# Characterization and Chromosomal Localization of Human Hair-Specific Keratin Genes and Comparative Expression During the Hair Growth Cycle

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**During anagen, cell proliferation in the germinative matrix of the hair follicle gives rise to the fiber and inner root sheath. The hair fiber is constructed from structural proteins belonging to four multigene families: keratin intermediate filaments, high-sulfur matrix proteins, ultra high-sulfur matrix proteins, and high glycine-tyrosine proteins. Several hair-specific keratin intermediate filament proteins have been characterized, and all have relatively cysteine-rich *N*- and *C*-terminal domains, a specialization that allows extensive disulfide cross-linking to matrix proteins. We have cloned two complete type II hair-specific keratin genes (ghHb1 and ghHb6). Both genes have nine exons and eight introns spanning about 7 kb and lying about 10 kb apart. The structure of both genes is highly conserved in the regions that encode the central rod domain but differs considerably in the *C*-terminal coding and noncoding sequences, although some conservation of introns does exist. These genes have been localized to the type II keratin cluster on chromosome 12q13 by fluorescence *in situ* hybridization. They, and their type I partner ghHa1, are expressed in differentiating hair cortical cells during anagen. In cultured follicles, ghHa1 expression declined in cortical cells and was no longer visible after 6 d, whereas the basal epidermal keratin hK14 appeared in the regressing matrix. The transition from anagen to telogen is marked by downregulation of hair cortical specific keratins and the appearance of hK14 in the epithelial sac to which the telogen hair fiber is anchored. Further studies of the regulation of these genes will improve our understanding of the cyclical molecular changes that occur as the hair follicle grows, regresses, and rests.** ***Key words: chromosome 12/DNA sequencing/FISH/hair growth cycle/intermediate filaments. J Invest Dermatol 110:158–164, 1998***

During the anagen phase of the hair cycle, cells of the germinative matrix proliferate rapidly and differentiate to generate the concentric cylinders of specialized keratinocytes which undergo terminal differentiation to form the hair fiber (cuticle, cortex, and medulla) and inner root sheath (cuticle, Huxley's layer, Henle's layer, and companion cells). The mechanism of cyclical hair formation and loss requires the regression and remodelling of the lower follicle, involving periodic reformation of a germinative matrix. The mesenchymal dermal papilla, which is retained from cycle to cycle, plays an instructive role in this process (Reynolds and Jahoda, 1991; Powell *et al*, 1991). This continual recapitulation of embryonic development makes the hair follicle a particularly interesting system for studies of complex tissue differentiation. Furthermore, developments in follicle maintenance and culture *in vitro* (Philpott *et al*, 1990) have made the hair follicle one of the few readily accessible human systems for studies of molecular differentiation.

Keratinocytes, which form the hair fiber, become committed to one of three major pathways of terminal differentiation (cuticle, cortex, or medulla), characterized by the synthesis of 50 or more specific structural proteins. These belong to at least four different multigene families: keratin intermediate filaments, high-sulfur matrix proteins, ultra high-sulfur matrix proteins, and high glycine-tyrosine proteins, which are activated during cortical cell differentiation (Kuczek and Rogers, 1987; Frenkel *et al*, 1989; Mackinnon *et al*, 1990; Powell *et al*, 1991, 1992; Fratini *et al*, 1993). Several human hair-specific keratin intermediate filament proteins have been described (hHa1–4, hHax, hHb1–4, hHbx) and these form a significant fraction of the dry mass of hair cortical cells (Heid *et al*, 1986; Lynch *et al*, 1986). They are homologous to but distinct from the keratin intermediate filament proteins expressed in the epidermis and other epithelia (Moll *et al*, 1982; Bowden, 1993). Hair-specific keratins are divided into type I acidic (40–48 kDa) and type II basic-neutral (58–65 kDa), are obligate heteropolymers, and show specific patterns of tissue coexpression (Bowden *et al*, 1987; Heid *et al*, 1988). Several hair-specific keratin genes have been characterized in sheep (Powell *et al*, 1992; Rogers and Powell, 1993), mouse (Bertolino *et al*, 1988, 1990; Kaytes *et al*, 1991), and man (Yu *et al*, 1993; Bowden *et al*, 1994; Rogers *et al*, 1995, 1996). These "hair-specific" keratins are also expressed in the nail, tongue, thymus, and rodent tail (Heid *et al*, 1988; Dhouailly *et al*, 1989).

There is recent evidence that expression of different type II keratin IF genes occurs progressively during cortical cell differentiation of sheep wool follicles (Powell *et al*, 1992), suggesting that an orderly sequence of expression of specific pairs of type I and II keratin genes

