Genomics **56,** 303–309 (1999)
Article ID geno.1998.5721, available online at http://www.idealibrary.com on IDEAL®

# The Genomic Organization of Type I Keratin Genes in Mice

Hajime Sato,*,† Tsuyoshi Koide,† Tomoko Sagai,† Sei-Ichi Ishiguro,* Makoto Tamai,* Naruya Saitou,‡ and Toshihiko Shiroishi†,[1]

*Department of Ophthalmology, Tohoku University School of Medicine, Seiryo-Machi 1-1, Sendai, Miyagi-ken 980-8574, Japan; †Mammalian Genetics Laboratory and ‡Laboratory of Evolutionary Genetics, National Institute of Genetics, Yata-1111, Mishima, Shizuoka-ken 411-8540, Japan

Received July 31, 1998; accepted December 7, 1998

**We isolated two new keratin cDNAs by screening a cDNA library constructed from poly(A)$^+$ RNA of the dorsal and abdominal skin of C57BL/10J mice with a probe of human *KRT14*. Due to its high sequence homology to human keratin 17 cDNA, one full-length cDNA is most likely to be mouse keratin 17 (*Krt1-17*) cDNA. The other is the putative full-length cDNA of a novel type I keratin gene, designated *Krt1-c29*. These two keratin genes were mapped to the distal portion of Chromosome 11, where the mouse keratin gene complex-1 (*Krt1*) is localized. To elucidate the genomic organization of *Krt1* in mice, we carried out genetic and physical analyses of *Krt1*. A large-scale linkage analysis using intersubspecific backcrosses suggested that there are two major clusters in *Krt1*, one containing *Krt1-c29, Krt1-10,* and *Krt1-12* and the other containing *Krt1-14, -15, -17,* and *-19*. Truncation experiments with two yeast artificial chromosome clones containing the two clusters above have revealed that the gene order of *Krt1* is centromere–*Krt1-c29–Krt1-10–Krt1-12–Krt1-13–Krt1-15–Krt1-19–Krt1-14–Krt1-17*–telomere. Finally, we analyzed sequence divergence between the genes belonging to the *Krt1* complex. The results clearly indicated that genes are classified into two major groups with respect to phylogenetic relationship. Each group consists of the respective gene cluster demonstrated by genetic and physical analyses in this study, suggesting that the physical organization of the *Krt1* complex reflects the evolutionary process of gene duplication of this complex.** © 1999 Academic Press

## INTRODUCTION

Keratins are a group of water-insoluble proteins constituting paired acidic and basic proteins that form intermediate filaments (IF) in epithelial cells. Coordinated expression of the acidic and basic proteins, which are encoded by the type I and type II keratin gene complexes, is essential for normal epithelial integrity. Characterization of the genomic organization is crucial for a better understanding of the regulation of keratin genes and the etiology of many genetic defects known to cause abnormal development of epithelial cells.

According to immunoreactivity and sequence homologies as well as relative charge, K10, K11, K12, K13, K14, K15, K16, K17, K18, and K19 belong to type I keratin, while K1, K2, K3, K4, K5, K6, K7, and K8 belong to type II keratin (Moll *et al.*, 1982; Eichner *et al.*, 1984). As is the case with other IF, a keratin chain consists of an amino-terminal end domain, an $\alpha$-helical rod domain, and a carboxyl-terminal end domain. In each group, the central $\alpha$-helical rod domain is remarkably conserved in its size, secondary structure, and sequence, while the amino- and carboxyl-terminal end domains differ in size and sequence (Steinert and Roop, 1988). Specific pairs of at least one type I and one type II keratin protein are coexpressed in a tissue- and differentiation-specific manner and are required for IF assembly. K1, K2, K5, K10, and K14 are expressed in normal epidermis; K3 and K12 are expressed in cornea; K4, K13, and K15 are expressed in esophagus and other nonkeratinizing epithelia; K8 and K18 are expressed in simple epithelia; K19 is expressed in periderm; and K9 is expressed in palmar and plantar tissue. K6, K16, and K17 are expressed mainly in the epidermis under pathological hyperproliferative states (Blumenberg, 1993).

It is reported that mutations in keratin genes cause many hereditary human diseases (Coulombe *et al.*, 1991; Cheng *et al.*, 1992; Reis *et al.*, 1994; McLean *et al.*, 1995; Richard *et al.*, 1995; Rugg *et al.*, 1995; Irvine *et al.*, 1997). Mutant keratin generates the abnormal keratin filament network that is visible as perinuclear keratin aggregates with keratin clumps. The disruption of the keratin network causes keratinocytes to be fragile, as was demonstrated in transgenic mice and knockout mice (Vassar *et al.*, 1991; Kao *et al.*, 1996). Further study of the keratin genes will be useful for diagnosis of inherited diseases of epithelia.

In humans, the type I keratin complex has been mapped to chromosome 17q12–q21. Recently, it was

[1] To whom correspondence should be addressed. Telephone: 81-559-81-6818. Fax: 81-559-81-6817. E-mail: tshirois@lab.nig.ac.jp.



0888-7543/99 $30.00

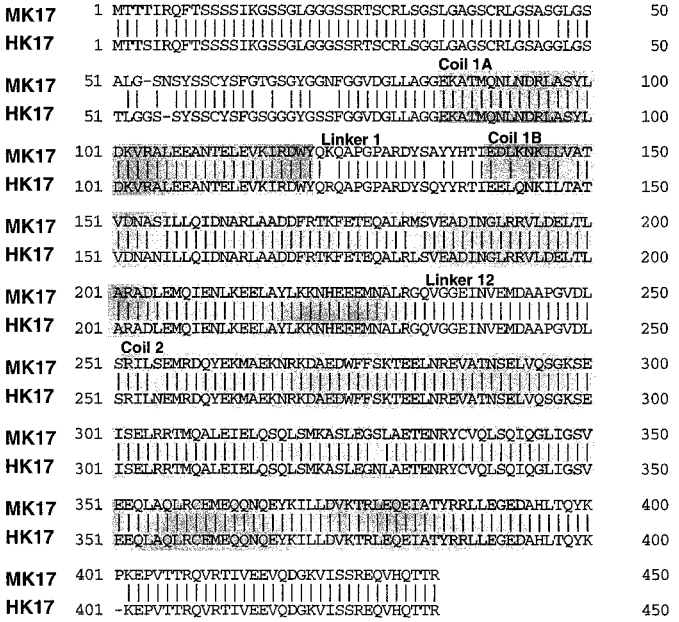


**FIG. 1.** Comparison of the amino acid sequences of mouse *Krt1-17* and human keratin 17 genes. Vertical lines denote identical amino acid residues. Note that the percentage of identical amino acid residues is 95%. The rod domain of the keratin chain consists of three $\alpha$-helical tracts termed Coil 1A, Coil 1B, and Coil 2, interrupted by linker 1 and linker 12. The boundary of these domains is based on the database. MK17, mouse keratin 17; HK17, human keratin 17.

