

*Communication*

**Cloning of the lymphoid enhancer binding factor-1 (Lef-1) cDNA from rat kidney: Homology to the mouse sequence<sup>⊙\*</sup>**

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**We have cloned and sequenced rat cDNA that encodes the Lef-1 protein. The cDNA, containing 1194 nt exhibits 94% similarity to the mouse Lef-1 cDNA. The deduced amino-acids sequence of rat Lef-1 protein, consisting of 397 amino acids, exhibited 98% homology with the known sequence of mouse Lef-1 protein.**

Mouse lymphoid enhancer binding factor-1 (Lef-1) is a 48 kDa nuclear protein, belonging to the family of high mobility group (HMG) proteins. It is expressed in pre-B and T-lymphocytes and binds to the consensus sequence 5'-CTTTGA/TA/T-3' (HK-1 motif) [1] that is functionally important for the T-cell receptor  $\alpha$  gene expression [2]. Lef-1 acts as an "architectural" factor, because it facilitates the binding of other transcription factors to the adjacent DNA sequences [3]. The downstream genes activated by Lef-1 which have been iden-

tified, include these of the cytokeratins important for the formation of hair follicles [4] and the genes encoding the cell adhesion molecule E-cadherin that maintains the interaction between the ectoderm and the mesenchyme [5].

Targeted inactivation of Lef-1 gene in mouse germ-line, results in a severe impairment in the development of tooth buds, whiskers, hair follicles and mammary glands [6]. Zhou *et al.* [4] have shown that promoters involved in the hair-specific gene expression contain the HK-1 motif and that Lef-1 expressed in the

\*The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number: **AF198533**.

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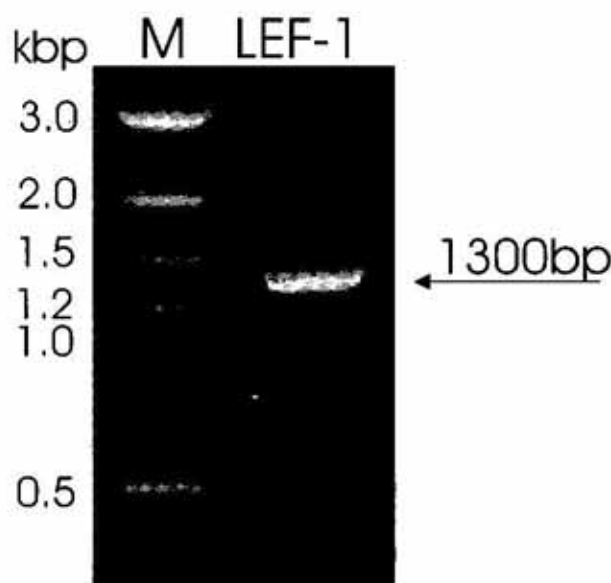
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hair follicles can bind to this sequence. Experiments in mice have demonstrated that Lef-1 protein associates with  $\beta$ -catenin, being a component of Wnt signaling pathway. In association with Lef-1,  $\beta$ -catenin can be transported to the nucleus and this complex can function as a conventional activator of transcription [5]. These results suggest that Lef-1 is an important regulatory protein in the epithelial-mesenchymal interactions during embryonic development.

With the use of the recombinant inbred strain of mice, Lef-1 gene was assigned to the distal chromosome 3 [7]. Mouse Lef-1 cDNA, containing 1194 nt, was cloned by Travis *et al.* [1]. It was demonstrated that the protein product, predicted from the nucleotide sequence, comprised 397 amino acids. The HMG domain, approximately 80 amino acid, was identified within this sequence.

The aim of this study was to clone and sequence Lef-1 cDNA derived from the rat and to compare the nucleotide sequence and the deduced amino-acids sequence of the protein product, with the mouse Lef-1 counterpart.

