catenin for their transcription [(1)]. *blimp1* also requires Otx(α) activity. Otx(α) is maternally inherited and localized to the 4th cleavage micromeres [(14,15)]. Thus, it is not clear why *blimp1* transcription occurs one cleavage after the start of *wnt8* transcription.

In the current model, the speed with which the cells behind the wave-front extinguish *blimp1*/*wnt8* expression is independent of the speed with which the wave-front propagates forward. Whether this is a true characteristic of the system, or an indication that additional interactions may coordinate the forward and rear boundaries of the moving wave remains to be explored. A related question is "what mechanisms regulate the distance by which the ring expands at each iteration?".

In providing a novel paradigm for dynamic spatio-temporal pattern formation through *cis*-regulatory logic, Smith et al may have initiated a wave of new questions and discoveries in molecular developmental biology.

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Fig. Captions

Fig. 1. Gene expression in cleavage-stage sea urchin embryos. (A) The cells in red indicate the domain of expression of *wnt8* and *blimp1* at the times indicated (hours post fertilization, hpf). The views here are looking directly at the vegetal pole. The timings given are for the Strongylocentrotus purpuratus (Sp) species. Expression starts in the micromeres shortly after the 4th cleavage. By 12hpf, expression has extinguished in the micromeres and instead started in the adjacent mesoderm progenitor cells. The ring of *wnt8*/*blimp1* expression next moves out to the Veg2 endoderm cells, and is extinguished in the mesoderm. (B) Cross sectional representations of the Sp embryo at 4th, 5th, 6th and 7th cleavage. Cell types at each stage are labeled. The endoderm comprises two distinct groups of cells labeled Veg2 and Veg1. Although the ring of *wnt8*/*blimp1* expression eventually extends all the way to the Veg1 endoderm, *wnt8* and *blimp1* regulatory interactions are different in these cells and not included in the current model (see text for details). The small micromeres (light blue cells, 5th cleavage onwards) do not take part in the processes of interest here. They are not shown in other figures, nor discussed in the text.

Fig. 2. Network views from the nuclei of different cells at different times. Light gray indicates inactive components. The "&" symbol below the *wnt8* gene emphasizes that *wnt8* expression requires both regulatory inputs. *blimp1* expression also requires both regulatory inputs to be active (logic not shown to avoid clutter). *blimp1* auto-repression over-rides any activity by its positive inputs. (A)–(C) The pattern of initiation, auto-regulation, and subsequent repression of *wnt8* and *blimp1* in the micromeres. Maternal factors initiate transcriptional activation. *blimp1* auto-repression shuts down both genes. The double-chevron symbol indicates inter-cellular signaling by the Wnt ligand. Details of the canonical Wnt signaling system are not shown. (D)–(F) The same process occurs in the progenitors of the mesoderm and endoderm, but instead of maternal factors, Wnt8 ligands from neighboring cells, combined with ubiquitously expressed Otx(α), initiate first *blimp1*, then *wnt8* transcription.

Fig. 3. Multiple roles of Wnt8 signaling. (A)-(C) show transcription in 5th cleavage micromeres. At this time, zygotic supplies of Otx(α), Blimp1 and nuclear β-catnin are replacing maternal factors. The view in (C) is a zoomed in version of the circled portion of (B), while (B) is a plan view of the circled portion in (A), looking down onto the vegetal pole (cf. Fig. 1A). The representation in (C) shows two micromeres (blue background color) and five macromeres (gray), not to scale. (C) emphasizes the two different roles of Wnt8 ligand diffusion: auto-regulatory positive feedback, and triggering activation in neighboring cells. (D) The effect of Wnt8 ligand-sharing by adjacent cells. For simplicity a one-cell-wide ring of cells is modeled. For illustrative purposes, the cells in the ring are displayed in a row (in the model, the two end-cells are neighbors). (i) Depicts a row of cells in which each cell is assigned a random '*wnt8* gene expression level' for illustrative purposes. Colors correspond to the scale shown in the color-bar key at the bottom. (ii) Shows the expression levels in the same group of cells after each cell has shared one quarter of its content with each of its neighbors, i.e.:

expression level in cell 'i' = ½(expression in 'i' + ½expression in 'i+1' + ½expression in 'i-1')

The result of this spatial averaging process is that cells exhibiting very high or very low levels of expression are brought closer to the average level (the colors are nearer the middle of the color-bar scale).

