Int. J. Dev. Biol. 39: 459-468 (1995)

Original Article

Comparison of expression of the *msx-1, msx-2, BMP-2* and *BMP-4* genes in the mouse upper diastemal and molar tooth primordia

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ABSTRACT The existence of transient putative tooth anlagen in the prospective mouse upper diastema region has been documented previously in morphological studies. By *in situ* hybridization we investigated the expression patterns of the *msx-1, msx-2, BMP-2* and *BMP-4* genes, supposed to regulate early tooth development, in day 10-14 mouse embryonic upper diastema and molar regions, using 49 series of frontal sections. On the basis of comparison of the temporo-spatial expression patterns in both diastemal and molar tooth primordia we conclude that each of the four genes was expressed at least for some period simultaneously and at a comparable developmental stage in the transient and persisting dental primordia. *BMP-2* and *BMP-4* expression was downregulated in the diastemal dental primordia during their regression starting at day 13. The temporo-spatial pattern of BMPs expression may be associated with the disappearance of diastemal rudiments. Contrary to the molar anlage, we did not detect *msx-2* gene expression in the diastemal dental rudiments after the stage of epithelial thickening. The deficiency of the *msx-2* gene products may play a role in the growth retardation of diastemal dental primordia resulting in their subsequent involution.

KEY WORDS: *msx, BMP, diastema, mouse, odontogenesis*

Introduction

A characteristic feature of mouse dentition is the presence of a large toothless diastema in each dental quadrant, separating one incisor and a group of three molars. It has been documented, however, that in mouse embryos a dental lamina and later three distinct rudimental epithelial anlagen originate in the prospective upper diastema, and that they reach maximally the bud stage before their disappearance. The two rudiments localized in front of the first upper molar may correspond to two premolars of fossil and some recent rodents (Peterková, 1983; Peterková *et al.*, 1993, 1995b). In the mandible, however, only an inconspicuous epithelial thickening without further differentiation extends mesially from the first molar anlage (Peterková *et al.*, 1995b). With regard to the simultaneous existence of both persisting and transient dental primordia, the mouse embryonic upper jaw constitutes an interesting tool to investigate molecular aspects of the control mechanisms involved during the early steps of dentition development (Peterková *et al.*, 1995a).

In the last few years, the expression patterns of a number of different molecules have been correlated with odontogenesis. The homeobox-containing genes *msx-1* and *msx-2*, as well as *BMP-2* and *BMP-4*, two growth factors in *TGF-ß* superfamily, have been proposed to have important roles in odontogenesis (MacKenzie *et al.*, 1991a,b, 1992; Jowett *et al.*, 1993; Vainio *et al.*, 1993; Bégue-Kirn *et al.*, 1994; Satokata and Maas, 1994; for recent reviews see Ruch, 1995; Sharpe, 1995; Thesleff, 1995).

In this paper we analyzed by *in situ* hybridization the expression patterns of *msx-1*, *msx-2*, *BMP-2* and *BMP-4* in day 10-14 mouse embryonic maxillae in order to: 1) ascertain whether these genes, involved earlier during the initiation and early morphogenesis of teeth, will also be expressed in the mouse transient diastemal rudiments; and 2) estimate which of these genes may be involved in the extinction of the transient rudiments during the mouse toothless diastema formation.

Results

Morphological aspects

Because of potential strain differences, as developmental speed is concerned, a survey of early odontogenesis in the CBAxNMRI mouse strain is described:

Abbrevations used in this paper: BMP, bone morphogenetic protein' PFA, paraformaldehyde.

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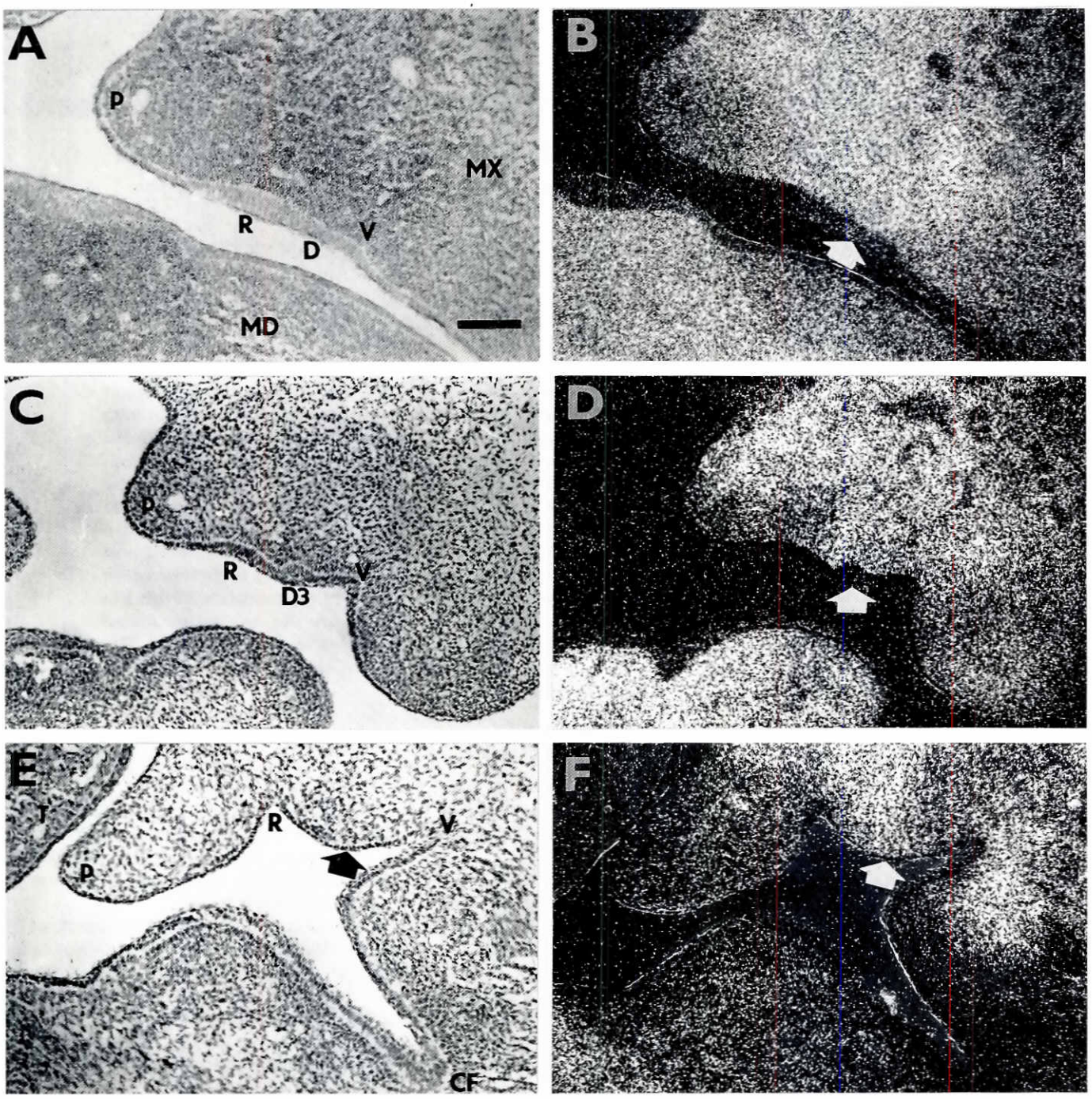

