Hyaluronan Is Inversely Correlated with the Expression of CD44 in the Dermal Condensation of the Embryonic Hair Follicle

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Previously, we have shown that CD44 (the hyaluronan receptor) was involved in the degradation of hyaluronan. In the present study, we examined the distribution of CD44 and hyaluronan in the skin of embryonic and mature mice. During embryonic development, CD44 was prominently expressed by the condensed mesenchymal cells involved in the formation of the hair follicles, but was absent from the surrounding interstitial cells. The cells of the dermal condensation expressed CD44 throughout the development of the hair follicle; however, once the hair follicle reached maturity, the mesenchymal cells of the dermal papilla no longer expressed this molecule. In contrast to the above, the distribution of hyaluronan was reversed from that of CD44. Hyaluronan was widespread throughout the embryonic dermis, but was conspicuously absent from the regions of the dermal condensation. This arrangement persisted through the development of the hair follicle; however, in the mature hair follicle, hyaluronan reappeared in the dermal papilla. Thus, in the embryonic dermis, the expression of CD44 and hyaluronan were complementary to each other. However, in the adult skin, only minor changes were detected in the levels of CD44 and hyaluronan associated with the cells of the dermal condensation during the hair cycle. When organ cultures of embryonic mouse skin were treated with *Streptomyces* hyaluronidase, the interstitial mesenchymal cells became compacted, indicating that the removal of hyaluronan leads to the condensation of these cells. The results of this study are consistent with the hypothesis that the expression of CD44 by the inductive mesenchymal cells allows them to degrade hyaluronan in a localized region, leading to formation and maintenance of the dermal condensation. Key words: hyaluronan/CD44/hair follicle/mesenchymal cells. *J Invest Dermatol 101:820–826, 1993*

One of the earliest recognizable events that occurs during the morphogenesis of the hair follicle is that the mesenchymal cells adjacent to the epidermal placode become more highly condensed than the surrounding interstitial cells [1–3]. This dermal condensation appears to induce a response in the overlying epidermis, causing it to migrate downward into the embryonic dermis, forming the hair follicle. Throughout this process, the cells of the dermal condensation remain at the leading edge of the growing hair follicle and eventually give rise to the dermal papilla of the mature hair.

Previous studies have suggested that in many cases the condensation of tissues is due to the loss of hyaluronan from the extracellular matrix [4,5]. For example, during the development of the cornea, hyaluronan is initially present, resulting in a swelling of the tissue, which facilitates the migration of mesenchymal cells from the periphery. Subsequently, the hyaluronan is lost and simultaneously the stroma of the cornea becomes condensed [4]. A similar situation has been observed in several other developing systems as well [5]. Thus, a major function of hyaluronan is to maintain the extracellular space between cells.

One mechanism for the removal of hyaluronan depends upon the hyaluronan receptor, which is identical to one of the isoforms of CD44 [6–8]. This receptor is responsible for binding hyaluronan to the cell surface so that it can be internalized and ultimately degraded by lysosomal enzymes. This process can be blocked by the addition of antibodies directed against the receptor [7].

In the present study, we have examined the distribution of hyaluronan and its receptor (CD44) in the skin of embryonic and mature mice. We have found that during embryonic development, CD44 was specifically expressed by the cells of the dermal condensation that were involved in the formation of the hair follicle, but absent from the surrounding interstitial cells. CD44 was expressed by the cells of the dermal condensation during the development of the hair follicle, but was greatly reduced or absent from these cells in mature follicles throughout the different stages of the hair cycle. Moreover, the expression of CD44 was correlated with the absence of hyaluronan in the region of the dermal condensation. One possible interpretation of these results is that the hyaluronan was degraded by the cells using a CD44-dependent mechanism. Alternatively, the relative absence of hyaluronan may have been due to a localized decrease in its rate of synthesis. In either case, the absence of hyaluronan in this region probably contributed to the condensation of the inductive mesenchymal cells.

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Abbreviations: b-KM-201, biotinylated KM-201 monoclonal antibody against mouse CD44; b-PG, biotinylated proteoglycan probe for hyaluronan; b-rat IgG, biotinylated rat antibody from non-immune serum; blotting buffer, 10% calf serum, 90% CMF-PBS, 0.05% tween 20; CMF-PBS, calcium and magnesium free phosphate buffered saline; MoAb, monoclonal antibody.

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MATERIALS AND METHODS

Preparation of Biochemicals KM-201 is a rat monoclonal antibody against mouse CD44, which was originally isolated by Miyake *et al* [9]. This MoAb blocks the interaction between hyaluronan and CD44 [6]. The antibody was isolated from ascites fluid of nude mice by chromatography on diethylaminoethyl Affi-gel blue columns as described previously [7]. It was coupled to biotin (b-KM-201) with sulfosuccinimidyl 6-(biotinamido) hexanoate (Pierce) using the methods of Updyke and Nicolson [10]. The rat IgG used as a control was purchased from Sigma and biotinylated in the same fashion (b-rat IgG).

The binding probe for hyaluronan (b-PG) was prepared from cartilage extracts as described previously [11]. This probe consists of a mixture of the link protein and a fragment of the proteoglycan core protein. Previous studies have shown that the b-PG probe binds to hyaluronan with high affinity and specificity [11,12].

