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Structure and site of expression of a murine type II hair keratin

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Abstract

We present here a 1770 bp-long cDNA which encodes a murine type II keratin. Sequence comparisons of the keratin with those of various type II keratins expressed in mouse epidermis and internal stratified epithelia reveal that the new keratin is unrelated to epithelial keratins. Rather the structural organization of its amino- and carboxyterminal domains and the high content of cysteine and proline residues in these regions suggest that the keratin represents a murine type II hair keratin. This assumption was confirmed by in situ hybridization which localized the mRNA of the keratin in upper cells of the hair cortex and in suprabasal cells of the central core unit of filiform papillae of the tongue. Hybrid selection analyses revealed that the keratin has a molecular weight of 58 kD. It remains to be seen whether the keratin corresponds to MHb 3 or MHb 4.

Introduction

The keratin multigene family consists of about 30 individual, however, structurally related members which can be grouped into epithelial-type keratins ('soft' α-keratins) and wool- and hair-type keratins ('hard' α-keratins) [1–3]. The great majority of these proteins belongs to the epithelial-type of keratins. As a rule, the mammalian hair follicle contains only a set of eight major 'hard' α-keratins [1–6]. Four of them, Hb1, Hb2, Hb3 and Hb4, belong to the basic to neutral, type II subfamily of keratins, whereas the remaining four keratins, Ha1-Ha4, represent acidic, type I keratins [1–6]. The occurrence of an equal number of type II and type I hair keratins indicates that their synthesis follows the principle of pairwise expression, although the exact composition of the sixteen possible hair keratin pairs has not yet been elucidated. Comparisons of the presently available amino acid sequence data of both wool and hair keratins with that of epithelial keratins have revealed that 'hard' and 'soft' keratins are rather homologous in their central α-helical rod domain, however, differ substantially in their non α-helical head and tail portions [3, 6–8]. Typically, the 'hard' keratins show an accumulation of cysteine and proline residues in their amino- and carboxyterminal domains [6–8] and the high content of cysteine is thought to essentially account for the rigidity and physical strength of the mature hair and wool fiber by extensive S-S-bridging of the constituent proteins. Immunohistochemical studies and protein investigations in different laboratories have shown that the eight hair type keratins are not only expressed in the hair follicle, but also occur in nail-forming cells, the filiform papillae of the tongue and the thymic reticulum [1, 2, 4, 5, 9].

It is evident that the knowledge of the amino acid squences of the individual hair keratins would essentially contribute to the understanding of the development and formation of hard, keratinized structures in these different anatomical locations. However, at present sequence information for wool and hair type keratins is relatively sparse and to our knowledge limited to that of a sheep wool type II keratin 7c [7] and to two murine type I hair keratins, MHa1 and MHa4 [6, 8].

In the course of our studies on keratin expression in various stratified and keratinized epithelia of the mouse, we have recently constructed a cDNA library with polyA$^+$RNA from adult mouse tail epidermis. This library was aimed to serve for the isolation of cDNA clones for keratins which are supposed to be expressed in the parakeratotic scale epidermis of this morphologically complex epithelium [10]. The screening of the library for type II keratin cDNAs yielded, besides several new keratin clones, one clone which turned out to encode a murine type II hair keratin. In this paper we present the almost complete amino acid sequence of this keratin and, by in situ hybridization, provide data on its tissue-specific expression.

Materials and methods

cDNA cloning and screening procedures

PolyA$^+$RNA of adult mouse tail epidermis, obtained after a short incubation of tail skin in 60 °C hot water [10], was isolated according to the method of Gough [11] and used for the construction of a cDNA library in λgt10 [12]. The library was screened by hybridization with a [^{32}P]-labeled 535 bp ClaI/KpnI fragment of the previously described cDNA clone pkt57 which encodes the type II keratin K4 of internal stratified mouse epithelia [13]. The fragment spans from position 601 of 1136 of pkt57 and thus contains a portion of the sequence coding for the α-helical domain of K4 [13]. Hybridization was carried out for 18 h at 42 °C (35% formamide, 5× SSPE) and subsequent washing was at 52 °C (final wash with 0.1× SSC, 0.1% SDS). The resulting positive phage clones were further hybridized with a mixture of [^{32}P]-labeled specific 3′ cDNA fragments of type II keratin clones pke70 [14], pktl-1 and pktl-5 [unpub. results]. Hybridization was performed for 18 h at 42 °C (50% formamide, 5× SSPE) and the final wash was at 68 °C (0.1× SSC, 0.1% SDS).

