Keratin 17 Expression in the Hard Epithelial Context of the Hair and Nail, and its Relevance for the Pachyonychia Congenita Phenotype

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The hard-keratin-containing portion of the murine hair shaft displays a positive immunoreactivity with an antibody against the soft epithelial keratin, K17. The K17-expressing cell population is located in the medulla compartment of the hair. Consistent with this observation, K17-containing cells also occur in the presumptive medulla precursor cells located in the hair follicle matrix. Western blot analysis of hair extracts prepared from a number of mouse strains confirms this observation and suggests that K17 expression in the hair shaft is a general trait in this species. The expression of K17 in human hair extracts is restricted to eyebrow and facial hair samples. These are the major sites for the occurrence of the *pili torti* (twisted hair) phenotype in the type 2 (Jackson-Lawler) form of pachyonychia congenita, previously shown to arise from inherited K17 mutations. Given that all forms of pachyonychia congenita show an involvement of the nail, we compared the expression of the two other genes mutated in pachyonychia congenita diseases, K6 and K16, with that of K17 in human nail. All three keratins are abundantly expressed within the nail bed epithelium, whereas K17 protein is expressed in the nail matrix, which contains the epithelial cell precursors for the nail plate. Our data suggest a role for K17 in the formation and maintenance of various skin appendages and directly support the concept that pachyonychia congenita is a disease of the nail bed. ***Key words: hair follicle/hard keratins/keratin 17/pachyonychia congenita. J Invest Dermatol 114:1101–1107, 2000***

Keratins are a group of more than 40 highly insoluble proteins that serve as the subunits for forming intermediate filament polymers in epithelial cells (O'Guin *et al*, 1990; Fuchs, 1995). The keratin protein family consists of two groups: the acidic or type I keratins and the basic or type II keratin proteins (Moll *et al*, 1982). A keratin filament is an obligatory heteropolymer, containing an equimolar amount of type I and type II proteins (Coulombe, 1993; Steinert, 1993). To meet this requirement, keratin genes are coordinately expressed as type I-type II pairs (Moll *et al*, 1982; O'Guin *et al*, 1990; Fuchs, 1995). The concept of pairwise regulation is supported by studies that demonstrate tissue- and differentiation-specific expression of keratin genes in the various epithelia of the body. The pairwise regulation of keratin gene transcription (Fuchs, 1995) determines, to a large extent, the resulting filament composition within a given epithelial cell. These filaments are organized into a prominent cytoplasmic network that is anchored at the surface of the nucleus as well as at cell-cell and cell-matrix adhesion complexes, and they typically span the entire cytoplasmic space in between. It is now firmly established, through studies conducted with transgenic mice and with patients suffering from inherited epithelial fragility disorders, that the major function of keratin filaments is to endow epithelial cells with the mechanical resilience they need to withstand the load of mechanical stress to which they are routinely subjected (Coulombe and Fuchs, 1994; McLean and Lane, 1995; Fuchs and Cleveland, 1998).

The notions of tissue- and differentiation-specific regulation of keratin genes suggest that these proteins may impart some degree of specialization to the various epithelia in which they are expressed. Detailed studies of keratin expression patterns support this concept (Moll *et al*, 1982; Tseng *et al*, 1982; Heid *et al*, 1986). Recent attempts to complement the phenotype of keratin 14 null mice, which die early after birth owing to extensive skin blistering, clearly showed that, even with their high degree of sequence homology, keratin proteins are only partially redundant at a functional level (Hutton *et al*, 1998; Paladini and Coulombe, 1999). From this it would appear that the multiplicity of keratin genes is related to the functional diversity of epithelial tissues. Thus, the characterization of keratin gene expression in normal and diseased epithelia will continue to play an important role for understanding the biology of complex epithelial systems.

The type II keratin K6 isoforms and the type I keratins 16 and/or 17 are coregulated in many complex epithelia, including all major skin appendages (e.g., hair, nail, glands; see McGowan and Coulombe, 1998b, for a review). Unlike most other keratin pairs, however, their distribution cannot be correlated with a well-defined epithelial context, leaving open the issue of their role(s) *in vivo*. Inherited missense mutations within the coding sequence of K6 isoforms, K16, or K17 cause various forms of ectodermal dysplasias, including pachyonychia congenita (PC). PC refers to a group of genodermatoses that invariably involve dyskeratotic

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Abbreviations: GFP, green fluorescence protein; LEF, lymphoid enhancer factor; PC, pachyonychia congenita; TCF, T cell factor.