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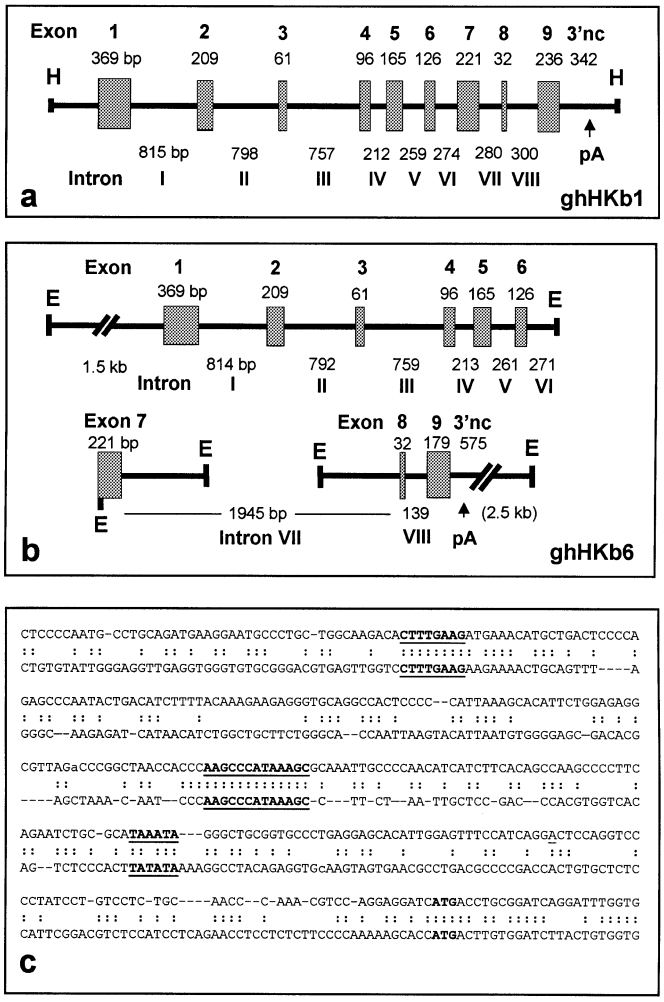


**Figure 1. Gene structure of ghHb1 and ghHb6 is highly conserved.** The gene structures of (*a*) ghHb1 and (*b*) ghHb6 are shown diagrammatically. There was only slight divergence in the intron sizes at the 5′ end of the gene but the introns at the 3′ end differed markedly. Exons 1–8 were identical in size but exon 9 differed between these two genes. Comparison of the 5′ noncoding sequences [(*c*) ghHb1 above and ghHb6 below] shows that the three promoter consensus sequences (HK1 box [Lef1], CAT box, and TATA box: *bold and underlined)* are highly conserved, as is the region around the initiation codon (ATG, *bold*) and the beginning of exon 1.

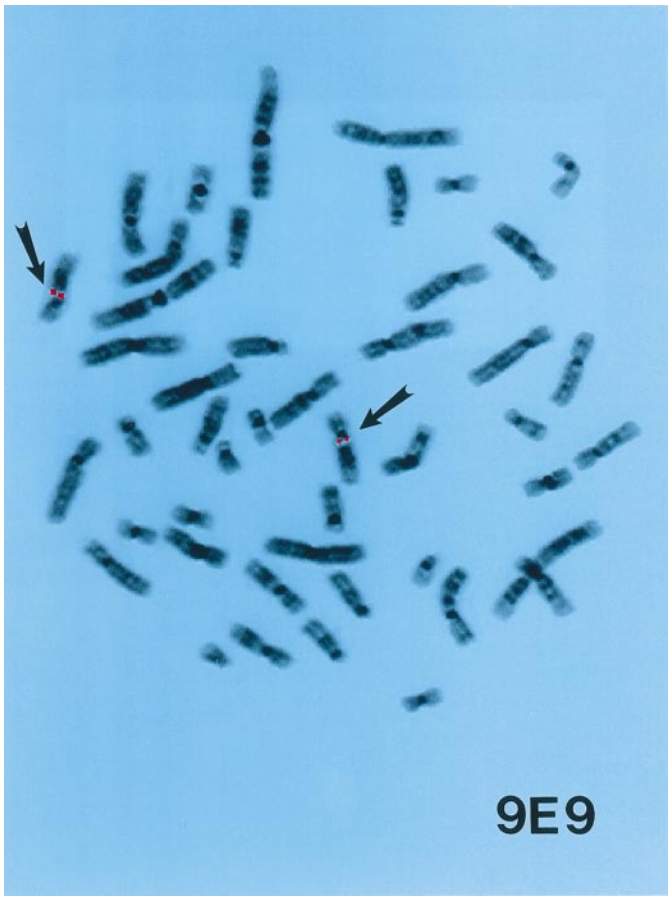


**Figure 2. Chromosomal in situ hybridization localizes ghHb6 to chromosome 12q13.** The 6-kb Eco RI fragment (9E9) of cosmid clone 9–1a was labeled with biotin-dUTP and used for fluorescence *in situ* hybridization on chromosome preparations from normal peripheral leukocytes. The probe fluoresced red when the chromosomes were counter-stained with DAPI. The probe clearly marks both copies of chromosome 12 just below the centromere (→), the location of the type II keratin gene cluster.

is important in cortical cell differentiation. A recent study has also shown that type II hair-specific keratins are differentially expressed in the human hair follicle (Rogers *et al*, 1997); however, there is still little information on activation of these genes at the onset of the growth cycle or their repression once the hair fiber reaches its definitive length and the follicle enters the resting stage (telogen). In the present study, we report the cloning, sequencing, and chromosomal localization of two complete type II human hair-specific keratin genes (ghHb1 and ghHb6). Additionally, we have examined hair-specific keratin intermediate filament expression with a previously cloned type I human hair-specific keratin probe (ghHa1, the presumptive partner of ghHb1) during the stages of active hair growth and follicle regression, by *in situ* hybridization *in vivo* and *in vitro*.

