Published in final edited form as:

Dev Dyn. 2011 July ; 240(7): 1737–1744. doi:10.1002/dvdy.22670.

Histomorphological study of palatal shelf elevation during murine secondary palate formation

Kai Yu and David M. Ornitz

Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110 USA

Abstract

During mammalian secondary palate development, the palatal shelves undergo dramatic morphological changes to elevate from a vertical to a horizontal plane in the oral-nasal cavity. We found that E14.5 mouse embryos displayed marked variations in shelf morphology that represent various intermediate states of the elevation process. With these variations, we reconstructed the sequence of shelf morphological changes that take place during the elevation process and discovered distinct patterns in different regions along the anterior-posterior (AP) axis. Moreover, our study revealed that during the elevation process, shelf morphological changes are accompanied by tongue morphological changes, which also show distinct characteristics along the AP axis. We further discuss how to divide the palate along the AP axis based on morphological criteria. Our study provides a framework that recognizes variation in timing of palatal morphogenesis along the AP axis that will aid in the investigation of the mechanisms regulating palatal shelf elevation.

Keywords

mouse; secondary palate; palatal shelf elevation; histomorphology

Introduction

The secondary palate, which separates the nasal cavity from the oral cavity, is formed by the fusion of two opposing shelves during embryonic development (Greene and Pratt, 1976; Ferguson, 1988). In mammals, after bulging out bilaterally from the maxillary process, the palate shelves grow rapidly away from the maxilla along the side of the tongue. Formation of the secondary palate requires the palatal shelves to undergo a process called shelf elevation or shelf reorientation, in which the palate shelves move from lateral of the tongue to above the dorsum of the tongue and change their orientation in the oral-nasal cavity from vertical to horizontal. After shelf elevation, the opposing horizontal shelves contact each other at the midline of the oral-nasal cavity. A midline epithelial seam (MES) is then formed by fusion of the medial edge epithelia (MEE) of the opposing palatal shelves. Subsequent degeneration of the MES allows fusion of opposing palate mesenchyme and complete formation of the secondary palate. Defects in any stage (growth, elevation and fusion) of palatogenesis result in cleft palate and incomplete separation of the nasal and oral cavities. Cleft palate is one of the most common birth defects in humans and is very often accompanied by cleft lip. For isolated cleft palate (without cleft lip), it has been estimated

that 90% of the cases are caused by defects related to the elevation process (Ferguson, 1977).

In the past two decades, mouse genetic approaches have been widely used to study palatogenesis. Although many mutant mice show defects in shelf elevation, these studies do not significantly advance the understanding of the mechanism of shelf elevation (Gritli-Linde, 2007; Gritli-Linde, 2008). The current consensus is still largely based on results of histomorphological studies (Walker and Fraser, 1956; Coleman, 1965) and various *in vitro* studies (Ferguson, 1978; Brinkley, 1980) that were conducted more than three decades ago. In the literature, the most comprehensive histomorphological study of palatal shelf elevation was conducted in rats (Diewert, 1978), which does not meet the needs of mechanistic studies using mouse genetic approaches. Here, we conducted a detailed histomorphological study in mice to reveal how the shelves change their morphology during the course of shelf elevation and along the whole length of the palate. This study, which builds on past histomorphological studies, provides a modernized developmental framework that will facilitate the analysis of mutant mice with defects in secondary palate development and make possible the integration and comparison of data derived from different studies.

Results

Methodology

In this study, we developed a histomorphological approach to examine how shelf elevation proceeds *in vivo*. The rationale is that shelf elevation is accomplished through tissue morphogenetic movements and thus can be studied by observing tissue morphological changes over time. In principle, morphological changes can be identified by observing and comparing tissue morphology displayed by specimens that are actively engaged in the elevation process. However, there are three potential problems associated with this method.