Poly-A<sup>+</sup> RNA isolated from rat kidney was reverse-transcribed and the cDNA was amplified with the use of primers specific for mouse Lef-1. A fragment of expected length (1300 bp) was obtained (Fig. 1) and cloned into the expression vector pQE32 (Qiagen), using the *KpnI* and *HindIII* sites. Three specific Cy5 labelled primers were used to determine the nucleotide sequence that allowed deducing the amino-acid sequence of the protein product (Fig. 2). The rat Lef-1 cDNA sequence consists of 1194 nt and shows a 94% identity to the nucleotide sequence of the mouse Lef-1 (68 nu-



**Figure 1. Identification of lymphoid enhancer binding factor-1 (Lef-1) cDNA.**

Poly-A<sup>+</sup> RNA was isolated from rat kidney homogenates and Lef-1 cDNA was prepared by reverse-transcription followed by PCR. The forward primer: 5'-TAC TTA GGT ACC TGC CCC AAC TTT CCG GAG-3', that contained *KpnI* restriction site (underlined), corresponded to nt 991-1008 of the mouse Lef-1 cDNA sequence. The reverse primer: 5'-GGG GTT TCA ACA AGC TTC CAT CTC CAG AAG-3', contained *HindIII* restriction site (underlined) and corresponded to nt 2246-2275 of the mouse Lef-1 cDNA. The amplification was conducted for 30 cycles involving: denaturation, annealing and elongation (94°C, 45 s; 65°C, 45 s; 72°C, 60 s). The reaction mixture contained in 25  $\mu$ l: 1 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 500 nM of each primer and 0.5 units of Taq DNA Polymerase (Qiagen). The PCR product was resolved in 1% agarose gel and visualised by ethidium bromide staining.

cleotides are different). The rat Lef-1 cDNA encodes a protein comprising 397 amino-acid

**Figure 2. Sequence alignment of the rat and the mouse Lef-1 cDNA and the predicted protein products.**

The amplified Lef-1 cDNA was cloned in *KpnI* and *HindIII* sites of an expression vector pQE32 (Qiagen). The selected clone was sequenced using Cy5 labeled primers: Vex F: 5'-CGG ATA ACA ATT TCA CAC AG-3', Lef-1F: 5'-AAC GAG TCT GAA ATC ATC CC-3' and Vex R: 5'-GGT CAT TAC TGG AGT CTT G-3'. The products of sequencing reactions were separated and analysed with the use of ALFexpress DNA sequencer (Pharmacia-LKB, Sweden). The nucleotides and amino acids were numbered as shown. Differences in the nucleotide and in the amino-acid sequence between the two species are indicated with shadowed boxes. The sequences of the HMG domains were printed in bold and boxed.

Mus- 1 ATG CCC CAA CTT TCC GGA GGA GGC GGC GGG GGG GAC CCG GAA CTC TGC GCC ACC GAT GAG ATG ATC CCC TTC AAG  
 Mus- 1 M P Q L S G G G G G G D P E L C A T D E M I P F K  
 Rat- 1 ATG CCC CAA CTT TCC GGA GGA GGC GGC GGG GGG GAC CCG GAA CTC TGC GCC ACC GAT GAG ATG ATC CCC TTC AAG  
 Rat- 1 M P Q L S G G G G G G D P E L C A T D E M I P F K  
  
 Mus- 76 GAC GAA GGC GAT CCC CAG AAG GAG AAG ATC TTC GCC GAG ATC AGT CAT CCC GAA GAG GAG GGC GAC TTA GCC GAC  
 Mus- 26 D E G D P Q K E K I F A E I S H P E E E G D L A D  
 Rat- 76 GAC GAA GGC GAT CCC CAG AAG GAG AAG ATC TTC GCC GAG ATC AGT CAT CCC GAA GAG GAG GGC GAC TTA GCC GAC  
 Rat- 26 D E G D P Q K E K I F A E I S H P E E E G D L A D  
  
 Mus- 151 ATC AAG TCA TCT TTG GTT AAC GAG TCC GAA ATC ATC CCA GCC AGC AAC GGG CAT GAG GTG GTC AGA CAA GCC CCG  
 Mus- 51 I K S S L V N E S E I I P A S N G H E V V R Q A P  
 Rat- 151 ATC AAG TCA TCT TTG GTT AAC GAG TCC GAA ATC ATC CCA GCC AGC AAC GGG CAT GAG GTG GTC GGA CAA ACA CAG  
 Rat- 51 I K S S L V N E S E I I P A S N G H E V V G Q I Q  
  