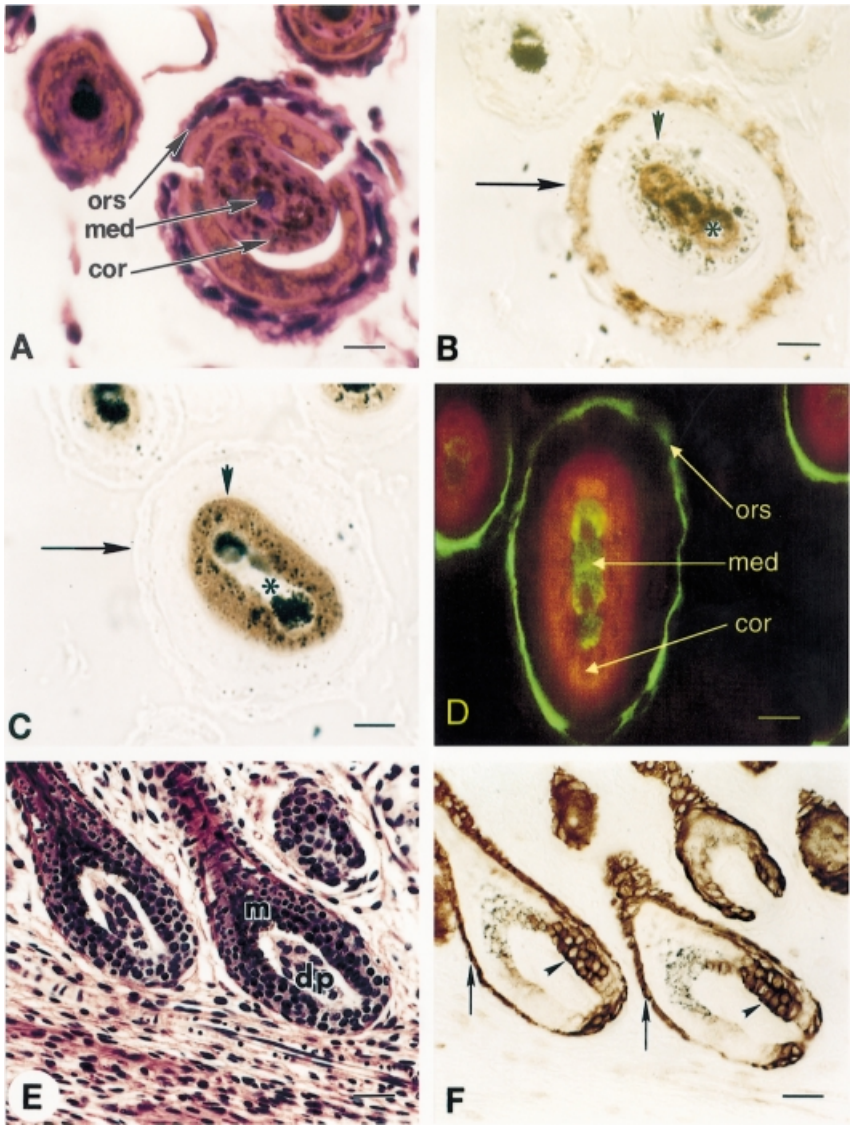

Figure 1. Immunostaining of sections from mouse backskin with antibodies to K17 and hard keratins (AE13). Paraffin-embedded tissue sections from mouse dorsum were processed for hematoxylin and eosin staining (*A* and *E*) or immunostaining (*B*, *C*, *D*, and *F*). Sections were prepared in a transverse orientation to display the outer root sheath (ORS), the medulla (med), and cortex (cor) compartments as indicated in (*A*). K17 immunostaining (*B*) was positive for two compartments of the hair follicle, the outer root sheath (*arrow*) and the medulla (*asterisk*), but negative in the cortex (*arrowhead*). AE13 antibody staining (*C*) indicates that the hard-keratin-containing components of the hair follicle reside predominantly in the cortex. Double immunofluorescence staining using rhodamine labeled antimouse IgG antibodies to reveal the AE13 staining (*red*) and a fluorescein isothiocyanate conjugated secondary antibody to indicate the K17-containing component (*green*) shows that the hard-keratin-containing component of the hair follicle and the K17 positive population do not colocalize (*D*). Three-day-old mouse backskin sections (*E*) stained with K17 (*F*) display positive immunoreactivity in the outer root sheath (*arrow*) and a distinct polarization within the matrix portion (*arrowhead*) to the side opposite the direction of hair follicle outgrowth. *Scale bar*: 100 μm.

changes in the nail and usually involve related alterations in palmoplantar epidermis and other stratified epithelia depending on the clinical variant (Feinstein *et al*, 1988). Jadassohn-Lewandowsky or type 1 PC disease (OMIM167200) is clinically distinct by the occurrence of oral leukoplakia (Feinstein *et al*, 1988; Dahl *et al*, 1995), and has been associated with mutations in the K6a or K16 sequences (Bowden *et al*, 1995; McLean *et al*, 1995; Lin *et al*, 1999). Jackson-Lawler or type 2 PC disease (MIM 167210) is distinct by the occurrence of neonatal teeth and subcutaneous cysts (Clementi *et al*, 1986; Feinstein *et al*, 1988), and has been associated with mutations in the K6b or K17 sequences (McLean *et al*, 1995; Smith *et al*, 1997, 1998; Fujimoto *et al*, 1998). Although the tissues affected in type 1 and type 2 PC patients bear an obvious relationship to the expression pattern of the gene affected, a closer look at the issue of genotype-phenotype correlation reveals several idiosyncrasies (McGowan and Coulombe, 1998b). For instance, mutations in the K16 sequence were also found in the context of a nonepidermolytic form of palmoplantar keratoderma (NEPPK), whereas mutations in K17 were discovered in several instances of steatocystoma multiplex (Shamsher *et al*, 1995; Smith *et al*, 1997). These disorders involve minimal alterations to the nail, if any. Thus, significant heterogeneity exists in the clinical picture associated with related mutations in these particular keratin genes, and the underlying basis for this remains unknown.