The first problem is how to identify specimens that are engaged in the elevation process. During mouse palatogenesis, shelf elevation occurs at approximately 14.5 days of embryonic development (E14.5). Among sixty-six E14.5 embryos that we collected, twenty-five embryos showed both shelves in the vertical direction, indicating that shelf elevation had not begun. Twenty-nine embryos showed that the opposing shelves fused to each other throughout the whole length of the AP axis, indicating that shelf elevation had finished. The rest (twelve embryos) showed shelf morphology (sometimes unilaterally) that was distinct from the other two groups, and thus were considered as specimens actively engaged in the elevation process.

The second problem is how to stage palatal development. We used a method that is based on shelf morphology, with the assumption that the elevation process is irreversible *in vivo*. Thus, a horizontal oriented shelf should be at a later stage than that of a vertical oriented shelf, and a shelf with its medial edges above the dorsum of the tongue should be at a later stage than that of a shelf with its ventral edge below the dorsum of the tongue. With this method, we divided the elevation process into six stages, with stage 1 defined as pre-elevation and stage 6 as post-elevation but prior to shelf fusion. Stages 2–5 represented transition stages with different degrees of progress in shelf elevation (Figure 2).

The third problem is how to compare morphology of specimens at different stages. It has already been proposed that shelf elevation is achieved through distinct mechanisms in different regions along the AP axis (Coleman, 1965). To completely understand the mechanism of shelf elevation, it is necessary to examine multiple regions. In this study, we established a new method in which six coronal planes along the AP axis (planes I–V from posterior to anterior and an additional one at the extreme posterior end of the palate) were

used to illustrate regional differences of the palate during shelf elevation (Figure 1A). The locations of planes I–V on the AP axis were correlated with tongue morphology along the AP axis. On midline sagittal sections, the dorsum of the tongue shows a unique flexure, which gradually ascends from the anterior tip, peaks in the middle, and then descends towards the posterior root. Moreover, the anterior portion of the tongue is not attached to the lower jaw. Thus, planes I and II are in the region in which the dorsum of the tongue shows a descending slope. Plane III is at an inflection point in which the dorsum of the tongue changes the orientation of its slope. Plane IV is at a transition zone in which the tongue starts to separate from the lower jaw. Plane V is in the region in which the tongue is no longer attached to the lower jaw. Coronal sections that represented comparable planes from different specimens were selected according to anatomical landmarks that included various craniofacial tissues and specific shelf and tongue morphology.

Extreme Posterior End

At the extreme posterior end (dashed arrow in Figure 1A), the palatal shelves do not need to undergo elevation since they are horizontally oriented from the beginning (Walker and Fraser, 1956; Ferguson, 1978). The anatomical landmark of this plane was the trigeminal ganglia above the cranial base. At E14 (Figure 1B), the shelves appeared as bulges positioned above the dorsum of the tongue. At E14.5 prior to shelf elevation (Figure 1C), the shelves showed expansion or growth toward the midline in a horizontal direction, but the most dramatic change was displacement of the tongue. At this stage, the root of the tongue was no longer present in the coronal plane that contained the extreme posterior end of the palate.

Plane I

Plane I was about 30 sections (120 μm) anterior to the extreme posterior end (Figure 1C). The anatomical landmark of this plane was the lateral border of the shelves. Since the shelves in this plane were fused with the maxilla dorso-laterally, the lateral borders of the shelves were rather short and appeared only in the ventral portion of the shelves prior to shelf elevation (Figure 2, I-1). At stage 1, the shelves were localized lateral to the tongue and the ventral edges of the shelves were below the dorsum of the tongue. At stage 2 (Figure 2, I-2), tongue volume/height was dramatically reduced and a space appeared between the cranial base and the dorsum of the tongue. The shelves dramatically increased their size through medial expansion into a vacant cavity space. The ventral edges of the shelves were expanded and turned into the ventral borders that were situated above the dorsum of the tongue. The shelf continually expanded medially at stage 3 (Figure 2, I-3, right side), but remained in a vertical direction through stage 5 (Figure 2, I-5, right side) when shelf elevation was completed in all other planes studied. At stage 6 (Figure 2, I-6), the shelves were oriented in a complete horizontal direction with fully developed ventral borders, but the medial edges of the opposing shelves remained separated by a gap.

