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Structure and Chromosomal Localization of the Human Renal Kallikrein Gene†

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ABSTRACT: Glandular kallikreins are a family of proteases encoded by a variable number of genes in different mammalian species. In all species examined, however, one particular kallikrein is functionally conserved in its capacity to release the vasoactive peptide, Lys-bradykinin, from low molecular weight kininogen. This kallikrein is found in the kidney, pancreas, and salivary gland, showing a unique pattern of tissue-specific expression relative to other members of the family. We have isolated a genomic clone carrying the human renal kallikrein gene and compared the nucleotide sequence of its promoter region with those of the mouse renal kallikrein gene and another mouse kallikrein gene expressed in a distinct cell type. We find four sequence elements conserved between renal kallikrein genes from the two species. We have also shown that the human gene is localized to 19q13, a position analogous to that of the kallikrein gene family on mouse chromosome 7.

Glandular kallikreins are a subgroup of the serine protease family that, unlike trypsin, demonstrate a high degree of substrate specificity. This property, in addition to the finding of a large multigene family in the mouse, led to the suggestion that different kallikreins may play diverse roles in the proteolytic activation of peptide hormones and growth factors (Bothwell et al., 1979; Schachter, 1980; Powers & Nasjletti, 1982; Mason et al., 1983). The major kallikrein found in all mammalian species cleaves the precursor kininogen to generate Lys-bradykinin, a vasoactive peptide (Schachter, 1980). In the mouse, several members of the family expressed in male salivary gland appear to be involved in the processing of nerve growth factor (Berger & Shooter, 1977) and epidermal growth factor (Frey et al., 1979). A general role for kallikreins in the specific processing of a range of growth factors seems unlikely, however, since the size of the gene family encoding these enzymes varies widely between different mammalian species.

The mouse carries an extensive multigene family, with 12 functional kallikrein genes and a further 12 pseudogenes (Evans et al., 1987; Drinkwater, 1987). A similar family of 15–20 kallikrein genes is found in the rat, where at least 4 genes are known to be expressed (Gerald et al., 1986; Ashley & MacDonald, 1985). In both rodent species, members of the kallikrein family are sufficiently homologous that a given gene cross-hybridizes with all other genes to a similar extent (Evans et al., 1987). On the other hand, genomic blot analysis of human DNA using a human renal kallikrein cDNA probe gives only three distinct bands (Fukushima et al., 1985; Baker & Shine, 1985). If there are more than three human kallikrein genes, they are far less conserved than those in mouse and rat.

Despite the large number of functional kallikreins in the mouse, only two patterns of gene expression are observed. A single gene, mGK-6, encoding the major kininogenase, is expressed in kidney, pancreas, and both the submandibular and parotid glands (van Leeuwen et al., 1986). Interestingly, this gene is also expressed in the pituitary-derived mouse cell line, AtT20 (Herbert et al., 1984). Expression of the remaining 11 kallikrein genes appears to be confined in adult mice to the male submandibular gland, with little or no expression in female salivary glands or in other tissues (Drinkwater, 1987). Within the submandibular gland, expression of mGK-6 occurs in a different cell type to that of the other kallikrein genes (van Leeuwen et al., 1987).

As in the mouse, the major human kininogenase, referred to here as renal kallikrein, is identical with the enzyme present in pancreas and salivary gland (Fukushima et al., 1985; Baker & Shine, 1985). A second member of the human kallikrein family is found only in the prostate gland (Watt et al., 1986; Stamey et al., 1987; Lundwall & Lilja, 1987). Two other human genomic sequences, hKK-3 (Fukushima et al., 1985) and hGK-1 (Schedlich et al., 1987), show 95% amino acid sequence homology and may represent polymorphic variants of a single gene. The expression of hKK-3 or hGK-1 has not been demonstrated in any human tissue.

To examine mechanisms behind the distinct patterns of tissue-specific expression, we have identified a genomic clone carrying the human renal kallikrein gene and determined its nucleotide sequence. This allows comparison of the promoter regions of the mouse (van Leeuwen et al., 1986) and human renal kallikrein genes, which show equivalent expression, with that of a related mouse gene (mGK-1; Mason et al., 1983), which is expressed in a distinct cell type. We have further characterized the human renal kallikrein gene by determining its chromosomal localization.

