Evaluation of BMP-mediated patterning in zebrafish embryos using a growing finite difference embryo model

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Abstract

Bone Morphogenetic Proteins (BMPs) play an important role in dorsal-ventral (DV) patterning of the early zebrafish embryo. BMP signaling is regulated by a network of extracellular and intracellular factors that impact the range and signaling of BMP ligands. Recent advances in understanding the mechanism of pattern formation support a source-sink mechanism, however it is not clear how the source-sink mechanism shapes patterns in 3D, nor how sensitive the pattern is along both the anteroposterior (AP) and DV axes of the embryo. We propose a new three-dimensional growing finite difference model to simulate the BMP patterning process during the blastula stage. This model provides a starting point to elucidate how different mechanisms and components work together in 3D to create and maintain the BMP gradient in the embryo. We also show how the 3D model fits the BMP signaling gradient data at multiple time points along both axes. Furthermore, sensitivity analysis of the model suggests that the spatiotemporal patterns of Chordin and BMP ligand gene expression are dominant drivers of shape in 3D and more work is needed to quantify the spatiotemporal profiles of gene and protein expression to further refine the models.

1. Introduction

Pattern formation by morphogens drives the normal development of various processes such as limb development and organogenesis in animals (Maini and Solursh 1991; Lecuit and Cohen 1998; Sansom and Livesey 2009; Rogers and Schier 2011; Rushlow and Shvartsman 2012; Bökel and Brand 2013; Tuazon and Mullins 2015). In zebrafish, patterns of gene expression along the dorsal-ventral (DV) body axis are regulated by Bone Morphogenetic Proteins (BMPs)(Tucker et al. 2008). BMPs are a member of the TGF (transforming growth factor β, TGF- β) superfamily. Very early in embryonic development, both invertebrates and vertebrates require BMP signaling

to pattern the DV axis(De Robertis and Sasai 1996; Holley and Ferguson 1997). Researchers have shown that BMPs pattern DV tissues of zebrafish, *Xenopus,* and *Drosophila* embryos by using a gradient-based mechanism, in which different levels of BMP signaling drive differential gene expression (Little and Mullins 2006).

BMP signaling is propagated by binding of BMP dimers to serine/threonine kinase receptors on the cell membrane. Type I and II receptors form higher order tetrameric complexes and phosphorylate intracellular Smads (P-Smad5 in zebrafish) that accumulate in the nucleus and regulate differential gene expression. BMP signaling is regulated by different molecules at multiple levels: extracellular, intracellular, and on the membrane(Wang et al. 2014). These regulators form a system that enhances, lessens or refines the level of BMP signaling. One group of regulators are the inhibitors of BMP signaling (Little and Mullins 2006; Umulis et al. 2009; Tuazon and Mullins 2015). BMP inhibitors include Twisted gastrulation, Chordin and Crossveinless2, Follistatin, Sizzled, and Noggin, (Khokha et al. 2005; Dal-Pra et al. 2006; Umulis et al. 2009; Wagner et al. 2010; Dutko and Mullins 2011), most of which act by binding BMP ligands, preventing them from binding their receptors. Downstream intracellular regulation of BMP signaling occurs throughout the BMP-Smad pathway(von Bubnoff and Cho 2001). Inhibitory Smads modulate BMP signaling, either by the function of interacting with Type I BMP receptors or by preventing R-Smads from binding Smad4. Others molecules, such as microRNAs and phosphatases may also act as intercellular modulators(Luo et al. 2015).

In our previous work, we developed a data-based 1-D modeling approach to investigate the mechanisms of BMP-mediated DV patterning in blastula embryos through 5.7 hpf before the initiation of BMP-mediated feedback. We quantified BMP signaling in wild-type (WT), *chordin* mutant, and *chordin* heterozygous embryos. In our previous model screen, we simulated BMP gradient formation along a 1D line at the embryo margin and used the quantitative measurements of P-Smad to inform our model selection. We concluded that the signaling gradient patterning the vertebrate DV axis is most consistent with a source-sink mechanism.

These models lay the ground work for our current study where we seek to add growth and 3D patterning to the model and test mechanisms of patterning in 3D. There is a significant need for spatially and temporally accurate 3D models of the early embryo to evaluate reaction-diffusion processes of chemical morphogens including BMP ligands. The development of accurate 3D models has been limited due to the complexity of embryo structure, and the computational resources needed to run parametric screens in 3D models. The work here builds off of previous work carried out by ourselves and others. Previous work in Drosophila focused on a three-dimensional organism-scale model of BMP and gap gene patterning in the *Drosophila* embryo (Umulis et al. 2010; Hengenius et al. 2011; Umulis and Othmer 2015). In zebrafish, the 3D structure shares some similarities but many differences with *Drosophila*, especially in regard to the growth during epiboly and the potential role of cell movement in shaping the gradient in the zebrafish. To date there are few mathematical models for zebrafish embryonic development and only one that developed a 3D approximation by Zhang et al. to study the role of Chordin in regulating BMP signaling in zebrafish (Zhang et al. 2007). This model focused on the blastula stage from 30% epiboly to shield stage (around 50% epiboly), however, it did not include growth, was not compared to data, and suggested a mechanism of BMP shuttling that has now been shown not to function in the embryo.