 Mus- 226 TCC TCT CAG GAG CCC TAC CAC GAC AAG GCC AGA GAA CAC CCT GAT GAA GGA AAG CAT CCA GAC GGA GGC CTG TAC  
 Mus- 76 S S Q E P Y H D K A R E H P D E G K H P D G G L Y  
 Rat- 226 TCC TCT CAG GAG CCC TAC CAC GAC AAG GCC AGA GAA CAC CCT GAT GAA GGA AAG CAT CCA GAC GGA GGC CTG TAC  
 Rat- 76 S S Q E P Y H D K A R E H P D D G K H P D G G L Y  
  
 Mus- 301 AAC AAG GGA CCC TCC TAC TCC AGT TAC TCT GGC TAC ATA ATG ATG CCC AAT ATG AAC AGC GAC CCG TAC ATG TCA  
 Mus- 101 N K G G P S Y S S Y S G Y I M M P N M N S D P Y M S  
 Rat- 301 AAC AAG GGA CCC TCC TAC TCC AGT TAC TCT GGC TAC ATA ATG ATG CCC AAT ATG AAC AGC GAC CCG TAC ATG TCA  
 Rat- 101 N K G G P S Y S S Y S G Y I M M P N M N S D P Y M S  
  
 Mus- 376 AAT GGG TCC CTT TCT CCA CCC ATC CCG AGG ACA TCA AAT AAA GTG CCC GTG GTG CAG CCC TCT CAC GCG GTC CAC  
 Mus- 126 N G S L S P P I P R T S N K V P V V Q P S H A V H  
 Rat- 376 AAT GGA TCC CTT TCT CCA CCC ATC CCG AGG ACA TCA AAT AAA GTG CCC GTG GTG CAG CCC TCT CAC GCG GTC CAC  
 Rat- 126 N G S L S P P I P R T S N K V P V V Q P S H A V H  
  
 Mus- 451 CCG CTC ACC CCC CTC ATC ACC TAC AGC GAC GAG CAC TTT TCT CCG GGA TCC CAC CCG TCA CAC ATC CCG TCA GAT  
 Mus- 151 P L T P L I T Y S D E H F S P G S H P S H I P S D  
 Rat- 451 CCG CTC ACC CCC CTC ATC ACC TAC AGC GAC GAG CAC TTT TCT CCG GGA TCC CAC CCG TCA CAC ATC CCG TCA GAG  
 Rat- 151 P L T P L I T Y S D E H F S P G S H P S H I P S F  
  
 Mus- 526 GTC AAC TCC AAG CAA GGC ATG TCC AGA CAC CCT CCA GCT CCT GAA ATC CCC ACC TTC TAC CCC CTG TCT CCG GGC  
 Mus- 176 V N S K Q G M S R H P P A P E I P T F Y P L S P G  
 Rat- 526 GTC AAC TCC AAG CAA GGC ATG TCC AGA CAC CCT CCA GCT CCT GAA ATC CCC ACC TTC TAC CCC CTG TCT CCG GGC  
 Rat- 176 V N P K Q G M S R H P P A P E M P T F Y P L S P G  
  
 Mus- 601 GGC GTT GGA CAG ATC ACC CCA CCC ATT GGC TGG CAA GGT CAG CCT GTT TAT CCC ATC ACG GGT GGA TTC AGG CAA  
 Mus- 201 G V G Q I T P P I G W Q G Q P V Y P I T G G F R Q  
 Rat- 601 GGC GTT GGA CAG ATC ACC CCA CCC ATT GGC TGG CAA GGT CAG CCT GTT TAT CCC ATC ACG GGT GGC TTC AGG CAA  
 Rat- 201 G V G Q I T P P L G W Q G Q P V Y P I T G G F R Q  
  
 Mus- 676 CCC TAC CCA TCC TCA CTG TCA GGC GAC ACT TCC ATG TCC AGG TTT TCC CAT CAT ATG ATT CCT GGT CCC CCT GGC  
 Mus- 226 P Y P S S L S G D T S M S R F S H H M I P G P P G  
 Rat- 676 CCC TAC CCA TCC TCA CTG TCA GGC GAC ACT TCC ATG TCC AGG TTT TCC CAT CAT ATG ATT CCT GGT CCC CCT GGC  
 Rat- 226 A Y P S S L S G D T S M S R F S H H M I P G P P G  
  
