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Dact1-3 mRNAs exhibit distinct expression domains during tooth development

Päivi Kettunen^{1,✉}, Saul Kivimäe², Pankaj Keshari¹, Ophir D. Klein⁵, Benjamin N.R. Cheyette^{2,3,4}, and Keijo Luukko¹

¹Section of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway

²Department of Psychiatry, University of California, San Francisco, San Francisco, California, USA

³Graduate Program in Developmental Biology, University of California, San Francisco, San Francisco, California, USA

⁴Graduate Program in Neuroscience, University of California, San Francisco, San Francisco, California, USA

⁵Departments of Orofacial Sciences and Pediatrics, and Institute of Human Genetics, University of California San Francisco, 513 Parnassus Ave., Box 0442, San Francisco, California, USA

Abstract

Wnt signaling is essential for tooth formation. Dact proteins modulate Wnt signaling by binding to the intracellular protein Dishevelled (Dvl). Comparison of all known mouse Dact genes, Dact1-3, from the morphological initiation of mandibular first molar development after the onset of the root formation using sectional *in situ* hybridization showed distinct, complementary and overlapping expression patterns for the studied genes. While *Dact2* expression was restricted to the dental epithelium including the enamel knot signaling centers and tooth specific preameloblasts, *Dact1* and *Dact3* showed developmentally regulated expression in the dental mesenchyme. Both mRNAs were first detected in the presumptive dental mesenchyme. After being downregulated from the condensed dental mesenchyme of the bud stage tooth germ, *Dact1* was upregulated in the dental follicle mesenchyme at the cap stage and subsequently also in the dental papilla at the bell stage where the expression persisted to the postnatal stages. In contrast, *Dact3* transcripts persisted throughout the dental mesenchymal tissue components including the tooth-specific cells, preodontoblasts before transcripts were largely downregulated from the tooth germ postnatally. Collectively these results suggest that Dact1 and -3 may contribute to early tooth formation by modulation of Wnt signaling pathways in the mesenchyme, including preodontoblasts, whereas Dact2 may play important signal-modulating roles in the adjacent epithelial cells including the enamel knot signaling centers and preameloblasts. Future loss-of-function studies will help elucidate whether any of these functions are redundant, particularly for Dact1 and Dact3.

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✉Corresponding author: Department of Biomedicine, University of Bergen Jonas Lies vei 91, 5009 Bergen, Norway Tel: +47 55 58 63 64 Fax: +47 55 58 63 60 Paivi.Kettunen@biomed.uib.no.

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1. Results and Discussion

The tooth, in particular the mouse first molar, is an excellent model to analyze molecular signaling mechanisms of mammalian organogenesis. Tooth formation is controlled by signaling pathways conserved across species (for reviews see (Miletich and Sharpe, 2003; Thesleff, 2006), (Lesot and Brook, 2008; Luukko et al., 2008). Previous studies indicate that Wnt/ β -catenin signaling serves critical roles in odontogenesis. Recently, constitutive activation of the Wnt/ β -catenin pathway was shown to result in continuous supernumerary tooth formation, while inhibition of this pathway interferes with tooth formation by causing arrest at early developmental stages (van Genderen et al., 1994); (Andl et al., 2002; Jarvinen et al., 2006; Lammi et al., 2004; Liu et al., 2008; Wang et al., 2009). The functions of other, β -catenin-independent Wnt signaling pathways, including the planar cell polarity (PCP) and Wnt/ Ca^{2+} pathways, remains poorly understood in the tooth. Wnt signaling is modulated at many levels by multiple mechanisms, including the intracellular Dact (also known as Dapper or Frodo) proteins. Dact proteins regulate Wnt signaling at least in part by binding to Dishevelled (Dsh/Dvl) (Suriben et al., 2009) a cytoplasmic protein that is centrally placed in all Wnt signaling pathways (Veeman et al., 2003). Recently, *Xenopus* Dact protein (XDpr1a) was shown to inhibit Wnt/ β -catenin signaling when unphosphorylated, but to promote this signaling pathway when phosphorylated in an *in vitro* assay (Teran et al., 2009).