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MATERIALS AND METHODS

Screening of a Human Parotid Gland cDNA Library. Poly(A) RNA was isolated from human parotid gland according to the procedures of Chirgwin et al. (1979) and Aviv and Leder (1972). A cDNA library of 50 000 clones in the λgt10 vector was constructed as described by van Leeuwen et al. (1986). The library was screened with an oligodeoxyribonucleotide probe (HK-1) designed to detect all human kallikrein genes. The probe corresponds to amino acids 85–107 predicted from the renal kallikrein cDNA (Fukushima et al., 1985). All oligodeoxyribonucleotide probes were synthesized in an Applied Biosystems Inc. Model 380A DNA synthesizer and purified by polyacrylamide gel electrophoresis.

Identification of the Human Renal Kallikrein Gene. The probe used (HRK-1) was a 30-mer corresponding to amino acids 142–151 of human renal kallikrein (Fukushima et al., 1985). The oligodeoxyribonucleotide was labeled with T4 polynucleotide kinase (Pharmacia) and [γ-^{32}P]ATP (Amersham Corp.).

Phage suspensions (1 μL) corresponding to 54 kallikrein-positive genomic clones (Fukushima et al., 1985) were spotted onto a lawn of LE392 cells and incubated at 37 °C overnight. Phage DNA was transferred to nitrocellulose, and the filters were prehybridized at 42 °C for 4 h in the 20% formamide buffer described by Ullrich et al. (1984). Following addition of the probe, hybridization was continued overnight at 42 °C; then the filters were washed in 2 × standard saline citrate at 60 °C and exposed at −80 °C with intensifying screens to Kodak XAR-5 film.

Restriction Mapping and Nucleotide Sequencing. Phage DNA from the genomic clone λHGK-43 was digested with a range of restriction enzymes (Pharmacia; see Figure 1), the fragments were separated on 0.9% agarose gels, and the DNA was transferred to nitrocellulose filters by the method of Southern (1975). The filters were hybridized as described above with either oligodeoxyribonucleotide probes or insert DNA from the human kallikrein cDNA labeled with random primers (van Leeuwen et al., 1986) and [α-^{32}P]dCTP (Bresa). Oligodeoxyribonucleotide probes used were HRK-1 (exon 4), HKE1B, a 20-mer complementary to the region in exon 1 corresponding to amino acids 10–16 of the signal peptide (Fukushima et al., 1985; see Figure 2), and HKE2B, a 20-mer complementary to the exon 2 sequence encoding amino acids 34–40 of human renal kallikrein (Fukushima et al., 1985). The exon positions of HRK-1, HKE1B, and HKE2B were predicted from the known organization of mouse kallikrein genes.

Templates for nucleotide sequencing were prepared by subcloning the cDNA insert or fragments from λHGK-43 into the M13 vectors mp18 and mp19 (Yanisch-Perron et al., 1985). DNA was sequenced by the chain termination method (Sanger et al., 1977).

Primer Extension Analysis. The oligodeoxyribonucleotide HKE2B was labeled at the 5′ end as described above; then 2 ng was hybridized for 1 h at 50 °C with 2.5 μg of poly(A) RNA from human parotid gland. The hybridization buffer and primer extension reaction were as described by Bodner and Karin (1987). Products were electrophoresed on a 7 M urea/6% polyacrylamide sequencing gel.

Chromosomal Localization of the Human Renal Kallikrein Gene. We mapped the renal kallikrein gene by two methods, both using insert DNA from the parotid gland cDNA clone described under Results. First, the cDNA was labeled with [α-^{32}P]dCTP and hybridized to Southern transfers of *Taq*I-digested DNA from a series of mouse/human hybrid cells (Callen, 1986) and from normal human leukocytes as controls.

