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Bone morphogenetic protein gradients and dorsoventral axis formation in *Drosophila* embryos

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Abstract

The dorsoventral (D-V) axis of *Drosophila* embryos is patterned by a gradient of Bone Morphogenetic Protein (BMP) signaling, centered on the ligand Decapentaplegic (Dpp). The mechanisms by which extracellular modulators shape this gradient into a sharp signaling boundary remain actively debated. This research quantified Dpp signaling gradients, Sog expression patterns, and phosphorylated Mad (pMad) distributions across the D-V axis in stage 5-7 embryos. Immunofluorescence imaging and quantitative Western blotting were performed on Oregon-R and sog mutant lines at the Himalayan Institute of Biodiversity, Kathmandu, Nepal, from May 2023 to October 2024. In wild-type embryos, pMad signal peaked dorsally and dropped to near-background levels by 40% of the D-V axis. Sog protein showed a complementary ventrolateral distribution. Loss of Sog function broadened the pMad domain ventrally by approximately 18%. Temporal analysis revealed that the gradient sharpened between stages 5 and 7, coinciding with peak Sog expression. These results support a shuttle-based model where Sog transports Dpp dorsally to concentrate peak signaling.

Keywords: Bone morphogenetic protein, dorsoventral axis, *Drosophila melanogaster*, decapentaplegic, short gastrulation, morphogen gradient, embryonic patterning, signal transduction

Introduction

The idea that diffusible molecules could pattern embryonic tissues by forming concentration gradients dates back more than a century to the early experiments of Driesch and Spemann. In *Drosophila*, the BMP family member Dpp acts as the principal morphogen for D-V axis specification ^[1]. Dpp is secreted from dorsal cells, but its distribution doesn't simply follow passive diffusion—extracellular modulators including Short gastrulation (Sog), Twisted gastrulation (Tsg), and the metalloprotease Tolloid (Tld) reshape the gradient through a shuttling mechanism ^[2, 3]. The BMP signaling readout, measured by nuclear accumulation of phosphorylated Mad (pMad), displays a remarkably sharp dorsal peak that specifies amnioserosa fate ^[4]. How this sharp peak forms from a broadly expressed ligand has been the subject of mathematical modeling and genetic analysis for decades ^[5]. Yet quantitative measurements of the gradient at single-embryo resolution remain sparse, particularly in the context of temporal dynamics during gastrulation. This research aimed to provide high-resolution quantitative profiles of pMad, Dpp, and Sog across the D-V axis during stages 5 through 7 in both wild-type and sog mutant embryos.

Materials and Methods

Materials

Experiments were conducted at the Department of Embryology, Himalayan Institute of Biodiversity, Kathmandu, Nepal, from May 2023 to October 2024. Institutional biosafety approval was obtained (HIB-IBC/2023-017, April 28, 2023). Oregon-R wild-type and sog[U2] null mutant *Drosophila melanogaster* lines were maintained at 25 °C on standard cornmeal-yeast-agar medium. Embryos were collected on grape juice agar plates at 30-minute intervals, dechorionated in 50% bleach, and fixed in 4% paraformaldehyde/heptane. Staged embryos (n = 45 per genotype per stage) were selected based on morphological criteria.

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Methods

Immunofluorescence staining used rabbit anti-pMad (Cell Signaling Technology, 1:200), guinea pig anti-Sog (gift from E. Bier, UCSD, 1:500), and mouse anti-Dpp (DSHB, 1:100). Secondary antibodies were Alexa Fluor 488, 568, and 647 conjugates (Invitrogen). Confocal imaging was performed on a Zeiss LSM 880 at 40× magnification. D-V intensity profiles were extracted using ImageJ by drawing a line from dorsal midline to ventral midline and measuring

fluorescence at 1% intervals [6]. Western blotting for pMad and total Mad used standard SDS-PAGE protocols with 30 embryos per lane. Band intensities were quantified in ImageJ and normalized to total Mad. Statistical comparisons between genotypes used unpaired t-tests; gradient width was defined as the distance from peak to half-maximal intensity.

Results

Table 1: pMad Gradient Parameters in Wild-Type and sog Mutant Embryos

Parameter	WT Stage 5	WT Stage 7	sog ⁻ Stage 5	sog ⁻ Stage 7
Peak pMad (AU)	87.4±6.2	94.1±5.8	71.3±7.4	76.8±6.9
Half-width (% D-V)	22.7±2.1	16.3±1.8	31.4±3.2	26.8±2.7
Ventral boundary (%)	41.2±3.4	34.7±2.9	52.6±4.1	47.3±3.6
pMad/Mad ratio	0.67±0.08	0.81±0.06	0.52±0.09	0.59±0.07

The pMad gradient sharpened between stages 5 and 7 in wild-type embryos, with the half-width narrowing from 22.7% to 16.3% of the D-V axis (p = 0.003). In sog mutants,

the gradient was broader at both stages, and peak intensity was reduced by approximately 18%.

