Remodeling of Desmosomal and Hemidesmosomal Adhesion Systems During Early Morphogenesis of Mouse Pelage Hair Follicles

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Early hair follicle morphogenesis proceeds with the formation of a hair placode, the downgrowth of the hair plug into the mesenchyme, and the development of an elongated hair follicle–processes that involve a series of exchange of messages between epithelium and mesenchyme. Regulation of epithelial cell adhesion during hair morphogenesis has been demonstrated in terms of the changing expression patterns of E- and P-cadherins. In this study, distribution patterns of several major components of desmosomes and hemidesmosomes, which are the most prominent cell adhesion systems in epidermal tissues, were examined during early morphogenesis of mouse pelage hair follicles. We found that both desmosomal and hemidesmosomal adhesion systems became downregulated in hair placodes and were much reduced or almost lost in hair plugs, which persisted in the region containing hair matrix. Downregulation of the adhesion systems in hair plugs was confirmed by electron microscopy. Similar distribution patterns of these molecules were obtained in the developing follicles in cultured skin. It may be that epidermal cells at the initial stages of hair development respond to the first mesenchymal message by grossly changing their cell adhesion systems and that the resultant changes in cell adhesivity underlie early hair follicle morphogenesis. ***Key words: desmosome/hair follicle/hemidesmosome/morphogenesis. J Invest Dermatol 114:171–177, 2000***

Hair follicle morphogenesis proceeds through a series of epithelial–mesenchymal interactions (Hardy, 1992; Philpott and Paus, 1998). Responding to the first mesenchymal message, the basal cells in the epidermis become thick to form a hair placode (stage 1) and grow down into mesenchyme to form a hair plug (stage 2). The epidermal message from the elongating follicle then initiates the formation of mesenchymal dermal papilla, which becomes surrounded by the bulbous base of the hair follicle (stage 3). Finally, the second mesenchymal message from the dermal papilla stimulates proliferation of the adjacent cells of the hair follicle, known as the hair matrix, and their differentiation into several types of epithelial cells of the hair follicle. Although various signaling molecules including bone morphogenetic proteins, Sonic hedgehog (Shh), and β-catenin have been implicated in hair follicle morphogenesis (Bitgood and McMahon, 1995; Zhou *et al*, 1995; Kratochwil *et al*, 1996; Oro *et al*, 1997; Gat *et al*, 1998; Oro and Scott, 1998; Philpott and Paus, 1998), how epidermal and mesenchymal cells respond to the message and what cellular mechanisms underlie hair follicle morphogenesis are poorly understood.

Adhesion of cells to each other and to the basal lamina, which primarily depends on cadherins and integrins, and its regulation play crucial parts in morphogenesis, sorting-out, and segregation of tissues (Takeichi, 1995; Gumbiner, 1996; Rubin *et al*, 1996; Steinberg, 1996). It has been reported that expression patterns of cadherin cell adhesion molecules change during early hair follicle morphogenesis; i.e., E-cadherin expression becomes reduced and P-cadherin expression is enhanced in the hair placode and matrix of developing pelage follicle (Fujita *et al*, 1992; Kaplan and Holbrook, 1994; Hardy and Vielkind, 1996) and cadherin-11 mRNA becomes to be expressed strongly in dermal papilla cells (Simonneau *et al*, 1995). E- and P-cadherins have been shown to be required not only for proper epithelial morphogenesis but for condensation of mesenchymal cells of developing vibrissa follicles (Hirai *et al*, 1989). Furthermore, expression patterns of neural cell adhesion molecule of the immunoglobulin superfamily and several subunits of integrin molecules such as the integrin α9 have also been demonstrated to be dynamically regulated during early hair follicle morphogenesis (Vielkind *et al*, 1995; Wang *et al*, 1995; Müller-Röver *et al*, 1996). It has been postulated that the differential expression of cell adhesion molecules, especially of E- and P-cadherins, may be an epidermal response to the first mesenchymal message and cause the sorting out of cells of hair placodes from the neighboring epidermal cells (Hardy and Vielkind, 1996; Müller-Röver and Paus, 1998).

In addition to E- and P-cadherins, certain members of the cadherin family, called desmocollins and desmogleins, also play crucial parts in intercellular adhesion in epidermal tissues. Those two members of cadherins together with the associated cytoplasmic molecules such as plakoglobin and desmoplakins are assembled into the desmosome, which actually is the most prominent intercellular adhesion system present in epidermal tissues (reviewed in Koch and Franke, 1994; Garrod *et al*, 1996). Cells in epidermal tissues also adhere stably to the basal lamina by hemidesmosomes, into which are assembled laminin-5 (previously called kalinin/nicein), its