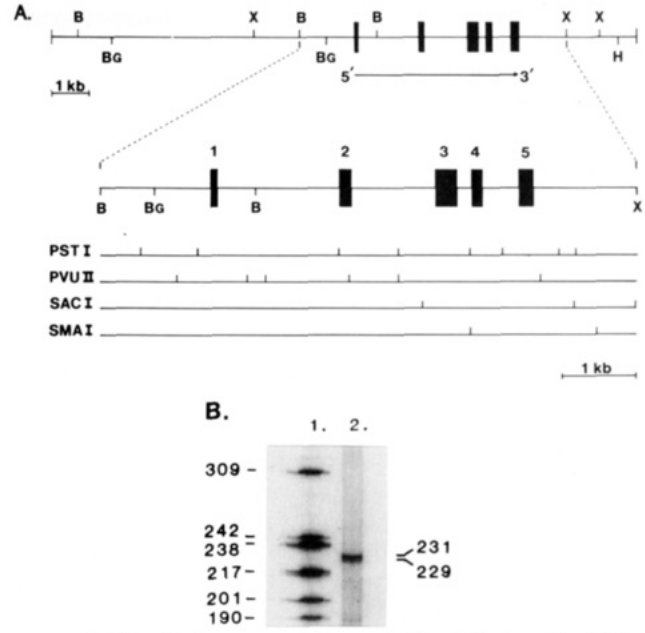


FIGURE 1: Restriction map and transcription initiation site of the human renal kallikrein gene. (A) The genomic clone λHGK-43 was mapped with the enzymes *Bam*HI (B), *Bgl*II (BG), *Xba*I (X), and *Hin*dIII (H); then subclones were characterized with the enzymes shown. The direction of transcription is shown by an arrow, and exons are numbered. (B) The 20-mer HKE2B (5′-AGCAGCTG-TGAGCACCCACT-3′) was annealed to 2.5 μg of poly(A) RNA from human parotid gland, at 50 °C for 1 h. Following primer extension, the products (lane 2) were electrophoresed on a denaturing 6% polyacrylamide gel. Markers (lane 1) are end-labeled *Hpa*II fragments of pBR322.

Details of the methods and the human chromosomes present in the hybrid cells are given in Sutherland et al. (1988). Second, the cDNA probe was tritium labeled to a specific activity of approximately 6.5 × 10^7 cpm/μg and hybridized at a concentration of 0.2 μg/mL to metaphases from two normal males. The autoradiographic emulsion was developed after 20 days of exposure.

RESULTS

Screening of a human parotid gland cDNA library with the oligodeoxyribonucleotide HK-1 gave six overlapping clones corresponding to a single mRNA species. One of these clones spanned amino acids 79–238 and was found to be identical in nucleotide sequence (data not shown) with the pancreatic and renal kallikrein cDNAs (Fukushima et al., 1985; Baker & Shine, 1985), with one exception. The glutamate at position 162 predicted by the latter sequences is replaced by a lysine, due to a change in codon from GAA to AAA. The virtual identity of cDNA clones from the three different tissues, however, confirms the previous observation that these kallikreins are encoded by a single human gene (Fukushima et al., 1985).

In order to isolate this gene, we reexamined 54 positive clones obtained by screening a human genomic library with the rat pancreatic kallikrein cDNA probe, prKK1 (Fukushima et al., 1985). We designed an oligodeoxyribonucleotide probe (HRK-1) based on a region of the human cDNA (Fukushima et al., 1985) showing low amino acid sequence homology with human prostate antigen (Watt et al., 1986). One of the 54 kallikrein-positive clones, λHGK-43, gave strong hybridization with this probe and was examined further by restriction mapping (Figure 1A) and nucleotide sequence analysis (Figure 2).

These data confirm that λHGK-43 carries the human renal kallikrein gene. The gene consists of five exons, with coding