Previous studies have reported that K17 expression in the mature hair follicle is limited to the outer root sheath (Moll *et al*, 1982; Stark *et al*, 1987; Troyanovsky *et al*, 1989; Panteleyev *et al*, 1997). Recently, we described K17 immunoreactivity in both the hard and soft portions of the murine hair follicle (McGowan and Coulombe, 1998a). In this report, we expand on this observation and we compare the cellular distribution of K6, K16, and K17 in the human nail. The results that we report have important implications for the function of K17 and the pathogenesis of PC.

MATERIALS AND METHODS

Materials Materials were obtained from the following sources: Protran nitrocellulose filters were purchased from Schleicher and Schuell (Keane, NH); alkaline phosphatase antibody detection kit was purchased from Bio-Rad (Hercules, CA). All other chemicals were reagent grade and were typically obtained from Sigma (St. Louis, MO).

Preparations of extracts Hair clippings were minced and incubated in a solution consisting of 8 M urea, 200 mM tri(hydroxymethyl)-aminomethane (Tris) HCl (pH 9.5), and 200 mM 2-mercaptoethanol for 2 h at 37°C. The samples were then homogenized with a polytron for 30 s and incubated for an additional 2 h at 37°C. Insoluble proteins were removed by centrifugation at 10,000 × *g* for 10 min and the supernatant was recovered and stored at –70°C until use (Lynch *et al*, 1986). All other tissue extractions were performed as described by Paladini *et al* (1999).

Antibodies The preparation and characterization of antisera to keratins 6, 16, and 17 have been described previously (Takahashi *et al*, 1994; McGowan and Coulombe, 1998a). The mouse monoclonal antibody against type I hair keratins (AE13) was the gift of Dr. Henry Sun (Lynch *et al*, 1986). Mouse monoclonal anti-K14 (LL001) was the gift of Dr. Irene Leigh (Purkis *et al*, 1990).

Immunologic analysis Immunoblot analysis was performed using 10 μg of hair extracts with recombinant keratin proteins as positive controls. The production of recombinant keratins has been described previously (Wawersik *et al*, 1997). Samples were electrophoresed and transferred to nitrocellulose; the blots were incubated with primary antisera diluted in blocking buffer (Tris-buffered saline with 0.5% Tween 20 and 5% powdered milk). Bound primary antibodies were revealed by alkaline-phosphatase-conjugated secondary antibodies as recommended by the manufacturer (Bio-Rad) or through enhanced chemiluminescence detection (Amersham).

Morphologic analysis Immunohistochemical analyses were performed on 5 μm sections prepared from either paraffin-embedded or fresh-frozen tissues. Tissues were fixed in Bouin's fixative overnight at 4°C. The fixed tissues were embedded in paraffin and 5 μm sections were stained with hematoxylin and eosin or immunostained. In addition, freshly isolated skin samples were washed in phosphate-buffered saline and quick frozen in liquid nitrogen using OCT (Sakura Finetek, Torrance, CA). The sections were incubated with the primary antisera and then revealed either with a peroxidase-based reaction (Kirkegaard and Perry Laboratories, Gaithersburg, MD) or by indirect immunofluorescence (Jackson Immunological Reagents, West Grove, PA).

Animal protocols All studies involving animals were reviewed by the Johns Hopkins University Animal Use and Care Committee. Hair samples from mouse, cat, monkey, and rat were obtained from animals housed at Johns Hopkins University. Human hair samples were provided by Dr. Stan Miller (Department of Dermatology, Johns Hopkins University). Pig and dog hairs were provided by Dr. Barbara Sollner-Webb (Department of Biological Chemistry, Johns Hopkins University).

RESULTS

K17 immunoreactivity in the medulla of the murine hair shaft Paraffin sections taken from mouse skin were immunostained with a rabbit polyclonal antibody to K17. These experiments revealed that, in addition to the previously reported staining in the outer root sheath (Moll *et al*, 1982; Stark *et al*, 1987; Troyanovsky *et al*, 1989; Panteleyev *et al*, 1997), the hair shaft also shows a positive immunostaining with the antibody (**Fig 1**). To localize this signal relative to the hard-keratin-expressing portion of the hair follicle these sections were stained with an antibody that recognizes type I hard keratins, AE13. This dual staining experiment revealed that the K17 and AE13 antigens are both expressed in the hair shaft, but do not colocalize in the subcompartments of this structure (**Fig 1*A-C***). Indeed, double-immunofluorescence staining of transverse sections clearly demonstrates that the K17 antigen is expressed in the medulla whereas the majority of hard keratin proteins are synthesized in the cortex (**Fig 1*D***). We did not detect K5 or K6 staining in this region (data not shown), keeping open the question as to what the type II partner for K17 may be in this compartment. We can readily detect K17 immunoreactivity in the matrix portion of the hair follicle (**Fig 1*E*, *F*).** The distribution of K17 protein in this portion of the hair displays a distinct polarity with the cluster of K17 positive cells located opposite to the direction of hair follicle outgrowth.

