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Short sequence-paper

Molecular cloning and expression analysis of MPPα-2, a novel mouse transcript detected in a differential screen of pituitary libraries

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Abstract

We identified a novel isoform transcript, MPPα-2, of the mouse Mg^{2+}-dependent protein phosphatase (MPP) alpha gene. The amino acid sequence encoded by MPPα-2 differs from the previously known MPPα-1 sequence only at the carboxyl terminal region. Northern and in situ hybridization analysis revealed differential expression patterns of these two transcripts in the embryo and in the adult organism, suggesting an elaborate regulation of the MPPα gene.
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Keywords: Protein phosphatase; Differential screening; *Lhx*3; Pituitary

The LIM homeodomain proteins *Lhx*3 and *Lhx*4 are important regulators of pituitary development [1–6]. The pituitary gland originates from two embryonic tissues. The anterior and intermediate lobes are derived from Rathke's pouch arising from the oral ectoderm, the posterior lobe from the neural ectoderm. The requirement for *Lhx*3 and *Lhx*4 during early organogenesis was proven by gene targeting [3,4]. They are essential for normal development of Rathke's pouch. In mutants that lack the function of either protein, there is an early arrest of pituitary development. In addition, both proteins are required for motor neuron development.

In an effort to identify genes that mediate the control of pituitary development exerted by *Lhx*3, we prepared cDNA libraries from E12.5 wild-type and *Lhx*3 null mutant pituitary tissue. E12.5 corresponds to the onset of pituitary differentiation. Briefly, total RNA isolated from a single wild-type and a single null mutant E12.5 pituitary were converted to cDNA and then amplified by LD-PCR. Shorter double-stranded cDNA fragments were generated by *Rsa*I digestion. cDNA fragments corresponding to mRNAs differentially expressed between the wild-type and the null mutant pituitary were selected by way of a denaturation/hybridization procedure. Differentially expressed cDNA was PCR-amplified using oligonucleotide primers specific for adaptors linked to their ends. The subtracted mixture was cloned into the pGEM-T Easy cloning vector (Promega). cDNA inserts from randomly picked clones were individually amplified. To identify clones that were differentially expressed, cDNA blot arrays were prepared in duplicate using the DNA amplified from each clone. The blots were hybridized with forward and reverse subtracted probes (refer to user's manual for details). Using the above procedure, we isolated an 800 bp clone. This clone was used to generate a probe in order to screen an E12.5 whole embryo cDNA phage library (Stratagene). The screening resulted in the isolation of three clones that were sequenced in both directions. A 5′ extension of one of these clones (Clonetech Marathon cDNA Amplification Kit) yielded cDNA with an open reading frame corresponding to a protein of 326 amino acids (Fig. 1). The first 317 codons correspond to MPPα-1, a member of a gene family known to encode Mg^{2+}-dependent protein phosphatases [7]. Thereafter, the sequences of our cDNA diverge abruptly, revealing a C-terminus that is distinct from that of MPPα-1. We use the term MPPα-2 to distinguish this putative protein from the one described earlier. An amino acid sequence comparison of the two gene products is shown (Fig. 2).

To elucidate the physiological role of MPPα-2, protein phosphatase assay was carried out. To generate the fragment of coding region of MPPα-2, PCR was performed with 5′ RACE product as a template. The oligonucleotides used to

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                                                                                                    TCAAGTCATA     10
ATGGGAGCATTTTTAGACAAGCCAAAGATGGAGAAGCATAATGCCCAGGGGCAGGGGAATGGGTTACGATACGGCCTAAGCAGCATGCAAGGTTGGCGAG    110
M  G  A  F  L  D  K  P  K  M  E  K  H  N  A  Q  G  Q  G  N  G  L  R  Y  G  L  S  S  M  Q  G  W  R  V

