Cell Reports

Supplemental Information

Axis Patterning by BMPs: Cnidarian Network Reveals Evolutionary Constraints

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Supplemental Figures

Figure S1- related to Figure 1.

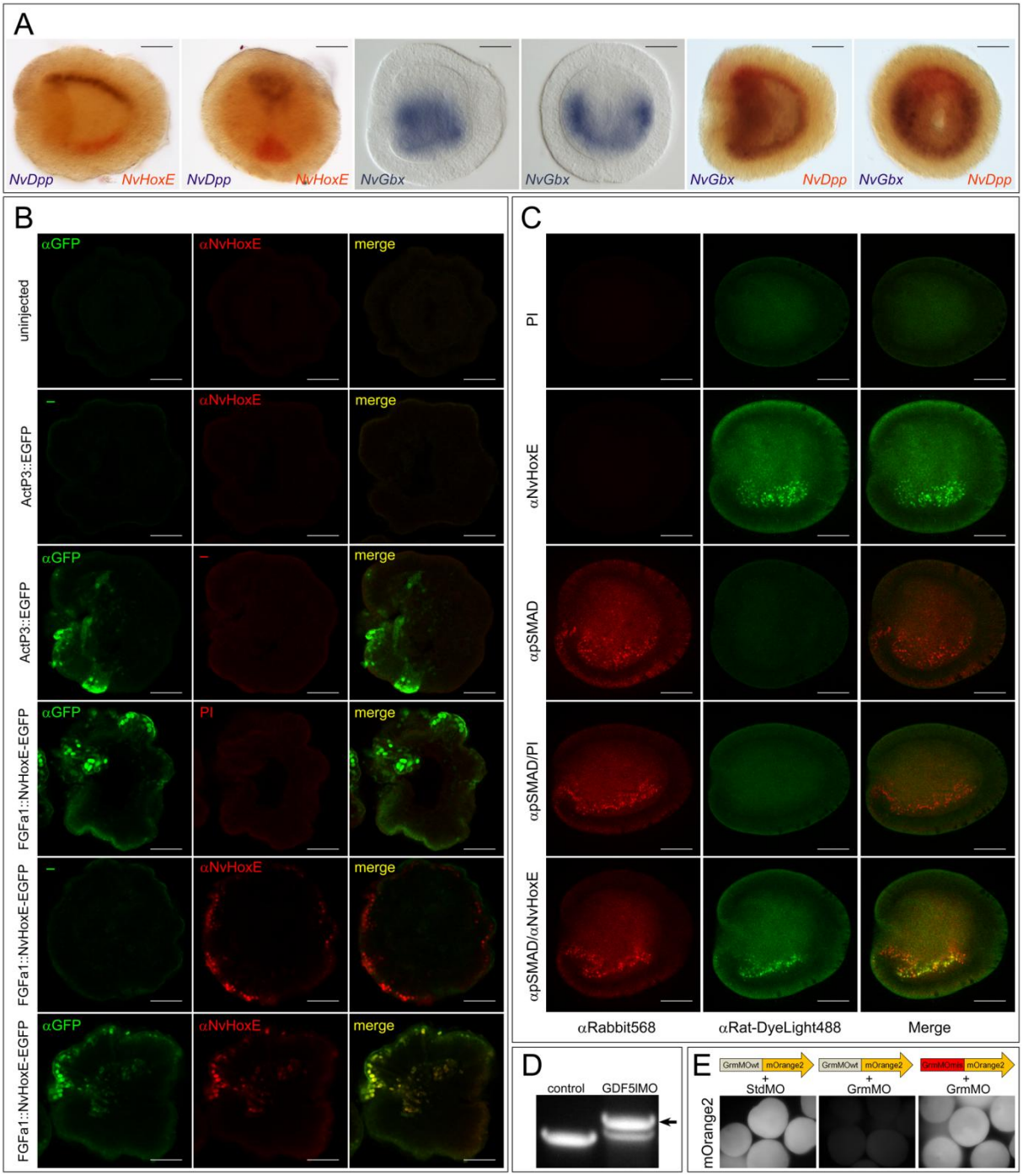

Figure S1 – related to Figure1. Controls for the determination of the BMP signaling side.

(A) *NvDpp* and *NvHoxE* are expressed on the opposing ends of the directive axis in early planula; *NvGbx* is expressed throughout the *NvDpp*-free part of the endoderm.

(B) Anti-NvHoxE antibody specifically stains NvHoxE. All stainings performed at gastrula stage, when no endogenous NvHoxE is expressed. Transgenic ActP3::EGFP - embryos mosaicly expressing EGFP under *Nematostella β-actin* promoter in a subset of cells. Transgenic FGFa1::NvHoxE-EGFP - embryos mosaicly expressing NvHoxE-EGFP fusion protein under *Nematostella FGFa1* promoter in a subset of cells. Both, anti-rat-DyLight549 and anti-rabbit-Alexa488 secondary antibodies were added to each sample. Primary antibodies are marked on the image. Minus sign stands for no primary antibody added. Uninjected embryos are not stained by either anti-GFP or anti-NvHoxE. EGFP expressing embryos are stained only by anti-GFP antibody and not by anti-NvHoxE antibody. NvHoxE-GFP expressing embryos stain with both anti-GFP and anti-NvHoxE antibody and do not stain with rat pre-immune serum (PI).

(C) Anti-NvHoxE antibody specifically stains endodermal nuclei on the pSMAD-positive side of the embryo. Combinations of the primary and secondary antibodies are marked. PI – rat preimmune serum.

(D) Injection of the GDF5lMO results in the retention of an intron with several stop codons in all reading frames in the majority of the *NvGDF5-like* transcripts as revealed by RT PCR. Arrow points at a PCR product containing an intron.

(E) GrmMO specifically inhibits the translation of the target mRNA with a fully complementary recognition sequence and not the one carrying 5 mismatches. Early planulae injected with the in vitro transcribed mRNA carrying the wild type recognition site for GrmMO (GrmMOwt) followed by *mOrange2* sequence are stronlgy fluorescent if co-injected with StdMO but not if co-injected with GrmMO. Early planulae co-injected with mRNA carrying the recognition site for GrmMO with 5 mismatches (GrmMOmis) followed by *mOrange2* sequence are strongly fluorescent and not affected by GrmMO.

Scale bars 50 μm.

Figure S2 - related to Figure 2.

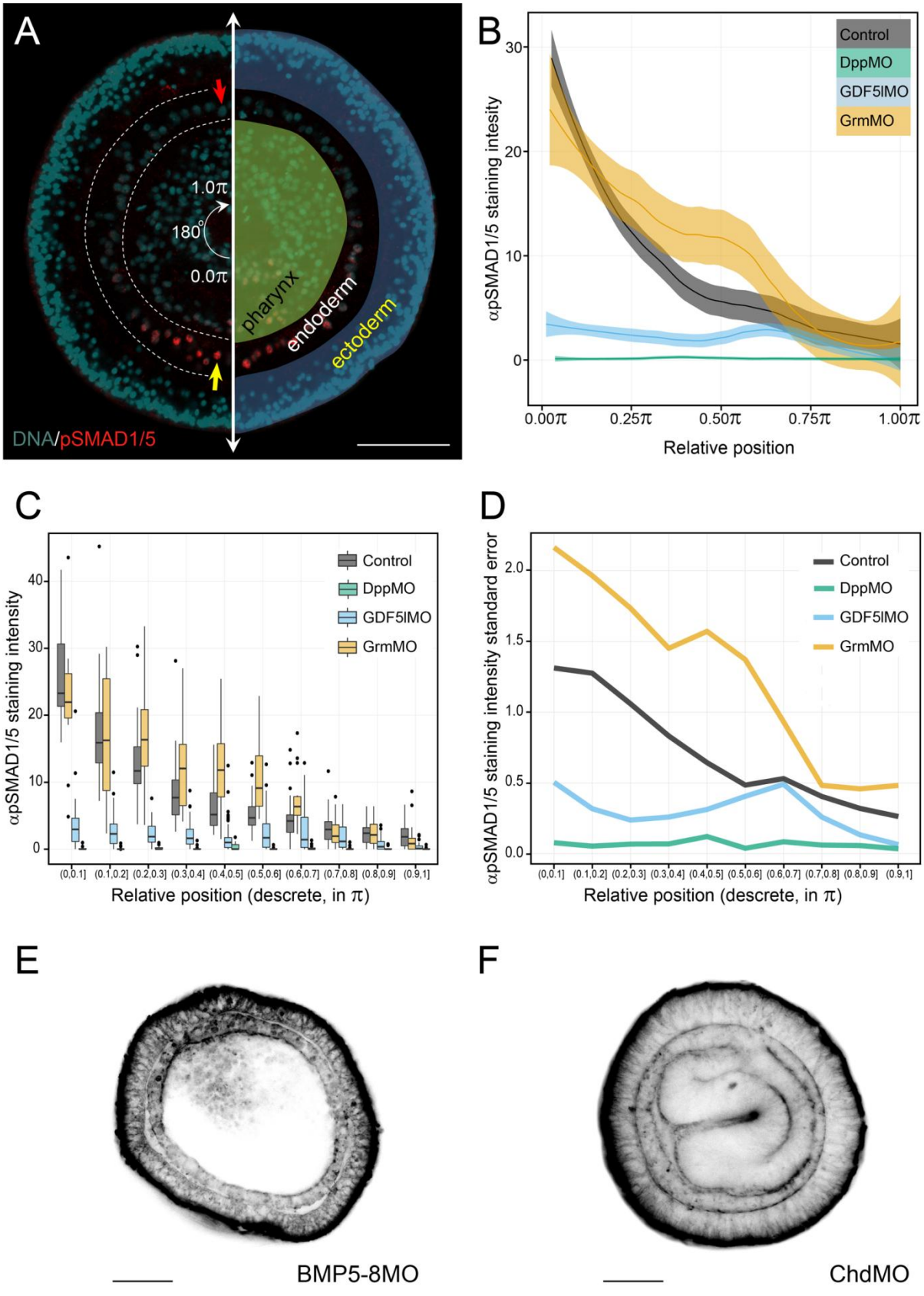

Figure S2, related to Figure 2. Statistical analysis of the BMP signaling gradient and the morphology of the BMP5-8MO and ChdMO late planulae.