Fig. 1

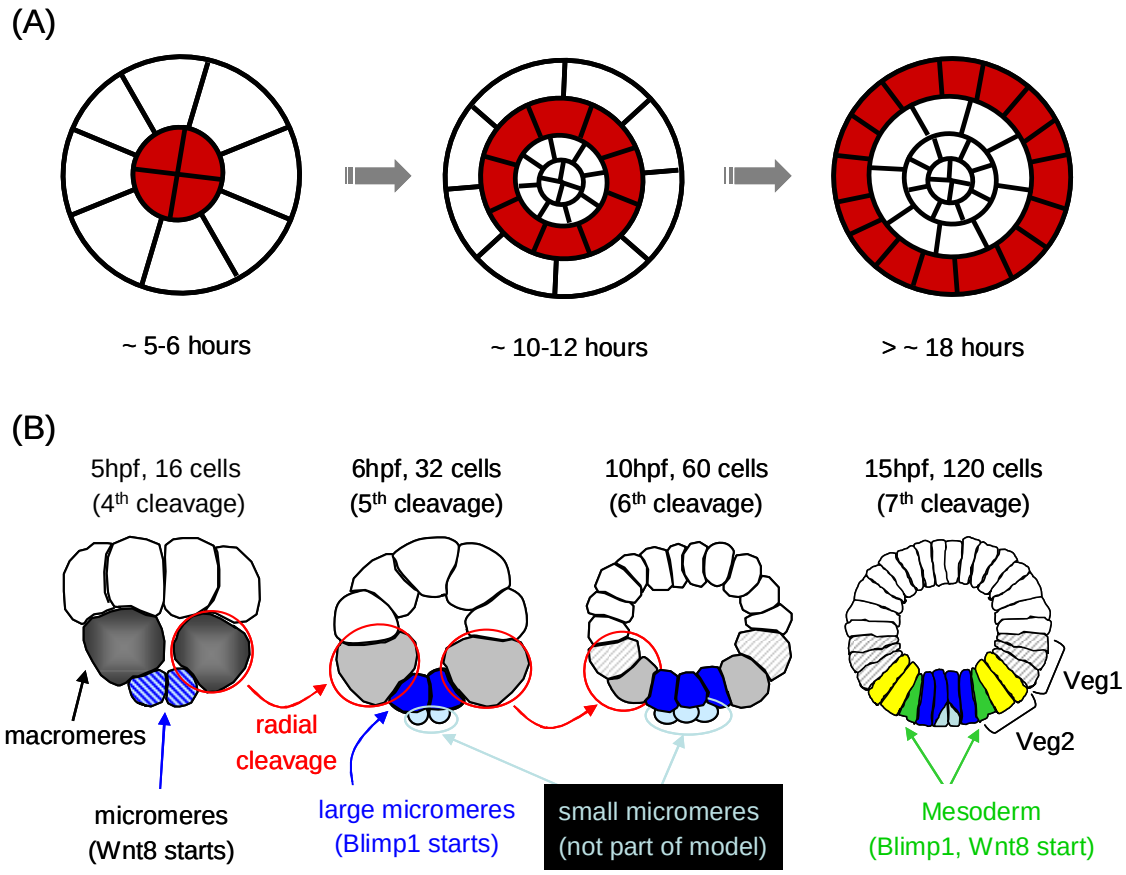
