Morphogenesis of Palatal Ridges in the Golden Hamster (*Mesocricetus auratus*, Rodentia)

M. BUCHTOVÁ[1,2], P. MATULOVÁ[3], K. WITTER[4], F. TICHÝ[1]

[1] Department of Anatomy, Histology and Embryology,
University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
[2] Department of Oral Health Sciences, Faculty of Dentistry,
University of British Columbia, Vancouver, BC, Canada
[3] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic,
Brno, Czech Republic
[4] Institute of Histology and Embryology, Department of Pathobiology,
University of Veterinary Medicine Vienna, Austria

Received December 17, 2004
Accepted August 30, 2005

Abstract

Buchtová M., P. Matulová, K. Witter, F. Tichý: Morphogenesis of Palatal Ridges in the Golden Hamster (*Mesocricetus auratus*, Rodentia). Acta Vet. Brno 2005, 74: 319-327.

Rugae palatinae (RP) – transversal mucosal ridges of the mammalian palate – are assumed to play a role during closure of the palate in embryos. The aim of this study was to assess the morphogenesis of palatal ridges in the golden hamster *(Mesocricetus auratus,* Rodentia*)* by light and scanning electron microscopy. Cell proliferation was detected by immunohistochemical staining of proliferating cell nuclear antigen (PCNA). In the hamster, three to four antemolar and three intermolar ridges were formed. In ED 11.0 (ED - embryonic day) embryos, RP1 and RP3 were at the epithelial thickening stage, RP2 was a primitive ruga in the rostral part of the palatal processes. In the caudal part of the palate, an epithelial placode represented the prospective RP4-RP7. At ED 12.5, the closed secondary palate bore six ridge primordia. Only RP2 protruded distinctly into the oral cavity. At ED 13.0-14.5 and ED15.0-15.5, the mesenchymal core of the antemolar and intermolar ridge primordia, respectively, started to develop. Strikingly, a local increase of proliferation activity does not seem to be the main process involved in palatal ridge formation and elevation. Although the palatal ridge formation and elevation is based on tissue volume enlargement, strikingly, the proliferation activity was higher in the interrugal epithelium than in the ridge primordia. Rather than the epithelial proliferation activity increase, the change in orientation of mitotic spindles of dividing cells seems to be a reason of palatal ridge formation.

Oral cavity, proliferation, embryo, foetus, development

Palatal ridges *(rugae palatinae)* are more or less transversal mucosal ridges of the hard palate, which are covered by a cornified stratified squamous epithelium. Number and arrangement of the palatal ridges is specific for the respective mammalian species.

Palatal ridges have an important function during pre- and postnatal development. They help to transport the food within the oral cavity (Zietzschmann et al. 1943) and take part in grinding the food between tongue and hard palate (Eisentraut 1975). Palatal ridges contain tactile and taste receptors, so they have also a sensory function (Scott and Symons 1967; Luke 1980). In embryos, they are assumed to stiffen and strengthen the palatal processes during formation of the secondary palate (Pourtois 1972; Brinkley and Vickerman 1982; Luke 1984; Bulleit and Zimmerman 1985).

Palatal ridge development has been studied mainly in the mouse. Primordia of the *rugae palatinae* are as well as dental and vestibular laminae derivatives of the odontogenic epithelial zone, an epithelial placode on the oral surface of the developing maxilla that can be recognised in ED 12.0 mouse embryos (Peterková 1985). The development of individual palatal ridges of the mouse starts at ED 13.0. Step by step eight rugal primordia occur. The

Address for correspondence:
MVDr. et Drvet. med. Kirsti Witter Ph. D.
Institute of Histology and Embryology
Department of Pathobiology
University of Veterinary Medicine Vienna
Veterinärplatz 1, A-1210 Wien, Austria

Phone: +43 1 25077 3403
Fax: +43 1 25077 3490
E-mail: kirsti.witter@vu-wien.ac.at.
http://www.vfu.cz/acta-vet/actavet.htm

adult mouse has three antemolar and five to six intermolar palatal ridges. In embryos and foetuses, six developmental stages of palatal ridges can be described: (I) epithelial thickening immersed into the underlying mesenchyme (rugal anlage); (II) flattening of the basement membrane, the thickened epithelium protrudes to the oral cavity (primitive ruga); (III) condensation of mesenchymal cells underneath the primitive ruga; (IV) formation of the fibrous stroma of the palatal ridge (rugal core), thin epithelium on the crest of the ridge; (V) ridge of fibrous tissue covered with cornifying epithelium of uniform thickness (definitive ruga), (VI) ruga as in adults (Peterková et al. 1987). The development of palatal ridges is probably initiated by nerves (Capon 1983). Knowledge on molecular interactions between palatal epithelium and mesenchyme during ridge formation is far from complete, although similarities with the reiterative signalling during dental lamina development (reviewed e.g. in Cobourne and Sharpe 2003; Thesleff 2003) can be expected.