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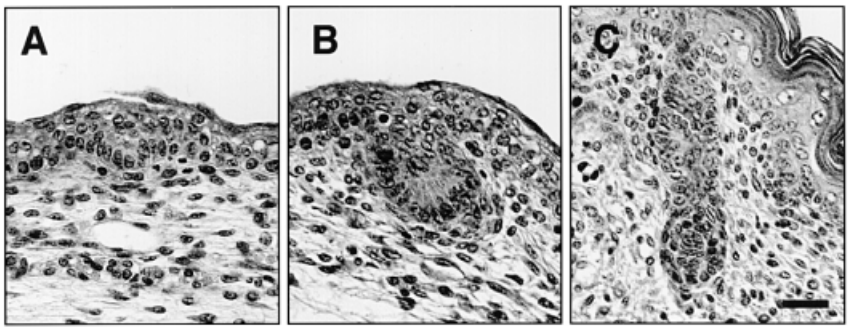

Figure 1. Histologic appearances of early epithelial morphogenesis in pelage hair follicles. Shown are hair placode (*A*), hair plug (*B*), and elongating hair follicle (*C*). Hematoxylin and eosin stain. *Scale bar*: 25 μm.

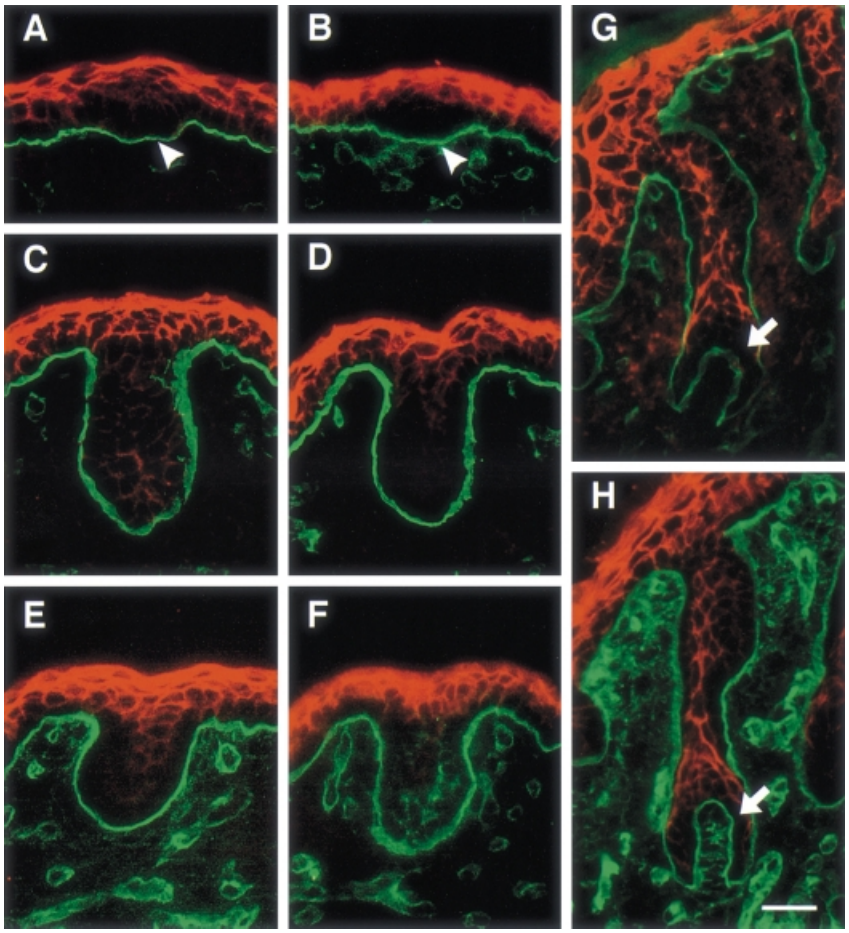

Figure 2. Immunofluorescence microscopy of desmosomal molecules in developing hair follicles. Note the specific loss of staining in hair placodes for desmoplakins and desmogleins (*arrowheads*; red in *A* and *B*, respectively) and in hair plugs for desmoplakins (*C*), plakoglobin (*D*), desmocollins (*E*), and desmogleins (*F*). Elongating hair follicle were strongly stained with antibodies to plakoglobin (*G*) and desmocollins (*H*). Note, however, the specific loss of their stainings at the hair matrix (*arrows*). Tissue sections were double-stained with antibodies to the basal lamina components (green; laminin-1 in *A–D*, *F*, and *G*, and heparan sulfate proteoglycan in *E* and *H*). *Scale bar*: 25 μm.

cellular receptor (α6β4 integrin), and another transmembrane protein [the collagenous, 180 kDa bullous pemphigoid antigen (BPAG) 2 (or collagen XVII)], and cytoplasmic proteins including HD1 (also called plectin) and 230 kDa BPAG1 (for reviews see Green and Jones, 1996; Burgeson and Christiano, 1997). It has also been reported that expression patterns of desmosomal molecules are spatiotemporally regulated in developing hair follicle (Nuber *et al*, 1996; Chidgey *et al*, 1997; Kurzen *et al*, 1998). In addition, desmosomal and hemidesmosomal adhesion systems are down-regulated in the hair matrix region of hair follicle and in basal cell carcinomas resulting from abnormal growth of developing hair follicles (McNutt, 1976; Kumakiri and Hashimoto, 1978; Luzi *et al*, 1987; Savoia *et al*, 1993; Fairley *et al*, 1995; Kore-eda *et al*, 1998). There is only limited information available, however, concerning regulation of desmosomal and hemidesmosomal adhesion systems during early hair follicle morphogenesis.