The 3D model developed here accounts for growth from 30% to 50% epiboly. Early zebrafish embryo development proceeds through a sequence of stages. The complete process includes involution, convergence, and extension until the cells cover the whole sphere-like

embryo(Kimmel et al. 1995). BMP signaling begins patterning the ventral tissues by the onset of gastrulation (Tucker et al. 2008). During gastrulation, coordinated cell movements organize the germ layers and establish the primary body axes of the embryo (Lepage and Bruce 2010). Epiboly begins during mid blastula stages (30% - 50% epiboly) and continues through the gastrula stage and entails a thinning and expanding of the cell layers from the animal pole, where the blastoderm lies, to cover the yolk cell over the vegetal pole(Warga and Kimmel 1990). While our final goal is to develop a complete advection-diffusion-reaction model that incorporates all stages of zebrafish embryos development, our current data and the development of the model herein that is tested against the data covers the first half of development up through 50% epiboly and also serves as a greater test of the source-sink mechanism. Herein, we find that a finite difference model for an embryo with growth through epiboly demonstrates that the source-sink mechanism patterns well in 3D, however, sensitivity analysis suggests that the prepatterns of mRNA expression of BMP ligands and the inhibitor Chordin play a large role in dictating the overall shape and dynamics of the BMP gradient. Additional work is needed to quantify the gene expression domains and map them into the 3D modeling environment to improve the model for greater understanding of the inhibitors roles in shaping the gradient in 3D.

2. Method

To perform a computational screen of mathematical models to fit the WT P-Smad5 gradient profiles, we first needed to determine the production domains of BMP, Chordin, Noggin, and Tolloid to use for the mathematical model. We used the RNAscope method on whole mount embryos to collect the profile of relative expression of *bmp*, *chd*, and *nog* mRNA throughout the smFISH embryo

The embryos were fixed at the desired developmental stages with 4% PFA at RT for 4 hours or at 4°C for 24 hours and washed with 0.1% PBSTween 3 times at RT, each for 10 min. 20-30 embryos were separated into 1.5ml RNase free tube. The chorions were removed, and the embryos gradually dehydrated from 25% methanol in PBST, 50% methanol in PBST, 75% methanol in PBST to 100% methanol, each for 5 min at RT. Store the embryos at -20 °C at least one day and up to 15 days. Two drops of Pretreat 3 (ACD, #320045) were added at RT for 15min to permeate the embryos. Counterstaining of the probes use the RNAscope Fluorescent multiplex detection reagents (ACD, #320851). The user's manual (323100-USM) is available online, however, we made some modifications. We performed probe hybridization at 40 °C for 16 hours, and C2 probes need to be diluted by C1 probes. Detailed information about the probes is shown in Table 1. All wash steps are performed three times using 0.2x SSCT for ten minutes each time. DAPI was used to stain the nuclei at 4 °C overnight. We chose AltC for Amp4 in the staining kit.

Embryos were mounted in 1% low melting agarose on 35mm glass bottom microwell dishes (Matek, P35G-1.5-10-C). Whole-mounted embryos were imaged with a 20×/1.0 Plan-Apochromat water immersion lens (D = 0.17 M27 75 mm). *chd* mRNA was imaged by excitation 555nm wavelength. *bmp2b* and *nog* mRNA were imaged by excitation 647nm wavelength.

Table 1, **RNA Probe Information**

probe	ACD catalog No.	Accession No.	Target Region	Probe dilution
Bmp2b-C2	456471	NM_131360.1	336 - 1238	1:20
Chd-C1	440081	NM_130973.2	387 - 1302	1:1
Nog-C2	476651-C2	NM_130983.2	2 - 1328	1:50

We approximated simple expression regions from confocal images to test the model. Various expression regions were tested for all three components (BMP, Chd, and Nog). Figure 1 illustrates a set of expression regions for BMP, Chd, and Nog that were tested. For the simulations, the image data were converted into binary regions of expression and no expression. As shown in Figure 1, BMP production region is limited to the ventral region, and Chd and Nog are limited to the dorsal side. As shown in Figure 1C, the Nog production region is smaller than the Chd production region.

