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Short sequence-paper

## Characterisation, chromosomal localisation and expression of the mouse *Kid3* gene<sup>1</sup>

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### Abstract

*Kid1* encodes a transcriptional repressor implicated in the differentiation of renal epithelial cells. Here we report the characterisation of *Kid3*, a novel mouse gene related to *Kid1*. *Kid3* encodes a C<sub>2</sub>H<sub>2</sub> zinc finger protein with an N-terminal KRAB transcriptional repression domain. It maps to chromosome 11, adjacent to *Kid1* and another related gene *Kid2*. Northern analysis shows that *Kid3* is highly expressed in embryonic and adult brain, with lower levels in adult and embryonic (E16.5) kidney, gut, lung and heart. Expression of *Kid3* in the kidney is developmentally regulated and suggests a role for *Kid3* in the early stages of nephrogenesis. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** KRAB; Zinc finger; Transcription factor; Kidney; Mouse

*Kid1* encodes a zinc finger protein which has been implicated in the differentiation of renal epithelial cells [1–3]. *Kid1* transcripts are detected predominantly in the adult rat kidney where they accumulate during post-natal kidney development. Expression of *Kid1* is modulated during kidney regeneration following injury, a process which is thought to involve de-differentiation, proliferation and subsequent differentiation of surviving renal epithelial cells. Following experimental renal injury, the levels of *Kid1* mRNA initially decline and then increase as the proliferating epithelial cells undergo subsequent differ-

entiation [1]. KID1 protein has recently been localised mainly in the nuclei of proximal tubule epithelial cells in the adult rat kidney, and its expression is also down-regulated in cystic epithelia and in renal cell carcinomas, both of which are characterised by proliferation of immature epithelial cells [3]. It has therefore been proposed that *Kid1* may play an important role during the differentiation of proximal tubule epithelial cells.

The *Kid1* gene encodes a C<sub>2</sub>H<sub>2</sub> zinc finger transcription factor with a highly conserved *Krüppel*-associated box (KRAB) domain at its amino-terminus. The KRAB domain is a 75 amino acid region which can be subdivided into KRAB A and KRAB B boxes [4]. In cell transfection studies, the KRAB A domain of KID1, and that of other KRAB-zinc finger proteins, has been shown to repress transcription from TATA-box-containing basal or activated promoters when fused to a heterologous DNA binding domain [5–7]. This activity is independent of the distance

Abbreviations: KRAB, *Krüppel*-associated box; UTR, untranslated region

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<sup>1</sup> Sequence data from this article have been deposited in the EMBL/GenBank data libraries with the accession number AF192804.