Plane II

Plane II was about 30 sections (120 μm) anterior to plane I. The anatomic landmarks of this plane were tooth germs and the lateral borders of the shelves. Since the shelves were completely separated from the maxillae, the lateral borders were greatly extended dorsally prior to shelf elevation when compared to that of plane I. At stage 1 (Figure 2, II-1), the shelves were positioned between the tongue and the oral-nasal cavity walls. The medial borders of the shelves were straight and vertically oriented, but the lateral borders of the shelves were not straight and contained epithelial invagination (green arrow) that changed orientation of the lateral borders of the shelves. The tongue showed a rectangular shape with straight lateral borders and a flat dorsal surface. Due to the presence of the shelves, the lateral borders of the tongue were not adjacent to the oral cavity walls but were adjacent to

the medial borders of the shelves. At stage 2 (Figure 2, II-2), shelf tissues bulged into the space between the cranial base and the dorsum of the tongue and thus formed a protrusion on the medial walls (medial wall protrusion, red arrows). Simultaneously, the tongue expanded laterally into the space below the ventral edges of the shelves. As its dorsal surface was reduced, the tongue displayed an unusual trapezoidal shape with its lateral borders adjacent to the oral cavity wall ventrally. At stage 3 (Figure 2, II-3, right side), the shelf expanded toward the midline in the cavity space above the dorsum of the tongue. Medial wall protrusion developed into a new medial growth tip, which was located above the dorsum of the tongue and pointed in a horizontal direction. The original ventral tip was expanded and became the ventral border of the shelf. At stage 4 (Figure 2, II-4, right side), the shelf continued to expand toward the midline through the medial tip, which now became the medial edge of the shelf. The shelves were above the dorsum of the tongue and oriented in a nearly horizontal position. The epithelial invagination on the lateral border of the shelf was straightened and turned into a part of the ventral border of the shelf. At stage 5 (Figure 2, II-5, right side), the shelf remained in a nearly horizontal orientation. At stage 6 (Figure 2, II-6), the shelves were in a complete horizontal orientation with fully developed ventral borders, but the medial edges of the opposing shelves remained separated by a gap. The tongue now occupied the oral cavity with its lateral borders adjacent to the oral cavity walls. The dorsal surface of the tongue was again flat but its width was increased when compared to that of stage 1 (Figure 2, II-1).

Plane III

Plane III was about 30 sections (120 μm) anterior to plane II. The anatomical landmarks of this plane were tooth germs, the cartilaginous primordium of the nasal septum and the primitive olfactory cavity (olfactory epithelium). Prior to shelf elevation, the lateral borders of the shelves were more horizontally oriented than that of plane II (Figure 2, III-1). During shelf elevation, the palate shelves and the tongue in plane III underwent similar morphological changes to those of plane II (Figure 2, III-2, III-3, right side and III-4, right side). However, such morphological changes were apparently delayed in plane III, likely resulting from a delayed onset of shelf tissue movement. Despite the delay, the shelf was in a complete horizontal orientation at stage 5 (Figure 2, III-5, right side) and the medial edges of the opposing shelves contacted each other at stage 6 (Figure 2, III-6), both earlier than that of plane II.

Plane IV

Plane IV was about 20 sections (80 μm) anterior to plane III. The anatomic landmarks of this plane were the nasal septum, which replaced the cranial base as the roof of the oral-nasal cavity, and the ventral borders of the tongue. As the tongue started to separate from the lower jaw, the lateral borders of the tongue extended ventrally to form the ventral borders that were adjacent to the oral cavity walls prior to shelf elevation. At this plane, the tongue was still connected with the lower jaw at the midline of the oral-nasal cavity. At stage 1 (Figure 2, IV-1), the medial borders of the shelves were not straight but showed a concave curvature. The lateral borders of the shelves showed similar orientation to that of plane III, but with an identifiable lateral epithelial invagination (green arrow). At stages 2 (Figure 2, IV-2) and 3 (Figure 2, IV-3, right side), both the shelves and the tongue showed little morphological changes, except that the medial borders of the shelves lost their concave curvature and became straight. At stage 4 (Figure 2, IV-4, right side), the shelf showed a medial wall protrusion (red arrow) that reduced the overlap distance between the ventral edge of the shelf and the dorsum of the tongue. However, the tongue in plane IV did not undergo a similar lateral expansion that accompanied the medial wall protrusion in planes II and III. At stage 5 (Figure 2, IV-5, right side), the shelf was above the dorsum of the tongue and in a complete horizontal orientation with a fully developed ventral border. The ventral