Here we report, based on immunofluorescence microscopy of their several major components and on ultrastructural observations, that desmosomal and hemidesmosomal adhesion systems become downregulated in hair placodes and are much reduced or almost lost in hair plugs, which condition persists in the region containing the hair matrix. Possible cellular mechanisms underlying early hair follicle morphogenesis are discussed in terms of gross remodeling of cell adhesion systems.

MATERIALS AND METHODS

Mouse embryos Embryos were obtained from DDY strain mice (Nihon SLC, Hamamatsu, Japan). The discovery of the vaginal plug was designated as embryonic day 0 (E0). The back skin was dissected from embryos at E13 through E17.

Antibodies Primary antibodies used were as follows: mouse monoclonal IgG antibodies to desmoplakins I/II (a mixture of clones DP2.15, 2.17, and 2.20; Cowin *et al*, 1985) and plakoglobin (clone PG5.1; Cowin *et al*, 1986), which were obtained from Progen (Heidelberg, Germany); rabbit antiserum recognizing all isoforms of desmocollins and mouse monoclonal IgM antibody recognizing desmogleins 1 and 2 (clone 33–3D), which were gifts

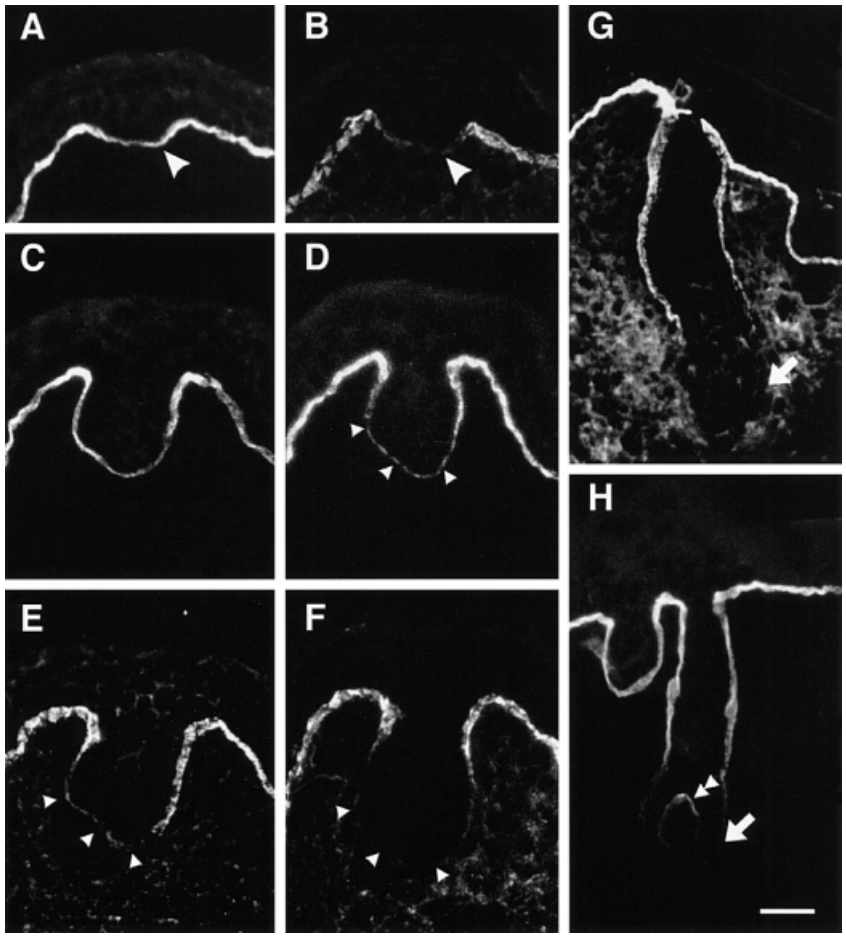

Figure 3. Immunofluorescence microscopy of hemidesmosomal molecules in developing hair follicles. Epidermal–dermal junction was clearly stained for integrin β4 (*A*) but was only weakly stained for HD1 (*B*) in hair placodes (*arrowheads*). In hair plugs the laminin-5 staining (*C*) was slightly reduced, whereas significant reduction of the integrin β4 staining (*D*) and near loss of staining for HD1 (*E*) and BPAG1 (*F*) were observed at the distal region (*small arrowheads*). Strong stainings for HD1 (*G*) and laminin-5 (*H*) were observed in elongating hair follicles except in the hair bulb region (*arrows*). Note, however, some staining for laminin-5 at the basal lamina region adjacent to the dermal papilla (*double arrowheads*). *Scale bar*: 25 μm.