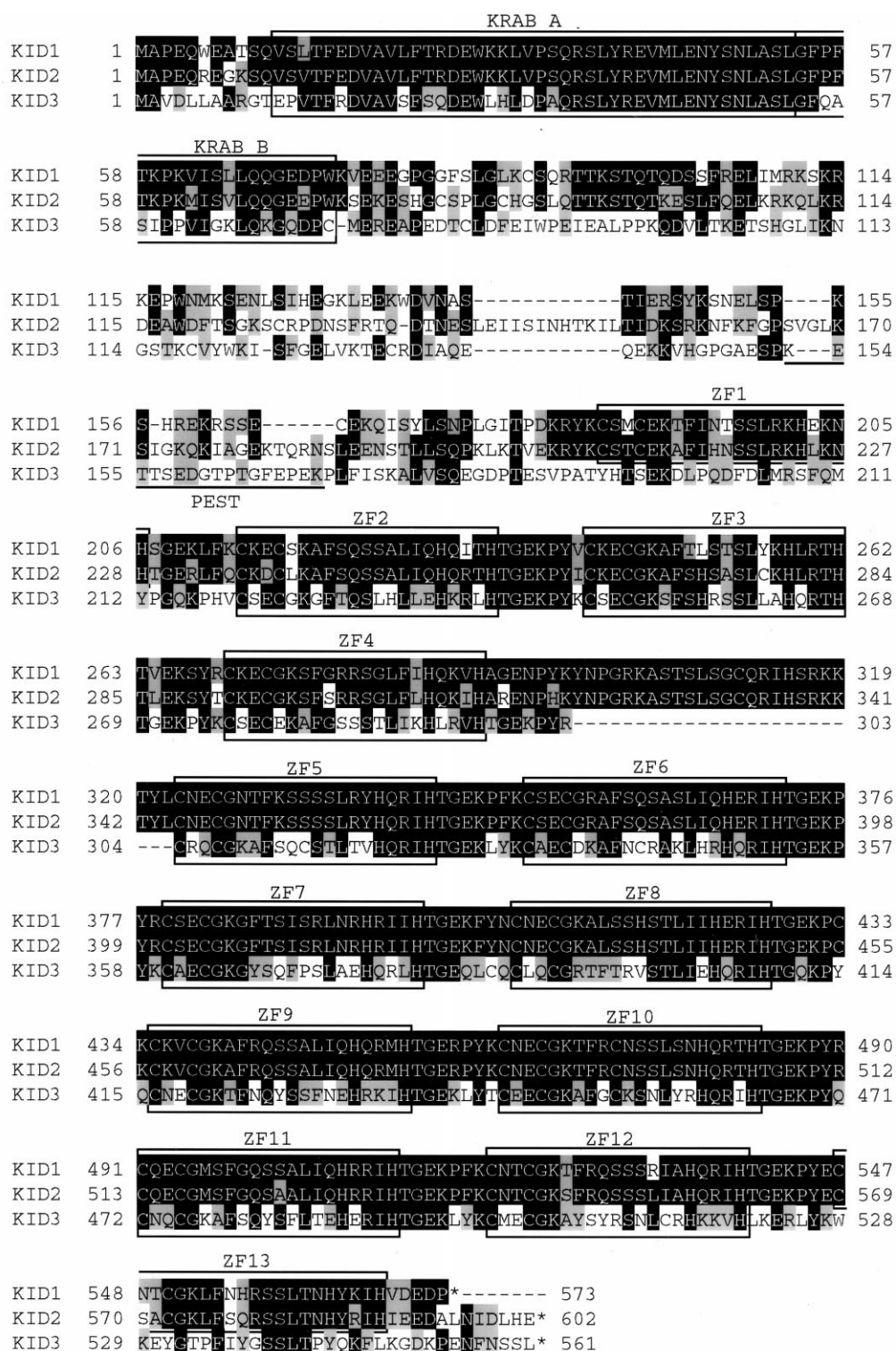


Fig. 1. Comparison of the deduced amino acid sequences of mouse KID1, KID2 and KID3. Identities are indicated by a black background and similar residues are shaded grey. KRAB A and B domains and zinc fingers are boxed and a possible PEST sequence is underlined.

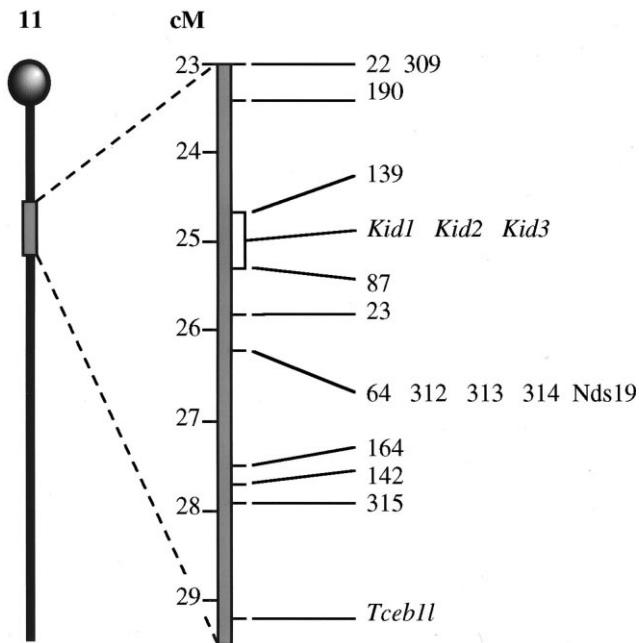


Fig. 2. Genetic map location of *Kid3* on mouse chromosome 11. *Kid3* maps to the same region of chromosome 11 as *Kid1* and *Kid2*, between markers D11Mit139 and D11Mit87. The positions of markers correspond to the EUCIB (BSB) mouse chromosome 11 linkage map.