RNA slot blot hybridization

Phage clones which did not react with the mixture of specific 3′-fragments of the clones coding for K1, K5 and the 70 kd keratin were subjected to RNA slot blot hybridization. RNA was isolated as described above from the epidermis of different skin sites, various internal stratified epithelia, epidermal tumors and cell lines. Slot blots were performed with Gene screen plus membranes (DuPont, Dreieich, Germany) using a minifold I system (Schleicher & Schüll, Dassel, Germany). Hybridization was carried out for 18 h at 42 °C (50% formamide, 1 M NaCl, 1% SDS, 50 mM Tris-Cl, pH 7.6, 10% dextrane sulfate) with [^{32}P]-labeled phage inserts. Membranes were washed stringently (0.1× SSC, 1% SDS at 68%) and exposed at −80 °C to Kodak X-Omat films. One phage clone, termed λktlII-4 was chosen for further characterization.

Subcloning and DNA sequencing

The insert of λktlII-4, attached to *Eco* RI adaptors, was cloned into the transcription vector Bluescript II KS + (Strategene; La Jolla, USA). Sequencing of both strands of this plasmid clone pktlII-4 was performed according to the dideoxy sequencing method of Sanger et al. [15], using first M13 and T3 primers and subsequently 17mer synthetic oligonucleotides as walking primers. To prepare a specific probe of 3′ noncoding region of clone pktlII-4, a 262 bp *Pst* I fragment was subcloned into Bluescript II KS + . This subclone was designated pktlII-4-3′.

In situ hybridization

The protocol used for in situ hybridization of frozen tissue sections (nominally 6 μm thick) was essentially as described previously [14, 16, 17], however, with some modifications [18, 19]. Upon linearization of pktlII-4-3′ with *Sma* I, riboprobes were obtained that were labeled with [^{35}S]-UTP by in vitro transcription with T3 RNA polymerase. A sense probe was used as negative control. Slides were dipped in Kodak NTB2 photoemulsion, developed after 2 to 5 days and stained with hematoxylin-eosin.

Hybrid selection analysis and translation in vitro

PolyA$^+$RNA obtained by oligo (dT) cellulose chromatography of total RNA of tail epidermis was hybridized to filter bound DNA of pktlII-4-3′ and the selected mRNA was translated in vitro in the presence of [^{35}S]-methionine. PolyA$^+$RNA from both footsole epidermis and tongue epithelium was also translated in vitro. One dimensional SDS PAGE of the in vitro translation products and autoradiography were performed as described [20].

Results and discussion

In an attempt to isolate a cDNA clone for a particular type II 65 kD keratin of adult mouse tail epidermis [10], we have screened a λgt10 library, constructed with polyA$^+$RNA of the epidermis of this skin site for the presence of type II keratin cDNAs. To this purpose we used an appropriately taylored DNA probe which was derived from the region coding for the α-helical domain of murine keratin K4 [13]. From the resulting bulk of keratin cDNAs, we first eliminated those clones which hybridized with the specific 3′noncoding ends of the previously isolated cDNAs of the 70 kD keratin [14] and of keratins K1 and K5 [unpub. data]. These keratins represent the major type II keratins expressed in adult mouse tail epidermis [10]. In order to preliminary characterize the remaining clones, these were subjected to slot blot hybridization analysis with RNA from epidermis of different skin sites, various internal stratified epithelia, benign and malignant epidermal tumors and a murine epidermal cell line. This analysis resulted in the identification of three clones which exhibited a strong hybridization signal with polyA$^+$RNA of tail epidermis, however, also showed a weak, but significant reaction with polyA$^+$RNA of tongue epithelium (Fig. 1). This intriguing expression characteristics prompted us to sequence the corresponding clones. Two of them turned out to be identical and were found to encode the desired type II 65 kD keratin whose properties will be reported elsewhere [Tobiasch et al., in prep.].