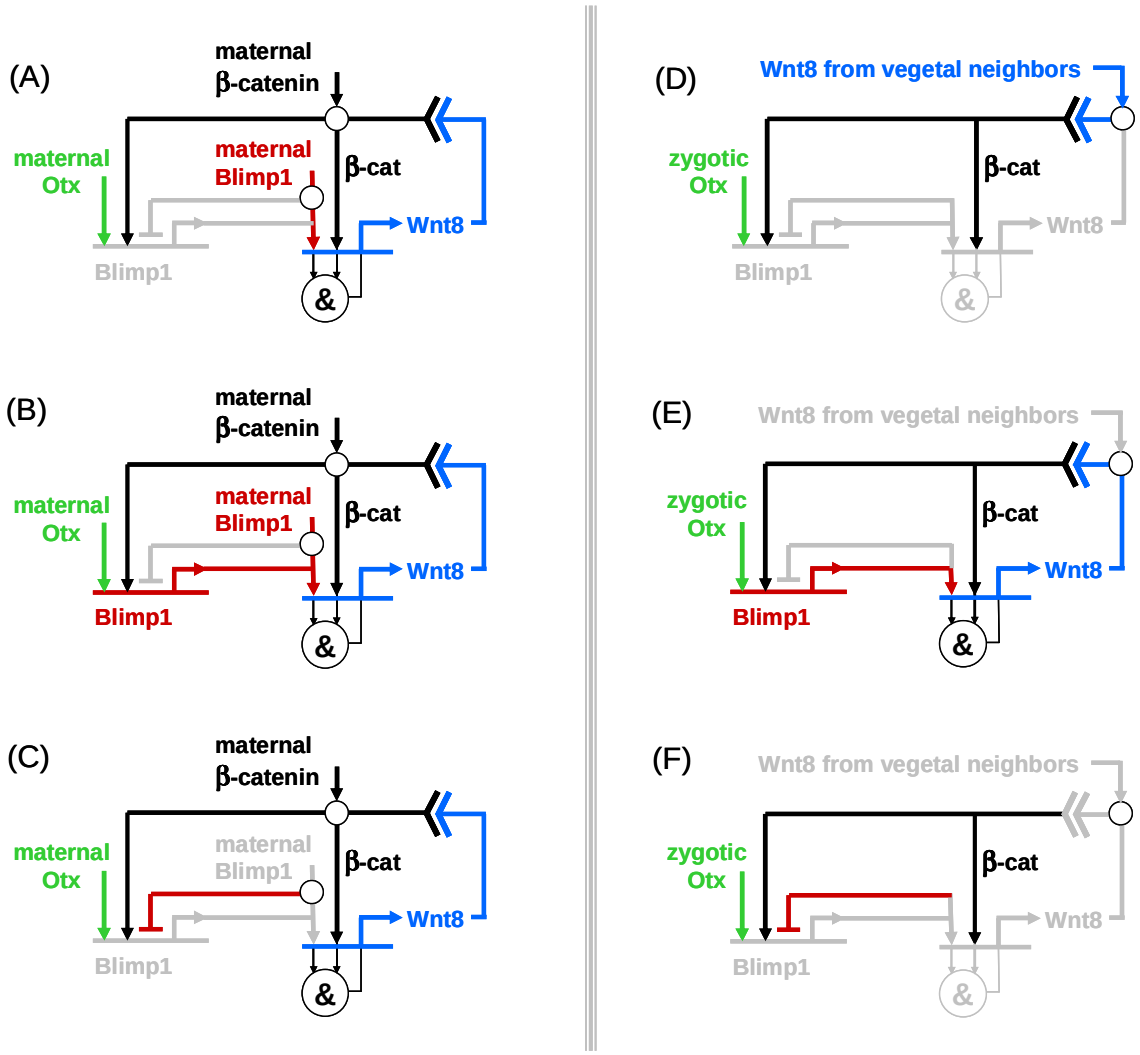