reported that the genes described below are organized within a 55-kb DNA fragment in the following order: 5′– *KRT19–KRT15–KRT17–KRT16–KRT14*–3′ (Milisavljevic *et al.*, 1996). In mice, the type I keratin complex (*Krt1*) was mapped to the distal portion of Chromosome 11, which is syntenic to human chromosome 17q12–q21. Although *Krt1-19, Krt1-15,* and *Krt1-13* are separated by 5–6 kb each and are in the same transcriptional orientation (Nozaki *et al.*, 1994; Filion *et al.*, 1994), the complete genomic organization of *Krt1* has not yet been determined.

In this study, we have isolated mouse *Krt1-17* and a novel type I keratin cDNA (*Krt1-c29*) from a skin cDNA library. We determined the comprehensive gene order of *Krt1* by fine linkage analysis and the physical organization of *Krt1* by analysis of YAC clones. Finally, we discuss the phylogenetic relationship of the cloned *Krt1* genes.

## MATERIALS AND METHODS

*Construction of the skin cDNA library.* Total RNA of dorsal and abdominal skin of adult C57BL/10J mice purchased from The Jackson Laboratory (Bar Harbor, ME) was extracted with guanidinium thiocyanate by a standard protocol (Sambrook *et al.*, 1989). Polyadenylated RNA was purified from the total RNA with a mRNA Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). cDNA was synthesized from 5 $\mu$g of mRNA with a cDNA synthesis module (Amersham Pharmacia Biotech, Buckinghamshire, UK) using anchored dT25 primers. The cDNA was methylated with *Eco*RI methylase and ligated with 5′-phosphorylated *Eco*RI linker. After digestion with *Eco*RI, it was size-fractionated with SizeSepTM400 Spun Columns (Amersham Pharmacia Biotech, Uppsala, Sweden) and ligated into the *Eco*RI site of Lambda ZAP II vector (Stratagene, La Jolla, CA). The ligated mixture was packaged with Gigapack II Gold Packaging Extract (Stratagene).

*Screening procedures and cDNA cloning.* The cDNA library was screened by plaque hybridization with $^{32}$P-labeled human keratin 14 as a probe by standard methods (Sambook *et al.*, 1989). The DNA fragment of *KRT14* was amplified by polymerase chain reaction (PCR) using specific primers (KRT14-F, 5-ATGACTACCTGCAGCCGCCA-3; KRT14-R, 5-TTGATCTCAGCAGGCCGCTG-3) (Marchuk *et al.*, 1985). Washing was performed in 2× SSC, 0.5% sodium dodecyl sulfate (SDS) for 1 h at room temperature followed by 0.1× SSC, 0.25% SDS for 10–15 min at 65°C. Seventeen independent clones were further analyzed.

*DNA sequence analysis.* The DNA sequence was determined for both strands by a 377 automated DNA sequencer (Perkin–Elmer Applied Biosystems, CT) using a dRhodamine Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin–Elmer Applied Biosystems) and analyzed by DNASIS version 3.0 (Hitachi Software, Japan). Homologous sequences were searched for in the database at the National Center for Biotechnology Information (NCBI) using the BLAST network service.

*Northern blot analysis.* Seven micrograms each of total RNA derived from several tissues was electrophoresed on a 1.2% formaldehyde gel by a standard method (Sambrook *et al.*, 1989) and blotted onto Hybond-N$^+$ nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). Hybridization was carried out with a $^{32}$P-labeled 628-bp *Eco*RI–*Pvu*II cDNA fragment of clone pKS29. Washing was performed at room temperature in 2× SSC, 0.5% SDS for 1 h and subsequently at 60°C in 0.1× SSC, 0.25% SDS for 10 min. Autoradiographs were analyzed with the BAS 2000 Bioimage Analyzer (Fuji Photo Film, Tokyo, Japan).

*Linkage analysis.* Linkage analyses were carried out using three different sets of mouse DNA panels previously constructed (Sagai *et al.*, 1998; Sato *et al.*, 1998). Microsatellite markers were purchased from Research Genetics (Huntsville, AL). The sequences of oligonucleotide primers for *Krt1-10* and *Krt1-12* were described previously (Sato *et al.*, 1998). The oligonucleotide primers set for *Krt1-14, Krt1-15, Krt1-17, Krt1-19,* and *Krt1-c29* were as follows: Krt1-14F, 5′-AGATCCGCACCAAGGTCATGG-3′ and Krt1-14R, 5′-GTGCAACTCAGAAAAAGAAGC-3′. The *Krt1-14* 3′-noncoding region probe was obtained from Dr. Dennis Roop (Knapp *et al.*, 1987). The sequences for Krt1-15F, 5′-GAGAGAAATCTAAGTGTCTGGTG-3′, and Krt1-15R, 5′-GATAACACCCTTTTAGAACTGG-3′ (Nozaki *et al.*, 1994), Krt1-17F, 5′-ACCACCCGTTAAGGACTCAG-3′, and Krt1-17R, 5′-CCATGGTCATTTATTTCAGTG-3′, were obtained from analyses of pKS11, pKS14, pKS17, pKS18, pKS20, and pKS28 clones in this study and the published sequence (Knapp *et al.*, 1987). Krt1-19F, 5′-CCAAGGCCATCTGAGCTACC-3′, and Krt1-19R, 5′-CCTTGGAGGATAGTTTTATTGAC-3′ (Lussier *et al.*, 1990), Krt1-c29F, 5′-ACAACGCCAACATCATCCTG-3′, and Krt1-c29R, 5′-TCTTGAGGTATGCCAGCTCC-3′, were obtained from the analysis of the pKS29 clone in this study. Simple sequence-length polymorphism and sin-