## MATERIALS AND METHODS

**Isolation, characterization, and subcloning of human cosmid clones** A human cosmid library (pWE15; Clontech, Palo Alto, CA) was screened with polymerase chain reaction (PCR)-cloned hair keratin genomic DNA (ghHKb2–1; Bowden *et al*, 1994) labeled by nick translation with [$\gamma^{32}$P]-dCTP (3000 Ci per mmole; Amersham, Little Chalfont, U.K.). Positive clones were purified to homogeneity, cosmid DNA isolated and digested with several restriction enzymes. Products were separated on 0.8% agarose gels, transferred to nylon membranes by Southern blotting, and hybridized to the original labeled hair keratin probe (ghHKb2–1). *Eco RI* and *Hind III* digests of cosmid DNA were ligated into pGEM-3Z (Promega, Southampton, U.K.) and were used to transform DH5α competent cells (Gibco BRL, Paisley, U.K.). Representative positive clones were identified by appropriate digestion of isolated plasmid DNA and agarose gel electrophoresis (Bowden *et al*, 1994).

**Sequencing of human cosmid subclones** DNA was isolated from human cosmid subclones by cesium chloride gradient centrifugation and sequenced by the dideoxy chain termination method (USB Sequenase, Amersham) and [α-$^{35}$S]-dATP (>3000 Ci per mmole; Amersham). The initial sequence reactions were primed with SP6 and T7 promoter primers and with exonic mouse hair-specific keratin primers, synthesized from published sequences (Bertolino *et al*, 1988, 1990; Kaytes *et al*, 1991; Yu *et al*, 1993). Sequence reactions were analyzed by electrophoresis on 6% and 8% denaturing polyacrylamide gels and visualized by autoradiography. Further sequencing of the cosmid clones was performed with overlapping synthetic oligonucleotide primers. All sequences were analyzed using IBI Pustell software (Cambridge, U.K.) on an IBM computer. The complete sequences have been submitted to the EMBL/Genebank nucleotide sequence database (accession numbers: ghHb1, Y13621, and ghHb6, AJ000263).

**Chromosomal *in situ* hybridization** Cosmid DNA subclones 9E7 (4.5 kb) and 9E9 (6 kb) were labeled with biotin- or digoxigenin-11-dUTP by nick translation. Fluorescence *in situ* hybridization was performed on chromosome preparations obtained from methotrexate synchronized normal peripheral leukocyte cultures as previously described (Zimonjic *et al*, 1994; Popescu *et al*, 1994). Biotin-labeled DNA was detected by fluorescein isothiocyanate conjugated avidin DCS and anti-avidin antibodies (Vector Laboratories, Burlingame, CA). Digoxigenin-labeled DNA probes were detected by anti-digoxigenin (mouse), anti-mouse Ig-digoxigenin, and anti-digoxigenin-rhodamine or anti-digoxigenin-fluorescein (Boehringer, Lewes, U.K.). Chromosomes were

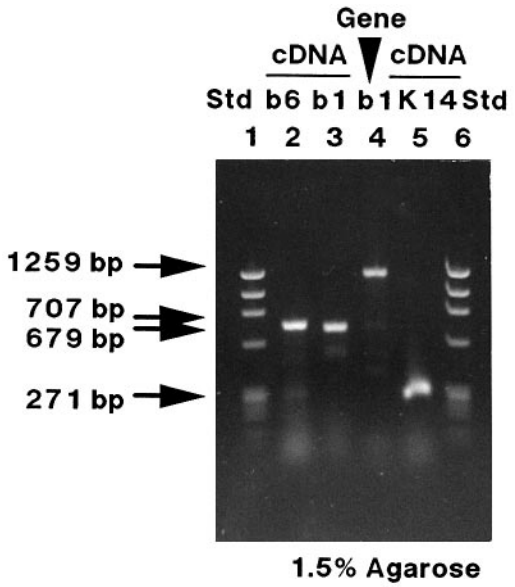


**Figure 3. ghHb1 and ghHb6 genes expressed in human hair follicles.** Total RNA was isolated from freshly plucked human anagen hair follicles and reverse transcribed to obtain total hair follicle specific cDNA. The cDNA was then amplified by PCR using ghHb1, ghHb6, and hK14 specific primers. The generated fragments (707 bp for ghHb6, *lane 2*; 679 bp for ghHb1, *lane 3*; and 271 bp for hK14, *lane 5*) were analyzed by agarose gel electrophoresis. Genomic DNA contamination was ruled out by amplifying over intron 8 that increases the size of the product from gDNA (1259 bp, *lane 4*). ϕX174 DNA digested with Hae III was used as a standard (std; *lanes 1* and *6*).

counter-stained with propidium iodide or 4′,6-diamino-2-phenoylindole dihydrochloride (DAPI) and examined with an Olympus BH2 epifluorescence microscope (Tokyo, Japan). Digital image acquisition processing and analysis and direct visualization of fluorescent signals to banded chromosomes were carried out as previously described (Zimonjic *et al*, 1995).