from Dr. Garrod; mouse monoclonal IgG antibodies to HD1 (clone HD-121; Hieda *et al*, 1992) and BPAG1 (clone 1E5; Owaribe *et al*, 1991), gifts from Dr. Owaribe; rat IgG antibody to integrin β4 subunit (clone 346–11A; Kennel *et al*, 1989), gift from Dr. Kennel; rabbit polyclonal antibody to laminin-5 (gift from Dr. Burgeson; Marinkovich *et al*, 1992); rabbit polyclonal antibody raised against EHS laminin (Collaborative Research, Bedford, MA); and rat monoclonal IgG antibody to the core protein of heparan sulfate proteoglycan (Seikagaku Kogyo, Tokyo, Japan). Secondary antibodies used were rhodamine-conjugated goat anti-mouse IgG and fluorescein isothiocyanate- or rhodamine-conjugated goat anti-rat IgG (Chemicon, Temewla, CA), and rhodamine-conjugated goat anti-mouse IgM and fluorescein isothiocyanate- or rhodamine-conjugated goat anti-rabbit IgG (Cappel, Durham, NC).

Immunofluorescence microscopy Freshly prepared skin fragments and cultured explants were embedded in OCT compounds and frozen in liquid nitrogen. Tissue sections of 6 μm thickness were air-dried and fixed in methanol at –20°C for 10 min or in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C for 10 min. The sections were treated with 0.5% Triton X-100 in PBS for 10 min and subsequently with 1% bovine serum albumin. Then they were incubated with primary antibodies for 1 h, washed in PBS, and incubated with secondary antibodies for 1 h. After a wash with PBS, the sections were mounted with PBS containing 50% glycerol and 0.1% p-phenylenediamine and examined under an Olympus BX-50 epifluorescence microscope. Photographs were processed on a Macintosh computer using Adobe Photoshop software and were printed with Pictrography (Fujifilm, Tokyo, Japan).

Histochemistry and electron microscopy For histologic examination, skin fragments and cultured explants were routinely fixed in Bouin's solution, dehydrated, and embedded in paraffin. Sections were processed for staining with hematoxylin and eosin.

Freshly prepared skin fragments for electron microscopy were fixed in 0.1 M cacodylate buffer (pH 7.3) containing 2.5% glutaraldehyde and 2% paraforaldehyde and were then immersed in 1% osmium tetraoxide in the same buffer. Dehydration in a gradient series of ethanol and propylene oxide was followed by embedding in PolyBed 812 (Polysciences, Warrington, PA). Ultrathin sections were cut on a Reichert ultramicrotome (Leica, Wien, Austria), stained with uranyl acetate and lead citrate, and observed with a JEOL 1200EX electron microscope.

Organ culture The back skin was taken from E13 embryos in Hanks' balanced salt solution and placed on an ultrathin Millipore filter (JHWP, 0.45 μm pore size) that covered the hole of a plastic plate. 199 medium containing 1% fetal bovine serum was applied as a hanging drop to the filter and the skin explants were cultured in organ tissue culture dishes (Falcon no. 3037; Becton Dickinson, Franklin Lakes, NJ) at 37°C and 5% CO_2. The medium was changed every 24 h.

RESULTS

Using immunofluorescence microscopy we first examined developmental changes in the desmosomal and hemidesmosomal adhesion systems in hair placodes of epidermal thickenings (Hardy's stage 1, **Fig 1*A***), the downgrowing plugs (stages 2–3a, **Fig 1*B***) and in the elongating follicles (stages 3c-4), when the epithelium has surrounded the condensed dermal papilla at its base (**Fig 1*C***).

For examination of the desmosomal adhesion system in developing hair follicles, we used antibodies to the four major molecules, that is, desmogleins, desmocollins, plakoglobin, and desmoplakins. Double stainings with antibodies to the basal lamina components, either EHS laminin or heparan sulfate proteoglycan, were performed to outline the developing epithelial tissues. All of the desmosomal molecules were expressed strongly in the upper epidermal cells and moderately in the basal epidermal cells (**Fig 2**). We noted, however, that staining for desmoplakins (**Fig 2*A***) and desmogleins (**Fig 2*B***) was significantly reduced in hair placodes. Similar results were obtained for plakoglobin and desmocollins (data not shown). The reduced intensity or almost complete loss of staining for these molecules became quite evident in the hair plugs (**Fig 2*C–F***). In the subsequent elongating follicles, staining for

