MOUSE *SERRATE-1* AND *NOTCH* GENES DIFFER IN THEIR RESPONSE TO SIGNALING MOLECULES DURING ODONTOGENESIS

Thimios A. MITSIADIS[1,2], Irma THESLEFF[3] and Urban LENDAHL[1]

[1]Department of Cell and Molecular Biology, Karolinska Institute, S-171 77 Stockholm, Sweden, [2]Institut de Biologie du Développement de Marseille, Université de la Méditerranée, Campus de Luminy Case 907, F-13288 Marseille Cedex 9, France, and [3]Institute of Biotechnology, P.O. Box 56, FIN-00014 University of Helsinki, Finland

Serrate-like genes encode transmembrane ligands to Notch receptors and control cell fate decisions during development. This signaling system appears to function in most multicellular organisms since Notch receptors and Delta/Serrate ligands have been found in both invertebrates and vertebrates (Artavanis-Tsakonas et al., 1995). Highly conserved homologue of the *Drosophila Serrate* have been recently identified in rat (Lindsell et al., 1995) and in mouse. *Jagged*, the rat *Serrate-1* homologue, is believed to participate in Notch signaling as it seems to activate Notch in myoblasts in culture, as reflected by an inhibition of their differentiation (Lindsell et al., 1995).

Little is known about regulation of vertebrate Notch receptors and ligands. As a step towards addressing this issue, we have analysed the expression and regulation of the mouse *Serrate-1* (*Ser-1*) gene during tooth development. Tooth develops as a result of sequential and reciprocal interactions between neural crest-derived mesenchyme and the oral ectoderm. Particular emphasis was given to tissue interactions, since it was previously shown that such interactions and exposure to specific signaling molecules are critical in organizing the expression of Notch receptors during odontogenesis (Mitsiadis et al., 1995).

Ser-1 mRNA is detected by in situ hybridization on tissue sections using a ^{35}S UTP-labeled riboprobe. *Ser-1* is first expressed the presumptive dental epithelium (E10.5-11) (Fig. 1A), where the inductive capacity for tooth formation resides. At E12-12.5 (Fig. 1B), expression is downregulated in epithelial cells contacting mesenchyme (asterisk) concomitant with an upregulation in dental mesenchyme, and this pattern of expression is maintained until E15.5 (cap stage). From E16.5 to E18.5 (bell stage), *Ser-1* mRNA is absent from dental papilla mesenchyme and epithelial preameloblasts, while expression is observed in other dental epithelial cells (Fig. 1C).

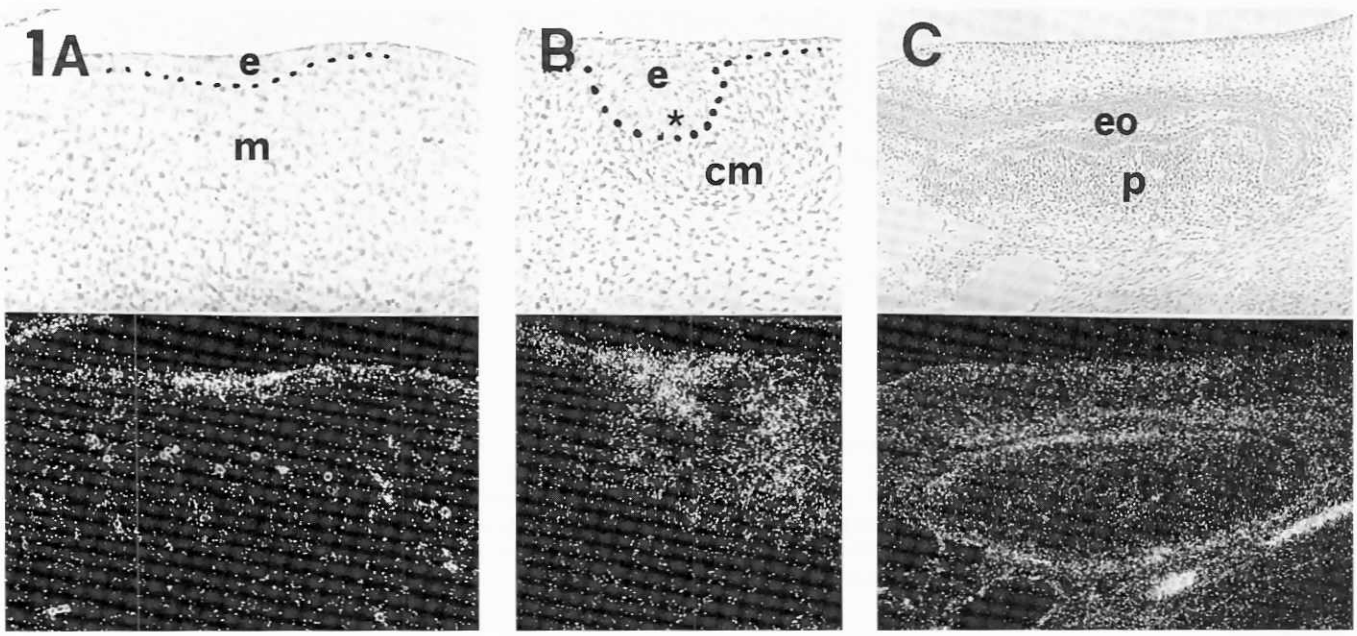

Figure 1. Expression of *Ser-1* during mouse molar tooth development. (A) *Ser-1* expression is restricted to presumptive dental epithelium at E11. (B) Bud staged tooth at E13. *Ser-1* mRNA is localized in both the dental epithelium and the condenced mesenchyme. Note the absence of transcripts from basal epithelial cells (asterisk). (C) At the early bell stage (E16), transcripts are detected only in epithelial cell of the enamel organ. Abbreviations: e, dental epithelium; m, mesenchyme; cm, condensed mesenchyme; p, dental papilla; eo, enamel organ.