(A) The αpSMAD1/5 staining intensity was measured along the arc of 180^0 in the body wall endoderm nuclei (between dashed white lines). The first nucleus to be measured (yellow arrow) is located closest to the centre of the intensively pSMAD1/5-positive domain; the last nucleus to be measured is located at the opposite side of the embryo (red arrow).

(B) αpSMAD1/5 staining intensity as a function of relative position (represented as fraction of the 180^0 arc) along the endoderm perimeter, described by a LOESS smoothed curve (solid line) with a 99% confidence interval for the mean (shade) based on individual measurements from multiple embryos for Control (n=12), GrmMO (n=7), GDF5lMO (n=10), and DppMO (n=8). Relative position allows compensating for different numbers of nuclei in different embryos and gives each nucleus its coordinate. It corresponds to the sequential number of each nucleus divided by total number of endodermal nuclei along the 180^0 arc in the particular embryo (as in A).

(C) pSMAD1/5 staining intensity as a function of relative position along the endoderm perimeter (represented as fractions of the 180^0 arc from 0 to π) in ten equally sized discrete windows, with box plots representing measurements from multiple embryos for Control (n=12), GrmMO (n=7), GDF5lMO (n=10), and DppMO (n=8). The pSMAD1/5 intensity was significantly higher in GrmMO than Control ($p<0.05$, Wilcoxon exact test) in the bins in the range (0.2,0.7]. The pSMAD1/5 intensity was significantly lower ($p<0.01$, Wilcoxon exact test) in GDF5lMO and DppMO treated embryos compared to Control in all positional bins.

(D) Bootstrap estimate of the standard error (SE) for pSMAD1/5 staining intensity for Control (N=12), GrmMO (N=7), GDF5lMO (N=10), and DppMO (N=8) treated embryos as a function of relative position along the endoderm perimeter (represented as fractions of the 180^0 arc) in equally sized discrete windows. The SE for pSMAD1/5 intensity is significantly higher in GrmMO than Control ($p<0.01$, F test) in the bins in the range (0.4,0.6]. A comparison of the residuals in linear model fits of pSMAD1/5 staining intensity to

logarithmic and linear independent variables representing relative position showed that globally, the variance is significantly higher for the GrmMO treated embryos than Control (p-value = 6.893e-07, F test).

(E-F) Oral views of the BMP5-8MO and ChdMO late planulae stained with fluorescent phalloidin. Morphants do not show any sign of mesentery formation.

Scale bars 50 µm.

Figure S3 - related to Figures 3 and 4.

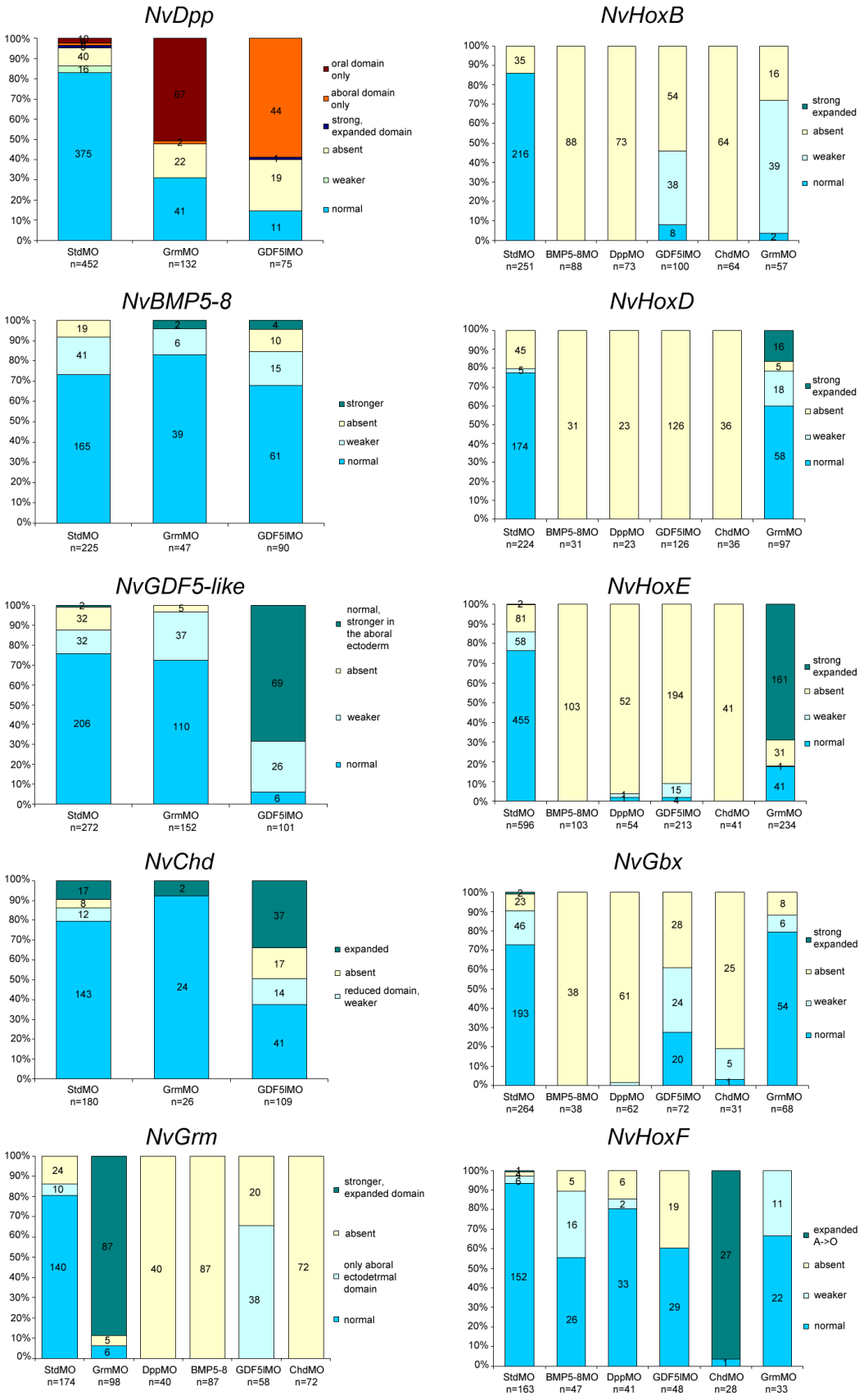

Figure S3 - related to Figures 3 and 4. Penetrance of the morpholino phenotypes

Penetrance of the morpholino phenotypes for the morpholino/gene combinations not reported in (Saina et al., 2009).

Figure S4 - related to Figure 4.