The nucleotide sequence of the 1770 bp insert of the third clone, pktlII-4, is shown in Fig. 2. It

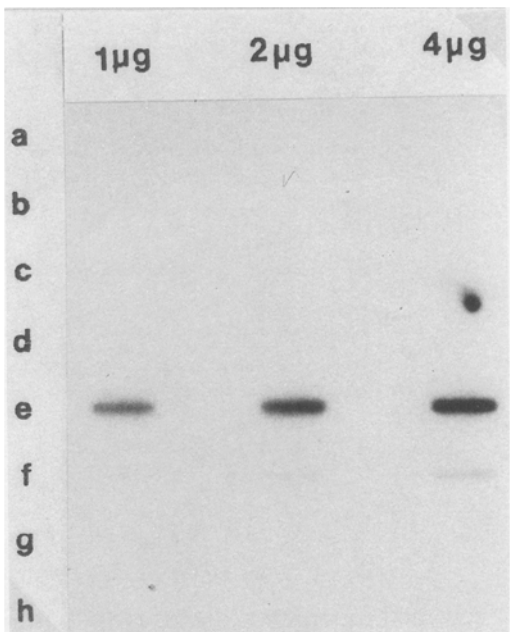

Fig. 1. RNA slot blot hybridization. Total RNA from (*a*) neonatal mouse epidermis, (*b*) adult mouse footsole epidermis (*c*) a DMBA/TPA-induced epidermal squamous cell carcinoma, (*d*) a papilloma-derived epidermal cell line, SP1 [26], (*e*) mouse tail epidermis, (*f*) mouse tongue epithelium, (*g*) mouse palate epithelium and (*h*) mouse brain was spotted onto a nylon membrane in concentrations of 1 μg, 2 μg and 4 μg each and hybridized with the phage clone λktl II-4 insert. Note the strong reaction with RNA of tail epidermis and the weak reaction with RNA of tongue epithelium (*e*, *f*).

