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Modeling and Analysis of Germ Layer Formations Using Finite Dynamical Systems

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Abstract

The development of an embryo from a fertilised egg to a multicellular organism proceeds through numerous steps, with the formation of the three germ layers (endoderm, mesoderm, ectoderm) being one of the first. In this paper we study the mesendoderm (the tissue that collectively gives rise to both mesoderm and endoderm) gene regulatory network for two species, *Xenopus laevis* and the axolotl (*Ambystoma mexicanum*) using Boolean networks. We find that previously-established bistability found in these networks can be reproduced using this Boolean framework, provided that some assumptions used in previously-published differential equations models are relaxed. We conclude by discussing our findings in relation to previous work modeling gene regulatory networks with Boolean network models.

Keywords: Bistability, Gene Regulatory Networks, Finite Dynamical Systems, Boolean Networks, Mesendoderm, Germ Layer Formation

1 Introduction

The development of an embryo from a fertilised egg to a multicellular organism proceeds through numerous steps, with the formation of the three germ layers (endoderm, mesoderm, ectoderm) being one of the first [1, 2]. In the development of these various germ layers, the interplay between signaling molecules and transcription factors yields the genes and morphology that distinguish the subsequent development of one type of gene layer from that of the others. The particular genes expressed within a particular cell are determined in part by the transcription factors and signals present within and around that cell. A gene encoding a transcription factor or a signal may encode other transcription factors or signals, leading to a gene regulatory network (GRN) [6]. A GRN, also called a genetic circuit, is a group of genes whose general protein products regulate one another's expression [3].

During the course of embryonic development, each germ layer gives rise to different tissue types in the developing embryo: endoderm (the inner layer) forms the digestive system and the lungs, mesoderm (middle layer) forms the muscle, blood and connective tissue and ectoderm (outer layer) forms the epidermis and nervous system. The tissue types that collectively give rise to both mesoderm and endoderm are called the mesendoderm [1].

In this paper we study the simplified mesendoderm gene regulatory network (mGRN) for two species, *Xenopus laevis* and *Ambystoma mexicanum* using Boolean networks [8, 9, 12, 10, 11]. Both *Xenopus* (the frog) and *Ambystoma mexicanum* (the axolotl) are amphibians, but from different orders, and these differences extend to the topology of the mGRN for both species [1]. Mathematical models for *Xenopus laevis* show that a negative feedback loop within the mGRN can reproduce various experimental observations, which in turn gives credibility to hypothesized mechanisms for the formation of different cell types [6]. Specifically, the modelers assume that there is a mutual repression of the *Mix.1* and *Brachyury* gene families via *Goosecoid* and the autoregulation of *Nodal*. The repression of *Mix.1* and *Brachyury* is the only source of competition between mesoderm and endoderm in the full mGRN [5, 4]. This negative feedback predicts the bistability seen in experimental observations of the network—certain external initial conditions elicit the formation of dorsal mesoderm (i.e., *Brachyury* expressing cells) and anterior mesendoderm (i.e., *Mix* and *Goosecoid* co-expressing cells). Initial conditions for their *in vitro* model corresponded to the size of a dose of Activin, while in their *in vivo* model initial conditions corresponded to initial concentrations of key maternal factor VegT [6]. Low/medium levels of Activin/VegT elicit the formation of dorsal mesoderm, while high levels elicit the formation of anterior mesendoderm—demonstrating the bistability present in the system.

Mathematical models for the axolotl have been able to reproduce this same bistable behavior, which has been observed in laboratory experiments as well [1]. That pa-

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per's *in vivo* model was able to reproduce the effect of the key maternal factor β-Catenin's concentration levels, with low concentrations eliciting the formation of the mesoderm and high concentrations able to induce the formation of the anterior mesendoderm. The similar bistability features of the axolotl models are despite the fact that *Mix* is required for the expression of *Brachyury* in its mGRN, while *Brachyury* is assumed to repress *Mix*. The bistability seen in models for both *Xenopus* and the axolotl, despite the differences in their mGRNs' network topologies, was viewed by [1] as a suprisingly stable feature of these networks.