Isolation of Tissue Timed-pregnant mice were obtained from Charles River (Wilmington, MA), Tyler Laboratories (Bellevue, WA), and the vivarium at the University of Washington (Seattle). The night of mating was defined as time 0 (i.e., the day after mating was considered day 0.5). At various times after mating, the mice were sacrificed and the embryos were surgically removed. For the biochemical analysis, the embryos were stored directly in 70% ethanol. For histochemical analysis, they were fixed overnight in 4% formaldehyde, 1.5% methanol, 0.08 M $NaHPO_4$, pH 7.0, and then stored in 70% ethanol. Similarly, samples of adult skin were removed from the backs of sacrificed mice, placed on a small piece of cardboard, and then fixed overnight as described above.

Western Blotting The back and flank skin was dissected from the mouse embryos, rinsed in calcium- and magnesium-free phosphate-buffered saline (CMF-PBS), and homogenized in 0.5% Na deoxycholate, 0.5 M NaCl, 0.02 M Tris pH 8.0. The concentration of protein in the extracts was determined using the bicinchoninic acid method (BCA Protein Assay Kit, Pierce, Rockford, IL). Samples containing 50 μg of protein were precipitated by the addition of 70% ethanol, and then redissolved in Laemmli sample buffer lacking β-mercaptoethanol. After placing in boiling water for 5 min, the samples, along with prestained molecular-weight standards (high molecular weight, Bio-Rad, Richmond, CA), were electrophoresed on a 10% SDS-polyacrylamide gel. The proteins were then electrophoretically transferred to a sheet of nitrocellulose (Immobilon, Millipore, New Bedford, MA) at 0.9 amp for 30 min using a transblot apparatus (Idea, Corvallis, OR). The sheet of nitrocellulose was incubated in 5% non-fat milk overnight to block residual protein binding sites. The sheet was then immunostained by incubation in the following solutions: 1) 4 μg/ml of b-KM-201 in 10% calf serum, 90% CMF-PBS containing 0.05% tween 20 (blotting buffer) for 1 h; 2) 1 to 500 dilution of peroxidase-labeled streptavidin (Kirkegaard and Perry, Gaithersburg, MD) in blotting buffer; and 3) a peroxidase substrate consisting of 0.03% H_2O_2 and 0.2 mg/ml 3-amino-9-ethyl carbazole in 0.05 M Na acetate pH 5.0 [13].

Histochemical Staining The embryos were first cut down the midsagittal plane. The embryos, as well as samples of adult skin, were dehydrated in a graded series of ethanol and water solutions, and then embedded in 90% polyester wax, 10% ethanol as described by Kusakabe *et al* [14]. Sections 8 μ thick were cut on a cryostat at −20°C, layered on a water bath at 4°C, and then taken up on slides subbed with egg albumin. The slides were dried overnight at 4°C, and stored at this temperature.

For staining, the sections were rehydrated in a graded series of ethanol and water solutions, and then incubated for 5–10 min in 10% H_2O_2 to inactivate endogenous peroxidase. The slides were incubated for 1 h with the primary reagent that was dissolved in 10% horse or calf serum, 90% CMF-PBS. For CD44, the primary reagent consisted of 4 μg/ml of b-KM-201, and the background was determined by substituting an equal concentration of control b-rat IgG. For hyaluronan, the primary reagent consisted of 4 μg/ml b-PG and the background was determined by premixing the b-PG with 100 μg/ml hyaluronan. After thorough washing, the sections were incubated for 15–30 min with peroxidase-labeled streptavidin diluted 1 to 500 in 10% serum, 90% CMF-PBS. Finally, the sections were incubated for 20–30 min in a peroxidase substrate consisting of H_2O_2 and 3-amino-9-ethyl carbazole. The sections were counterstained for 1 min in Mayer's hematoxylin followed by 0.5 M Tris pH 8.6 for 5 min, and then preserved in Crystal/Mount (Biomeda, Foster City, CA). Coverslips (1½ weight) were attached using Histomount (National Diagnostics, Highland Park, NJ).

Some aspects of the b-PG stain for hyaluronan should be noted [11]. First, agents that precipitate hyaluronan (e.g., cetyl pyridinium chloride) were not used in this protocol, as they generally reduced the intensity of staining (unpublished observations). Consequently, hyaluronan, which is not associated with protein, may be leached out during processing of the tissue. And secondly, hyaluronan, which is complexed with large amounts of protein, such as in cartilage, will not be stained, as it is unable to interact with the b-PG probe.