Identification of K17 in protein extracts prepared from hair clippings To further characterize the results obtained in tissue sections, Western blot analysis was performed on protein extracts prepared from mouse hair samples. To avoid contamination by outer root sheath keratins, the extracts were prepared from hair clippings rather than plucked hair. Additionally, to test that this result is not limited to the B6C3F1 mouse strain (**Fig 1**), hair clippings were obtained from a variety of mouse strains and subjected to Western blot analysis with several antibodies (**Fig 2**, *upper gel*). Independent of the mouse strain used, the hair extracts contain an immunoreactive band that comigrates with recombinant mouse K17 protein. The mouse strains examined include three nonpigmented (albino) hair samples and the first hair coat produced by hairless (HRS-/–) mice. Additionally, we do not observe any immunoreactivity with an anti-K14 antibody indicating that any contamination with outer root sheath components was minimal (data not shown). Likewise, antibodies to keratins 4, 5, and 6 fail to detect these proteins in the hair extracts (data not shown).

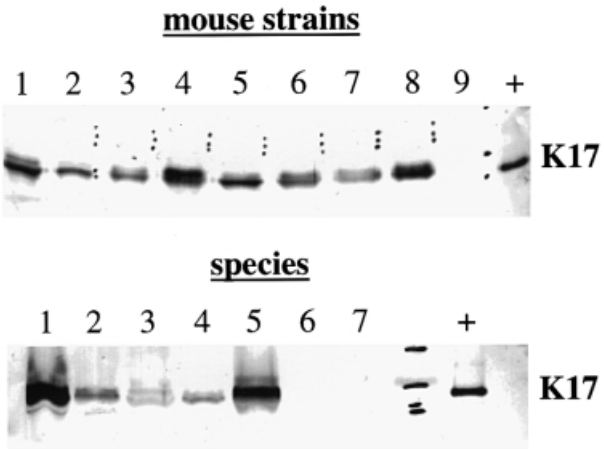

Figure 2. Western blot analysis of protein extracts prepared from hair clippings. Protein extracts were prepared from dorsal hair clippings obtained from a variety of mouse strains (*top gel*) and animal species (*bottom gel*). Extracts were prepared as described in the *Materials and Methods* section. These samples were loaded at a concentration of 5 μg per lane, electrophoresed, transferred to nitrocellulose membrane, and reacted with the K17 antibody. The top gel, containing the various mouse strains tested, was loaded as follows: *lane 1*, CD-1; *lane 2*, FVB; *lane 3*, ICR; *lane 4*, C57Bl6; *lane 5*, hairless; *lane 6*, C3B6F1; *lane 7*, C3B6F1 (K17-GFP); *lane 8*, 129 SvJ; *lane 9*, human scalp hair; (+) 50 ng recombinant human K17. The CD-1, FVB, and ICR strains are nonpigmented samples. The hairless [*hrs* (–/–)] mouse sample was obtained from the initial hair coat produced by these mice prior to the onset of hair loss. A similar analysis was performed on hair extracts obtained from several species of animals (*lower gel*). All hair samples, with the exception of the human scalp hair sample, were obtained from the dorsum of these animals. The samples were loaded as follows: *lane 1*, cat; *lane 2*, dog; *lane 3*, monkey; *lane 4*, mouse; *lane 5*, rat; *lane 6*, human scalp; *lane 7*, pig; (+) 50 ng recombinant human K17. All samples stained positively for the AE13 antibody to verify that the lack of K17 staining in the human and pig samples was not due to poor extraction (not shown). Identical blots did not stain positively with antibodies to either K14 or K6 (not shown) indicating that contamination of the preparations with outer root sheath proteins was minimal.

K17 expression in protein extracts prepared from other species Hair extracts were prepared from rat, dog, cat, monkey, pig, and human hairs using the identical procedure described above for mouse. Western blot analyses (**Fig 2**, *lower gel*) show that the hair extracts from all of these species, with the exception of human and pig, contain an antigen related to K17. K17 protein is readily detectable in the outer root sheath of human and pig hair follicles when applied to paraffin-embedded tissue sections (data not shown), confirming that the lack of reactivity in these samples is not due to a failure of the antibody to react with human or pig K17 proteins.