The models employed in [6] and [1] were both systems of differential equations, which have been most commonly used in the mathematical modeling of biological processes [9]. However, for most realistic biological systems these models are sufficiently complex to the point where they can only be analyzed using numerical/visual simulations, which can sometimes yield limited biological insights. Additionally, differential equation models require parameter values that are often difficult to estimate empirically, and thus modelers often choose parameter values that qualitatively fit the outcomes found in experimental settings, which can limit the models' abilities to extrapolate biological outcomes stemming from initial conditions that are outside of previously-observed experimental states [1, 6, 9]. One way to avoid this complexity problem is to use Boolean, or finite dynamical system, models [11]. For many GRNs, one can simplify the state variables by assuming they can only take on the values 0/1, "off/on" or "not expressed/expressed", as opposed to a real number giving its level of expression or concentration, which are often poorly estimated or are sensitive to various model parameters and/or assumptions. The interactions between these states (referred to as the system's *network topology*) can be preserved by imposing logical (AND/OR/NOT) operations on the variables. It has been shown that these discrete models can capture key dynamic features of biological networks and can be used for hypothesis generation ([9], [11]). Finally, these models tend to be more intuitive and easility accessible to life scientists, aiding in their increasing popularity in systems biology [8].

In this paper we show that the *in vitro* results found in [6] (for *Xenopus*) and [1] (for the axolotl) can also be found when modeled by Boolean Networks. We show, however, that the autorepressive effect of *Goosecoid* in both settings forces us to make simplifying assumptions about the system in order to reproduce the qualitative state space and steady state dynamics found using the differential equation models in those papers, which were verified experimentally. Additionally, for the axolotl model in [1] the negative feedback loop involving *Mix* and *Brachyury* needed to be removed to elicit the desired state space dynamics, calling into question either (a) the importance of the *Mix*/*Brachyury* interaction in driving the dynamics in the mGRNs for at least the axolotl, or (b) the efficacy of using simple Boolean network models to model all GRNs found in nature.

2 Model

A *finite dynamical system* that models a GRN with N variables is a difference equation of the form

$$\vec{x}(t+1) = \vec{f}(\vec{x}(t)), \tag{1}$$

where the i^{th} element of $\vec{x}(t)$ is an ordinal variable, which can take values in the finite set $[[0, m_i]] := \{0, 1, ..., m_i\}$. Here, each of the elements in $[[0, m_i]]$ models different concentration levels for x_i. For example, if $m_i = 1$, then $x_i(t)$ can take the value 0, modeling "low" concentrations, or 1, modeling "high" concentrations. The system updates during each time-step via the function $\vec{f}$, which is a vector-valued function from the *state space* $\mathcal{S} := [[0, m_1]] \times [[0, m_2]] \times \cdots \times [[0, m_N]]$ to itself. If $\mathcal{S} = [[0, 1]]^N$, then (1) is a *Boolean network* [10].

A GRN's "network topology" refers to the connectivity structure of the network [12], with the network's *wiring diagram* defined as the graph with N vertices with an edge from j to i if and only if f_i depends on x_j. The edge is assigned a positive (resp. negative) sign if and only if f_i is increasing (resp. decreasing) with respect to x_j. If f_i is neither increasing or decreasing with respect to x_j, the edge is unassigned. For Boolean networks we use the Boolean operators $\vee$, $\wedge$, and $\neg$ (logical OR, AND, and NOT, respectively). Thus, an edge from j to i is positive (represented by a regular arrow) if and only if x_j appears in the function f_i, and negative (represented by a blunt arrow) if and only if $\neg x_j$ appears in the function f_i.

A vector $\vec{x}^*$ such that $\vec{f}(\vec{x}^*) = \vec{x}^*$ is called a *fixed point* or *steady state* of the Boolean network (1).

To create Boolean models for both *Xenopus* and the axolotl in the *in vitro* setting we use the assumptions in [6] and [1], respectively, which manifest themselves in the systems of differential equations used to model their mGRNs. For *Xenopus*, the *in vitro* differential equation model (see equation (9) in [6]) is given by

$$\begin{aligned}
B' &= \lambda_{A,B} H(A)(1 - H(G + M)) - B \\
G' &= \lambda_{M,G} H\left(\frac{M}{\theta_{M,G}}\right)\left(1 - H\left(\frac{G}{\theta_{G,G}}\right)\right) - \mu_G G \\
M' &= \lambda_{A,M} H\left(\frac{A}{\theta_{X,M}}\right)\left(1 - H\left(\frac{B}{\theta_{B,M}}\right)\right) - \mu_M M \\
A(0) &= A_0, \quad B(0) = G(0) = M(0) = 0.
\end{aligned} \tag{2}$$