Localised proliferation, cell death, cell migration and differentiation within the involved tissues can be assumed to shape the oral surface of the hard palate. During prenatal development of palatal ridges in the mouse, proliferating cells have been detected mainly in the interrugal region (Takanosu et al. 2002) as well as in the thick epithelium of the developing rugae (Luke 1984). In contrast, apoptotic cells could be found mainly in the epithelium of ridge anlagen at early developmental stages (Turečková et al. 1996; Takanosu et al. 2002).

Palatal ridge development in other mammals than the mouse has been studied only rarely (e.g., Gegenbaur 1878; Meller et al. 1980; Schüpbach et al. 1983; Thomas 1984; Amasaki et al. 1991; Thomas and Rossouw 1991; Ikemi et al. 1995; Takanosu et al. 1996ab; Buchtová et al. 2003).

Neither the temporo-spatial shaping process of palatal ridges during prenatal development, nor the role of proliferation in this process is fully understood. Therefore, the aim of this work was 1) to determine the time programme of development and morphogenesis of the *rugae palatinae* in a further model organism – the golden hamster, 2) to detect proliferating cell populations in both epithelium and mesenchyme of the palatal ridge primordia, and 3) to estimate the role of proliferation during shaping of the oral surface of the hard palate.

Materials and Methods

Embryos and foetuses

Golden hamsters *(Mesocricetus auratus,* Rodentia) were individually housed under monitored conditions in standard cages for laboratory animals. The animals were fed a commercial diet for hamsters and water *ad libitum*. Adult females were mated in the evening hours for approximately 30 min, the exact time of copulation was registered. Embryos and foetuses were harvested at ED 10.5-16.0 in intervals of 12 hours. For this purpose, females of the respective gestation stage were anaesthetised with chloroform and killed by craniocervical dislocation. Their uteri were removed. Embryos and foetuses were prepared in cooled phosphate buffered saline, weighed, measured (crown-rump-length) and fixed in a buffered 4% formaldehyde solution.

Anatomical examination

Palates of selected adult females killed for harvesting embryos were examined after dissection of the oral cavity. Number and localisation of palatal ridges were assessed.

Scanning electron microscopy

In order to study the development of the secondary palate, three heads of embryos and foetuses at each ED 11.0-16.0 were processed for scanning electron microscopy. After removing of the mandibles of the fixed specimens, the heads were washed in distilled water, dehydrated in graded ethanol solutions, critical point dried, coated with gold, and viewed and photographed by the Tesla BS 300 scanning electron microscope (TESLA ELMI Inc., Brno, Czech Republic).

Light microscopy

Two heads per stage at each ED 10.5-16.0 were decalcified if necessary and embedded in paraffin. Sagittal and transversal serial sections (5 μm) were prepared and stained with hematoxylin-eosin. In selected sections, reticulin, collagen and elastin were stained according to Gomori, van Gieson, and with orcein, respectively. Mallory staining was performed to demonstrate the cornification of the palatal epithelium.

All sections were examined with a light microscope. For better orientation, the rugae (RP - *ruga palatina*) and their primordia were numbered from RP1 to RP7 in rostro-caudal direction. The development of the palatal ridges was described according to the nomenclature of Peterková et al. (1987).

Immunohistochemistry

In order to distinguish proliferating cells in the developing palatal ridges, PCNA (proliferating cell nuclear antigen) was detected immunohistochemically.

Deparaffinised and rehydrated sagittal sections near the midline of the head were incubated with primary polyclonal anti-PCNA antibodies (a kind gift from Dr. Bořivoj Vojtěšek, Masaryk Memorial Cancer Institute, Brno, Czech Republic) at 4 °C overnight (1:1000). Products of the immunoreaction were detected using the Vectastain ABC Elite kit (Vector Laboratories, Burlingame, California, USA) and visualised with DAB, Liquid (Dako, Glostrup, Denmark) following the manufacturer's instructions. Sections were counterstained with haematoxylin.

Results

The definitive palate of the hamster was formed by primary and secondary palate. The morphologically detectable development of palatal ridges started at ED 11.0. In the adult hamster, three to four antemolar and two to three intermolar ridges were formed.

ED 11.0-12.0 (Plates I, II, Figs 1A, 2A and 2B)

The definitive palate was not yet developed. The lateral palatal processes were vertical (Plate I, Fig. 1A). In their rostral part, three epithelial thickenings, each formed by three cell layers, could be detected. In these regions, the basal epithelial cells were high, cylindrical with oval nuclei. The intermediate epithelial layer was formed by polygonal cells with round nuclei and the superficial layer by flat cells with flat nuclei. At ED 11.0, RP1 and RP3 were invaginated into the mesenchyme (rugal anlage). The RP2 protruded to the oral cavity (primitive ruga). The primitive ruga stage was reached by all three thickenings at ED 11.5-12.0 (Plate I, II, Figs 1A and 2A). At this stage, the palatal ridges were formed by four to five epithelial cell layers.