K17 expression in protein extracts prepared from various human body sites Protein extracts prepared from hair clippings obtained from various human body sites including the eyebrow, pubic, underarm, body, and whisker regions were analyzed for K17 protein via Western blot analysis (**Fig 3**, *upper panel*). The results indicate that the extracts prepared from eyebrow and whisker hairs contained detectable amounts of K17. The other body sites that were tested were lacking in K17 protein. Samples typical of those used to obtain protein extracts were analyzed by light microscopy

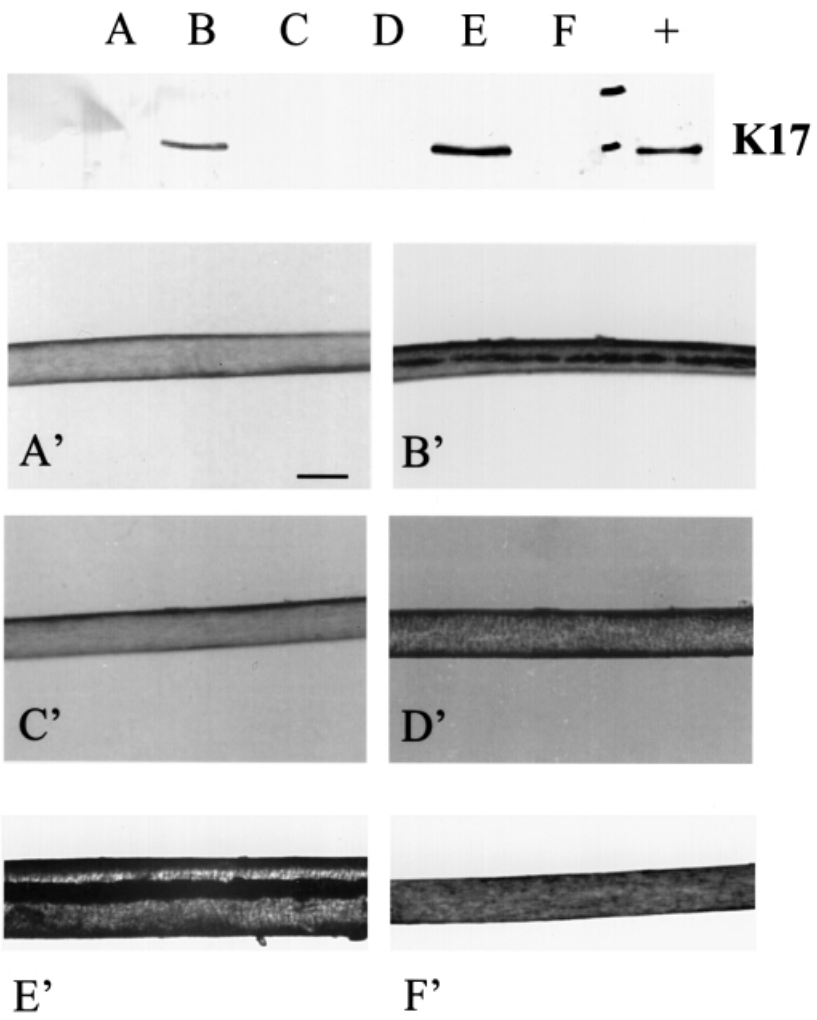

Figure 3. K17 expression in hair protein extracts prepared from various human body sites. Human hair extracts prepared from a variety of body sites were tested for K17 protein via Western blot analysis (*upper panel*). The samples were obtained from a single individual and were loaded (25 μg per lane) as follows: *lane A*, body hairs (back/chest/leg); *lane B*, eyebrow; *lane C*, pubic; *lane D*, scalp; *lane E*, whiskers/beard; *lane F*, arm axillae; (+) 50 ng recombinant human K17 (the marks between the lanes F and + are pen marks indicating molecular weight references). This analysis shows that extracts prepared from eyebrow and whisker hairs contained K17. *Lower panel*, hair follicles representative of those used for the preparation of the protein extracts were examined using a light microscope: *A′*, body hairs (back/chest/leg); *B′*, eyebrow; *C′*, pubic; *D′*, scalp; *E′*, whiskers/beard; *F′*, arm axillae. Both eyebrow (*B′*) and whisker (*E′*) hairs contain a visible medulla compartment seen as a dark-appearing band. *Scale bar*: 300 μm.

(**Fig 3**, *lower panel*). Both the eyebrow and whisker specimens contained a visible, continuous medulla compartment (**B′**, **E′**).

K17 expression in eyebrow and facial hair specimens The samples used for **Fig 3** were obtained from a single individual; therefore we tested additional eyebrow and facial hair specimens from several individuals (**Fig 4**). All of the samples tested contained K17 as detected by Western blotting. It should be noted that the level of K17 expression in the eyebrows was not uniform across the population that we tested. This lack of uniformity strongly paralleled the degree to which the medulla compartment was visible in these samples when examined by light microscopy (data not shown). The levels of K17 in the whisker preparations were more uniform among the test population and corresponded to the consistent appearance of a medulla compartment in this hair type (data not shown).