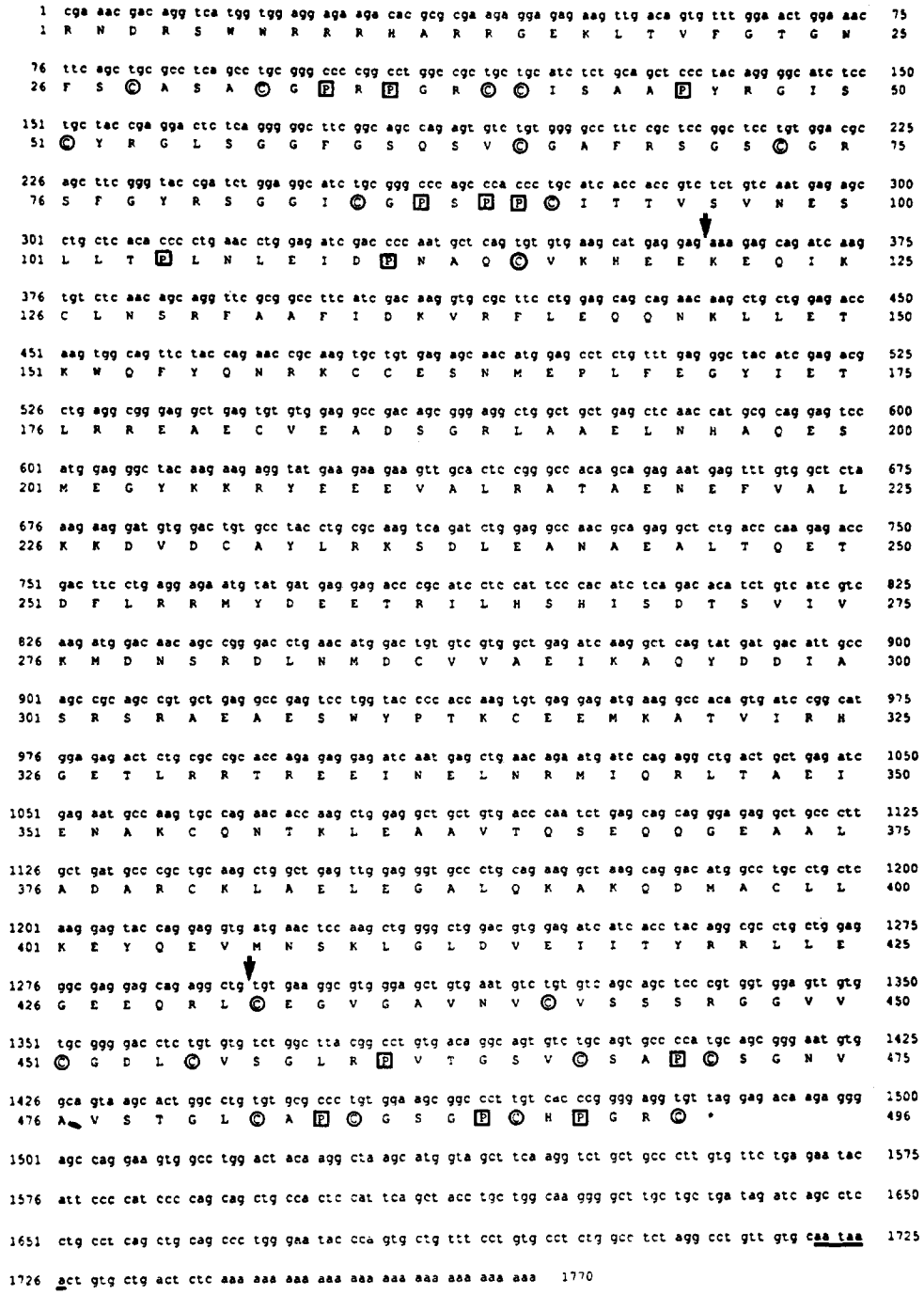

Fig. 2. Nucleotide sequence of the pktl II-4 insert and deduced amino acid sequence of the encoded type II keratin. The nucleotide sequence is shown in the 5′ to 3′ direction of the mRNA. The stop codon is marked by an asterisk, the polyadenylation signal is underlined. Arrows denote the region coding for the α-helical domain. In the non α-helial domains, cysteine residues are encircled and proline residues are boxed.

contains the complete 3′ noncoding region and almost the complete coding region of the mRNA of a keratin whose amino acid sequence is also indicated in Fig. 2. Sequence alignments with various type II murine keratins expressed in the epidermis and in internal stratified epithelia (shown here for keratin K4 in Fig. 3) readily reveal that the keratin encoded by pktlII-4 is unrelated to these keratins. The α-helical rod domains of the two keratins exhibit a sequence homology of about 55%. However, there is evidence for an only poorly conserved H1 subdomain [21] in the aminoterminal region of the new keratin, whereas a distinct H2 subdomain in its carboxyterminus cannot be detected. In addition, although both the head and tail regions of the new keratin are relatively rich in serine residues, the absence of the typical accumulation of GGG and GGX motifs (with X being predominantly serine) [21] in the central part of both regions, does not allow to delineate distinct V1 and V2 subdomains [21]. Instead both, the amino- and the carboxyterminus of the new keratin contain a high percentage of cysteine and proline residues (10 cysteines, 8 prolines in the aminoterminus, 10 cysteines and 5 prolines, in the carboxyterminus). Especially in the carboxyterminus, these amino acids frequently appear as PC motifs (Fig. 2). Interestingly, all these properties are also a typical feature of the sheep wool type II keratin 7c [7] and of two murine type I hair keratins, MHa1 and MHa4, recently described by Bertolino et al. [6, 8]. It therefore appears that clone pktlII-4 encodes a type II murine hair keratin.

This assumption is confirmed by sequence comparison of the carboxyterminal regions of the new keratin and sheep wool type II keratin 7c (Fig. 4). Except for the penultimate parts of the carboxytermini, such an alignment demonstrates an almost complete homology of the two sequences. Moreover, in situ hybridization with the insert of subclone pktlII-4-3′ to frozen sections of adult mouse tail epidermis and newborn mouse back epidermis reveals hybridization signals specifically over hair follicles. In longitudinal sections of hair follicles (Fig. 5a), it can be seen that the hybridization signals occur only over upper cortex cells, whereas cells of the outer and inner root sheaths and matrix cells in the bulbar region are free of label. This particular distribution of the mRNA of the new keratin could also be confirmed in cross sections through different levels of the hair follicle (Fig. 5b–d). The site of expression of the mRNA of the new hair keratin is therefore in agreement with numerous studies in which the localization of hair keratin proteins in the follicles of different species has been investigated by indirect immunofluorescent staining techniques [1, 2, 4, 5, 6, 8].