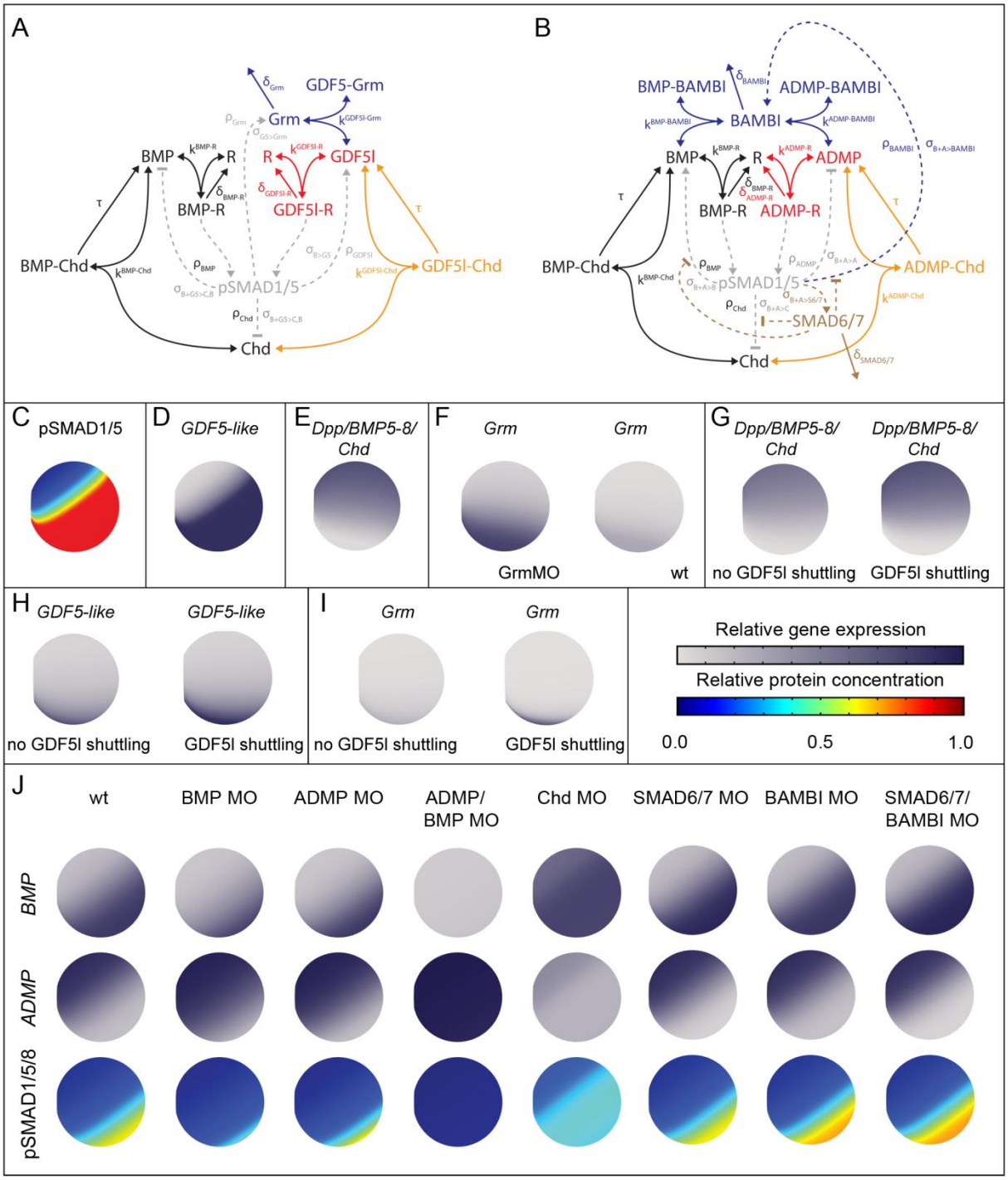

Figure S4 - related to Figure 4. The regulatory interactions of the *Nematostella* and *Xenopus* models.

A-B The regulatory interactions of the core *Drosophila* model (Mizutani et al., 2005) (black arrows) and the model extensions specific for *Nematostella* (A, coloured arrows) and *Xenopus* (B, coloured arrows). Parameter names are written next to the interactions they belong to.

pSMAD1/5 (grey) is included for clarity but was not modelled explicitly. The parameter name above the arrows from pSMAD1/5 to the targets indicates which ligand-receptor complex impacts on the downstream components. Note that in the *Nematostella* model some regulatory interactions only depend on one of the two ligand-receptor complexes, while in *Xenopus* BMP-R and ADMP-R have the same targets.

(C-I) Impact of the sequential incorporation of signalling modules in the *Nematostella* network. Parameter values are as in the standard model, with parameter values of non-incorporated parts set to zero.

(C) The core model (black arrows) results in a stable BMP signaling domain opposite to the *NvChd* expression domain. This domain corresponds to the *NvGDF5-like* expressing domain (D).

(E) Including the negative feedback of both GDF5l-Receptor and BMP-Receptor signaling on BMP and Chordin expression into the model results in the restriction of both the *NvDpp*, *NvBMP5-8* and the *NvChd* expression domains.

(F) Adding NvGrm as an antagonist of NvGDF5-like into the model results in the upregulation of *NvGrm* expression in the NvGrmMO compared to wild type.

(G-I) Shuttling of NvGDF5-like, although not essential, sharpens the expression domains of *BMP* and *NvChd* (G) , *NvGDF5-like* (H) and *NvGrm* (I) compared to the alternative model where only NvDpp/NvBMP5-8 is allowed to shuttle.

(J) Xenopus model. Simulation of the *BMP4/BMP7* (*BMP*) and *ADMP/BMP2* (*ADMP*) mRNA expression patterns and pSMAD1/5/8 localization in wild type and upon knockdown of different BMP network components in *Xenopus*. Relative expression (shown in colour code as ISH) and relative pSMAD1/5 concentrations (shown as heat maps) are plotted onto the Brachet's cleft.

Figure S5 - related to Figure 5

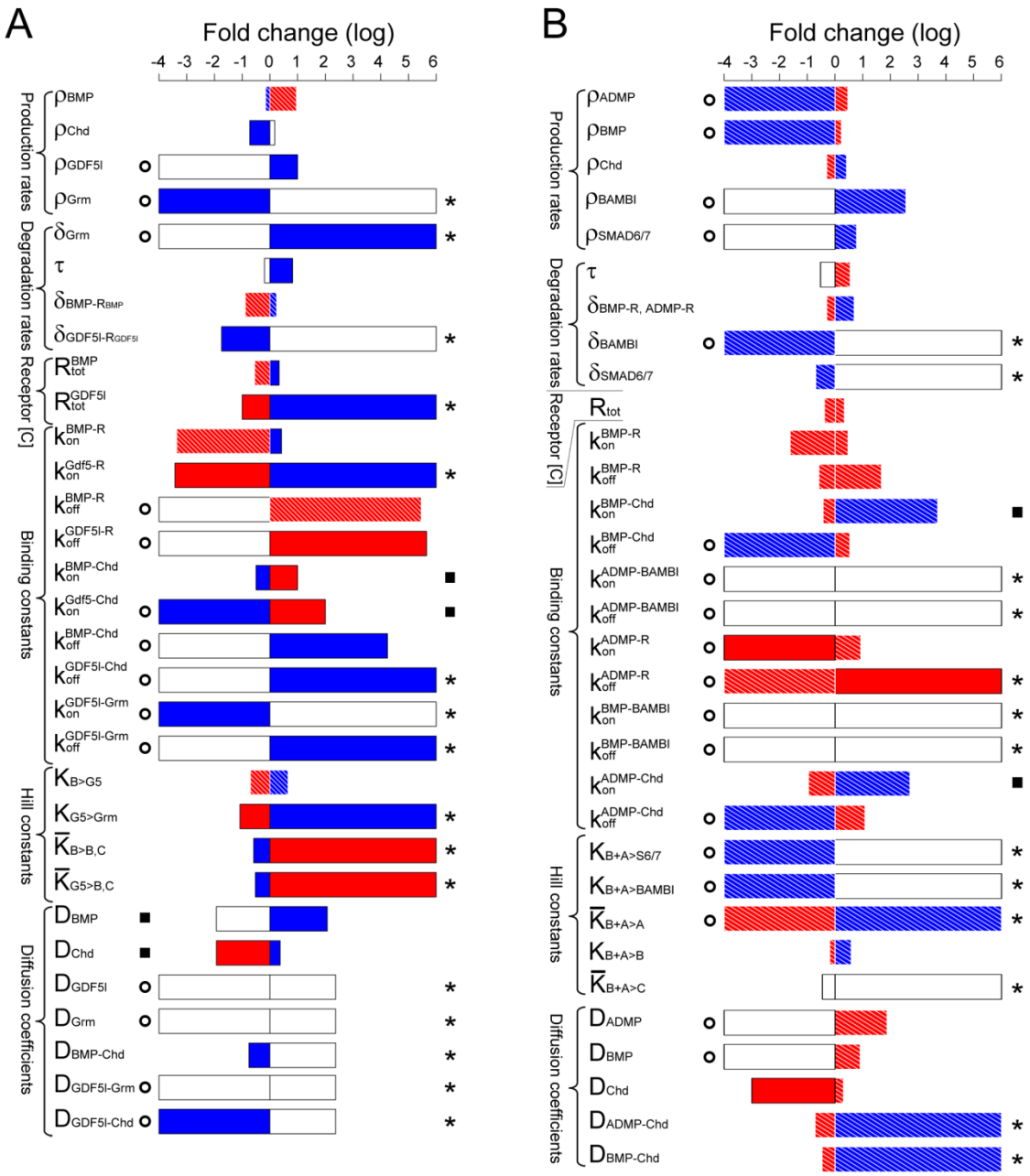

Figure S5 - related to Figure 5. Sensitivity of the asymmetric BMP signaling pattern to the parameter values in the *Nematostella* (A) and *Xenopus* (B) model.

For each parameter, as indicated at the bottom of each bar, we report the relative fold change from the standard value used in the model (indicated by zero) on a logarithmic scale that still permits the emergence of an asymmetric BMP signaling pattern as defined in the Supporting Information. For parameter values outside the bar, asymmetric BMP signaling becomes

symmetric or unstable in that it oscillates over time. A zero at the bottom of the bar indicates that an asymmetric BMP signaling is observed even when the parameter value is set to zero, i.e. when the reaction is absent from the model. A star at the top of a bar indicates that asymmetric BMP signaling was still observed at very large parameter values (infinite values cannot be tested in a simulation framework). A filled square indicates that wider parameter spaces could not be tested due to numerical limitations. Red bars indicate stronger asymmetric BMP signaling; blue bars indicate weaker asymmetric BMP signaling. Red-white bars indicate a shift of the asymmetric BMP signaling domain towards the *NvChd* expression domain in the case of *Nematostella* (A) or a ventralization in the case of the frog (B); blue-white bars indicate a shift in the opposite direction away from the *NvChd* expression domain (A) or a dorsalization (B). White bars indicate that none of these changes in the asymmetric BMP signaling pattern are observed as the parameter values are changed.