Fig. 1. Bright field (A,C,E) and dark field (B,D,F) photomicrographs of *msx-1* expression in frontal sections of the mouse embryonic maxilla. *Exclusively mesenchymal expression in the diastema region:* **(A,B)** *stage 11(20);* **(C,D)** *stage 12(20);* **(E,F)** *stage 14(8). Abbreviations: MX, maxilla; MD, mandible; D, diastemal dental lamina; D3, the third diastemal anlage; V, vestibular lamina; R, palatal ruga; P, palatal shelf margin; CF, cheek furrow (in the place of connection between maxillary and mandibular processes); T, tongue. Black and white arrow* (**E** and **F**, respectively) *indicates position of the disappeared second diastemal primordium (D2), white arrow* **(B,D)** *indicates the diastemal dental anlagen. Scale bar, 70 um (A,B,E,F) and 100 um (C,D).*

Stage 10(20): continuous thickening of the maxillary oral epithelium included both the prospective diastema and molar regions.
Stage 11(20)-12(8): the diastemal and molar dental laminae arose from the epithelial thickening.

Stage 12(20): in the first molar anlage, the epithelium had formed a bud which was surrounded by a large region of condensed mesenchyme. The second (D2) and the third (D3) diastemal dental primordia were maximally developed: the epithelial buds

(much smaller in comparison with molar) were surrounded by a narrow region of condensed mesenchyme.

Stage 13(8)-14(8): a small diastemal dental primordium (D1) was apparent along the primary choana at the stage 13(8). During stages 13(8) and 14(8), the diastemal dental structures were regressing, while the first molar epithelial anlage progressed into the early cap stage surrounded by a concentrically (on frontal sections) arranged mesenchyme.

Gene expression patterns

No significant hybridization signals were detected with control sense probes at any developmental stages investigated (data not shown). The antisense probes allowed us to identify very specific time-space related distribution patterns.

Distribution of msx-1 transcripts

Msx-1 expression was restricted to mesenchymal cells. At each stage within days 11-14, a significant signal for *msx-1* was detected in a large continuous area from the primary choana as far as the isthmus faucium, including the prospective diastema and molar regions. The area of expression gradually narrowed in the mesio-distal direction from both buccal and palatal sides. In the lip region, the signal spread buccally as far as the facial epithelium, whereas in the cheek region it disappeared approximately at the level of the cheek furrow. The palatal margin of the expression area reached mesially the apex of the palatal shelf and distally approached to the palatal margin of the molar epithelium (Figs. 1 and 6).

At stage 14(8), the *msx-1* hybridization signal was strong in the mesenchyme of the prospective diastema segment, although most D2 diastemal epithelial rudiments had already disappeared at this stage (Fig. 1E,F). *Msx-1* expression was intense in the concentrically arranged mesenchyme around the molar epithelium (data not shown).

Distribution of msx-2 transcripts

Diastema region: at stage 10(20), both oral epithelium and mesenchyme exhibited a diffuse hybridization signal in the mesial pole of the maxillary process. Behind the primary choana, the palatal borders of the epithelial and mesenchymal expression areas rapidly curved disto-buccally. Consequently, *msx-2* expression was found only in the buccal part of the diastemal epithelial thickening and in the adjacent mesenchyme and it progressed as far as the facial surface of the maxillary process (Figs. 2A,B and 6).

From stage 11(20) onwards, no significant signal was detected in the diastemal dental epithelium (Fig. 2E,F); the area of expression included only the epithelium of the lip furrow and oral surface of the lip (Figs. 2I,J and 6), and was interconnected with the expression area in the molar region until stage 12(20). During days 13-14, this signal disappeared in a disto-mesial course (Fig. 6).

At stage 11(20), a weak mesenchymal signal was still present above the lip furrow epithelium and in the upper lip (Figs. 2E,F and 6). During days 12-14, the mesenchyme did not exhibit any hybridization signal (Fig. 2I,J).

Molar region: at stage 10(20), expression of *msx-2* was not detected in the epithelial thickening. The area of mesenchymal expression, in continuity with the diastema region, was localized above the buccal part of the molar epithelial thickening and progressed into the upper lip or cheek throughout the whole mesio-distal course of the molar anlage (Figs. 2C,D and 6).