Here A_0, the initial concentration of Activin, is the parameter of interest in the *in vitro* setting, while the values of the remaining (nondimensional) parameters used by [6] are given in Table 2 of that paper. The constant A is the concentration of the signaling molecule Activin, while the variables B, M and G are the concentrations of the transcription factors *Brachyury, Mix* and *Goosecoid*, respectively. The function H is the Hill function

$$H(y) = \frac{y^m}{y^m + 1},$$

which turns into a step function as $m \to \infty$. To construct the wiring diagram in Figure 1 we assume that if $H(x)$ is present in the right-hand side of a differential equation for y, then x is an activator for y in a Boolean network. On the other hand, if $1 - H(x)$ is present in a differential equation for y, then x is an inhibitor of y (giving rise to the NOT operator in a Boolean network). We assume multiplication in a differential equation model corresponds to the AND operator in a Boolean network, while addition corresponds to the OR operator. The differential equation model (2) gives rise to the the Boolean network

$$\begin{aligned} f_B &= A \wedge \neg(G \vee M) \\ f_G &= M \wedge \neg G \\ f_M &= A \wedge \neg B, \end{aligned} \tag{3}$$

for the *Xenopus* mGRN. Notice that, while the Boolean variables B, G and M evolve in time, A, the concentration of Activin stays its initial value for the entire time course of the system. Also note that $-B$, $-\mu_G G$, and $-\mu_M M$ terms on the right-hand side of (2) do not need to be taken explicitly into account in (3), since the exponential decline in B, G and M in the absence of activation in (2) simply occurs in one time-step in (3).

For the axolotl, we use the same procedure as above to create the wiring diagram in Figure 2. The *in vitro* differential equation model (see equation (7) in [1]) is given by

$$\begin{aligned} B' &= \lambda_{AM,B} H(A)H(M)(1 - H(G)) - B \\ G' &= \lambda_{M,G} H\left(\frac{M}{\theta_{M,G}}\right)\left(1 - H\left(\frac{G}{\theta_{G,G}}\right)\right) - \mu_G G \\ M' &= \lambda_{A,M} H\left(\frac{A}{\theta_{A,M}}\right)\left(1 - H\left(\frac{B}{\theta_{B,M}}\right)\right) - \mu_M M \\ A(0) &= A_0, \quad B(0) = G(0) = M(0) = 0. \end{aligned} \tag{4}$$

The parameter values used by [1] are found in Table 2 in that paper. The differential equation model (4) gives rise to the Boolean network

$$\begin{aligned} f_B &= A \wedge M \wedge \neg G \\ f_G &= M \wedge \neg G \\ f_M &= A \wedge \neg B, \end{aligned} \tag{5}$$

where A again is the (constant) concentration of the signaling molecule Activin, while B, M, and G are the concentrations of the transcription factors *Brachyury*, *Mix*, and *Goosecoid*, respectively. For a more detailed biological background for these systems, see [6] and [1], as well as the references therein.

Here, for each t, the state of the system is given by $\vec{x}(t) = (A, B, G, M)^T$ for both models. Notice again that, while there are similarities between the networks, the key difference between the two is that *Brachyury* is inhibited by the presence of *Mix* in the *Xenopus* mGRN and activated by the presence of *Mix* in the axolotl mGRN (as long as *Goosecoid* is absent). To see if these are plausible models for these two species' *in vitro* mGRNs, we seek to reproduce the qualitative features seen in Figures 5 and 5 in [6] and [1], respectively. In both cases, low/medium initial levels of Activin yield high concentrations of *Brachyury*/low levels of *Mix* and *Goosecoid*, signaling the formation of mesoderm, while high levels of Activin yields high concentrations of *Mix* and *Goosecoid*/low levels of *Brachyury*, signaling the formation of anterior mesendoderm. From the point of view of Boolean networks, this bistability manifests itself by eliciting a state space that takes the initial condition $\vec{x}_0 = (A, B, G, M) = (0, 0, 0, 0)^T$ to the stable steady state $\vec{x}^* = (0, 1, 0, 0)^T$, while taking the initial condition $\vec{x}_0 = (1, 0, 0, 0)^T$ to the stable steady state $\vec{x}^* = (1, 0, 1, 1)^T$. In the next section we show that the models (3) and (5) are incapable of achieving such results, and pose alternative models that are able to reproduce this bistability.

