

Mouse tooth development time sequence determination for the ICR/Jcl strain

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Abstract: To establish the normal dental development pattern of the ICR/Jcl strain of mouse, we analyzed a significant number of observations of the different developmental stages of the first mandibular molar, accurately recording the chronology of their daily embryonic development. Proliferation of the dental sheet began at day 12.5 in utero (E-12.5), the bud stage appeared at days E-13.5 and E-14.5, the cap stage was observed at days E-14.5, E-15.5 and E-16.5 and the early bell stage at day E-17.5. The presence of pre-dentin was observed at day E-18.5 and dentin was observed 1 and 2 days after birth (D-1 and D-2). The late bell stage with presence of enamel was detected more than 3 days after birth. Embryonic and dental development in the ICR/Jcl strain of mouse is faster than in other well-known strains. The establishment of this developmental pattern will be useful for future investigations of transgenic mice. (J. Oral Sci. 46, 135-141, 2004)

Key words: mice odontogenesis; ICR/Jcl strain; first mandibular molar.

Introduction

Following Spemann's pioneering work on neural induction, it has been clearly established that the embryonic development of vertebrates rests on a succession of interactions between different groups of cells (1,2). Except for the central nervous system, all organ development

starts from epithelial and mesenchymal tissues, whose reciprocal and sequential interactions govern the main stages of organogenesis (3-6).

Tooth development constitutes a particularly interesting model for studying such epithelio-mesenchymal interactions (7,8) and is an excellent subject for evolutionary studies (9-11). The different induction stages that precede the morphogenesis and differentiation of the teeth result from reciprocal interactions among stomodeal epithelial cells and mesenchymal cells derived from neural crests (ectomesenchyme) (12,13). The identification of numerous growth differentiation factors (14-17), transcription factors (18-21) and molecules of adhesion (22-24) expressed in the course of the development of the tooth have revealed associations of multiple genes with tooth morphogenesis. Studies on the functions of signals and tissue interactions in cultured tissue explants and in mutant mouse embryos have revealed inductive signaling and hierarchies in downstream transcription factors (25).

The main lines that govern dental development are generally the same in all mammals; however, features specific to each species exist, and those of each strain should be established and thoroughly analyzed when genetic and developmental studies are to be carried out (26,27).

The mouse, *Mus musculus*, is the main species used for studies of mammalian development because of its advantages with regard to feeding, reproduction, and genetic and embryonic manipulation (28,29). It has a short period of gestation (18 to 21 days) and a long period of reproductive activity (2 to 14 months); therefore, it is ideal for experimentation in mammals (28). The various strains of laboratory mice available have a number of genetic and phenotypic advantages. The ICR/Jcl strain is characterized by its high reproductive efficiency (30), and

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its tooth morphogenesis has not yet been described.

The formation of teeth can be divided into three major stages: initiation, morphogenesis and differentiation. The first morphological sign of tooth development is a thickening of the oral ectoderm. This is followed by budding of the epithelium and condensation of the neural crest-derived mesenchymal cells around the bud. The bud then undergoes folding morphogenesis and develops into a cap-like structure (the dental epithelium is subsequently called the enamel organ), and the final shape of the tooth crown (the part of the tooth in the oral cavity) develops during the bell stage. After the completion of crown formation, roots develop and the teeth erupt into the oral cavity (31,32).

Mice have only two tooth families, one incisor in the front and three molars in the back of each half of the jaws. The incisors and molars are separated by an area with no teeth, the diastema. However incipient abortive formation of dental germs is distinguishable in that space (33).

The object of this study was to describe tooth morphogenesis in the ICR/Jcl strain and to compare it with the developmental stages in other previously analyzed strains. This description will be based mainly on the first mandibular molar (M1), which has been the most extensively studied, from the budding of the oral epithelium on prenatal day 11 (E-11) to the appearance of the pre-enamel of the crown on postnatal day 3 (D-3). This will allow us to establish the timing of the normal dental development pattern for this strain, and to use it in future investigations of transgenic mice.