**Hair keratin expression [reverse transcriptase polymerase chain reaction (RT-PCR)]** Plucked human anagen scalp hair follicles were placed into RNAzol (Biogenesis, Poole, U.K.), homogenised, and total RNA extracted (Promega Kit). Total cDNA was generated with oligo-dT primed AMV reverse transcriptase and human hair-keratin cDNA were amplified by PCR using specific primers: 9E8p46 (5′-GAGTACCAGGAGGTGATGAACTCC-3′) and 9E8p8R (5′-AACACAGATCAAGAGCAG-3′) for ghHb1, and 9E7p9 (5′-AGGCGTCGGCTCGGTGAATGTCTG-3′) and 9E7p10R (5′-TTTCA-GAAGCTCTTGAGGGCAGCC-3′) for ghHb6. The specific nature of the *C*-terminal and 3′ noncoding sequences of ghHb1 and ghHb6 allowed these primer sets to specifically detect these genes and their encoded mRNA. The primer sets were designed over at least one intron so genomic DNA contamination of the hair follicle cDNA preparations could be detected. Amplified DNA was analyzed on agarose gels and stained with ethidium bromide.

**Follicle organ culture** Isolated scalp hair follicle cultures were based on an established technique (Philpott *et al*, 1990) using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 mM HEPES, 4 mM L-glutamine, 10 μg gentamycin per ml, and 2.5 μg fungizone per ml. Skin cut into 2 mm thick slices (axis parallel to emergent hair) washed in Hanks-HEPES solution containing gentamycin and fungizone was transferred to DMEM-HEPES and dissected under a stereo microscope in a laminar flow hood. Large anagen hair follicles were maintained individually in 1.0 ml supplemented DMEM (24 well plates) at 37°C in 5% $CO_2$/air. Follicles were observed and photographed at daily intervals.

**Tissue in situ hybridization** Sense and anti-sense [$^{35}$S]-riboprobes specific activity $>10^8$ cpm RNA per μg) were transcribed by SP6 and T7 RNA polymerases from previously cloned riboprobes (pGEM-3Z, Promega) containing *C*-terminal and 3′ noncoding fragments of either a human type I hair-specific keratin gene (ghHa1) or a human K14 (Bowden *et al*, 1994). *In situ* hybridization was carried out as described (Millan *et al*, 1991) on normal human scalp skin or isolated hair follicles fixed overnight in 4% paraformaldehyde-PBS. Five micometer sections were digested with proteinase K (20 μg per ml), acetylated, and hybridized overnight at 52°C (10 μl solution containing $3 \times 10^5$ cpm probe), washed with 50% formamide in 2 × sodium citrate/chloride buffer at 65°C, digested with RNase A, and washed again in 0.1 × sodium citrate/chloride buffer. The slides were dipped in Ilford K5 emulsion, developed after 7 d, and counter-stained with hematoxylin and eosin. Slides were dehydrated and mounted with DPX resin.

## RESULTS

**Characterization of cosmid clones** Screening of a human cosmid library with an 800 bp PCR-generated hair-specific keratin probe (ghHKb2–1; Bowden *et al*, 1994) yielded several positive signals and restriction enzyme digestion showed that two of these clones (5–2a and 9–1b) were closely related but not identical. Southern blotting with the PCR probe (ghHKb2–1) identified two *Eco RI* fragments (11 kb and 1.2 kb) that contained sequences homologous to exons 7–9 of the type II keratin probe (Bowden *et al*, 1994), inferring the presence of at least two keratin genes on this 40 kb cosmid clone.

Digestion of the cosmid clone 9–1b with *Eco RI* gave six fragments (11 kb, 6 kb, 4.5 kb, 2.5 kb, 1.2 kb, and 0.9 kb) in addition to the vector (9 kb) whereas *Hind III* gave nine fragments (11 kb, 7.5 kb, 5 kb, 4 kb, 3 kb, 1.4 kb, 1.0 kb, 0.8 kb) including the vector. These *Eco RI* and *Hind III* fragments were subcloned into pGEM-3Z and sequenced from the SP6 and T7 promoter primers. Those fragments suspected to contain keratin genes were also sequenced with internal primers to mouse hair keratin cDNA (Bertolino *et al*, 1988, 1990; Kaytes *et al*, 1991). Four *Eco RI* and two *Hind III* subclones of cosmid 9–1b DNA were found to contain keratin encoding sequences.

A complete hair-specific keratin gene (ghHb1) was found on the 11 kb *Eco RI* fragment (subclone 9E8) within a 7.5 kb internal *Hind III* fragment. The complete structural gene consisted of nine open reading frames bordered by consensus exon-intron splice sites spanning 5552 bp from the ATG to the poly A site (EMBL/Genebank Accession # Y13621). The predicted transcript from ghHb1 is 1.9 kb, giving rise to a protein of 505 amino acid residues. The predicted molecular weight of the protein is 54,949 and the pI is 6.14.

