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RESEARCH ARTICLE

Jun YIN, Jinquan LI, Yanjun ZHANG, Changqing LI

Sequence of a Cashmere goat type I hair keratin gene and its expression in skin

Abstract The keratin family includes the epithelial (soft) keratins and the hair (hard) keratins, which can be divided into two subfamilies—the acidic type I and the basic or neutral type II. A cDNA is isolated and sequenced from a cDNA library constructed with poly (A)+RNA derived from the goat skin. Because the gene sequence of the known type I hair keratins *8C1* is in accordance with that of the *hHa1*, this keratin can be identified as the goat hair keratin gHa1 cDNA (GenBank Accession No. AY510110.1). The 413 amino acid sequence derived is compared with the Sheep 8C1 type I mRNA and they exhibit the highest homology (97.8%). In *in situ* hybridization, it has revealed a strong expression in the cortex of both primary and secondary hair follicles.

Keywords Cashmere goat, hair keratin, *in situ* hybridization

1 Introduction

Cashmere produced from the Cashmere goat (*Capra hircus*) is one of the softest, warmest and longest-lasting materials on the market today. It is an important export commodity of Inner Mongolia Region, China. Its fiber properties are of commercial importance in the textile industry because their excellent quality. Cashmere fiber mainly consists of keratin. It is important to know the characteristics of the keratin gene in explaining the quality of cashmere.

The wool keratins are of a complex and diverse protein group which may amount to as many as 100 types (Powell, and Rogers, 1986). The keratin multigene family comprises the cytokeratins (or soft) α-keratins, which are expressed in various types of epithelia, and the hair (or hard α-) keratins, which are involved in the formation of hard keratinized structures (Hanukoglu and Fuchs, 1982). Both of them can be divided into type I (acidic) and type II (basic-neutral) proteins that form the 8–10-nm intermediate filament network of epithelial cells through obligatory association of equimolar amounts of type I and type II keratins (Dowling et al., 1983, 1983; Steinert et al., 1985). Although initial studies of hair keratin proteins of several species have indicated the existence of eight major types of hair keratins, including four type I members, termed as Ha1–Ha4, and four type II members, termed as Hb1–Hb4, as well as one minor hair keratin pair, termed as Hax/Hbx (Heid et al., 1986, 1988), the latest studies show that the hair keratin family is much more complex (Langbein et al., 1999, 2001).

In the course of characterization of the goat hair keratins and their expression patterns, we have discovered a full-length cDNA from the goat skin. The present study attempts to expand and analyze the *gHa1* cDNA in order to know more about the characteristics of the goat type I hair keratin and reveal its function in skin.

2 Materials and methods

2.1 Tissue collection and preparation

Midflank skin (anagen hair follicles wiped out) was excised from a living adult goat. The tissue samples were sterilized in tincture of iodine and deiodined in ethyl alcohol. Part of the tissue samples was quickly frozen in liquid nitrogen for subsequent RNA extraction while the rest were fixed in 4% phosphate-buffered paraformaldehyde saline (pH 7.2) for 14 h at 4°C and embedded in paraffin.

2.2 Cloning of the cDNA sequence of hair keratin

A cDNA library was built up with the RNA extracted from the skin tissue (Yin et al., 2004). The library was

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Jun YIN
College of Biotechnology, Inner Mongolia Agricultural University, Huhhot 010018, China

Jinquan LI (✉), Yanjun ZHANG, Changqing LI
College of Animal Science and Medical Science, Inner Mongolia Agricultural University, Huhhot 010018, China
E-mail: lijinquanh@yahoo.com

constructed with a plasmid library produced by inserting oligo (dT) sequences. The library has 1.8×10^5 clones. The average insertion is 1.1 kbp. Three hundred and ninety two expressed sequence tags (ESTs) were generated from sequencing the 5′ ends of randomly selected skin cDNA clones. The ESTs were sequenced and the result was compared with that in the NCBI database (nr) using the Blast-N programs (http://www.ncbi.nlm.nih.gov/BLAST).