Supplemental tables

Table S1, related to Figure 1. TGFβ molecules and BMP antagonists found in *Nematostella vectensis*

Gene name	Accession numbers	Suggested homologues in Bilateria	Asymmetric expression	Knockdown phenotype	References
NvDpp	AY391716 AY363391	*BMP2/4, decapentaplegic*	Yes, at symmetry break	Radialization of *NvDpp*, *NvBMP5-8* and *NvChd*; disappearance of pSMAD1/5; expression of *NvGDF5-like*, *NvGrm*, *Hox* genes and *NvGbx* is abolished	(Finnerty et al., 2004; Rentzsch et al., 2006a; Saina et al., 2009) This paper
NvBMP5-8	DQ358699	*BMP5-8, Screw*	Yes, at symmetry break	Radialization of *NvDpp*, *NvBMP5-8* and *NvChd* expression; disappearance of pSMAD1/5; expression of *NvGDF5-like*, *NvGrm*, *Hox* genes and *NvGbx* is abolished	(Matus et al., 2006b; Saina et al., 2009) This paper
NvGDF5-like	AY496945, AY391717	*Gdf5, Gdf6*	Yes, at symmetry break	Expansion of *NvChd*; reduction of pSMAD1/5; expression of *NvGrm*, *Hox* genes and *NvGbx* is abolished or repressed	(Rentzsch et al., 2006a; Technau et al., 2005) This paper
mstn/gdf11	KC662373	*myostatin, Gdf8/11*	No	N/A	(Saina and Technau, 2009)
NvActivin	ABF61781	*activin*	No	N/A	(Matus et al., 2006a)
NvAdmp-related	JQ959545	*ADMP*	N/A after symmetry	N/A	(Röttinger et al., 2012)

			break		
NvChd	DQ286294 DQ358700	*Chordin, short gastrulation (sog)*	Yes, at symmetry break	Radialization of *NvChd*, disappearance of pSMAD1/5, disappearance of expression of *NvDpp*, *NvBMP5-8*, *NvGDF5-like*, *NvGrm*, *Hox* genes and *NvGbx*	(Matus et al., 2006b; Rentzsch et al., 2006a; Saina et al., 2009) This paper
NvGremlin	DQ517924	*Gremlin*	No	N/A	(Matus et al., 2006a)
NvGrm	DQ471325	*Gremlin*	Yes, at symmetry break	Delay in symmetry break; expansion of pSMAD1/5; expansion of the *NvGrm* and *NvHoxE* expression	(Rentzsch et al., 2006a) This paper
NvFollistatin-like	DQ517921	*Follistatin*	No	N/A	(Matus et al., 2006a)
NvFollistatin	DQ517920	*Follistatin*	No	N/A	(Matus et al., 2006a)
NvNoggin1	DQ517922	*Noggin*	Yes, after symmetry break	N/A	(Matus et al., 2006a)
NvNoggin2	DQ517923	*Noggin*	No	N/A	(Matus et al., 2006a)

N/A – data not available

Table S2, related to Figure 3. Different nomenclature of the *Nematostella vectensis Hox* genes with confirmed expression

This study	Chourrout et al., 2006 (Chourrout et al., 2006)	Ryan et al., 2007 (Ryan et al., 2007)
NvHoxA	*NvHoxA*	*Anthox6*
NvHoxB	*NvHoxB*	*Anthox6a*
NvHoxC	*NvHoxC*	*Anthox7*
*NvHoxD**	*NvHoxDa*	*Anthox8a*
	NvHoxDb	*Anthox8b*
NvHoxE	*NvHoxE*	*Anthox1a*
NvHoxF	*NvHoxF*	*Anthox1*

* *NvHoxDa* and *NvHoxDb* have been shown to be different transcripts originating from the same locus by alternative splicing (Moran et al., 2014). In this study, we name this gene *NvHoxD*.

Supplemental Experimental Procedures

1. Morpholino injection and generating the constitutively active NvBMPRI.

Morpholino oligonucleotides were injected at following concentrations: DppMO (GTAAGAAACAGCGTAAGAGAAGCAT), BMP5-8MO (GTAACAGGTCTCGTATTCTCCGCAT) and ChdMO (GATCCACTCACCATCTTTGCGAGAC) at 300µM concentration as in (Saina et al., 2009) (see also for controls of MO specificity); GDF5LMO (AGGTTATTTAGCCTGACCTTGATCG), GrmMO (CTCAACAGCTTCTTCAATGATCCGT) and standard control morpholino StdMO (CCTCTTACCTCAGTTACAATTTATA) - at 500µM concentration. The functionality of the GDF5lMO was assessed by PCR on cDNA from GDF5l morphants and control embryos (Fig. S1D) followed by sequencing the PCR products; the specificity of the GrmMO, which is constructed against a region in the 5' UTR of the *NvGrm*, was tested by co-injecting mRNA carrying either a wild type sequence (ACGGATCATTGAAGAAGCTGTTGAG) recognized by GrmMO or the one with 5 mismatches (ACTGATGATTGACGAAGTTGTTAAG) upstream of the *mOrange2* coding sequence with StdMO and GrmMO (Fig. S1E). *Nematostella BMPRI* sequence (KJ948110) was mutated by template switching PCR to substitute the Q209 residue by a D residue in the resulting protein to generate a constitutively active BMPRI (Wieser et al., 1995). Then NvBMPRIQ_{209}>D sequence was fused through a Gly4Ser spacer to the EGFP coding sequence and cloned downstream of the 3.4 kb *NvFGFa1* promoter, which has an early ubiquitous activity and drives mosaic transgene expression in the early F0 embryos. Microinjection of the plasmid was carried out as described in (Renfer et al., 2010).

2. NvHoxE protein expression, antibody production, staining and imaging

NvHoxE coding sequence was cloned downstream of the GST and His-tag coding sequence in a pET21 vector. The protein was expressed in Rosetta™(DE3)pLysS Competent Cells (Novagen) and isolated under native conditions on a GSTrap column (GE Healthcare) using the BioRad BioLogic DuoFlow FPLC machine. The tags were cleaved off by AcTEV protease (Invitrogen) according to the manufacturer's protocol, and isolated GST-His tag was trapped from the solution with Ni-NTA agarose (Qiagen). The sequence of the purified protein was confirmed by MSMS. The rat anti-NvHox antibody was produced at BioGenes (Berlin, Germany). The specificity of the antibody (Fig. S1B) was assayed by transiently expressing the NvHoxE-EGFP fusion protein under control of the 3.4 kb *NvFGFa1* promoter, and performing the staining with the anti-NvHoxE and anti-GFP antibodies at the gastrula stage (when endogenous *NvHoxE* is not yet expressed). Embryos injected with EGFP coding sequence downstream of the 1.1 kb *ActP3 Nvβ-actin* promoter were used as control.

For whole mount antibody staining, the embryos were fixed in 4% MEM/PFA containing 0.2% Tween 20 and 0.2% TritonX100 for 30 minutes, then washed 5 times for 5 minutes in 1xPBS containing 0.2% Tween 20 and 0.2% TritonX100 (PBSTT) and blocked in a 4:1 mixture of 1%BSA/PBSTT and heat inactivated sheep serum for 2 hours. The rat anti-NvHoxE antibody (1:500), the rabbit anti-Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad8 (Ser426/428) antibody (Cell Signaling, 9511) (1:100), rabbit anti-GFP antibody (abcam, ab290) (1:1000) and mouse anti-GFP antibody (Life Technologies, A11120) (1:500) were preabsorbed in the blocking solution for the time of the blocking. The embryos were stained with primary antibodies overnight at 4^0 and then washed 5 times for 25 minutes with PBSTT at room temperature. The secondary antibody staining and washing was done in the same way as described for the primary antibody. The following secondary antibodies were used: goat anti-rabbit-Alexa Fluor 568 (Life Technologies, A11011), goat anti-rabbit-Alexa Fluor 488 (Life Technologies, A11008), goat anti-rabbit-Alexa Fluor 633

(Life Technologies, A21070), goat anti-rat DyLight549 (Jackson, 112-505-143), goat anti-rat DyLight488 (Rockland, 612-141-120). For imaging, embryos were embedded in Vectashield (Vector Labs). Confocal imaging was done with a Leica TCS SP5X. *In situ* images were taken with Nikon DS-Fi1 camera attached to Nikon Eclipse 80i microscope. Image processing was done in Adobe Photoshop CS5.

3. Quantification of the pSMAD1/5 gradient

Intensity of αpSMAD1/5 staining was quantified on 16 bit images of confocal optical sections (oral views) of early morphant and control planulae stained with DAPI and αpSMAD1/5. Staining intensities of all endodermal nuclei along an arc of 180^0 starting from the centre of the pSMAD1/5-positive domain were collected in ImageJ. Nuclei were detected based on DAPI staining. Each counted nucleus was given a sequential number. To correct for the different numbers of endodermal nuclei in different planulae, the sequential number of each counted nucleus was divided by a total number of counted nuclei on the optical section resulting in a relative position of the nucleus along the 180^0 arc (from 0 to π). Background staining values were calculated for each embryo by measuring and averaging αpSMAD1/5 staining intensity in several endodermal positions, which did not overlap with nuclei. These average background values were subtracted from all the staining intensities prior to analysis. No additional normalization of the data was performed.

4. Mathematical Model

Since the BMP signaling network involves multiple players and the interactions are very complex, a number of mathematical models have been proposed to explain various aspects of D-V patterning (Ben-Zvi et al., 2008; Eldar et al., 2002; Inomata et al., 2013; Lee et al., 2009; Mizutani et al., 2005; Paulsen et al., 2011; Shimmi et al., 2005; Umulis et al., 2006; Umulis et al., 2010) . Yet, to date, there is no mechanistic understanding of the BMP signaling networks outside Bilateria. In our paper, we combine loss of function experiments and mathematical modelling to understand the general rules of BMP signaling-dependent axis patterning in animals.