TTGAAATGGAGGACGCACACACGGCTGTGATCGGTTCGCCAAGTGGACTTGAGACATGGTCATTCTTTGCTGTATATGATGGGCATGCTGGTTCTCAGGT    210
   E  M  E  D  A  H  T  A  V  I  G  S  P  S  G  L  E  T  W  S  F  F  A  V  Y  D  G  H  A  G  S  Q  V

TGCCAAATACTGCTGTGAGCACTTGTTAGATCACATCACCAATAACCAGGATTTCAGAGGATCTGCAGGAGCACCTTCTGTGGAGAACGTAAAGAATGGA    310
 A  K  Y  C  C  E  H  L  L  D  H  I  T  N  N  Q  D  F  R  G  S  A  G  A  P  S  V  E  N  V  K  N  G

ATCAGAACAGGGTTTCTGGAGATTGATGAACACATGAGAGTTATGTCAGAGAAGAAACATGGTGCAGATAGAAGCGGGTCAACAGCTGTGGGCGTCTTAA    410
I  R  T  G  F  L  E  I  D  E  H  M  R  V  M  S  E  K  K  H  G  A  D  R  S  G  S  T  A  V  G  V  L  I

TCTCTCCCCAACATACTTATTTCATTAACTGTGGAGACTCGAGAGGTTTACTTTGTAGGAATAGAAAAGTTCACTTCTTCACACAAGACCATAAACCAAG    510
   S  P  Q  H  T  Y  F  I  N  C  G  D  S  R  G  L  L  C  R  N  R  K  V  H  F  F  T  Q  D  H  K  P  S

TAACCCGCTGGAAAAAGAACGAATTCAGAATGCAGGGGGCTCGGTGATGATTCAGCGTGTCAATGGCTCTCTGGCTGTATCGAGGGCCCTTGGGGATTTC    610
 N  P  L  E  K  E  R  I  Q  N  A  G  G  S  V  M  I  Q  R  V  N  G  S  L  A  V  S  R  A  L  G  D  F

GATTACAAATGTGTCCATGGAAAAGGTCCCACAGAGCAGCTTGTCTCCCCAGAGCCCGAAGTCCATGATATTGAAAGGTCTGAAGAAGATGACCAGTTCA    710
D  Y  K  C  V  H  G  K  G  P  T  E  Q  L  V  S  P  E  P  E  V  H  D  I  E  R  S  E  E  D  D  Q  F  I

TCATCCTTGCATGCGATGGCATCTGGGACGTCATGGGGAACGAAGAGCTCTGTGACTTTGTGAGATCCAGACTTGAAGTCACTGATGACCTTGAGAAAGT    810
   I  L  A  C  D  G  I  W  D  V  M  G  N  E  E  L  C  D  F  V  R  S  R  L  E  V  T  D  D  L  E  K  V

TTGCAATGAAGTAGTCGACACCTGCTTGTATAAGGGAAGTCGAGACAACATGAGTGTGATTTTGATCTGTTTTCCAAGTGCACCCAAAGTCTCGGCAGAG    910
 C  N  E  V  V  D  T  C  L  Y  K  G  S  R  D  N  M  S  V  I  L  I  C  F  P  S  A  P  K  V  S  A  E

GCGGTGAAGAAGGAGGCGGAGCTGGACAAGTACCTGGAGAGCAGAGTAGAAGGTGGATCATTAACAAAAATAAATAAGTAGCTTTCTTTCAAAACAAAAC   1010
A  V  K  K  E  A  E  L  D  K  Y  L  E  S  R  V  E  G  G  S  L  T  K  I  N  K  *