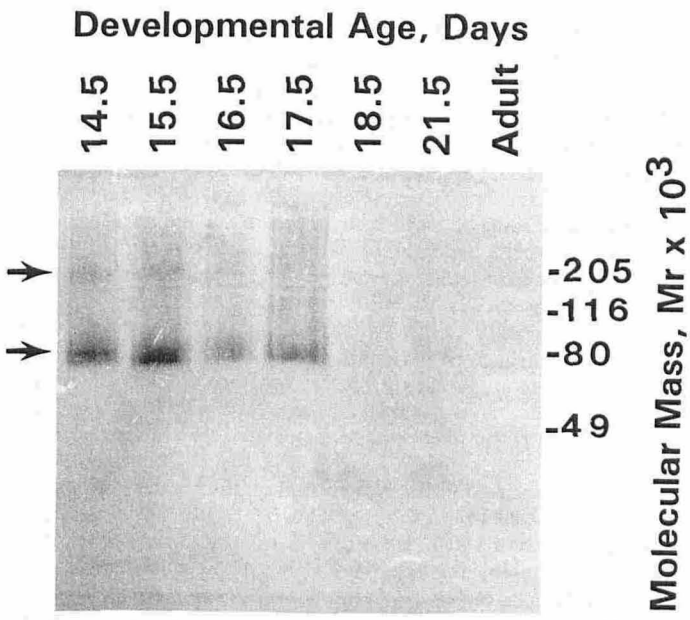

Figure 1. Western blot analysis of CD44 from mouse skin at different stages of development. Skin was removed from both embryonic and adult mice and extracted in Laemmli sample buffer in the absence of β-mercaptoethanol. Samples of each extract containing 50 μg of protein were electrophoresed on a 10% sodium dodecylsulfate–polyacrylamide gel and then transferred to nitrocellulose. The blot was stained for CD44 with the b-KM-201 MoAb. *Arrows,* the position of two diffuse bands at M_r 80 and 180 × 10^3, which were apparent in the samples from embryos of 14.5–17.5 d. The positions of prestained molecular weight standards are shown on the right.

Organ Culture The cheek pads of 12.5-d embryos were removed and placed directly on metal screens that, in turn, were placed in organ culture chambers. The wells were filled with 5% fetal calf serum, 95% DMEM, such that the tissue was at the air-medium interface. *Streptomyces* hyaluronidase (type IX; Sigma, St. Louis, MO) was added to the medium of the test cultures at a final concentration of approximately 25 units/ml. The cultures were maintained for 2 d in a 10% CO_2 incubator at 37°C, fixed in formalin, and then embedded in polyester wax as described above. Following sectioning, the slides were stained for hyaluronan using the b-PG probe or with hematoxylin alone.

RESULTS

The Different Isoforms of CD44 in Mouse Skin In initial experiments, the different isoforms of CD44 present in the embryonic mouse skin were examined by Western blotting. Figure 1 shows that two major species were apparent following staining with the KM-201 MoAb. The major immunoreactive band of 80 × 10^3 M_r corresponds to the CD44H isoform, associated with mesenchymal cells, whereas the minor band of approximately 180 × 10^3 M_r corresponds to the CD44E isoform, associated with epidermal cells [8,15,16]. In addition, another high-molecular mass form (>200 × 10^3) could be observed in the sample of adult skin when run on a lower percentage gel (data not shown).

The relative concentration of CD44 changed as a function of embryonic development. CD44 immunoreactivity was present in the skin prior to embryonic day 13 (data not shown). The amount of CD44 relative to protein reached a maximum value between days 14.5 and 17.5. Thereafter, the relative amount of CD44 immunoreactivity decreased and only small amounts were apparent in the adult skin. Part of this decrease in CD44 immunoreactivity may reflect the large increase in the collagenous component of the skin

with age, which reduces the proportion of the cell-associated proteins, including CD44.

Localization of CD44 in Embryonic Skin The distribution of CD44 in the pelage skin at different stages of development was then examined by immunocytochemistry. The specific staining of CD44 with the b-KM-201 MoAb and control staining with rat IgG are illustrated in Figs 2*a,b*, respectively. Figure 2*a* shows that positive staining was associated with four different cell types. Firstly, the fibroblast-like cells beneath the skeletal muscle layer, panniculus carnosus, showed a weak staining that was present only during early embryonic stages (cells labeled *F* in Fig 2*a*).

Secondly, positive staining was associated with round cells in the dermis that appear to be macrophages (labeled *M* in Fig 2*a*). These cells were relatively sparse during early stages of development and increased in number as a function of age. Similar observations have been made with the macrophages associated with the lung tissue [7,11,17].

Thirdly, immunostaining was present on the keratinocytes of the epidermis (labeled PD in Fig 2*a*). These cells probably express the CD44E isoform of 180×10^3 M_r, which may or may not be able to interact with hyaluronan [16,17]. In the embryos, the expression of CD44 by the keratinocytes changed with development. It was absent from the skin during initial stages (Fig 2*c*), and first appeared on the more superficial layers of the epidermis (Fig 2*a,d*). In later stages of development, it was present in the spinous layer of the skin (*small arrowheads* in Fig 2*f,g*) and was maintained in this region in the adult [12]. This distribution may be a reflection of the fact that CD44 is most prominently expressed by proliferating epithelial cells [12]. On the other hand, it was also prominent along the inner root sheath of the hair follicles (*small arrowhead* in Fig 2*h*), which are generally considered to be differentiated cells.