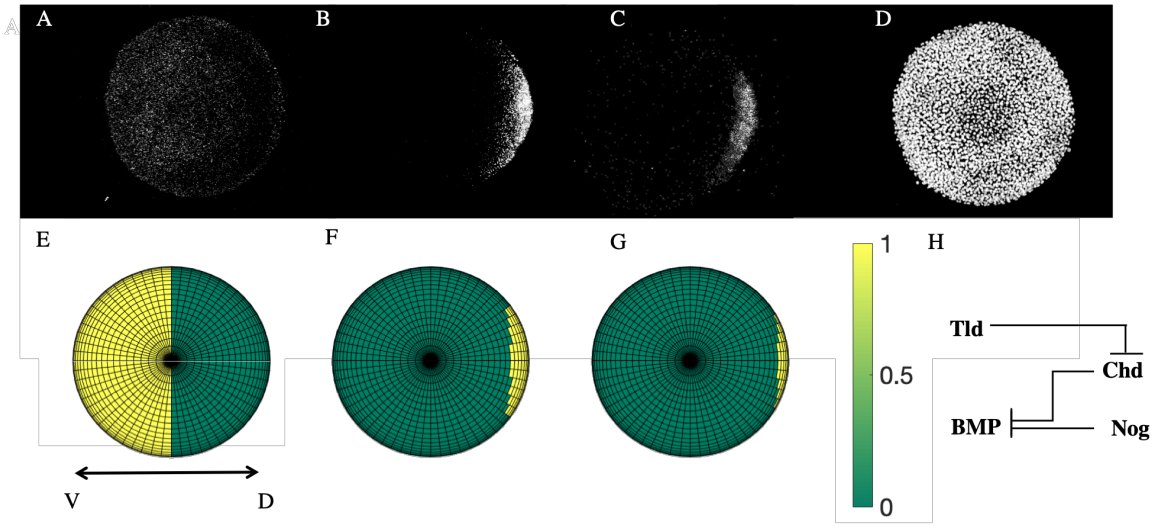

Figure 1, Whole mount experimental result of bmp2b (A), chd (B), nog(C) mRNA expression and nucleus positions (D), view from the animal pole. (E-G) Simulation input of expression area in WT for BMP Chd and Nog, respectively. Yellow color indicates an area of production for different species (H) Simplified BMP regulation network using in this study.

For early blastula development, we first estimated the potential role of advection in shaping the gradient based on our estimates for diffusion and the rates of cell movement in early development. We quantified the cell movement during epiboly by using the digital embryo data presented by Keller et al. (Keller et al. 2008) and quantified individual cell traces from 30% to 50% epiboly. We estimated the average Péclet number for diffusive vs advective transport and found that the Péclet number is much less than 1, supporting diffusion dominant transport prior to 50% epiboly. Later during gastrulation, we found that the Peclet number is approximately 1, suggesting a need for simultaneous advection and diffusion, and this is discussed in greater detail later herein. Thus, for the blastula stage embryo we can assume this problem as a moving domain non-advection problem. The zebrafish embryo is approximated as a perfect hemisphere and the reaction-diffusion process happens on the surface of the sphere. Using a hemisphere allows us to discretize the model using the spherical coordinate system. We solved the five-coupled non-linear partial differential equations (PDEs) for BMP ligand, Chordin, Noggin, and the complexes of BMP-Chordin, BMP-Noggin in MATLAB. The model solves the spatio-temporal diffusion problem in spherical coordinates using the derivative in expanded form:

$$\frac{\partial C}{\partial t} = D\nabla^2 C$$

into:

$$\frac{\partial C}{\partial t} = \frac{D_C}{r^2}\left(\frac{\partial}{\partial r}\left(r^2\frac{\delta c}{\delta r}\right) + \frac{1}{sin^2\theta}\frac{\partial^2 C}{\partial \emptyset^2} + cot\theta\frac{\partial C}{\partial \theta} + \frac{\partial^2 C}{\partial \theta^2}\right)$$

Note, C here represents a generic concentration, where C in the following equations represents the concentration of Chordin.
For each species:

$$\frac{\partial B}{\partial t} = \frac{D_B}{r^2}\left(\frac{1}{sin^2\theta}\frac{\partial^2 B}{\partial \phi^2} + cot\theta\frac{\partial B}{\partial \theta} + \frac{\partial^2 B}{\partial \theta^2}\right) + \phi_B(x) \; -k_1 B\cdot C + \; k_{-1}BC - k_2 B\cdot N + k_{-2}BN + \lambda_1 Tld\cdot BC - k_b B$$