The smaller 6 kb *Eco RI* fragment (subclone 9E9) contained six open reading frames encoding exons 1–6 of a type II hair-specific keratin gene bordered by consensus splice sites. The adjacent 1.2 kb *Eco RI* fragment downstream (subclone 9E12) was positive on the Southern blot and sequencing located exon 7 and a large intron. The next *Eco RI* fragment downstream was subclone 9E7 (4.5 kb), which contained the 3′ end of the cosmid insert including part of the cosmid vector. Sequencing of this subclone revealed exons 8 and 9 together with the 3′ noncoding portion of a second type II hair-specific keratin gene (ghHb6). Combining the sequence from these three adjacent subclones gave a total size of 7117 bp from the ATG to the polyadenylation signal (EMBL/Genebank accession #AJ000263). The predicted transcript from ghHb6 is 2.1 kb, which encodes a protein of 486 amino acid residues with a predicted molecular weight of 53,478 and a pI of 6.22.

Comparison of these two keratin genes showed that they were closely related but not identical (**Fig 1*a*,*b***). In particular, not only were exons 1–7 of both genes almost identical in size and sequence, but so were the intervening introns (I–VI); however, a sharp divergence occurred at intron 7 and exon 9, which encodes the *C*-terminal domain of the protein. This region was divergent between these two related hair-specific keratin genes, a phenomenon observed for many functionally related keratin genes. The helical encoding sequences were partially homologous to published human type II epithelial keratins and the complete coding sequences were highly homologous to human, mouse, and sheep type II hair-specific keratins.

The larger *Eco RI* fragment (subclone 9E8) not only contained a complete type II hair-specific keratin gene (ghHb1), but also had 3 kb of upstream regulatory sequences and 1.6 kb of downstream noncoding sequence, making this clone ideal for studies of gene regulation. In addition, 15 kb of cosmid sequence upstream of this gene still remains uncharacterized. The upstream regulatory sequences (**Fig 1*c***) not only contained TATA and CAT box motifs but also had the HK1 box (LEF1) consensus sequence (CTTTGAAG) found in other hair-keratin genes. The exon and intron sequences of 9E8 were 100% homologous to the original PCR probe (hHKb2–1) and almost 100% homologous to a published hair-specific keratin cDNA (Hb1; Rogers *et al*, 1995).