CTTCCACACAAAGTCTAACCTTTTAGAAAGGTTTTAGTCCATTACATGCCTTTAGCATGTAGTACTAGTGTGTAAAAGTGGAAAGGACGTTCTGTAGTAA   1110
CCATTTGTTTAGCTGTGTTCCCTGGGCCCTGTGCACTATGGTTAGGAAACTTCCAAGTGACTGAAGCATGGCTCTAAGATTCTTGCTAAACCCACTGGCA   1210
TGTGTAATGTCATGCCAGGCAAGCCTGAGTATCCTCCTCTGAAAAGACGTACTGCAAGTCTCTGGAATATTTTTAATTACCTGTGTTTGGTTGGTTGGAT   1310
TTTGGGTGGTTTTGTTTTGTTGGTTTTGGTTTTGAGAACGTGAAGGTCAAATGTTTAGCAACAACTGTCGGGTCCCACAGCTTTGAAACTCTGCTTTCAG   1410
CTGGTTCACGGCTTTCCTCCTGAGCCATGAGCAGTTTGAGCTTACAACACAGACCTGGCAGGAATTCCCAGATGAATCAAGAAAGTAGCTTGCAACCCCA   1510
GGAGTGTGAATCTGATGTCTCTGCAGATCACTTTAGAGTAGAACAAATGGAAAGTGTTTGTACCTCAAAAAAAAAAAAAAGCAATGCATTTAAGTATTTG   1610
GAGAAATTAATCTTTTGTTGGTGGGGTTTGGTTACTTGGTTGTGGAACTTGGGACCAAATCTAAGGTTTTTTGCACAGAAGGGAAGTATTTCCCTCTGTG   1710
GCACACCTCGGCCCTTACGTGATCTTTGATAATGCTGTTCACCACGCATAAAATAAGCATTGTCTCCCCTGTGCGTACACACAATGCATATGGATCATGT   1810
AGGCCCTATAAGCAGAAACACTTCCATGTAAAAAGTGTGTTTGACTCTTCTGATACCATAATTGGCATGCAAGCTTCAATCTGCCATGTCCTTGCATCTC   1910
ATTGTGTCTTCATTATTAAAATTAGTGTGCATCTTAATTTTATTTTCTCCATTTCAGTTGCTTGATTTCATTTGTAATCTGCCTATTAGCATCATGTTTG   2010
GTTTTGGTTGTCAGTGTTTCTAGAGACCGGAGACGGTCTTGTTCTTCTTACCCCCTCCCTCCTTGTATGGTAATGTCTGTGTTTGTACTGTTTAGTATTT   2110


