Scaling of BMP gradients in *Xenopus* embryos

Arising from: D. Ben-Zvi *et al.* *Nature* 453, 1205–1211 (2008)

Metazoan organisms can 'scale', that is, maintain similar proportions regardless of size. Ben-Zvi *et al.*[1] use experiments in *Xenopus* to support a quantitative model that explains morphological scaling as the result of scaling of a gradient of bone morphogenetic protein (BMP) signals. We believe that the evidence for scaling in *Xenopus* is misinterpreted, and that their model for embryonic patterning disagrees with prior data. The experiments they present supporting their model admit alternative interpretations.

The authors' model (box 1 of ref. 1) is built around the (BMP inhibitor) Chordin-facilitated transport of two members of the BMP family of ligands, BMP (BMP2/4/7), the total amount of which is preserved, and ADMP, which is produced only in the dorsal organizer but concentrates ventrally and scales along the dorsal–ventral axis at blastula stage.

The paper is based on the assumption that dorsal embryonic halves produce well-proportioned (scaled) tadpoles, which is in contrast to existing data. Kageura *et al.*[2] show that removing ventral cells from the eight-cell blastula (series 15, 17) results in normal heads attached to a small body. This is in accordance with standard fate maps that assign most of what is conventionally called ventral in the blastula to posterior tissue in the tadpole[3]. It is right–left half-embryos that will reproduce correctly proportioned half-size tadpoles[2]. The quoted paper by Cooke[4] examined only mesoderm patterning in transverse sections of tailbud embryos. Therefore, dorsal half-embryos do not scale in the sense defined by the authors. We henceforth focus on molecular evidence contradicting the presented theory construed as a model for embryonic patterning.

In the frog a twofold change in morphogen levels can elicit different cell fates[5]. Because ligands cannot be directly measured, nuclear Smad1/5/8 transcription factor is the best measure for total BMP signalling[6]. Experiments in frog[7] and fish[8] show at most a fourfold variation versus the 10^2 to 10^4 range required for scaling in the model.

The model requires that total BMP activity derives predominantly from ADMP, yet BMP depletion (figure 2H in ref. 9) has a stronger phenotype than ADMP-depletion (figure 2H in ref. 10), resulting in embryos with disproportionately large heads[8,9] similar to dorsal half-embryos (figure 3C in ref. 10). BMP4 injection significantly ventralizes the embryo (figure 1H in ref. 9), yet the model does not constrain the total amount of BMP or its initial location, because 'shuttling' actively concentrates it on the ventral side.

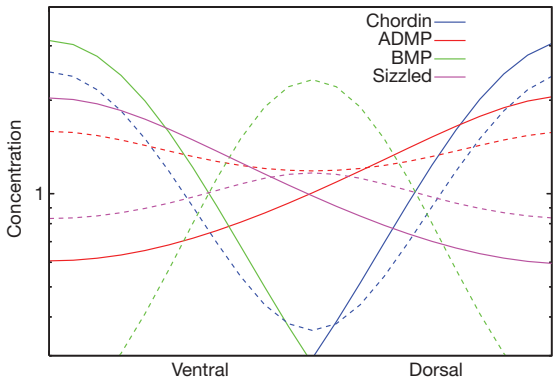

Figure 1 | Morphogen profiles after axes duplication (dashed lines) compared with the wild-type embryo (solid lines) from a reaction diffusion model[14]. The gene network is adapted from figure 3L in ref. 10 and involves self-sustained ventral and dorsal centres. The concentration of the ventral BMP marker in the lateral region between the duplicated axes is clearly reproduced. (The inhibitors, ADMP and Sizzled, diffuse rapidly, the other species slowly. Activation and repression are modelled as Hill functions and summarize more complex biochemistry. The equations and parameters that were solved to produce Fig. 1 are available from the authors.

Experiments in figure 3 of ref. 1 were performed to demonstrate Chordin-dependent shuttling. *BMP4* is used instead of *ADMP*, and the protein distribution is shown in mid–late gastrula, although BMP must act before early gastrulation to affect dorsal–ventral patterning significantly[11]. Labelled BMP4 is localized in endoderm and not ventral mesoderm as in the schematic of figure 3a of ref. 1. Other explanations for the localization of injected *BMP*4, such as secretion into the blastocoel cavity and ectopic uptake[12], need to be addressed. The Chordin-depleted embryos used as controls still show movement of injected *BMP4*, and the phenotype undercuts the larger message given that such embryos have well-defined axes[13]. The complete model that addresses these questions (supplementary information 6a–h of ref. 1) contains over 30 free constants to explain essentially qualitative data; a number so large as to render the predictions questionable.

Axis duplication experiments (figure 4 of ref. 1) are taken as evidence for shuttling: the authors assert that the (well-known) expression of ventral markers between the two axes is evidence for their mode of transport. However, there must be a maximum in BMP signalling between the two axes because it is suppressed in each. Reaction diffusion models[14,15] show that ordinary diffusion, as opposed to facilitated diffusion through shuttling, can generate patterns consistent with the qualitative data presented (our Fig. 1).

In summary, we feel it is incorrect to appeal to qualitative data beyond the onset of gastrulation to support a model for blastula patterning, because other layers of regulation may intervene.

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Reply to Francois et al.

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Francois *et al.*[1], commenting on our paper[2], argue that (1) scaling does not occur, (2) our model is inconsistent with existing experiments, and (3) our experiments are not conclusive. We disagree.

The ability of amphibian embryos to scale pattern with size is evident from the large variability in egg size, and is a pre-assumption of our study. In his 1938 monograph, H. Spemann reports his manipulation of newt embryos: ''...when the two halves are completely separated, the dorsal half develops into a small embryo of normal proportions''[3] (Fig. 1a). This experiment is cited in standard textbooks[4] and reproduced in laboratories[5] and teaching courses. Clearly, not all half-embryos develop normally, but this is not surprising given the likelihood of secondary damage. The key point is that surviving embryos maintain mesodermal ventral tissues (for example, blood and heart), expected to be lost in the absence of scaling.