With the demonstration that clone pktlII-4 encodes a type II hair keratin, the positive reaction with tongue epithelial polyA$^+$RNA in the slot blot hybridization analysis of Fig. 1 becomes understandable. Recent investigations in different laboratories have shown that hair type keratins are expressed in the central core unit of the filiform tongue papillae [5, 9]. In this compartment of the morphologically complex murine filiform papillae [22–24] the synthesis of these keratins is thought to be responsible for the formation of the posteriorly inclined, hook-shaped solid spine which consists of an extremely condensed keratin material [9]. As shown in Fig. 6, the mRNA of the new type II hair keratin is clearly expressed in the central core unit of the filiform papillae. The label is concentrated over living, suprabasal cells, whereas basal cells are free of label (Fig. 6b). We have previously shown that basal cells of the filiform papillae express keratins K5 and K14 [17].

The finding that clone pktlII-4 encodes a type II hair keratin, requires an explanation for its strong reaction with mRNA of tail epidermis and the negative reaction with mRNA of both newborn mouse epidermis and adult mouse footsole epidermis in the slot blot hybridization analysis of Fig. 1. We have previously shown that by gently lifting tail epidermis from the dermis of tail skin incubated for a short period in hot water, hair follicles quantitatively remain in the dermis [25]. In the present investigation, however, tail epidermis was scraped off from the dermis with forceps after incubating the skin in hot water. By this manipulation, large quantities of hair follicles are removed together with the epidermis. In contrast