between the binding site and the transcription initiation site [8] and may be mediated by the nuclear protein, TIF1 $\beta$  (or KAP-1/KRIP-1) [9–11]. This protein has been shown to interact with the KRAB A domain and also with the putative heterochromatin-associated factors HP1 $\alpha$ , MOD1 and MOD2 [12,13]. These findings suggest that KRAB-mediated transcriptional repression may involve alterations in chromatin structure. Although no specific DNA recognition sequence has yet been identified for any KRAB-zinc finger protein, the zinc fingers of KID1 have been shown to recognise heteroduplex DNA and to localise KID1 in the nucleolus [14,15].

We have recently cloned the mouse homologue of *Kid1* and shown that its expression is not confined to the adult mouse kidney, but can be detected in a wide variety of adult and embryonic tissues including brain, lung, and heart, suggesting additional roles for this gene during mouse development [16]. We have also identified a gene highly homologous to *Kid1*, called *Kid2*, and propose that *Kid1* may form part of a family of highly related genes conserved across mammals [16]. Here we report the isolation and char-

acterisation of the genomic locus and cDNA of a novel mouse gene called *Kid3*, the third member of the *Kid* gene family. We demonstrate that *Kid3* maps adjacent to the other family members on chromosome 11, and describe its expression pattern in a variety of embryonic and adult mouse tissues.

The *Kid3* gene was isolated from a 129/Sv lambda 2001 genomic library screened with a 172 bp *EcoRI/NcoI* KRAB A-encoding fragment from the rat *Kid1* cDNA (GenBank accession number M96548). From  $1.25 \times 10^6$  plaques screened, 20 lambda clones that hybridised to this probe were isolated and purified to homogeneity. Clones containing *Kid1* and *Kid2* genomic sequences were identified by Southern blotting or by PCR using gene-specific primers and eliminated. Of the remaining clones, three were found to contain overlapping fragments of the *Kid3* locus, the positions of exons conserved with *Kid1* being determined by Southern analysis and DNA sequencing. Three *Kid3* cDNAs were also isolated from a mouse embryonic E15.5 cDNA library screened with the KRAB A probe. Sequencing of these cDNAs identified the extent of the 5' untranslated region (UTR) and confirmed the position of exon/intron boundaries. A 1683 bp open reading frame encoding a putative 560 amino acid protein was assembled from the genomic clones and the isolated cDNAs (GenBank accession number AF192804).

The structure of *Kid3* is similar to the other members of the *Kid* family isolated to date, consisting of five exons spanning over 14 kb of genomic DNA. Exon I and part of exon II are non-coding; comparison of three independent cDNAs gives the extent of the 5' UTR as at least 151 bp. The translation initiation codon in exon II is conserved in all the *Kid* genes and is in agreement with the Kozak consensus sequence [17]. Exons III and IV encode the KRAB A and B domains respectively. Exon V encodes 11 C<sub>2</sub>H<sub>2</sub> zinc fingers which, in contrast to *Kid1* and *Kid2*, are tandemly repeated in one continuous block. Exon V also encodes a unique spacer region linking the KRAB and zinc finger domains. The extent of the 3' UTR has not been mapped, but from the Northern analysis described below, is approximately 3 kb in length.

