

## Two type II keratin genes are localized on human chromosome 12

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**Summary.** Human genomic DNA containing two type II keratin genes, one coding for keratin 1 (K1, a 68-kD basic protein) and another closely linked type II gene 10–15 kb upstream (K?, gene product unknown), was isolated on a single cosmid clone. *EcoRI* restriction fragments of the cosmid were subcloned into pGEM-3Z, and specific probes comprising the C-terminal coding and 3' noncoding regions of the two genes were constructed. The type II keratin genes were localized by in situ hybridization of the subcloned probes to normal human lymphocyte chromosomes. In a total of 70 chromosome spreads hybridized with the K? probe (gHK?-3', *PstI*, 800 bp), 36 of the 105 grains observed were on chromosome 12, and 32 of these were clustered on the long arm near the centromere (12q11–13). In 100 labeled metaphases hybridized with the K1 probe (gHK1-3', *BamHI-PstI*, 2100 bp), 53 grains localized to chromosome 12 and 46 of these were found in the same region (q11–13). Therefore, both the gene for human keratin 1, a specific marker for terminal differentiation in mammalian epidermis, and another closely linked unknown type II keratin gene (K?, 10–15 kb upstream of K1) are on the long arm (q11–13) of human chromosome 12.

Evidence has demonstrated that keratins are linked in type-specific clusters (Powell et al. 1986; RayChaudhury et al. 1986; Blumenberg and Savtchenko 1987; Bowden et al. 1987b), but the distribution of these keratin clusters throughout the genome has not been determined. The other related IF genes so far studied have been localized to different chromosomes, desmin on chromosome 2 (Quax et al. 1985), vimentin on the short arm of chromosome 10 (Quax et al. 1985; Ferrari et al. 1987), and the light neurofilament chain (NF-L) on the short arm of chromosome 8 (Hurst et al. 1987).

Recently, two closely linked type I keratin genes (K14 and K16) were localized to both the long and short arms of human chromosome 17, probably the result of a duplication event (Rosenberg et al. 1988). Preliminary results from another laboratory have localized a type II keratin gene (K4) on chromosome 12 and a type I gene (K13) on chromosome 17 (Romano et al. 1987). In the present study, two other type II keratin genes (K1 and K?, a linked keratin gene upstream of K1), which were subcloned from a single 40-kb cosmid insert, have been localized to the long arm of chromosome 12, close to the centromere (q11–13).

### Introduction

Keratin genes are expressed in all epithelial cells in a differentiation-specific manner and form 10-nm intermediate filaments (IF), an integral part of the mammalian cytoskeleton (Anderton 1981; Quinlan et al. 1985; Steinert and Parry 1985). Specific alterations in epidermal keratin gene expression occur during terminal differentiation. Cells in the proliferative basal layer of the epidermis have a filament system composed mainly of K5 (keratin 5, a 60-kD basic IF protein) and K14 (keratin 14, a 51 kD acidic IF-protein: see Moll et al. 1982). As cells become committed to terminal differentiation and migrate into the spinous layer above, the K5 and K14 genes are down-regulated while the K1 and K10 genes are strongly up-regulated in these supra-basal cells (Sun et al. 1983; Bowden et al. 1984; Schweizer et al. 1984). Thus, K1 and K10 are characteristic of terminally differentiating epidermal cells (Sun et al. 1985; Bowden et al. 1987a).

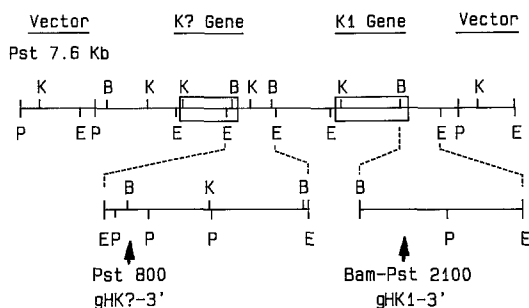
### Materials and methods

#### *Probe isolation*

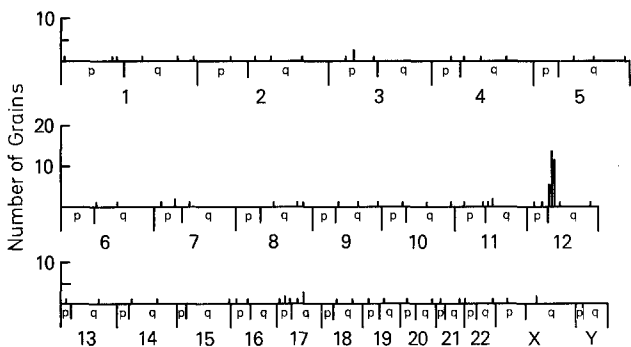
The keratin probes were subcloned from a human cosmid clone (14-1a, see Bowden et al. 1987b), which contained two closely linked type II keratin genes. The cosmid DNA was restricted with *EcoRI* and the six fragments (11.2 kb, 9.8 kb, 7.8 kb, 5.6 kb, 5.1 kb, 4.9 kb) were subcloned into *EcoRI*-cut pGEM-3Z vector (Promega, Madison, Wis 53711). The subclones containing keratin genes were identified by colony hybridization to keratin cDNA clones (see Steinert et al. 1985a). Three subclones (p4E11, insert 5.1 kb; p4D11, insert 4.9 kb and p4D1, insert 11.2 kb) were identified, mapped (Fig. 1), and partially sequenced (P. E. Bowden, unpublished data). The C-terminal and 3' noncoding regions of both genes were then further subcloned into pGEM by isolating fragments from low-melting-point agarose: p4D11 (K?), *PstI* piece (800 bp), and p4D1 (K1), *BamHI-PstI* piece (2100 bp). DNA was isolated from these subclones by cesium gradient centrifugation (Lofstrand Laboratories, Gaithersburg, Md 20879) and adjusted to a concentration of 1 mg/ml for in situ hybridization.