border was straight without epithelial invagination. The tongue expanded laterally in the cavity below the horizontally oriented shelves and increased its dorsal width. At this stage, the medial edges of the opposing shelves remained separated by a gap. At stage 6 (Figure 2, IV-6), the medial edges of the opposing shelves contacted each other at the midline. The tongue now occupied the oral cavity with its lateral/ventral borders adjacent to the oral cavity walls. From stage 1 to stage 6, the tongue shape changed dramatically from a mushroom-like shape into an inverted triangle shape.

Plane V

Plane V was about 20 sections (80 μm) anterior to plane IV and about 30 sections (120 μm) posterior to the primary palate. The anatomical landmarks of this plane were the precartilaginous primordium of the nasal capsule, the vomeronasal organ and the ventral border of the tongue, which completely separated the tongue from the lower jaw. At stage I (Figure 2, V-1), the lateral epithelial invagination observed in stage V was more prominent (green arrow). Compared to other planes, the shelves in plane V did not form a medial wall protrusion and the tongue did not show morphological changes from stage 2 to stage 4 (Figure 2, V-2, V-3, right side and V-5, right side). Nevertheless, the overlap distance between the ventral edge of the shelf and the dorsum of the tongue was significantly reduced at stage 4 (Figure 2, V-4, right side). At stage 5 (Figure 2, V-5, right side), the shelf was above the dorsum of the tongue and in a completely horizontal orientation with fully developed ventral borders. The ventral border was straight without epithelial invaginations. The tongue was expanded laterally in the cavity below the horizontally oriented shelves and increased its dorsal width, which was similar to that of plane IV. At stage 6 (Figure 2, V-6), the medial edges of the opposing shelves remained separated by a gap.

Morphological transition zone along the AP axis

It appeared that shelf elevation in planes II and III was completed through gradual morphological changes, which spanned several intermediate stages. On the other hand, shelf elevation in plane V was completed in the short interval between stage 4 and 5 without showing detectable intermediate morphology. To understand how such a transition happened, we examined additional sections that flanked plane IV and one additional stage between stages 3 and 4 (Figure 3). We found that the regions flanking plane IV represented a morphological transition zone in which the shelves gradually lost the ability to form a medial wall protrusion toward the anterior. This transition zone also marked a major structural change in the oral cavity; separation of the tongue from the lower jaw toward the anterior. Posterior to this transition zone (planes II and III) where the tongue is fully connected with the lower jaw, we found that the medial wall protrusion of the shelves was accompanied by simultaneous tongue lateral expansion during shelf elevation (Figure 2, II-2 and III-2). However, as the tongue starts to separate from the lower jaw in this transition zone, tongue lateral expansion no longer immediately followed medial wall protrusion of the shelves (Figure 3). In fact, tongue lateral expansion in planes IV and V did not occur until completion of shelf elevation (Figure 2, IV-5 and V-5). It should be noted that tongue lateral expansion did not change the anatomic structures (full connection, partial connection or complete separation) that appeared between the ventral portion of the tongue and the lower jaw in each plane. Thus, such an anatomic difference can be used to distinguish different planes along the AP axis.