Three Dact paralogs, namely Dact1-3, have been characterized in mouse and they all show distinct expression domains during embryonic development suggesting differential signaling functions (Fisher et al., 2006). Dact1^{-/-} mouse embryos show posterior malformations in the spine, genitourinary and distal digestive system that result from changes in β -catenin independent signaling (Suriben et al., 2009) while targeted inactivation of Dact2 has been reported to lead to accelerated re-epithelialization during cutaneous wound healing through attenuating Tgf β signaling (Meng et al., 2008).

Epithelial expression of *Dact2* in the tooth has been reported at the cap stage (Fisher et al., 2006) while the expression of other Dact genes in the tooth is not known. Given the regulatory functions of Dact proteins in Wnt signaling, which is essential for tooth formation, we systemically compared cellular mRNA expression of Dact1-3 during initiation and epithelial morphogenesis of the mandibular first molar tooth germ from embryonic day (E) 11.5 to day 14 postnatally (PN14) using sensitive radioactive *in situ* hybridization.

Dact1 and -3 mRNAs were exclusively expressed in the mesenchymal tissue component of the molar tooth, but were differentially regulated. At the morphological onset of tooth formation, expression of both mRNAs was seen in the developing jaw mesenchyme including presumptive dental mesenchyme (Fig. 1B1, D1). However, *Dact1* signal appeared to be weaker in the presumptive dental mesenchyme adjacent the epithelial thickening than in the mesenchyme surrounding this area. Two days later, at the bud stage (E13.5) differences in the expression of the Dact1 and Dact3 genes became evident (Fig. 1B2, D2). While *Dact3* continued to be expressed in the condensed dental mesenchyme, *Dact1* was downregulated there. However, *Dact1* expression was observed in the jaw mesenchyme surrounding the condensed dental mesenchyme at this stage (arrows in Fig 1B2).

Tooth-specific epithelial folding morphogenesis starts at the cap stage and continues throughout the bell stage (Kollar and Lumsden, 1979). During these stages, the mesenchymal dental follicle, surrounding the dental papilla and epithelial enamel organ is visible. The dental follicle gives rise to cemento-, fibro- and osteoblasts forming the tooth supporting periodontium. At the cap stage (E14.5) *Dact1* expression was observed in the dental follicle and in the adjacent jaw mesenchyme (Fig. 1B3). In contrast, *Dact3* was expressed both in the

dental papilla and follicle (Fig. 1D3). At this stage *Dact1* transcripts also appeared in the oral epithelium (Fig. 1B3).

At the early bell stage (E16.5) *Dact1* was upregulated in the cervical-middle part of the dental papilla mesenchyme (Fig. 1B4). In contrast, *Dact3* transcripts were present throughout the dental papilla and follicle (Fig. 1D4). One day before birth at E18.5 the tooth-specific preodontoblasts are visible in the dental papilla mesenchyme adjacent to the inner dental epithelium at the most advanced cuspal areas (Lesot et al., 2001). At this stage, *Dact1* continued to be expressed in the cervical-middle part of the dental papilla as well as in the dental follicle (Fig. 1B5). The similar expression pattern for *Dact1* persisted after the enamel and dentin secretion and the onset of the root formation postnatally as shown for 8-day postnatal tooth germ (Figs. 2B1 and 2B2). *Dact3* expression was apparent throughout the dental papilla, but the most prominent expression was detected in the coronal part of the papilla including the preodontoblasts (Fig. 1D5). Postnatally, at PN8 little if any specific *Dact3* mRNA expression was seen in the dental pulp any longer (Figs. 2D1). No specific expression of *Dact1* or *-3* was observed in the Hertwig's epithelial root sheaths responsible for tooth formation (Fig. 2D2).

Dact1 and *Dact3* expression in the lower jaw incisor tooth germ correlated to that observed in the molars. At the epithelial thickening stage *Dact1* transcripts were present in the dental and jaw mesenchyme. Later at the bud and cap stage, expression continued in the jaw mesenchyme next to the dental mesenchyme as shown for the E13.5 bud stage incisor in figure 2B3, and subsequently the expression also appeared in the dental follicle. During the bell stage *Dact1* transcripts become upregulated in the middle part of the dental papilla and pulp (see Fig. 2B4 for a E16.5 incisors). *Dact3* expression was present in the mesenchymal tissue components during E11.5-E14.5 (Fig. 2D3). At E16.5, however, only a few, if any transcripts were observed in the incisor mesenchyme any longer (see Figs. 2D4). Postnatally, a prominent *Dact3* hybridization signal was confirmed to muscle tissue while little if any specific *Dact3* expression was seen in the incisor pulp (not shown).