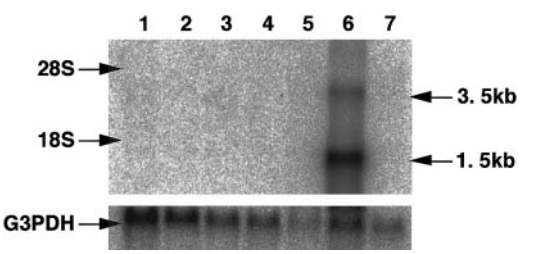


**FIG. 2.** Northern blot of *Krt1-c29*. The *Krt1-c29* probe detected two signals of approximately 1.5 and 3.5 kb in skin. The intensity of signals suggested that the 1.5-kb mRNA is the major transcript. G3PDH is the internal control. Lanes **1,** whole embryo (11.5 days postcoitus (dpc); **2,** whole embryo (12.5 dpc); **3,** placenta (12.5 dpc); **4,** liver (adult); **5,** testis (adult); **6,** skin (adult); **7,** lung (adult).

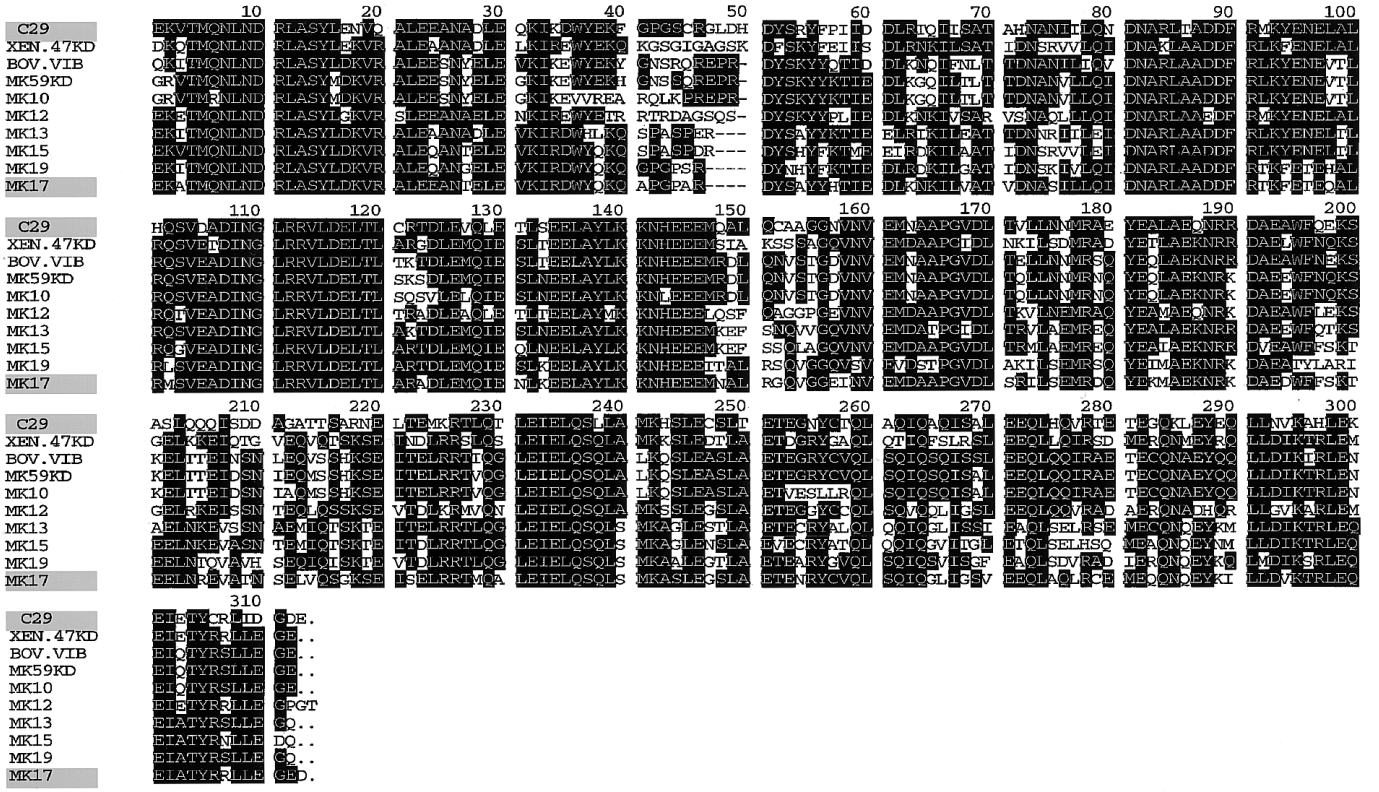


**FIG. 3.** Comparison of the amino acid sequences of type I keratin rod domains. The amino acid sequences are optimally aligned. Dashes denote deletions introduced to obtain optimal alignment. White letters with a black background represent identical amino acid residues. There is 70–80% identity among the genes. XEN.47KD, *Xenopus laevis* 47-kDa type I keratin; BOV.VIB, bovine type I keratin VIB; MK59KD, mouse 59-kDa type I keratin; MK, mouse keratin.

gle-strand conformation polymorphism for *Krt1-10*, *Krt1-15*, *Krt1-19*, and *Krt1-c29* were performed as previously described (Sato *et al.*, 1998). The PCR products amplified with *Krt1-14* primers were visualized by staining with ethidium bromide by 16% polyacrylamide gel electrophoresis. The linkage analysis was performed with Map Manager v2.6.5 and the QTB11 program (Manly, 1993).

*Isolation of the yeast artificial chromosome (YAC) clone.* A genomic YAC library (Research Genetics) was screened by PCR with specific primers for each keratin gene. Clone YAC120F10 (approximately 430 kb), clone YAC185F10 (approximately 530 kb), and clone YAC112A6 (approximately 580 kb) were isolated and further analyzed.