$$\frac{\partial C}{\partial t} = \frac{D_C}{r^2}\left(\frac{1}{sin^2\theta}\frac{\partial^2 C}{\partial \phi^2} + cot\theta\frac{\partial C}{\partial \theta} + \frac{\partial^2 C}{\partial \theta^2}\right) + \phi_C(x) \; -k_1 B\cdot C + \; k_{-1}BC - \lambda_2 T\,ld\cdot C - k_c C$$

$$\frac{\partial N}{\partial t} = \frac{D_N}{r^2}\left(\frac{1}{sin^2\theta}\frac{\partial^2 N}{\partial \phi^2} + cot\theta\frac{\partial N}{\partial \theta} + \frac{\partial^2 N}{\partial \theta^2}\right) + \phi_N(x) \; -k_2 B\cdot N + \; k_{-2}BN - k_n N$$

$$\frac{\partial BC}{\partial t} = \frac{D_{BC}}{r^2}\left(\frac{1}{sin^2\theta}\frac{\partial^2 BC}{\partial \phi^2} + cot\theta\frac{\partial BC}{\partial \theta} + \frac{\partial^2 BC}{\partial \theta^2}\right) + \; k_1 B\cdot C - \; k_{-1}BC - \lambda_1 Tld\cdot BC - k_{BC}BC$$

$$\frac{\partial BN}{\partial t} = \frac{D_{BN}}{r^2}\left(\frac{1}{sin^2\theta}\frac{\partial^2 BN}{\partial \phi^2} + cot\theta\frac{\partial BN}{\partial \theta} + \frac{\partial^2 BN}{\partial \theta^2}\right) + k_2 B\cdot N - \; k_{-2}BN - k_{BN}BN$$

Since we limited the domain on the sphere surface, the diffusion in the r direction can be ignored. The above equation holds in the spatial domain Ωt for all 0 < t < T. B, C, N, BC, BN and Tld represent the concentration of BMP, Chordin, Noggin, BMP-Chordin complex, BMP-Noggin complex, and Tolloid, respectively. *Di* represents the diffusion rate for each species, S_i is the constant term for original expression of the specific gene, k_n and k_{-n} are the association and dissociation constant for reactive ligands, λ_n is the Tld processing rate for Chd and BC complex, and k_i is the decay rate for specific ligands. The finite difference decentralization scheme is based on elevation and azimuth angles in spherical coordinates. Since the experimental result indicates that the development of zebrafish embryo is symmetrical during the blastula and gastrula stage, we only calculate our model in a partial hemisphere domain to decrease the computation and storage load. No-flux boundary conditions are applied for all species on both the ventral and dorsal boundaries. To avoid the singularity that happens on the top points where elevation angle θ is equal to zero, we used a hollow-shaft approximation method presented by Thibault et al.(Thibault et al. 1987) In which mesh point $\theta = 0$ is eliminated by introducing a small but finite interior surface $\theta = f_\theta \Delta\theta$, we have tested and used $f_\theta = 0.1$ in this study. The simulation domain will increase in size by adding a layer in the elevation direction to reflect embryo changes during epiboly.

To test the finite difference models developed herein, we used the previously published point cloud data for P-Smad5 (readout of BMP signaling) that is available online at Zinski et al. (Zinski et al. 2017). To apply these data to our model, we processed the data by fitting the original data to a standard size hemisphere with different coverage along the elevation direction based on the stage of embryo development. Different sets of experimental data in the same stage were averaged on the sample points over the globular domain to obtain the representative P-Smad level for each stage, Figure 2B.

We adopted the mathematical model from our previous 1D margin-based model ((Zinski et al. 2017)) to our 3D whole embryo growth domain model. To evaluate the system over a wide range of possibilities, we developed a computational model-based screen of over 300,000 combinations of biophysical parameters of the major extracellular BMP modulators. Each parameter combination was then re-simulated without Chordin to predict the BMP signaling gradient in a *chordin* loss-of-function (LOF) scenario. Based on previous studies (Pomreinke et al. 2017)(Zinski et al. 2017), the diffusion rate and decay of ligand BMP and Chd are fixed as constant, 4.4 $\mu m^2/s$ and 8.7×10^{-5} /s for BMP, and 7 $\mu m^2/s$ and 9.6×10^{-5} /s for Chd, respectively. The parameters space for the rest of unknown parameters consist of the range used in Zinski et al's study (see

Table 2). The model is solved for the developmental window that spans from 3.5 to 5.7 hpf, and all measurements of model error are calculated at 4.7 hpf, 5.3 hpf, and 5.7 hpf.