4.1 Model of directive axis patterning in *Nematostella*

Towards the end of gastrulation two striking events occur: a symmetry break in the expression domains and the emergence of the BMP signaling domain at the opposite site of the *NvDpp* expression domain. To explain the emergence of this counterintuitive signaling pattern of BMP from the asymmetric expression patterns we built a mathematical model for axis patterning in *Nematostella* that is based on the known regulatory interactions between the key regulatory proteins NvDpp/NvBMP5-8/NvGDF5-like, the BMP receptor, the BMP antagonists Chordin (NvChd) and Gremlin (NvGrm), and the protease Tolloid (Figure S7A). The regulatory proteins that have been identified in *Nematostella* are strikingly similar to those involved in dorso-ventral patterning in *Drosophila* and the model therefore builds on a previously published shuttling mechanism for *Drosophila* (Iber and Gaglia, 2007; Mizutani et al., 2005). Since *NvDpp* and *NvBMP5-8* are co-expressed in *Nematostella* and their morpholino phenotypes are indistinguishable (Saina et al., 2009), we assume in the model that NvDpp and NvBMP5-8 heterodimerize to form NvDpp/NvBMP5-8 complex. Interestingly,

heterodimers of *Drosophila* Dpp/Screw and vertebrate BMP4/BMP7 signal stronger (Shimmi et al., 2005; Suzuki et al., 1997) and are shuttled by Chordin preferentially over homodimers (Shimmi et al., 2005).

As a starting point of the model, we mimicked the situation after the symmetry break and, therefore, restricted the expression of NvChd in the initial time steps until the regulatory network can self-maintain the asymmetric expression patterns. After gastrulation, *NvDpp* and *NvBMP5-8* are expressed in a stripe in the endoderm, while *NvChd* remains expressed in the overlying ectoderm, separated by the mesogloea, the extracellular matrix separating ectoderm and endoderm. Although so far, we could not directly show the secretion site of the corresponding proteins, our knockdown experiments suggest that their diffusion and physical interactions most likely occur in the mesogloea, similar to the situation in the frog (Plouhinec et al., 2013). We therefore simplified the geometry by modelling only the mesogloea. As a result, we did not distinguish between the endodermal expression of *NvDpp* and *NvBMP5-8* and the ectodermal expression of *NvChd*, but rather considered NvDpp, NvBMP5-8 and NvChd to be co-produced and co-secreted in the same domain. In doing so, we generated a minimal viable model demonstrating the system of the BMP signaling gradient maintenance in *Nematostella* planula.

4.1.1 Geometry

The geometry of this early developmental stage is similar in *Drosophila* and *Nematostella* in that the embryo is a spherical structure with a diameter of about 200 μm, and in both cases the proteins can diffuse in a fluid-filled cavity (periviteline fluid and mesogloea respectively). In *Drosophila* it surrounds a 1-layered epithelium that secretes the proteins into this space and which harbours the receptors; in case of *Nematostella*, it is sandwiched between two protein secreting 1 cell thick cell layers: the ectoderm on the outside and the endoderm on the inside.

The oral opening can be described as a small interruption in the ectodermal and endodermal cell sheets. In the simplest approximation we can use a 1-dimensional domain with an approximate length of 500 μm and periodic boundary conditions at the ends of the domain. In the more realistic 3-dimensional model, based on measurements of the dimensions of multiple embryos, we represent the mesogloea as the surface of a hollow sphere with a radius of 80 μm and an oral opening of radius 45 μm at one side of the sphere. In this case we use zero flux boundary conditions at the oral pole.

4.1.2 Regulatory interactions

The model for axis patterning in *Nematostella* (Figure S5A) is an adaptation of the model by Mizutani and co-workers for Drosophila (Iber and Gaglia, 2007; Mizutani et al., 2005). The original model for dorso-ventral patterning in *Drosophila* included Dpp ligand, the Dpp ligand-receptor complex, Sog, Tsg, the Sog/Tsg complex, and the Sog/Tsg/Ligand complex. There is no homologue of Tsg in *Nematostella*, and we therefore modified the original model in that we removed Tsg and allowed the heterodimeric NvDpp/NvBMP5-8 ligand (henceforth called BMP) to bind the Sog-homologue Chordin directly without binding of additional proteins, which was shown to be possible in vertebrates(Rentzsch et al., 2006b). In summary, the *Nematostella* model has four components: the Dpp/BMP5-8 ligand (BMP), the ligand-receptor complex (BMP-R), Chordin (Chd), and the Dpp/BMP5-8-Chd complex (BMP-Chd). All components can diffuse at speed D, resulting in a set of reaction-diffusion equations of the form

$$\frac{\partial c_i}{\partial t} = D\Delta c_i + \mathcal{R}(c_i)$$

As the receptor is bound to the surface and cannot diffuse the differential equation for the ligand-receptor complex (BMP-R) reads

$$\frac{\partial[BMP-R]}{\partial t} = \mathcal{R}(BMP-R)$$

The biological processes are translated into the following reaction terms

$$\mathcal{R}(BMP) = \rho_{BMP}\,\Lambda_{BMP}(x) - k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} + \left(k_{off}^{BMP-Chd} + \tau\right)c_{BMP-Chd}$$
$$- k_{on}^{BMP-R}(R_{tot} - c_{BMP-R})\,c_{BMP} + k_{off}^{BMP-R}\,c_{BMP-R}$$
$$\mathcal{R}(BMP-R) = k_{on}^{BMP-R}\,(R_{tot} - c_{BMP-R})c_{BMP} - (k_{off}^{BMP-R} + \delta_{BMP-R})\,c_{BMP-R}$$
$$\mathcal{R}(Chd) = \rho_{Chd}\,\Lambda_{Chd}(x) - k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} + k_{off}^{BMP-Chd}\,c_{BMP-Chd}$$
$$\mathcal{R}(BMP-Chd) = k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} - (k_{off}^{BMP-Chd} + \tau)\,c_{BMP-Chd}$$

The model has been described in detail before (Iber and Gaglia, 2007; Mizutani et al., 2005). In brief, the total BMP receptor concentration R_0 is assumed to be constant over time and space, such that $R_{tot} = c_{BMP-R} + c_R$. The expression of BMP is spatially restricted according to $\Lambda_{BMP}(x)$. BMP-receptor binding and unbinding occurs at rate k_{on}^{BMP-R} and k_{off}^{BMP-R}, respectively. Upon BMP-receptor binding, the ligand is endocytosed and degraded at rate $\delta_{BMP\text{-}R}$, which has been determined experimentally for *Drosophila* embryos. In *Nematostella*, BMP can bind at rate $k_{on}^{BMP-Chd}$ to Chordin, and the BMP-Chordin complex, BMP-Chd, can then dissociate at rate $k_{off}^{BMP-Chd}$. Chordin is expressed in a restricted part of the domain according to $\Lambda_{Chd}(x)$ and can be cleaved by the protease Tolloid at rate τ when bound to the BMP ligand.

As motivated in the main text, in the 3D model we further included the signaling protein NvGDF5-like (GDF5l) and its receptor, as well as Gremlin (Grm), an antagonist of GDF5l. GDF5l is a member of the BMP family, and we therefore allow it to bind to Chordin with the same kinetics as the BMP ligand, i.e. binding at rate $k_{on}^{GDF5l-Chd}$ and unbinding at rate $k_{off}^{GDF5l-Chd}$. Gremlin is a GDF5l antagonist much as Chordin, but it is not cleaved by Tolloid.

We therefore allow it to bind to Gdf5 at the same rate as Chordin, i.e. binding at rate $k_{on}^{GDF5l-Grm}$ and unbinding at rate $k_{off}^{GDF5l-Grm}$. GDF5l expression is induced by BMP-receptor signaling, while Gremlin expression is induced by GDF5l. Both GDF5l and BMP signalling can inhibit Chordin and BMP expression. This translates into the following reaction terms:

$$\mathcal{R}(BMP) = \rho_{BMP}\,\bar{\sigma}_{G5>B,C}\bar{\sigma}_{B>B,C} - k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} + \left(k_{off}^{BMP-Chd} + \tau\right)c_{BMP-Chd} - k_{on}^{BMP-R}\left(R_{tot}^{BMP} - c_{BMP-R}\right)c_{BMP} + k_{off}^{BMP-R}\,c_{BMP-R}$$

$$\mathcal{R}(BMP-R) = k_{on}^{BMP-R}\left(R_{tot}^{BMP} - c_{BMP-R}\right)c_{BMP} - (k_{off}^{BMP-R} + \delta_{BMP-R})\,c_{BMP-R}$$

$$\mathcal{R}(Chd) = \rho_{Chd}\,\Lambda(x,t) - k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} - k_{on}^{GDF5l-Chd}\,c_{GDF5l}\,c_{Chd} + k_{off}^{BMP-Chd}\,c_{BMP-Chd} + k_{off}^{GDF5l-Chd}\,c_{GDF5l-Chd}$$

$$\mathcal{R}(BMP-Chd) = k_{on}^{BMP-Chd}\,c_{BMP}\,c_{Chd} - (k_{off}^{BMP-Chd} + \tau)\,c_{BMP-Chd}$$