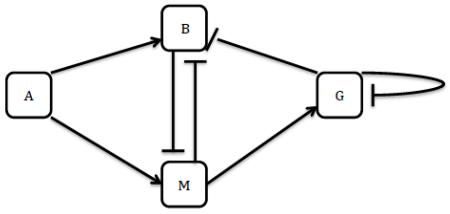

Figure 1: The wiring diagram for *Xenopus laevis*, using the network topology assumed in [6]. Here regular arrows indicate activation, while blunt arrows indicate inhibition/downward regulation.

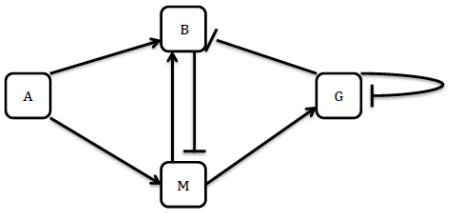

Figure 2: The wiring diagram for the axolotl, using the network topology assumed in [1]. Here regular arrows indicate activation, while blunt arrows indicate downward inhibition/downward regulation.

3 Results

Due to the self-regulation of *Goosecoid* in both (3) and (5), there are no steady state solutions for these systems wherein *Goosecoid* is activated. Thus, neither (3) or (5) model the steady-state production of anterior mesendoderm. Furthermore, since *Brachyury* requires the presence of Activin, if $A = 0$, then $B = 0$ for all t, and thus neither model predicts the the development of mesoderm, either.

Simply relaxing the self-regulation nature of *Goosecoid* in both of the models is not sufficient to obtain the desired bistability, however, since at least some Activin is required to activate *Brachyury*. Thus, we need (at least) three levels of Activin in our model. In lieu of straying from the Boolean framework, we let A_m and A_h be two Boolean variables that model medium and high levels of Activin, respectively. In this formulation, the ordered pairs $(A_m, A_h) = (0,0), (1,0)$ and $(1,1)$ indicate low, medium and high levels of Activin, respectively. With the differentiated levels of Activin, and without the self-regulation of *Goosecoid*, our models become

$$\begin{aligned} f_B &= A_m \wedge \neg(G \vee M) \\ f_G &= M \\ f_M &= (A_m \wedge A_h) \wedge \neg B \end{aligned} \tag{6}$$

for *Xenopus* and

$$\begin{aligned} f_B &= A_m \wedge M \wedge \neg G \\ f_G &= M \\ f_M &= (A_m \wedge A_h) \wedge \neg B \end{aligned} \tag{7}$$

for the axolotl. The model (6), whose wiring diagram is given by Figure 3 predicts bistability for *Xenopus*, as the initial condition $\vec{x}_0 = (A_m, A_h, B, G, M)_0 = (1,0,0,0,0)$ is in the basin of attraction for the steady state $\vec{x}^* = (1,0,1,0,0)$ corresponding to the formation of mesoderm, while the initial condition $\vec{x}_0 = (1,1,0,0,0)$ is in the basin of attraction for the steady state $\vec{x}^* = (1,1,0,1,1)$ corresponding to the formation of anterior mesendoderm (Figure 4). Thus, medium levels of Activin yield the development of mesoderm, while high concentrations of Activin yield the formulation of anterior mesendoderm, as sought.

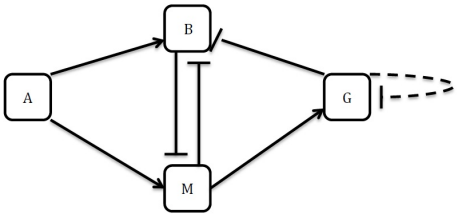

Figure 3: Updated wiring diagram for *Xenopus laevis*. Solid edges are activations and downward regulations from [6] still modeled in our Boolean network, and dashed edges represent those omitted from our updated model.

The activation of *Brachyury* by *Mix* assumed in (7) prohibits the existence of the bistability desired for the axolotl, as *Brachyury* is incapable of appearing at high levels in the absence of *Mix*, and hence the formation of mesoderm cannot be a steady state for (7). Simply omitting this activation is not enough to elicit the bistability, either, as the initial condition $\vec{x}_0 = (1,1,0,0,0)$ for this model would yield $\vec{x}_1 = (1,1,1,0,1)$, which would yield $\vec{x}_2 = (1,1,1,1,0)$, which in turn would come back to $\vec{x}_0 = (1,1,0,0,0)$, resulting in a stable three-cycle as opposed to an equilibrium. However, omitting both the activation of *Brachyury* by *Mix* and the inhibition of *Mix* by *Brachyury* (Figure 5), which results in the model

$$\begin{aligned} f_B &= A_m \wedge \neg G \\ f_G &= M \\ f_M &= A_m \wedge A_h, \end{aligned} \tag{8}$$

elicits the desired bistability (Figure 6), where medium levels of Activin yield the formation of mesoderm, while high levels of Activin yield the formation of anterior mesendoderm.