Results

We identified the simulations generating BMP profiles that fit the measured P-Smad5 gradient at 4.7, 5.3, and 5.7hpf using the normalized root mean squared deviation (NRMSD) to validate our simulation results for both WT and Chd LOF. Figure 2 demonstrates a simulation result for a WT case at these time points. The simulated BMP concentration level and measured P-Smad5 profiles are normalized between 0 and 1 to calculate the relative error between each profile on the entire domain (Figure2B). In our current simulations, we find good agreement throughout the embryo, except on the ventral-anterior side and the middle region between ventral and dorsal end, that exhibits a relative error of ~12% (Figure 2C). The current best-fitted parameter set is listed in Table 2. Compared with the 1D results of Zinski et al., based on the current best-fitted parameters, and BMP and Chd expression range, the result is consistent with the source-sink mechanism in which BMP diffuses from its source of ventral production to a sink of dorsal Chd (Figure 2A). The simulations result in a wider range of BMP accumulation in the ventral region than the relative P-Smad5 level. As the simulation processes, the difference accumulates in the ventral-anterior region and at the lateral portion of the margin for later stages. The likely source for this error is the assumption of binary expression domains (Figure 1E-G).

Table 2, Parameters range

Parameters	Range	Best Fit	Unit
D_B (BMP Diffusivity)	4.4	4.4	$\mu m^2/s$
D_C (Chd Diffusivity)	7	7	$\mu m^2/s$
D_N (Nog Diffusivity)	10^{-2}~10^{2}	0.58	$\mu m^2/s$
D_{BC} (BC Diffusivity)	10^{-2}~10^{2}	25.89	$\mu m^2/s$
D_{BN} (BN Diffusivity)	10^{-2}~10^{2}	5.09	$\mu m^2/s$
k2 (Association Constant for BMP and Chd)	10^{-4}~1	0.14	nM
k_2 (Dissociation Constant for BMP-Chd)	= k2	0.14	nM
k3(Association Constant for BMP and Nog)	10^{-4}~1	2.56 x10^{-4}	nM
k_3(Dissociation Constant for BMP-Nog)	= 0.1k3	2.56 x10^{-5}	nM
k_B (Decay rate of Ligand BMP)	8.7x10^{-5}	8.7x10^{-5}	1/s
k_C (Decay rate of Ligand Chd)	9.6x10^{-5}	9.6x10^{-5}	1/s
k_N(Decay rate of Ligand Nog)	10^{-1}~10^{-5}	0.05	1/s
k_{BC} (Decay rate of Ligand BC)	10^{-1}~10^{-5}	0.0015	1/s
k_{BN} (Decay rate of Ligand BN)	10^{-1}~10^{-5}	6.87x10^{-4}	1/s
S_B (Production rate of BMP)	10^{-2}~10^{2}	0.41	nM/s
S_C(Production rate of Chd)	10^{-2}~10^{2}	17.34	nM/s
S_N (Production rate of Nog)	10^{-2}~10^{2}	0.02	nM/s
λ_C (tld processing rate of Chd)	1~10^{-4}	0.0016	1/s
λ_{BC} (tld processing rate of BC)	1~10^{-4}	0.0022	1/s

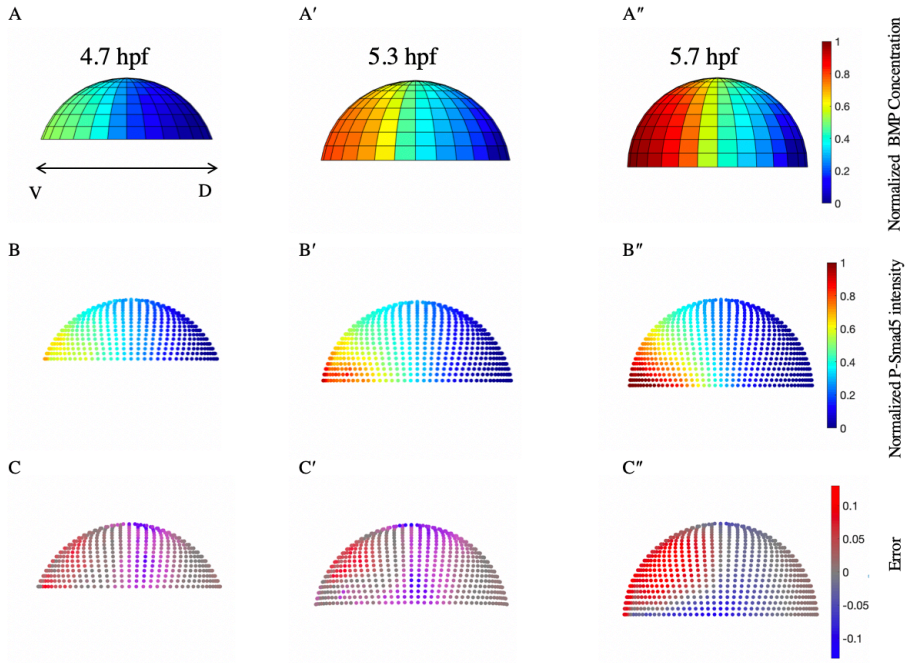

Figure 2 A, A' and A", Normalized simulation result of a wild type case results of 4.7hpf, 5.3 hpf, and 5.7hpf. B, B' and B", Averaged and normalized P-Smad5 profile of 4.7hpf, 5.3 hpf, and 5.7hpf. C,C' and C", Relative differences between simulation results and P-Smad5 level. Positive error indicates the expreimental data is higer than simulaiton results, negtive error indicates the expreimental data is lower than simulaiton results.