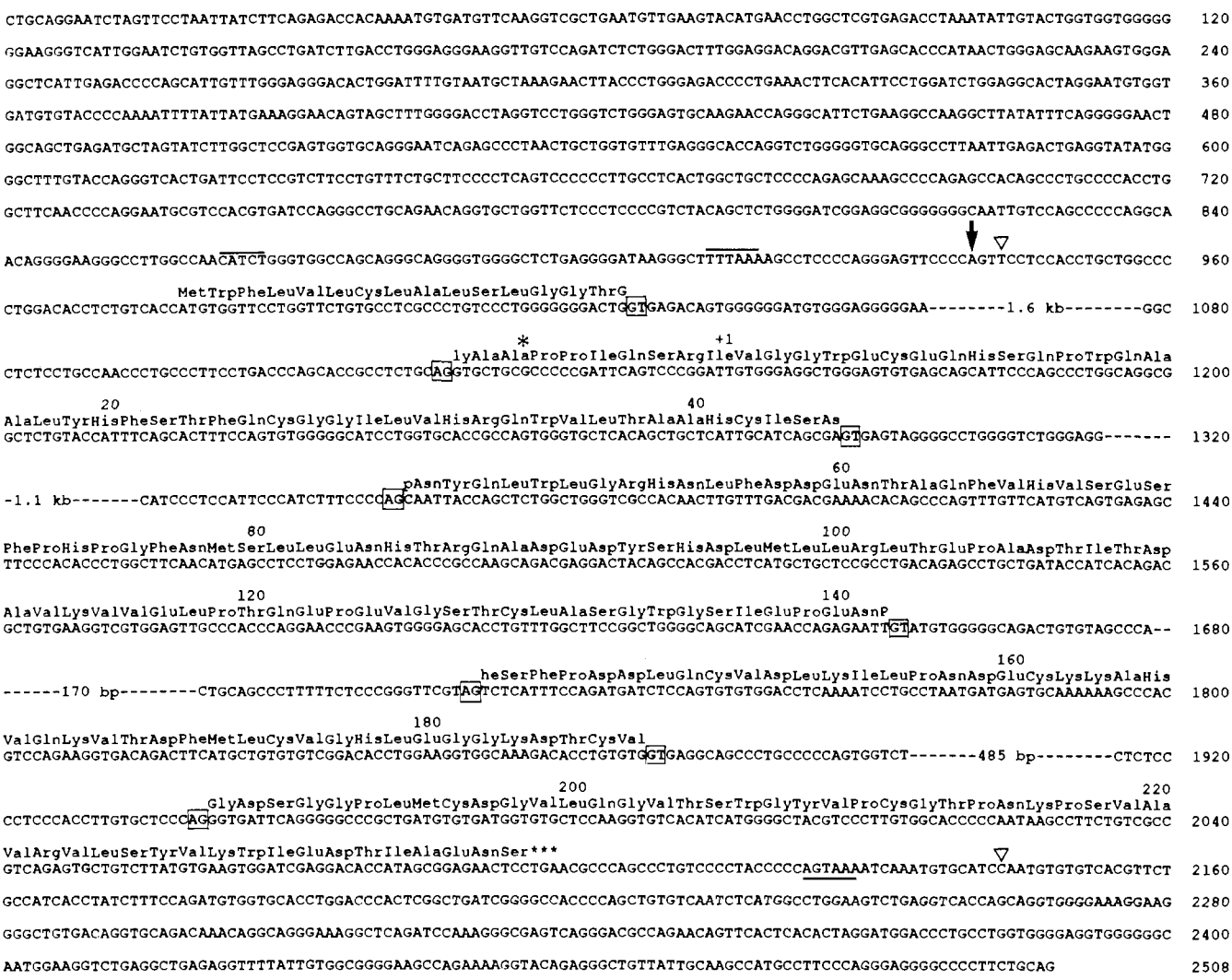
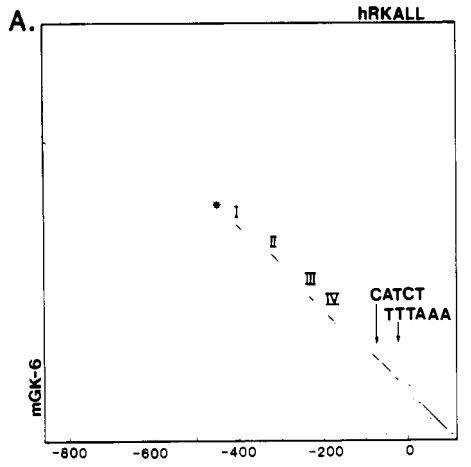