In contrast to the ridge primordia, the interrugal epithelium consisted of only two cell layers with cuboidal basal and flat superficial cells. The height of the rugal epithelium was 20-30 μm, of the interrugal epithelium 9-11 μm.

In the caudal part of the lateral palatal processes, a large epithelial area was immersed into the underlying mesenchyme. This epithelial placode consisted of four cell layers and was 30-35 μm thick. The basal layer was formed by cylindrical cells. Some of the superficial cells had cupola-like prominences on their oral surface (Plate II, Fig. 2B).

The mesenchymal cells underlying the palatal epithelium were condensed, but distributed uniformly without local differences (Plate II, Fig. 2A). In developmentally advanced ED 12.0 specimens, the density of the deep mesenchymal layer decreased. Near the epithelial surface, the distribution of mesenchymal cells remained unchanged.

In the rostral part of the palatal processes, PCNA-positive cells could be detected mainly in the epithelium. They were localised in all epithelial layers, in the ridge primordia as well as in the interrugal epithelium (Plate II, Fig. 2A). In contrast to the rostral part, PCNA-negative cells prevailed in the thickened caudal epithelial area (Plate II, Fig. 2B). Only few positive cells were found in the subjacent palatal mesenchyme.

ED 12.5 (Plates I, II, Figs 1B and 2C)

The secondary palate was closing by horizontalisation of the lateral palatal outgrowths (Plate I, Fig. 1B), starting in the middle part of the developing palate and proceeding rostrally and caudally.

A total of six palatal ridge primordia could be detected on the lateral palatal processes. RP1 was located near the primitive choana (Plate I, Fig. 1B). All rugal primordia had the shape of epithelial ridges (thickness 19-30 μm) consisting of three cell layers (Plate II, Fig. 2C). Even in transversal sections, the thick ridge primordia were readily distinguishable

from the thin adjacent epithelium (two to three cell layers, thickness 7-9 μm). The developing mesenchymal core of the RP2 primordium caused a protrusion of the ridge epithelium to the oral cavity.

The mesenchyme subjacent to the antemolar ridge primordia was condensed and formed concentrically aligned layers (Plate II, Fig. 2C). In contrast, the mesenchyme underlying the intermolar ridge primordia did not condense markedly. The deep layer of the developing palate consisted of sparse mesenchymal tissue.

PCNA-positive cells could be found mainly in the interrugal epithelium and the epithelium of the slopes of the ridge primordia. The edge of the ridges was more or less PCNA-negative (Plate II, Fig. 2C). The palatal mesenchyme contained more immunopositive than negative cells.

ED 13.0-13.5 (Plates I, II, Figs 1C and 2D)

The primitive choanae were closed. The only remaining communication between nasal and oral cavity was the *ductus incisivus* (Plate I, Fig. 1C).

On the hard palate, six to seven ridge primordia could be distinguished (Plate I, Fig. 1C). RP1, located near the incisive duct, was at the primitive ruga stage (thickness of the epithelium 22 μm). RP2-RP5 were 30-45 μm high ridges with well developed mesenchymal core (Plate II, Fig. 2D). Their epithelium had a constant thickness of 22-30 μm and consisted of five to six cell layers. RP6 and RP7 were low ridges without rugal core, formed by three layers of epithelial cells. The basal cells had a cylindrical, cells of both superficial layers a cuboidal shape. The interrugal epithelium consisted of two to three cell layers and was 12-15 μm high (Plate II, Fig. 2D).

The epithelium of the edge of the ridge primordia contained mainly PCNA-negative cells. Positive cells could be found on the slopes of the ridge, in basal as well as in superficial epithelial layers. In the interrugal epithelium, PCNA-positive cells were uniformly distributed. The developing core of the rostral ridge primordia consisted mainly of PCNA-negative cells (Plate II, Fig. 2D).

ED 14-14.5 (Plate II, Fig. 2E)

The secondary palate was definitively closed. The incisive duct represented a narrow channel. On the hard palate, six to seven *rugae palatinae* could be distinguished. The antemolar RP1-RP4 had already a distinct mesenchymal rugal core.

RP1 was localised near the *ductus incisivus*. Its epithelium, which consisted of five to six cell layers, was thicker than in the other antemolar ridges (up to 30 μm on the edge as well as on the flanks of the ridge). RP2-RP4 were up to 60 μm high (Plate II, Fig. 2E). The RP2 epithelium was formed by two to three cell layers. On the RP2 edge, it was thinner than on its slopes (15 μm and 20 μm, respectively) due to the different shape of its basal cells. The superficial epithelial cells cornified. The intermolar RP5-RP7 were represented by epithelial thickenings (thickness 18-21 μm) without rugal core.