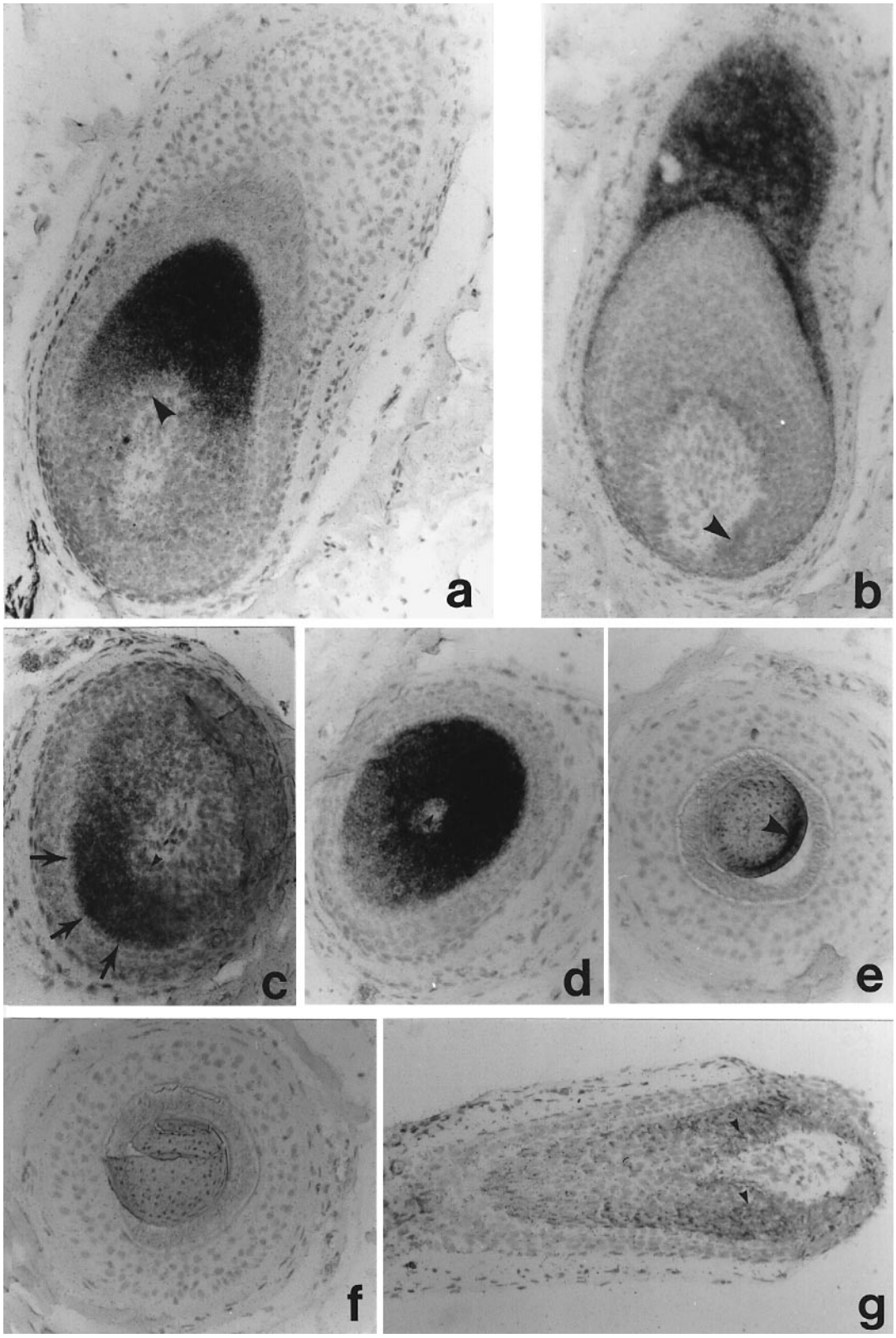


**Figure 4. Human anagen hair follicles express hHa1 in keratogenous zone.** Bright field autoradiographs showing sites of probe hybridization as black silver grains on a counter-stained background (hematoxylin and eosin). (*a*) Longitudinal section of a large anagen follicle with an anti-sense probe to hHa1 demonstrates labeling of hair cortical cells (keratogenous zone) but no labeling in the germinative matrix and cells adjacent to the dermal papilla (*arrowhead*). (*b*) In comparison, labeling with a hK14 probe shows labeling of the outer root sheath but not the germinative matrix (*arrowhead*) or the keratogenous zone. (*c*) Transverse section close to the apex of the dermal papilla (*small arrowhead*) showing hHa1 expression in cortical cells (*arrows*). (*d*) Transverse sections distal from the dermal papilla showing hHa1 expression throughout the cortex but absent from the central medulla (*small arrowhead*). (*e*) Transverse section at the upper limit of the keratogenous zone showing hHa1 expression restricted to the outer rim of the cortex (*arrowhead*). (*f*) Transverse section of an anagen follicle treated with a sense probe to hHa1 (negative control). (*g*) Longitudinal section treated with the hHa1 sense probe (negative control). The black deposits are due to melanin deposition (*small arrowhead*).

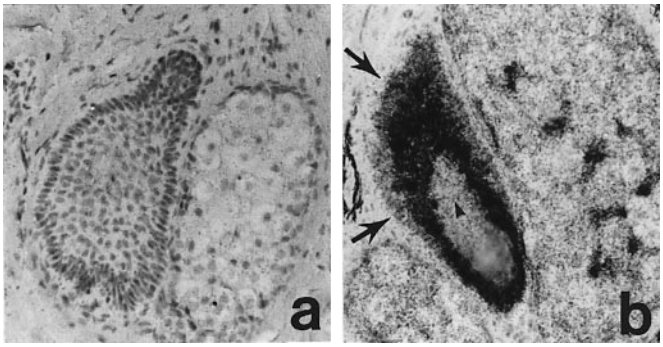


**Figure 5. No hair-specific keratin expression in telogen hair follicles.** (*a*) Longitudinal section of a telogen hair follicle shows no hHa1 hybridization in any part of the hair follicle or the adjacent sebaceous gland. (*b*) The same telogen hair follicle hybridized to a hK14 anti-sense probe showing a strong signal in cells of the epithelial sac surrounding the keratinized hair root (*small arrowhead*). The hK14 signal appears weaker in the basal cells (*arrows*).

The type II hair-specific keratin gene (ghHb6) found on subclones 9E9–9E12–9E7 was similar to ghHb1 but intron VII was very large (1945 bp), both intron VIII and exon 8 were smaller, and the 3′ noncoding was larger. Clone 9E9 contained 2 kb of 5′ upstream regulatory sequences and the same three consensus motifs [TATA box, CAT box, and HK1 box (LEF1)] were found immediately upstream of the ATG (–106, –154, and –265, respectively). The unique portions of this keratin gene were compared with all known hair-specific and epithelial keratin genes (human, mouse, sheep) and homology was found to a partial sheep wool sequence (sWKII-11; Powell *et al*, 1992) and a human cDNA sequence (Hb6) cloned from hair follicles (Rogers *et al*, 1997).

**Chromosomal localization of type II hair-specific keratin genes** Genomic DNA from subclones 9E9 (exons 1–6) and 9E7 (exons 8–9 plus 3′ noncoding) of ghHb6 was used for *in situ* hybridization on normal human prophase and metaphase chromosomes. Both probes produced specific signals on chromosome 12q. In preparations hybridized with labeled 9E9 probe, fluorescent signal was detected in 93 of 120 randomly selected chromosome spreads (77.5%) with low nonspecific fluorescein isothiocyanate background. Hybridization consisting of symmetrical fluorescent spots on the long arm of both chromatids of chromosome 12 was observed in 72.5% of the chromosome spreads. In 54 cases (45%), both copies of chromosome 12 were labeled (**Fig 2**). The same results were obtained in two independent experiments and hybridization signals on both sister chromatids were not observed on any other chromosome. Similar results were obtained with 9E7 (data not shown) and in both cases chromosome identity was determined by re-hybridization with a painting probe specific for chromosome 12. On this basis, the human type II hair keratin locus was assigned to chromosome 12q13.