$$\mathcal{R}(GDF5l) = \rho_{GDF5l}\,\sigma_{B>G5} - k_{on}^{GDF5l-Chd}\,c_{GDF5l}\,c_{Chd} + (k_{off}^{GDF5l-Chd} + \tau)\,c_{GDF5l-Chd} - k_{on}^{GDF5l-R}\,(R_{tot}^{GDF5l} - c_{GDF5l-R1})\,c_{GDF5l} + k_{off}^{GDF5l-R}\,c_{GDF5l-R1} - k_{on}^{GDF5l-Grm}\,c_{GDF5l}\,c_{Grm} + k_{off}^{GDF5l-Grm}\,c_{Grm-GDF5l}$$

$$\mathcal{R}(GDF5l-R) = k_{on}^{GDF5l-R}\left(R_{tot}^{GDF5l} - c_{GDF5l-R1}\right)c_{GDF5l} - (k_{off}^{GDF5l-R} + \delta_{GDF5l-R1})\,c_{GDF5l-R1}$$

$$\mathcal{R}(GDF5l-Chd) = k_{on}^{GDF5l-Chd}\,c_{GDF5l}\,c_{Chd} - (k_{off}^{GDF5l-Chd} + \tau)\,c_{GDF5l-Chd}$$

$$\mathcal{R}(Grm) = \rho_{Grm}\sigma_{G5>Grm} - \delta_{Grm}c_{Grm} - k_{on}^{GDF5l-Grm}\,c_{Grm}\,c_{GDF5l} + k_{off}^{GDF5l-Grm}\,c_{Grm-GDF5l}$$

$$\mathcal{R}(Grm-GDF5l) = k_{on}^{GDF5l-Grm}\,c_{Grm}\,c_{GDF5l} - k_{off}^{GDF5l-Grm}\,c_{Grm-GDF5l}$$

4.1.3 Parameter Values

<u>1D model:</u>

The parameter values are the same as previously (Iber and Gaglia, 2007; Mizutani et al., 2005), with exception of the Tolloid activity, τ, which we lowered about 10-fold compared to the previous model, to compensate for the removal of Tsg.

Parameter	**Value**	**Explanation**	**Ref**
ρ_{BMP}	0.001 μM s^{-1}	Production rate BMP	(Iber and Gaglia, 2007;

			Mizutani et al., 2005)
ρ_{Chd}	0.08 μM s^{-1}	Production rate Chd	(Iber and Gaglia, 2007; Mizutani et al., 2005)
R_{tot}	3 μM	Concentration of BMP receptor	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$k_{on}^{BMP-Chd}$	95 μM^{-1} s^{-1}	Binding rate of BMP to Chordin	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$k_{off}^{BMP-Chd}$	$4*10^{-6}$ s^{-1}	Unbinding rate of the BMP-Chd complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
k_{on}^{BMP-R}	0.4 μM^{-1} s^{-1}	Binding rate of BMP to its receptor	(Iber and Gaglia, 2007; Mizutani et al., 2005)
k_{off}^{BMP-R}	$4*10^{-6}$ s^{-1}	Unbinding rate of the BMP-receptor complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
δ_{BMP-R}	$5*10^{-4}$ s^{-1}	Degradation rate of BMP-receptor complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)

τ	0.05 s^{-1}	Tolloid-mediated degradation rate of BMP-Chd complexes	
D	85 μm^2 s^{-1}	Diffusion coefficient of BMP, Chd and BMP-Chd complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
L	500 μm	Length of the 1D domain	measured

The production of Chordin is restricted to parts of the domain, using

$$\Lambda_{Chd}(x) = \begin{cases} 1 & \text{if} \quad 0 < x < \frac{\pi}{2} \quad \vee \quad \frac{3\pi}{2} < x < 2\pi \\ 0 & \text{if} \quad \frac{\pi}{2} < x < \frac{3\pi}{2} \end{cases}$$

As we investigate the effects of three different BMP expression domains three different values are assigned to $\Lambda_{BMP}(x)$:

$\Lambda_{BMP}(x) = \Lambda_{Chd}(x)$ if BMP expression is on the same site as Chordin expression.

$\Lambda_{BMP}(x) = 1 - \Lambda_{Chd}(x)$ if BMP expression is on the opposite site as Chordin expression.

$\Lambda_{BMP}(x) = \frac{1}{2}$ if BMP expression homogeneous.

3D model:

The parameter values are the same as in the 1D model unless otherwise indicated in the Ref column. The constants without an entry in the Ref column were adjusted to match the observed expression patterns.

Parameter	Value	Explanation	Ref
ρ_{BMP}	0.001 μM s^{-1}	Production rate BMP	(Iber and Gaglia, 2007; Mizutani et al., 2005)
ρ_{Chd}	0.08 μM s^{-1}	Production rate Chd	(Iber and Gaglia, 2007; Mizutani et al., 2005)
ρ_{GDF5l}	0.001 μM s^{-1}	Production rate GDF5l	Assumed to be the equal to ρ_{BMP}
ρ_{Grm}	0.003 μM s^{-1}	Production rate Grm	
δ_{Grm}	0.001 s^{-1}	Degradation rate Grm	
τ	0.05 s^{-1}	Tolloid-mediated degradation rate of Ligand-Chd complexes	Same as in 1D model
$\delta_{BMP\text{-}R}$	$5*10^{-4}$ s^{-1}	Degradation rate of BMP-receptor complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$\delta_{GDF5l\text{-}R1}$	$5*10^{-4}$ s^{-1}	Degradation rate of GDF5l-receptor complex	Assumed to be equal to $\delta_{BMP\text{-}R}$
R_{tot}^{BMP}	3 μM	Concentration of BMP receptor	(Iber and Gaglia, 2007; Mizutani et al., 2005)
R_{tot}^{GDF5l}	3 μM	Concentration of GDF5l receptor	Assumed to be equal to R_{tot}^{BMP}
k_{on}^{BMP-R}	0.4 μM^{-1} s^{-1}	Binding rate of BMP to its receptor	(Iber and Gaglia, 2007;

			Mizutani et al., 2005)
$k_{on}^{\mathrm{GDF5l}-R}$	0.4 μM^{-1} s^{-1}	Binding rate of GDF5l to its receptor	Assumed to be equal to k_{on}^{BMP-R}
k_{off}^{BMP-R}	$4*10^{-6}$ s^{-1}	Unbinding rate of the BMP-receptor complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$k_{off}^{\mathrm{GDF5l}-R}$	$4*10^{-6}$ s^{-1}	Unbinding rate of the GDF5l-receptor complex	Assumed to be equal to k_{off}^{BMP-R}
$k_{on}^{BMP-Chd}$	95 μM^{-1} s^{-1}	Binding rate of BMP to Chordin	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$k_{on}^{\mathrm{GDF5l}-Chd}$	95 μM^{-1} s^{-1}	Binding rate of GDF5l to Chordin	Assumed to be equal to $k_{on}^{BMP-Chd}$
$k_{off}^{BMP-Chd}$	$4*10^{-6}$ s^{-1}	Unbinding rate of the BMP-Chd complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$k_{off}^{\mathrm{GDF5l}-Chd}$	$4*10^{-6}$ s^{-1}	Unbinding rate of the GDF5l-Chd complex	Assumed to be equal to $k_{off}^{BMP-Chd}$
$k_{on}^{\mathrm{GDF5l}-Grm}$	95 μM^{-1} s^{-1}	Binding rate of GDF5l to Gremlin	Assumed to be equal to $k_{on}^{BMP-Chd}$
$k_{off}^{GDF5l-Grm}$	$4*10^{-6}$ s^{-1}	Unbinding rate of the GDF5l-Gremlin complex	Assumed to be

			equal to $k_{off}^{BMP-Chd}$
$K_{B>G5}$	1 μM	Activating Hill constant of the BMP-R complex	
$K_{G5>Grm}$	0.6 μM	Activating Hill constant of the GDF5l-R1 complex	
$\bar{K}_{B>C,B}$	0.5 μM	Inhibiting Hill constant of the BMP-R complex	
$\bar{K}_{G5>C,B}$	0.1 μM	Inhibiting Hill constant of the GDF5l-R1 complex	
D_{BMP}	85 $\mu m^2 s^{-1}$	Diffusion coefficient BMP	(Iber and Gaglia, 2007; Mizutani et al., 2005)
D_{Chd}	85 $\mu m^2 s^{-1}$	Diffusion coefficient Chd	(Iber and Gaglia, 2007; Mizutani et al., 2005)
D_{GDF5l}	85 $\mu m^2 s^{-1}$	Diffusion coefficient GDF5l	Assumed to be equal to all other diffusion constants
D_{Grm}	85 $\mu m^2 s^{-1}$	Diffusion coefficient Grm	Assumed to be equal to all other diffusion constants
$D_{BMP-Chd}$	85 $\mu m^2 s^{-1}$	Diffusion coefficient BMP-Chd complex	(Iber and Gaglia, 2007; Mizutani et al., 2005)
$D_{GDF5l-Grm}$	85 $\mu m^2 s^{-1}$	Diffusion coefficient GDF5l-Grm complex	Assumed to be equal to all other diffusion constants

$D_{GDF5l\text{-}Chd}$	85 $\mu m^2 s^{-1}$	Diffusion coefficient GDF5l-Chd complex	Assumed to be equal to all other diffusion constants
r_{ball}	80 µm	Measured radius of the mesogloea in the embryo	Measured
r_{mouth}	45 µm	Measured radius of the oral opening	Measured