The interrugal epithelium consisted of three to five cell layers and was 10-17 μm thick. The basal and superficial cells were cuboidal and flat, respectively. The superficial layers of the interrugal epithelium cornified as well as the ridge epithelium.

PCNA-positive cells could be found only in the interrugal epithelium, in the epithelium of the caudal part of the palate (behind the ridge primordia), and in the deep mesenchymal layers. The epithelium of the developing palatal ridges as well as their mesenchyme were immunonegative.

ED 15.0-15.5

The palate was closed and exhibited six to seven ridge primordia.

The antemolar RP1-RP4 had a distinct rugal core and protruded to the oral cavity. The total height of the ridges was approximately 60 μm. The height of the epithelium on the ridge

edges was 13-23 μm, on the slopes 13-15 μm. The surface of the epithelium was covered with a 6 μm thick cornified layer. RP5-RP6 (RP7) had a less developed rugal core compared to the antemolar ridge primordia. Their total height reached approximately 30 μm, the thickness of the epithelium was maximally 23 μm. The basement membrane of the ridge epithelium had a wavy appearance. However, the underlying connective tissue did not form papillary prominences. The rugal core with its uniformly distributed cells contained many blood vessels. The interrugal region was covered with a thin (7-9 μm) epithelium consisting of three cell layers. In contrast to the cuboidal basal cells, the superficial layers were cornified.

The cell-rich mesenchyme of the developing antemolar ridges was very dense. In caudal direction, a dense superficial layer adjacent to the epithelium was distinguishable from a deep layer with low cell density, which contained large blood vessels.

Unlike the superficial cell layers of the palatal epithelium, the germinative basal layer showed PCNA-positive immunoreaction in the rugal as well as in the interrugal region. Only the epithelium on the edge of the ridge primordia was PCNA-negative. In the mesenchymal rugal core, mainly PCNA-negative and few uniformly distributed immunopositive cells could be detected.

ED 16.0 – newborn (Plate II, Fig. 2F)

Six to seven *rugae palatinae* were apparent. The antemolar ridges protruded distinctly to the oral cavity. Their overall height reached up to 135 μm, the thickness of their epithelium 23-30 μm. The cells of the basal epithelial layer had a cylindrical shape, these of the two to three superficial layers were cuboidal or flat. The cornified superficial layer detached in some places (Plate II, Fig. 2F). The intermolar palatal ridges were lower than the antemolar ones (37-45 μm, thickness of the epithelium 23 μm). Compared to the previous developmental stage, their mesenchymal core was reduced. Caudally from RP7, the epithelium thickened and reached a height of up to 53 μm. The basement membrane of the palatal epithelium was corrugated, however, fibrous papillae were not yet formed. The rugal core consisted of uniformly distributed mesenchymal cells and contained blood capillaries.

High proliferation activity of palatal tissues was typical for this developmental stage. Most PCNA-positive cells could be found in the germinative layer of the epithelium. In superficial direction, the number of immunopositive cells decreased. Fewer PCNA-positive cells could be found on the edges of the antemolar palatal ridges (Plate II, Fig. 2F), similarly as in the medial parts of the low intermolar ridges. In the caudal part of the palatal epithelium, the basal epithelial cell layer was PCNA-negative. In this region, positive cells were scattered in the middle layers of the epithelium. In the mesenchymal rugal core, the few immunopositive cells were distributed uniformly.

Discussion

Time programme of palatal ridge development

In the golden hamster, the morphologically detectable development of palatal ridges started at ED 11.0, i.e. before horizontalisation and fusion of the lateral palatal processes. However, an earlier onset of initiation processes can be expected, similarly to the dental lamina (Ferguson et al. 1998; Hardcastle et al. 1999; Peters and Balling 1999; Dassule et al. 2000). As in the mouse (Peterková et al. 1987; Sakamoto et al. 1989), the rat (Thomas and Rossouw 1991), and the European pine vole (Buchtová et al. 2003), three antemolar ridge primordia were initially formed in the hamster. At this early stage, the developing palatal ridges could stiffen the lateral palatal processes, as suggested by Pourtois (1972). The intermolar ridges of the hamster did not develop until the lateral palatal processes had shifted into horizontal position. During closure of the secondary palate, all palatal ridge primordia were present.

The material for the intermolar ridges, which developed during caudal prolongation of the lateral palatal processes at later developmental stages, was probably provided by the thickened epithelial placode in the caudal part of the hamster palate. Such a placode has been described before only in the European pine vole (Buchtová et al. 2003).

The rugal anlagen of the hamster passed the same developmental stages as Peterková et al. (1987) described for the mouse. The most advanced primordium was at all developmental stages RP2. This palatal ridge is located centrally in the diastema region and could therefore play postnatally the most important role during transport and comminution of the food within the oral cavity (Scott and Symons 1967; Eisentraut 1975).