**Hair keratin gene expression (RT-PCR)** RT-PCR of human hair follicle RNA with ghHb1 specific primers (9E8p46 and 9E8p8R) yielded a cDNA product of 679 bp and PCR with genomic DNA yielded a product of 1259 bp (**Fig 3**). A cDNA product of 707 bp was obtained from hair follicle total RNA (**Fig 3**) with ghHb6 specific primers (9E7p9 and 9E7p10R). Sequencing of both these cDNA products showed 100% homology to the exons of the cloned genes (data not shown). Furthermore, these type II hair-specific keratin primers gave no product with epidermal cDNA (data not shown).

***In situ* hybridization of ghHa1 and hK14 in skin sections** A detailed study of the expression pattern of the ghHa1/ghHb1 pair was conducted using a previously described riboprobe recognizing ghHa1 (Bowden *et al*, 1994). This hair-specific keratin was abundantly expressed in the differentiating cortex of growing (anagen) hair (**Fig 4**). Expression appeared to be restricted to the keratinocytes of the hair cortex (**Fig 4*a***) and was absent from inner root sheath and medulla (**Fig 4*d*,*e***). The onset of ghHa1 expression commenced about 2–3 cells above the apex of the dermal papilla (**Fig 4*a*,*c***), indicating that keratinocytes immediately below are not committed to the cortical pathway of differentiation. The sense control probes showed no signal (**Fig 4*f*,*g***). The epidermal type I keratin (hK14) was strongly expressed in the outer root sheath of anagen follicles but not in the germinative matrix, inner root sheath, or hair (**Fig 4*b***); however, hK14 was observed in keratinocytes surrounding the club hair during telogen when ghHa1 expression was no longer seen (**Fig 5*a*,*b***). At the onset of anagen ghHa1 expression was not seen before differentiation of hair cortex and inner root sheath was apparent. At this stage hK14 mRNA, although persisting in cells close to the old hair fiber, appeared to be downregulated in the newly forming germinative matrix.

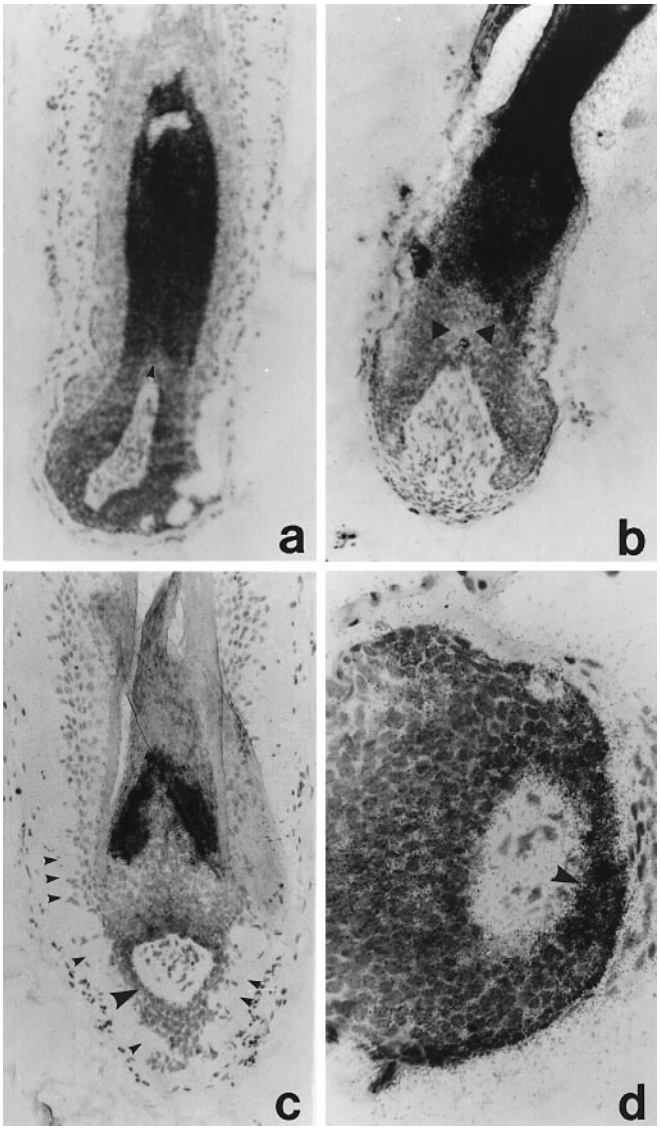


**Figure 6. Isolated hair follicles cultured *in vitro* have limited expression of hair-specific keratins.** (*a*) An anti-sense probe shows expression of hHa1 in a human anagen hair follicle after 24 h in culture. The cortex is well labeled but the cell layers (*small arrowhead*) adjacent to the dermal papilla are not. (*b*) After 48 h in culture, labeling of the germinative matrix shows signs of regression away from the dermal papilla apex (*arrowheads*). (*c*) At 6 d, the cultured hair follicle shows very little hHa1 expression. The germinative matrix has regressed (*arrowhead*) and a prominent wrinkled glassy membrane has formed around the outer root sheath (*small arrowhead*). (*d*) After 48 h in culture, the hair follicles show hK14 expression in the lower germinative matrix (*arrowhead*) adjacent to the dermal papilla.