BMP is expressed homogenously, while Chordin is restricted to the following domain:

$$\Lambda(x,t) = \begin{cases} 1.4 * x + z > 110\ \mu m & if\ t \leq 2\ h \\ \bar{\sigma}_{G5>B,C}\ \bar{\sigma}_{B>B,C} & if\ t > 2\ h \end{cases}$$

Activating Hill terms are denoted $\sigma_{C1>C2}$, where C1 is the component that is signaling and thereby inducing the expression of component C2. Inhibiting Hill terms are denoted $\bar{\sigma}_{C1>C2}$, where C1 is the component that is signaling and thereby inhibiting the expression of component C2:

$$\sigma_{C1>C2} = \frac{C1^n}{C1^n + {K_{C1>C2}}^n}$$

and

$$\bar{\sigma}_{C1>C2} = \frac{{\bar{K}_{C1>C2}}^n}{C1^n + {\bar{K}_{C1>C2}}^n}$$

4.2 Model of DV patterning in the Frog

4.2.1 Geometry

The geometry of this early developmental stage is similar, but much larger than in *Nematostella* , in that the embryo is a spherical structure with a radius of about 550 μm. Extracellular proteins can diffuse in the Brachet's cleft, a thin layer of extracellular matrix between ectoderm and endomesoderm (Plouhinec et al., 2013). In our 3-dimensional model, we represent the Brachet's cleft as the surface of a hollow sphere with a radius of 550 μm and a blastopore opening of radius 150 μm on one side of the sphere. We use zero flux boundary conditions at the blastopore opening.

4.2.2 Regulatory interactions

The *Xenopus* model has 6 main components: the BMP4/7 ligand (BMP), the ADMP/BMP2 ligand (ADMP), Chordin (Chd), the BMP/ADMP receptor (R), BAMBI and SMAD6/7. For simplicity, we absorbed the effects of Twisted gastrulation protein, which promotes diffusion of the Chd-BMP complex (Oelgeschlager et al., 2000; Wang et al., 2008), into the parameter values. We also did not model the effects of the important modulators of the Tolloid activity – the dorsally expressed ONT1 (Inomata et al., 2008) and the ventrally expressed Sizzled (Inomata et al., 2013), which keep Tolloid activity highest at intermediate BMP signaling levels, - but rather absorbed them in the Tolloid activity (τ) directly. We did not consider CV2, which is a ventrally expressed protein exhibiting pro-BMP and anti-BMP activities depending on the context (Ambrosio et al., 2008). We also assumed Chordin to be the sole dorsally expressed BMP inhibitor, thus ignoring Noggin and Follistatin (Khokha et al., 2005), which are not regulated by Tolloid cleavage.

In brief, the total BMP/ADMP receptor concentration R_{tot} is assumed to be constant over time and space, such that $R_{tot} = c_{BMP-R} + c_{ADMP-R} + c_R$. Upon BMP-receptor or ADMP-receptor

binding , the ligand is endocytosed and degraded at rate $\delta_{BMP\text{-}R}$ or $\delta_{ADMP\text{-}R}$ respectively. Both BMP and ADMP can bind at rate $k_{on}^{BMP-Chd}$ (Piccolo et al., 1996) and $k_{on}^{ADMP-Chd}$ to Chordin (Reversade and De Robertis, 2005). The BMP-Chordin (BMP-Chd) and ADMP-Chordin complex (ADMP-Chd), can then dissociate at rate $k_{off}^{BMP-Chd}$ and $k_{off}^{ADMP-Chd}$. Chordin is expressed in a restricted part of the domain according to $\Lambda_{Chd}(x)$ and can be cleaved by the protease Tolloid (Lee et al., 2006; Lee et al., 2009) at rate τ when bound to the BMP or ADMP ligand.

We further included a dominant negative BMP receptor BAMBI (Onichtchouk et al., 1999) and inhibitory SMAD6/7 (Imamura et al., 1997; Nakao et al., 1997), which are negative feedback antagonists of BMP/ADMP signaling (Paulsen et al., 2011). Both BMP and ADMP bind to BAMBI at rate $k_{on}^{BMP-BAMBI}$ and $k_{on}^{ADMP-BAMBI}$ and unbind at rate $k_{off}^{BMP-BAMBI}$ and $k_{off}^{ADMP-BAMBI}$. As BAMBI is only a derivative of the BMP receptor, we decided to use the same degradation rates $\delta_{BMP\text{-}R}$ and $\delta_{ADMP\text{-}R}$ for the BMP/ADMP bound BAMBI receptors. Production of SMAD6/7 is induced upon BMP/ADMP signaling and SMAD6/7 inhibits BMP/ADMP signaling by competitive inhibition (Paulsen et al., 2011). Both, ADMP and BMP signaling, inhibit Chordin and ADMP expression, and induce BMP, BAMBI and SMAD6/7 expression (for review see (Plouhinec et al., 2011). This translates into the following reaction terms:

$$\mathcal{R}(BMP) = \rho_{BMP}(0.1 + \sigma_{B+A>B}) - k_{on}^{BMP-Chd} c_{BMP}\, c_{Chd} + (k_{off}^{BMP-Chd} + \tau)\, c_{BMP-Chd} - k_{on}^{BMP-R}(R_{tot} - c_{BMP-R} - c_{ADMP-R})\, c_{BMP} + k_{off}^{BMP-R}\, c_{BMP-R} - k_{on}^{BMP-BAMBI} c_{BMP}\, c_{BAMBI} + k_{off}^{BMP-BAMBI}\, c_{BMP-BAMBI}$$

$$\mathcal{R}(BMP-R) = k_{on}^{BMP-R}(R_{tot} - c_{BMP-R} - c_{ADMP-R}) c_{BMP} - (k_{off}^{BMP-R} + \delta_{BMP-R})\, c_{BMP-R}$$

$$\mathcal{R}(Chd) = \rho_{Chd}\, \Lambda(x) \bar{\sigma}_{B+A>C} - k_{on}^{BMP-Chd} c_{BMP}\, c_{Chd} + k_{off}^{BMP-Chd}\, c_{BMP-Chd} - k_{on}^{ADMP-Chd} c_{ADMP}\, c_{Chd} + k_{off}^{ADMP-Chd}\, c_{ADMP-Chd}$$

$$\mathcal{R}(BMP-Chd) = k_{on}^{BMP-Chd} c_{BMP}\, c_{Chd} - (k_{off}^{BMP-Chd} + \tau)\, c_{BMP-Chd}$$

$$\mathcal{R}(ADMP) = \rho_{ADMP}\, \bar{\sigma}_{B+A>A} - k_{on}^{ADMP-Chd} c_{ADMP}\, c_{Chd} + (k_{off}^{ADMP-Chd} + \tau)\, c_{ADMP-Chd} - k_{on}^{ADMP-R}(R_{tot} - c_{BMP-R} - c_{ADMP-R})\, c_{ADMP} + k_{off}^{ADMP-R}\, c_{ADMP-R} - k_{on}^{ADMP-BAMBI} c_{ADMP}\, c_{Bambi} + k_{off}^{ADMP-BAMBI}\, c_{ADMP-BAMBI}$$

$$\mathcal{R}(ADMP-R) = k_{on}^{ADMP-R}(R_{tot} - c_{BMP-R} - c_{ADMP-R}) c_{ADMP} - (k_{off}^{ADMP-R} + \delta_{ADMP-R})\, c_{ADMP-R}$$

$$\mathcal{R}(ADMP - Chd) = k_{on}^{ADMP-Chd} c_{ADMP}\, c_{Chd} - (k_{off}^{ADMP-Chd} + \tau)\, c_{ADMP-Chd}$$

$$\mathcal{R}(BAMBI) = \rho_{BAMBI}\, \sigma_{B+A>BAMBI} - \delta_{BAMBI}\, c_{BAMBI} - k_{on}^{BMP-BAMBI} c_{BMP}\, c_{BAMBI} + k_{off}^{BMP-BAMBI}\, c_{BMP-BAMBI} - k_{on}^{ADMP-BAMBI} c_{ADMP}\, c_{BAMBI} + k_{off}^{ADMP-BAMBI}\, c_{ADMP-BAMBI}$$

$$\mathcal{R}(BMP - BAMBI) = k_{on}^{BMP-BAMBI} c_{BMP}\, c_{BAMBI} - k_{off}^{BMP-BAMBI}\, c_{BMP-BAMBI} - \delta_{BMP-R}\, c_{BMP-BAMBI}$$

$$\mathcal{R}(ADMP - BAMBI) = k_{on}^{ADMP-BAMBI} c_{ADMP}\, c_{BAMBI} - k_{off}^{ADMP-BAMBI}\, c_{ADMP-BAMBI} - \delta_{ADMP-R}\, c_{ADMP-BAMBI}$$

$$\mathcal{R}(SMAD6/7) = \rho_{SMAD}\, \sigma_{B+A>S6/7} - \delta_{SMAD}\, c_{SMAD6/7}$$

4.2.3 Parameter Values

In contrast to *Nematostella*, some quantitative data is available for *Xenopus*.

1. Spatially averaged Chordin concentration of around 33 nM (Lee et al., 2006)
2. BMP4/BMP7 concentration in the range between 0.1 nM and 2.5 nM (Piccolo et al., 1996)
3. KD value for BMP4/7 and Chd = 3.2 ± 0.6*10-10 M (Piccolo et al., 1996).
4. KD value for BMP4 and BMP receptor = 2.5 – 9 *10-10 M (Graff et al., 1994; Penton et al., 1994).