A ClustalW multiple sequence alignment [18] of the deduced amino acid sequences of KID1, KID2 and KID3 is shown in Fig. 1. The overall homology

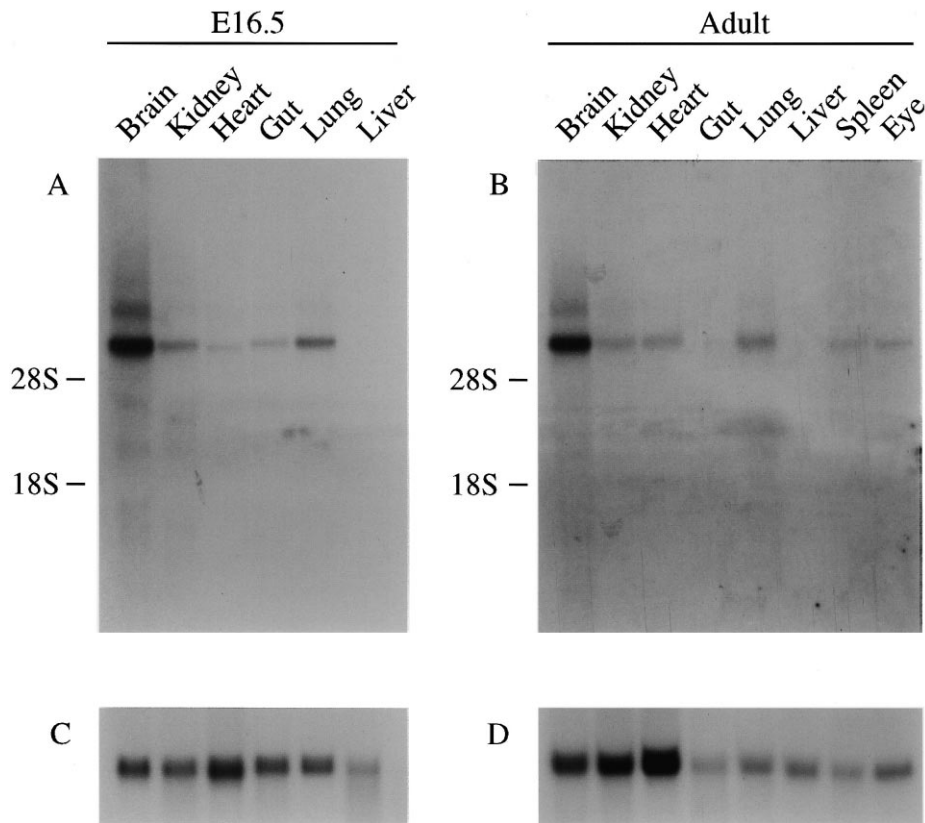


Fig. 3. Northern blot analysis of *Kid3* transcripts in adult and embryonic mouse tissues. 20  $\mu$ g of total RNA from various embryonic E16.5 (A) and adult (B) mouse tissues was loaded in each lane. The gels were blotted and the filters hybridised with a *Kid3* specific probe. To control for RNA integrity, the blots were re-hybridised with a control probe derived from the *Gapdh* gene (C, D). Locations of the 28S and 18S ribosomal bands are indicated.

of KID3 to KID1 is considerably lower than that demonstrated for KID2. This is most notable in the zinc finger domain where both the arrangement and sequence of the fingers is significantly different. Whereas both KID1 and KID2 are nearly identical in the sequences of their 13 zinc fingers, KID3 has only 11 zinc fingers which are quite divergent in sequence (54% identity to zinc fingers 2–12 of KID1). A high degree of homology is, however, conserved within the KRAB A domains of all three proteins: over the entire KRAB A domain there is 98% amino acid identity (100% similarity) between KID1 and KID2 and 74% amino acid identity (86% similarity) between KID1 and KID3. However, this rises to 100% for all three proteins in the 19 amino acids (position 35–53) at the carboxy-terminus of this domain. The high degree of conservation of the KRAB A domain between the KID proteins suggests that they may share a common pathway of gene regula-

tion, but it is likely that their target genes are different, given the extent of divergence seen between the zinc fingers of KID3 and KID1/KID2.

Analysis of the KID3 amino acid sequence using the program PESTfind (<http://www.at.embnet.org>) [19] reveals a possible PEST protein instability sequence at amino acids 153–169, suggesting that the protein may undergo rapid turnover in vivo [20]. In addition, analysis of the Prosite database using the software Motif (<http://www.motif.genome.ad.jp>) identifies 15 putative casein kinase II phosphorylation sites and six potential protein kinase C phosphorylation sites within the deduced KID3 protein. The presence of such structural elements suggests that KID3 activity may be modulated by phosphorylation.

