Int. J. Dev. Biol. 38: 553-556 (1994)

Short Contribution

Integrin regulatory switching in development: oscillation of $ß_5$ integrin mRNA expression during epithelial-mesenchymal interactions in tooth development

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ABSTRACT Integrin receptors for extracellular matrix molecules are thought to play important roles in morphogenesis since they mediate aspects of embryonic cell adhesion and migration. Using *in situ* hybridization, the mRNA expression pattern of the $ß_5$ integrin receptor subunit was examined during murine tooth development, a classical system for studying morphogenesis. In developing tooth, high-level expression of $ß_5$ integrin mRNA alternates from epithelium to mesenchyme and back to epithelium. Each switch in localization occurs within one day. These results demonstrate that an integrin mRNA can be precisely and rapidly up- and down-regulated over unexpectedly short time spans, and that expression can oscillate between adjacent, interacting epithelial and mesenchymal tissues during morphogenesis. This rapid modulation of mRNA expression suggests a potential regulatory role for the $ß_5$ integrin receptor in morphogenesis.

KEY WORDS: *integrin, tooth development, mouse embryos, in situ hybridization*

The integrins are a large, multifunctional family of heterodimeric receptors that bind to extracellular matrix molecules and certain cell surface proteins. Each integrin consists of a noncovalently associated α and ß subunit, with each subunit contributing to specificity (Akiyama *et al.*, 1990; Albelda and Buck, 1990; McDonald and Mecham, 1991; Hynes, 1992). Some integrins have been shown to be both regulated and required during embryogenesis. During vertebrate development, α_5 and $ß_1$ integrins exhibit relatively broad patterns of expression in a wide variety of tissues; expression of both subunits decreases as development is completed (Chen *et al.*, 1986; Muschler and Horwitz, 1991). The presence of α_5 integrin has been demonstrated to be required for development, since murine α_5 integrin-null embryos show severe posterior and extraembryonic mesodermal defects and die early in gestation (Yang *et al.*, 1993). In contrast to the broad expression patterns of α_5 and $ß_1$ integrins, the α_1, α_6, and α_8 integrins show more tissue-specific distribution and greater variation in expression levels during development (Bossy *et al.*, 1991; Bronner-Fraser *et al.*, 1992; Duband *et al.*, 1992). However, rapid changes in integrin expression levels or localization, such as that observed for certain growth factors and their receptors (e.g. see Lehnert and Akhurst, 1988; Partanen, 1990), have not been reported.

We demonstrate here that an integrin mRNA can undergo (a) rapid oscillation in expression levels within a single tissue, with transition periods of less than a single day, and (b) a coordinated switching of expression from one tissue component of an epithelial-mesenchymal interaction system to the other, and then back again. These novel conclusions are based on *in situ* hybridization of α_5 integrin mRNA during murine tooth morphogenesis. The $ß_5$ subunit associates with α_v to form the $\alpha_v ß_5$ integrin, which binds to vitronectin and probably fibronectin (Ramaswamy and Hemler, 1990; Smith *et al.*, 1990). $ß_5$ integrins have been reported in a wide variety of cultured cell types, except lymphoid cells, and are found prominently in epithelial tissues of human thymus, kidney, and skin (Pasqualini *et al.*, 1993 and references therein).

In this study, we have examined the expression pattern of $ß_5$ integrin during tooth development, a well-defined system for studying morphogenesis. *In situ* hybridization with digoxigenin-labeled, single-stranded antisense $ß_5$ RNA probe exhibited a specific hybridization pattern compared to control experiments with sense strand RNA in 13.5-day embryo maxilla and mandible sections (Fig. 1B and C). Hybridization and washing were performed at high stringency to avoid cross-hybridization with related integrin mRNAs, such as $ß_3$. In addition, two cDNA probes were used: MB5.4, a 0.9 kb clone that spans the 3' untranslated region and approximately 400 bp of translated mRNA, and MB5.Ac, which is 1.3 kb long and is derived from the translated region of the mRNA (Fig. 1A). The RNA probes derived from these clones revealed the same expression patterns and hybridization signal intensities (data not shown).