Discussion

Histomorphological studies in the past allowed researchers to formulate basic models about the mechanism of palatal shelf elevation. For example, Coleman, who used rats in his study, suggested that shelf elevation in the anterior and posterior portions of the palate occurred

through different mechanisms (Coleman, 1965). Walker et al., who used mice in their studies, suggested that shelf elevation is achieved through shelf tissue “remodeling” around the tongue (Walker and Fraser, 1956). However, due to inherent anatomical differences between species, studies with different animal models also lead to conflicting conclusions. In the literature, there is disagreement about where the elevation process begins and how it proceeds along the AP axis. Both Coleman and Ferguson, who used rats in their studies, suggested that shelf elevation begins rostrally (anteriorly) and proceeds caudally (posteriorly) (Coleman, 1965; Ferguson, 1978). Walker and Fraser, who used mice in their studies, suggested the opposite (Walker and Fraser, 1956). Our study also found that during murine palatogenesis, shelf elevation in the anterior region (plane V) occurs at a later stage than that of the more posterior region (planes II and III). Thus, the general consensus that shelf elevation initiates anteriorly or at the level of the second ruga palatina (Gritli-Linde, 2008) should not be considered as the common mechanism of mammalian palatogenesis.

Dynamic morphological changes during shelf elevation

In a previous study, Walker and Fraser examined shelf morphological changes during murine palatal shelf closure and used two coronal planes to show regional differences along the AP axis (Walker and Fraser, 1956). They concluded that shelf elevation is achieved through shelf tissue remodeling movement consisting of protrusion of the medial wall and regression of the ventral wall of the vertical shelves, which carries the shelves dorsal to the tongue. They also suggested that such an elevation process starts at the posterior end of the palate, where the shelves are horizontally oriented from the beginning, and proceeds in a wave-like manner toward the anterior end. We find that two coronal planes used in Walker and Fraser’s study for the anterior and posterior palate approximately corresponds to planes V and II, respectively. Although shelf elevation in plane II occurs earlier than that of plane V, shelf elevation in plane I, which represents the region between the extreme posterior end and plane II, lags behind that of plane II and is not completed until shelf elevation is completed in plane V. Our histomorphological study further suggests that the remodeling mechanism of shelf elevation might be operative only in planes II, III and to some extent plane IV, but unlikely in either plane I or V. Thus, during murine palatogenesis, shelf elevation along the AP axis does not simply follow a posterior-to-anterior order as Walker and Fraser suggested, but rather shows a dynamic pattern that results from different elevation mechanisms employed by different regions along the AP axis.

In addition to shelf morphological changes, our study also revealed tongue morphological changes during the course of shelf elevation. It has been proposed for a long time that tongue morphogenetic movement is important for shelf elevation (Greene and Pratt, 1976). However, in the literature, there are no detailed studies about tongue movement during shelf elevation. Our finding of displacement between the root of tongue and the extreme posterior end of the palate (Figure 1B,C) suggests that the tongue could move anteriorly along the AP axis prior to shelf elevation. Such tongue movement is likely due to growth of the mandible (or Meckel’s cartilage) in the lower jaw to which the root of the tongue is attached. We also found that during shelf elevation, the tongue expands laterally in the oral cavity, which increases the dorsal width of the tongue along the medial-lateral (ML) axis, but at the same time reduces tongue height along the dorsal-ventral (DV) axis. In the literature, there is one uncharacterized tongue morphological change that occurs during shelf elevation and is described as tongue flattening (Wragg et al., 1972; Ferguson, 1978). Ferguson suggested that tongue flattening reduces the dorsal arch of the tongue, creates space (common nasal passage) above the dorsum of the tongue to accommodate the elevated shelves and causes the tip of the tongue to protrude out of the oral cavity (Ferguson, 1978). We suggest that tongue flattening could result from tongue lateral expansion, which actually occurs throughout the entire palate (despite being less prominent in plane I). Moreover, we found

that the timing of tongue lateral expansion is different along the AP axis. In planes II and III, tongue lateral expansion occurs prior to completion of shelf elevation (Figure 2, II-3 and III-4), but occurs in planes IV and V after completion of shelf elevation (Figure 2, IV-5 and V-5). Thus, in addition to differences in morphological changes, the timing of morphological changes also contributes to regional heterogeneity that is displayed along the AP axis during shelf elevation.