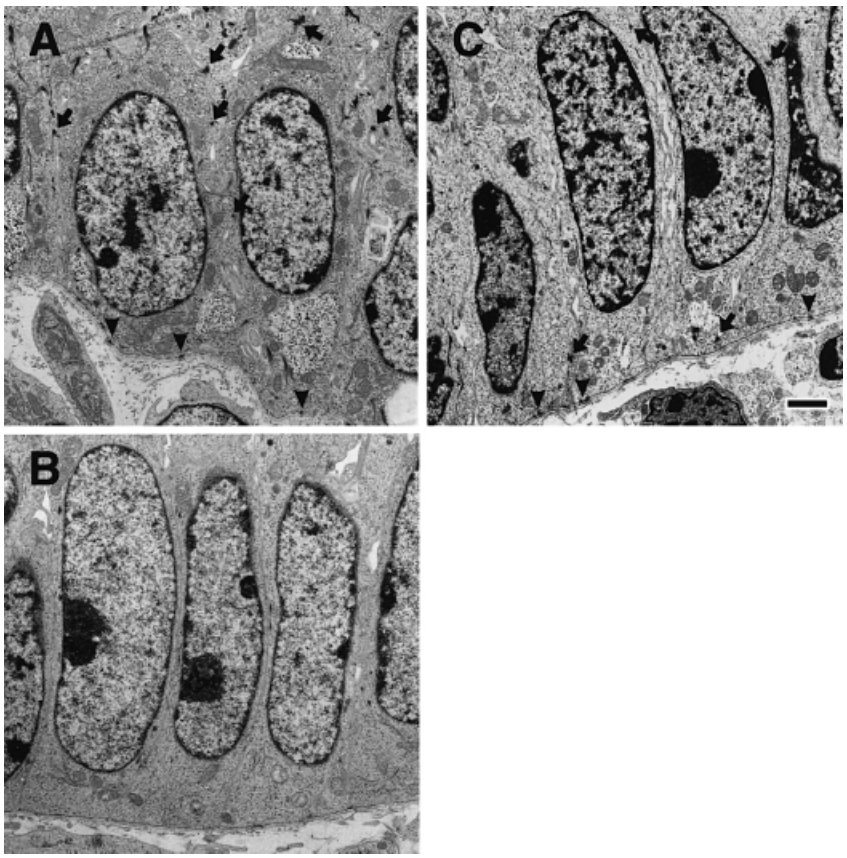

Figure 4. Ultrastructural differences between interfollicular epidermis and developing hair follicles. Shown are basal cells of the epidermis at E15 (*A*), peripheral cells of hair plug at E15 (*B*) and of the middle portion of elongating hair follicle at E17 (*C*). Definite structures of desmosomes and hemidesmosomes are indicated by *arrows* and *arrowheads*, respectively. Note loss of the adhesive structure in the hair plug. *Scale bar*: 1 μm.

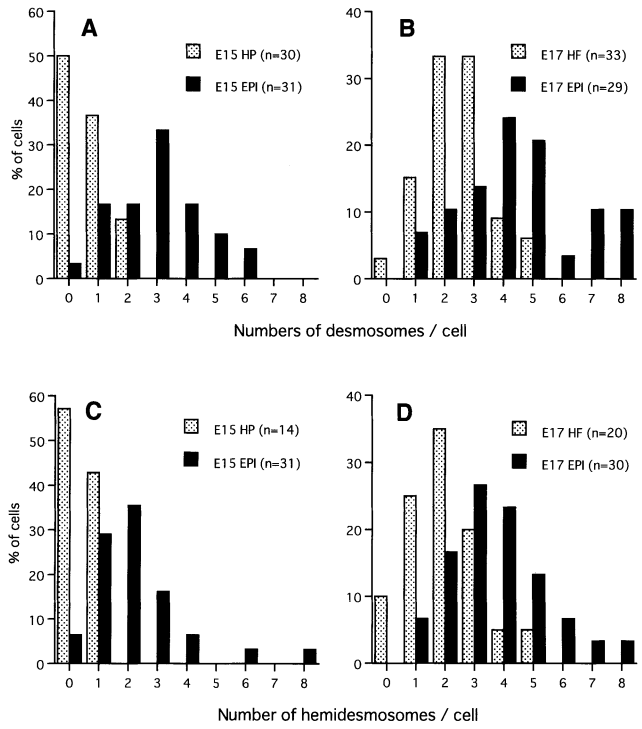

Figure 5. Decreases in the number of desmosomes and hemidesmosomes in the hair plug. Two representative electron micrographs of E15 and E17 skin were examined to count desmosomes and hemidesmosomes in cells of hair plug (E15 HP), of the middle portion of elongating hair follicle (E17 HF), and of interfollicular epidermal basal layer (E15 EPI and E17 EPI). In E15 HP and E17 HF, both the peripheral and inner cells were examined for desmosomes, and the peripheral cells only, for hemidesmosomes. The vertical axis presents the percentage of cells in the total population examined (indicated by n) having certain numbers of the junctions.

plakoglobin (**Fig 2*G***) and desmocollins (**Fig 2*H***) was detectable except in the hair matrix region. Similar results were obtained for desmoplakins and desmogleins (data not shown).