(A)
~ 5-6 hours
~ 10-12 hours
> ~ 18 hours
(B)
5hpf, 16 cells
(4th cleavage)
6hpf, 32 cells
(5th cleavage)
10hpf, 60 cells
(6th cleavage)
15hpf, 120 cells
(7th cleavage)
macromeres
micromeres
(Wnt8 starts)
radial
cleavage
large micromeres
(Blimp1 starts)
small micromeres
(not part of model)
Veg1
Veg2
Mesoderm
(Blimp1, Wnt8 start)

Fig. 2


(A)
maternal
β-catenin
maternal
Otx
maternal
Blimp1
β-cat
Wnt8
Blimp1
&
(B)
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β-catenin
maternal
Otx
maternal
Blimp1
β-cat
Wnt8
Blimp1
&
(C)
maternal
β-catenin
maternal
Otx
maternal
Blimp1
β-cat
Wnt8
Blimp1
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(D)
Wnt8 from vegetal neighbors
zygotic
Otx
β-cat
Wnt8
Blimp1
&
(E)
Wnt8 from vegetal neighbors
zygotic
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β-cat
Wnt8
Blimp1
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(F)
Wnt8 from vegetal neighbors
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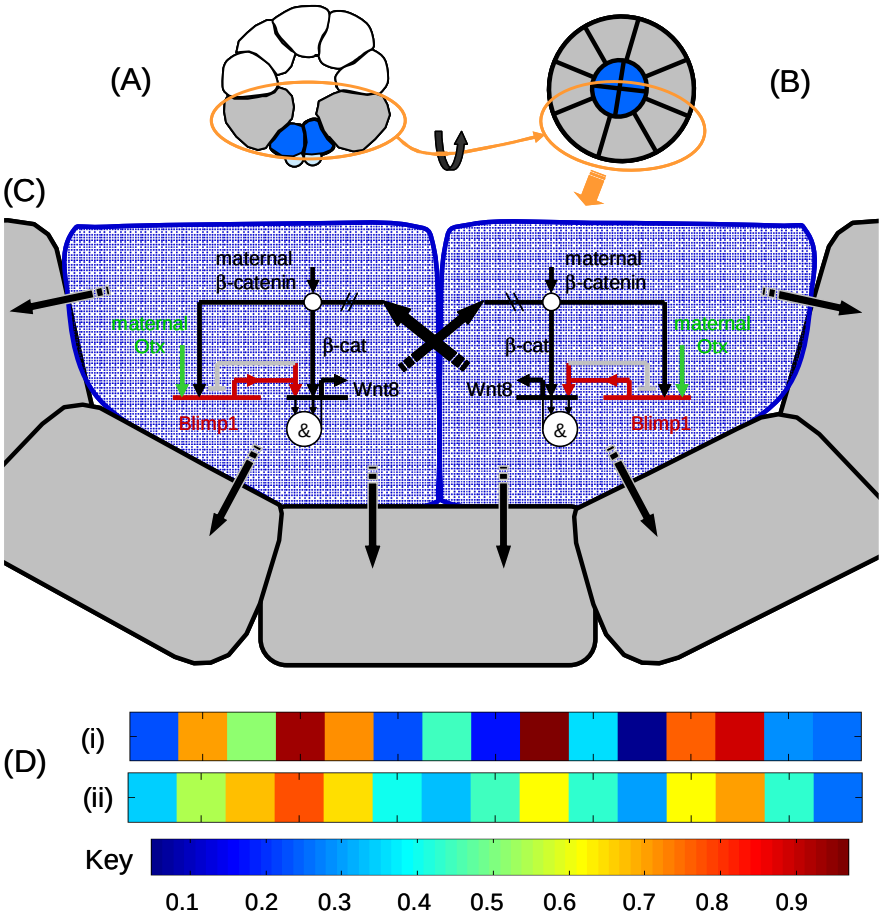
Fig. 3


(A)
(B)
(C)
maternal
β-catenin
maternal
Otx
β-cat
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Blimp1
&
(D)
(i)
(ii)
Key
0.1
0.2
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