And finally, the cells of dermal condensation also expressed CD44 (labeled DC in Fig 2*a*), in contrast to the surrounding interstitial cells. The expression of CD44 by the cells of the dermal condensation also changed during development. It was absent from the dermis prior to hair follicle induction (Fig 2*c*), and first appeared in the pre-germ stage on the mesenchymal cells immediately below the developing placode of the epidermis (*large arrowhead* in Fig 2*d*). In some cases, the expression of CD44 appeared to precede the actual condensation of the mesenchymal cells. Cells of the dermal condensation continued to express CD44 as hair follicle development progressed through the hair-germ, hair-peg, and bulbous-peg stages (*large arrowheads* in Figs 2*e,f,* and *g*). However, in the mature hair follicle of a 4-d-old pup, the expression of CD44 was greatly reduced or absent from the dermal papilla (labeled DP in Fig 2*h*). Similar observations were made with the developing vibrissa hair, which begin development earlier than pelage hair. Thus, it appears that the expression of CD44 by the inductive mesenchymal cells is directly correlated with the formation of the hair follicle.

Localization of Hyaluronan in Embryonic Skin The distribution of hyaluronan in the pelage skin of mice at different stages of development was examined by staining with b-PG, a specific hyaluronan-binding probe derived from cartilage [11]. Figures 3*a,b* show a comparison of the specific and background staining obtained with this probe on skin from a 15.5-d mouse embryo. As demonstrated in Fig 3*a*, hyaluronan was present in both the dermis and epidermis. In the dermis, it was particularly abundant immediately next to the epidermis, but reduced below the level of the skeletal muscle (Fig 3*a,c*). In the epidermis, hyaluronan was initially observed in the periderm and internal layers (*small arrowheads* in Figs 3*a,d*), but, subsequently, relocated to the spinous layer (*small arrowheads* in Figs 3*e,f,g*), where it remained in the mature skin [12,18]. This is similar to the translocation that occurred in CD44 of the epidermis described above.

Most interestingly, hyaluronan was greatly reduced or absent from the region of the dermal condensation (*large arrowhead* in Fig 3*a*). Whereas hyaluronan was evenly distributed throughout the dermis prior to hair follicle induction (Fig 3*c*), discontinuities where the hyaluronan was absent became apparent during the pre-germ stage (*large arrowhead* in Fig 3*d*). These sites were located next to the developing epidermal placodes prior to the formation of the dermal condensation. Hyaluronan was also absent from the dermal condensation during the subsequent hair-germ, hair-peg, and bulbous-peg stages (*large arrowheads* in Figs 3*e,f,g*). However, in the mature hair follicle (Fig 3*h*), hyaluronan was observed to be present in the dermal papilla. A similar developmental sequence was apparent with both pelage and vibrissa hair follicles, except that the rate of development was different. These results indicate that in the inductive mesenchymal cells, the presence of hyaluronan was inversely correlated with the expression of the receptor on the mesenchymal cells, both spatially and temporally.

CD44 and Hyaluronan of the Dermal Condensation During the Hair Cycle of Adult Skin Skin from adult mice was examined to determine whether similar changes occurred during the hair cycle. Figures 4*a,c,* and *e* show the distribution of CD44 in hair follicles during anagen, catagen, and telogen, respectively. In most cases, little or no CD44 was detected in the dermal condensation (indicated by the *arrowheads*) throughout the different stages of the hair follicle. Similarly, Figs 4*b,d,* and *f* show the distribution of hyaluronan in hair follicles during equivalent stages of the hair cycle. In general, low levels of hyaluronan were associated with the dermal condensation during these stages. It should be noted that there were exceptions in which CD44 was detected in the dermal condensation and hyaluronan was totally absent. Thus, the possibility that CD44 and hyaluronan undergo changes during the hair cycle cannot be eliminated. However, if such changes occur, the magnitude is far less than that seen during the embryonic development of the hair follicles.

Effects of Hyaluronidase on Embryonic Mouse Skin To determine whether condensation of the mesenchymal cells associated with the developing hair follicle could be due to localized degradation of hyaluronan, the cheek pads of 12.5-d mouse embryos were cultured in the presence and absence of *Streptomyces* hyaluronidase, an enzyme that specifically degrades hyaluronan [20]. After 2 d in this medium, the samples of skin were processed for histology. Staining with the b-PG probe showed that hyaluronan was abundant in the extracellular matrix of the control tissue, but was totally absent from the hyaluronidase-treated tissue (data not shown). In addition, the mesenchymal cells in the hyaluronidase-treated tissue were highly condensed, whereas those of the control tissue were still dispersed (Fig 5*a,b*). Similar results have been reported by other researchers when intact embryos were treated with hyaluronidase [21,22]. These results show that the removal of hyaluronan alone can result in the condensation of mesenchymal cells.

DISCUSSION

The results of the present study demonstrate that there is a close correlation between the expression of CD44 and the absence of hyaluronan. The cells of the dermal condensation expressed CD44 only during the formation of the hair follicle, corresponding to the interval when hyaluronan was conspicuously absent from this region. Once the hair follicle reached maturity, the mesenchymal cells of the dermal papilla no longer expressed CD44 and hyaluronan returned to this region. This situation continued in the mature hair follicles of adult skin throughout the different stages of the hair cycle. Thus, both spatially and temporally, the expression of CD44 was correlated to the absence of hyaluronan.

