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Expression of *Drosophila* forkhead transcription factors during kidney development

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ABSTRACT

The *Drosophila* forkhead (*Dfkh*) family of transcription factors has over 40 family members. One *Dfkh* family member, BF2 (aka FoxD1), has been shown, by targeted disruption, to be essential for kidney development. In order to determine if other *Dfkh* family members were involved in kidney development and to search for new members of this family, reverse transcriptase polymerase chain reaction (RT-PCR) was performed using degenerate primers of the consensus sequence of the DNA binding domain of this family and developing rat kidney RNA. The RT-PCR product was used to probe RNA from a developing rat kidney (neonatal), from a 20-day old kidney, and from an adult kidney. The RT-PCR product hybridized only to a developing kidney RNA transcript of ~2.3 kb (the size of BF2). A lambda gt10 mouse neonatal kidney library was then screened, using the above-described RT-PCR product as a probe. Three lambda phage clones were isolated that strongly hybridized to the RT-PCR probe. Sequencing of the RT-PCR product and the lambda phage clones isolated from the developing kidney library revealed *Dfkh* BF2. In summary, only *Dfkh* family member BF2, which has already been shown to be essential for nephrogenesis, was identified in our screen and no other candidate *Dfkh* family members were identified.

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1. Introduction

In mammals, the metanephroi, or permanent kidneys, form as a result of an epithelial–mesenchymal interaction between the ureteric bud and the metanephric mesenchyme. When the ureteric bud and the metanephric mesenchyme come into contact at embryonic day 11 (E11) in mice (rat equivalent E13, human equivalent E35), a reciprocal induction occurs that continues, at least in rodents, for several days after birth and results in formation of the metanephric kidney [6].

Many crucial genes including WT-1, PAX-2, Wnt-4, c-ret, GDNF, BMP-7, PDGF β, and *Drosophila* forkhead (*Dfkh*) BF2 are involved in metanephric kidney formation as shown by the fact that targeted disruptions of these genes result in non-functional kidneys (as reviewed in [5]).

The *Drosophila* forkhead (*Dfkh*) family of transcription factors has a winged helix DNA binding domain. Over 40 family members have been described [4]. One *Dfkh* family member, BF2 (aka FoxD1, GenBank # L38607), has been shown, by targeted disruption, to be crucial for kidney development. BF2 (−/−) mutants all died within 24 h of birth with markedly abnormal kidneys, as BF2 seemed to be necessary for normal development of the stromal mesenchyme [2].

In order to determine if other *Dfkh* family members were involved in kidney development, we performed a non-biased RNA-based screen of the developing kidney based on the consensus DNA-binding sequence of the *Dfkh* family as described below. We found BF2 expression, but no other *Dfkh* transcription factor expression during kidney development.

2. Methods

2.1. Kidney RNA

Rat pups were sacrificed at the ages described, and RNA was obtained using the RNA Stat kit (Tel Test, Inc.). Briefly, rate kidneys were dissected out, and homogenized using RNA STAT (1 ml per 50–100 mg tissue). RNA was then extracted using 1 volume of homogenate + 0.2 volumes of chloroform. RNA precipitation was then performed using 0.5 volumes of isopropanol, with an RNA wash of 75% ethanol.

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2.2. Cloning and sequencing

The RT-PCR products obtained from the rat kidneys were cloned into the pBluescript II KS(+) plasmid (Stratagene). DH5α cells were transformed by this plasmid and grown. The plasmid DNA was isolated using the Wizard Miniprep (Promega) and sequenced (Applied Biosystems, Inc.).

2.3. Reagents

A lambda gt10 neonatal mouse kidney cDNA library, which was a kind gift from Dr. Yashpal Kanwar, was screened, using the RT-PCR product as a probe.

3. Results

RNA was obtained from neonatal rat kidney using the RNA Stat kit (Tel Test, Inc.). Reverse transcriptase polymerase chain reaction (RT-PCR) was then performed on the neonatal rat kidney RNA using degenerate primers of the consensus sequences of the DNA binding domain as described by Clevidence and colleagues [1] (Fig. 1).

The RT-PCR product was cloned into the pBluescript II KS(+) plasmid (Stratagene). DH5α cells were transformed by this plasmid and grown. The plasmid DNA was isolated using the Wizard Miniprep (Promega). Using the RT-PCR product as a probe, a Northern blot was performed with RNA from a developing rat kidney (neonatal), from a 20-day rat kidney, and from an adult rat kidney. The probe hybridized only to a developing kidney transcript of ~2.3 kb (Fig. 2).

A lambda gt10 neonatal mouse kidney cDNA library, which was a kind gift from Dr. Yashpal Kanwar [7], was then screened, using the RT-PCR product as a probe. Three lambda phage clones were isolated that strongly hybridized to the probe. These clones appeared to be an aggregation of a 1 kb fragment and a 2 kb fragment (Fig. 3).

Sequencing of the RT-PCR product and the lambda phage clones revealed only *Dfkh*, BF2 (Fig. 4).

4. Discussion

Several *Dfkh* family members have been found to be expressed in adult kidney including HFH-1, HFH-3, fkh-6, and MFH-1 [4]. BF2 was cloned by Hatini et al. in 1994 and was expressed in some mesenchymal cells outside the brain including the metanephric blastema of the developing kidney, but not in the condensed mesenchyme of the developing kidney [3]. As noted in the Introduction, targeted disruption of BF2 demonstrated the essential role of BF2 in metanephric kidney development, which seemed to act via the stromal mesenchyme [2].