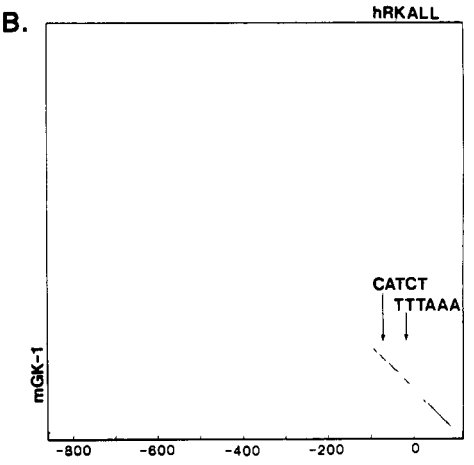
FIGURE 2: Nucleotide sequence of the human kallikrein gene. The sequence of the sense strand is shown. Numbers at the end of each line refer to nucleotides, while those above the amino acid sequence give the position relative to the N-terminal isoleucine of mature renal kallikrein. The alanine marked with an asterisk is the first amino acid of the zymogen peptide (Evans & Richards, 1985). The CATCT and TTTAAA boxes are overlined, and the polyadenylation signal AGTAAA is underlined. The major transcription initiation site determined by primer extension (Figure 1B) is marked with an arrow, and triangles show the beginning and end of the pancreatic kallikrein cDNA clone described by Fukushima et al. (1985).

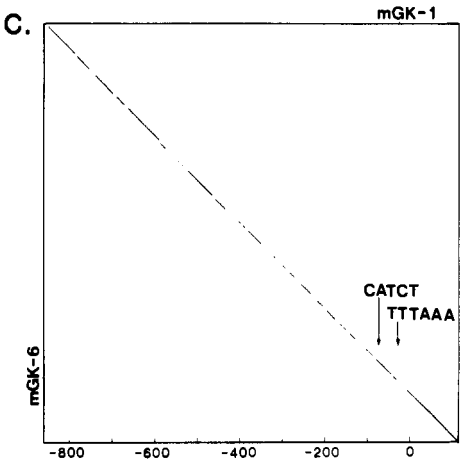
regions interrupted in positions identical with those observed in the mouse renal kallikrein gene, mGK-6 (van Leeuwen et al., 1986), all other mouse kallikrein genes analyzed (Evans et al., 1987), and the human gene hGK-1 (Schedlich et al., 1987). The exon sequences of the human renal kallikrein gene are identical with those reported for the pancreatic and renal cDNA clones (Fukushima et al., 1985; Baker & Shine, 1985), except that the same change to lysine at position 162 occurs in the gene as in the salivary gland cDNA. This discrepancy is somewhat surprising, given that not even silent nucleotide changes are seen in the remainder of the coding region. The transition to lysine, however, is conservative in the sense that a charged residue is retained and is unlikely to affect the overall structure or function of the active protein since amino acid 162 is found on the surface of the molecule (Bode et al., 1983; Chen & Bode, 1983). It is thus possible that position 162 represents a common polymorphic site in the human kallikrein gene.

Sequences flanking exons 1 and 5 of the kallikrein gene include the signals required for eukaryote gene expression (Figure 2). The 3′ untranslated region contains the variant polyadenylation signal, AGTAAA, observed in the full-length cDNA of Fukushima et al. (1985). No other possible polyadenylation signal is seen in a further 380 nucleotides of flanking DNA. The 5′ flanking region contains the element CATCT (Benoist et al., 1980) as well as the variant TTTAAA box seen in mouse kallikrein genes (Mason et al., 1983; Evans & Richards, 1985).

We carried out primer extension analysis to determine the cap site for the human renal kallikrein gene (Figure 1B). Using parotid gland poly(A) RNA as the template, we obtained a major band corresponding to the A residue marked in Figure 2. There is a second minor band resulting from a transcription product that is shorter by two nucleotides; however, it is unclear whether this is due to heterogeneity in the 5′ ends of kallikrein mRNAs or simply represents premature termination of the reverse transcriptase reaction. The pancreatic cDNA clone isolated by Fukushima et al. (1985) begins a further nucleotide downstream, at the T residue marked in Figure 2. It seems most likely that the sequence AGTTCC represents a single transcription start site in both pancreas and salivary gland. This sequence is repeated 8 base pairs (bp) upstream, but there is no corresponding band observed in the primer extension experiment. The finding of a single cap site is in contrast to the mouse kallikrein gene, mGK-1, where both homologues of a similar repeated element, AGCTC, are involved in transcription initiation (Mason et al., 1983). In this case, however, the 5′ element is 22 bp from the sequence







D.

