

Human Hair Keratin-Associated Proteins

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Hair keratin-associated proteins (KAP) are a major component of the hair fiber, and play crucial roles in forming a strong hair shaft through a cross-linked network with keratin intermediate filaments (KIF), which are produced from hair keratins. Recently, the study of human KAP has advanced significantly. So far, five clusters of human KAP genes have been characterized, leading to the identification of more than 80 individual human KAP genes. *In situ* hybridization studies have demonstrated sequential and spatial expression patterns of these KAP members in differential portions of the hair fiber cortex and cuticle. Furthermore, several human KAP genes have size polymorphisms that are mainly because of variable numbers of cysteine-rich repeat segments, and the patterns of some of these size variants are distinct between different human populations.

Key words: KAP/KIF/size polymorphism

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Definition and Classification of Keratin-Associated Proteins (KAP)

Hair is a strongly keratinized tissue formed within the hair follicle. Growth of the hair originates in matrix cells located in the bulb region. As trichocytes pass through the keratogeneous zone, keratinization occurs actively and a rigid hair shaft is produced. Hair fiber consists of an external cuticular layer, an inner cortex, and occasionally a central lying medulla. In the cross-section of the mature human hair cortex, the packing arrangements of keratin intermediate filaments (KIF) are observed in the cytoplasm of the cortical cells, and each KIF, about 8–10 nm in diameter, is surrounded by an amorphous space called matrix. The major structural components of hair fiber are hair keratins and hair KAP. The hair keratins form the KIF in trichocytes. The hair KAP, termed KAP, are located in the matrix around the KIF, and are therefore also called matrix proteins. KAP are encoded by a large number of multigene families. Generally, each KAP gene consists of a single exon without any introns. KAP are relatively small, hydrophobic proteins, and often have characteristic repeat structures. KAP are a major component of the matrix between the KIF and may be responsible for forming the rigid hair shaft through extensive disulfide bond cross-linking with the KIF (Powell and Rogers, 1997).

On the basis of their amino acid composition, KAP were originally classified into three groups: high sulfur (<30 mol% cysteine content), ultrahigh sulfur (>30 mol% cysteine content), and high glycine/tyrosine KAP. As several KAP were identified, however, the nomenclature became complicated. To resolve this problem, Rogers and Powell (1993) proposed a unified nomenclature for KAP. In this no-

menclature, KAP are subdivided into families based on their sequence homology and the nature of the repeat structures (Rogers and Powell, 1993). Each KAP has been given a name in the form “KRTAPm-n”, whose is “KAPm.n”; “m” refers to the family and “n” refers to a number identifying the component. To date, more than 100 KAP have been isolated from sheep, mice, rats, rabbits, and humans, and classified into a total of 23 families. Of these, families 1–3, 10–16, and 23 belong to high-sulfur KAP, families 4, 5, 9, and 17 represent ultrahigh-sulfur KAP, and families 6–8 and 18–22 are high glycine/tyrosine KAP (Rogers and Powell, 1993; Powell and Rogers, 1997; Rogers *et al*, 2001, 2002).

Clusters of Human KAP Genes

During the last three decades, KAP have mainly been studied in sheep and mouse. Recent advances in the human genome project, however, have led to the identification of large domains of human KAP genes. So far, five clusters of human KAP genes have been reported (Rogers *et al*, 2001, 2002, 2004; Yahagi *et al*, 2004) (Table I). As a rule, most KAP families consist of several genes and pseudogenes that share high nucleotide sequence homology with each other (Rogers *et al*, 2001, 2002, 2004). These multiple family members might have arisen by gene duplication events, perhaps in response to high evolutionary pressure that favored producing more rigid hair.

The first cluster of human KAP genes that was identified is located on chromosome 17q12–21 (Rogers *et al*, 2001). It is composed of four high-sulfur (KAP1–3 and 16) and three ultrahigh-sulfur (KAP4, 9, and 17) KAP families (Rogers *et al*, 2001). Interestingly, this large domain, which harbors 34 KAP genes and four KAP pseudogenes, is inserted into the type I keratin gene cluster (Rogers *et al*, 2001). Generally, high- and ultrahigh-sulfur KAP contain many cysteine-rich pentapeptide repeat structures (Rogers *et al*, 2001). *In situ*

Abbreviations: KAP, keratin-associated proteins; KIF, keratin intermediate filaments