Materials and Methods

The study was approved by the Animal Welfare Committee of the Dental School of the University of Chile. *Mus musculus* ICR/Jcl embryo heads (Laboratory Clea Japan, Japan) were dissected from E-10.5 to D-2 (midnight before vaginal plug observation = D-0) at 0900 h each day and fixed in 4% paraformaldehyde-PBS buffer (pH 7.4) for 8 h at 4°C (Paraformaldehyde, Merck, Santiago, Chile). The heads were measured with calipers and 30 similar-sized heads were analyzed for each day of development (approximately three litters per embryonic stage). The embryo heads were rinsed overnight in PBS buffer at 4°C. The D-1 and D-2 embryo heads were demineralized in 0.125 M EDTA at 4°C for 2 weeks. The tissues were embedded in paraffin (Histowax, ProLab, Chile) and serially sectioned. The 7-µm serial sections, cut both frontally and sagittally, were stained with hematoxylin-eosin and examined with a DLMS Leica light microscope (Equilab, Chile).

Values were assigned from 0 to 7 according to the

morphologic aspects observed in the dental developmental stage: any morphologic change: 0; proliferation of the dental sheet: 1; bud stage: 2; cap stage: 3; early bell stage: 4; presence of pre-dentin: 5; presence of pre-dentin and dentin: 6; late bell stage (presence of pre-dentin, dentin and enamel): 7.

The frequency of occurrence of each morphological stage in tooth development was calculated as a percentage of the total number of observations for each day of the gestation period. The strains were maintained in mouse housing with a P 2 security level.

Results

Morphological analysis

All ICR/Jcl mice were born at day E-19.5 of gestation, considered day 1 (D-1). A summary of development stages of the mandibular M1 in the ICR/Jcl strain is presented in Table 1. The first morphological features of M1 ICR/Jcl mouse odontogenesis were initiated at E-12.5 from the ectoderm covering the maxillary, frontonasal (maxillary) and mandibular processes forming a single row in the upper and lower jaws. A thickened epithelial stripe marked the future dental arch (Fig. 1-A). At E-13.5 the outgrowth of the epithelium into the ectomesenchyme was prominent. A condensation of mesenchymal cells, probably neural crest-derived cells, was observed around the bud, comprising the bud stage (Fig. 1-B). A tooth bud of increased size with a fold at its tip was observed at E-14.5, marking the transition of the bud to the cap stage with enamel organ formation. The folding of the bud end resulted in the formation of cervical loops, which grew rapidly downwards. The dental mesenchyme cells that condensed around the bud formed the dental papilla between the cervical loops and the dental follicle surrounding the epithelium (Fig. 1-C and D).

At E-15.5 further growth lengthwise and widthwise, together with the folding of the epithelium, gave rise to the cap stage. A basal membrane separated the enamel organ from the dental papilla. The epithelial cap was made up of two epithelial sheets enclosing a group of cells that started to separate and form an enamel knot in the internal dental epithelium of the enamel organ. The vascular elements increased in the dental papilla. The separation between the central cells of the enamel organ increased at E-16.5. The external dental epithelium was formed by several layers of cubical cells and the internal dental epithelium by only one layer. Bone formation around the follicle was observed.

At E-17.5 the external dental epithelium cells were smoother, while the internal dental epithelium cells had acquired a lengthened form with a clear intracellular

Table 1 Summary of the developmental and morphological features of the ICR/Jcl first mandibular molar (D-0=vaginal plug)

Morphological stage	Day of development	% of tooth morphological stage observation
Proliferation of the dental sheet	E-12	86.67 %
Bud stage	E-13 and E-14	67 %
Cap stage	E-15 and E-16	80%
Early bell stage	E-17	53.3%
Presence of pre-dentin at the top of the tip	E-18	63%
Presence of pre-dentin and dentin	D-1 and D-2	46.7%
Late bell stage (presence of pre-dentin, dentin and enamel)	>3 days	

material. The stellate reticulum intercellular spaces increased and an intermediate stratum appeared between the stellate reticulum and the internal dental epithelium. The cervical loop was more marked. This was the beginning of the bell stage (Fig. 1-E). The tooth crown shape then began to be distinguishable. The internal dental epithelium folded at the sites of the tips of future tooth cusps and the secondary enamel knots became distinguishable. The stellate reticulum became differentiated, the smoothed cells of the external dental epithelium remained joined to the oral epithelium through an epithelial bridge and the dental follicle spaces increased. At the tip of the principal cusps, the internal dental epithelium cells were higher and thinner and their nuclei were polarized consistent with their differentiation into pre-secretor ameloblasts. In the dental papilla the external cells in contact with the basal membrane assumed a columnar shape while the central cells had an undifferentiated appearance and were surrounded by numerous blood vessels.