Hemidesmosomal adhesion systems in developing hair follicles were examined by using antibodies to laminin-5, integrin β4, and the cytoplasmic proteins HD1 and BPAG1. All the antibodies specifically stained the epidermal–dermal junction of skin (**Fig 3**). Whereas integrin β4 (**Fig 3*A***) and laminin-5 (data not shown) were also localized at the junctional region of hair placodes, a significant decrease in the staining intensity was noticed for HD1 (**Fig 3*B***) and BPAG1 (data not shown). In hair plugs, however, a slight reduction of the staining intensity at the distal region was detected for laminin-5 (**Fig 3*C***); and the reduction was more obvious for integrin β4 (**Fig 3*D***). Furthermore, HD1 (**Fig 3*E***) and BPAG1 (**Fig 3*F***) were almost lost. All of the hemidesmosomal molecules examined were found to be localized at the epithelial–mesenchymal boundary of the elongating follicles except in the hair bulb region (**Fig 3*G***, ***H***). Some staining for laminin-5 was reproducibly observed in the basal lamina region adjacent to the dermal papilla.

Next, in order to confirm the immunostaining observations, we examined changes in the occurrence of desmosomal and hemidesmosomal structures in the developing hair follicles by electron microscopy. Desmosomes and hemidesmosomes, which appear electron-dense cytoplasmic plaques, and keratin tonofilaments were easily observed in the interfollicular epidermis, and there were numerous interdigitations between the cells (**Fig 4*A***). In the hair plug, however, cell surfaces were smooth with less interdigitations and definite structures of desmosomes and hemidesmosomes and keratin filaments were rarely detected (**Fig 4*B***), although rudimentary intercellular junctions were occasionally present. In elongating hair follicles, desmosomes and hemidesmosomes reappeared in addition to rudimentary junctions (**Fig 4*C***).

The ultrastructural observations were supported by statistical analyses of two representative electron micrographs (**Fig 5**). The mean number of desmosomes per cell was 0.6 (±0.7 SD) in hair plug of E15 skin (E15 HP), whereas that in the interfollicular

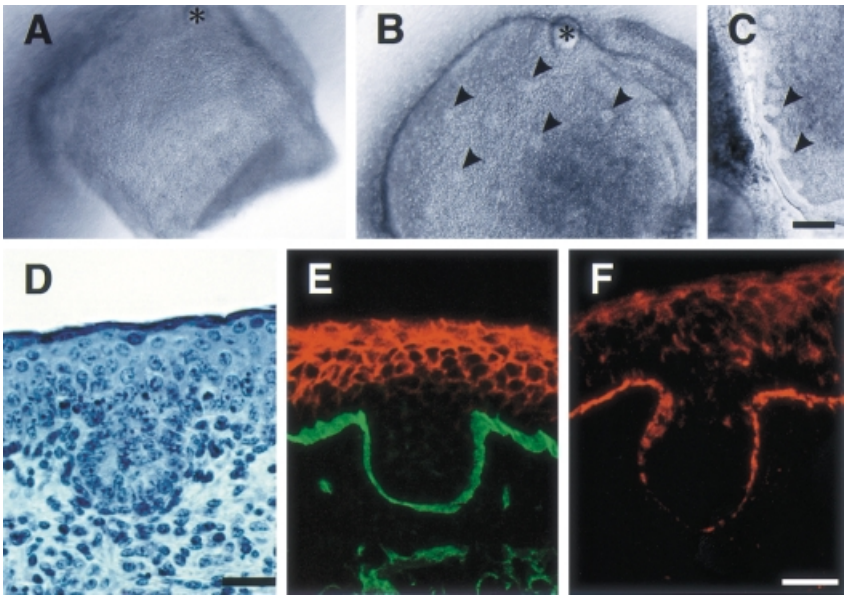

Figure 6. Development of hair follicles in culture and distribution of desmosomal and hemidesmosomal molecules. Light microscopic appearances of skin fragment with no hair germs from an E13 embryo (*A*) and of the fragment cultured for 2 d (*B*, *C*). Note several light spots seen at regular intervals and epidermal buds in the cultured skin explants (*arrowheads*). Asterisks indicate mammary rudiments. (*D*) Histologic section of the cultured skin explants showing epidermal downgrowth similar to that by hair plugs. Immunofluorescence microscopy showing downregulation of plakoglobin (*E*) and HD1 (*F*) in the epidermal downgrowth. *Scale bar*s: 200 μm (*A–C*); 25 μm (*D–F*).