To clarify the differences between simulation results and P-Smad profiles, we isolated the profiles along the embryo's margin and central region for WT and Chd LOF in Figure 3 for the current best-fit model. The central region is defined as the region connecting the ventral and dorsal ends through the animal pole. Thus, for different growth stages, the number of indices measured radially along the margin remains the same, but the central region covers increasing radial portions with epiboly. The relative level of BMP ligands on the margin region agree with the experimental P-Smad5 profile for different time points (Figure 3A, 3C). However, the central profile shows a gap for the Chd LOF model. Compared to the experimental profile, which has a nearly linear drop from ventral to dorsal, the simulation results' profiles show a stronger sigmoidal shape. Again, this may be caused by the sharp boundary of BMP production in the lateral region between ventral and dorsal or the sudden appearance of inhibiting Chd ligands. Based on the current data (Zinski et al. 2017) the P-Smad5 gradient in *chordin* mutants showed a statistically significant increase in lateral regions of the embryo during these time points. Our results demonstrate the WT model has relatively better fitness on the central line compared to the Chd LOF model (Figure 3 A-D). The fitness at 4.7, 5.3, and 5.7 hpf for WT exhibits an overall consistency and solutions lay within one standard deviation of the measured mean, however, the earlier 4.7 hpf timepoints between the model predictions and PSmad data for the Chordin mutants show greater differences along the line that travels through the animal pole (central, Figure 3D).