### In situ hybridization

In situ hybridization of human metaphase and prometaphase preparations from methotrexate-synchronized normal peripheral lymphocyte cultures and banding procedures from grain localization were carried out as previously described (Harper and Saunders 1981; Popescu et al. 1985).



**Fig. 1.** Restriction map of a human cosmid clone (14-1a) containing about 40 kb of genomic DNA, which codes for two type II keratin genes. The identity of the upstream gene is unknown (gHK?) but the downstream gene codes for human keratin 1 (gHK1). The K? gene exists on two *EcoRI* fragments (4E11, 5.1 kb and 4D11, 4.9 kb) and the gHK?-3' probe was made by subcloning an 800-bp *PstI* piece of 4D11. The K1 gene lies on an 11.2-kb *EcoRI* fragment (4D1) and the gHK1-3' probe was made by subcloning a 2100-bp *BamHI* to *PstI* piece. Both probes cover unique C-terminal coding and 3' noncoding sequences



**Fig. 2.** Histogram representing the grain distribution in chromosome spreads hybridized to the genomic DNA probe gHK?-3' (p4D11 *PstI*, 800 bp)

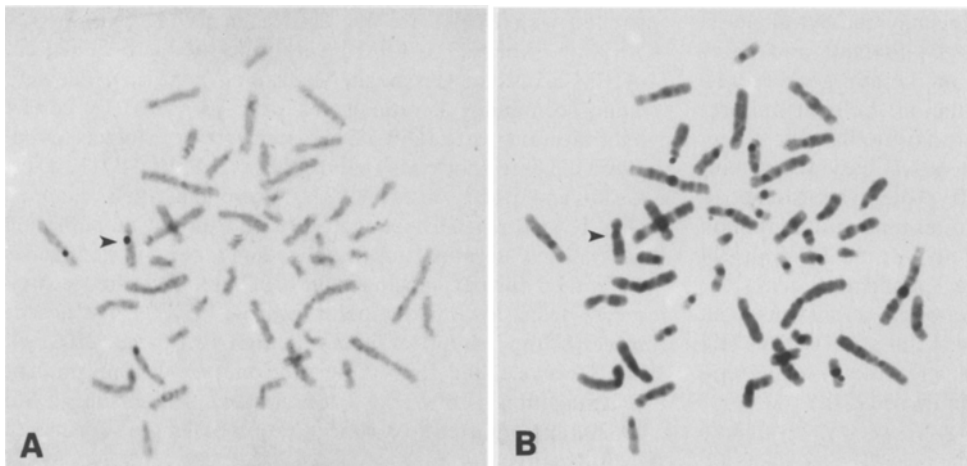
### Results

The chromosomal location of two keratin genes (type II) was determined by in situ hybridization of  $^3\text{H}$ -labeled plasmids containing a 3' probe (Fig. 1) for either an unknown type II human keratin gene, gHK?-3' (p4D11, *PstI*: 800 bp) or a human keratin 1 gene, gHK1-3' (p4D1, *BamHI-PstI*: 2100 bp) on human chromosomes from a normal donor. A total of 70 labeled chromosome spreads were examined for the gHK?-3' probe and 105 grains were localized on a 400-band ideogram (Fig. 2). This resulted in the specific labeling of chromosome 12 (Fig. 3) and 32 of the 36 grains were clustered on the long arm (bands 11-13) near the centromere. The second probe, gHK1-3', isolated from the same cosmid clone, was also localized to the same region of chromosome 12 (Fig. 4). A total of 201 grains from 100 metaphases were examined and 53 grains (26%) were located on chromosome 12 with the largest accumulation (46 of 53) in the region 12q11-13. In addition, 5 grains were observed on the short arm of chromosome 12 with the gHK1-3' probe and 3 of these were found at 12p15. The remaining grains were distributed randomly over the rest of the chromosomes.