The developing tooth starts as an invaginating epithelial bud surrounded by condensed mesenchyme, undergoing morphogenetic change regulated by reciprocal epithelial-mesenchymal interactions (Ruch, 1984; Lumsden, 1988). During early developmental stages, $ß_5$ mRNA exhibited dramatic changes of expres-

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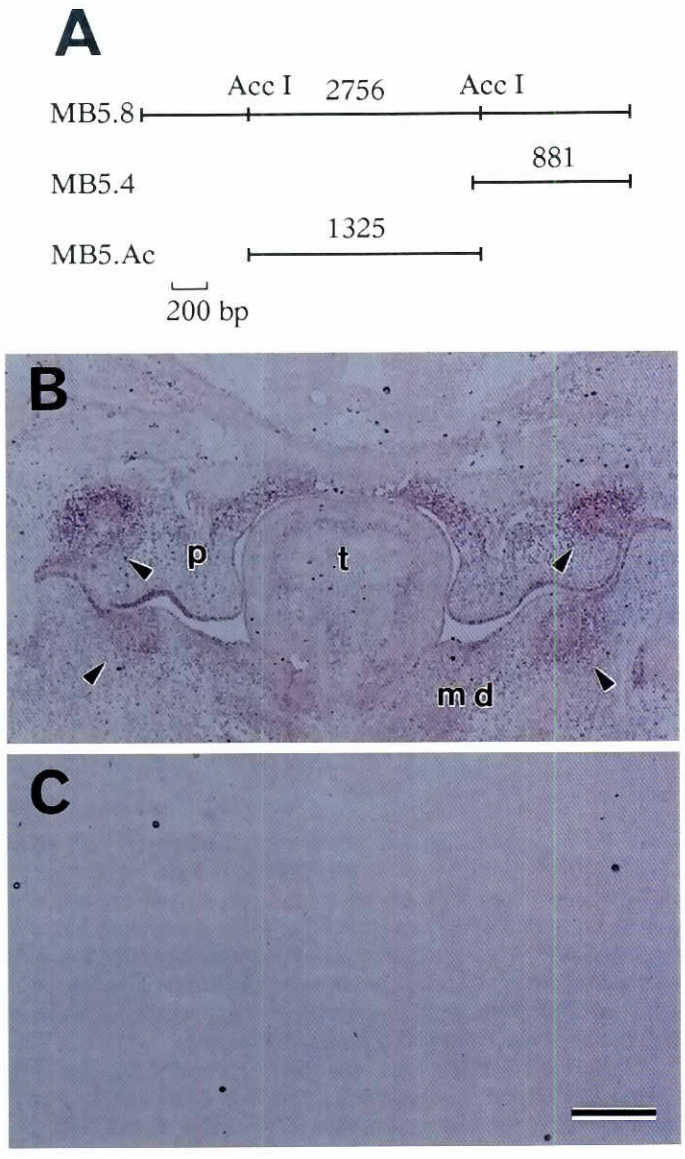

Fig. 1. Cloned mouse cDNA fragments of β_5 integrin and expression pattern in the craniofacial region. (A) *Schematic representation of the mouse β_5 cDNA sequences used for in situ hybridization. The 0.9 kb and 1.3 kb fragments of mouse β_5 integrin subunit were subcloned into pBluescript SK- (Stratagene), and termed MB5.4 and MB5.Ac, respectively.* **(B)** *Expression of β_5 integrin in the craniofacial region in a 13.5-day embryo. A frontal section hybridized to β_5 antisense probe.* **(C)** *A section adjacent to* **(B)** *hybridized to β_5 sense probe. Tongue (t), mandible (md), palate (p). Arrowheads point to tooth germs at bud stage. Scale bar, 400 μm.*