*Truncation of YAC clones.* Truncation vectors pB1F and pB1R, which carry a mouse B1 repeat sequence in different orientations, a copy of the yeast lysine-2 (*Lys2*) gene, and the telomere sequence, were kindly provided by J. Edmondson and R. Rothstein. Isolated YAC clones were transformed with pB1F or pB1R vector, which was linearized by digestion with *Sal*I using an alkali cation yeast transformation kit (Bio 101, Inc., CA), and transformed yeast cells were plated on synthetic complete (SC) medium lacking tryptophan and lysine. After 2 days, colonies were plated in duplicate on SC (Lys-, Trp-) and SC (Lys-, Ura-) plates. Those colonies that grew only on SC (Lys-, Trp-) plates were further analyzed (Lewis *et al.*, 1992).

*Pulsed-field gel electrophoresis and Southern blot analysis.* Selected colonies were examined by PCR with the above primers to determine whether there were any *Krt1* genes and microsatellite markers. The sequences of oligonucleotide primers for *Krt1-13* were as follows: Krt1-13F, 5′-GTTTCTCCAGCCTAAGCACC-3′; Krt1-13R, 5′-TCCAATCAGAAGAGATGGGG-3′ (Filion *et al.*, 1994). DNA from successfully truncated YAC clones was prepared in low-melting-point agarose (SeaPlaque GTG agarose; FMC BioProducts, Rockland, ME) according to the protocol provided at the training course at Cold Spring Harbor Laboratory (Analysis and Genetic Manipulation of YACs, 1993). Agarose plugs were loaded onto a 1% gel and size-fractionated in a CHEF Mapper XA Pulsed Field Electrophoresis System (Bio-Rad). Gels were blotted onto Hybond-$N^+$ nylon membrane, and Southern hybridization was performed with a $^{32}$P-labeled pBR322 probe that hybridizes with the left arm of the YAC vector. Washing and autoradiographs were performed as described above.

*Phylogenetic tree analysis.* The neighbor-joining method (Saitou and Nei, 1987) was used for constructing phylogenetic trees of mouse type I keratin genes. CLUSTAL W (Thompson *et al.*, 1994) was used for multiple alignment and tree construction.

## RESULTS

### *Isolation of Two New Type I Keratin cDNAs*

In this study, we isolated two type I keratin cDNAs from a skin cDNA library. One full-length cDNA was highly homologous to human keratin 17 cDNA (Troyanovsky *et al.*, 1992) and was designated mouse keratin 17 (*Krt1-17*) cDNA. The complete nucleotide sequence of the *Krt1-17* cDNA was determined by the analysis of six independent clones, pKS11, pKS14, pKS17, pKS18, pKS20, and pKS28. This sequence has an open reading frame (ORF) of 1299 bp and encodes a protein of 433 amino acids (DDBJ Accession No. AB013608). The deduced amino acid sequence is 95.4% identical with human keratin 17 over 433 amino acids (Fig. 1). The second was the cDNA of a novel keratin gene, which we designated *Krt1-c29*. Its nucleotide sequence was determined by analysis of the clone pKS29.

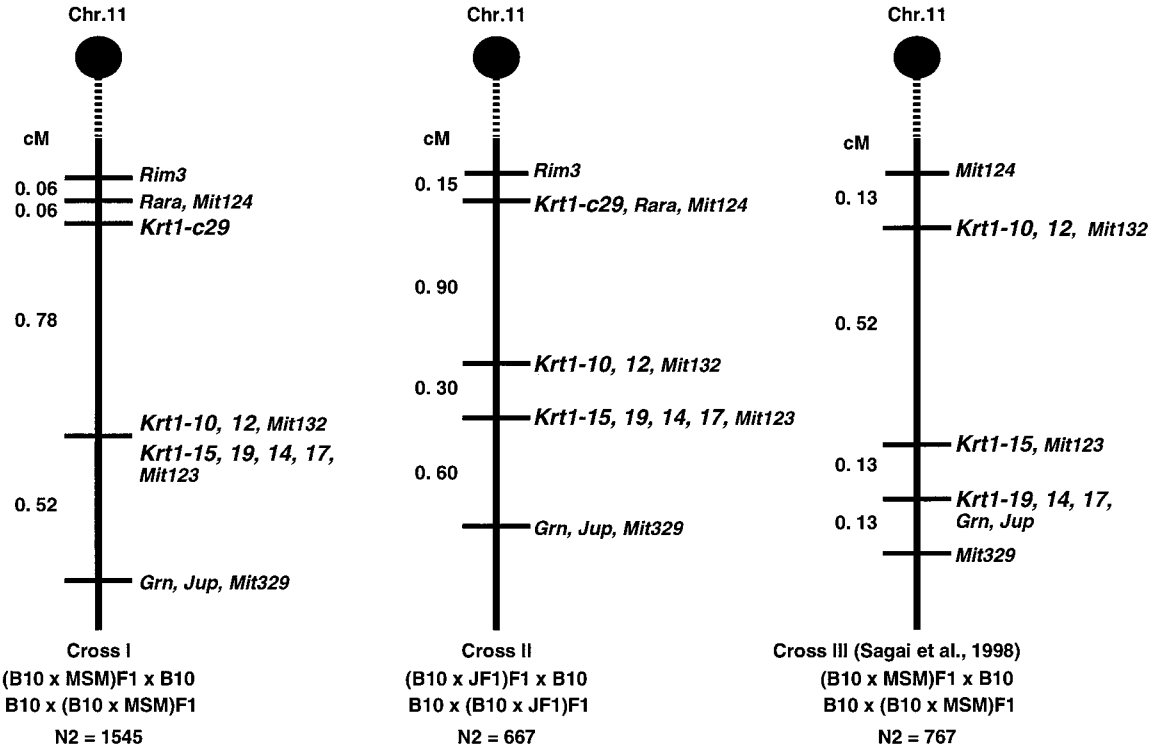


**FIG. 4.** Linkage analysis of mouse type I keratin genes. Recombination distances between loci in centimorgans are shown to the left of the chromosome, and microsatellite markers and gene loci are shown to the right. Note that there are two clusters in *Krt1*, one containing *Krt1-c29*, *Krt1-10*, and *Krt1-12* and the other containing *Krt1-14*, *-15*, *-17*, and *-19*. *Rim3*, recombination-induced mutation 3; *Rara*, retinoic acid receptor $\alpha$; *Grn*, granulin; *Jup*, junctional plakoglobin.