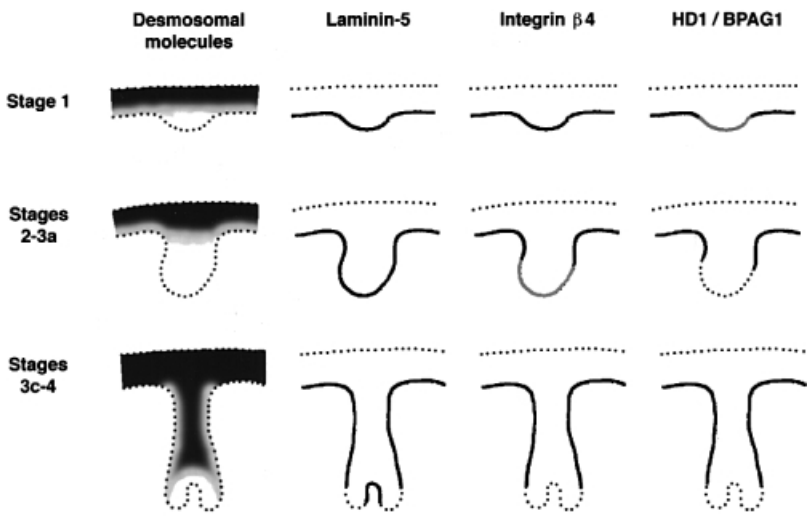

Figure 7. Schematic illustration of the expression changes of desmosomal and hemidesmosomal molecules examined during early hair follicle morphogenesis. Different levels of immunostaining for the molecules are represented by different levels of gray.

epidermis (E15 EPI) was 3.0 (±1.5). At E17, these values were 2.5 (±1.1) and 4.4 (±2.0), for the middle portion of elongating hair follicle (E17 HF) and E17 EPI, respectively. Hemidesmosomes were 0.4 (±0.5) for E15 HP, whereas these in E15 EPI, E17 HF, and in E17 EPI were 2.2 (±1.6), 2.0 (±1.3) and 3.7 (±1.7), respectively.

Downregulation of desmosomal and hemidesmosomal adhesion systems in hair plugs *in vivo* was found to be reproduced *in vitro*. For this purpose, E13 skin fragments with no hair germs (**Fig 6*A***) were cultured on a membrane filter. Two days thereafter, some light spots and epidermal buds could be observed in the skin explants (**Fig 6*B***, ***C***). Histologic observation (**Fig 6*D***) also revealed that epidermal downgrowth just like that by hair plugs occurred and that, as previously reported (Kashiwagi *et al*, 1997), the epidermis in the explants became thicker than the intact 15 d epidermis. Expression of desmosomal and hemidesmosomal molecules was reduced in the epidermal downgrowth as seen in the intact hair plugs (**Fig 6*E***, ***F***).

DISCUSSION

Previous studies have demonstrated that early hair follicle morphogenesis accompanies dynamic regulation of expression patterns of cell adhesion molecules including E- and P-cadherins, neural cell adhesion molecules, and integrins (Fujita *et al*, 1992; Kaplan and Holbrook, 1994; Vielkind *et al*, 1995; Wang *et al*, 1995; Hardy and Vielkind, 1996; Müller-Röver *et al*, 1996). As for desmosomal cadherins, it was reported that desmoglein 2 was strongly expressed in hair placodes of fetal human scalp skin (Kurzen *et al*, 1998) and that desmocollin 3 was upregulated in the stage 3a hair follicle of the mouse (Chidgey *et al*, 1997). We showed in this study, however, that not only desmosomes but also hemidesmosomes, which are the most prominent adhesion systems present in epidermal tissues, were rather dynamically remodeled during the early morphogenesis of mouse pelage hair follicles (**Fig 7**). This was confirmed by repetitive and careful examination and, as far as we examined, no interindividual and interfollicular fluctuations were observed in the observed immunostaining patterns. The remodeling was highlighted by the specific downregulation of both adhesion systems in hair placodes and, more evidently, in hair plugs. It is most likely that the early follicle morphogenesis involves gross remodeling of epithelial cell adhesion systems, although patterns of the remodeling may be different between species and/or between regions of the body.

Hair follicle morphogenesis at the earliest stages, during which epidermal cells form the thickened hair placode and the downgrowth of the hair plug, requires that the epidermal cells respond to the first mesenchymal message. Not only differential expression of E- and P-cadherin (Hardy and Vielkind, 1996), but also gross remodeling of cell adhesion systems including downregulation of desmosomal and hemidesmosomal adhesion systems may be the epidermal responses. How is the remodeling of cell adhesion systems related to follicle morphogenesis? It has been well established that the cadherin family of molecules plays primary roles in intercellular adhesion and that two populations of cells expressing different types of cadherins or expressing different amounts of cadherins, even the same type of cadherins, sort out or segregate (Steinberg and Takeichi, 1994; Takeichi, 1995; Steinberg, 1996). On the basis of differential expression patterns of E- and P-cadherins, Hardy and Vielkind (1996) have suggested that cells of hair placodes undergo changes in intercellular adhesivity and become sorted out from the neighboring interfollicular epidermal cells. This is supported and strengthened by the present observation showing that the desmosomal adhesion system, in which the desmosomal cadherins of desmocollins and desmogleins function, is downregulated in hair placodes. As desmosomes are the most prominent adhesive structures present in the epidermal tissues, the data suggest that intercellular adhesivity between cells of hair placodes becomes weaker than that between the neighboring epidermal cells.