The expression of K17, K16, and K6 in the human nail The matrix portion of the nail, which contains the precursor cell population for the hard-keratin-containing nail plate, stains positively for K17 (**Fig 5*D***). The expression of K6 and K16 is nearly negligible in this portion of the nail (**Fig 5*B*, *C***). Contrarily, K6 and K16 immunoreactivity can be readily detected in the ventral portion of the proximal nail fold, whereas K17 synthesis is considerably less in this region. The expression patterns for K6, K16, and K17 display much greater overlap in the nail bed epithelium (**Fig 5*F-H***). Whereas K17 immunoreactivity is detectable throughout the entire nail bed including the basal

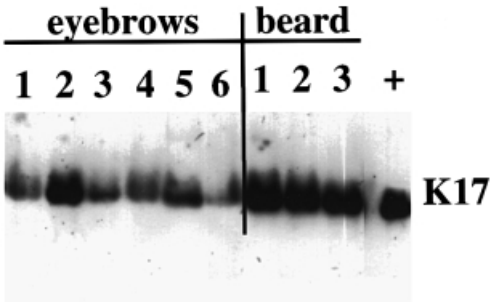

Figure 4. K17 expression in eyebrow and facial hair specimens. Given that the samples shown in **Fig 3** were obtained from a single source, a similar analysis was performed on eyebrow and whisker/beard protein extracts prepared from additional individuals. Equivalent amounts of protein extracts (40 μg per lane) prepared from six separate eyebrow samples (*lanes 1–6*) and three separate beard samples (*1–3*) were tested for K17 protein via Western blot analysis. The amount of K17 detectable in eyebrow samples displayed great variability (compare *lanes 2* and *6*) whereas the levels of K17 were more uniform in beard samples. Light microscopy of the individual eyebrow samples indicates that the medulla compartment of this hair type is generally more fragmented and discontinuous. It should be noted that the use of the ECL detection method (Amersham) for this experiment reflects the low abundance of K17 protein found in these extracts. The positive control (+) lane for these gels contains approximately 500 pg of recombinant human K17.