As in mice (Sakamoto et al. 1989), the intermolar palatal ridges of the hamster are smaller during development and in adult animals in comparison with the antemolar ridges, which are localised in the diastema region of the upper jaw. Interestingly, not all palatal ridge primordia of the hamster passed the respective developmental stages in a linear manner. The intermolar rugal anlagen RP5-RP7 de-differentiated after ED 15.0, so that in ED 16.0 foetuses could be detected in this region only primitive rugae, i.e. epithelial thickenings without distinct mesenchymal core. Prenatal regression of initially well-developed caudal palatal ridge primordia has also been described in humans (Gegenbaur 1878).

In general, the time programme of palatal ridge development in the hamster resembles that in other rodents (Peterková et al. 1987; Sakamoto et al. 1989; Thomas and Rossouw 1991; Buchtová et al. 2003).

Epithelium and mesenchyme of the palatal ridge primordia

Similarly as in odontogenesis, the epithelium of the palate seems to play an important role during the first stages of ridge development. The first morphological sign of the ridge primordia are epithelial thickenings immersed to the palatal mesenchyme. Although *rugae palatinae* as well as teeth can be considered as repetitively homologue structures, they probably do not derive from a single lamina as tooth primordia. The epithelial placode in the caudal part of the palate, from which the intermolar ridges of the hamster derived, seems to be rather an exception. However, it is not possible to exclude the existence of a primary signalling region in the palatal epithelium, in which are later induced single segmental thickenings.

Initially, the epithelial thickenings representing the palatal ridge primordia were immersed into the subjacent mesenchyme. Later on, they protruded to the oral cavity. After development of the rugal core, the epithelial height seems to change in a species-specific manner. In the European pine vole, the basement membrane of the rugal epithelium has a wavy structure and forms interdigitations with the mesenchyme. In this mammal, the epithelium of the rostral slope is thicker than on the caudal slope (Buchtová et al. 2003). In contrast, during the prenatal period of the hamster, the thickness of the rugal epithelium was identical on both rostral and caudal slopes of the ridge primordia. In the mouse, the ridge primordia are described to bend during development in caudal direction, what is accompanied by thickening of the epithelium on the rostral slope (Takanosu et al. 2002). Peterková et al. (1987), however, indicated that the thickness of the mouse palatal epithelium is uniform on both slopes of the ridge primordia as well as in the interrugal region.

At postnatal developmental stages, the epithelium on the edge of hamster palatal ridges was thinner than that on the ridge slopes. Similar configuration of the epithelium has been described also in adult mice and could be related to the localisation of tactile bodies near the ridge edge (Luke 1980).

First signs of re-arrangement of the ridge mesenchyme could be distinguished after protrusion of the ridge epithelium to the oral cavity (primitive ruga stage). It is possible that

the molecular background of this process is a similar shift of instructive capacity from epithelium to mesenchyme as described in tooth primordia (for review, see e.g. Maas and Bei 1997; Pispa and Thesleff 2003). Whether the primary signal inducing palatal ridge formation originates from epithelium or underlying mesenchyme and whether ridge development is indeed underlined by iterative interaction between these tissues remains to be studied.

Proliferation activity and morphogenesis

Although palatal ridge formation is based on volume enlargement first of epithelium and later of mesenchyme, epithelial proliferation in the ridge areas seems to play in these processes a smaller role than primarily expected.

Strikingly, the proliferation activity was higher in the thin interrugal epithelium, which practically did not thicken at all, than in the ridge primordia. A similar distribution of proliferating cells has also been found in the mouse palate (Luke 1980; Takanosu et al. 2002), where epithelial cells on the ridge edges showed the lowest proliferation activity. The primary thickening of the epithelium in rugal anlagen and primitive rugae seems to be a result of a change in the orientation of mitotic spindles of dividing cells rather than of increased proliferation activity. Similar processes have been described also during formation of the primary dental lamina (TenCate 1998).

The mesenchyme condensed adjacent to the ridge primordia at early developmental stages. In the mesenchymal core of the ridges at later developmental stages, the few PCNA-immunopositive cells were distributed more or less uniformly. Therefore, neither mesenchymal cell proliferation seems to play a major developmental role after initiation of ridge elevation.

Whether increased epithelial apoptosis within the primitive palatal rugae as described in the mouse (Turečková et al. 1996; Peterková et al. 1998; Amasaki et al. 2002; Takanosu et al. 2002;) plays a role in palatal ridge formation and elevation, remains to be studied.

In conclusion, knowledge on the role of morphogenetic processes, such as proliferation, cell death, differentiation and migration, in formation, development and shaping of palatal ridges is far from complete. Regarding the great clinical significance of correct development of the palate, further studies on this topic are necessary.