These results suggest, but do not prove, that the expression of CD44 by the inductive mesenchymal cells leads to the localized removal of hyaluronan. Indeed, previous studies have shown that macrophages and other cells that express CD44 have the capacity to degrade hyaluronan [7]. CD44 appears to bind hyaluronan to the cell surface, where it can be taken up inside the cell and then degraded by lysosomal hydrolases. Presumably, the mesenchymal cells of the dermal condensation have a similar capacity. However, it is also possible that the absence of hyaluronan is due to a decrease in its synthesis. The synthesis of hyaluronan is directly related to the rate of cell proliferation [23,24], which is particularly low in the dermal

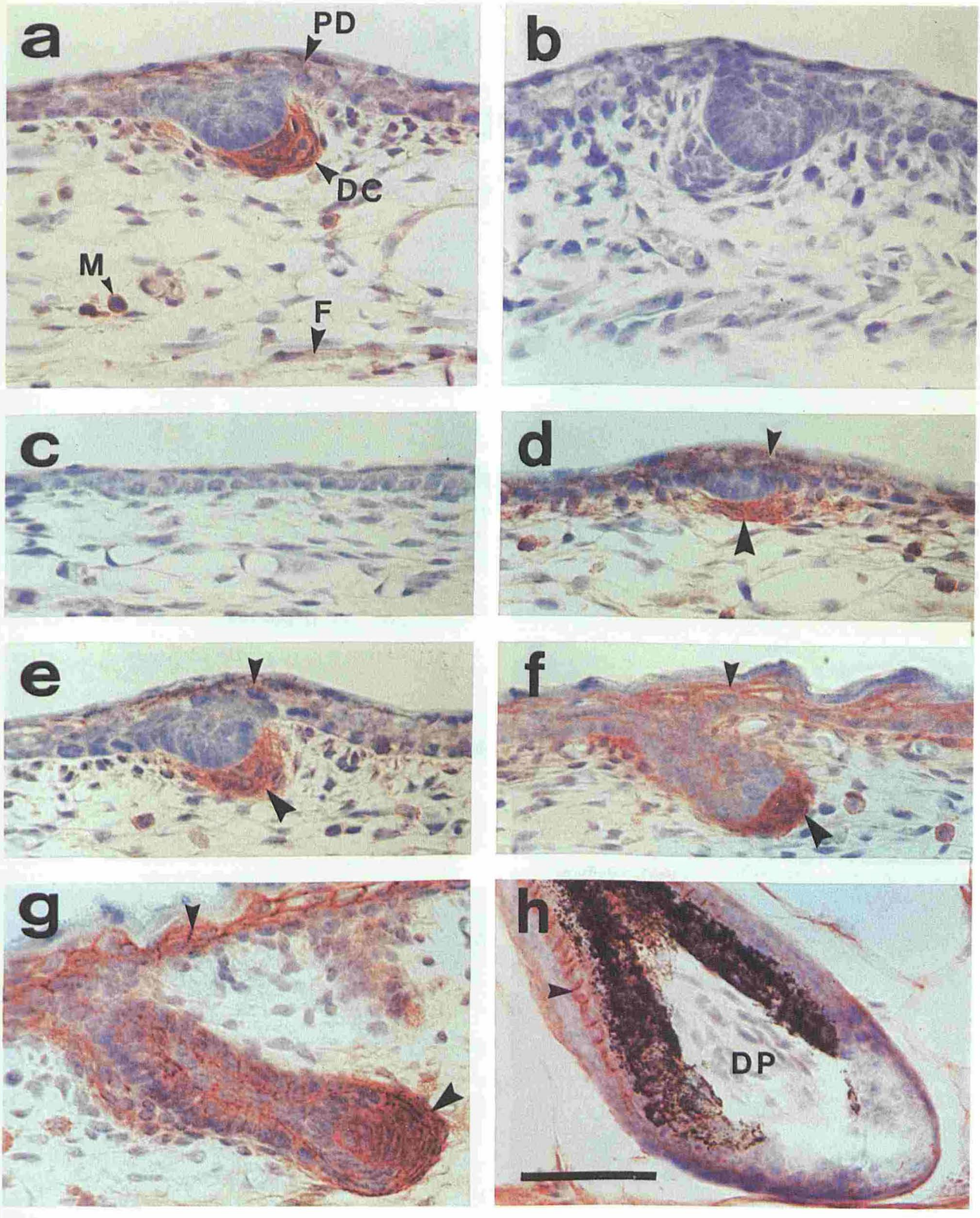

Figure 2. The distribution of CD44 in mouse skin at different stages of development. Sections *a* and *c* through *h* were stained for CD44 with the b-KM-201 MoAb *(red staining)* and then counterstained with hematoxylin *(blue staining)*. *a*) A section from a 15.5-d mouse embryo shows that CD44 immunostaining was associated with the following structures: *DC*, dermal condensation; *M*, macrophage; *PD*, periderm; and *F*, fibroblast-like cell below the muscle layer, panniculus carnosus. *b*) The control staining was obtained by treating an equivalent section (15.5-d embryo) with b-rat IgG followed by counterstaining with hematoxylin. *c*) The skin from a 14.5-d mouse embryo shows the absence of CD44 immunostaining prior to the onset of hair follicle initiation. *d*) A 15.5-d mouse embryo shows the presence of CD44 immunostaining on both the outer layer of the epidermis *(small arrowhead)* and on the mesenchymal cells next to the hair placode in the pre-germ stage *(large arrowhead)*. *e*) A 15.5-d embryo shows positive CD44 immunostaining associated with the dermal condensation of a hair-germ. *f*) The skin from a 16.5-d embryo shows the presence of CD44 on the dermal condensation of a follicle in the hair-peg stage. *g*) The skin from a 17.5-d mouse embryo shows CD44 immunostaining in the dermal condensation of the bulbous-peg stage hair follicle *(large arrowhead)* and in the spinous layer of the epidermis *(small arrowhead)*. *h*)The bulb of a mature hair follicle (anagen) from a 4-d old mouse pup shows that the cells of the dermal papilla (DP) do not express CD44, whereas the germinal layer of the epithelium is positive for CD44 *(small arrowhead)*. *Black pigment,* melanin granules in the germinal layer. *Bar,* 50 μm.

