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Cell communication with the neural plate is required for induction of neural markers by BMP inhibition: evidence for homeogenetic induction and implications for *Xenopus* animal cap and chick explant assays

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ABSTRACT

In *Xenopus*, the animal cap is very sensitive to BMP antagonists, which result in neuralization. In chick, however, only cells at the border of the neural plate can be neuralized by BMP inhibition. Here we compare the two systems. BMP antagonists can induce neural plate border markers in both ventral *Xenopus* epidermis and non-neural chick epiblast. However, BMP antagonism can only neuralize ectodermal cells when the BMP-inhibited cells form a continuous trail connecting them to the neural plate or its border, suggesting that homeogenetic neuralizing factors can only travel between BMP-inhibited cells. *Xenopus* animal cap explants contain cells fated to contribute to the neural plate border and even to the anterior neural plate, explaining why they are so easily neuralized by BMP-inhibition. Furthermore, chick explants isolated from embryonic epiblast behave like *Xenopus* animal caps and express border markers. We propose that the animal cap assay in *Xenopus* and explant assays in the chick are unsuitable for studying instructive signals in neural induction.

Introduction

Since the discovery of neural induction by Spemann and Mangold in 1924 (Spemann and Mangold, 1924), there has been considerable interest in identifying the signals responsible. Relatively little progress was made until about a decade ago, when the "default model" was proposed (Hemmati-Brivanlou and Melton, 1997a,b; Harland, 2000; Muñoz-Sanjuán and Brivanlou, 2002). This model states that Bone Morphogenetic Proteins (BMP) are initially active throughout the entire ectoderm. As gastrulation starts, the organizer and dorsal mesoderm secrete BMP antagonists generating a dorso-ventral gradient of BMP activity. Consequently neural tissue, neural crest and epidermis arise in the ectoderm at progressively higher levels of BMP activity as they are situated further away from the dorsal mesoderm. Since the default model was first proposed there has been considerable controversy concerning whether or not it provides an adequate explanation for neural induction. Recent experiments in chicken and *Xenopus* embryos indicate more complexity to the establishment of a functional neural plate (Streit et al., 1998; Streit and Stern, 1999c,b; Streit et al., 2000; Linker and Stern, 2004; De Almeida et al., 2008). In particular, one set of experiments in the chick raised the possibility that not all of the ectoderm, as the default model predicts, but only cells close to the neural/epidermal border are sensitive to BMP and its antagonists (Streit et al., 1998; Streit and Stern, 1999b). We therefore re-examined this issue in *Xenopus* and chick to determine whether the two systems behave in a comparable way. In both, we find that non-neural ectoderm can be neuralized by BMP inhibition only when the BMP-inhibited cells form a continuous trail from the neural plate or its border. This suggests that homeogenetic (induction of like by like — in this case induction by the neural plate; (Mangold and Spemann, 1927; Mangold, 1929, 1933; Nieuwkoop et al., 1952; Servetnick and Grainger, 1991) inducing signals from the neural plate can only travel between BMP inhibited cells. We wondered whether the animal cap, which is easily neuralized by BMP inhibitors, might be equivalent to the neural–epidermal border. Detailed fate maps reveal that even the smallest

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caps contain cells fated to contribute to this border. Finally we show that chick epiblast explants express markers consistent with a border-like identity and behave like *Xenopus* animal caps.

Materials and methods

Xenopus embryology

Fertilization, staging, injections, lineage tracing, animal cap assays and in situ hybridization were performed as described (Linker and Stern, 2004). mRNA was transcribed from *Smad6-pCS2+* (Linker and Stern, 2004). *CerberusShort-pCS2+* was kindly provided by E. de Robertis (Piccolo et al., 1999), *ΔSmad7-pCS2+*, *TEV2GR-pCS2+* by M. Whitman (Wawersik et al., 2005), *FGF8a-pCS2+* by R. Harland (Fletcher et al., 2006) and *eFGF-pCS2+* (*Xenopus* FGF4) by J. Slack (Isaacs et al., 1994). Nuclear-*LacZ* mRNA or 5–10 ng lysine-fixable-fluorescein (FDX, 40,000 M_r; Molecular Probes) were used as lineage tracers. Where noted, dexamethasone (DEX) was added (final: 10 μM).

Animal caps of different sizes were transplanted from FDX-injected embryos into uninjected hosts (stages 8.5–9; (Nieuwkoop and Faber, 1967). Embryos were allowed to heal in 3/4 Normal Amphibian Medium (NAM) for 1 h and grown overnight (to stage 19) in 1/10 NAM at 14 °C. After healing, fluorescent and bright-field pictures of animal views of the embryos were taken. From these, the projected surface area of the transplanted tissue was calculated using ImageJ. Transplants were categorized as smaller or larger than a "typical" animal cap (Sive et al., 2000) and fate maps generated for each of these. Standardized outlines of embryos at stages 9 and 19 were created by averaging the outlines of 10 embryos at each stage. Fluorescence and bright-field photographs were taken after transplantation, just before fixation and after processing for *Sox3* expression. Images of the embryos were then morphed to the standard outline and the overlap between transplanted areas in different embryos calculated.