It also has a long ORF of 1344 bp and encodes a protein of 448 amino acids (DDBJ Accession No. AB013607). Northern blot analysis demonstrated the presence of a major 1.5-kb transcript in adult mouse skin but not in any other tissues examined (Fig. 2). Additionally, the deduced amino acid sequence in the keratin rod domain has 70–80% identity with other type I keratin genes (Fig. 3).

### *Linkage Analysis of Mouse Type I Keratin Genes*

We carried out a linkage analysis of *Krt1-17* and *Krt1-c29* and other mouse type I keratin genes, based on three different crosses. *Krt1-17* and *Krt1-c29* were mapped to the distal portion of mouse Chromosome 11, where other *Krt1* genes are located (Fig. 4). The linkage analysis in Cross I revealed that *Krt1-c29* is located proximal to other *Krt1* genes. In Cross II, *Krt1-10* and *Krt1-12* were segregated from *Krt1-15*, *Krt1-19*, *Krt1-14*, and *Krt1-17* and mapped proximal to them. The result of Cross III showed that *Krt1-15* is located proximal to the *Krt1-19*, *Krt1-14*, and *Krt1-17* genes. Finally, these linkage analyses indicated that the gene order of *Krt1* is centromere–*Krt1-c29*–(0.81 cM)– *Krt1-10*, *Krt1-12*–(0.42 cM)–*Krt1-15*–(0.13 cM)–*Krt1-19*, *Krt1-14*, *Krt1-17*–telomere.

### *Physical Organization of Mouse Type 1 Keratin Genes*

To determine the gene order in *Krt1* independently, YAC truncation was performed using a pB1F or pB1R vector. Physical separation of *Krt1-c29*, *Krt1-10*, and *Krt1-12* by the truncation of YAC120F10 (Fig. 5) indicated that they are localized in following order: centromere–*Krt1-c29*–*Krt1-10*–*Krt1-12*–telomere and suggested that the distance between *Krt1-c29* and *Krt1-10* is within 80 kb and that *Krt1-10* is located within 40 kb of *Krt1-12*. Additionally, separation of *Krt1-19*, *Krt1-14*, and *Krt1-17* by the truncation of YAC185F10 (Fig. 5) indicated that they are localized in the following order: centromere–*Krt1-13*, *Krt1-15*–*Krt1-19*–*Krt1-14*–*Krt1-17*–telomere and suggested that *Krt1-19* and *Krt1-14* are separated by a distance of 20–100 kb and *Krt1-17* is situated within a distance of 130 kb from *Krt1-14*. The failure to separate *Krt1-13*, *Krt1-15*, and *Krt1-19* suggested that these genes are tightly clustered, which is consistent with previous reports (Filion *et al.*, 1994). The result of the truncation of YAC112A6 was the same as that of YAC185F10 except for the distance between *Krt1-19* and *Krt1-14*. The distance between *Krt1-19* and *Krt1-14* was estimated to be 20–100 kb in YAC185F10 and 100–180 kb in YAC112A6. This difference is possibly due to an artifactual deletion in the clone YAC112A6. The physical organization of the *Krt 1* complex is summarized in Fig. 6.

### *Phylogenetic Tree Analysis of Mouse Type I Keratin Genes*

We constructed a phylogenetic tree of the genes to explore the evolution of mouse type I keratin genes