Morfogeneze patrových lišt u křečka zlatého (*Mesocricetus auratus*, Rodentia)

Rugae palatinae (RP) – transverzální slizniční lišty na patře savců – pravděpodobně hrají úlohu při uzavírání patra u embryí. Cílem této studie byl popis morfogeneze patrových lišt u křečka zlatého (*Mesocricetus auratus*, Rodentia) na základě vyšetření světelným a rastrovacím elektronovým mikroskopem. Buněčná proliferace byla stanovena imunohistochemickým průkazem proliferating cell nuclear antigen (PCNA). U křečka byly na patře vytvořeny tři až čtyři antemolární a tři intermolární patrové lišty. U embryí v ED 11,0 (ED – den embryonálního vývoje) představovaly RP1 a RP3 epiteliální ztluštění, RP2 primitivní patrovou lištu v rostrální části patrových výběžků. V kaudální části patra se nachází epiteliální ztluštění, které je oblastí dávající základ budoucím patrovým lištám RP4-RP7. V ED 12,5 bylo sekundární patro uzavřené a mělo na svém povrchu základy šesti patrových lišt. Pouze RP2 vyčníval zřetelně do dutiny ústní. Vývoj mezenchymálního jádra začal v ED 13,0-14,5 u antemolárních a v ED15,0-15,5 u intermolárních patrových lišt. Přestože tvorba patrových lišt je provázena zvětšením objemu tkáně, proliferační aktivita byla překvapivě vyšší v oblastech mezi lištami. Zvýšená lokální proliferační aktivita tedy pravděpodobně není hlavním procesem v průběhu vývoje patrových lišt, ale spíše orientace mitotického aparátu dělících se buněk.

Acknowledgements

We wish to thank Prof. Drahomír Horký for his help with the scanning electron microscope and Ms. Marie Míšková for her technical assistance. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (COST project B23.001) and the Fond of the Development of Universities of the Czech Republic (projects FRVS1489/2004 and 1487/2004).

References

AMASAKI H, MATSUMOTO S, TAKANOSU M, DAIGO M 1991: Distributions of extracellular matrix and carbonic anhydrase-III during bovine palatine ridge development. J Vet Med Sci **53**: 1031-1036

AMASAKI H, OGAWA M, NAGASAO J, MUTOH K, ICHIHARA N, ASARI M 2002: Distribution of apoptotic cells and apoptosis-related molecules in the developing murine palatine rugae. J Vet Med Sci **64**: 1103-1106

BUCHTOVÁ M, TICHÝ F, PUTNOVÁ I, MÍŠEK I 2003: The development of palatal rugae in the European pine vole, Microtus subterraneus (Arvicolidae, Rodentia). Folia Zool **52**: 127-136

BRINKLEY L, VICKERMAN M 1982: The effects of chlorcyclizine-induced alterations of glycosaminoglycans on mouse palatal shelf elevation in vivo and in vitro. J Embryol Exp Morphol **69**: 193-213

BULLEIT RF, ZIMMERMAN EF 1985: The influence of the epithelium on palate shelf reorientation. J Embryol Exp Morphol **88**: 265-279

CAPON AE 1983: The role of the innervation in the development of the rugae in the mouse. J Dent Res 62: 415

COBOURNE MT, SHARPE PT 2003: Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. Arch Oral Biol **48**: 1-14

DASSULE HR, LEWIS P, BEI M, MAAS R, MCMAHON AP 2000: Sonic hedgehog regulates growth and morphogenesis of the tooth. Development **127**: 4775-4785

EISENTRAUT M 1975: Das Gaumenfaltenmuster bei afrikanischen Sciuriden. Z Säugetierk **40**: 133-141

FERGUSON CA, TUCKER AS, CHRISTENSEN L, LAU AL, MATZUK MM, SHARPE PT 1998: Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev **12**: 2636-2649

GEGENBAUR C 1878: Die Gaumenfalten des Menschen. Morphol Jahrb **4**: 573-583

HARDCASTLE Z, HUI CC, SHARPE PT 1999: The Shh signalling pathway in early tooth development. Cell Mol Biol **45**: 567-578

IKEMI N, KAWATA M, YASUDA M 1995: All-trans-retinoic acid-induced variant patterns of palatal rugae in Crj:SD rat fetuses and their potential as indicators for teratogenicity. Reprod Toxicol **9**: 369-377

LUKE DA 1980: Distribution of mitoses in mouse palatal epithelium. Acta Anat (Basel) **108**: 98-101

LUKE DA 1984: Epithelial proliferation and development of rugae in relation to palatal shelf elevation in the mouse. J Anat **138**: 251-258

MAAS R, BEI M 1997: The genetic control of early tooth development. Crit Rev Oral Biol Med 8: 4-39

MELLER SM, PAOLA DP, BARTON LH, MANDELLA RD 1980: Secondary palatal development in the New Zealand white rabbit: a scanning electron microscopic study. Anat Rec **198**: 229-244

PETERKOVÁ R 1985: The common developmental origin and phylogenetic aspects of teeth, rugae palatinae, and fornix vestibuli oris in the mouse. J Craniofac Genet Dev Biol **5**: 89-104