2.3 *In situ* hybridizations

In situ hybridization with digoxigenin (DIG)-labeled cRNA probes was performed using a modification of the protocol described by Tohyama et al. (1994). Paraffin blocks were serially cut into 7 mm sections. After being deparaffinized (xylene) and rehydrated (100%–50% ethanol then phosphate-buffered saline), the sections were digested for optimal probe penetration with proteinase K (10 mg·mL^{-1}) for exactly 10 min at 37°C. To minimize nonspecific background signals, the sections were acetylated at room temperature for 15 min. The sections were hybridized with sense- or antisense-labeled DIG probes at 50°C for 16 h in the presence of 50% formamide, 4 × sodium citrate/chloride buffer, 1 × Denhardt's, 50% dextran sulfate, and 50 mg·mL^{-1} yeast tRNA. T7 and T3 polymerases were used to synthesize sense and antisense transcripts labeled with DIG-labeling kit (Boheringer Mannheim, Mannheim Germany) from goat Ha1 cDNA template. The unbound cRNA probes were removed from the sections after an initial wash with 2 × sodium citrate/chloride buffer/50% formamide solution, digestion with 20 mg·mL^{-1} RNase A at 37°C for 15 min, and a final wash with 1 × sodium citrate/chloride buffer/50% formamide at 50°C for 10 min. The sections were incubated for 30 min in 5% normal heat-activated serum in 100 mmol·L^{-1} Tris–150 mmol·L^{-1} NaCl solution, rinsed and again incubated for a further 60 min in 0.5% DIG blocking reagent (Roche Diagnostics) in Tris–NaCl. The bound probes reacted with anti-DIG antibody conjugated to alkaline phosphatase for 60 min and were visualized using the standard color development solution of 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium. Then the sections were immersed in 100 mmol·L^{-1} Tris–10 mmol·L^{-1} EDTA, pH 8.0; and then the reaction stopped. The sections were dyed in Haematoxylin. Finally, photomicrography was performed with an OLYMPUS photomicroscope.

3 Results

3.1 Cloning of the cDNA sequence of hair keratin

BLAST analysis indicates that there is a 94% match between the EST data (GenBank CD052028) and the sequence of sheep *8C1* (*s8C1*, GenBank AF227758.1). After a sequence analysis of the whole inserted fragments of plasmid, BLAST identified a novel full-length 1598-bp cDNA that encoded hair type I keratin, termed as *Capra hircus* hair acidic keratin 1 (*gHa1*, GenBank AC055715).

3.2 Multiple sequence alignments of hair keratin

gHa1 shares identities in nucleotide sequence with *s8C1*, *hHa1*, *hHa3a*, *hHa3b* and *hHa4a*, by 97%, 88%, 88%, 88%, 86%, 87%, respectively. Figure 1 shows the nucleotide sequences of *gHa1*.

Comparing the derived amino acid sequence of the *gHa1* with the known sheep wool micro fibril component 8C1 type I and the human hHa1, it is found that they exhibit a very high homology (97.8%) with sheep hair keratin 8C1 and 86%with human hair acidic keratin 1. Besides the fact that the rod domains is conserved, the homologous regions comprising the entire head domains as well as the 10 amino acid residues long carboxyl-terminal sequences adjacent to the end of the α-helical rod domains are also strictly conserved in size (Fig. 2). Since the carboxy terminal subdomain is the most specific for a single keratin, this data shows that the identification of the goat keratin as the ortholog of s8C1, hHa1 and mKrt1-1.The gHa1 possesses 413 amino acid residues, with calculated molecular masses being 46.87 kDa and isoelectric point being 4.61.

3.3 Skin tissue *in situ* hybridization

ISH with the *gHa1* cRNA probe on the primary hair follicles reveals strong expression of the *gHa1* mRNA (Fig. 3 (a), (b) and (c)), leading to a prominent, well circumscribed label comprising the matrix compartment as well as the lower cortex region above the vertex of the dome-shaped dermal papilla; but near the matrix compartment there is no *gHa1* expression in the medulla region (Fig. 3(a)). The *gHa1* transcripts can first be seen above the matrix cell pool, as shown in the longitudinal sections (Fig. 3(a)) and the cross-sections through different levels of the hair shaft (Fig. 3 (c)). ISH with the *gHa1* cRNA probe on the secondary hair follicles also reveals strong expression that begins from the transition of the matrix and the cortex, and remains prominent throughout the lower- and mid-cortex region until it gradually vanishes in the middle cortex cells (Fig. 3(b) and (c)). Cells lining the apex of the dermal papilla are free of *gHa1* transcripts as shown in the longitudinal skin sections (Fig. 3(a) and (b)).