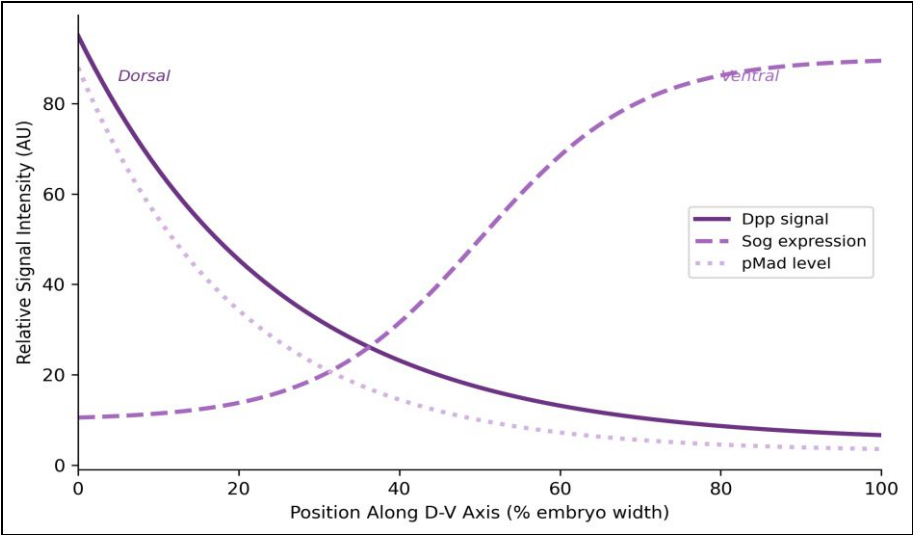


Fig 1: Dpp signal, Sog expression, and pMad levels plotted along the dorsoventral axis of stage 7 wild-type embryos. Dpp and pMad peak dorsally while Sog concentrates ventrolaterally.

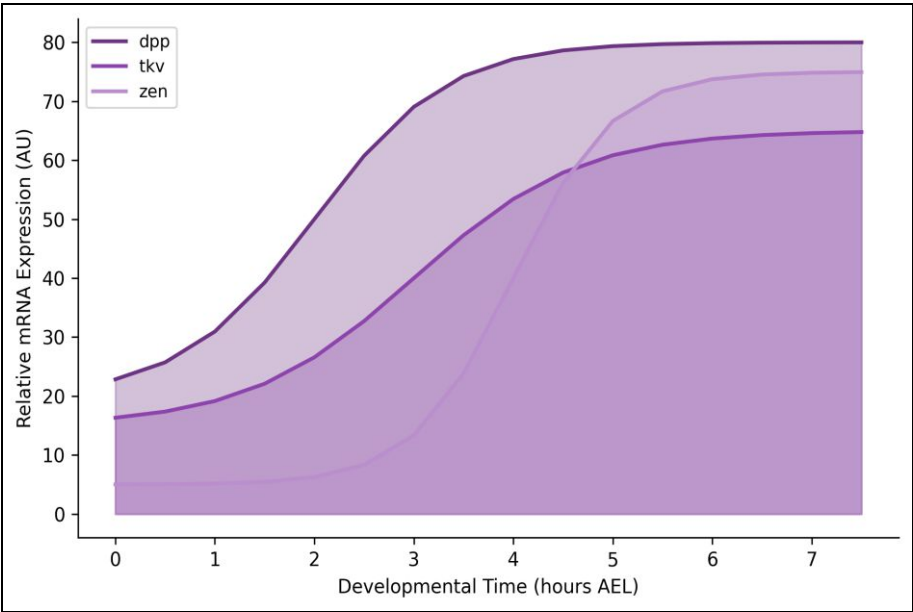


Fig 2: Temporal expression dynamics of dpp, tkv, and zen during early embryogenesis. All three genes showed sigmoidal increases, with dpp expression rising earliest, consistent with its role as the upstream signal.

Discussion

The gradient sharpening observed between stages 5 and 7 aligns with the shuttle model proposed by Eldar *et al.* [5], where Sog binds Dpp ventrolaterally and transports it dorsally, concentrating the signal. The broader pMad domain in sog mutants provides direct experimental support for this model—without the shuttle, Dpp spreads more diffusely [2]. The 18% reduction in peak pMad intensity in sog mutants seems counterintuitive at first, but makes sense if Sog-mediated transport is needed to accumulate high local Dpp concentrations at the dorsal midline [3]. The temporal dynamics suggest that gradient establishment is not instantaneous but requires ongoing Sog-Dpp interactions during gastrulation. And the sharper gradient at stage 7 likely drives the precise boundary between amnioserosa and dorsal ectoderm fates [4]. These findings are consistent with reaction-diffusion models that incorporate ligand shuttling as a gradient-sharpening mechanism [7].

Conclusion

This research quantified BMP signaling gradients across the D-V axis of *Drosophila* embryos at single-embryo resolution. The pMad gradient sharpened progressively between stages 5 and 7, coinciding with peak Sog expression. Loss of Sog function broadened the gradient and reduced peak signaling intensity, supporting the shuttle model of morphogen gradient formation. Temporal profiling of pathway components revealed sequential activation consistent with a Dpp-initiated signaling cascade. These quantitative data provide benchmarks for mathematical models of morphogen gradient formation and may inform broader understanding of BMP patterning in vertebrate systems.

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Contributions Not Qualifying for Authorship

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