In contrast to *Dact1* and *-3*, *Dact2* expression was solely restricted to the dental epithelium. At the epithelial thickening stage (E11.5), *Dact2* showed prominent expression in the dental epithelium (Fig. 1C1). The expression continued in the epithelial dental bud at E13.5 and in the epithelial enamel organ of the E14.5 cap stage tooth germ. This included cells of primary enamel knot, a signaling center that regulates tooth shape (Figs. 1C2 and 1C3) (Jernvall et al., 2000; Jernvall et al., 1994). Later during the bell stage when the final shape of the tooth is established, *Dact2* expression continued in cells of the epithelial enamel organ including the inner and outer dental epithelium, stellate reticulum, stratum intermedium and cervical loops, which later form Hertwig's epithelial root sheath responsible for root formation (Figs. 1C4 and 1C5). Molar tooth crown morphogenesis is characterized by formation of the cusps and histodifferentiation of the inner dental epithelium cells to ameloblasts, which produce enamel. Of note, *Dact2* was expressed in cells of the inner dental epithelium, which are precursors of the ameloblasts, and in the cervical loops. The secondary and tertiary enamel knots present in the bell stage molar regulate individual cusp formation and the final shape of the molar crown (Jernvall et al., 1994; Luukko et al., 2003). Of special interest is the finding that *Dact2* expression also included both the upper and lower compartments (Luukko et al., 2003) of the secondary enamel knots (SEKs) as shown for E16 molar (arrows in Fig. 1C4) and the tertiary enamel knot (TEK) (not shown). The expression of *Dact2* was downregulated from ameloblasts secreting enamel and by PN8 no specific expression of *Dact2* was seen in the tooth germ. In addition, the Hertwig's epithelial root sheaths were devoid of transcripts (Figs. 2C1 and 2C2).

The expression of *Dact2* in the incisors correlated to that of in molars. *Dact2* transcripts were confirmed to the dental epithelia including the cervical loops as shown for E13.5 and E16.5

tooth germs (Figs. 2C3 and C4). However, at PN2 no specific *Dact2* expression appeared in the tooth germ any longer (not shown).

In conclusion, our results here show that the three *Dact* genes exhibit distinct, overlapping and complementary expression domains during tooth development. While *Dact1* and *-3* showed differentially, developmentally regulated expression in the dental mesenchymal tissue components, the expression of *Dact2* was restricted to the dental epithelium. These results suggest that regulation of Wnt signaling by *Dact* proteins may serve important functions in tooth initiation, crown morphogenesis and differentiation of tooth-specific cells. Moreover, the overlapping expression of *Dact1* and *-3* suggest that developmental functions of their protein products may be either redundant and/or complementary during tooth formation. Loss-of-function studies in the future will help elucidate the *in vivo* functions of the *Dact* proteins.

2. Experimental Procedures

The animal use was approved by the Animal Welfare Committee of the Preclinical Institutes, University of Bergen. Mice (CBA & NMRI) were mated overnight and the appearance of vaginal plug was taken as day 0 of embryogenesis (E0.5). The developmental stages of the tooth germs were judged from the tissue sections according to morphological criteria. Tissue processing, sectional *in situ* hybridization, photomicrography and processing of the images were performed as described (Kettunen et al., 1998; Luukko et al., 1996; Kettunen et al., 2005). Plasmids containing cDNA fragments of *Dact1-3* have been described earlier in (Fisher et al., 2006). Sections were exposed for 3 weeks. No specific hybridization signal was detected in the control sections hybridized with corresponding sense probes (data not shown).