How to divide the palate along the AP axis

In the literature, there is no consensus about how to divide the palate and how to identify different regions along the AP axis. Traditionally, the palate can be divided into up to four regions prior to shelf elevation. From anterior to posterior, they are anterior, middle and posterior portions of the presumptive hard palate that forms the anterior two thirds of the palate and the presumptive soft palate that forms the posterior one third of the palate (Brinkley and Vickerman, 1979; Bulleit and Zimmerman, 1985; Okano et al., 2006). It has been found that the presumptive hard palate could elevate without connection with the presumptive soft palate but that the presumptive soft palate could not elevate without connection with the presumptive hard palate (Brinkley and Vickerman, 1979; Bulleit and Zimmerman, 1985). However, it is not clear whether the definitive hard palate (containing maxillary bones and palatine bones) and soft palate (containing muscles and soft tissues) truly develop from the presumptive hard palate and soft palate that are defined according to shelf elevation behaviors. Since osteogenesis or myogenesis in shelf mesenchyme does not occur at the time of shelf elevation, we suggest that prior to shelf elevation, different regions along the AP axis be named strictly according to their positions on the AP axis. Thus, from anterior to posterior, the palate can be divided into anterior, middle, posterior regions and a posterior end zone that includes the extreme posterior end and corresponds to the presumptive soft palate referred to in the literature.

In past studies, Brinkley used anatomical landmarks to identify histological sections that represent different regions along the AP axis (Brinkley and Vickerman, 1982; Brinkley, 1984). Based on Brinkley's descriptions, we found that planes V, IV and III approximately correspond to the anterior, middle and posterior presumptive hard palate in Brinkley's studies, which we rename as the anterior, middle and posterior region, respectively. We also found that the coronal plane used by Brinkley to represent the posterior end zone (presumptive soft palate in Brinkley's studies) is located between planes I and II. Our histomorphological study indicates that shelf elevation in planes I and II is very different. Shelf elevation in plane II not only proceeds in a similar way to that of plane III, but also occurs prior to that of plane III. Moreover, shelf elevation in planes II and III can be achieved to a large degree prior to that of the anterior portion (Figure 2, II-4 and III-4). Thus, plane II can also be considered as a part of the posterior region, which spans both planes II and III. On the other hand, shelf elevation in plane I is clearly delayed and might depend on that of the anterior portion (Figure 2, I-5 and I-6). Thus, we used plane I to represent the posterior end zone. We also examined the plane that is used in Brinkley's studies for the posterior end zone and found that the shelves display intermediate morphology between that of planes I and II (data not shown). This suggests that the posterior end zone (plane I) and the posterior region (planes II and III) are not separated by a clear boundary but rather by a morphological transition zone, much like the middle region (plane IV) that separates the posterior region (planes II and III) and the anterior region (plane V).

Caveats for molecular genetic study of palatogenesis

The mouse model is widely used in molecular genetic studies of palate development and pathogenesis. Since shelf elevation occurs approximately at E14.5 of mouse embryonic

development, it is routine to examine and compare shelf morphology at this stage to determine whether shelf elevation is normal in genetic mutant mice. However, our study indicated that during normal development, E14.5 embryos, even from the same litter, would display a variety of shelf morphologies that range from completely unelevated to completely fused. This is likely due to normal variations in time of conception for embryos at the same chronological age determined by the method of observing a vaginal plug. Thus, the presence of the vertically oriented shelves in E14.5 embryos does not necessarily indicate delay or failure of shelf elevation that results from defects in palatogenesis. On the other hand, if shelf elevation is delayed, the shelves would remain vertically oriented and normal variations in shelf morphology at E14.5 should not be observed. Thus, the percentage of E14.5 embryos with vertically oriented shelves can be used to evaluate whether shelf elevation is delayed. Previously, we studied cleft palate phenotypes in mice carrying a Crouzon syndrome mutation (Snyder-Warwick et al., 2010). We found that this percentage in wild type, heterozygous and homozygous mice is 20%, 50% and 100%, respectively, and thus concluded that shelf elevation is delayed in mice homozygous for the Crouzon syndrome mutation.

