

Developmental mechanisms underlying sharp yet robust border formation in the forebrain

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1 Introduction and Background

The Choroid plexus (CP) forms in the dorsal midline (DM) of the embryonic forebrain and secretes the cerebrospinal fluid that fills the ventricles and bathes the central nervous system, but little is known about the development of this important tissue [3]. We have found that the anterior border of the CP extends into the rostral midline in the presence of excess Bone Morphogenetic proteins (BMPs) in mouse forebrain explant cultures. The rostral midline is a known signaling center during forebrain development that expresses Fibroblast growth factors (FGFs), the Bmp antagonist Chordin, and Tolloid proteases [2, 3, 6, 7]. We therefore examined the relationship between these rostral signals and BMPs during the formation of the CP border in the rostradorsal midline using computational and experimental approaches.

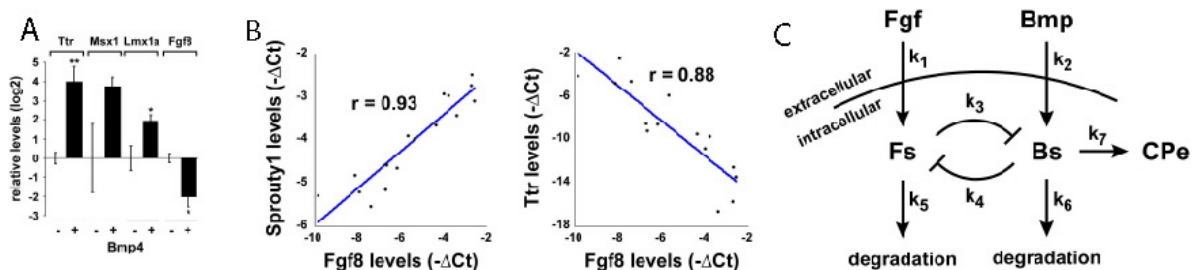


Figure 1: **Bmp-Fgf interactions.** (A) Real-time qRT-PCR analysis, rostral portions of E9.5 explants with and without Bmp4. (B) Scatterplots, real-time qRT-PCR data from all explants examined for Fgf, Fgf downstream signalling element Sprouty, and Ttr. (C) Single cell model of Bmp-Fgf interactions. Fs and Bs are the Fgf and Bmp intracellular signalling elements

Experiments and previous studies provide evidence for two interacting mechanisms that form and position the anterior CP border. First, we show that Bmps induce transthyretin (Ttr) expression (the specific CP marker) while Fgfs inhibit it. This mutual inhibition interaction forms a positive feedback loop that could translate small spatial differences in BMP

and FGF concentrations into a sharp border of cell responsiveness (Fig. 1). We hypothesize the presence of the second mechanism of facilitated transport (FT) in DM development analogous to the mechanism observed in early *Drosophila* embryos, where it serves to concentrate BMPs towards their source [4, 5]. In the forebrain FT model, Chordin expressed away from the DM diffuses, complexes with BMPs, then releases free BMPs primarily at the DM upon cleavage by Tolloid-related proteases. The evidence for the presence of FT in the forebrain comes from studies showing expression of Bmp, Chordin, and Tollids in the required positions. Additionally, results from explant bead experiments and Chordin mutant studies are consistent with model predictions. Taken together, we show through mathematical modelling that the two mechanisms function in concert to generate a sharp and robust anterior border of CP in the forebrain DM.

2 Methods

We model the BMP morphogen and its interaction with other proteins at two scales: extracellular and intracellular. The morphogenetic interactions between BMP, FGF, Chordin, and Tolloid occur in extracellular space and are modelled as PDEs in one dimension (approximating the thin cortical sheet to a wire along the DM). The intracellular interactions are modelled as ODEs and include the mutual antagonism of the BMP and FGF pathways. The model output is the Ttr concentration along the DM. The kinetic, diffusion, and binding parameters were taken from comparable studies in *Drosophila* and other mice models.

3 Results

We use modelling to study the interaction dynamics between the BMP-FGF positive feedback loop and FT in CP border formation and positioning. First, modelling confirms earlier studies of the FT model which show an effective localization of the Bmp ligand at the source. Second we show that the positive feedback loop functions as a great sensitivity detector in positioning the borders over small changes in protein concentrations. But, when combined with the FT mechanism the sensitivity of the border position to fluctuations in BMP or FGF production becomes more robust (Fig. 2). We propose that the performance objective [1] of such a system is robustness of the CP border position.

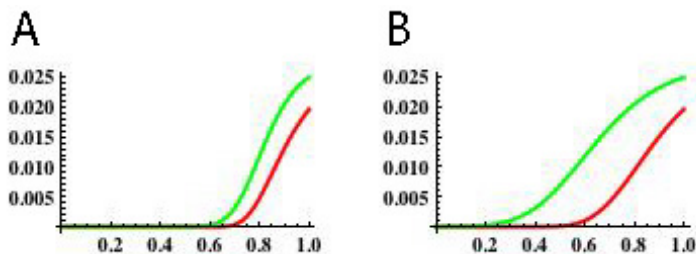


Figure 2: **Robustness mechanism.** Red and white lines indicate the intracellular Bmp signalling strength with Bmp dosages of 2x and 1x. (A) With robustness mechanism, (B) Without robustness mechanism. The X axis represents the dorsal midline with a normalized length of 1. The Bmp source lies at 1 and the Fgf and Chordin sources are at 0. The Y-Axis shows signal strength normalized to maximum extracellular Bmp concentration.

References

- [1] A. Lander, Morpheus Unbound: Reimagining the Morphogen Gradient. *Cell*, Volume 128, Issue 2, Pages 245-256.
- [2] Furuta, Y., D.W. Piston, and B.L. Hogan, Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development*, 1997. 124(11): p. 2203-12.
- [3] Currle, D.S., et al., Direct and indirect roles of CNS dorsal midline cells in choroid plexus epithelia formation. *Development*, 2005. 132(15): p. 3549-59.
- [4] Mizutani CM, Nie Q, Wan FY, Zhang YT, Vilmos P, Sousa-Neves R, Bier E, Marsh JL, Lander AD. Formation of the BMP activity gradient in the *Drosophila* embryo. *Dev Cell*. 2005 Jun;8(6):915-24.
- [5] D.M. Umulis, M. Serpe, M.B. O'Connor and H.G. Othmer, Robust, bistable patterning of the dorsal surface of the *Drosophila* embryo, *Proc. Natl. Acad. Sci. USA* 103 (2006), pp. 11613-11618 Published online July 24, 2006.
- [6] Anderson RM, Lawrence AR, Stottmann RW, Bachiller D, Klingensmith J, Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development*. 2002 Nov;129(21):4975-87.
- [7] Ge G, Greenspan DS, Developmental roles of the BMP1/TLD metalloproteinases. *Birth Defects Res C Embryo Today*. 2006 Mar;78(1):47-68.