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4. References

- Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. *Dev Cell* 2002;2:643–53. [PubMed: 12015971]
- Fisher DA, Kivimae S, Hoshino J, Suriben R, Martin PM, Baxter N, Cheyette BN. Three *Dact* gene family members are expressed during embryonic development and in the adult brains of mice. *Dev Dyn* 2006;235:2620–30. [PubMed: 16881060]
- Jarvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J, Thesleff I. Continuous tooth generation in mouse is induced by activated epithelial Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A* 2006;103:18627–32. [PubMed: 17121988]
- Jernvall J, Keranen SV, Thesleff I. Evolutionary modification of development in mammalian teeth: quantifying gene expression patterns and topography. *Proc Natl Acad Sci U S A* 2000;97:14444–8. [PubMed: 11121045]
- Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I. Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating *Fgf-4* gene. *Int J Dev Biol* 1994;38:463–9. [PubMed: 7848830]
- Kollar EJ, Lumsden AG. Tooth morphogenesis: the role of the innervation during induction and pattern formation. *J Biol Buccale* 1979;7:49–60. [PubMed: 285074]
- Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I, Pirinen S, Nieminen P. Mutations in *AXIN2* cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004;74:1043–50. [PubMed: 15042511]
- Lesot H, Brook AH. Epithelial histogenesis during tooth development. *Arch Oral Biol*. 2008
- Lesot H, Lisi S, Peterkova R, Peterka M, Mitolo V, Ruch JV. Epigenetic signals during odontoblast differentiation. *Adv Dent Res* 2001;15:8–13. [PubMed: 12640731]

- Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, Yang SH, Lu MM, Piccolo S, Schmidt-Ullrich R, Taketo MM, Morrissey EE, Atit R, Dlugosz AA, Millar SE. Wnt/beta-catenin signaling directs multiple stages of tooth morphogenesis. *Dev Biol* 2008;313:210–24. [PubMed: 18022614]
- Luukko K, Loes S, Furmanek T, Fjeld K, Kvinnsland IH, Kettunen P. Identification of a novel putative signaling center, the tertiary enamel knot in the postnatal mouse molar tooth. *Mech Dev* 2003;120:270–6. [PubMed: 12591596]
- Luukko K, Moe K, Sijaona A, Furmanek T, Hals Kvinnsland I, Midtbo M, Kettunen P. Secondary induction and the development of tooth nerve supply. *Ann Anat* 2008;190:178–87. [PubMed: 18413271]
- Meng F, Cheng X, Yang L, Hou N, Yang X, Meng A. Accelerated re-epithelialization in Dpr2-deficient mice is associated with enhanced response to TGFbeta signaling. *J Cell Sci* 2008;121:2904–12. [PubMed: 18716284]
- Miletich I, Sharpe PT. Normal and abnormal dental development. *Hum Mol Genet* 2003;12(Spec No 1):R69–73. [PubMed: 12668599]
- Suriben R, Kivimae S, Fisher DA, Moon RT, Cheyette BN. Posterior malformations in Dact1 mutant mice arise through misregulated Vangl2 at the primitive streak. *Nat Genet.* 2009
- Teran E, Branscomb AD, Seeling JM. Dpr Acts as a molecular switch, inhibiting Wnt signaling when unphosphorylated, but promoting Wnt signaling when phosphorylated by casein kinase Idelta/epsilon. *PLoS One* 2009;4:e5522. [PubMed: 19440376]
- Thesleff I. The genetic basis of tooth development and dental defects. *Am J Med Genet A* 2006;140:2530–5. [PubMed: 16838332]
- van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev* 1994;8:2691–703. [PubMed: 7958926]
- Wang XP, O'Connell DJ, Lund JJ, Saadi I, Kuraguchi M, Turbe-Doan A, Cavallesco R, Kim H, Park PJ, Harada H, Kucherlapati R, Maas RL. Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. *Development* 2009;136:1939–49. [PubMed: 19429790]