4 Discussion

Wilson et al. (1988) have suggested that the closest transcription of sheep 8C1 gene is from the 59-nt upstream,

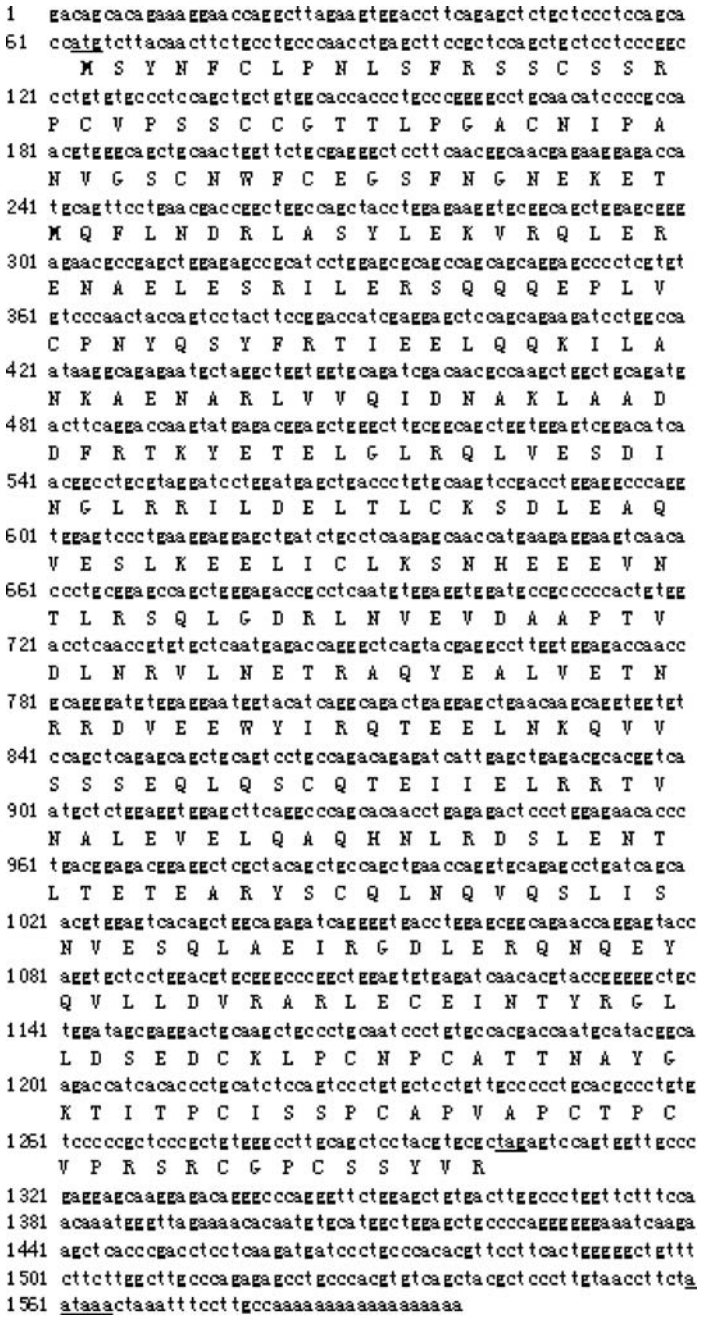

Fig. 1 Nucleotide sequences of cashmere goat type I hair keratin *gHa1* and derived amino acid sequences of encoded keratin
Note: Start codon, stop codon and the polyadenylation signals are underlined.

the starting point of translation. Comparing the 5′ non-translated region of *gHa1* with that of *s8C1*, it is found that they have equivalent nucleotides, but the sequences of N-terminal 47 upstream, the 5′ non-translated starting point, do not share many identities (Fig. 4). Further research is still needed to identify whether this affects their expression. At the end of the 3′ region of the gene, the polyadenylation signal AATAAA usually begins from the 20-nt upstream polyadenylation. The polyadenylation signal AATAAA of the *gHal* is from the 1560-nt upstream polyadenylation, which implies that *gHa1* may be a full-length cDNA.

In the present study, we have isolated a cDNA clone that contains sequence information of goat type I hair keratin. Sequence homology comparison of the known type I hair keratins has clearly shown that the chain specificity of a single hair keratin is essentially determined by the sequence and length of carboxyl terminus (Bertolino et al., 1990; Kaytes et al., 1991). Moreover, there are hard evidences that this keratin subdomain is also highly con-

Fig. 2 Multiple sequence alignments of hair keratins gHa1, sheep 8C1 and hHa1
Note: Asterisks below the amino acid sequences indicate sequence identities. Inverted solid triangles demarcate borders of the α-helical. domains.

served in both length and sequence in the orthologous hair keratins of different species. Thus, gHa1 and sheep type I hair keratins 8C1 share a highly homologous37-amino-acid–long carboxyl terminus which is also found in the human type I hair keratin component hHa1 with three more amino acids (Kaytes et al., 1991; Winter et al., 1994). Based on these particular features of hair keratins, it is found that the new goat type I keratin cDNA represents the ortholoy of the sheep hair keratin *8C1* and *hHa1* through sequence homology comparison.