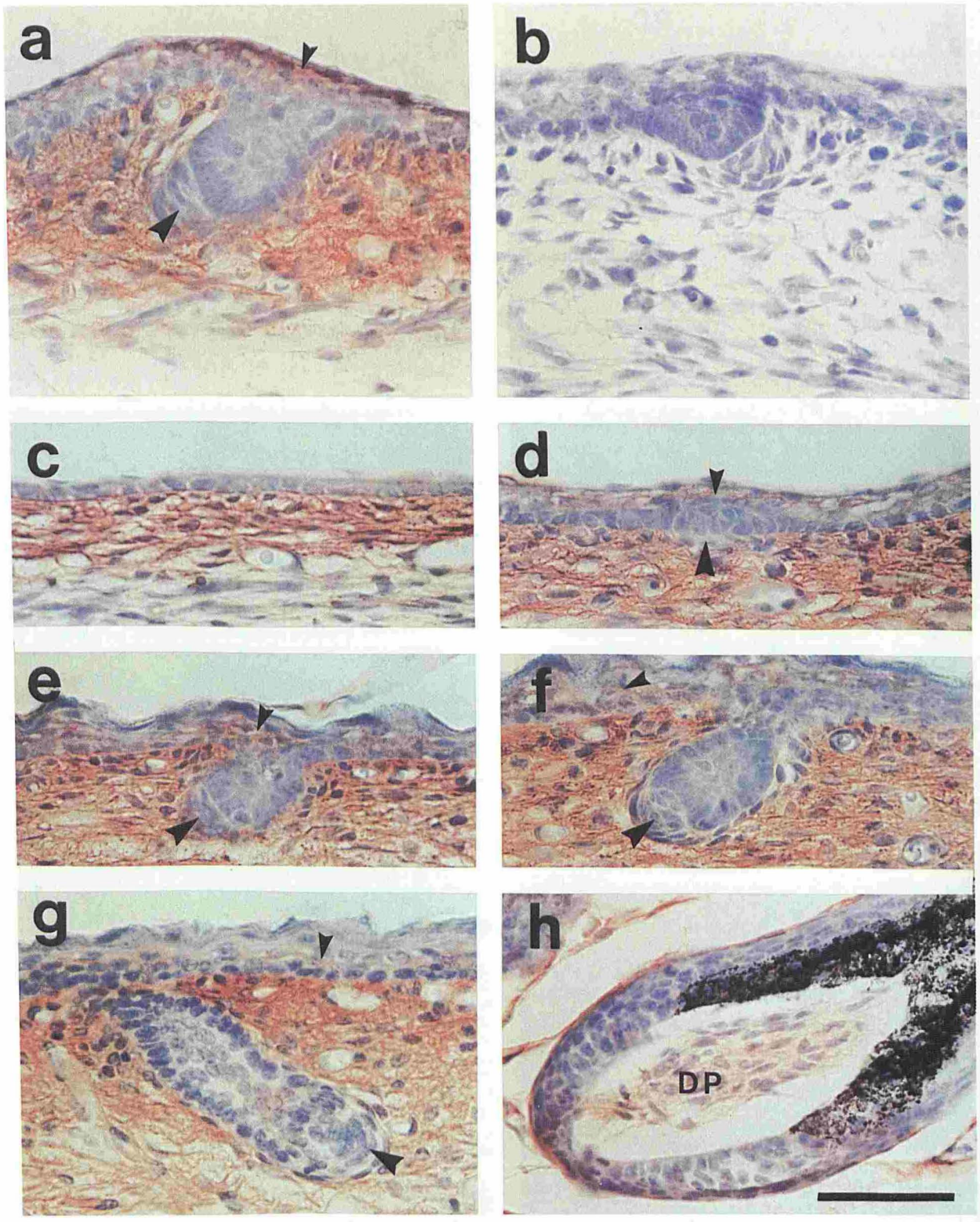

Figure 3. The distribution of hyaluronan in mouse skin at different stages of development. Sections *a* and *c* through *h* were stained for hyaluronan with the b-PG probe *(red staining)* followed by counterstaining with hematoxylin *(blue staining)*. *a*) A section of skin from a 15.5-d mouse embryo shows that hyaluronan was present in the periderm and intermediate layers of the epidermis *(small arrowhead)* and throughout the dermis extending to the region below the muscle layer. Hyaluronan was greatly reduced or absent from the dermal condensation *(large arrowhead)*. *b*) An equivalent section of a 15.5-d embryo was treated with the b-PG probe that had been preincubated with an excess of free hyaluronan to demonstrate background staining. *c*) Skin from a 14.5-d embryo shows that hyaluronan is distributed uniformly below the epidermis prior to hair follicle initiation. *d*) The skin from a 15.5-d mouse embryo shows the diminished presence of hyaluronan in the dermis immediately below the placode of a pre-germ follicle *(large arrowhead)*. *e*) Skin from a 16.5-d embryo shows the absence of hyaluronan in the dermal condensation associated with a hair germ. *f*) A section of a 16.5-d embryo again shows the absence of hyaluronan in the dermal condensation of a developing hair follicle in the hair-peg stage *(large