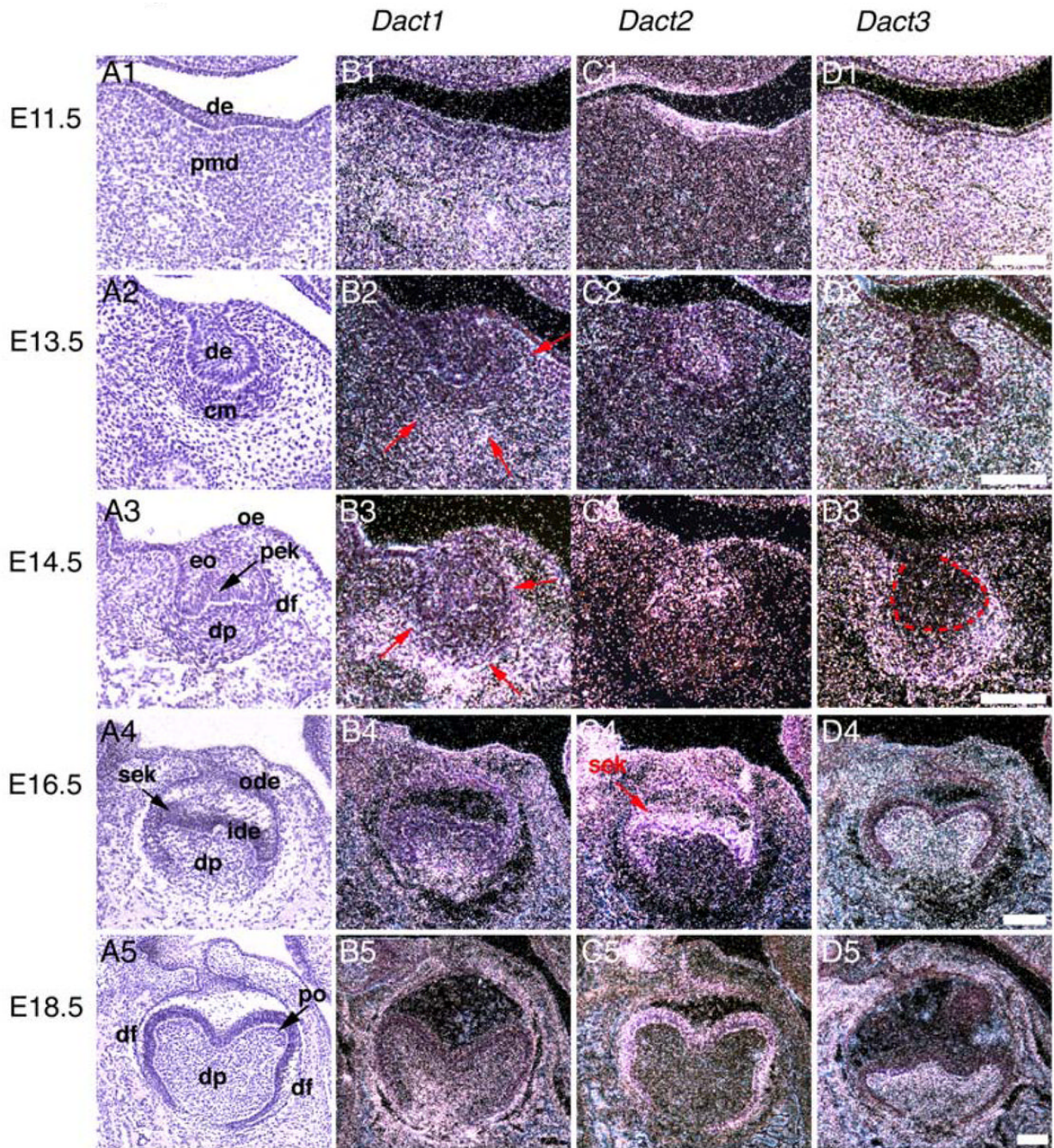


Figure 1.

Localization of *Dact1-3* mRNAs during development of the mouse tooth. Frontal sections of the first mandibular molars. Arrows in B2 indicate jaw mesenchyme surrounding the epithelial bud and condensed dental mesenchyme, while arrows in B3 indicate the mesenchymal dental follicle. Buccal and lingual side is to the left and right, respectively. Basement membrane is marked with a dashed line in D3. Abbreviations: cm, condensed dental mesenchyme; de, dental epithelium; df, dental follicle; dp, dental papilla mesenchyme; E, embryonic age; eo, enamel organ; ide, inner dental epithelium; oe, oral epithelium; ode, outer dental epithelium; pek, primary enamel knot; pdm, presumptive dental mesenchyme; po, preodontoblasts; sek, secondary enamel knot. Scale bars: 100 μ m.