The possibility that scaling results from over-growth was ruled out by J. Cooke, who demonstrated that proportionate assignment of trunk mesodermal cells to tissues is maintained in embryos in which as much as ~70% of the cells are removed[6]. The tissues examined depend on bone morphogenetic protein (BMP) and thus provide readout of the early gradient.

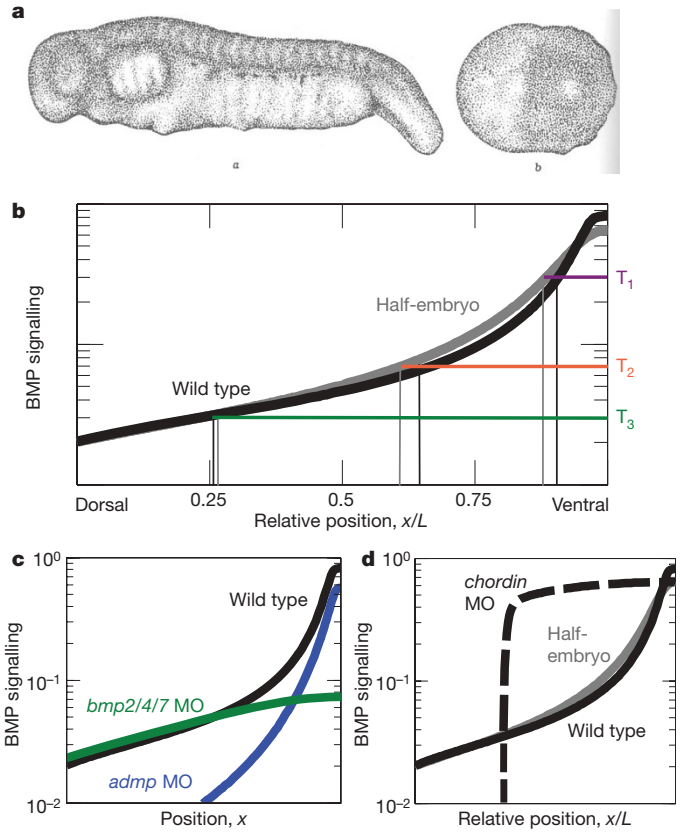

Figure 1 | Scaling pattern with size. a, A newt dorsal half-embryo (*a*, left) develops into a small embryo of normal proportions, in contrast to the ventral half (*b*, right). (Panel **a** is from ref. 3.) **b**, The BMP activation profile predicted by our model for wild-type (black line) and half-sized (grey line) embryos. *L* is the length of the dorsal-ventral axis and T_1, T_2 and T_3 are arbitrary thresholds differing by less than tenfold. **c**, Numerical simulation of profiles in a wild-type embryo (black line), embryo depleted of Admp by *admp* morpholino (MO) (blue line) or depleted of Bmp2/4/7 (green line). **d**, Numerical simulation of profiles in wild-type (black line) and half-sized (grey line) embryos, and of an embryo depleted of Chordin (dashed black line, *chordin* morpholino). The same parameters were used in all figures (contact the authors for further information on parameters).

Scaling in our model[2] is robust to the parameter choice, but the range of the gradient depends on parameters. We do not attempt to predict the *in vivo* parameters. The ~100-fold concentration difference shown (Fig. 1b) is comparable with the estimated range of the Dpp gradient in the significantly smaller *Drosophila* wing imaginal disc[7]. Quantitative measures of the pSmad1 profile in the opaque *Xenopus* embryo are still limited (see ref. 8 for example).

Our model is consistent with existing experiments. The severe phenotype of Bmp2/4/7 depletion (Fig. 1c) reflects the positive feedback on *bmp4* expression, which increases ventral Bmp4 levels. Similarly, the remaining polarity of Chordin-depleted embryos (Fig. 1d) is explained by the additional BMP inhibitors (for example, Noggin), which are structurally different from Chordin, and are not likely to be cleaved by Xlr or to mediate shuttling. Both properties were included in our original simulations and do not alter the robustness of the scaling mechanism.

Our model is robust to ligand production rate, but we do not expect this robustness to hold for arbitrarily high levels which can override available Chordin. Importantly, our conclusion that dorsally produced Admp accumulates ventrally was in fact tested: Bmp2/4/7-depleted embryos retain dorsal–ventral polarity[9] and this polarity is abolished only when Admp is also depleted[5].

Francois *et al.*[1] are concerned that separation of Bmp4-Myc from its site of injection may be due to ligand secretion into the blastocoel, and non-uniform uptake by ventral cells. However, separation was abolished when Chordin was depleted, with no apparent reason to assume that such secretion and uptake requires Chordin.

The novel point of our secondary-axis experiment is the removal of Admp from the secondary organizer. This eliminates an auxiliary source of a BMP ligand, which could contribute to the increase of BMP in mid-embryo. Francois *et al.* imply that a BMP profile that peaks in the centre of the embryo to a level similar to the normal 'lateral' level is sufficient to obtain *sizzled* expression. Clearly, this is not the case, because *sizzled* expression requires high BMP levels normally found in ventral positions.

Our model refers to the gastrulation stage, when BMP functions in dorsal–ventral patterning, and not the pre-gastrula stage, when BMP represses the organizer[10]. Francois *et al.* suggest that patterning can be explained by a different reaction–diffusion model. Their underlying assumptions[11], however, do not reflect the known network topology[12], and the resulting profiles are inconsistent with the system properties. Hence, we do not find their model to be a valid alternative.

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