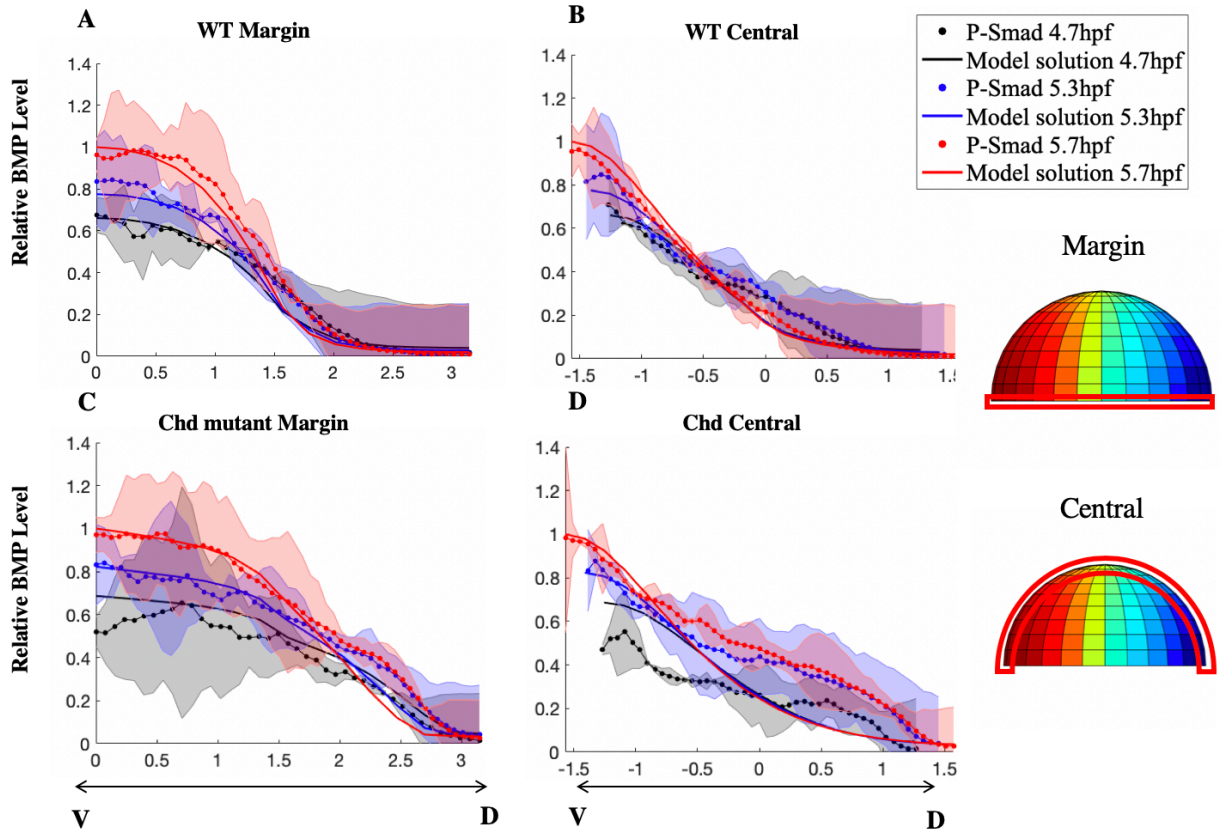

Figure 3, BMP distribution of a single modeling simulation result compared with P-Smad 5 profile. A, WT case profile in the margin region. B, WT case profile in the central region. C, Chordin LOF case on margin region D, Chordin LOF case on the central region. All graphs are from ventral to dorsal as indicated in the bottom row. Black color represents 4.7 hpf, Blue color represents 5.3hpf, Red color represents 5.7hpf. The shaded region indicates standard deviation for individual experimental data, represented by the dotted line. The x-axis indicates the radial position measured every degree.

To determine the sensitivity of the solutions to model parameters and identify the likely contributors to the data-model mismatch, we developed a local-sensitivity analysis. Figure 4 A-D shows the parameter space for different unknown parameters vs. error between normalized simulation results and P-Smad5 averaged intensities. The best parameters among 100,000 random sets were chosen as the starting point to test the local sensitivity. Compared to other unknown parameters, the production-related parameters demonstrate the greatest sensitivity for the simulation error. As shown in Figure 4A, the model fitness changed most in response to BMP and Chd production rate changes, where the smallest error of 5% occurred with BMP production at 0.4 nM/s and Chd at 17.7 nM/s. Interestingly, model fitness does not vary with Nog production rate. The current best fit parameter set is a Chd dominant model, where the production rate of Chd (17.34 nM/s) is much higher than the Nog production rate (0.02 nM/s). Thus, the WT model sensitivity shows that Chd dominates the sharp BMP gradient profile rather than Nog, consistent with previous 1D modeling results(Zinski et al. 2017). Since the decay rates and diffusivities of BMP and Chd are fixed based on measurements(Pomreinke et al. 2017; Zinski et al. 2017), the local sensitivity for the decay rates and diffusivities of Nog, BMP-Chd complex, and BMP-Nog complex are compared in Figure 4B and 4C. The model shows limited sensitivity to the decay rate of BMP-Chd. The model fitness is not sensitive to the decay rate and diffusivity of Nog. The Tld processing rate of Chd and the BMP-Chd complex show similar and relatively large sensitivities. The best Tld processing rate of Chd appears at 1.6×10^{-3}/s, and model fitness worsens considerably

when Tld processing rate of BMP-Chd complex increases beyond 4.1×10^{-3}/s. Also, the model shows less sensitivity to the association constant for BMP/Chd and is insensitive to the association constant for BMP/Nog. Together, the parameters related to Noggin show minimal effects on model fitness based on our current results, since the current best fit model is a relatively Chd dominate model. This also indicates that a precise production region of the major components (BMP, Chordin, Noggin and Tld) and production rate of them could be the critical factors that increases the fitness quality of our current model. Identifying a global best fit is also limited by sparse parameter sampling.

To relate the model to the observed phenotypes, we tested how the expression range of BMP, Chd, and Nog will influence the maximum concentration level of BMP ligands by using the same best-fit parameter set. Shown in Figure 4E, as the expression range of BMP increases, the maximum BMP ligand concentration increases. On the other hand, as Chd expression decreases, the maximum concentration level of BMP ligand increases and leads to a dramatic increase when Chd expression range is less than 10% of the embryo length. As BMP expression widens, the BMP ligand concentration profile widens on the margin but does not lead to a substantial difference on the central region (Figure 4F). Alternatively, as Chd expression widens, it sharpens the BMP ligand concentration profile and limits the BMP ligand range for both margin and central region (Figure 4G).