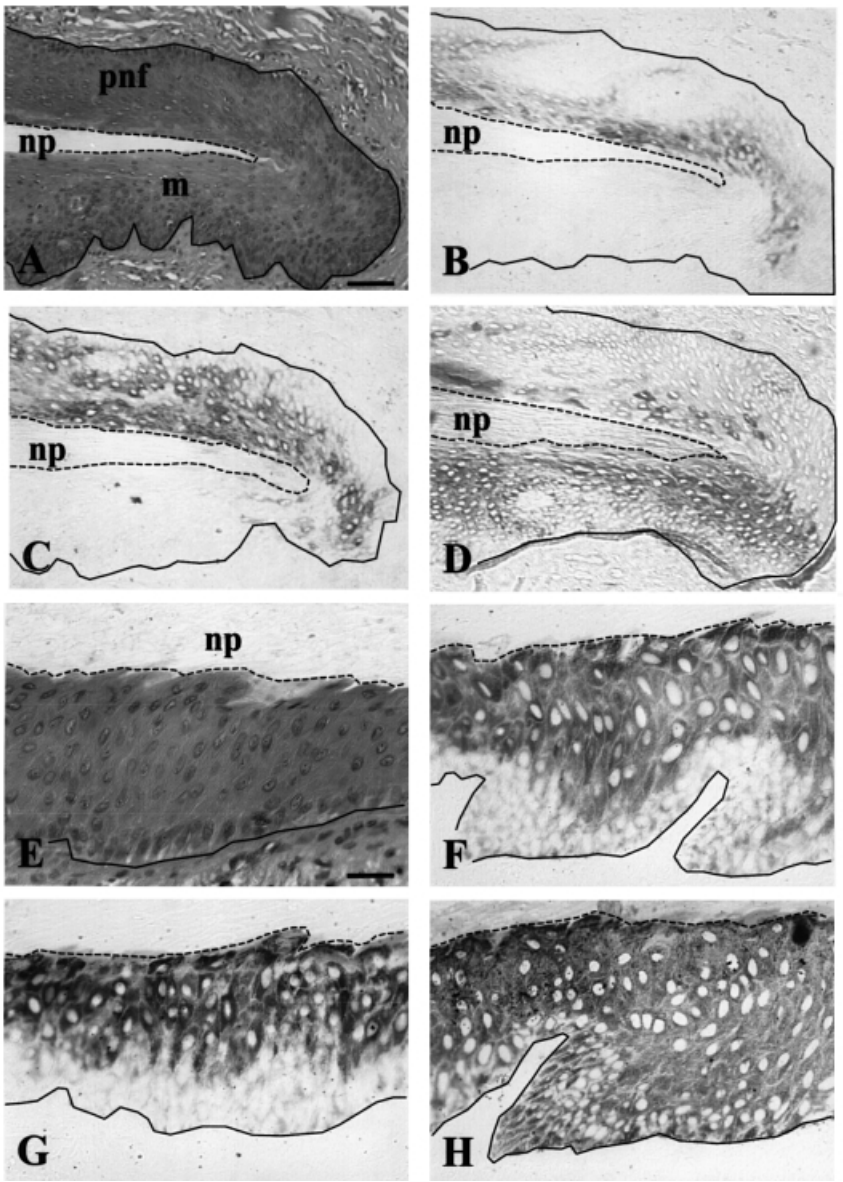

Figure 5. **Immunostaining of human nail for keratins 6, 16, and 17.** Paraffin-embedded tissue sections from a human nail sample were processed for hematoxylin and eosin staining (*A* and *E*) or immunostaining with antibodies against K6 (*B* and *F*), K16 (*C* and *G*), and K17 (*D* and *H*). *Panel (A)* shows the hard-keratin-containing nail plate (np) located between the nail matrix (m) and the ventral portion of the proximal nail fold (pnf). The dermal–epidermal boundary is indicated by a solid line and the interface between the nail plate and the adjoining epithelia is indicated by a dashed line. Keratins 6 and 16 (*B* and *C*) are detectable in the proximal nail fold but are absent from the nail matrix. Conversely, a positive immunostaining for K17 is observed primarily in the matrix region with a few scattered positive cells in the proximal nail fold (*D*). Hematoxylin and eosin staining of the nail bed epithelium is shown in (*E*). Keratins 6 and 16 (*F* and *G*) are expressed throughout the suprabasal compartment of this epithelium including cells directly adjacent to the nail plate (np) but are not detectable in the basal layer. K17 expression is more extensive (*H*) and includes the basal compartment (*arrowhead*). *Scale bar*: (*A-D*) 500 μm; (*E-H*) 200 μm.

layer, K6 and K16 are restricted to the suprabasal, postmitotic compartment. A similar phenomenon occurs in the outer root sheath of mouse hair follicles (McGowan and Coulombe, 1998a).

K17 GFP mice express green fluorescence protein (GFP) in their hair shafts We have produced a line of transgenic mice harboring a transgene consisting of the mouse K17 promoter driving the expression of the enhanced GFP. A complete characterization of these mice will be reported elsewhere**.** Hair clippings from these mice were used to produce protein extracts for Western blot analysis with an anti-GFP antibody (**Fig 6**). The GFP was detectable in these extracts, where it migrated as a doublet when compared with a recombinant control, suggesting that the expression of K17 in the hair shaft is controlled by elements in its promoter.

DISCUSSION

The predominant clinical feature of PC is, as the name suggests, a severe dystrophy of the nail. Although there has been much debate about the origin of this lesion (Fleckman, 1985), it is now widely accepted that the nail phenotype in all forms of PC disease arises from hyperkeratosis of the nail bed (Kelly and Pinkus, 1958) and not from alterations in the matrix. In fact, Kelly and Pinkus (1958) reported that the nail plate and matrix appear normal in PC. Our results, which demonstrate the coexpression of the K6, K16, and K17 genes in the nail bed epithelium, coupled with the similarity of nail alterations in type 1 and type 2 PC diseases, would support this conclusion. Similar to what we are reporting for the hair follicle, we observed K17 staining in the hard-keratin-synthesizing precursor cells located in the nail matrix. Our data further indicate that K6 and K16 are not synthesized in this portion of the nail but can be detected in the ventral nail fold in close proximity to the dorsal nail plate. It is interesting to note the close association between a hard-keratin-containing structure and the expression of K6, K16, and K17 that occurs in both the hair shaft and nail plate. Zaias (1965) noted that most of the nail bed epithelium remains attached to the nail plate following avulsion. Additionally, labeling studies demonstrated the growth rate and movement of both structures to be identical (Zaias, 1967, 1980; Zaias and Alvarez, 1968). A better understanding of the nature of this relationship, in particular whether it is dependent upon K6, K16, and K17 expression, will contribute significantly to the understanding of the nail phenotype in all forms of PC disease.

The distinction in the PC phenotypes produced by inherited mutations in the K17 versus K16 mutations results largely from differences in the expression patterns for these genes. As proof of

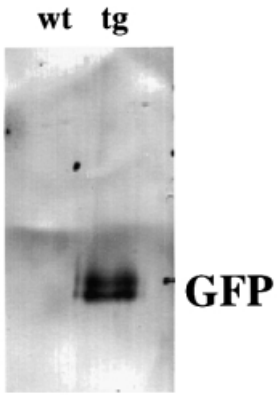

Figure 6. Western blot analysis for GFP expression in hair extracts prepared from K17 GFP mice. Hair extracts were prepared from dorsal hairs taken from mice harboring a transgene consisting of the mouse K17 promoter driving the expression of enhanced GFP. Ten micrograms of protein extracts prepared from a control littermate (wt) or a transgenic mouse (tg) were electrophoresed, blotted, and probed with a mouse monoclonal antibody to GFP. A doublet band was observed in the extract prepared from the transgenic mouse but not from the control. The expression of K17 in these samples is shown in **Fig 2** (*lanes 6* and 7, *upper gel*). The migration of GFP was consistent with its predicted molecular weight of 27 kDa and was comparable to a recombinant standard (not shown). The detected band migrated as a doublet, perhaps as the result of post-translational modifications occurring in the hair shaft.

this principle, we have recently described the expression of K17 in the early stages of epidermal appendage development (McGowan and Coulombe, 1998a). This observation substantiates a role for K17 in this context and may help explain some of the typifying features of type 2 or Jackson-Lawler PC disease such as the presence of neonatal teeth (Clementi, 1986; Feinstein *et al*, 1988). This report extends this principle by describing a unique aspect of the K17 gene, its expression in the medulla portion of the hair shaft. This pattern of expression may help to explain a defining feature of type 2 PC disease, the hair follicle phenotype.