At each stage within days 11-14, *msx-2* was expressed only in the buccal side of the mesial (developmentally more advanced) part of the molar epithelial anlage, and the density of grains increased with advancing age (Figs. 2 and 6). The expression also progressed buccally into the adjacent oral epithelium, but the margin of this expression area was approaching the buccal slope of the molar epithelial anlage during days 11-14 (Fig. 6).

On day 11, the area of *msx-2* expression in the mesenchyme corresponded approximately to that in the epithelium. During days 12-14, mesenchymal expression was localized around the molar dental lamina or bud. During this period, however, the whole expression area shifted in a distal direction, as the signal was falling off mesially and progressing distally (Fig. 6).

Distribution of BMP-2 transcripts

At stage 10(20), no significant signal was detected in the maxillary oral epithelium and mesenchyme. During later periods, distinct signals were found in the diastema and molar regions.

Diastema region: the oral epithelial thickening did not exhibit any signal on day 11. At stages 12(8) and 12(20), a diffuse continuous signal was observed in the epithelium of both the distal part of D2 and in the D3 rudiment (Figs. 3C,D and 6). There was no continuity between diastemal and molar expression area in the epithelium. At stage 13(8) expression was no more apparent in the degenerating diastemal dental epithelium.

During days 11-13, no significant signal was detected in the mesenchyme adjacent to the oral epithelium situated between the edge of the palatal shelf and lip-furrow (Figs. 3 and 6).

Molar region: during days 11-12, the *BMP-2* signal was found only mesially in the molar epithelium, but the shape of the expression area changed (Figs. 3A,B and 6). As the length of the molar anlage increased, the mesio-distal length of the labeled area became relatively shorter. On day 13, the *BMP-2* signal was apparent in the middle of the convexity of the epithelial bud, in the segment of the highest developmental progress of the molar anlage (Fig. 6, compare with Fig. 5G,H).

In the mesenchyme surrounding the molar epithelium, we did not observe any specific signal within days 11-13. From stage 12(20), only the mesenchyme adjacent to the epithelium of the lip oral surface or cheek furrow exhibited the *BMP-2* signal (Fig. 6).

Distribution of BMP-4 transcripts

Diastema region: at stage 10(20), the epithelial expression area involved the whole maxillary oral surface behind the primary choana, narrowing in distal direction from both buccal and palatal sides (Figs. 4A,B and 6).

On day 11, *BMP-4* transcripts were detected in epithelial cells at the presumptive site of the distal part of the prospective diastemal dental lamina (Fig. 4C,D).

At stage 12(8), the signal was diffuse in the D2 and D3 epithelia in sections through the area of their maximal development (Fig. 4G,H).

At stage 12(20), the signal was concentrated to the central flat cells in the distal part of D2 and continued in the same manner into the mesial part of D3 (Fig. 5C,D). At day 13, no labeling was detected anywhere in the diastemal epithelia.