PETERKOVÁ R, KLEPÁČEK I, PETERKA M 1987: Prenatal development of rugae palatinae in mice: scanning electron microscopic and histologic studies. J Craniofac Genet Dev Biol **7**: 169-189

PETERKOVÁ R, PETERKA M, VONESCH JL, TUREČKOVÁ J, VIRIOT L, RUCH JV, LESOT H 1998: Correlation between apoptosis distribution and BMP-2 and BMP-4 expression in vestigial tooth primordia in mice. Eur J Oral Sci **106**: 667-670

PETERS H, BALLING R 1999: Teeth. Where and how to make them. Trends Genet **15**: 59-65

PISPA J, THESLEFF I 2003: Mechanisms of ectodermal organogenesis. Dev Biol **262**: 195-205

POURTOIS M 1972: Morphogenesis of the primary and secondary palate. In: SLAVKIN HC, BARETTA LA (Eds.): Developmental aspects of oral biology. Academic Press, London, pp. 81-108

SAKAMOTO MK, NAKAMURA K, HANDA J, KIHARA T, TANIMURA T 1989: Morphogenesis of the secondary palate in mouse embryos with special reference to the development of rugae. Anat Rec **223**: 299-310

SCOTT JH, SYMONS NBB 1967: Introduction to dental anatomy. E. and S. Livingstone, London, 422 p.

SCHÜPBACH PM, CHAMBERLAIN JG, SCHROEDER HE 1983: Development of the secondary palate in the rat: a scanning electron microscopic study. J Craniofac Genet Dev Biol **3**: 159-177

TAKANOSU M, AMASAKI H, MATSUMOTO S, KIMATA K 1996a: Distribution of chondroitin sulphate proteoglycans and peanut agglutinin-binding molecules during bovine fetal palatine ridge formation. J Anat **189**: 109-115

TAKANOSU M, AMASAKI H, MIURA T, ASARI M, SUZUKI K 1996b: Distribution of chondroitin sulfate proteoglycans during bovine fetal ruminal papillae formation. Acta Anat **156**: 283-288

TAKANOSU M, AMASAKI H, IWAMA Y, OGAWA M, HIBI S, SUZUKI K 2002: Epithelial cell proliferation and apoptosis in the developing murine palatal rugae. Anat Histol Embryol **31**: 9-14

TENCATE R 1998: Oral Histology. Development, structure, and function. Mosby, Toronto, 497 p.

THESLEFF I 2003: Developmental biology and building a tooth. Quintenssence Int **34**: 613-620

THOMAS CJ 1984: The prenatal developmental microscopic anatomy of the palatal rugae. J Dent Assoc S Afr **39**: 527-533
THOMAS CJ, ROSSOUW RJ 1991: The early development of palatal rugae in the rat. Aust Dent J **36**: 342-348
TUREČKOVÁ J, LESOT H, VONESCH JL, PETERKA M, PETERKOVÁ R, RUCH JV 1996: Apoptosis is involved in the disappearance of the diastemal dental primordia in mouse embryo. Int J Dev Biol **40**: 483-489
ZIETZSCHMANN O, ACKERKNECHT E, GRAU H 1943: Handbuch der vergleichenden Anatomie der Haustiere. Springer Verlag, Berlin, 1155 p.