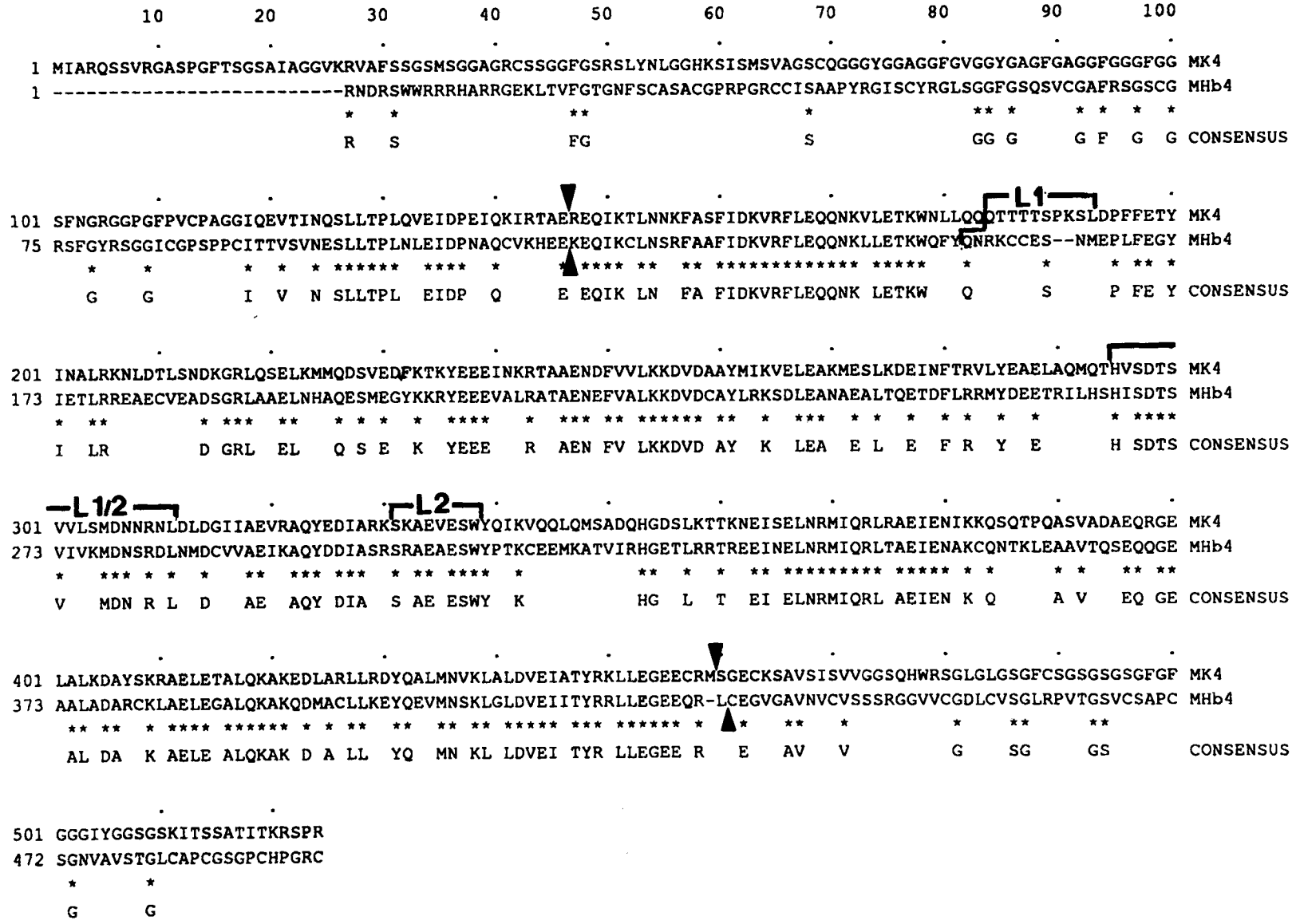

Fig. 3. Comparison of the amino acid sequences of the murine type II epithelial keratin K4 (first row) and the keratin encoded by clone pktl II-4 (second row). Also indicated is the consensus sequence between the two keratins (fourth row). The arrowheads denote the cental α-helical domains in which the non coiled-coil linker regions are indicated.

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          .          .          .          .          .
LCEGVGAVNVCVSSSRGGVVCGDLCVSGLRPVTGSVCSAPCSGNVAVSTG  MK
||||||||||||||||||||||||||||| ||||||||||||||| |||||
LCEGVGAVNVCVSSSRGGVVCGDLCVSGSRPVTGSVCSAPCSGNLAVSTG  SK
          .          .
LCAPCGSGPCHPGRC.......
||||||       |
LCAPCGQLNTTCGGGSCSLGRC
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Fig. 4. Sequence comparison of the carboxyterminal regions of the new murine type II keratin encoded by clone pktl II-4 (MK) and sheep wool type II keratin 7c (SK) [7].