sion. In 12.5-day embryos, during dental lamina formation of the first molar, diffuse hybridization of β_5 transcripts was present in both the epithelial and dental mesenchyme; hybridization generally appeared slightly higher in epithelium than mesenchyme (Fig. 2A). As bud formation of the molar enamel epithelium proceeded in 13.5-day embryos, β_5 hybridization signals became abundant in the dental mesenchyme, but remained low in epithelium (Fig. 2B). In the cap stage of 14.5-day embryos, when the epithelial enamel organ begins to surround the mesenchymal cells of the dental papilla, intense β_5 mRNA expression was observed in the enamel epithelium, especially in the lingual aspect of the inner enamel epithelium (Fig. 2C). However, expression of β_5 was low in mesenchymal cells, as well as in epithelial cells of the stellate reticulum and outer enamel epithelium. This same expression pattern was seen in the mandibular molar tooth germ (data not shown). By the early bell stage of 15.5-day embryos, β_5 mRNA levels were reduced but still largely confined to the inner enamel epithelium (Fig. 2D). At the late bell stage of 18.5-day embryos, a similar pattern of β_5 expression was observed (data not shown).

To quantitate the differences in β_5 mRNA expression between mesenchyme and epithelium, the intensity of hybridization signals was measured using video densitometry. This quantitation was used to calculate the ratio of β_5 expression between epithelial tissue and its adjacent mesenchyme at different stages in tooth development (Fig. 3). In 12.5-day embryos, the dental lamina stage, β_5 hybridization was higher in epithelium than in mesenchyme. One day later, at the bud stage, the ratio of labeling was reversed, so that mesenchyme hybridization was higher than epithelium. In the cap stage of 14.5-day embryos, labeling ratios were reversed again with hybridization to epithelium two- to three-fold higher than to mesenchyme. This difference was especially pronounced in the lingual aspect of the molar; however, at other stages in development, no differences were observed between the lingual and buccal aspects of the tooth. In 15.5-day embryos, the level of β_5 mRNA had decreased, but remained higher in epithelial tissue than in mesenchyme.

The alternating levels of β_5 mRNA expression observed within embryonic dental epithelium and mesenchyme, and the associated coordinated oscillation of β_5 mRNA localization between these tissues, accompany a complex set of reciprocal epithelial-mesenchymal interactions during odontogenesis (Ruch, 1984; Lumsden, 1988). At the bud stage, very high levels of β_5 expression appear in mesenchyme; the peak of expression occurs at the time of early differentiation of mesenchymal cells to odontoblast precursors. One day later, the dramatic switch to high levels of β_5 mRNA expression in the inner enamel epithelium of the cap stage corresponds temporally to the time of early differentiation of ameloblast precursors.

These switches in β_5 expression can be compared to the changes observed for the extracellular matrix proteins, tenascin and syndecan (Thesleff *et al.*, 1990; Vainio *et al.*, 1991) during the transition from bud through bell stages of tooth development. The changes in β_5 expression are more complex than those for tenascin and syndecan, involving an unprecedented seesaw or oscillatory pattern. Moreover, the distribution of a putative ligand for $\alpha_v\beta_5$, fibronectin, is relatively uniform throughout the loose jaw mesenchyme and in basement membrane, and does not show marked stage-specific changes (Thesleff *et al.*, 1979); the distribution of vitronectin, the other ligand for $\alpha_v\beta_5$, is unknown.

In comparison, the expression patterns for certain growth factors and their receptors more closely resemble the dynamics of β_5 mRNA. For example, an assay of EGF bound to its receptors shows an initial elevation in epithelium, then a switch to mesenchyme at the bell stage (Partanen, 1990). TGF-β1 expression during embryonic tooth morphogenesis also exhibits a seesaw regulation (Lehnert and Akhurst, 1988; Vaahtokari *et al.*, 1991). Furthermore, the expression of bone morphogenetic protein 4 (BMP-4), a member of TGF-β superfamily, shifts from dental epithelium to mesenchyme and leads to mesenchymal induction, implicated in the regulation of epithelial-mesenchymal interactions during tooth