At E-18.5, the dental organ had acquired the form of the crown, and the dental lamina joining the tooth germ and the oral epithelium had broken up. The basal membrane between the epithelium and mesenchyme disappeared at the tip of the principal cusps and was replaced by a thin and clear eosinophilic layer, corresponding to pre-dentin (non-mineralized dentin) (Fig. 1-F). The odontoblasts left behind a cell process that would later become embedded in the dentin matrix and subsequently occupy a dentinal tubule. At D-1 (postnatal day 1), dentinogenesis was still progressing. A thin mineralized dentin layer was observed at the tip of the cusps (Fig. 1-G), and at D-2 (postnatal day 2), although the characteristics more frequently observed

corresponded to those of D-1, and sometimes a thin layer of pre-enamel was observed at the tip of the cusps (Fig. 1-H).

Statistical Analysis

The frequency of occurrence of each morphological stage of tooth development was calculated as a percentage of the total number of observations for each day of the gestation period. The results are shown in Fig. 2. It can be clearly seen that on day E-12.5, 86.67% of the observed samples showed proliferation of the dental sheet, the first morphological sign of the initiation of odontogenesis. Fig. 2 and Table 1 show the days on which a high percentage of samples displayed features of each morphological stage of dental development. Between D-1 and D-2, less than 50% of the samples featured dentin rather than pre-dentin, indicating that the presence of dentin should be expected at a more advanced developmental stage in this strain. The same could be expected for the presence of enamel in the mandibular molars.

Discussion

This study investigated the stages of tooth development up to the appearance of the pre-enamel to establish the normal development pattern of the ICR/Jcl strain of mouse. We observed that the period of gestation for the ICR/Jcl strain is 19.5 days, which is significantly shorter than the 20 days described for the albino strain (34). The development of the ICR/Jcl molar showed similar embryonic stages to those observed in humans, other species of rodents such as the mouse *Mus caroli*, the rat *Rattus rattus* and the hamster *Cricetus* sp., and in bovine