Table I. Clusters of the human KAP genes

KAP family	Chromosomal location	Total number of KAP genes and pseudogenes on each cluster
KAP1–3 and 16 (high sulfur) KAP4, 9, and 17 (ultrahigh sulfur)	17q12–21	34 genes and four pseudogenes
KAP11, 13, 15, and 23 (high sulfur), KAP6–8 and 19–22 (high glycine/tyrosine)	21q22.1	24 genes and nine pseudogenes
KAP10 and 12 (high sulfur)	21q23	16 genes and two pseudogenes
KAP5 (ultrahigh sulfur)	11q15	Six genes
KAP5 (ultrahigh sulfur)	11q13	Five genes

KAP, keratin-associated proteins.

hybridization studies demonstrated that the transcripts of six high-sulfur (*KAP1.1B*, *KAP1.3*, *KAP1.4*, *KAP1.5*, *KAP2.4*, and *KAP3.3*) and two ultrahigh-sulfur (*KAP4.3* and *KAP9.2*) KAP members in this domain are expressed predominantly and strongly in the highly differentiated portions of the middle and upper keratogeneous zone in the hair cortex (Rogers *et al*, 2001; Shimomura *et al*, 2002a, b, 2003). Furthermore, indirect immunofluorescence using a pan-KAP1 antibody revealed that the KAP1 proteins are expressed exactly in the same region as their transcripts (Shimomura *et al*, 2002b). Note that a pan-antibody must often be used as it may be impossible to render an antibody specific to an individual KAP protein, because KAP members in the same family share such high amino acid sequence homology.

The second cluster of human KAP genes identified is located on chromosome 21q22.1 (Rogers *et al*, 2002). This domain contains 24 KAP genes and nine KAP pseudogenes, which are classified into four high-sulfur (KAP11, 13, 15, and 23) and seven high glycine-tyrosine (KAP6–8 and 19–22) KAP families (Rogers *et al*, 2002). *In situ* hybridization revealed that the initiation point and the signal intensity of their transcripts are variable, and some of these genes are expressed not only in the cortex but also in the cuticle layer (Rogers *et al*, 2002). The most interesting observation was that the expression of KAP6 and KAP8 family members in the cortex is vertically compartmentalized (Rogers *et al*, 2002). Several sheep KAP showed a similar expression pattern (Fratini *et al*, 1993; Powell and Rogers, 1997). In sheep, this phenomenon is apparently correlated with the bilateral asymmetry of para- and ortho-cortical cells, which have different ultrastructural organizations and appear to contain different proportions of KIF and KAP (Rogers and Powell, 1993). As no similar bilateral segmentation of these

two cell types is observed in human hair, the cause and consequence of the asymmetric distribution of KAP in human hair remain unknown.

The third cluster of human KAP genes to be identified, which lies on chromosome 21q23, harbors 16 KAP genes and two KAP pseudogenes (Rogers *et al*, 2004). High-sulfur KAP 10 and KAP12 family members are located in this domain (Rogers *et al*, 2004). *In situ* hybridization demonstrated the predominant expression of their transcripts in a narrow region of the middle portion of the hair fiber cuticle (Rogers *et al*, 2004). Furthermore, most recently, clusters of human ultrahigh-sulfur KAP5 genes have been identified at chromosomes 11p15 and 11q13 (Yahagi *et al*, 2004). These two clusters contain a total of 11 KAP5 genes (Yahagi *et al*, 2004). Previously performed *in situ* hybridization for one of the KAP5 members showed a cuticular expression pattern that was similar to the expression patterns of the KAP10 and KAP12 family members (MacKinnon *et al*, 1990). The morphology of the cuticle cells shows three distinct layers: outermost A layer, exocuticle, and endocuticle (Rogers, 2004). In the future, it should be confirmed in which sub-cellular compartment of the hair cuticle these KAP exist, even though they have been suggested to be located in the exocuticle (Rogers, 2004).

In humans, more than 80 individual KAP members are expressed differentially in the hair cortex and cuticle during hair fiber formation. Some KAP begin to be expressed in the lower cortex or even in the matrix of the bulb region (Rogers *et al*, 2002) (Table II). The expression of more KAP, however, initiates in the middle and upper keratogeneous zone of the cortex and/or cuticle (MacKinnon *et al*, 1990; Rogers *et al*, 2001, 2002, 2004; Shimomura *et al*, 2002a, b, 2003) (Table II), where most hair keratins have been already generated (Langbein *et al*, 1999, 2001). It seems reasonable to