CATTCATTGAGTGTACTAGTCAGTGCATTTTTTCTCACTATTTTAAGGTTCTTGTAACTTTTTATATCTTAAGTAATTTCAATATGTTATTTGTAGATAA   2210
AACAGTAAAGTTAAAAATACTTAGAATGTTACAGGGTGAGTGGTCTAAATTGCTGATCGGTAGAAAAATATGCAGCCAAAAAAATATGTATAGGGTTAAG   2310
TTCCTATTGATGTCTTGGCACTTTAACCTGGGCATATGTTTTATTGGTATAAAGAATTAATAAATACCTTAGTATTTTTACTGTGAAGTGATATCCAGTT   2410
TTTGGAATGAATTGCTATTTTTCTCTTTAAGCGTGCTTTATTTTAAAGTAAATTTTTGGTGGAATTATCAAACATTCACTACATACATATAACTTTATAA   2510
GTTTTATATTTCATACTATCAAGTGAGAGTTGCCATCTGGGCAGAGTTTACATTAGTAGTCAATATTTTTGCTATATTCAAGTGTTTTAACATGCAGTTG   2610
GAGGTAATGTCATAAATTAAGATGCAACTAGAGAGAAAATGATGTTCGGTAAAGCTGCTGCTATTTAAAGGCATAGAATGTGACTTTGCACTGTAAATGA   2710
TGTATGTCTTAAATGAAGATCACAGCTTCTAAAGCAGGCACCAAATACCTTTTTAAATACATGCCTTGACTAAGTTGTTGTGGCCCTACTAAGCCTCATT   2810
GATTTGCTGTCGACCGTTTTGGCCTGTGTGACTCCACCATTGGGAAGGATAATGCTTCCCTCCTGCTGCTGTGTCCGCCTTCACTGAAGGCTGCTTGTCC   2910
TTATGAAGGCGGAGTGCTGCTGTGCTGCGGTCCTTGGGCCCTTTAAGGTCATGCTTACCTGCGTGACCTTCCCACCACTGCTACTCACCTGGCTCCCTGA   3010
AAAGTTGCTAATGTGAGGGAGCACTTTATTAAGGAAGGTGTGGGGGGGGATTATGTTTGTGTCTGTGTTCATGAAACATAAGAGAATTTCGTGGCATACA   3110
TATTCTTTATGAAGGTATCAAGTCTTTTACAGTCTGTTAAATTGTATAGTCCAAGAACAATTAAAAGAATTTCCTTCTCATTCAATATTTTCTAGAAGCC   3210
AAAGGAAGAGGGAGGGATAGTGTAATTGTTCAGTTTAAATGATTGTCCCACATCAAATAAGAGATTCTAATTGTTTCTAAAGCTTTTATTATTCATAAAA   3310
TATTTTTCCTGTATTCTTCACAATTAAAATATCCTAACATTTTCGTGAAGATGTAAGCTACATCAGATACAGTGGGGTTTTTACTTAGGACTGAAACCAT   3410
CATTGGTTAAATTTATCATCAGTGTTCATTTCTCTTGTATCTAGAGCTCTAATCAAATGATTGTATTAGCTTAGTAGACACTGTGTTGTGTGTTACAGTG   3510
TTAGATTGTGAACTTGTGTAGCTTAGGTAAGCATCAAACCCCTTTATTTTTGTATAGTAGATTT                                          3574
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Fig. 1. Nucleotide sequence and predicated amino acid sequence of the MPPα-2 gene. The nucleotide sequence has been deposited in GenBank under access number AF369981.

amplify cDNA were: 5′-CGGGATCCATGGGAGCATTTT-TAGACAAGCC-3′ and 5′-GGGGCGGCCGCCTTATTTA-TTTTTGTTAATGATCCACCTTC-3′. The amplified product was digested with *Not*I/*Bam*HI and cloned into pET21b vector (Novagen). The recombinant plasmid was transferred to the *E. coli* expression strain BL21 (DE3). MPPα-2 protein was purified and Western blot analysis was carried out with anti-His antibody as a primary antibody. A band with a molecular mass of 41 kDa was detected (data not shown). Protein phosphatase assay was performed using a Serine/Threonine Phosphatase Assay System (Promega) according to the manufacturer's instructions. The reaction mixture contained 50 mM imidazole (pH 7.2), 0.2 mM EGTA, 0.02% β-mercaptoethanol, 0.1 mg/ml BSA, 0.1 mM phosphopeptide and 5 mM $MgCl_2$ or 1 mM EDTA. Base level activity (< 0.1 pmol phosphate/min/μg protein) was measured in the control (vector alone). By contrast, the extract containing the vector with the MPPα-2 insert exhibited substantial activity (3.16 pmol phosphate/min/μg protein) in the presence of 5 mM $MgCl_2$. This activity was reduced to base levels (< 0.1 pmol phosphate/min/μg protein) in the presence of 1 mM EDTA.

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MPPα1  MGAFLDKPKMEKHNAQGQGNGLRYGLSSMQGWRVEMEDAHTAVIGLPSGL   50
MPPα2  MGAFLDKPKMEKHNAQGQGNGLRYGLSSMQGWRVEMEDAHTAVIGSPSGL   50

MPPα1  ETWSFFAVYDGHAGSQVAKYCCEHLLDHITNNQDFRGSAGAPSVENVKNG  100
MPPα2  ETWSFFAVYDGHAGSQVAKYCCEHLLDHITNNQDFRGSAGAPSVENVKNG  100