The large number of *Dfkh* family members and the crucial importance of at least one family member in kidney development, spurred us to do the present study. We identified only *Dfkh* family member BF2, which has already been shown to be essential for nephrogenesis, in our non-biased developing kidney screen. We cannot completely rule out the involvement of other *Dfkh* family members in kidney development. For example, due to sequence

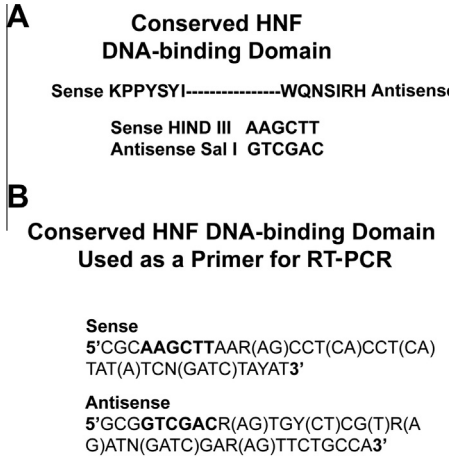

Fig. 1. Conserved domains of the *Dfkh* transcription factor family. (A) Conserved *Dfkh* family DNA binding domain consensus sequence at the protein level. (B) Degenerate primers of the consensus sequence of the DNA binding domain used for reverse transcriptase PCR (RT-PCR).

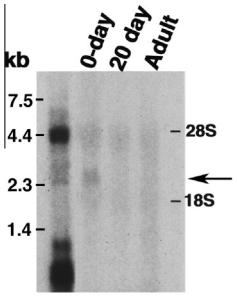

Fig. 2. Northern blot using the RT-PCR product as a probe. Kidney tissue was obtained from a newborn rat (at which point nephrogenesis is still ongoing), from a 20-day old rat, and from an adult rat. RNA was isolated and equal amounts of RNA (~20 μg), were loaded onto each lane as assessed by ethidium bromide staining of the gel (data not shown). The RT-PCR product obtained from developing (0-day) kidney, using the degenerate primers of the consensus sequence of the DNA binding domain described in Fig. 1 was used as a probe, and hybridized only to a kidney transcript of ~2.3 kb in 0-day kidney (arrow).

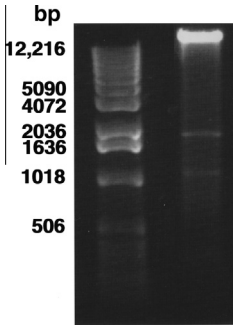

Fig. 3. Library screening using the RT-PCR product as a probe. A lambda gt10 neonatal mouse kidney cDNA library was screened, using the RT-PCR product obtained from developing (0-day) kidney as a probe. Three clones appeared to be an aggregation of a 1 kb fragment and a 2 kb fragment and were identical sizes.

Fig. 4. Sequencing of the products of the RT-PCR reaction and the library screen. Sequencing revealed *Dfkh* BF2. The one amino acid difference between the RT-PCR product we obtained and the BF2 sequence (amino acids in bold italics) is explained by the fact that BF2 was cloned from a mouse cDNA library, while the RT-PCR product is of rat origin. When compared to the DNA binding domain of rat BF2 (Genbank # Q63251), the two sequences were identical.

divergence the degenerate primers we used for RT-PCR would not have hybridized to FOXO family members. In addition, expression of some *Dfkh* family members during kidney development may have been too low to identify. Nevertheless, this study makes it much less likely that other *Dfkh* family members are involved in kidney development.

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References

[1] D.E. Clevidence, D.G. Overdier, W. Tao, X. Qian, L. Pani, E. Lai, R.H. Costa, Identification of nine tissue-specific transcription factors of the hepatocyte nuclear factor 3/forkhead DNA-binding-domain family, Proc. Natl. Acad. Sci. USA 90 (1993) 3948–3952.

[2] V. Hatini, S.O. Huh, D. Herzlinger, V.C. Soares, E. Lai, Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of winged helix transcription factor BF2, Genes Dev. 10 (1996) 1467–1478.

[3] V. Hatini, W. Tao, E. Lai, Expression of winged helix genes, BF-1 and BF2, define adjacent domains within the developing forebrain and retina, J. Neurobiol. 25 (1994) 1293–1309.

[4] R. Hromas, R. Costa, The hepatocyte nuclear factor-3/forkhead transcription regulatory family in development, inflammation, and neoplasia, Crit. Rev. Oncol. Hematol. 20 (1995) 129–140.

[5] J.H. Lipschutz, The molecular development of the kidney: a review of the results of gene disruption studies, Am. J. Kid. Dis. 31 (1998) 383–397.

[6] L. Saxen, Organogenesis of the Kidney, Cambridge University Press, Cambridge, 1987.

[7] J. Wada, Z.Z. Liu, K. Alvares, A. Kumar, E. Wallner, H. Makino, Y.S. Kanwar, Cloning of cDNA for the alpha subunit of mouse insulin-like growth factor I receptor and the role of the receptor in metanephric development, Proc. Natl. Acad. Sci. USA 90 (1993) 10360–10364.