The chromosomal location of the *Kid3* gene was determined using the EUCIB *Mus musculus*/*Mus spretus* backcross facility [21]. The *M. musculus* and

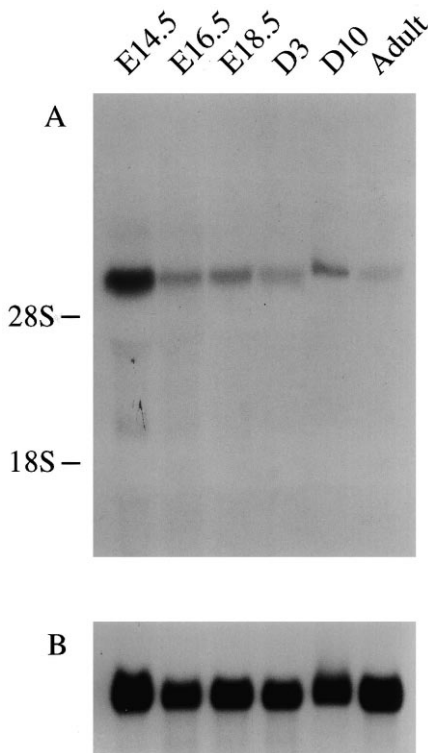


Fig. 4. Northern blot analysis of *Kid3* expression in the developing mouse kidney. 30  $\mu$ g of total RNA was prepared from E14.5, E16.5, E18.5, newborn day 3 (D3), day 10 (D10) and adult kidneys. The filter was probed initially with the *Kid3*-specific probe (A) and then re-hybridised with a *Gapdh* control probe (B). Locations of the 28S and 18S ribosomal bands are indicated.

*M. spretus* alleles were distinguished by PCR and restriction enzyme digestion of the PCR products. The data is available on <http://www.hgmp.mrc.ac.uk/MBx/MBxHomepage.html>. *Kid3* maps adjacent to *Kid1* and *Kid2*, between the markers D11Mit139 and D11Mit87 on mouse chromosome 11, having a recombination frequency of 8.7% with the primary anchor locus D11Nds19 (lod score of 11.92) (Fig. 2). The chromosomal location of *Kid3*, and its high degree of structural homology with the other *Kid* genes, is consistent with the previously observed clustering of homologous KRAB zinc finger genes [22] and indicates that the *Kid* family of genes arose from a series of gene duplication events.

The tissue and developmental expression of *Kid3* was analysed by Northern blot analysis of total RNA. The probe used was a 0.5 kb cDNA fragment containing sequences from the 3' end of exon III, exon IV and the non-zinc finger region of exon V.

Southern blot analysis of mouse genomic DNA showed that this probe hybridises to a single copy sequence in the mouse genome and is therefore specific for the *Kid3* gene (data not shown). When hybridised to Northern blots of total RNA it detected a single transcript of approximately 5 kb in a variety of embryonic and adult mouse tissues (Fig. 3). In embryonic day 16.5 (E16.5) tissues, high levels of *Kid3* mRNA are detected in brain, with lower levels in the kidney, lung, gut and heart (Fig. 3A). In the adult, the major site of *Kid3* expression is brain, with transcripts also present at a lower level in the kidney, heart, lung, spleen and eye (Fig. 3B). These sites of *Kid3* expression overlap with those of *Kid1* and *Kid2* in all embryonic and adult tissues investigated by Northern analysis [16].

In view of the potential role of *Kid1* in renal cell differentiation, the level of *Kid3* mRNA was determined at various stages of kidney development. In contrast to *Kid1* and *Kid2* which are expressed highly in adult kidney [1,16], *Kid3* is down-regulated during kidney maturation (Fig. 4). It is expressed highest in embryonic E14.5 kidneys, with mRNA levels decreasing in late embryonic, new born and adult kidneys. This suggests that *Kid3* may have a role to play in the early stages of nephrogenesis.

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