Plate I
Buchtová M. et al.: Morphogenesis.... pp. 319-327

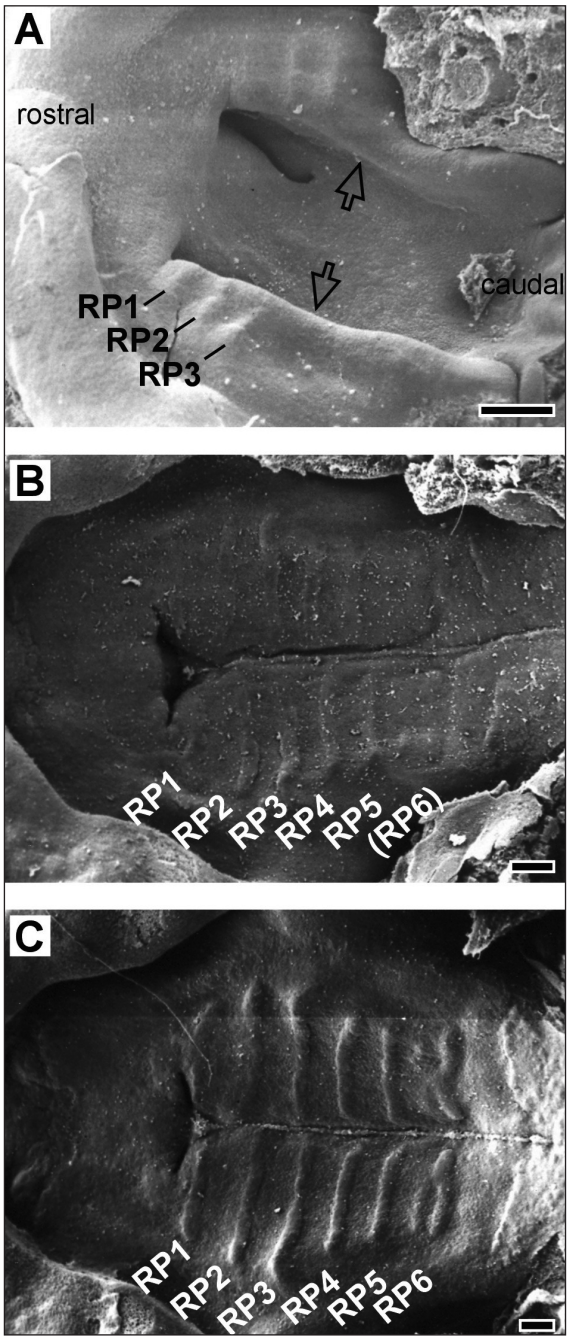

Fig. 1. Development of the secondary palate in the hamster. (A) Vertical palatal shelves (arrows) with three palatal ridge primordia (RP1-RP3) in an ED 11.5 embryo. (B) Fusion of the palatal shelves with six indistinct palatal ridge primordia (RP1-RP6) in an ED 12.5 foetus. (C) Six prominent palatal ridges (RP1-RP6) on the closed secondary palate of an ED 13.0 hamster foetus. Scanning electron micrographs, bar = 200 μm.

Plate II

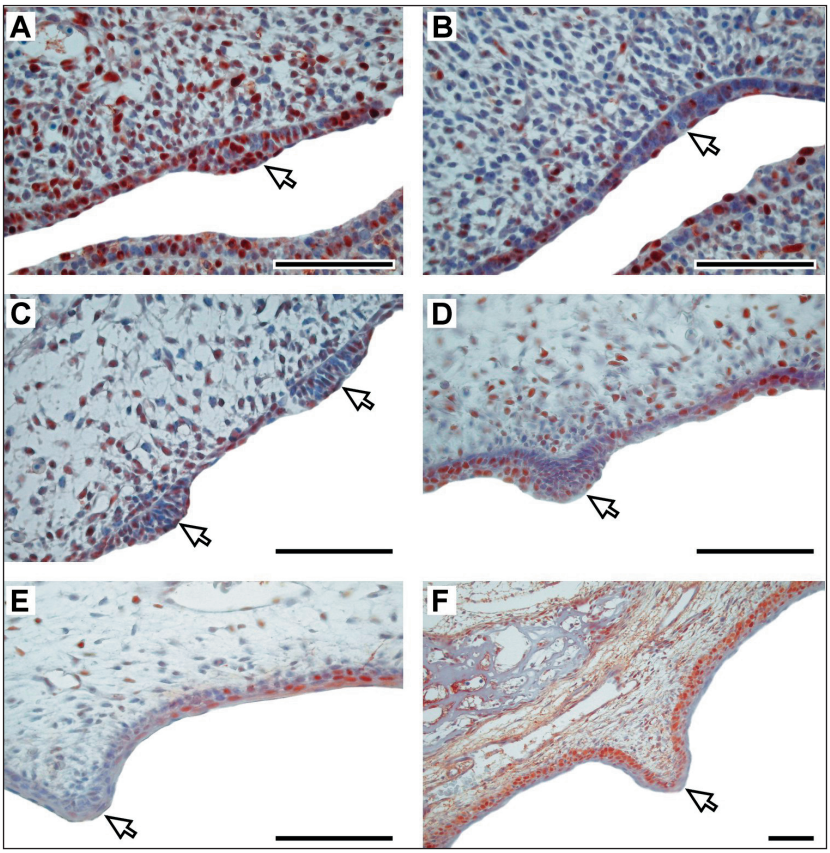

Fig. 2 Distribution of proliferating cells in the developing palatal ridges of the hamster. (A) Primitive ruga stage of the antemolar ridges (arrow) in an ED 11.5 embryo. Many uniformly distributed PCNA-positive cells in epithelium and mesenchyme. (B) Epithelial placode, from which the intermolar ridges develop (arrow), in an ED 11.5 embryo. Few PCNA-positive cells. (C) Condensation of mesenchyme underlying the primitive antemolar rugae in an ED 12.5 foetus. PCNA-positive cells mainly in the interrugal epithelium. (D) Onset of rugal core development in an ED 13.5 foetus. The edge of the palatal ridge is PCNA-negative. (E) Well developed rugal core in an ED 14.5 foetus. PCNA positive cells only in the interrugal epithelium. (F) Well developed palatal ridge in an ED 16.0 foetus. PCNA-positive basal cell layer of the complete oral epithelium. Sagittal sections of the palate, immunohistochemical detection of PCNA, detection of the immunoreaction with horseradish peroxidase-diaminobenzidine, counterstaining haematoxylin, bar = 50 μm.