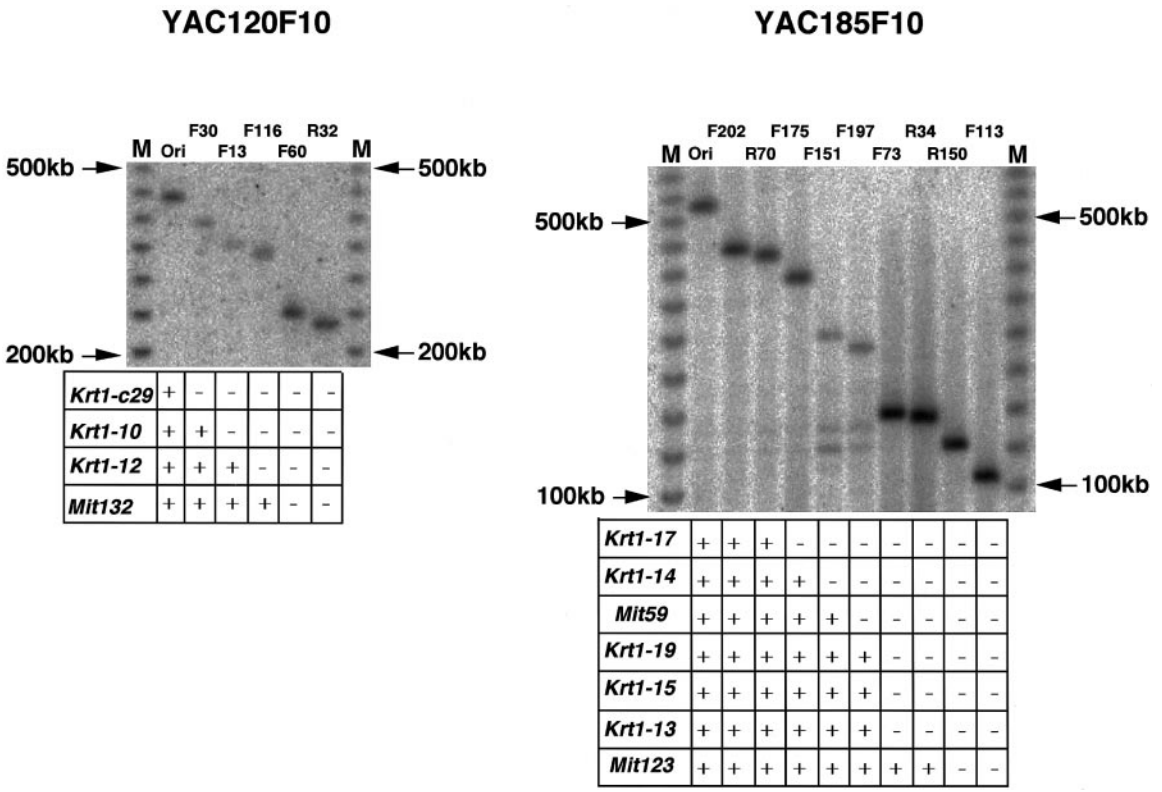


**FIG. 5.** Southern hybridization of serial truncated YAC clones. Southern hybridization with a YAC left-arm probe revealed that YAC 120F10 and YAC 185F10 were successfully truncated with pB1F or pB1R. F or R indicates a clone truncated with pB1F or pB1R, respectively. The lower panels represent the result of PCR. *Krt1* genes and microsatellite markers are shown to the left. Lane **M,** 50-kb lambda ladder marker; **Ori,** original YAC clone.

(Fig. 7). The result clearly showed that *Krt1* genes are classified into two major groups, one group including *Krt1-10*, *Krt1-12*, and *Krt1-c29*, and the other including *Krt1-13*, *-15*, *-19*, *-14*, and *-17*. Each member of the two groups might have arisen from ancestral genes by gene duplication. The grouping based on this phylogenetic tree showed a good correlation with the two clusters of *Krt1* genes in the mouse genome.

## DISCUSSION

We have presented here the sequence data of two cDNAs of type I keratin genes, *Krt1-17* and *Krt1-c29*, which were cloned from a skin cDNA library. It is highly likely that *Krt1-17* is the mouse orthologue of human *KRT17*, because of the high percentage of amino acid sequence identity and the similar expres-

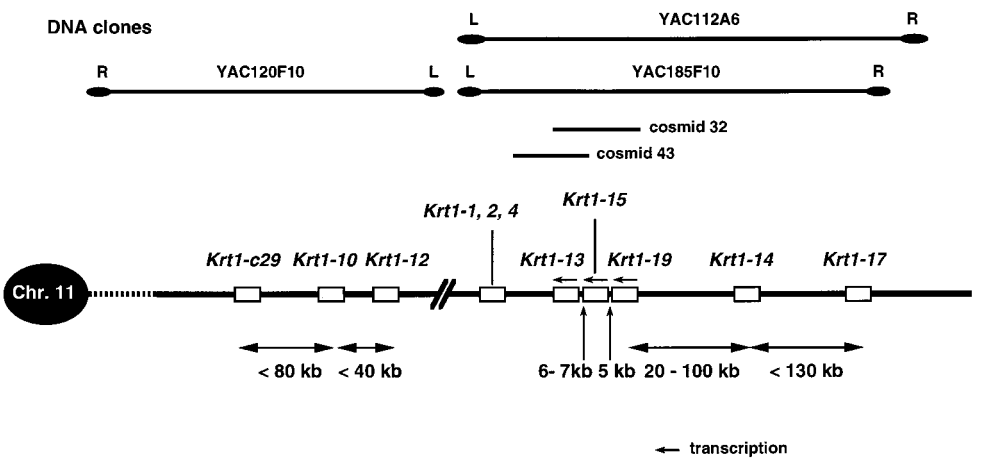


**FIG. 6.** Organization of the mouse *Krt1* genes. The gene order and the distance between adjacent genes denoted above and below the chromosome, respectively, are based on the result of the serial truncation of the YAC clones. Those of *Krt1-13*, *-15*, and *-19* are based on the physical map of cosmid clones. DNA clones used in this study are shown above the chromosome. Arrows at *Krt1-13*, *-15*, and *-19* indicate the transcriptional orientation. R, right arm of YAC; L, left arm of YAC.

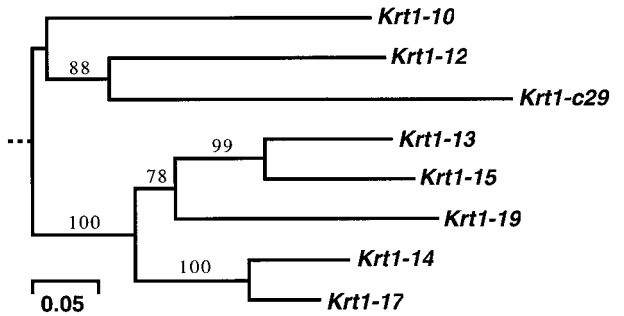


**FIG. 7.** A phylogenetic tree of eight mouse keratin genes. The location of the root (designated as a broken line) was estimated by using the mouse desmin sequence as the outgroup. Numbers on interior branches are bootstrap probabilities (%). Scale is number of amino acid substitutions per site.

sion pattern (Knapp *et al.*, 1987). Knapp and co-workers presented data for the sequence of pkSCC50, which seemed to be a partial cDNA fragment of the mouse orthologue of human *KRT17*. The sequence of pkSCC50 appears to be identical to a 0.8-kb 3′-region of *Krt1-17* cDNA. They also reported that the expression of kSCC50 was detected in normal hair follicles, hyperproliferative epidermis, and squamous cell carcinoma, where human *KRT17* is expressed as well (Moll *et al.*, 1982; Troyanovsky *et al.*, 1992). While this article was in review, another description of mouse *Krt1-17* reporting a nucleotide sequence identical to that of ours was published (McGowan and Coulombe, 1998).

On the other hand, *Krt1-c29* is a novel gene that belongs to *Krt1* as suggested by the alignment of the type I keratin rod domain (Fig. 3). Northern blot analysis in this study (Fig. 2) demonstrated that the size of the *Krt1-c29* major transcript was approximately 1.5 kb. In addition, since the sequence ACCATGT flanking the AUG initiator codon of *Krt1-c29* cDNA has homology to the Kozak consensus sequence, ACCATGG (Kozak, 1986, 1987), *Krt1-c29* is probably a full-length cDNA. The presence of a minor transcript approximately 3.5 kb in size indicates that there is an alternative promoter or alternative splicing in the *Krt1-c29* gene, because genomic Southern hybridization with a 0.6-kb 5′-fragment of the *Krt1-c29* cDNA as a probe gave a unique strong signal.