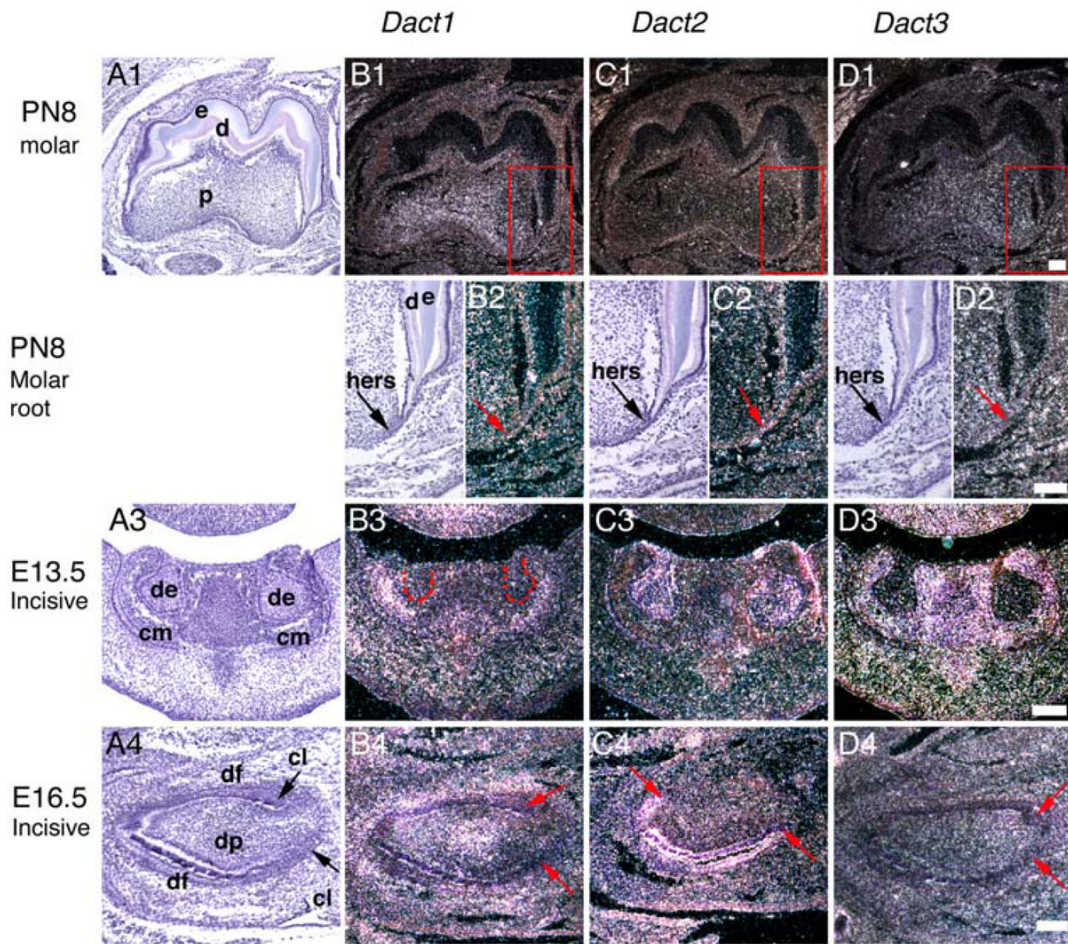


Figure 2.

Localization of *Dact1-3* mRNAs during development of the mouse tooth. Sagittal (A1-D2, A4-D4) and frontal (A3-D3) sections of the first mandibular molars (A1-D2) and mandibular incisors (A3-D4). Figures B2-D2 are higher magnifications from the areas in figures B1-D1 marked by red boxes. In frontal sections, buccal and lingual side is to the left and right, respectively. In sagittal sections rostral and dorsal side is to left and right, respectively. Basement membrane is marked with a dashed line in B3. *Dact3* does not show specific expression in tooth in D1. Abbreviations: cl, cervical loop; cm, condensed dental mesenchyme; d, dentin; de, dental epithelium; df, dental follicle; dp, dental papilla mesenchyme; e, enamel; hers, Hertwig's epithelial root sheath. Scale bars: 100 μ m.