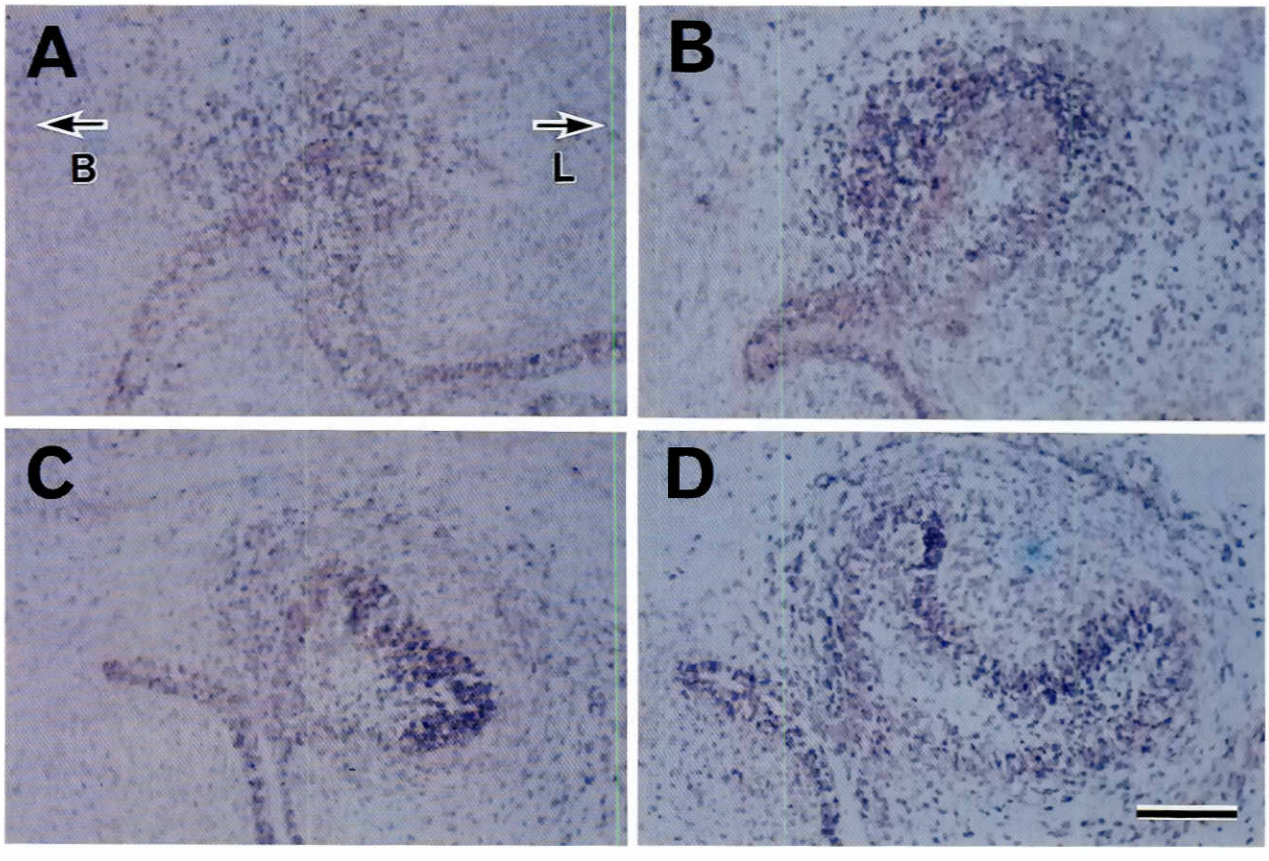

Fig. 2. Expression of $ß_5$ integrin of maxillary first molar tooth germs at various developmental stages. *Photomicrographs are frontal sections hybridized to $ß_5$ antisense probe.* **(A)** *Dental lamina in a 12.5-day embryo.* **(B)** *Bud stage in a 13.5-day embryo.* **(C)** *Cap stage in a 14.5-day embryo.* **(D)** *Early bell stage in a 15.5-day embryo. Left and right arrows represent buccal and lingual sides, respectively. Scale bar, 100 μm.*

development (Vainio *et al.*, 1993). Whether the apparently even more complex $ß_5$ mRNA expression pattern we observe is controlled by an independent program or is due to a particularly sensitive reactivity to changes in growth factors or their receptors remains to be determined. It should be emphasized, however, that no pattern of growth factor or receptor expression described to date matches the $ß_5$ mRNA expression program. In addition, whether expression of the $ß_5$ protein mirrors mRNA expression cannot be determined at present due to the unavailability of anti-mouse $ß_5$ antibodies.