MPPα1  IRTGFLEIDEHMRVMSEKKHGADRSGSTAVGVLISPQHTYFINCGDSRGL  150
MPPα2  IRTGFLEIDEHMRVMSEKKHGADRSGSTAVGVLISPQHTYFINCGDSRGL  150

MPPα1  LCRNRKVHFFTQDHKPSNPLEKERIQNAGGSVMIQRVNGSLAVSRALGDF  200
MPPα2  LCRNRKVHFFTQDHKPSNPLEKERIQNAGGSVMIQRVNGSLAVSRALGDF  200

MPPα1  DYKCVHGKGPTEQLVSPEPEVHDIERSEEDDQFIILACDGIWDVMGNEEL  250
MPPα2  DYKCVHGKGPTEQLVSPEPEVHDIERSEEDDQFIILACDGIWDVMGNEEL  250

MPPα1  CDFVRSRLEVTDDLEKVCNEVVDTCLYKGSRDNMSVILICFPSAPKVSAE  300
MPPα2  CDFVRSRLEVTDDLEKVCNEVVDTCLYKGSRDNMSVILICFPSAPKVSAE  300

MPPα1  AVKKEAELDKYLESRVEEIIKKQVEGVPDLVHVMRTLASENIPSLPPGGE  350
MPPα2  AVKKEAELDKYLESRVEGGSLTKINK*                         326

MPPα1  LASKRNVIEAVYNRLNPYKNDDTDSASTDDMW*                   382
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Fig. 2. Comparison of the deduced amino acid sequences encoded by MPPα-1 and MPPα-2 transcripts, respectively. Asterisks show the positions of stop codons. Bold face indicates sequence divergence.

In higher eukaryotes, four distinct MPP genes (PP2Cα, PP2Cβ, PP2CϒΤ and Wip1) have been reported [8–12]. Regulation of these genes seems complex as indicated by the fact that at least four mRNA isoforms are derived from the PP2Cβ gene, each with a distinct spatial and temporal pattern of expression. The encoded proteins differ from one another only in their C-terminal regions [13–15]. Recent studies demonstrated that the alternative usage of two promoters within the PP2Cβ gene regulate the tissue-specific expression pattern of PP2Cβ mRNAs [16]. Recently, the human PP2Cα which inhibits the human stress-responsive p38 and *Jun*-N-terminal kinase (JNK) mitogen-activated protein kinase (MARK) pathways was isolated [17]. Our discovery of a second isoform of MPPα mRNA suggests that the transcriptional regulation of this gene may be elaborate as well.

Expression profiles of the MPPα-1 and MPPα-2 transcripts were established by section in situ hybridization of the E12.5 pituitary and surrounding tissues. Sagittal sections (5 μm) of wax-embedded embryos were hybridized to ^{33}P-labeled riboprobes, according to published procedures [18]. RNA probes were generated using SP6 or T7 polymerase (Ambion), using the manufacturer's instructions. Exposure time for radioactive signal detection was 21 days. Riboprobes corresponded to distinct 3′ regions of the two MPPα transcripts. MPPα-2 was prominently expressed in the E12.5 wild-type pituitary and the adjacent floor of the diencephalon but not in the *Lhx*3 null mutant pituitary (Fig. 3A, B), thereby validating the approach that led to the isolation of this transcript. By contrast, MPPα-1 transcripts were detected in the E12.5 pituitaries of both wild-type and *Lhx*3 null mutant embryos (Fig. 3C, D). This implies that the transcriptional regulation of the MPPα gene is part of the pituitary differentiation program controlled by *Lhx*3.