Variations in shelf morphology at E14.5 can also be used in mechanistic studies of palatogenesis. Currently, almost all studies of gene expression prior to shelf elevation are conducted at E13.5, almost one day before shelf elevation actually occurs *in vivo*, which is not ideal for understanding gene functions during shelf elevation. Another drawback of using E13.5 embryos is that it would miss any defects that developed after E13.5 in mutant mice. These problems can be solved by selecting E14.5 embryos that have vertically oriented shelves, which allow gene expression or phenotypes to be studied at a time immediately (up to several hours) before shelf elevation.

The five coronal planes defined in our study can serve as a reference for gene expression analysis to accurately position gene expression domains onto the AP axis and directly link gene functions to distinct morphological changes that occur in different regions of the palate during shelf elevation. This type of analysis, in combination with genetic mutations that affect palatogenesis will be essential to understand how shelf elevation is regulated in different regions along the AP axis and how genetic defects could lead to cleft palate.

Experimental Procedures

Mice were maintained on a C57BL/6J $\times$ 129X1/SvJ mixed genetic background. Female mice were sacrificed at 14 days or 14.5 days post-coitum. The morning when a vaginal plug was observed was defined as embryonic day 0.5 (E0.5). Embryos were dissected from the uterus in PBS, fixed in 4% paraformaldehyde overnight at 4°C, and stored in 70% ethanol before embedding. Embryo heads were severed from bodies, embedded in paraffin, sectioned (4 μ m) and stained with hematoxylin and eosin. Specimens were sectioned either sagittally or coronally. The sagittal plane is parallel to the anterior-posterior (AP) axis (the rostral-caudal axis of the mouse head). The coronal plane is perpendicular to the AP axis that we defined and is parallel to the dorsal-ventral (DV) axis (Figure 1). Tissue movements were also examined along the medial-lateral (ML) axis, which is perpendicular to the DV axis in the coronal plane and extending from the midline of the oral-nasal cavity laterally (Figure 1). For shelf orientation in the oral-nasal cavity, the terms “vertical” and “horizontal” refer to parallel to the DV axis and the ML axis, respectively.

Acknowledgments

We would like to thank Joan Richtsmeier for critical comments and valuable suggestions to improve the manuscript. We thank Craig Smith for technical help. This work was supported by NIH grant HD049808.

References

- Brinkley, L. In vitro studies of palatal shelf elevation. In: Pratt, RM.; Christiansen, RL., editors. *Current Research Trends in Prenatal Craniofacial Development*. New York: Elsevier-North-Holland; 1980. p. 203-220.
- Brinkley LL. Changes in cell distribution during mouse secondary palate closure in vivo and in vitro. I. Epithelial cells. *Dev Biol*. 1984; 102:216–227. [PubMed: 6698305]
- Brinkley LL, Vickerman MM. Elevation of lesioned palatal shelves in vitro. *J Embryol Exp Morphol*. 1979; 54:229–240. [PubMed: 528868]
- Brinkley LL, Vickerman MM. The effects of chlorcyclizine-induced alterations of glycosaminoglycans on mouse palatal shelf elevation in vivo and in vitro. *J Embryol Exp Morphol*. 1982; 69:193–213. [PubMed: 6126516]
- Bulleit RF, Zimmerman EF. The influence of the epithelium on palate shelf reorientation. *J Embryol Exp Morphol*. 1985; 88:265–279. [PubMed: 3935750]
- Coleman RD. DEVELOPMENT OF THE RAT PALATE. *Anat Rec*. 1965; 151:107–117. [PubMed: 14278705]
- Diewert VM. A quantitative coronal plane evaluation of craniofacial growth and spatial relations during secondary palate development in the rat. *Arch Oral Biol*. 1978; 23:607–629. [PubMed: 281896]
- Ferguson MW. The mechanism of palatal shelf elevation and the pathogenesis of cleft palate. *Virchows Arch A Pathol Anat Histol*. 1977; 375:97–113. [PubMed: 143115]
- Ferguson MW. Palatal shelf elevation in the Wistar rat fetus. *J Anat*. 1978; 125:555–577. [PubMed: 640958]
- Ferguson MW. Palate development. *Development*. 1988; 103(Suppl):41–60. [PubMed: 3074914]
- Greene RM, Kochhar DM. Palatal closure in the mouse as demonstrated in frozen sections. *Am J Anat*. 1973; 137:477–482. [PubMed: 4730464]
- Greene RM, Pratt RM. Developmental aspects of secondary palate formation. *J Embryol Exp Morphol*. 1976; 36:225–245. [PubMed: 1033980]
- Gritli-Linde A. Molecular control of secondary palate development. *Dev Biol*. 2007; 301:309–326. [PubMed: 16942766]
- Gritli-Linde A. The etiopathogenesis of cleft lip and cleft palate: usefulness and caveats of mouse models. *Curr Top Dev Biol*. 2008; 84:37–138. [PubMed: 19186243]
- Okano J, Suzuki S, Shiota K. Regional heterogeneity in the developing palate: morphological and molecular evidence for normal and abnormal palatogenesis. *Congenit Anom (Kyoto)*. 2006; 46:49–54. [PubMed: 16732762]
- Snyder-Warwick AK, Perlyn CA, Pan J, Yu K, Zhang L, Ornitz DM. Analysis of a gain-of-function FGFR2 Crouzon mutation provides evidence of loss of function activity in the etiology of cleft palate. *Proc Natl Acad Sci U S A*. 2010; 107:2515–2520. [PubMed: 20133659]
- Walker BE, Fraser FC. Closure of the Secondary Palate in Three Strains of Mice. *J Embryol Exp Morphol*. 1956; 4:176–189.
- Wragg LE, Diewert VM, Klein M. Spatial relations in the oral cavity and the mechanism of secondary palate closure in the rat. *Arch Oral Biol*. 1972; 17:683–690. [PubMed: 4558302]