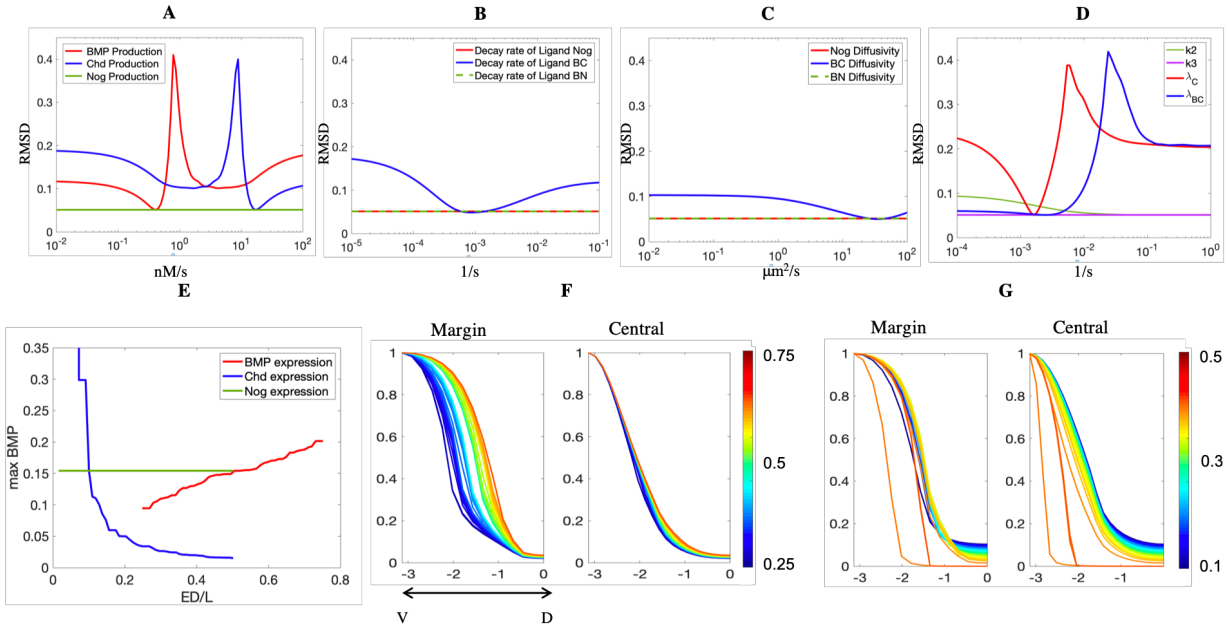

Figure 4, Local sensitivity analysis for unknown parameters. A, production rate for BMP, Chd, and Nog. B, Decay rate for Nog, BMP-Chd complex, and BMP-Nog complex. C, Diffusivity of Nog, BMP-Chd complex, and BMP-Nog complex. D, Tld processing rate of Chd and BMP-Chd complex, Association constant for BMP/Chd (k2) and BMP/Nog (k3). For Figure A-D, the x-axis represents the parameters' value in log scale. The y-axis represents the error of the simulation result for the normalized BMP ligand concentration compared to the normalized experimental P-Smad intensity. E, Maximum BMP concentration changes as expression domains change with BMP, Chd and Nog. X-axis represents the proportion of expression domain vs embryo length. F. Normalized BMP profile on margin and central line changed by proportional expression range of BMP. G. Normalized BMP profile on margin and central line changed by proportional expression range of Chd. X-axis represents the radial position from Ventral (left) to Dorsal (right). Red line indicates a wider range of BMP or Chd expression, respectively.

Discussion

One advantage of the 3D geometry proposed here is that it enables observation of the variation of the level through the whole embryo domain. Though morphogen activity along the margin has offered preliminary insight into DV axis patterning, differences in model fitness are observed along the margin vs. the meridian line that passes through the animal pole.

The major challenge for this study is the vast unknown parameter space and computationally expensive simulation. The 3D growing embryo scheme is considerably more complex compared to a fixed 1D or 3D model. Using the finite difference scheme, the model growth increments are at a constant radial rate along the longitudinal direction and also along a regular shape, which limits our capability to introduce a more realistic embryo shape and incorporate the advection caused by the cell mobility during later epiboly. However, by controlling the mesh size and growth duration in a 3D fashion that considers both the spatial and temporal aspects of embryo-scale modeling, this paradigm will still provide a powerful framework on which to test different mechanisms that 1D and fixed domain models cannot support.

Local sensitivity analysis provides direct evidence that the BMP gradient is sensitive to the gene expression inputs for BMP ligand, Chordin, and Tld processing. Thus, for future studies, we are collecting *bmp*, *chordin*, *noggin*, and *tld* quantitative mRNA wholemount expression profiles at different stages. This work contributes to our long-term goal of developing 3D models of the embryo with growth and advective cell movement, quantitative gene expression, and feedback to determine the interplay of these processes on pattern formation. The study here, is the first step and provides a 3D mathematical model on a growing domain and provides a computational framework to elucidate how the components work together through the whole embryo domain to establish the BMP gradient at multiple time-points in the blastula embryo.

Acknowledgements

We would like to acknowledge funding from NIH grants R01HD073156 to DMU, and NIH R01GM056326 to MCM, and editing help from Matt Thompson.

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