The in vivo expession pattern indicates that the *Ser-1* gene is regulated by tissue interactions. Tissue interactions can be studied in dissected pieces of dental epithelium and mesenchyme cultured as recombinants, and here we analysed *Ser-1* expression in E13-14 dental explants. When dental epithelium is cultured together with dental mesenchyme *Ser-1* transcripts are detected in the mesenchymal cells adjacent to the dental epithelium after 20 hours of culture (Fig. 2A). While epithelium express *Ser-1*, epithelial cells contacting the *Ser-1*-positive mesenchyme do not express the gene.

The transient upregulation of *Ser-1* expression in dental mesenchyme in vivo and in tissue explants in vitro, suggest that *Ser-1* expression in mesenchyme is activated by epithelial-derived signals present at these developmental stages (E12.5-15.5). Beads releasing signaling molecules can be implanted into dissected dental tissue to analyse gene regulation (Mitsiadis et al., 1995). Diffusible molecules that may mediate this signal include members of the FGF and BMP families of growth factors. An increase in *Ser-1* expression is observed when FGF-4 beads were implanted in dental mesenchyme cultured for 20 hours (Fig. 2A). The appearance of a translucent area around the beads confirm that biologically active protein is secreted from the bead. In contrast, the level of *Ser-1* expression around BMP-2 or/and BMP-4 releasing beads is not increased above background (not shown). We next tested whether FGFs and BMPs would regulate *Notch* expression in a similar way. No increase in *Notch* expression is observed in dental mesenchyme after implantation of beads releasing FGF-4 (not shown). In contrast, *Notch* transcription is increased around BMPs releasing beads in dental mesenchyme (Fig. 2B).

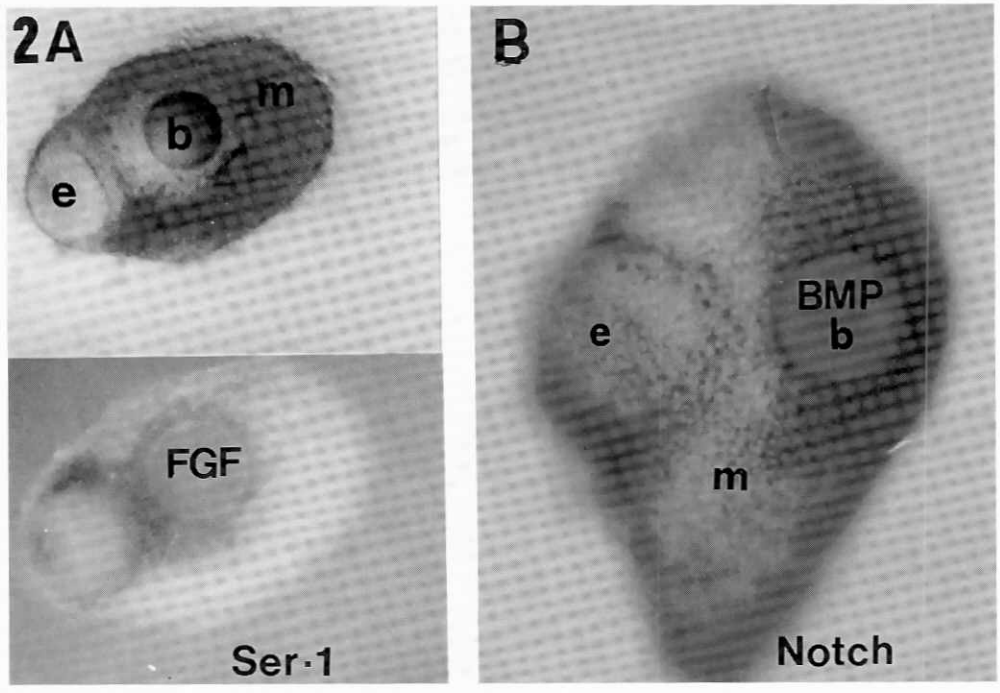

Figure 2. Expression of *Ser-1* in explants of recombined E13.5-14.5 dental epithelium and mesenchyme and/or after exposure to signaling molecules. (A) Effects of dental epithelium and FGFs on *Ser-1* expression in dental mesenchyme. In situ hybridization using a digoxigenin-labeled probe for *Ser-1*. Note the upregulation of the gene in mesenchume close to epithelium and around a bead containing 100 μg/ml FGF-4. (B). Implantation of a bead containing 250 μg/ml BMP-4 results in an upregulation of *Notch 3* expression in E13.5 dental mesenchyme. Abbreviations: b, bead; e, epithelium; m, mesenchyme.

The fact that BMPs induce *Notch* gene expression, but did not stimulate cell proliferation, may indicate that BMPs act instructively to influence the fate of dental cells. FGFs upregulates *Ser-1* expression and stimulate cell proliferation, suggesting that FGFs act rather selectively to support survival of lineage-committed progenitors.

In conclusion, it appears that two major induction pathways are involved in regulating the expression of members of the Notch signaling pathway in the developing tooth, but affect Notch ligands and receptors differently. The developing tooth organ may therefore utilize growth factors in different ways to generate cellular diversity.

References

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