A mouse multiple tissue Northern blot (Clontech) was hybridized with 242 and 800 bp probes generated by PCR

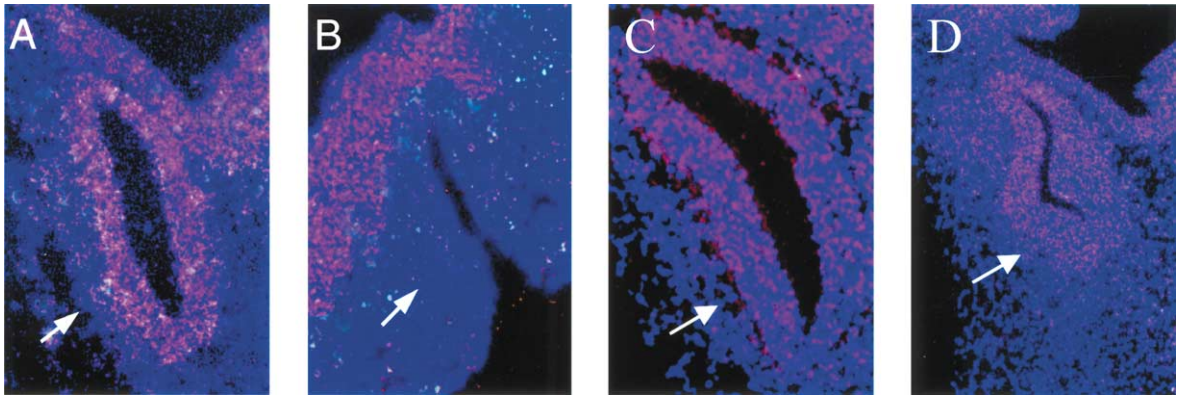

Fig. 3. E12.5 pituitary (arrows) expression patterns of MPPα-2 (A, B) and MPPα-1 (C, D) in wild-type (A, C) and *Lhx*3 null mutant embryos (B, D), respectively.

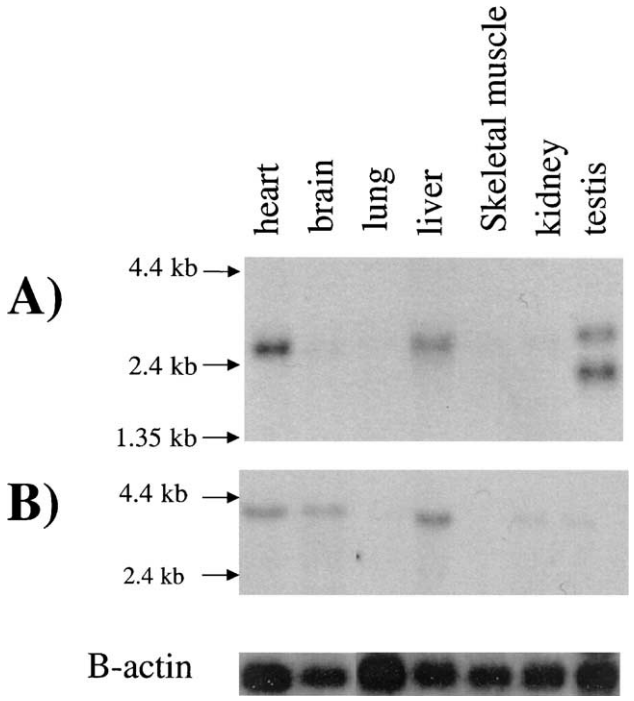

Fig. 4. Northern blots of RNA corresponding to select adult mouse tissues, hybridized with MPPα-1 (A) and MPPα-2 (B) probes, respectively.

from 3′ sequences specific for the MPPα-1 and the MPPα-2 transcript, respectively. Differential regulation of MPPα transcription is clearly not restricted to the developing pituitary. A Northern analysis of select adult tissues revealed the presence of MPPα-1 mRNA in heart, liver and testis (Fig. 4A), and that of MPPα-2 mRNA in heart, brain and liver (Fig. 4B).

In conclusion, our results show a novel isoform of PP2Cα. PP2Cα-2 is expressed during wild-type pituitary development, but this expression is not detected in the *Lhx*3 mutant. This differential expression pattern is not seen with PP2Cα-1 indicating distinct transcriptional control mechanisms for these isoforms.

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