All the other parameter values were adjusted to obtain the published expression patterns and observe the four measured concentrations and affinities listed above. We note that given the change in the decay rates, also the diffusion coefficients needed to be adjusted. The diffusion coefficients of 85 μm^2/s chosen by Mizutani and co-workers are at the high end of what has been measured for proteins diffusion in tissue (Yu et al., 2009). The new 3.4-fold lower diffusion coefficients are thus if anything more realistic (Kalwarczyk et al., 2012; Muller et al., 2012; Zhou et al., 2012). We note that the model also works over a broad range of parameters closer to the ones used in *Nematostella*. However it would then not observe the measured values above.

Parameter	**Value**	**Explanation**
ρ_{BMP}	$3*10^{-7}$ μM s^{-1}	Production rate BMP

ρ_{Chd}	$1.5*10^{-5}$ μM s^{-1}	Production rate Chd
ρ_{ADMP}	$1*10^{-7}$ μM s^{-1}	Production rate ADMP
ρ_{BAMBI}	$1.8*10^{-6}$ μM s^{-1}	Production rate BAMBI
ρ_{SMAD}	$3*10^{-4}$ μM s^{-1}	Production rate SMAD
τ	0.0017 s^{-1}	Tolloid-mediated degradation rate of Ligand-Chd complexes
$\delta_{BMP\text{-}R}$, $\delta_{ADMP\text{-}R}$	$5*10^{-4}$ s^{-1}	Degradation rate of BMP-receptor / ADMP-receptor complex (as in (Iber and Gaglia, 2007; Mizutani et al., 2005))
δ_{BAMBI}	$1*10^{-3}$ s^{-1}	Degradation rate of BAMBI
δ_{SMAD}	$1*10^{-4}$ s^{-1}	Degradation rate of SMAD6/7
R_{tot}	$1.5*10^{-3}$ μM	Concentration of BMP/ADMP receptor
k_{on}^{BMP-R}	8 μM^{-1} s^{-1}	Binding rate of BMP to its receptor
k_{on}^{ADMP-R}	8 μM^{-1} s^{-1}	Binding rate of ADMP to its receptor
k_{off}^{BMP-R}	$4*10^{-3}$ s^{-1}	Unbinding rate of the BMP-receptor complex
k_{off}^{ADMP-R}	$4*10^{-3}$ s^{-1}	Unbinding rate of the ADMP-receptor complex
$k_{on}^{BMP-Chd}$	14 μM^{-1} s^{-1}	Binding rate of BMP to Chordin
$k_{on}^{ADMP-Chd}$	14 μM^{-1} s^{-1}	Binding rate of ADMP to Chordin
$k_{off}^{BMP-Chd}$	$4.5*10^{-3}$ s^{-1}	Unbinding rate of the BMP-Chd complex
$k_{off}^{ADMP-Chd}$	$4.5*10^{-3}$ s^{-1}	Unbinding rate of the ADMP-Chd complex
$k_{on}^{ADMP-BAMBI}$	8 μM^{-1} s^{-1}	Binding rate of ADMP to BAMBI
$k_{on}^{BMP-BAMBI}$	8 μM^{-1} s^{-1}	Binding rate of BMP to BAMBI
$k_{off}^{ADMP-BAMBI}$	$4*10^{-3}$ s^{-1}	Unbinding rate of the ADMP-BAMBI complex
$k_{off}^{BMP-BAMBI}$	$4*10^{-3}$ s^{-1}	Unbinding rate of the BMP-BAMBI complex
$K_{B+A>B}$	$2.25*10^{-4}$ μM	Activating Hill constant on BMP expression
$K_{B+A>BAMBI}$	$1.5*10^{-3}$ μM	Activating Hill constant on BAMBI expression
$K_{B+A>S6/7}$	$1.5*10^{-3}$ μM	Activating Hill constant on SMAD6/7 expression
$\overline{K}_{B+A>C}$	$7.5*10^{-4}$ μM	Inhibiting Hill constant on Chordin expression

$\bar{K}_{B+A>A}$	$2.25*10^{-4}$ μM	Inhibiting Hill constant on ADMP expression
D_{BMP}	25 $\mu m^2 s^{-1}$	Diffusion coefficient BMP
D_{Chd}	25 $\mu m^2 s^{-1}$	Diffusion coefficient Chd
D_{ADMP}	25 $\mu m^2 s^{-1}$	Diffusion coefficient ADMP
$D_{ADMP\text{-}Chd}$	25 $\mu m^2 s^{-1}$	Diffusion coefficient ADMP-Chd complex
$D_{BMP\text{-}Chd}$	25 $\mu m^2 s^{-1}$	Diffusion coefficient BMP-Chd complex
r_{ball}	550 μm	Radius of the Brachet's cleft in the embryo
$r_{blastopore}$	150 μm	Radius of the blastopore opening

Production of Chordin is restricted to part of the domain, using

$$\Lambda(x) = 1.3 * x + z > 300\ \mu m$$

In the frog model, all Hill terms depend on both ADMP and BMP signaling. This signaling induces SMAD6/7 activation, which acts as a competitive inhibitor. Activating Hill terms are therefore as denoted $\sigma_{B+A>C1}$, where BMP and ADMP are the components that are signaling and thereby inducing the expression component C1. Inhibiting Hill terms are denoted $\bar{\sigma}_{B+A>C1}$, where BMP and ADMP are the components that are signaling and thereby inhibiting the expression of component C1:

$$\sigma_{B+A>C1} = \frac{(c_{BMP-R} + c_{ADMP-R})^n}{(c_{BMP-R} + c_{ADMP-R})^n + (K_{B+A>C1}\,(1 + c_{SMAD6/7}))^n}$$

and

$$\bar{\sigma}_{B+A>C1} = \frac{(\bar{K}_{B+A>C1}\,(1 + c_{SMAD6/7}))^n}{(c_{BMP-R} + c_{ADMP-R})^n + (\bar{K}_{B+A>C1}\,(1 + c_{SMAD6/7}))^n}$$

4.3 Model analysis

4.3.1 Comparison of simulation results with experimental data

Antibody stainings detect proteins, and thus provide a measure of protein concentrations. We therefore compare the antibody stainings to the values of the variables in our model (which represent protein concentrations).

In situ hybridization (ISH) experiments provide a (semi-quantitative) measure of the mRNA concentration, i.e. of the protein expression rate (rather than the protein concentration). To compare ISH results with our simulations, we thus compare them to the production terms in the model (rather than the variables, which would represent concentrations).

4.3.2. Simulation of Morphants

We model Morpholino experiments by reducing the production rates of the relevant proteins. In the *Nematostella* simulations of the Chordin, GDF5l and Gremlin morphants, the production rate was reduced to 10% of the standard values (Table 2). In the BMP morphant we reduced the production rate to 5% of the original value to avoid oscillations.

MO knockdowns in the frog show in general a less extreme phenotype compared to *Nematostella*, possibly due to partial compensation by redundant BMP proteins. In our simulations all knockdowns were therefore modeled by a reduction of the production rates or by setting the Tolloid cleavage rate to 20% of the standard values in order to reproduce the less severe MO effects.

4.3.3 Sensitivity Analysis

We conducted a sensitivity analysis to determine the range of parameter values for which the pattern of interest would be observed. We report an upper and lower boundary for each parameter value; parameter values outside of these boundaries result in a loss of pattern. A key event in the simulation is the emergence of an asymmetric BMP5-8/Dpp signaling pattern in *Nematostella* and the emergence of an asymmetric BMP4/7 signaling pattern in *Xenopus*. Thus, we define the lack of an asymmetric BMP5-8/Dpp or BMP4/7 signaling pattern as a loss of pattern.

In particular, an asymmetric BMP5-8/Dpp signaling pattern is considered absent if one of the following three conditions is met:

1. The maximum of $\sigma_{B>G5}$, the Hill function inducing GDF5l expression, is below 0.3.
2. The ratio between the maximal and minimal GDF5l expression is less than 3-fold.
3. The system is unstable and oscillates.

In the frog, an asymmetric BMP4/7 signaling pattern is considered absent if one of the following three conditions is met:

1. The maximum of $\sigma_{B+A>B}$, the Hill function inducing BMP expression, is below 0.3.
2. The ratio between the maximum and minimum of the Hill function $\sigma_{B+A>B}$ is less than 3-fold.
3. The system is unstable and oscillates.

We further report if increasing or decreasing a parameter value leads to stronger (red) or weaker (blue) expression domains.

In *Nematostella*, in a few cases the *NvGDF5-like* expression domain is expanded while the *NvDpp/NvBMP5-8/NvChd* expression domain is reduced. This results in a shift of the *NvGDF5-like* expression domain towards the *NvChd* expression domain (red with white lines).

The opposite case, a reduced *NvGDF5-like* and an expanded *Dpp/BMP5-8/Chd* expression domain, results in a shift of *NvChd* expression towards the *NvDpp/NvBMP5-8/NvChd* expression domain (blue with white lines).

These cases are similar to dorsalization (blue with white lines) and ventralization (red with white lines) in the frog.

Expansions/Reductions of the expression domains were judged by eye.
In some cases we were not able to increase or decrease the parameter range any further due to numerical problems with the stiff set of equations. These cases are denoted by a filled black square. The results of the sensitivity analysis are presented on Figure S6.

4.4 Materials and methods

The PDEs were solved with finite element methods as implemented in COMSOL Multiphysics 4.3b as previously described (Germann et al., 2011).

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