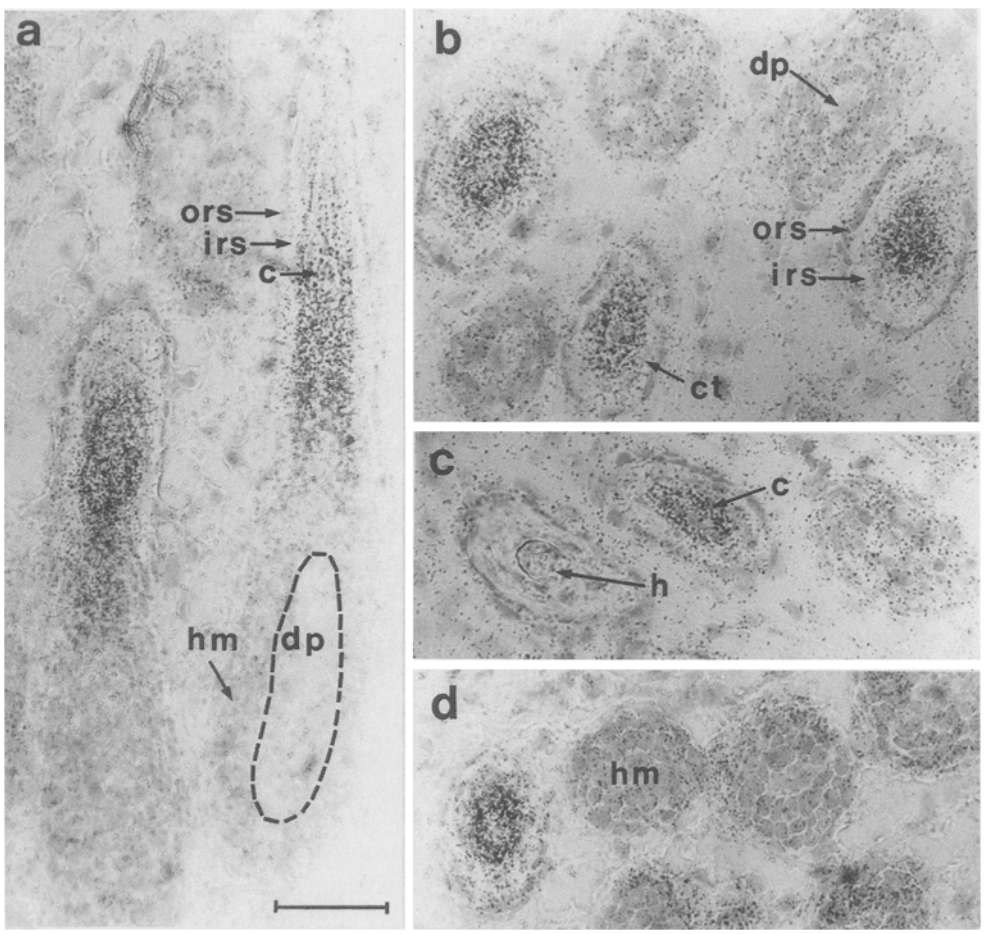

Fig. 5. In situ hybridization of the [^{35}S]-labeled specific riboprobe of subclone pktl II-4-3′ to frozen sections of (*a*) adult mouse tail skin and (*b–d*) neonatal (3 days old) mouse back skin. In (*a*) a longitudinal section and in (*b–d*), cross sections of hair follicles are shown. ors, outer root sheath; irs, inner root sheath; c, cortex; hm, hair matrix; ct, cuticle; h, hair; dp, dermal papillae. Bar = 100 μm throughout.

newborn mouse epidermis was gently peeled off from the dermis and in the case of footsole epidermis, only the glabrous skin portion was used for the heat isolation of epidermis. It is thus conceivable that these differences in tissue separation may account for the presence of hair keratin mRNAs in tail epidermis.

Finally, in order to investigate to which of the

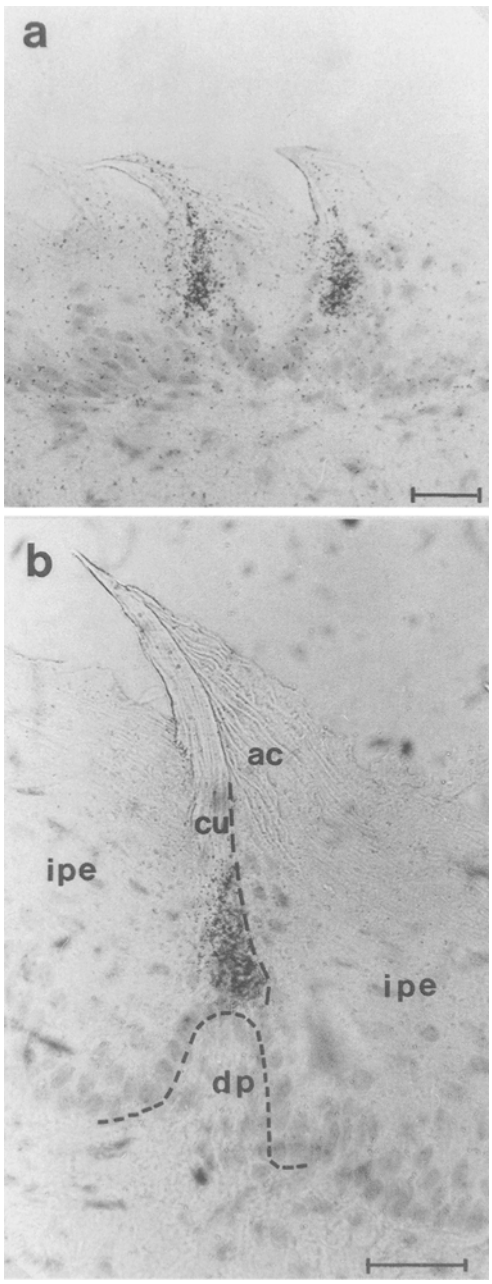

Fig. 6. In situ hybridization of the [^{35}S]-labeled specific riboprobe of subclone pktl II-4-3′ to frozen sections of mouse tongue (*a, b*). Note the specific localization of hybridization signals in suprabasal cells of the central core unit of the filiform papillae (*b*). fp, filiform papillae; ac, anterior compartment; cu, central core unit; ipe, interpapillary epithelium; dp, dermal papillae. Bar = 100 μm in (*a*) and (*b*).