The high-resolution linkage analyses in this study (Fig. 4) revealed that the gene order in *Krt1* is centromere–*Krt1-c29*–*Krt1-10*, *Krt1-12*–*Krt1-15*–*Krt1-19*, *Krt1-14*, *Krt1-17*–telomere and suggested that there are two major clusters in the *Krt1* complex, one containing *Krt1-c29*, *Krt1-10*, and *Krt1-12* and the other containing *Krt1-15*, *Krt1-19*, *Krt1-14*, and *Krt1-17*. The mouse type I hair keratin 2 and 3 genes *Krt1-4* and *Krt1-2* (Winter *et al.*, 1994) did not recombine with *D11Mit123*. The oligonucleotide primers for *D11Mit123* are set within *Krt1-1* (data not shown). In conjunction with these data, the fact that *Krt1-1*, *Krt1-4*, and *Krt1-2* are present in YAC185F10 and YAC112A6, but not in cosmids 32 and 43 (Fig. 6), suggests that the mouse type I hair keratin genes are located between *Krt1-12* and *Krt1-13*. It has been proposed that *Krt1* genes are responsible for mouse mutations with skin and hair anomalies, such as *Re*, *Bsk*, and *Rim3* (Eicher and Varnum, 1986; Lyon and Zenthon, 1986, 1987; Nadeau *et al.*, 1989; Liu *et al.*, 1994; Sato *et al.*, 1998). Linkage analysis in this study, however, indicated that the *Krt1* complex including type I hair keratin genes are excluded as candidates for *Rim3*, which may be allelic to $Re^{den}$.

We have determined the genomic organization of the *Krt1* complex in mice using the YAC truncation method (Fig. 6). The isolation of a clone truncated between *D11Mit59* and *Krt1-19* in YAC185F10 (Fig. 5) determined the transcriptional orientation of *Krt1-19*, because *D11Mit59* is located within the first intron of *Krt1-19* and the PCR primer set for *Krt1-19* used in this study is located at the 3′ noncoding region. Because there are several B1 repetitive sequences in the first intron of *Krt1-19*, a recombination could occur in one of them (Lussier *et al.*, 1990). We constructed a restriction map of two overlapping cosmid clones isolated from a mouse cosmid library (Clontech, Palo Alto, CA). The combined size of the two clones was approximately 55 kb, and they contained *Krt1-13*, *Krt1-15*, and *Krt1-19* (data not shown). This is consistent with the study of Filion *et al.* (1994) and suggested that *Krt1-14* and *Krt1-17* are located at least 5 kb from the 5′-region of *Krt1-19*. Although we need to obtain a more precise physical map and to determine the transcriptional orientation of each gene, it is likely that the genome organization of *Krt1-13*, *Krt1-15*, *Krt1-19*, *Krt1-14*, and *Krt1-17* is different from that in the human genome (Milisavljevic *et al.*, 1996). Conversely, it would be intriguing to analyze the genomic organization of human orthologues of *Krt1-c29*, *Krt1-10*, and *Krt1-12*.

A phylogenetic analysis of the *Krt1* complex clearly showed that the genes are classified into two subclusters, and the clustering is well correlated to the genomic organization. This suggests that each subcluster arose independently from a separate ancestral gene. It seemed likely that members of the same subcluster of a multigene family tend to have similar expression patterns and functions. In fact, *Krt1-13*, *-15*, and *-19* are expressed in the same epithelial tissues, such as oral cavity and esophagus, and are expected to have similar functions. Likewise, *Krt1-14* and *-17* are commonly expressed in the epithelia of hair follicle and mammary gland ducts. On the other hand, *Krt1-10* and *-12*, which constitute another subcluster, are not expressed in the same tissues.

## ACKNOWLEDGMENTS

We are grateful to Dr. Dennis Roop of Baylor College of Medicine for his donation of the 3′-noncoding region probe of the mouse *Krt1-14* gene. This study was supported by a Grant in Aid from the Ministry of Education, Science and Culture of Japan. This paper is contribution No. 2135 from the National Institute of Genetics, Japan.

## REFERENCES


Blumenberg, M. (1993). Molecular biology of human keratin genes. *In* "Molecular Biology of the Skin: The Keratinocyte" (M. Darmon, *et al.*, Eds.), pp. 1–24, Academic Press, New York.

Cheng, J., Syder, A. J., Yu, Q.-C., Letai, A., Paller, A. S., and Fuchs, E. (1992). The genetic basis of epidermolytic hyperkeratosis: A disorder of differentiation-specific epidermal keratin genes. *Cell* **70:** 811–819.

Coulombe, P. A., Hutton, M. E., Letai, A., Hebert, A., Paller, A. S., and Fuchs, E. (1991). Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: Genetic and functional analyses. *Cell* **66:** 1301–1311.

Eicher, E. M., and Varnum, D. (1986). Allelism of Den and Re. *Mouse News Lett.* **75:** 29–30.

Eichner, R., Bonitz, P., and Sun, T-T. (1984). Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. *J. Cell. Biol.* **98:** 1388–1396.

Filion, M., Sarafian, V., Lussier, M., Belanger, C., Lapointe, L., and Royal, A. (1994). Arrangement of a cluster of three mouse type I keratin genes expressed sequentially during esophageal-type epithelial cell differentiation. *Genomics* **24:** 303–310.

Irvine, A. D., Corden, L. D., Swensson, O., Swensson, B., Moore, J. E., Frazer, D. G., Smith, F. J. D., Knowlton, R. G., Christophers, E., Rochels, R., Uitto, J., and McLean, W. H. I. (1997). Mutations in cornea-specific keratin *K3* or *K12* genes cause Meesmann's corneal dystrophy. *Nat. Genet.* **16:** 184–187.