Table II. Summary of the mRNA expression of the human KAP

KAP family	Onset of the mRNA expression
KAP1, KAP2, KAP3, KAP4, KAP9, KAP7, KAP19 , KAP6	Middle and upper keratogeneous zone in the hair fiber cortex
KAP10, KAP12, KAP5 , KAP13, KAP15, KAP19, KAP21, KAP23	Middle and upper keratogeneous zone in the hair fiber cuticle
KAP8, KAP11 , KAP13, KAP15, KAP19, KAP20, KAP21, KAP23	Matrix or precortex

Bold letters reflect the strong expression level. For understanding a detailed expression pattern of an individual KAP member, see references MacKinnon *et al* (1990), Rogers *et al* (2001, 2002, 2004), Shimomura *et al* (2002a, b, 2003).

KAP, keratin-associated proteins.

conclude that, at the late stage of the keratinization, large amounts of KAP are gradually synthesized and play essential roles in forming a strong hair shaft through a cross-linked network with KIF.

Size Polymorphisms in Human *KAP* Genes

We previously isolated two human *KAP1* genes: *KAP1.6* and *KAP1.7* (Shimomura *et al*, 2002a). We originally reported them as novel genes, although their gene loci were unknown (Shimomura *et al*, 2002a). Further studies, however, proved that both clones are actually size polymorphisms of the *KAP1.1B* gene (Shimomura *et al*, 2002b), which is one of the high-sulfur KAP1 family members located on chromosome 17q12–21 (Rogers *et al*, 2001). Comparisons of their nucleotide sequences showed that these molecules share identical sequences in their noncoding regions and differ in their open-reading frames (Shimomura *et al*, 2002a). The alignment of the deduced amino acid sequences of the three protein products showed that the main difference among them is in the number of cysteine-rich repeat segments, indicating that these size polymorphisms may have been generated by an intragenic deletion and/or duplication event of the repeat structures during evolution (Shimomura *et al*, 2002b). In addition, we have identified several size polymorphisms in the *hKAP1.3* gene as well (Shimomura *et al*, 2002b). We have further demonstrated that the allele frequencies of these polymorphic variants are different between Japanese and Caucasian populations (Shimomura *et al*, 2002b).

The existence of size polymorphisms in human KAP1 family members prompted us to analyze other KAP families. As a next step, we focused on the KAP4 family, which is an ultrahigh-sulfur family on chromosome 17q12–21 (Rogers *et al*, 2001). The analysis detected numerous size polymorphisms among the members of this family (Kariya *et al*, 2005). Like KAP1 members, the differences in size of these KAP4 gene variants almost depend on differences in the length of their open-reading frames; these different reading frames encode variable numbers of cysteine-rich pentapeptide repeat structures in the proteins (Kariya *et al*, 2005). Furthermore, our preliminary study revealed that some KAP4 members have population-specific polymorphisms, whereas others show similar frequencies among human populations (unpublished data). Some polymorphic alleles may have already been generated before the divergence of the human lineage. The analysis of size polymorphisms of KAP in other species, especially in our closest relatives among the primates, will provide an insight into the evolution of the human genome.

Current Questions and Future Research

The study of KAP has recently advanced. Many questions, however, remain to be answered. First, we are interested in the mechanism by which the differential expression of human KAP is regulated. Only limited analyses of the promoter regions of human *KAP* genes have been performed so far. Second, at present, we do not know of any congenital hair

disorders caused by mutations in the *KAP* genes. Several animal models (Powell and Rogers, 1990; Tkatchenko *et al*, 2001) and analyses of trichothiodystrophy (Gummer *et al*, 1985; Venning *et al*, 1986) indicate that the downregulation of KAP causes hair abnormalities. But further studies are needed to confirm the pathogenicity of mutated *KAP* genes. Third, it is not yet clear how KIF and KAP interact with each other. The proteins are believed to interact mainly through disulfide bonds and/or hydrophobic connections. In addition, there may be specific interactions between them. Finally, the reason for the presence of the size polymorphisms in human *KAP* genes is unknown. As described above, several human KAP family members have size polymorphisms because of variations in the number of cysteine-rich repeat segments, which results in their having different cysteine contents (Shimomura *et al*, 2002b; Kariya *et al*, 2005). Cysteine is essential for the formation of disulfide bonds. Therefore, it is possible that the difference in cysteine content of these size polymorphisms may change the interactions between the KIF and KAP, resulting in the differences observed in the structure of the hair fiber among individuals as well as in different human populations. After the complete sets of size polymorphisms among the KAP have been identified, the phenotypic consequences of these polymorphisms can be assessed.

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