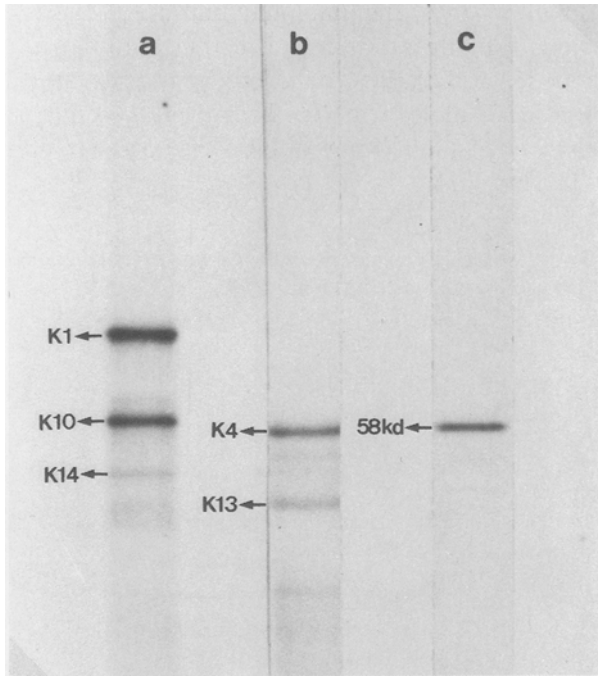

Fig. 7. Hybrid selection analysis. The mRNA-species selected by hybridization of polyA $^+$RNA of tail epidermis to filter-bound DNA of subclone pktl II-4-3′ was translated in vitro in the presence of [^{35}S]-methionine. The translation product was resolved by one dimensional SDS-PAGE on a 9% gel (lane *c*). Lanes *a* and *b* represent the keratin profiles obtained by in vitro translation of polyA $^+$RNA of footsole epidermis (*a*) and tongue epithelium (*b*). The molecular weight of the keratin in lane *c* was estimated relative to the molecular weights of keratins K1 (67 kD), K10 (60 kD), K14 (52 kD) in lane *a* and K4 (57 kD) and K13 (47 kD) in lane *b*.

four type II murine hair keratins the new keratin might correspond, we performed a hybrid selection experiment with polyA$^+$RNA of tail epidermis. As shown in Fig. 7, subclone pktlII-4-3′ selected a mRNA which on release and in vitro translation yielded a single protein (Fig. 7, lane c). Using the in vitro translated keratins of footsole epidermis and tongue epithelium polyA$^+$RNA as a reference, the size of the protein could be assessed to 58 kD. Calculation of the molecular weight of the presented part of the pktlII-4 encoded keratin yields a value of 55 kD, indicating that the sequence of the keratin lacks only few

amino acids of the aminoterminal domain. Previous experiments had shown that both murine type II hair keratins MHb3 and MHb4 migrate in the 58 kD molecular weight range [6, 8]. Further experiments are required to definitely assign our new keratin within the type II murine hair keratin subfamily.

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Note

After submission of our paper, Yu et al. published a full length cDNA clone encoding a murine type II hair keratin. By means of Western blotting with a monospecific antibody directed against the last 18 amino acids of the carboxyterminus of the encoded keratin, the authors showed that the keratin corresponds to MHb4 (Yu et al., J. Invest. Dermatol. 97, 354–361, 1991). Except for a short sequence area coding for the penultimate aminoterminal region, the clone published by Yu et al. and our clone are completely identical. The break between the two sequences occurs upstream from position 61 (triplet ttt in Fig. 2). We have re-examined the sequence area 1–61 of our clone and found it to be correct. The calculated molecular weight of the keratin encoded by the clone of Yu et al. is 52 kD. This value is far below the SDS-PAGE size estimates of about 57–58 kD for MHb4 (Bertolino et al., J. Invest. Dermatol. 94, 297–303, 1990). As a rule molecular weights of keratins calculated from cloned sequences are slightly larger than those estimated from SDS-PAGE. This is also true for type I hair keratins (Bertolino et al., J. Invest. Dermatol. 91, 541–546, 1988). It, therefore, appears that the aminoterminal domain of the clone published by Yu et al. may be too short.

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