The seesaw pattern of expression of $ß_5$ suggests that regulation of this integrin subunit, rather than of one of its ligands, fibronectin, may control cell adhesion during tooth development; in addition, such precise and rapid switching may represent additional regulatory functions for this particular integrin. Regardless of such additional functions, the capacity for rapid regulation of tissue-specific expression of adhesion receptors, as well as of their extracellular matrix ligands, provides an additional layer of control and flexibility of regulation for mediating morphogenesis.

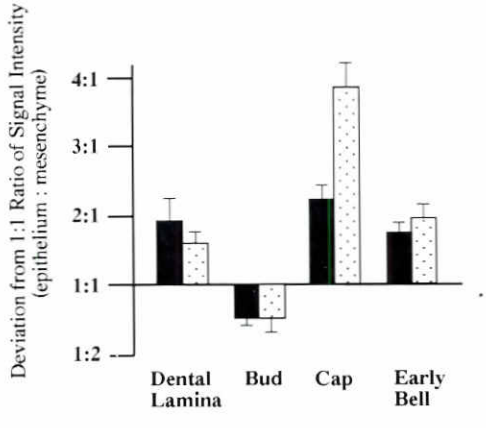

Fig. 3. Semi-quantitation of $ß_5$ transcripts in the tooth germ at different embryonic stages. *Values represent ratios of signal intensities measured in 100 μm² zones of tooth epithelium and the opposite mesenchyme located an equal distance across the epithelial-mesenchymal junction. Data were obtained separately from lingual (solid bars) and buccal (stippled bars) aspects of each tooth germ. Bars represent the average± SEM (standard error of the mean) for fifteen values from three different sections for each stage.*

Experimental Procedures

Embryos and Tissues

FVB/N inbred mice were used. The day of the vaginal plug was designated as day 0. Frozen sections for *in situ* hybridization were prepared from heads of 12.5-, 13.5-, 14.5-, 15.5-, and 18.5-day embryos as de-

scribed (Hogan *et al.*, 1986) except that 6 μm sections were mounted on 3-aminopropyltriethoxysilane-coated slides.

In situ hybridization

Prehybridization treatment was performed essentially as described (Hogan *et al.*, 1986) with the following modifications: sections were digested by 15 μg/ml proteinase K for 10 min at room temperature. After fixation in 4% paraformaldehyde, sections were treated with 0.2 M HCl for 10 min at room temperature to inactivate endogenous alkaline phosphatase. Mouse β_5 integrin subunit cDNA fragments of 0.9 kb and 1.3 kb (K.E. Brown, S. Yamada and K.M. Yamada, in preparation) were subcloned into pBluescript SK-(Stratagene), and termed MB5.4 and MB5.Ac, respectively (Fig. 1A). Digoxigenin UTP-labeled single-stranded sense and antisense RNA probes were prepared as described by the manufacturer (Genius System, Boehringer Mannheim), then reduced to an average size of 150 bases by limited alkaline hydrolysis (Cox *et al.*, 1984), and used for hybridization at a final concentration of 0.5 μg/ml. Hybridization and high stringency washing conditions were essentially as described (Cox *et al.*, 1984; Hogan *et al.*, 1986; Holland *et al.*, 1987). Immunohistochemical staining with anti-digoxigenin conjugated to alkaline phosphatase followed the manufacturer's protocol (Boehringer Mannheim). Stained sections were washed in 10 mM Tris-Cl, pH 7.6/1 mM EDTA, rinsed in distilled water and mounted.

Quantitation of hybridization

Densitometry of 100 μm^2 zones of tooth epithelium and corresponding mesenchyme located an equal distance across the epithelial-mesenchymal junction was performed using the Measure Area Brightness Function of Image-1 video image analysis software (Universal Imaging Corp., West Chester, PA, USA) using an inverted microscope (Model ICM 405, Carl Zeiss) equipped with a video camera (Newvicon Model C2400, Hamamatsu Photonics). Background density was subtracted prior to the calculations of the ratios of epithelial to mesenchymal values.

Acknowledgments

We thank Dr. Shintaro Nomura, Osaka University School of Medicine, for excellent technical advice concerning in situ hybridization.

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Accepted for publication: May 1994