I

```
mGK-6     TAACTGCTGGTTTCT AGGG
          :::::::::::: : : ::::
hRKALL    TAACTGCTGGTGTTTGAGGG
          :::::::    : : :  ::
mGK-1     TAACTGCAACTTTCTG GGA
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II

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mGK-6     GGGTCACTGGCTCCTCCACCTTCCT
          :::::::::  ::::::  ::::::
hRKALL    GGGTCACTGATTCCTCCGTCTTCCT
               ::::   ::: :   :  ::
mGK-1     ACAGCACTTGCTCCACTACCCACCA
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III

```
mGK-6     CCCTGCCCCACC
          ::::::::::::
hRKALL    CCCTGCCCCACC
             :::::  ::
mGK-1     AATTGCCCTCCC
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IV

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mGK-6     AGGGCCCACAGGAGAGCAGGTGC
          ::::::  ::: : : :::::::
hRKALL    AGGGCCTGCAG A A CAGGTGC
          :: :::: : : : :   :::::
mGK-1     AGAGCCTTCTGGACAGTGGGTGC
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FIGURE 3: Conserved elements in the promoters of the human and mouse renal kallikrein genes. Parts A–C show graphic displays derived from the DIAGON program of the Staden database system (Staden, 1982; Melbourne University version modified by Dr. A. Kyne, Walter and Eliza Hall Institute of Medical Research, Melbourne). The parameters used were a span length of 15 and a proportional matching score of 11. Numbers below each diagram refer to nucleotide position relative to the transcription start site. The region between the asterisk (diagram A) and the transcription start site is required for expression of mGK-6. The nucleotide sequences corresponding to elements I–IV (diagram A) for each of the three genes are shown in part D, with matches indicated by colons. hRKALL, human renal kallikrein gene; mGK-6, mouse renal kallikrein gene; mGK-1, a mouse kallikrein gene expressed only in submandibular gland.

TTTAAA, whereas in the human gene, the first of the repeated elements is only 14 bp downstream from the TTTAAA box while the second element is 22 bp downstream.

To identify conserved elements that may determine the tissue-specific expression of renal kallikrein genes, we have compared the promoter of the human gene with those of mouse kallikrein genes that have similar (mGK-6) or distinct (mGK-1) expression phenotypes. Diagrams of the dot matrix obtained by comparison of 870 bp of the 5′ flanking region plus exon 1 from each pair of genes are shown in Figure 3. All three genes show extensive homology in the vicinity of the CATCT and TTTAAA boxes. If we consider the mouse and human renal kallikrein genes, there are an additional four distinct regions of homology upstream from the CATCT box (elements I–IV, Figure 3A). A comparison of the human renal kallikrein gene with mGK-1, on the other hand, indicates very little homology between nucleotides −400 and −100 (Figure 3B). The actual sequences of the four elements in the renal kallikrein genes and mGK-1 are shown in Figure 3D. Elements I and IV require gaps for optimal alignment between the human kallikrein gene and mGK-6, whereas elements II and III show either perfect homology or differences involving

FIGURE 4: Distribution of silver grains over chromosomes in 40 metaphases after in situ hybridization with the salivary gland kallikrein cDNA probe.

purine to purine or pyrimidine to pyrimidine changes. In all four cases, the homology of the human kallikrein gene with mGK-6 is clearly greater than with mGK-1. Also, despite the overall similarity between the two mouse promoters (Figure 3C), the four elements show greater homology between the human renal kallikrein gene and mGK-6 than between mGK-6 and mGK-1.

We have examined the chromosomal localization and environment of the human renal kallikrein gene. First, the genomic clone λHGK-43 carries 8.1 kb of 5′ flanking DNA and 3.2 kb of 3′ flanking DNA, neither region showing any evidence of another kallikrein gene. The long intergenic region 5′ to the human gene contrasts with the mouse, where the renal kallikrein gene mGK-6 is located only 4.5 kb downstream from another gene, mGK-5 (van Leeuwen et al., 1986).