The expression of K17 in the medulla may help to resolve two key issues concerning this aspect of the disease: how the twisted hair phenotype arises and how it is restricted to certain hair types. The various cell types that make up the hair follicle are derived from precursor cells located in the hair follicle matrix. Consistent with our observation that K17 is expressed in the medulla, we can detect K17 positive cells among the precursor cell population located in the matrix (**Fig 1*E-F***). Interestingly, the K17 positive cell population displays a polarized pattern of expression in the matrix that is similar to that reported by several groups for the sonic hedgehog molecule (Bitgood and MaMahon, 1995; Gat *et al*, 1998). Gat *et al* (1998) have suggested that disrupting the polarization of the sonic hedgehog molecule in the matrix impacts the orientation of hair follicles relative to the skin surface. Given that we observe that K17 expression is polarized in a similar manner and that mutations in the K17 gene result in a twisted hair phenotype, we would predict that this keratin plays a role in the proper function of this subset of matrix epithelial cells. A second feature of the hair follicle phenotype associated with PC type 2 is that it is more pronounced in the eyebrow and other coarse body hairs (McLean *et al*, 1995). It is somewhat difficult to reconcile this regional phenotype with the more general expression pattern of K17 in the outer root sheath. Our findings suggest that this aspect of the phenotype may originate from K17 expression in the medulla compartment. Our survey of body hairs suggests that only the coarser body hairs contain an extensive medulla compartment. Interestingly, these samples corresponded to those sites most often implicated in type 2 PC disease. Whether there is a direct correspondence between K17 protein and the presence of a medulla remains an open question. A thorough analysis of hair specimens from type 2 PC patients would provide an interesting test of this hypothesis.

The relationship between K17 gene expression and the type 2 PC disease phenotype suggests that the regulation of K17 gene expression is an important component in the pathogenesis of the disease. Panteleyev and colleagues examined the distribution of K17 mRNA during various stages in the murine hair cycle using *in situ* hybridization (Panteleyev *et al*, 1997). This group found that K17 message was present in the isthmus, suprainfundibulum, and bulge region, although previous immunostaining experiments did not report the synthesis of K17 protein in these regions. Based upon this lack of correlation between mRNA and protein it was proposed that K17 gene expression may be regulated at a post-transcriptional level via differential mRNA stability (Panteleyev *et al*, 1997). We have observed K17 protein in the regions in question, however: isthmus, suprainfundibulum, and bulge (McGowan and Coulombe, 1998a). Thus, we believe that the regulation of K17 gene expression is largely transcriptional. Previous studies have implicated members of the lymphoid enhancer factor/T cell factor (LEF/TCF) family of transcriptional complexes as key regulators of epidermal appendage development (Powell *et al*, 1991; van Genderen *et al*, 1994; Zhou *et al*, 1994). A recent study by Das Gupta and Fuchs (1999) clearly defines the temporal and spatial activity of these transcription factors in the epidermis. In their study, mice harboring a transgene containing a β-galactosidase gene under the control of an LEF/TCF inducible promoter demonstrated the activation of this promoter in the hard keratin precursor cells located in the hair follicle matrix. The K17 promoter contains binding sites for this family of transcription factors. We have previously shown that the ectopic expression of LEF-1 in the basal layer of the epidermis leads to K17 synthesis in this compartment (McGowan and Coulombe, 1998a), suggesting that K17 expression may be an LEF/TCF responsive gene. Similar to the experiments of Das Gupta and Fuchs (1999), we have constructed a transgene containing 2.0 kb of the mouse K17 promoter regulating the expression of the GFP cDNA. Hair extracts prepared from these mice contained detectable amounts of the reporter transgene product, indicating that the 5′ upstream region of the mouse K17 gene is transcriptionally active within the hair shaft and provides a molecular mechanism for K17 expression in this context.

We have provided experimental evidence that the soft keratin, K17, can also be expressed in the predominantly hard keratin portion of the hair shaft. The expression of K17 in this context may reflect a requirement for this protein in determining hair shape and orientation. Additionally, K17-containing filaments may provide unique mechanical properties required of coarser body hairs. Whether K17 plays an essential role in the formation and maintenance of these structures awaits the results of gene inactivation experiments and the function of K17 protein in the medulla remains an open question. Given its diverse pattern of expression, compared with other keratin genes expressed in the hair follicle, K17 may be uniquely suited for addressing the important questions of how keratin genes are regulated and how they impart functional diversity to epithelial tissues.

Note added in proof After the submission of our manuscript we became aware of a paper entitled ''Keratin expression in the normal nail unit: markers of regional differentiation'', by De Berker, Wojnarowska, Sviland, Westgate, Dawber and Leigh, which appeared in the *British Journal of Dermatology* 142:89–96, 2000. We refer readers to this paper for additional information regarding keratin gene expression in the nail.

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