Notice that, for both species' mGRNs, it appears as though the concentration of Activin is the driving force in the ultimate outcome of the system, with the downward regulation of *Brachyury* by *Goosecoid* accounting for the bistability seen both in the differential equation models in [6] and [1], confirming experimental observations. In fact, with the exception of the basin of attraction containing the single state $(A_m, A_h, B, G, M) = (1,1,1,0,0)$ in *Xenopus laevis*, the basins of attraction for each of the steady states discussed in this section are determined completely by the initial levels of Activin in the system. In both species the size of the basin of attraction for the zero equilibrium (where neither mesoderm or anterior

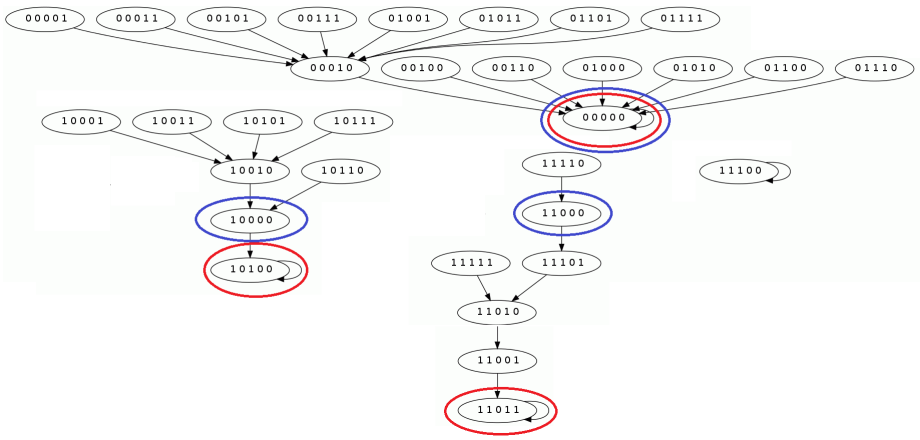

Figure 4: State space diagram for our updated model for *Xenopus laevis*, using the wiring diagram in Figure 3. Equilibria representing the lack of mesoderm/anterior mesendoderm formation (right), the formation of mesoderm (left), and the formation of anterior mesendoderm (bottom) stemming from low, medium, and high Activin levels are enclosed in the red ovals, with their corresponing intial conditions enclosed by blue ovals. This figure was created using Virginia Bioinformatics Institute's Anaysis of Dynamic Algebraic Models (ADAM) program [13].

mesendoderm are formed) is the largest, with the sizes of the basins of attraction for the other two roughly the same. The interaction between *Mix* and *Brachyury*, the key difference between the mGRNs of *Xenopus* and the axolotl, plays little to no role as to which initial conditions give rise to which equilibria, but does play a role in how quickly the state $(A_m, A_h, B, G, M) = (1, 1, 0, 1, 1)$ (modeling the formation of anterior mesendoderm) is reached. In the *Xenopus laevis* model it takes four time periods for this to occur, while in the axolotl it takes just two. In the differential equation models in [6] and [1] the concentration of *Brachyury* stays above zero for some time before *Goosecoid* and then *Mix* increase to their equilibrium values. This doesn't happen in either of our models, where the model trajectories pass through the state $(A_m, A_h, B, G, M) = (1, 1, 1, 0, 1)$ prior to converging to $(A_m, A_h, B, G, M) = (1, 1, 0, 1, 1)$.

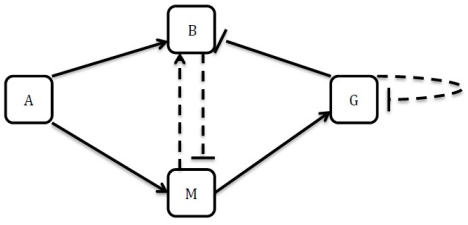

Figure 5: Updated wiring diagram for the axolotl. Solid edges are activations and inhibitions/downward regulations from [1] still modeled in our Boolean network, and dashed edges represent those omitted from our updated model.