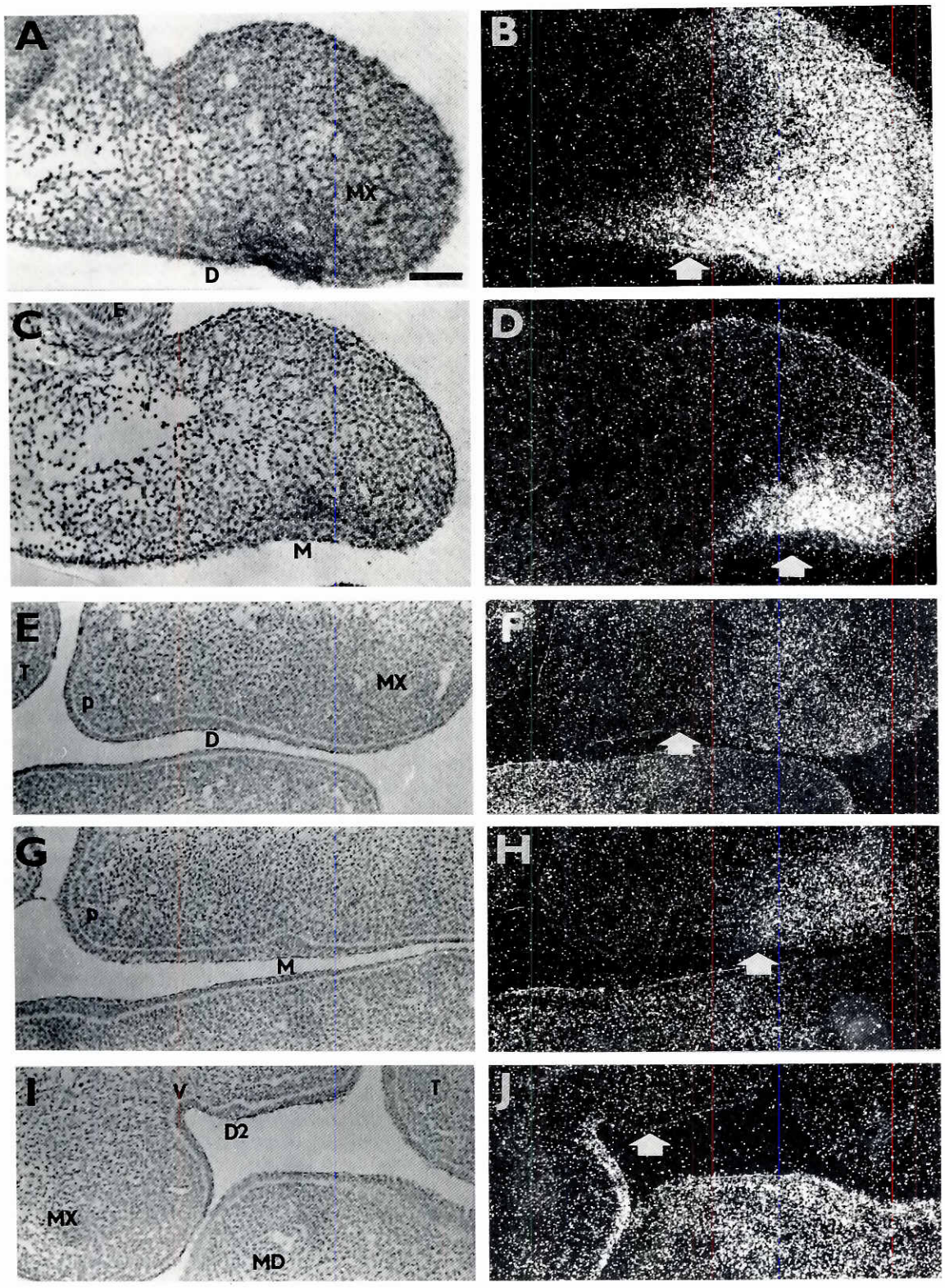

Fig. 2. *Msx-2* gene expression in the mouse maxilla between days 10-13. Bright field (A,C,E,G,I) and dark field (B,D,F,H,J) micrographs of frontal sections. *Prospective diastema region:* **(A,B)** *stage 10(20);* **(E,F)** *11(20);* **(I,J)** *stage 13(8). Molar region:* **(C,D)** *stage 10(20);* **(G,H)** *stage 11(20). Note that the signal in the epithelial thickening is detectable in the diastema and missing in the molar region on day 10. From day 11 onwards, the situation is reversed. E, eye; M, molar anlage. White arrow indicates position of dental anlage. For explanation of other abbreviations, see Fig. 1. Scale bar, 70 um (A,B) and 100 um (C-J).*

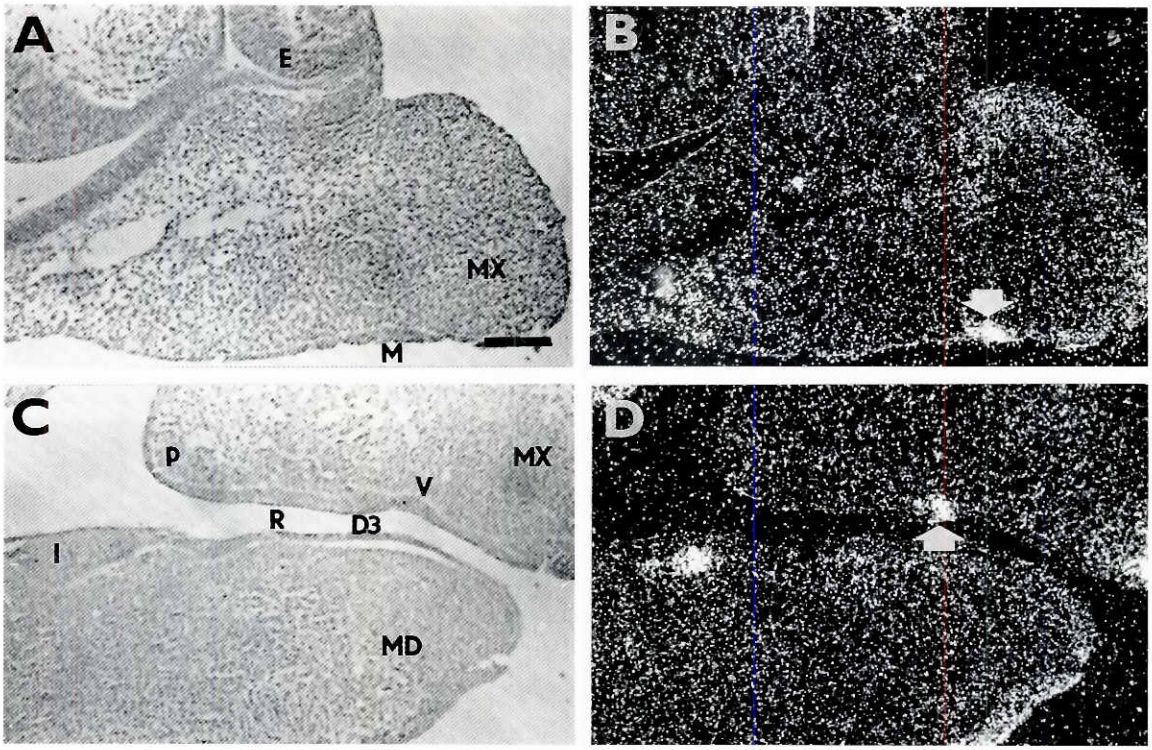

Fig. 3. Expression pattern of *BMP-2* gene on frontal sections: diastema region at stage 12(8) (C,D) and molar region at stage 11(20) (A,B). *Bright field* **(A,C)** *and dark field* **(B,D)** *micrographs. The signal is restricted to the dental epithelium (white arrow). I, incisor; M, molar anlage. For other abbreviations, see Fig. 1. Scale bar, 100 um.*

At stage 10(20), a mesenchymal signal was observed above the prospective site of diastemal dental lamina extending buccally as far as the facial epithelium. The density of grains decreased in distal direction (Fig. 6).

At days 11-12, the mesenchymal expression spread above the D2 and D3 epithelial anlagen and proceeded buccally into the upper lip and distally to the molar region (Figs. 4 and 5).

At stage 13(8), the signal in diastemal mesenchyme disappeared.

Molar region: on day 10, *BMP-4* signal was detected neither in the epithelium nor in the mesenchyme of the molar region (data not shown). At stages 11(20) and 12(8), the signal was located in the mesial part of the molar anlage, extending palatally in a distal course (Figs. 4E,F; 5A,B and 6). No expression was seen in the molar epithelium at stage 12(20) (Fig. 5E,F).