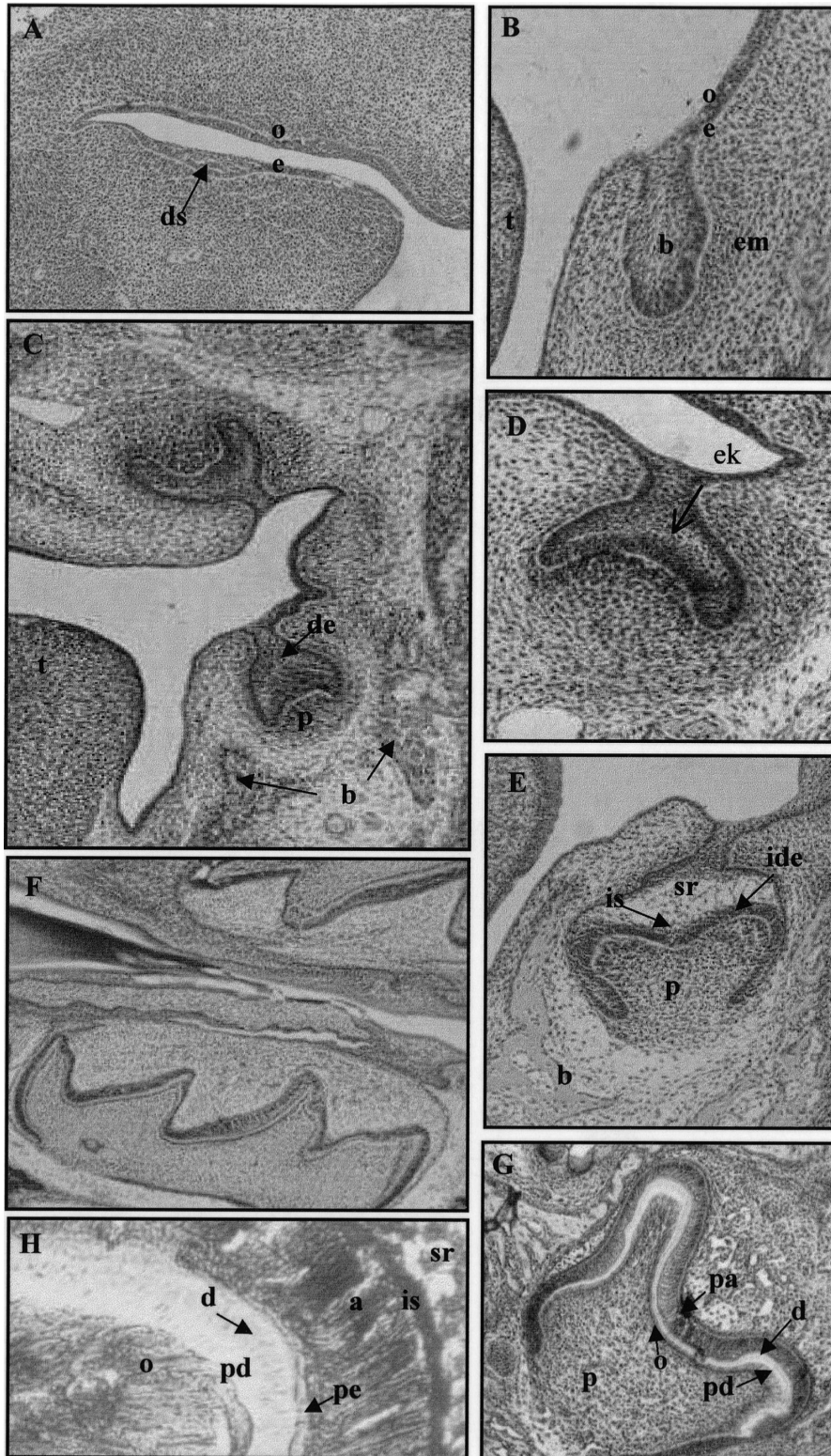


Fig. 1 HE stain

A: ICR/Jcl mouse odontogenesis at E-12. (ds) dental sheet (oe) oral epithelium Magnification $\times 10$ B: Bud stage at E-14. (b) bud (em) ectomesenchyme, (t) tongue Magnification $\times 10$ C: E-15 Cap stage. (de) dental epithelium, (p) dental papilla, (b) bone Magnification $\times 4$ D: Cap stage. (ek) enamel knot Magnification $\times 10$ E: Bell stage E-17. (sr) stellate reticulum, (is) intermediate stratum, (ide) internal dental epithelium, (p) pulp, (b) bone Magnification $\times 10$ F: D-1 sagittal view. Magnification $\times 4$ G: D-2 Frontal view. (pa) preameloblasts, (o) odontoblasts, (pd) predentin, (d) dentin, (p) pulp Magnification $\times 10$ H: D-2. (a) ameloblasts, (pe) pre-enamel, (o) odontoblasts, (pd) predentin, (d) dentin, (is) intermediate stratum, (sr) stellate reticulum Magnification $\times 40$

species. Furthermore Keranen et al. (35) showed that similar molecular cascades are present in the early budding of tooth germs when comparisons of gene expression patterns and morphologies are made between different species (mouse and vole). This suggests that the mouse molar can be used as an odontogenesis model to understand the developmental biology and pathology of human teeth and other calcified tissues. Although the expression patterns of certain genes are similar in the first stages of tooth formation, even among different animal species, the mechanisms that regulate the number of teeth and their shape, specific to each species, have not yet been elucidated (25).

In this study we observed that in comparison with the albino strain of mouse as described by Cohn (34), the gestation period of the ICR/Jcl strain is shorter and the beginning of molar odontogenesis is earlier (day E-12.5). This suggests that the initiation of odontogenesis in each species may be directly related to the period of gestation and the size of the animal. Other studies have shown a good correlation between tooth morpho-histodifferentiation and age/weight staging (27). Although cases of faster and slower odontogenesis were observed for each day, the percentages calculated for each morphological stage of

dental development are statistically representative for the observations carried out.

Other studies of postnatal development should be performed to analyze the details of enamel formation. Our results are consistent with those of Lesot et al. (36), who analyzed the development of the first mandibular molar from the cap to the early bell stage and reported that in the ICR strain the cap stage was at E-14.5, when the enamel knot also appeared, and that cuspidogenesis began at E-16.5.