4 Discussion

In this paper we constructed and analyzed Boolean network models for the mGRN in both *Xenopus laevis* and the axolotl in the *in vitro* setting. In both settings the initial Boolean network models are incapable of predicting the bistatilbity referred to in [6] and [1], that medium levels of the signalling molecule Activin elicit the formation of mesoderm, while high levels of this signalling molecule elicit the formation of anterior mesendoderm. In order to achieve the desired bistability, we needed to omit the assumption that *Goosecoid* is self-regulatory in all four settings, along with allowing for three levels of Activin (low, medium and high). We also needed to remove the interactions between *Mix* and *Brachyury* in wiring diagram for the axolotl mGRN, which was its key difference between its network topology and that of *Xenopus*, cited by [1].

One of the main themes in [1] was that, despite the fact that mGRNs governing the development of germ layers in the *Xenopus* and the axolotl have different network topologies, both species exibited the discussed bistability. Our results seem to suggest that the activation of *Brachyury* by *Mix* is not a substantial driver of the dynamics of the axolotl mGRN, and neither is the inhibition of *Mix* by *Brachyury*, since we were only able to achieve the desired bistability by omitting these interactions from our wiring diagram. This may be due to the fact that there is only one *Mix* gene in the axolotl mGRN, while there are seven in that of *Xenopus* [1, 2]. Additional research has modeled the fact that the spatio-temporal dynamics of *Mix* and *Brachyury* differ considerably between *Xenopus* and the axolotl, meaning the synchrony

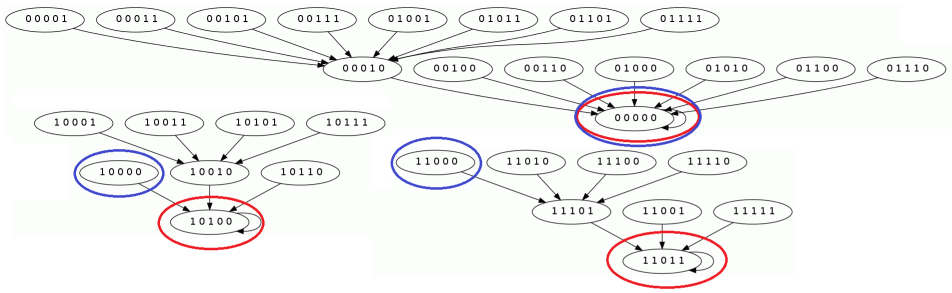

Figure 6: State space diagram for our updated model for the axolotl, using the wiring diagram in Figure 4. Equilibria representing the lack of mesoderm/anterior mesendoderm formation (upper right), the formation of mesoderm (left), and the formation of anterior mesendoderm (lower right) stemming from low, medium, and high Activin levels are enclosed in the red ovals, with their corresponding initial conditions enclosed in the blue ovals. This figure was created using Virginia Bioinformatics Institute's Anaysis of Dynamic Algebraic Models (ADAM) program [13].

assumed by simple Boolean or finite dynamical systems models may not be warranted [2]. Future work should include an analysis of similar models using asynchronous update rules as in [7].

Our results also appear to suggest that the self-regulatory nature of *Goosecoid* is also not a strong as the other interactions in either of the species' the wiring diagram. This highlights a limitation with using Boolean networks—as inhibition/regulation is often considered absolute. The presence of *Goosecoid* one time step turns it off the next, when often this self-regulation causes convergence to an equilibrium rather than eliciting a cycle. Moving from the Boolean framework to a more general finite dynamical system will eventually remove this issue, as [11] prove, but there is the increasing cost of model complexity in this case. While Theorem 3.4 in [11] says there is a bijection between the number of steady states of a finite dynamical system and those of its analogous ordinary differential equation models, the number of discrete states necessary to characterize the concentration of each of the state variables may be significantly larger than those illustrated in Figures 4 and 6.

In conclusion, we showed that Boolean network models are capable of reproducing bistable dynamics in two mesendoderm gene regulatory networks for *Xenopus laevis* and the axolotl, provided that some, possibly less influential, interactions are omitted from the network topology. The resulting network topologies for both species indicate that the concentration of Activin is the substantial driver of the mGRN dynamics, with the downward regulation of *Goosecoid* on *Brachyury* causing the differentiation between the development of mesoderm and anterior mesendoderm stemming from the different Activin levels. The differences between the mGRNs—the interaction between *Mix* and *Goosecoid*—play a far less substantial role in the equilibrium dynamics of these systems, which is possibly why similar bistability properties are seen in both systems.

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