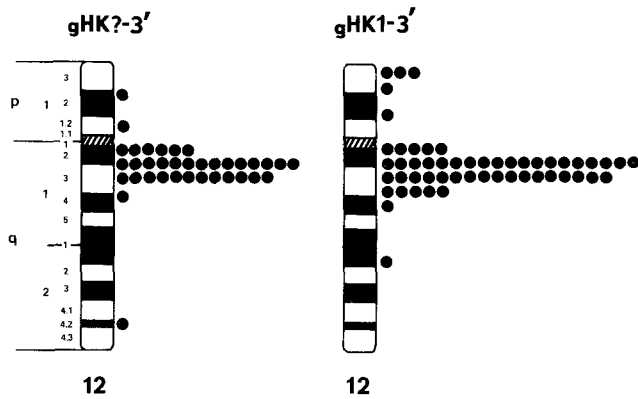
Combining these two sets of data and considering that the two probes used came from different type II keratin genes, which according to cosmid cloning data lie about 10-15 kb apart in the genome, at least two type II keratin genes are located on chromosome 12 at 12q11-13.

### Discussion

Keratin genes appear to be linked in a type-specific manner (RayChaudhury et al. 1986; Powell et al. 1986; Bowden et al. 1987b; Blumenberg and Savtchenko 1987), but neither the size variation of the individual gene clusters nor their distribution through the genome has yet been determined. However, recent studies suggest that, unlike other IF genes, keratin genes reside on at least two different chromosomes, K4 (type II) on chromosome 12 (Romano et al. 1987) and K13, K14, and K16 (all type I) on chromosome 17 (Romano et al. 1987; Rosenberg et al. 1988). The present in situ data localizes two type II keratin genes to the long arm (q11-13) of human chromosome 12. The two genes were originally subcloned from a single human cosmid clone (Bowden et al. 1987b; 1988) and are separated by 10-15 kb. The upstream gene has not yet



**Fig. 3A, B.** Sequential analysis of a human metaphase spread (A) after in situ hybridization with the genomic DNA probe gHK?-3' (p4D11 *PstI*, 800 bp) and (B) after G-banding to identify the individual chromosomes. Arrowheads indicate chromosome 12 with label on the long arm (q11-13)



**Fig. 4.** Distribution of autoradiographic silver grains shown diagrammatically on chromosome 12 for both genomic probes: *left* gHK?-3' (p4D11 *Pst*I, 800 bp); *right* gHK1-3' (p4D1 *Bam*HI-*Pst*I, 2100 bp). The largest number of grains are on the long arm at q11-13

been identified but the sequence of the probe (gHK?-3'), which is highly specific for this gene by Southern hybridization, codes for type II helical sequences and a glycine-rich C-terminal domain (P. E. Bowden, unpublished results). The sequence information so far available has ruled out the possibility that this is K4 or K1 and there is no evidence to suggest that this is a pseudogene. The downstream gene has been identified by mapping and sequencing as a polymorphic variant of human keratin 1.

Other closely related IF genes have been localized elsewhere, desmin on chromosome 2, vimentin on chromosome 10, and a neurofilament gene (NF-L) on chromosome 8. Thus, while different types of IF gene appear to be dispersed throughout the genome, the individual members comprising each type or class of IF genes may well be clustered on a single chromosome. There are still at least fourteen other epithelial keratin genes as well as several hair-specific keratin genes to map, so it cannot yet be ruled out that keratin genes are localized on chromosomes other than 12 and 17. However, from the observations of clusters on genomic clones from several laboratories, it is tempting to speculate that large clusters of keratin genes occur at defined loci on these two chromosomes.

Several genes have now been localized to the q11-13 region of human chromosome 12—melanoma-associated antigen gene ME491 (Hotta et al. 1988), human histone gene family 4 (Tripputi et al. 1986; Tanguay et al. 1988) and the gene for human lactalbumin (Davies et al. 1987). The Int-1 proto-oncogene (Van't Veer et al. 1984; Turc-Carel et al. 1987) and the GLi gene isolated from a human glioma (Kinsler et al. 1987) are both found at 12q11-13. Furthermore, a heritable fragile site exists at 12q13.1 (Sutherland and Hinton 1981), which may facilitate the integration of human papilloma virus (HPV type 18) into this region in the SW756 cervical carcinoma cell line (Popescu et al. 1987). This region of chromosome 12 is also nonrandomly involved in structural alterations in several malignancies such as mixed liposarcoma (Limon et al. 1986; Turc-Carel et al. 1986), seminoma (Atkin and Baker 1982), and teratocarcinoma (Andrews et al. 1984). As changes in keratin expression often occur in epithelial abnormalities, especially in tumors, alterations in type II keratin expression may be indicative of deletions or rearrangements involving chromosome 12. Studies of possible interactions be-

tween keratin genes and other nearby genes, as well as the influence of specific chromosome alterations on their structure or expression would therefore be relevant to the process of cell differentiation and tumor development.

Finally, the human homeobox gene C8, which is homologous to the murine Hox 1.1 locus (Cannizzaro et al. 1987), and other members of the human HOX-3 locus (Simeone et al. 1988) are present in this region of chromosome 12. Homeobox genes are involved in cellular differentiation and spatial organization during development (Snow 1986). Specific changes in keratin gene expression have also been found during embryogenesis (Shuler and Schwartz 1986; Viebahn et al. 1988) and the co-localization of these two gene families may be important for co-ordinate regulation during embryonic development. It will be interesting to see how many other type II keratin genes localize to this and other regions of chromosome 12, and to pursue further the genomic structure and regulation of this important multigene family.

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