Active role of embryonic facial epithelium: New evidence of cellular events in morphogenesis

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SUMMARY

Epithelial cells of the C57B1/6J mouse embryo participate in a temporal sequence of events associated with the approximation, fusion and consolidation of components of the facial primordia into a definitive structure. These cells lose their surface microvilli, and after a brief period of quiescence they begin to fill the grooves separating facial constituents by producing a series of surface projections that increase in size and complexity as the process of fusion nears termination. Cessation of surface activity and the restoration of epithelial microvilli indicate the end of the temporal sequence. Significantly, the epithelial cells of primary palates of embryos with genetically- and phenytoin-induced cleft lip remain unchanged and do not participate in fusion. This epithelial sequence has not been described previously and we suggest that all of its steps may be critical to the normal development of the mammalian face.

INTRODUCTION

The development of the face, a complex process that begins early in embryonic life (Johnston, Hassell & Brown, 1975), results from closely timed steps in growth and fusion of four bilateral tissue outgrowths (Johnston et al. 1975; Trasler & Fraser, 1979) called facial prominences, processes or elevations (Slavkin, 1979); the medial nasal, lateral nasal, maxillary and mandibular prominences (Fig. 1a). There is abundant evidence that these prominences remodel and approach each other, but information regarding the mechanisms involved in these steps is fragmentary and incomplete (Trasler & Fraser, 1979). Cell differentiation, division, migration and specific interactions between epithelium, mesenchyme and extracellular matrix are believed to be key elements in facial morphogenesis (Johnston et al. 1975; Trasler & Fraser, 1979). The fusion of these prominences shortly after contact consolidates the individual outgrowths into a more definitive facial structure.

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We have studied facial development in C57B1/6J mouse embryos by methods of scanning and transmission electron microscopy and discovered a sequence of events exhibited by the epithelial cells located between facial prominences during periods of consolidation.

Clefts of the upper lip with or without cleft palate are among the most common developmental defects in humans. Therefore, although we studied all of the primordia which contribute to the face, this report will emphasize the epithelial events occurring between the facial prominences of the embryo which give origin to the upper lip.

**MATERIALS AND METHODS**

We removed gravid uteri from ten pregnant C57Bl/6J mice between 5 p.m. on gestational day 10 and 8 a.m. on day 11 (day of plug = 0) and made small uterine incisions over each implantation site before placing the tissues in refrigerated paraformaldehyde–glutaraldehyde (Waterman, 1974) fixative. One hour later, we dissected the embryos free from the membranes and staged them by the number of tail somites posterior to the genital tubercle. At the time the first tail somite appears, the embryo has approximately 28 body somites. Heads of staged embryos were immersed in fresh paraformaldehyde–glutaraldehyde and refrigerated overnight. Specimens were rinsed in 0-1 M cacodylate buffer and post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer for 90 min, followed by rapid dehydration through ethanol and ethanol-1,1,2-trichlorotrifluoroethane. Embryonic heads were critical-point dried from monochlorotrifluoromethane, positioned on aluminium mounts, and sputter coated with gold–palladium. A total of 72 specimens (from ten litters) were observed by scanning electron microscopy. In addition, several specimens were studied by transmission electron microscopy.

