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Short Report

Study of novel genes involved in odontoblast and ameloblast differentiation

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ABSTRACT Teeth are organs which develop by inductive interactions between dental epithelium and subjacent mesenchyme. In order to identify genes involved in odontoblast and ameloblast differentiation, we have constructed a cDNA library from E19.5 mouse molars. In this work, we report partial DNA sequences of 10 non-characterized genes. In addition, we show cell expression of the transcripts in mouse embryo molars by *in situ* hybridization. We discuss the role of these genes in cell differentiation.

Introduction

Signaling molecules, their receptors and target genes from pathways and networks regulate the development of the tooth from initiation through cell differentiation (Thesleff 2000). In particular, odontoblasts are post-mitotic polarized cells that differentiate in mouse molars at E18 bell stage and they are involved in production of predentin-dentin matrix. In addition, ameloblasts differentiate at late bell stage (E19-20) and synthesize and secrete enamel matrix.

Although there is a vast information about genes involved in tooth initiation and morphogenesis, odontoblast and ameloblast differentiation remains as complex and unclear processes. This complex series of events is regulated by growth factors, receptors, transcription factors and cell substrate and cell adhesion molecules (Zeichner-David *et al.,* 1995; Ruch and Lesot, 2000; Unda *et al.,* 2000). In order to identify genes involved in odontoblast and ameloblast differentiation, we have prepared a cDNA library from E19.5 lower first mouse molars and studied novel genes by partial sequencing of cDNA clones and *in situ* hybridization.

Materials and Methods

Construction of the cDNA library from microdissected embryo molars

First lower molars from E19.5-day old mouse embryos were excised and Poly A^+ RNA (5 μg) was obtained by use of oligo dT cellulose. A cDNA was constructed in the λZAP II vector by use the Poly A^+ RNA and the Uni-Zap XR kit (Stratagene). The average size of cDNA inserts was approximately 1.5-2 kb. The paghe cDNA library was converted into a pBluescript phagemid cDNA library by *in vivo* excision by the ExAssist/SOLR system (Stratagene), as described in the manufacturer's protocol. The pBluescript cDNA library was plated on LB plates with X-gal, IPTG, and ampicilin, and white colonies were selected for sequencing.

DNA sequencing and homology search

Selected clones were used to prepare plasmid DNA. One μg of DNA was used for fluorescent-tag dideoxy sequencing reactions with AmpliTaq DNA polymerase and M13 reverse primer from the polylinker region of pBluescript SK (+/-) phagemid. Cycle DNA sequencing was performed by means of a Gene Amp PCR System. The sample was analyzed on a 6% acrylamide-8 M urea gel by an automated DNA sequencer (Applied Biosystems, model 373A). The average length of DNA sequence was 500 base. Homology searches for each sequence were performed on the GenBank database by Blast Search program via Internet connection to the National Center for Biotechnology (Benson *et al.,* 2000).

In situ *hybridization in whole tissues*

In order to prepare antisense RNA probes, FL probe was linearized by EcoRI, whereas I, FC and FM were linearized with BamHI. Digoxigenin-UTP labeled, single-stranded antisense RNA probe were prepared with T7 RNA polymerase using a RNA labeling kit (Boehringer Mannheim, Indianapolis, IN, USA). E19.5 first lower molars were fixed in 4% paraformaldehyde/PBS overnight, dehydrated in methanol and kept at –20°C until analyzed. Whole-mount RNA *in situ* hybridization was carried out according to Nieto (1996).

TABLE 1

CLONES WITH LIMITED REGIONS HOMOLOGY TO SEQUENCES IN THE GENEBANK DATABASE

Clone	Putative identification	Score
F2	Human HLA-B-associated transcript 2 gene	133
F3	Mus musculus, clone 187J17	44
F5	Plasmodium falciparum MAL3P1	116
D	Anolis carolensis Brain-2 gene	147
I	Mouse beta-glucuronidase	183
FC	Staphylococcus capitis epr gene	129
FE	Human cosmid LL12NCO1-N-184C4	163
FL	Iduronate-2-sulfatase	152
FM	C. elegans cosmid F46F6	156
FT	C. elegans cosmid C11E	168

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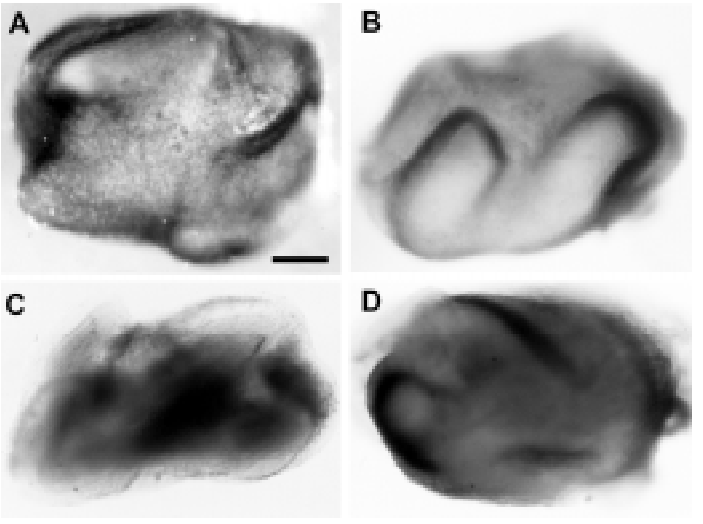

Fig. 1. Whole mount *in situ* hybridization in the first mouse molar at 19.5 days post coitum. *Expression of transcripts for I* **(A)**, *cranial view, and FC* **(B)**, *FL* **(C)** *and FM* **(D)**, *sagittal view. Scale bar, 50 μm.*

Results and Discussion

Sequence analysis and predictions of cDNA clones

We randomly selected 50 bacterial white colonies from LB-agar plates containing X-gal, and plasmid DNA was prepared. The partial reverse sequences (500 base length) from these cDNA clones we searched for sequence homology. The predicted coding sequences of 40 genes (80%) were found to exhibit significant similarities to known genes (at least one match with a score greater than 300). The remaining clones (20%) showed less homology to genes in the databases (scores all less than 200). The results of the classification of these genes are summarized in Table 1. Clones whose sequence was very similar to enamel protein known as enamelin were found twice. In addition, we found Ptx1 gene, a member of the small bicoid family of homeobox-containing genes, which has been involved in Treacher-Collins-Franceschetti Syndrome, a facial abnormality consisting of micrognathia, and other deformities of the head.

Expression of novel genes in the developing tooth

In order to obtain further information of non-characterized genes shown in Table 1, we examined their expression profiles in E19.5 mouse molars by *in situ* hybridization. In this context, I gene was detected in stratum intermedium and stellate reticulum. Dental mesenchyme cells displayed very faint signal (Fig. 1A). FC gene was restricted to dental epithelia cells. Preameloblast/ameloblast cells exhibited a strong expression and dental cusps clearly expressed the transcripts for this gene. This location suggests a role for ameloblast differentiation (Fig. 1B). On the contrary, FL gene was expressed in dental papillae cells, whereas dental epithelium was negative (Fig. 1C). FM expression was observed both in dental epithelium and mesenchyme (Fig. 1D). The remaining unidentified genes (F2, F3, F5, D, FE and FT) were not detected in tooth tissues by using antisense-digoxigenin labeled RNA probes.

These novel genes are being further analyzed in depth for their possible biological significance during odontogenesis and craniofacial development.

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