The salivary gland kallikrein cDNA was used for Southern blot analysis of a panel of mouse–human hybrid cell lines carrying human chromosomes 1, 3, 4, 7, 8, 10, 12, 13, 16, 17, 20, 21, 22, X, and 11pter–11q14 (Sutherland et al., 1988). Whereas total human DNA digested with *Taq*I gave bands at 3.1, 0.8, and 0.5 kb, none of the hybrid cell lines showed any signal (data not shown). This result excludes the renal kallikrein gene from the above chromosomes. The in situ hybridization results (Figure 4) show that from 40 metaphases on which there were 237 silver grains, 34 (16.7%) were on the long arm of chromosome 19. The presence of silver grains on other chromosomes was found to be statistically insignificant and in most cases corresponded to positions that had been excluded by data from the hybrid cell lines. Similar results were obtained from hybridization to the chromosomes of the second male (data not shown). The distribution of the silver grains suggests that the renal kallikrein gene is at or close to 19qter, most likely in the region q13.2–q13.4. The kallikrein cDNA used as a probe cross-hybridizes with the prostate antigen and hKK-3 genes, although somewhat weakly (Baker & Shine, 1985; Fukushima et al., 1985; our unpublished data). Thus the finding of the only one region of in situ hybridization suggests that all human kallikrein genes may be localized in a single locus on chromosome 19.

Discussion

The glandular kallikreins provide a system in which to study mechanisms underlying the differential tissue-specific expression of a set of closely related genes. We have undertaken an analysis of the human renal kallikrein gene, since this provides us with a comparison of the same gene from two different species, as well as the comparison with other mouse kallikrein genes showing a distinct expression phenotype. We intend to use the isolated promoter regions from these genes to study transcriptional activation mediated by the binding of cell-specific factors. On the basis of nucleotide sequence comparisons, however, we can make predictions on elements that may interact with such factors.

As outlined in Figure 3, the mouse and human renal kallikrein genes share four sequence elements upstream from the CATCT box that show much greater homology than the corresponding regions between the human gene and mGK-1. The conservation of elements II and III is particularly striking. All four elements lie within the region between nucleotide −460 and the TTTAAA box, which is necessary and sufficient for tissue-specific expression of the mouse renal kallikrein gene mGK-6 in AtT20 cells (J. Close and R. Richards, unpublished data).

We can place these findings in context by considering the example of the growth hormone gene. Comparison of the human (DeNoto et al., 1981) and rat (Barta et al., 1981) promoter sequences indicates four regions upstream from the TATAAA box that show significant homology. It is interesting that two of these regions have been shown to bind the cell-specific factor GHF-1 and to be necessary for transcriptional activation (Bodner & Karin, 1987). We propose that the four elements shared by the human and mouse renal kallikrein gene promoters similarly represent strong candidate sequences for the regulation of tissue-specific expression.

We have mapped the human renal kallikrein gene to the long arm of chromosome 19. This region carries a number of genetic markers, including glucosephosphate isomerase (GPI), cytochrome P-450 (CYP1), and transforming growth factor β (TGFB; Naylor et al., 1985). These markers all appear on chromosome 7 in the mouse and, in the case of Gpi-1 and Coh (corresponding to GPI and CYP1), map to a region adjacent to the Tam-1, or kallikrein gene locus (Searle et al., 1987; Lalley & McKusick, 1985; Evans et al., 1987). The human renal kallikrein gene, and possibly the entire gene family, is thus localized in a chromosomal region analogous to the position of the kallikrein locus in mouse. The major

difference between kallikreins in the two species is therefore the extensive duplication and gene conversion occurring within the mouse gene family. It will be interesting to determine whether the change in chromosomal environment imposed by the close linkage of mGK-6 with other mouse kallikrein genes has any higher order effects on expression compared to the human renal kallikrein gene.

Finally, although chromosome 19 in humans is relatively well mapped (Naylor et al., 1985), there are only a few probes defining restriction fragment length polymorphisms (RFLP's; Donis-Keller et al., 1987). The localization of the human renal kallikrein gene to 19q13.2–q13.4, as well as observed RFLP's (Baker & Shine, 1985), provides a useful addition to the repertoire of chromosome 19 markers.

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