Kao, W. W.-Y., Liu, C.-Y., Converse, R. L., Shiraishi, A., Kao, C. W.-C., Ishizaki, M., Doetschman, T., and Duffy, J. (1996). Keratin 12-deficient mice have fragile corneal epithelia. *Invest. Ophthalmol. Vis. Sci.* **37:** 2572–2584.

Knapp, B., Rentrop, M., Schweizer, J., and Winter, H. (1987). Three cDNA sequences of mouse type I keratins. *J. Biol. Chem.* **262:** 938–945.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44:** 283–292.

Kozak, M. (1987). At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. *J. Mol. Biol.* **196:** 947–950.

Lewis, B. C., Shah, N. P., Braun, B. S., and Denny, C. T. (1992). Creation of a yeast artificial chromosome fragmentation vector based on lysine-2. *GATA* **9:** 86–90.

Liu, C-Y., Zhu, G., Converse, R., Kao, C. W-C., Nakamura, H., Tseng, S. C-G., Mui, M-M., Seyer, J., Justice, M. J., Stech, M. E., Hansen, G. M., and Kao, W. W-Y. (1994). Characterization and chromosomal localization of the cornea-specific murine keratin gene *krt1.12. J. Biol. Chem.* **269:** 24627–24636.

Lussier, M., Filion, M., Compton, J. G., Nadeau, J. H., Lapointe, L., and Royal, A. (1990). The mouse keratin 19-encoding gene: Sequence, structure, and chromosomal assignment. *Gene* **95:** 203–213.

Lyon, M. F., and Zenthon, J. F. (1986). Close linkage of bareskin (Bsk) and rex (Re). *Mouse News Lett.* **74:** 96.

Lyon, M. F., and Zenthon, J. F. (1987). Relationship between bareskin, denuded and rex. *Mouse News Lett.* **77:** 125.

Manly, K. (1993). A Macintosh program for storage and analysis of experimental genetic mapping data. *Mamm. Genome* **4:** 301–314.

Marchuk, D., McCrohon, S., and Fuchs, E. (1985). Complete sequence of a gene encoding a human type I keratin: Sequences homologous to enhancer elements in the regulatory region of the gene. *Proc. Natl. Acad. Sci. USA* **82:** 1609–1613.

McGowan, K. M., and Coulombe, P. A. (1998). Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. *J. Cell Biol.* **143:** 469–486.

McLean, W. H. I., Rugg, E. L., Lunny, D. P., Morley, S. M., Lane, E. B., Swensson, O., Dopping-Hepenstal, P. J. C., Griffiths, W. A. D., Eady, R. A. J., Higgins, C., Navsaria, H. A., Leigh, I. M., Strachan, T., Kunkeler, L., and Munro, C. S. (1995). Keratin 16 and keratin 17 mutations cause pachyonychia congenita. *Nat. Genet.* **9:** 273–278.

Milisavljevic, V., Freedberg, I. M., and Blumenberg, M. (1996). Close linkage of the two keratin gene clusters in the human genome. *Genomics* **34:** 134–138.

Moll, R., Franke, W. W., Schiller, D. L., Geiger, B., and Krepler, R. (1982). The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors, and cultured cells. *Cell* **31:** 11–24.

Nadeau, J. H., Berger, F. G., Cox, D. R., Crosby, J. L., Davisson, M. T., Ferrara, D., Fuchs, E., Hart, C., Hunihan, L., Lalley, P. A., Langley, S. H., Martin, G. R., Nichols, L., Phillips, S. J., Roderick, T. H., Roop, D. R., Ruddle, F. H., Skow, L. C., and Compton, J. G. (1989). A family of type I keratin genes and the homeobox-2 gene complex are closely linked to the rex locus on mouse chromosome 11. *Genomics* **5:** 454–462.

Nozaki, M., Mori, M., and Matsushiro A. (1994). The complete sequence of the gene encoding mouse cytokeratin 15. *Gene* **138:** 197–200.

Reis, A., Hennies, H.-C., Langbein, L., Digweed, M., Mischke, D., Drechsler, M., Schrock, E., Royer-Pokora, B., Franke, W. W., Sperling, K., and Kuster, W. (1994). Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK). *Nat. Getet.* **6:** 174–179.

Richard, G., Laurenzi, V. D., Didona, B., Bale, S. J., and Compton, J. G. (1995). Keratin 13 point mutation underlies the hereditary mucosal epithelia disorder white sponge nevus. *Nat. Genet.* **11:** 453–455.

Rugg, E. L., McLean, W. H. I., Allison, W. E., Lunny, D. P., Macleod, R. I., Felix, D. H., Lane, E. B., and Munro, C. S. (1995). A mutation in the mucosal keratin K4 is associated with oral white sponge nevus. *Nat. Genet.* **11:** 450–452.

Sagai, T., Koide, T., Endo, M., Tanoue, K., Kikkawa, Y., Yonekawa, H., Ishiguro, S., Tamai, M., Matsuda, Y., Wakana, S., and Shiroishi, T. (1998). *rim2* (recombination-induced mutation 2) is a new allele of pearl and a mouse model of human Hermansky–Pudlak syndrome (HPS): Genetic and physical mapping. *Mamm. Genome* **9:** 2–7.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sato, H., Koide, T., Masuya, H., Wakana, S., Sagai, T., Umezawa, A., Ishiguro, S., Tamai, M., and Shiroishi, T. (1998). A new mutation *Rim3* resembling *Reden* is mapped close to retinoic acid receptor alpha (*Rara*) gene on mouse chromosome 11. *Mamm. Genome* **9:** 20–25.

Steinert, P. M., and Roop, D. R. (1988). Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* **57:** 593–625.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.

Troyanovsky, S. M., Leube, R. E., and Franke, W. W. (1992). Characterization of the human gene encoding cytokeratin 17 and its expression pattern. *Eur. J. Cell Biol.* **59:** 127–137.

Vassar, R., Coulombe, P. A., Degenstein, L., Albers, K., and Fuchs, E. (1991). Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* **64:** 365–380.

Winter, H., Siry, P., Tobiasch, E., and Schweizer, J. (1994). Sequence and expression of murine type I hair keratins mHa2 and mHa3. *Exp. Cell Res.* **212:** 190–200.