arrowhead)*. Also note a small amount of hyaluronan staining in the spinous layer of the epidermis *(small arrowhead)*. *g*) Skin of a 17.5-d mouse embryo shows the lack of hyaluronan staining in the dermal condensation of a hair follicle in the bulbous-peg stage *(large arrowhead)*. *h*) The bulb of a mature hair follicle from a 4-d old mouse pup shows the reappearance of hyaluronan in the cells of the dermal papilla. *Black pigment,* melanin granules in the germinal layer. *Bar,* 50 μm.

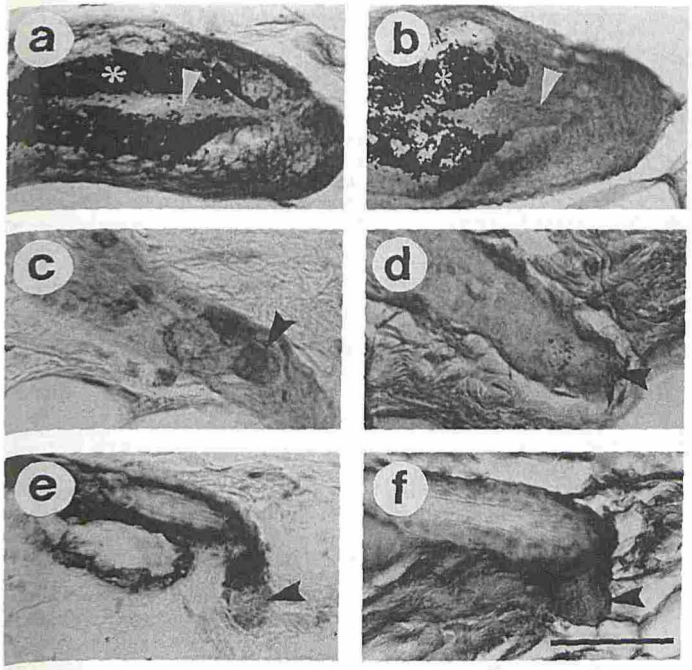

Figure 4. The distribution of CD44 and hyaluronan in the different stages of the hair cycle in adult skin. Sections *a,c,* and *e* were stained for CD44 with the b-KM-201 MoAb and sections *b,d,* and *f* were stained for hyaluronan with b-PG. All of the sections were counterstained with hematoxylin. *a,b)* The dermal papilla *(large arrowheads)* from hair follicles in anagen or growing phase express little or no CD44 and contain detectable levels of hyaluronan (the *black pigment indicated by the asterisk,* melanin granules). *c,d)* The dermal condensations of hair follicles in catagen or the involution phase generally express small amounts of CD44 and contain hyaluronan. *e,f)* The dermal condensations of hair follicle in telogen or the resting phase, again, do not express CD44 and are associated with hyaluronan. *Bar,* 50 μm.