**RESULTS**

Our observations indicate that C57Bl/6J embryos possess bilateral facial depressions (nasal pits) by the stage of two to four tail somites. The primary palate begins to close at six tail somites and fusion is completed by ten tail somites. These embryonic events take place over a period of 12 h in utero. Prior to the initiation of primary palate closure, individual epithelial cells on the surface of the facial prominences are outlined by rows of microvilli at the cell margins (Fig. 1b). Detailed examination of nasal prominences revealed that a specific group of 10–20 epithelial cells located at the lower portion of the nasal pit (between the medial and lateral nasal prominences) undergoes transformation beginning with the disappearance of the microvilli. These cellular alterations coincide with the initiation of primary palate closure at six tail somites. At the bottom of the nasal pit, there is a triangular area (Fig. 1a) of
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smooth-surfaced cells (Fig. 2a), but soon this quiescent area will be the site for a sequence of events that may be critical to the successful closure of the primary palate. By seven tail somites it is possible to detect the first signs of surface activity in this initially smooth area. Ridges begin to form on the smooth triangular surface (Fig. 2b); these ridges (about 0.1 μm across and about 10–20 μm long) appear in locations previously occupied by rows of microvilli and course over the epithelial surface. The triangular bottom of the nasal pit narrows after the appearance of these ridges, and this morphogenetic rearrangement brings the medial and lateral nasal prominences in closer apposition. At a slightly later stage epithelial filopodia of about 0.1–0.3 μm in diameter begin to span from both medial and lateral nasal prominences establishing the initial bridges (Figs. 3a and 4) which seem to guide the surface activity that follows. Our observations with scanning and transmission electron microscopy suggest that these initial filopodia anchor into the surface by penetrating between surface cells (Fig. 3a, b). Embryos observed at the stage of seven to eight tail somites display larger cellular extensions (0.5–0.7 μm in diameter) which bridge the area between nasal prominences (Fig. 5a). Since these secondary extensions are in close contact with the initial small-diameter filopodia, it is tempting to speculate that secondary bridges are guided across the gap by the previously established filopodia. At this stage of primary palate development, the epithelial connexions between nasal prominences consist of small and large diameter cellular extensions wrapped around each other (Fig. 5a). We have observed that secondary extensions often display enlargements (2–3 μm) that terminate in filopodia (Fig. 6). We have also observed intercellular junctions between the enlargements in the secondary bridges and the epithelial surface of the nasal prominences (Fig. 5b).

In the last 2–3 h of primary palate fusion (about nine tail somites) flattened cells from the surface of the medial and lateral nasal prominences appear to move down into the inferior portion of the nasal pit filling the gap between these prominences (Figs 4, 5a and 6). At this stage of development, we noted that these migrating cells are in close contact with the initial filopodial and secondary extensions (Figs 4, 5a and 6). We found spheroidal particles, 1–3 μm in diameter (possibly representing cellular debris) on the epithelial surface of primary palates undergoing active epithelial adhesion as early as six tail somites and becoming more numerous in the final stages of fusion (Figs 4, 6 and 7). Finally, by the stage of ten tail somites the surface of the pre-closure epithelium is re-established. Before the activity described above ceases, rows of epithelial microvilli reappear forming a pattern different from that observed before the primary palate began closing. The new rows of microvilli are symmetrically aligned longitudinally and transversely to the line of primary palate fusion (Fig. 7). The above developmental steps overlap in time, and fit into the sequence illustrated in Fig. 8.
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DESCRIPTION OF FIGURES 1-7

FIGURE 1

(a) Overall SEM view of facial primordia of a mouse embryo. Triangle at the junction of the medial nasal (MNP), lateral nasal (LNP) and maxillary (MxP) prominences indicates the area of initial fusion of the primary palate. MnP: mandibular prominence; E: eye primordium.
(b) Higher magnification of surface epithelium demonstrates rows of microvilli (Mv) at cell margins.

FIGURE 2

(a) Nasal pit of a mouse embryo of six tail somites. A triangular area (dotted lines) on the floor of the nasal pit has lost the rows of microvilli. The transition between altered and normal epithelium is best illustrated on the left margin of the triangle. LNP: Lateral nasal prominence; MNP: medial nasal prominence.
(b) The smooth triangular floor of the primary palate begins to exhibit surface ridges (SR).

FIGURE 3

(a) Filopodial projections (FP) 0.1 μm in diameter frequently establish the initial bridges between medial (MNP) and lateral nasal prominences (LNP) in embryos of seven tail somites. These surface projections seem to anchor into the apposing nasal prominences by penetrating between epithelial cells (asterisk).
(b) TEM view of filopodial projections (FP) penetrating between cells (asterisk) of the surface epithelium (SE).