On day 13, only the middle of the convexity of the epithelial bud was labeled in the segment of the highest developmental progress of molar anlage (Fig. 5G,H). This localization of the epithelial signal was similar to that of *BMP-2.*

During days 11-12, the strong mesenchymal hybridization signal was situated above the buccal part of molar epithelium and buccally to it. At the stage 13(8), *BMP-4* was expressed in the mesenchymal cells surrounding molar epithelial anlage. The mesenchymal expression area always extended as far as the isthmus faucium within days 11-13 (Figs. 4, 5 and 6).

Comparison of gene expression patterns between the upper diastema and molar region.

In Figure 6, the time-space related expression patterns are schematically summarized. When the diastema and the molar regions are compared, the most interesting data are:

Msx-1 expression showed no differences between the diastema and molar areas during days 11-13. On day 14, the signal was strong in the mesenchyme concentrically arranged around the molar epithelium, while it remained diffuse in the mesenchyme of the diastema.

Msx-2 gene expression exhibited the most significant differences between the diastema and molar regions. On day 10, at the stage of epithelial thickening, the mesenchymal expression was apparent in both the diastema and molar regions, while the epithelial signal was found only in the diastema.

The *msx-2* signal in the molar epithelium was detected as late as from day 11. However, at dental lamina and bud stages, neither the diastemal dental epithelium nor the adjacent mesenchyme exhibited any positive *msx-2* signal, whereas the epithelium and mesenchyme of the molar anlage expressed *msx-2* intensely.

BMP-2 as well as *BMP-4* were expressed both in the diastema and molar regions during a limited developmental period. In comparison with molar, the onset of the expression in the epithelium of diastemal rudiments was either delayed *(BMP-2)* or accelerated *(BMP-4).* The *BMP* signals in the diastemal epithelium lasted even at stage 12(20), when the significant signals in the molar epithelium were either restricted *(BMP-2)* or absent *(BMP-4).* On day 13, strong signals of *BMP-2* and *BMP-4* reappeared in the molar epithelium, but in different locations than at younger stages, being no longer detected either in the diastema epithelium or mesenchyme.

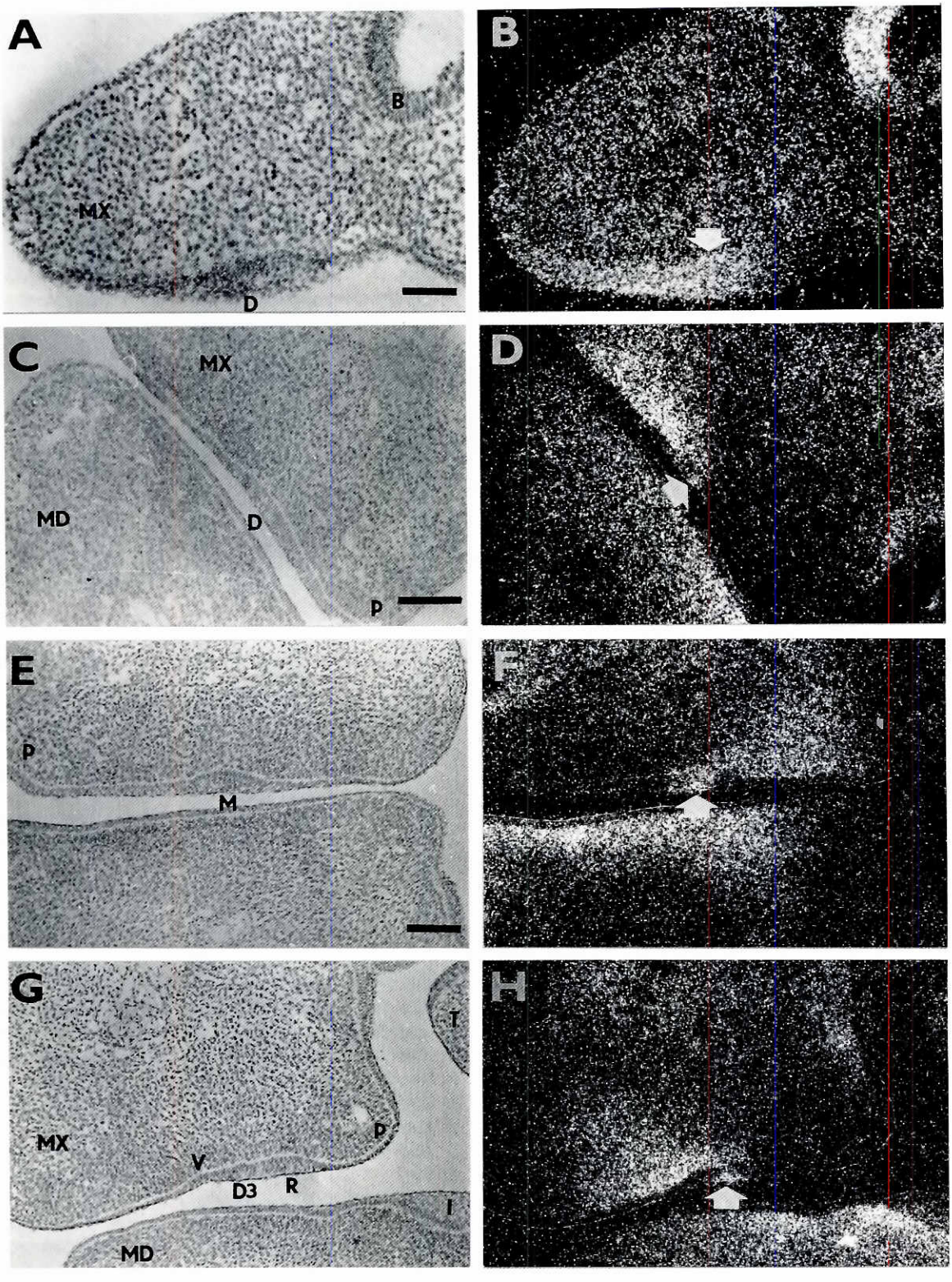

Fig. 4. Bright field (A,C,E,G) and dark field (B,D,F,H) micrographs of *BMP-4* gene expression pattern on frontal sections: *diastema region at stage 10(20)* **(A,B)**; *stage 11(20)* **(C,D)** *and stage 12(8)* **(G,H)** *(note the expression signal is localized rather in superficial cell layers of the epithelial primordium at the latter stage). Molar region at stage 11(20)* **(E,F)**. *White arrow indicates position of dental anlage. M, molar anlage. For explanation of other abbreviations, see Fig. 1. Scale bars, 50 um (A,B), 70 um (C,D) and 100 um (E-H).*