***In situ* hybridization of ghHa1 and hK14 during follicle culture** Scalp hair follicles maintained in unsupplemented DMEM continued to produce a hair fiber for 4 d before cell division in the germinative matrix ceased and the follicle entered a state closely

resembling catagen. Noticeable features of similarity between *in vivo* and *in vitro* catagen follicles were the pronounced wrinkling of the outer root sheath and thickening of the basal lamina and glassy membrane, regression of the germinative matrix, and condensation of the dermal papilla. At the onset of catagen *in vitro*, ghHa1 expression was strong in the cortex to within two or three cells of the dermal papilla (**Fig 6*a***); however, as the germinative matrix regressed ghHa1 was lost from the lowest rows of cortical cells, although further along the hair shaft the signal remained strong even when the matrix had almost completely regressed (**Fig 6*b*,*c***). hK14 appeared in the regressing matrix and in keratinocytes adjacent to the dermal papilla (**Fig 6*d***).

## DISCUSSION

Hair-specific keratin IF genes encode a family of proteins closely homologous to, but distinct from, the other epithelial keratins. In both cases, type II keratin genes exist on chromosome 12q and type I on chromosome 17q with K18 being an exception (Rosenberg *et al*, 1988; Popescu *et al*, 1989; Yoon *et al*, 1994). The present data show that the hair-specific keratin IF genes, ghHb1 and ghHb6, are localized at chromosome 12q13 with the other type II keratin genes.

In terms of size, ghHb1 is smaller than ghHb6, largely because of the size of intron VII. Both genes have similar promoter motifs immediately 5′ of the translational start codon (ATG) and in both genes introns I–III are quite large (815 bp, 798 bp, and 757 bp). A similar structure has been reported for the homologous sheep wool genes (Powell *et al*, 1992). Thus, it is clear from the chromosomal localization, intron-exon structure, presence of promoter elements, and polyadenylation signals that these two genes are probably functional. Further evidence for expression comes from the independent cloning of cDNA for both genes (Rogers *et al*, 1996, 1997) and our own RT-PCR data. In addition to generating specific signals from hair follicle cDNA, we also sequenced the cDNA products, confirmed they were 100% homologous to the genes, and showed that the splice sites for exons 8 and 9 were correct. These are the first two complete human type II hair-specific keratin genes to be characterized and the sequence information will be useful in designing primers to investigate mutations in genetic diseases of the hair and nail (Healy *et al*, 1995; Stevens *et al*, 1996; for review see Rothnagel, 1996)

Although the hair follicle is an epidermal derivative, it appears that expression of hair-specific and epidermal keratin genes is to some extent mutually exclusive. The epidermal basal cell keratins (K5 & K14) are strongly expressed in the outer root sheath of the anagen follicle but not in the germinative matrix, hair fiber, or inner root sheath. In contrast, the hair-specific keratin genes identified to date are expressed only in cells which are committed to form hair cortex and cuticle (present data; Powell *et al*, 1992; Rogers *et al*, 1996, 1997). This absence of typical epidermal and hair keratin expression in the matrix is consistent with the ultrastructural appearance of those cells that contain very few IF bundles (our unpublished data). Accumulating evidence supports the view that at the start of each hair growth cycle, new matrix cells are recruited from elements of outer root sheath that remain after regression of the lower follicle (Cotsarelis *et al*, 1990; Rochat *et al*, 1994). Thus, it appears that repression of epidermal keratin synthesis is an essential prerequisite before expression of the hair-specific keratin phenotype is activated. This would be consistent with the observation that expression of a mouse type I or type II hair-specific keratin in Hela cells produced partial collapse of the endogenous epithelial keratin IF network (Yu *et al*, 1991).

In follicle organ culture, hair-specific keratin expression persists in cortical cells, which are about 10 or more cells above the dermal papilla, indicating either that the mRNA is long lived or that cortical cells become irreversibly committed to ghHa1/ghHb1 expression until terminal differentiation is complete; however, hHa1 mRNA disappears from cells between two and 10 cell layers above the papilla when fiber formation ceases. This restriction of hHa1 at the end of anagen may explain why Rogers *et al* (1997) only observed its partner hHb1 in cortex cells 10–15 layers distal to the apex of the dermal papilla.

An obvious feature of catagen *in vitro* is the appearance of hK14 mRNA in cells of the regressing matrix, expression that eventually extends around the base of the follicle epithelium adjoining the dermal papilla. This would be consistent with migration of outer root sheath cells around the base of the club hair and could explain why hK14 expression has occasionally been observed to extend from the outer root sheath to the cells around the dermal papilla (Coulombe *et al*, 1989).

It is clear that further work is needed to understand the changes between hair-specific and epidermal-specific keratin gene expression during the hair growth cycle. The total number of hair-specific keratin cDNA is still incomplete and there are very few full-length human gene sequences available, a strong indication that further cloning and sequencing of this interesting gene family is required. Once the regulatory regions of these and other hair-specific keratin genes have been defined, the molecular mechanisms required for switching from hair-specific to epidermal-specific gene expression (and vice versa) during the hair growth cycle will be better understood.

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