Figure 1.

Regions examined along the anterior-posterior axis during palatal shelf elevation. (A) Midline sagittal section of an E14.5 head showing positions of five (I–V) coronal planes and the extreme posterior end (dashed arrow) along the anterior-posterior axis. (B) Coronal section showing the extreme posterior end at E14. Note that the shelves (arrow) are in a horizontal direction above the dorsum of the tongue. (C) Coronal section showing the extreme posterior end at E14.5 prior to shelf elevation (stage 1). The section comes from the same specimen used in Figure 2 (stage 1). Note that the shelves (arrow) expand horizontally but the tongue is no longer present below the shelves. A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral; t, tongue; tga, trigeminal ganglia.

Figure 2.

Morphological changes during palatal shelf elevation. The elevation process is examined at six stages. In each stage, sections that represent five coronal planes come from a single E14.5 specimen. Note that the section of stage 4 plane I was not collected during sectioning. Stage 3, 4 and 5 shelves only appear unilaterally in specimens examined and are shown on the right side, and the shelves on the left side have already progressed to stage 6. Images of stage 5 were flipped horizontally to match those of stages 3 and 4. Note that the open space in the oral-nasal cavity appearing in planes II–V of stage I are artifacts resulting from the paraffin embedding process (Greene and Kochhar, 1973). Red arrows indicate medial wall protrusion. Green arrows indicate epithelial invaginations. mc, meckel's cartilage; nc, nasal capsule; ns, nasal septum; oe, olfactory epithelium; ps, palatal shelf; t, tongue; tg, tooth germ; vo, vomeronasal organ.

Figure 3.

Morphological transition zone. (A–C) Coronal sections showing a stage 3 shelf (right side) from a single E14.5 specimen. (B) is identical to IV-3 in Figure 2. (D–F) Coronal sections showing a shelf (right side) between stage 3 and stage 4 from a single E14.5 specimen. (G–I) Coronal sections showing a stage 4 shelf (right side) from a single E14.5 specimen. (H) is identical to IV-4 in Figure 2. (A, D, G) are in a plane between planes III and IV. (C, F, I) are in a plane between planes IV and V. Note that shelves on left side have already progressed to stage 6. Red arrows indicate medial wall protrusion. tg, tooth germ; ns, nasal septum.