Knowledge of the remodeling of desmosomal and hemidesmosomal adhesion systems may also provide insight into the cellular mechanisms of the subsequent downgrowth of hair plugs into the dermal mesenchyme and the surrounding of the dermal papilla by the elongating follicle. In the epidermis, the basal cells detach from the basal lamina to stratify, which may involve downregulation of integrins including the $\alpha 6\beta 4$ integrin (Tannenbaum *et al*, 1996). In addition, transformation by ras of MDCK cells, which normally grow as a monolayer sheet, induces downregulation of the integrin $\beta 1$ expression to result in multilayering of the cells (Schoenberger *et al*, 1991, 1994). It is therefore likely that, also in the early developing hair follicles, the epidermal cells are promoted to detach from the basal lamina because of downregulation of integrin $\beta 4$ and associated cytoplasmic proteins, with the resultant loss of the hemidesmosomes that serve as the major structure for adhesion to the basal lamina. It should be noted here that whereas desmosomes become more abundant as epidermal cells move up to stratify, they become downregulated in cells of the hair plug growing down to the dermal mesenchyme, as shown in this study. Downgrowth of the epidermal cells to form the hair plug may be driven by segregation or exclusion of the detaching and proliferating cell population with weaker intercellular adhesivity from the overlying, more tightly adhering epidermal cells.

Desmosomal and hemidesmosomal adhesion systems were shown to subsequently recover in the developing hair follicles. Elongation of the cylinder-like hair follicles might result from rearrangements and alignments along the basal lamina of cells undergoing restoration of their cell adhesion systems. On the other hand, this study and previously reports (McNutt, 1976; Kumakiri and Hashimoto, 1978; Luzi *et al*, 1987; Savoia *et al*, 1993; Fairley *et al*, 1995; Kore-eda *et al*, 1998) demonstrate that these adhesion systems remain downregulated in the distal bulb region containing the hair matrix, which surrounds the dermal papilla. It is of note here that the dermal papilla is a population of closely packed cells, which express cadherin-11 (Simounneau *et al*, 1995). Cells of the bulb region might spread to engulf the dermal papilla, just as seen in the behavior of aggregation of cells with weaker intercellular adhesivity apposed to those with stronger intercellular adhesivity (Steinberg, 1978, 1996).

At present, the mechanisms underlying the regulation of the cell adhesion systems during early hair follicle morphogenesis remain unknown. It is worth noting, however, that molecules associated with the Sonic hedgehog (Shh) signaling pathway are expressed during early hair follicle morphogenesis, that hair follicle morphogenesis is blocked at the hair placode stage in the skin of *Shh*−/− mutant mice and that aberrant activation of the Shh pathway lead basal cell carcinomas, which result from abnormal growth of developing hair follicles and accompany downregulation of desmosomal and hemidesmosomal adhesion systems (Hahn *et al*, 1996; Johnson *et al*, 1996; Dahmane *et al*, 1997; Fan *et al*, 1997; Oro *et al*, 1997; Xie *et al*, 1998; Chiang *et al*, 1999). The Shh pathway might be involved in the remodeling of cell adhesion systems during early follicle morphogenesis. Also, although it remains unknown to what extent, if any, cell adhesion systems during early follicle morphogenesis are related in a regulatory fashion to each other, the differential expression of E- and P-cadherins might be associated with the desmosomal downregulation as it has been reported that P-cadherin expression may be incompatible with desmosome formation (Marrs *et al*, 1995) and that plakoglobin association with E-cadherin is necessary for desmosome organization (Lewis *et al*, 1997). Regulation of the classic cadherin-mediated cell adhesion might be an event associated with transient stabilization of β-catenin (Gat *et al*, 1998), which is a molecule closely related to plakoglobin and is involved in cell adhesion mediated by the cadherins (Takeichi, 1995; Gumbiner, 1996, 1997).

Finally, it is of interest that the remodeling of desmosomal and hemidesmosomal adhesion systems takes place during early development of not only hair follicles but also some other organ rudiments whose epithelial morphogenesis requires tissue interaction with mesenchyme. For instance, it has been demonstrated that desmosome assembly and expression of the component molecules are suppressed in the branching epithelium at early developmental stages of submandibular glands (Redman, 1987; Kadoya and Yamashina, 1993; Hieda *et al*, 1996; Hieda and Nakanishi, 1997) and that the submandibular mesenchyme has the ability to disorganize epithelial cell-to-cell adhesion systems (Iwai *et al*, 1998). Furthermore, expression of laminin-5 or integrin $\beta 4$ is downregulated during the early development of submandibular and mammary glands (our unpublished data). It may be that the remodeling of cell adhesion systems is a shared cellular mechanism underlying early epithelial morphogenesis of several organ rudiments.

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