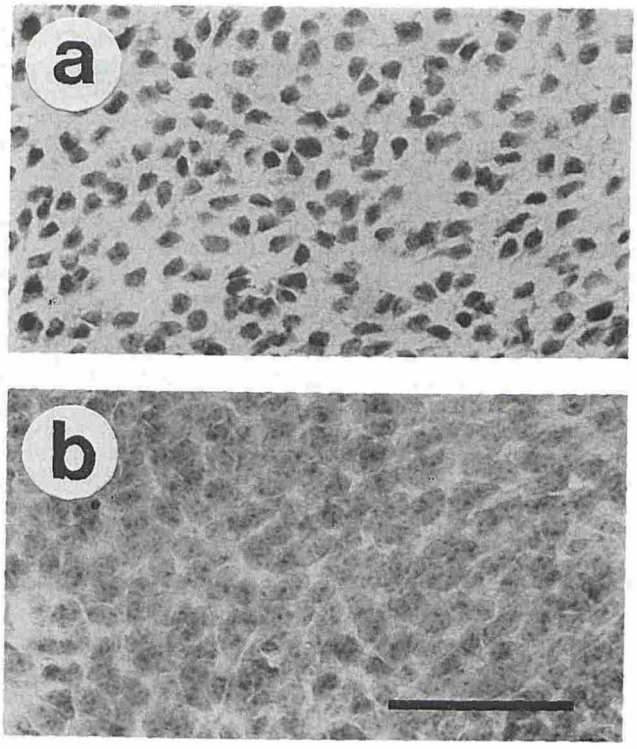

Figure 5. The effects of treatment with *Streptomyces* hyaluronidase on the mesenchymal cells of organ cultures of mouse skin. The upper cheek pads from 12.5-d mouse embryos were placed in culture in the presence and absence of *Streptomyces* hyaluronidase. After 2 d, the tissues were fixed and examined histochemically. *a)* In control tissue, hyaluronan is present and the mesenchymal cells are clearly separated from one another. *b)* In hyaluronidase-treated tissue, hyaluronan is absent and the mesenchymal cells are highly condensed. Interestingly, the nuclei were larger than in the control sample. The reason for this difference is unclear. *Bar,* 50 μm.

condensation [25]. Thus, it is possible that increased degradation and/or decreased synthesis contribute to the absence of hyaluronan in the region of the dermal condensation.

The removal of hyaluronan can lead to the condensation of the mesenchymal cells. In the present study, this was demonstrated by the fact that the treatment of cultured cheek pad with *Streptomyces* hyaluronidase leads to the collapse of the intercellular spaces, causing the condensation of the mesenchymal cells, reminiscent of the dermal condensation. Several other studies have made similar observations concerning the collapse of the extracellular matrix in intact embryos, following treatment with hyaluronidase [21,22]. Clearly, hyaluronan is responsible for maintaining these extracellular spaces, which is an important aspect of morphogenesis.

The relationship between the expression of CD44 and the absence of hyaluronan has been observed in several other systems as well. For example, during lung development, the loss of hyaluronan was directly related to an increase in the number of macrophages expressing CD44 [17]. Furthermore, when new-born mice were injected with blocking antibodies to CD44, the hyaluronan content of the lungs increased significantly. Presumably, the antibody prevented the breakdown of hyaluronan by the macrophages. A similar inverse relationship between CD44 and hyaluronan has been noted in the developing tooth, liver, and lymphoid tissues (Underhill, unpublished observations). And finally, in the present study we noted that the hyaluronan in the dermis extended to the level of the skeletal muscle, below which the fibroblasts express CD44. Thus, the inverse relationship between hyaluronan and the expression of CD44 by mesenchymal cells is a general phenomenon.

However, this inverse relationship did not hold up in the case of the epidermis, where the expression of CD44 and hyaluronan were directly correlated with one another. Both molecules were present in the periderm during early stages of development and subsequently moved to the spinous layer as the epidermis matured. This codistribution of CD44 and hyaluronan has been noted in several other epithelia as well [12,19,26]. However, the situation in the epidermis may be fundamentally different from that of the mesenchymal cells, because the keratinocytes express the CD44E isoform of 180×10^3 M_r [15]. This isoform contains an additional insert in the extracellular domain that may prevent it from interacting with hyaluronan [16], although there is some controversy about this point [18]. Thus, the epidermis may or may not be able to participate in the degradation of hyaluronan. Whereas the function of the CD44E isoform has not yet been determined, it may be involved in maintaining the extracellular spaces between adjacent epidermal cells [12].

In addition to hyaluronan, several other components of the extracellular matrix are conspicuously absent from the dermal condensation during hair follicle development. For example, there is a diminution of fibrillin and collagens type I, III, V, and VI surrounding the hair follicle, and simultaneously, there is an increase in the expression of interstitial collagenase [27,28]. Similarly, there is a decrease in the bulbous pemphigoid antigen, a molecule that is closely associated with hemidesmosomes [29] and a dermatan-sulfate–containing proteoglycan that may be identical to decorin.* And finally, tenascin, a molecule associated with epithelial-mesenchymal interactions between cells, also undergoes dynamic changes during hair follicle morphogenesis. It is initially present beneath the pre-germ, but is transiently lost from the dermal condensation during the hair-peg stage, and reappears during subsequent stages.* In contrast, most of the components of the basal lamina (laminin, type

* Kaplan ED, Underwood RA, Holbrook KA: Cell aggregation in follicle morphogenesis during fetal skin development (abstr). *Anat Rec* 229:47a, 1991.

IV collagen, fibronectin, and heparan sulfate proteoglycan) do not appear to change during development of the hair follicle [29–31]. The mechanism that accounts for the distribution of these components is unclear. It is possible that the loss of the extracellular matrix underlying the basal lamina may facilitate the migration of the epidermal cells into the dermis during the formation of the hair follicle.

In addition to CD44, the cells of the dermal condensation express several other proteins that carry out a variety of functions. For example, these cells also express syndecan, a molecule that binds to both collagen and fibronectin [32–34]. It is interesting to note that CD44 and syndecan are co-expressed in several other tissues, including the epithelium, tooth, and crypts of Lieberkuhn [12,34–36]. Similarly, the cell adhesion molecule N-CAM is also expressed by the cells of the dermal condensation [37].* And finally, the mesenchymal cells secrete epimorphin, which is involved in the signaling between the mesenchyme and the epidermis [38]. Thus, the expression of CD44 and the associated loss of hyaluronan should be considered as one of a number of events that occur during morphogenesis of the hair follicle.

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