Scaling of BMP gradients in *Xenopus* embryos

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 find that existing observations contradict the biological premises of the paper, experiments do not prove essential elements of the model, and the full mathematical formulation has too many free parameters to be predictive. We conclude that understanding scaling requires quantitative data, molecular markers, and a predictive theory.

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.

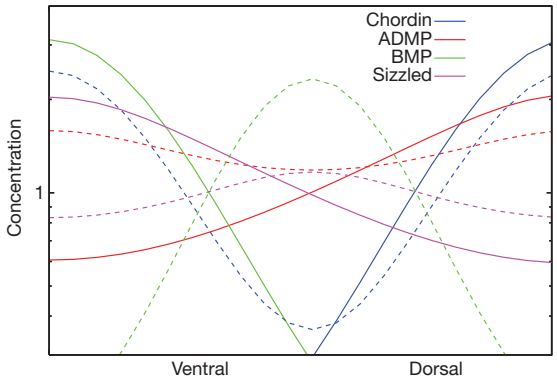

Figure 1 | Morphogen profiles after axes duplication (dashed lines) compared with the wild-type embryo (solid lines) from a reaction diffusion model[14]. The gene network is adapted from figure 3L in ref. 10 and involves self-sustained ventral and dorsal centres. The concentration of the ventral BMP marker in the lateral region between the duplicated axes is clearly reproduced, unlike in the authors' figure 4b (ref. 1). (The inhibitors, ADMP and Sizzled, diffuse rapidly, the other species slowly. Activation and repression are modelled as Hill functions and summarize more complex biochemistry. See http://xenopus.rockefeller.edu/scaling for additional *Xenopus*-related information or http://www.physics.rockefeller.edu/~pfrancois/scaling.html for the equations and parameters that were solved to produce Fig. 1, and a movie.

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 significantly affect dorsal–ventral patterning[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 *BMP4* 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 conclusion, the paper uses an inappropriate example of scaling (dorsal halves), does not clearly prove the existence of the process which underpins the model (BMP shuttling), and the mathematical formulation is too accommodating to be predictive. It is also incorrect to appeal to data beyond the onset of gastrulation to support a model for blastula patterning, because other layers of regulation may intervene.

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Received 14 November 2008; accepted 24 June 2009.

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doi:10.1038/nature08305

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