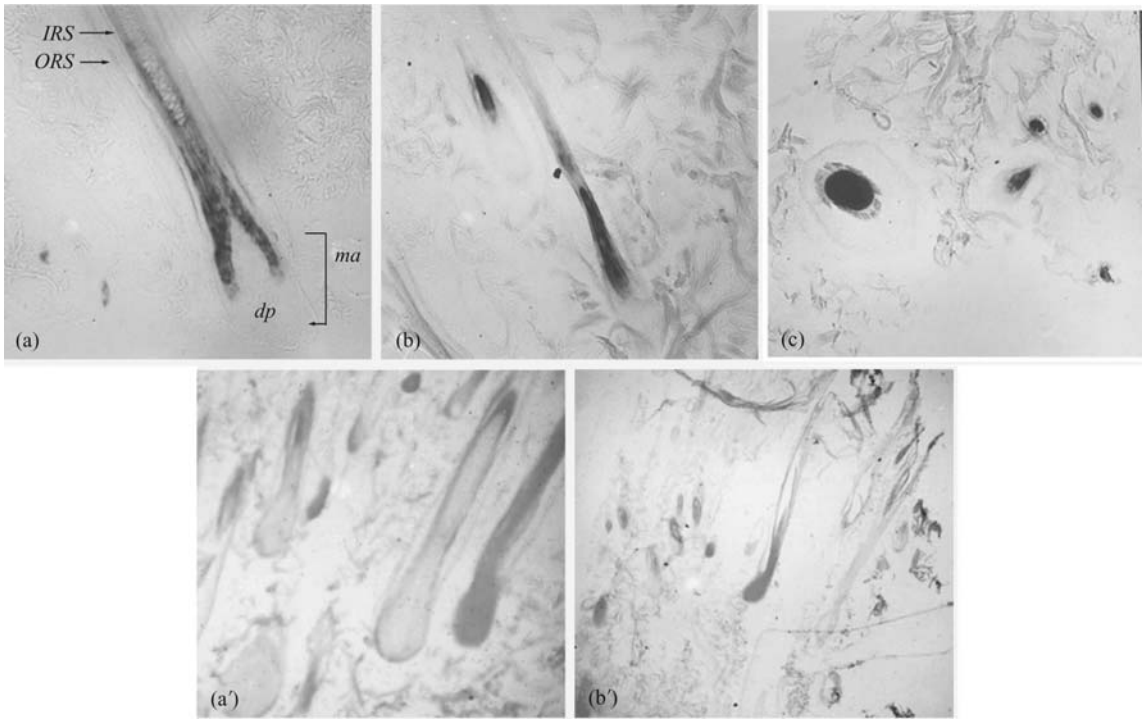

Fig. 3 The expression of *gHa1* mRNA in skin
Note: (a) represents antisense probe on longitudinal section of primary hair follicle; (a′) represents sense probe of control for primary hair follicles; (b) represents antisense probe on longitudinal section of secondary hair follicle; (b′) represents sense probe of control for secondary hair follicle; (c) represents antisense probe on longitudinal section of primary and secondary hair follicles. ORS, IRS, ma and dp represent outer root sheath, inner root sheath, matrix and dermal papilla, respectively. Purple blue color indicates the positive signal.

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gHa1:  gacagcacagaaaggaaccaggcttagaagtggaccttcagagctctgctccctccagcaccatg 65
       ||||          | | |             |  || |  ||||||||||||||||||
s8C1:  gacaagctccttggagaacttggactctgtgttcagtcaggaacctcgctccctccagcaccatg 65
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Fig. 4 The nucleotide sequences comparison of the 5′ non-translated regions of *gHa1* and *s8C1*
Note: The start codons are underlined. The short lines between the nucleotide sequences indicate the shared identities.

The expression of gHa1 on the primary and the secondary follicles is apparently strong, which means it is the gHa1 that constitutes the cortex keratin of the cashmere hair and cashmere. However, because the cuticle layer is very thin, it is hard to identify whether gHa1 is expressed or not. Hair keratins of most species exhibit an extraordinarily high sequence homology, as sequentially expressed in the cortex of the hair and the wool follicles according to their given order, respectively (Langbein et al., 1999; Langbein et al., 2001). The elucidation of the structure and function of cashmere type I hair keratin enables us to make an exact description of the maturation of the terminal anagen cashmere on the base of a thorough study of its most important structural proteins. At present, we have only known a single goat hair keratin gene, the characteristics of other type I hair keratins are not known yet. Next, we need to make further studies of the expression patterns of other type I and type II hair keratins.

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