Similarly, Dassule and MacMahon (37), analyzing Swiss mice, observed that tooth morphogenesis was first apparent between E-11 and E-12, when important epithelial signaling molecules were specifically expressed in the epithelium (BMP2, Shh, Wnt10B and Wnt10a). We conclude that odontogenesis of the first mandibular molar in the ICR/Jcl strain begins morphologically at E-12.5. It has been shown that the expression of Pax 9, a paired box transcription factor, specifically marks the regions at the prospective sites of all teeth prior to any morphological manifestations, and Pax 9 mesenchymal expression is found in the prospective molar region from E-10 until E-16.5 (38). A number of transcription factors, signaling molecules, growth factor receptors and extracellular matrix molecules

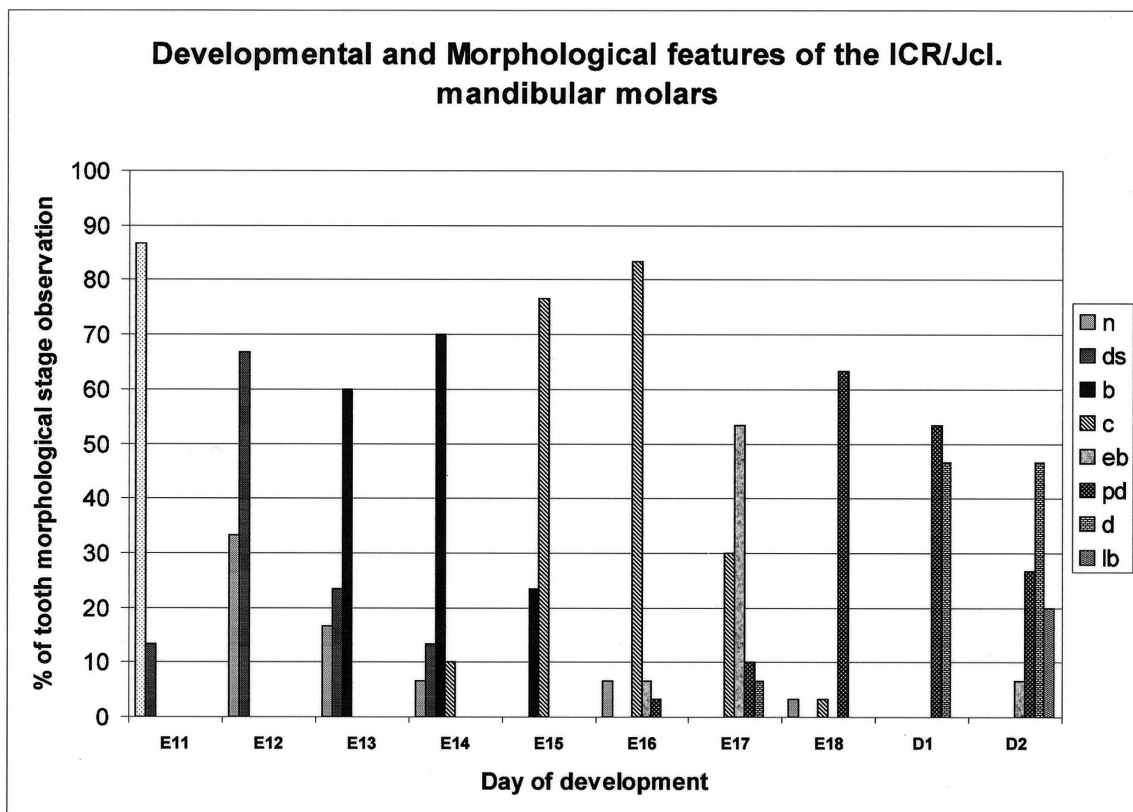


Fig. 2 Graph showing developmental and morphological features of the ICR/Jcl first mandibular molar. (n) any morphological changes, (ds) dental sheet, (b) bud stage, (c) cap stage, (eb) early bell stage, (pd) predentin, (d) dentin, (lb) late bell stage

are expressed in the mesenchyme of the first branchial arch in spatially and temporally regulated patterns. It is thought that bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) control the expression of Pax 9, which in turn would determine the formation of the tooth buds (39). However the Pax 9 knockout mouse showed the beginnings of bud formation, indicating that other genes may intervene in the initiation of odontogenesis (40).

The techniques of DNA recombination allow the manipulation of genes that can be permanently inserted inside the germinal line to produce transgenic mice, producing an important tool for studying the function of genes during development. Similarly, experiments involving gene targeting can produce a knockout mouse that has lost the expression of a specific gene (29).

Our study allows us to conclude that all the stages of normal odontogenesis are present in ICR/Jcl mice, with durations specific to this strain, and that these periods have been well established. Further investigations should focus on tooth-specific gene expression in the ICR/Jcl strain.

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