Chick experiments

Fertilized hens' eggs (Brown Bovan Gold; Henry Stewart) were incubated at 38 °C. Factors were delivered at stage $3^+/4$ (Hamburger and Hamilton, 1951) by electroporation, by grafting transfected COS cells or as proteins adsorbed to heparin-coated acrylic beads. Electroporation was performed (Sheng et al., 2003) using the

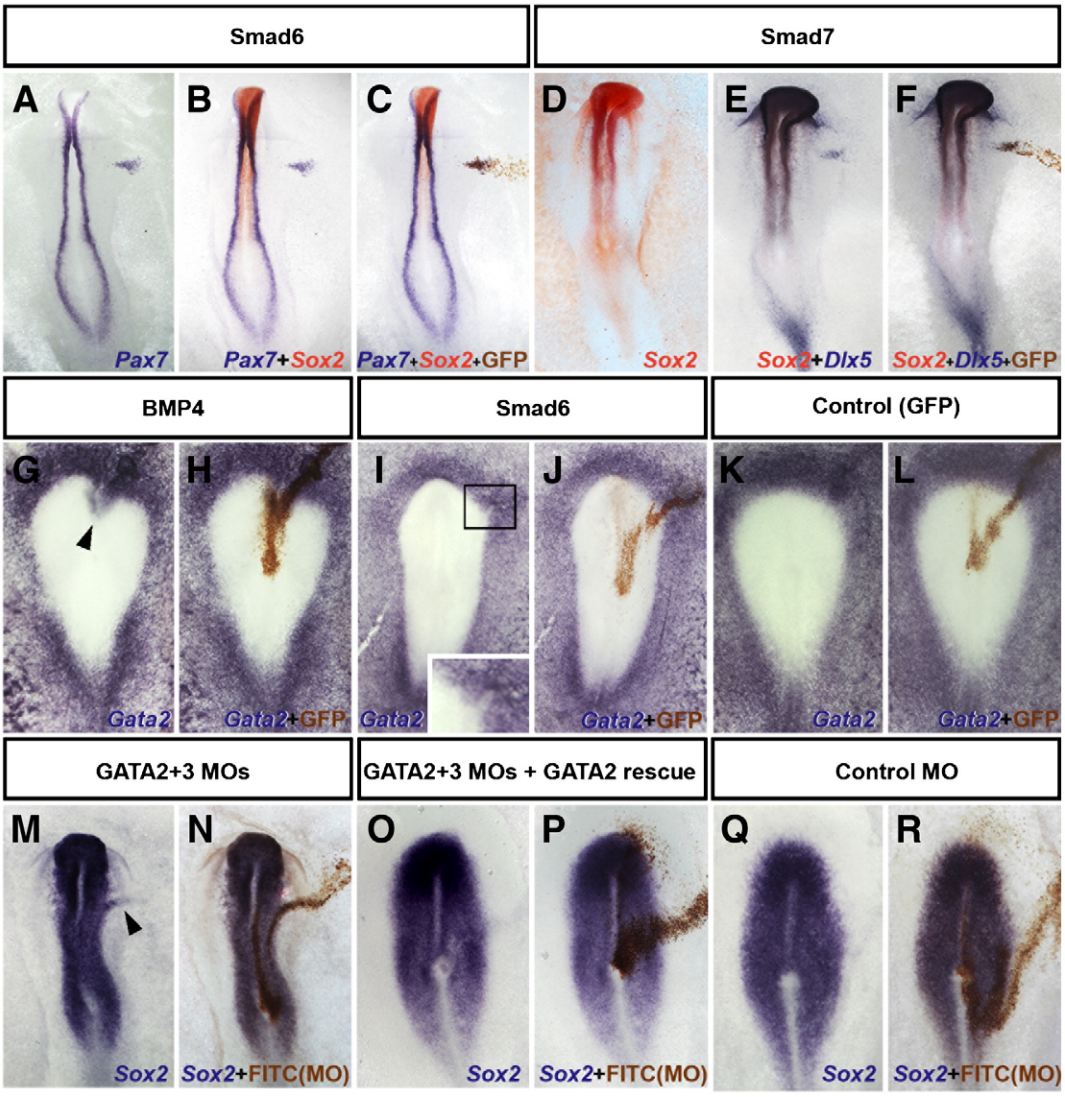

Fig. 1. BMP inhibitors induce neural plate border markers in chick. (A–L) Electroporation of Smad6 or Smad7 in prospective epidermis induces *Pax7* (A–C) and *Dlx5* (E, F) in the absence of *Sox2* (B, C and D, F). Electroporation of *BMP4* induces *Gata2* in the neural plate (G, H). Inhibition of BMP by Smad6 inhibits *Gata2* at the neural border (I, J). GFP (control) does not affect *Gata2* (K, L). (M–R) Gata-2/-3 morpholinos expand *Sox2* into the non-neural territory (M, N) (arrowhead), which is rescued by *Gata2* (O, P), the slight down-regulation of Sox2 in the neural plate is an electroporation artefact; control morpholino has no effect (Q, R). Electroporated cells were stained with anti-GFP antibody (C, F, H, J, L, for the embryos to their left) or with anti-FITC antibody (N, P, R).