Discussion

Comparison of our findings with published data

The distribution of *msx-1*, *msx-2*, *BMP-2* and *BMP-4* have been reported during early development of the facial processes and first molar development (MacKenzie *et al.*, 1991a,b, 1992; Jowett *et al.*, 1993; Vainio *et al.*, 1993; Satokata and Maas, 1994). Although our findings concerning molar region are mostly in line with the earlier data, some differences were observed.

MacKenzie *et al.* (1991b) reported a low signal for *msx-1* in the distal part of the molar mesenchyme on days 11 and 12. We observed a strong hybridization signal both in the diastema and molar regions during days 11-14 (Fig. 1). The signal always extended as far as the isthmus faucium.

Concerning *BMP-2* expression, Vainio *et al.* (1993) detected a significant signal in molar epithelium on day 14, whereas we found a strong signal already on day 11 (Fig. 3A,B).

Our observations on *BMP-4* expression in the molar epithelium are in agreement with those of Vainio *et al.* (1993) but contrary to those of Jones *et al.* (1991): *BMP-4* transcripts were detected very early, i.e. on day 11 (Fig. 4E,F).

Vainio *et al.* (1993) reported a shift of the *BMP-4* expression between the molar epithelium and mesenchyme during days 11-13: *BMP-4* is expressed only in the epithelium on day 11, then both in the epithelium and mesenchyme for a short period (days 12-13) and from day 13 the signal shifts completely to the mesenchyme.

A simultaneous expression of *BMP-2* and *BMP-4* transcripts is apparent as late as in the course of odontoblast differentiation and very transiently in ameloblasts. We found in the molar region a simultaneous epithelial and mesenchymal signal for *BMP-4* during days 11-13 and simultaneous expression of both the *BMP-2* and *BMP-4* genes already from day 11. At stage 12(20), the epithelial expression transiently either disappeared *(BMP-4)* or was strongly reduced *(BMP-2)*.

A one day delay in the expression of *msx-2* and *BMP-4* in epithelium, and of *BMP-4* in mesenchyme, was apparent in the molar region (day 11) as compared with the diastema (day 10). This mesio-distal course in the progress of expression might be related to the general mesio-distal course of maxillary development (Gaunt, 1964; Peters and Straßburg, 1970; Lumsden, 1979), and may reflect the progress of neural crest cell migration (Nichols, 1986).

Molecular implications of msx-1, msx-2, BMP-2 and BMP-4 in early steps of odontogenesis

The transcription patterns and some functional investigations have suggested that the expression of *msx-1*, *msx-2*, *BMP-2* and *BMP-4* are required for tooth patterning, initiation, histomorphogenesis and cytodifferentiation (MacKenzie *et al.*, 1991a,b, 1992; Jowett *et al.*, 1993; Vainio *et al.*, 1993; Bégue-Kirn *et al.*, 1994; Satokata and Maas, 1994; for recent reviews see Ruch, 1995; Sharpe, 1995; Thesleff, 1995). At least for some period, each of these four genes was expressed simultaneously and at a comparable stage in the diastemal and molar anlagen during the initial steps of their development. The differences observed in gene expression patterns during the dental lamina and bud stages in the diastema as compared to the molar region might be correlated with the involution of the diastemal dental primordia.

Disruption of the *msx-1* gene in transgenic mice results in the absence of incisor primordia and arrest of molar development at bud stage (Satokata and Maas, 1994). The authors attributed the latter developmental defect to an insufficient amount of condensed dental mesenchyme in the mice lacking *msx-1* function. According to Cohn (1957), the cap stage is characterized by the concentric arrangement of mesenchymal cells around the molar epithelium. Such concentric arrangement is discernible already at the bud stage (Peterková, 1974) before the formation of the epithelial cap. This step of molar mesenchyme differentiation might be disturbed in the mutant mice and could explain their developmental arrest before the cap stage.

In opposition to the *msx-1* knock-out mouse molars, the transient dental primordia in the upper diastema region of normal mice stopped their development at less advanced bud stage and then disappeared independently on *msx-1* expression (Fig. 1E,F). Therefore, although the development of molars in the Msx-1 deficient mice as well as that of the diastemal dental primordia in normal mice is arrested at the bud stage, the reasons for the respective developmental behavior are different.

Although staging of the diastemal and molar dental anlagen is comparable during days 10-12, the diastemal primordia are smaller than those of the persisting teeth (molar, incisor) from the beginning of their formation (Peterková *et al.*, 1993, 1995b). Quantitative aspects of *BMP* gene products (Ferguson and Anderson, 1992) might play a role in mesenchyme condensation (Kingsley, 1994), i.e. the amount of BMPs in the diastemal dental epithelium might be low and associated with low amount of adjacent condensed mesenchyme.

Expression of *BMP-2* and *BMP-4* in the diastemal rudiments disappeared on day 13, when they began to degenerate. The downregulation of *BMP*s expression as late as on day 13 might play a role in the final involution of the diastemal primordia.