 Mus- 751 CCC CAC ACA ACT GGC ATC CCT CAT CCA GCT ATT GTA ACA CCT CAG GTC AAA CAG GAG CAC CCC CAC ACG GAC AGT  
 Mus- 251 P H T T G I P H P A I V T P Q V K Q E H P H T D S  
 Rat- 751 CCC CAC ACA ACT GGC ATC CCT CAT CCA GCT ATT GTA ACA CCT CAG GTC AAA CAG GAG CAC CCC CAC ACG GAC AGT  
 Rat- 251 P H T T G I P H P A I V T P Q V K Q E H P H T D S  
  
 Mus- 826 GAC CTA ATG CAC GTG AAG CCT CAA CAC GAA CAG AGA AAG GAG CAG GAG CCC AAA AGA CCT CAT ATT AAG AAG CCT  
 Mus- 276 D L M H V K P Q H E Q R K E Q E P K R P H I K K P  
 Rat- 826 GAC CTA ATG CAC GTG AAG CCT CAA CAC GAA CAG AGA AAG GAG CAG GAG CCC AAA AGA CCT CAT ATT AAG AAG CCT  
 Rat- 276 D L M H V K P E H E Q R K E Q E P K R P H I K K P  
  
 Mus- 901 CTG AAT GCT TTC ATG TTA TAT ATG AAA GAA ATG AGA GCG AAT GTC GTA GCT GAG TGC ACG CTA AAG GAG AGT GCA  
 Mus- 301 L N A F M L Y M K E M R A N V V A E C T L K E S A  
 Rat- 901 CTG AAT GCT TTC ATG TTA TAT ATG AAA GAA ATG AGA GCG AAT GTC GTA GCT GAG TGC ACG CTA AAG GAG AGT GCA  
 Rat- 301 L N A F M L Y M K E M R A N V V A E C T L K E S A  
  
 Mus- 976 GCT ATC AAC CAG ATC CTG GGC AGA AGA TGG CAC GCC CTC TCC CGG GAA GAG CAG GCC AAA TAC TAT GAA CTA GCA  
 Mus- 326 A I N Q I L G R R W H A L S R E E Q A K Y Y E L A  
 Rat- 976 GCT ATC AAC CAG ATC CTG GGC AGA AGA TGG CAC GCC CTC TCC CGG GAA GAG CAG GCC AAA TAT TAT GAA TTA GCG  
 Rat- 326 A I N Q I L G R R W H A L S R E E Q A K Y Y E L A  
  
 Mus- 1051 CCG AAA GAG AGA CAG CTA CAC ATG CAG CTT TAT CCA GGC TGG TCA GCG CGA GAC AAT TAT GGC AAG AAG AAG AAG  
 Mus- 351 R K E R Q L H M Q L Y P G W S A R D N Y G K K K K  
 Rat- 1051 CCG AAA GAA AGA CAG CTA CAC ATG CAG CTT TAT CCA GGC TGG TCA GCG CGA GAG AAT TAT GGC AAG AAG AAG AAG  
 Rat- 351 R K E R Q L H M Q L Y P G W S A R D N Y G K K K K  
  
 Mus- 1126 AGG AAG AGA GAG AAG CTA CAG GAG TCG ACT TCA GGT ACA GGT CCC AGA ATG ACA GCT GCC TAC ATC TGA  
 Mus- 376 R K R E K L Q E S T S G T G P R M T A A Y I \*  
 Rat- 1126 AGG AAG AGA GAG AAG CTA CAG GAA TCG ACT TCA GGT ACA GGT CCC AGA ATG ACA GCT GCC TAC ATC TGA  
 Rat- 376 R K R E K L Q E S T S G T G P R M T A A Y I \*

residues that is 98% identical to the amino-acid sequence of the mouse Lef-1 protein product and differs only in 9 amino acids. It was found that the HMG domain exhibited 100% identity to the mouse sequence.

High homology of the rat transcription factor Lef-1 to its mouse counterpart may suggest similarities in the epithelial-mesenchymal interactions during embryonic development of the two different species.

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