FIGURE 4

Sheets of flattened cells (FC) and filopodial projections (FP) actively fuse the area between the lateral nasal (LNP) and medial nasal prominences (MNP). Spheroidal particles (S) are very common in areas of intense surface activity.

FIGURE 5

(a) The fusion area of this 8-tail-somite embryo displays filopodial projections (FP) and secondary projections (SP) wrapped around each other. Note the sheets of flattened cells (FC) progressing toward the center of the fusion area between the lateral nasal (LNP) and medial nasal prominences (MNP).
(b) Intercellular junctions (IJ) are present at points of contact between secondary projections (SP) and surface epithelium (SE).

FIGURE 6

Secondary projections (SP) of this 9 tail somite embryo have 2–3 μm enlargements (E) that terminate in filopodial projections (FP). Two of these enlargements are twisted around each other. Note advancing edge of flattened cells (FC) and spheroidal particles (S) present in the fusion area between the lateral nasal (LNP) and medial nasal prominences (MNP).

FIGURE 7

At the conclusion of the fusion events the rows of epithelial microvilli (Mv) are reestablished in very symmetrical patterns, and surface activity ceases. Spheroidal particles (S) remain after fusion is completed.
It is probable that the epithelial events described in this report are critical for facial morphogenesis since we have recently reported preliminary observations that CL/Fr mouse embryos (genetically predisposed, in our colony, to spontaneous cleft lip with or without cleft palate) do not exhibit the sequence of epithelial involvement typical of the C57B1/6J embryos (Millicovsky & Johnston, 1980a). Similarly, we observed that the epithelium of embryos from A/J mice treated with the anticonvulsant phenytoin (producing 90% cleft lip, with or without cleft palate) exhibits a depressed ability to participate in bridging (Millicovsky & Johnston, 1980b). Our studies of genetically- and phenytoin-induced cleft lip and palate indicate that the primary palate epithelium of six tail somite embryos is already different from control, suggesting that the predisposing changes leading to facial clefts can be detected early in development. The epithelial cells in these two abnormal models remain relatively unchanged during the stages of primary palate development.

Our observations suggest that facial consolidation and the filling of grooves between prominences occurs by a temporal sequence of events which include the loss of microvilli by the epithelial cells followed by a period of morphologic quiescence in the depth of the grooves. Next, the presence of filamentous ridges integrated into the smooth surface of these grooves may be associated with the reduction in area of these depressions. As the result of morphogenetic rearrangements and differential rates of cell division (Minkoff & Kuntz, 1977), prominences grow in size and appose the neighbouring prominences. At this stage of development, filopodial and secondary projections span the distance between prominences. It is significant that filopodial projections are also present in the fusion of the free edges of the neural tube of chick (Bancroft & Bellairs, 1975; Santander & Cuadrado, 1976; Schoenwolf, 1979), hamster (Waterman, 1975, 1976), and mouse (Waterman, 1975, 1976). Short projections
were also observed in the contact of mouse primary palate at later stages of development (Gaare & Langman, 1977). In addition, obscured cellular boundaries and appearance of surface filamentous material are characteristic of secondary palate morphogenesis in mouse and man (Waterman, Ross & Meller, 1973; Waterman & Meller, 1974). As the consolidation of facial segments nears its end, sheets of cells seem to complete the filling of facial grooves, and the epithelial cells re-establish their microvilli. We have found that steps resembling those in the epithelium between the medial and lateral nasal prominences occur in all of the other grooves separating facial prominences during their stage of consolidation.

In conclusion, we believe that the consolidation of the embryonic face is an active phenomenon involving a sequence of events dependent on the participation of epithelial cells. We also suggest that the absence of any of the components of this sequence may be associated with conditions leading to facial clefts.

Based on existing evidence we must speculate that since similar sequences of cellular activity may exist in other animal models and in other anatomical areas, it is possible that we are describing a generalized process permitting the coalescence of embryonic primordia.

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