Msx-2 transcripts, which may provide time-specific signals during tooth development, were not detected in the diastemal dental anlagen from day 11 onwards. *In vitro* inhibition of *msx-2* expression in explanted branchial arches has been reported to either prevent tooth initiation or alter tooth morphogenesis (Sharpe, 1995). Downregulation of *msx-2* gene expression might play an important role during the growth retardation ending in the involution of dental anlagen in the prospective upper diastema in mouse. Accordingly, a deficiency of *msx-2* gene expression might be part of the evolutionary mechanism of teeth extinction and determination of species specific functional dental patterns.

Further studies on the diastema region concerning the expression of other genes and gene products (e.g. EGF and its receptors, tenascin, syndecan) implicated in the regulation of tooth development are necessary. The identification of target genes of the homeoproteins and understanding the functional relationships between *msx-1*, *msx-2* and *BMP*s are also challenging problems. Further comparisons of temporo-spatial expression patterns between the upper diastemal rudiments and the incisor primordium as well as between the upper and lower diastema could bring some interesting results, too.

Odontogenesis in the mouse diastema, which represents a model of hypodontia of evolutionary origin (Peterková *et al.*, 1995b) should be compared with the development of hypodontia, either induced experimentally in transgenic mice, or spontaneously appearing in natural mouse mutants and in some human genetic conditions.

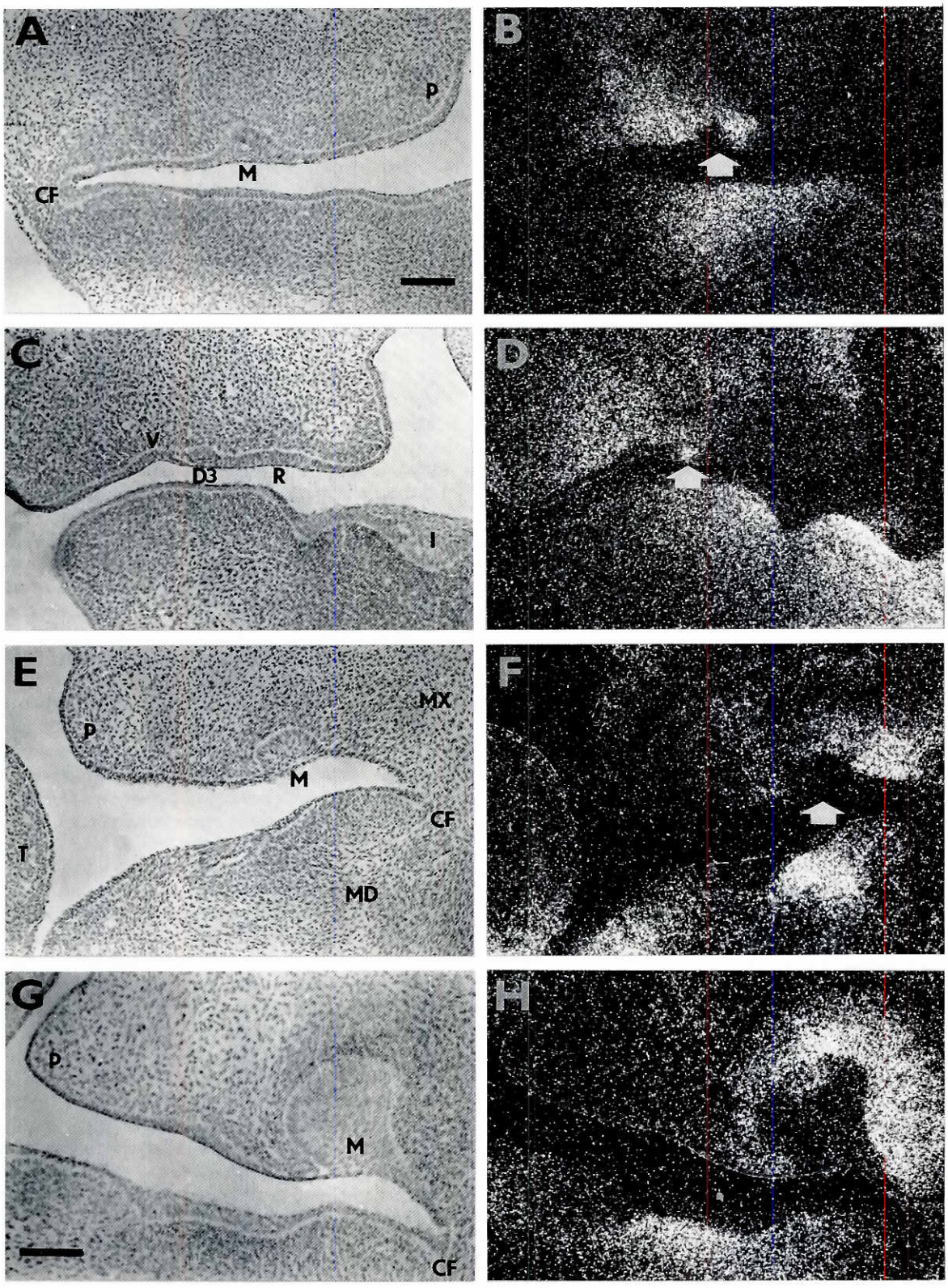

Fig. 5. Bright field (A,C,E,G) and dark field (B,D,F,H) micrographs of *BMP-4* gene expression. *In the diastema region at stage 12(20)* **(C,D)**, *the epithelial signal is localized preferentially in the central and superficial cell layers.* **(A,B)** *Molar region at stage 12(8). The signal is present in the lingual part of the epithelium.* **(E,F)** *Molar region staged 12(20) (there is no significant signal in the epithelium). At stage 13(8)* **(G,H)**, *the epithelial signal is apparent in the middle of the convexity of the first molar bud. White arrow indicates position of a dental anlage. M, molar primordium. For explanation of other abbreviations, see Fig. 1. Scale bars, 100 um (A-F) and 70 um (G,H).*

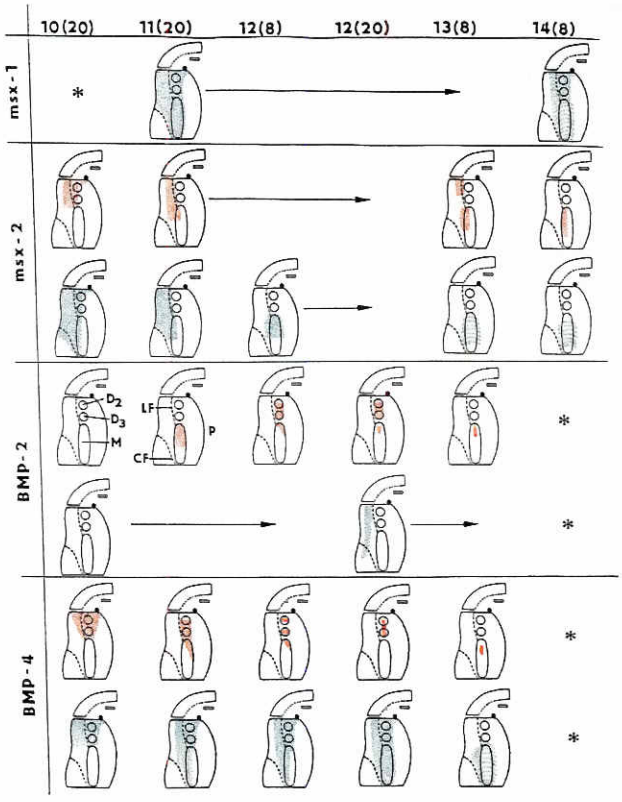

Fig. 6. A schematic representation of the expression patterns of the *msx-1*, *msx-2*, *BMP-2* and *BMP-4* genes in the maxilla of mouse embryos at stages 10(20), 11(20), 12(8), 12(20), 13(8) and 14(8), in the mesenchyme (green) and epithelium (red). *The black point indicates the position of the primary choana. The region in front of the primary choana with the incisor anlage (rectangle) was not investigated. D2 and D3 represent the second and third diastemal primordium during days 12-13, or their supposed and previous position on days 10-11 and 14, respectively. (The first diastemal primordium D1 is not indicated). M, molar anlage; LF and CF, lip and cheek furrow, respectively; P, margin of the palatal shelf. The horizontal arrows indicate the period during which the expression patterns did not change. Stars indicate omitted stages. Fine modifications in timing may be expected reflecting strain differences in developmental speed.*

Materials and Methods

Preparation of tissues

Pregnant female mice (CBAxNMRI strain) were killed by cervical dislocation at 8th and/or 20th h of days 10-14 of pregnancy (day of vaginal plug= day 0), i.e. at stages 10(20), 11(20), 12(8), 12(20), 13(8) and 14(8) (the figure in parentheses indicates an hour of the appropriate embryonic day).

Embryos were removed in phosphate-buffered saline (PBS) and weighed. As morphological staging (Grüneberg, 1943; Theiler, 1972) has proved to be too crude for detailed study of early odontogenesis, the embryos at each chronological stage were distributed into 25-mg weight classes (Peterková *et al.*, 1993). On the basis of a preliminary morphological study of dental development in CBAxNMRI mouse strain, the most representative weight classes were selected at each chronological stage and embryos of these classes were analyzed by *in situ* hybridization. Embryos of days 10-13, 11-14, and 10-14 were analyzed for expression of *BMP*s, *msx-1*, and *msx-2*, respectively. Heads of the embryos were fixed for 2 days in 4% PFA in PBS at 4°C. They were dehydrated and embedded in paraffin. Serial sections (7 um thick) were placed on silanized slides and stored in tight boxes at 4°C until further processing.

In situ hybridization

Murine *msx-1*, *msx-2*, *BMP-2* and *BMP-4* antisense and sense probes were used. Single stranded (^{35}S)-UTP-labeled (Amersham) RNA probes were synthesized by *in vitro* transcription from the following DNA fragments: a 600 bp fragment of *msx-1* cDNA and a 850 bp fragment of *msx-2* cDNA subcloned into plasmid pSP72 – gifts from P. Sharpe (MacKenzie *et al.*, 1991b, 1992), a 240 bp fragment of *BMP-2* cDNA and 285 bp fragment of *BMP-4* cDNA inserted into pGEM3 – gifts from Genetics Institute, Cambridge, USA (Vainio *et al.*, 1993).

In situ hybridization of paraffin sections was done essentially as described by Wilkinson and Green (1990). Labeling was detected after 10 or 12 day exposure using msx and BMP probes, respectively.

Microscopic evaluation

At least two complete series of frontal sections representing each of the chronological stages within days 10-14 were labeled independently with each probe. The only exceptions were *BMP-2* at stages 11(20) and 12(8), and *msx-2* and *BMP-4* at stage 10(20), where only one series of good quality sections was available. The total number of series was 49.

In the upper jaw, gene expression was evaluated in the molar region and in the antemolar diastema segment where two more distally situated rudimental anlagen (D2 and D3) develop (Peterková *et al.*, 1995b). The most mesial diastemal anlage (D1), adjacent buccally to the primary choana, was omitted, as it exists very transiently and is smaller in comparison with the other two (Peterková *et al.*, 1995b). Therefore, the anterior and posterior margins of the investigated area were situated behind the primary choana and in front of the isthmus faucium, respectively.

Acknowledgments

We thank Dr. Paul Sharpe (Guy's Hospital, London) for the msx probes and Dr. Elizabeth Wang (Genetics Institute, Cambridge, MA) for the BMP probes. This work was supported by the Academy of Finland and by grants 304/93/0594 (Grant Agency of Czech Republic) and 645109 (Academy of Sciences of Czech Republic).

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Accepted for publication: March 1995