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FAST TRACK

Molecular Characterization and Chromosomal Localization of Mouse Purα Gene

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Abstract Purα is a 39-kDa sequence-specific single-stranded DNA/RNA binding protein with the ability to modulate transcription of several genes containing the Pur element in their promoter region. Human and mouse Purα exhibit an extraordinary degree of conservation with only two changes at amino acid residues 49 and 306. A 15-kb genomic clone encompassing the mouse Purα gene was isolated by screening the mouse genomic library, using a PCR-amplified fragment from human Purα cDNA. Results from sequencing analysis confirmed the isolated genomic clone to be Purα and not the other members of the Pur family, including Purβ. Characterization of the mouse Purα gene by restriction analysis/Southern blotting and sequencing revealed that the Purα gene contains only one intron within the 5′ UTR and the open reading frame was intact. Using chromosomal markers, the Purα gene was mapped to chromosome 18 in mouse and rat. J. Cell. Biochem. 77:1–5, 2000.

Key words: Purα; 5′ UTR; open reading frame

Pur is a family of DNA and RNA binding proteins that recognize purine-rich single-stranded molecules in a sequence-specific manner. cDNA for two members of this family, Purα and Purβ, have been identified and their complete DNA sequences determined [Bergemann and Johnson, 1992; Kelm et al., 1997]. Human Purα protein contains a modular structure in which its central region is composed of three 23 amino acid class I repeats interspersed with two 26-amino acid class II repeats. The central repeat region of Purα is important for binding to its single-stranded DNA target sequence [Chen et al., 1995; Johnson et al., 1995]. The class I repeat that contains conserved phenylalanine or tyrosine residues as well as several basic amino acids is present in Purβ. However, the C-terminus of Purα contains a glutamine glutamate-rich domain that does not exist in Purβ. Sequence analysis of human and murine Purα demonstrates significant homology between human and mouse protein. In fact, the mouse Purα differs from its human homologue in lacking Gly at positions 49 and Ala to Thr substitutions at residue 306.

Several lines of study have suggested that Purα is a transcriptional factor. Earlier studies have shown that Purα binds specifically to the proximal regulatory element of the mouse myelin basic protein (MBP) gene and enhances its activity in in vitro cell-free and in vivo cell culture systems [Haas et al., 1993, 1995]. Several other viral and cellular genes have been shown to be transcriptionally regulated by Purα, including the human smooth muscle α-actin [Kelm et al., 1997], the neuron-specific FE65 gene promoter [Zambrano et al., 1997], the β-subunit of neuronal nicotonic acetylcholine receptor genes [Du et al., 1997], human neurotropic JCV promoters [Chen et al., 1995], and human immunodeficiency virus type 1 (HIV-1) [Chepenik et al., 1997]. In avian fibroblasts infected with Rous sarcoma virus (V-src), a Pur element functions as an enhancer for the clusterin gene [Hereault et al., 1993]. Purα has also been implicated in the process of DNA replica-

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tion. Evidently, by binding to the viral early protein, T-antigen, Purα modulates the level of JCV DNA replication in the cells [Chang et al., 1996]. It has also been suggested that Purα may have a regulatory role in cellular DNA replication either by binding to Pur elements positioned in close proximity of replication origin and/or by associating with proteins involved in the control of cellular replication. Of interest, Purα associates with the hypophosphorylated form of pRb, a cellular protein that controls cell cycle progression in S phase [Johnson et al., 1995]. Thus, the ability of Purα to bind to pRb suggests a role for Purα in cell proliferation and growth regulation. In accord with these observations, mapping studies revealed chromosomal localization of the Purα gene in humans at chromosome 5q31 [Ma et al., 1995], where the loss of heterozygosity at this region is frequently associated with myelodysplastic syndrome and particularly myeloid leukemia [Pederson, 1993]. In this report, we describe the isolation of mouse genomic sequence of Purα, characterization of the genomic organization, and its chromosomal location in mouse and rat.

Approximately one million plaques of recombinant λ EMBL3 phages containing 129 SvJ mouse genomic library were screened with a 215-bp polymerase chain reaction (PCR)-amplified DNA probe, designated "e" domain that represents nucleotides 845–1060 of human Purα. Five genomic clones were isolated and, on the basis of their DNA digestion pattern

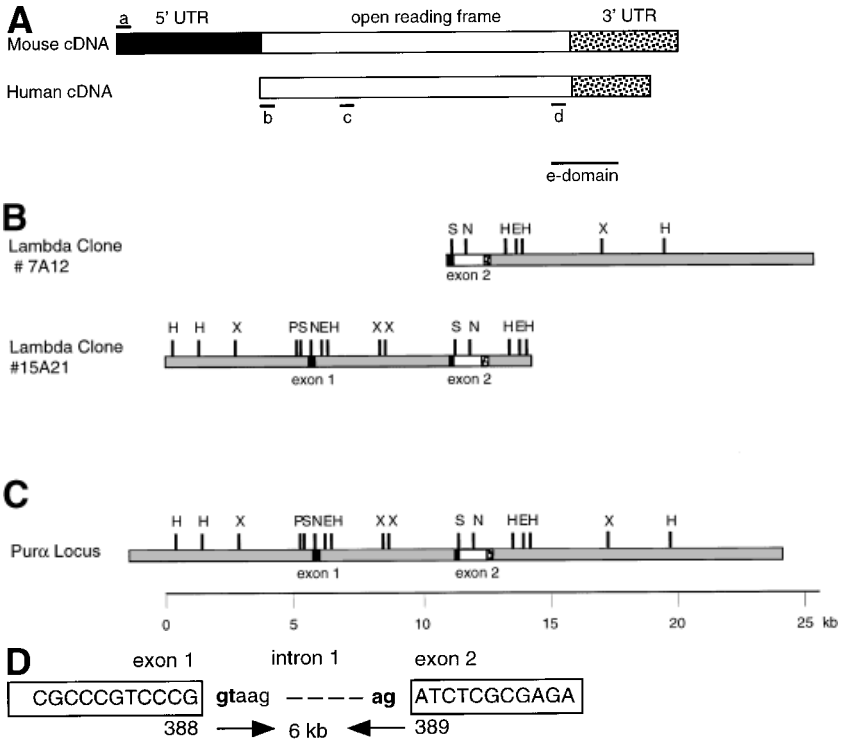

Fig. 1. **A:** Structural organization of Purα cDNA and the positions of the DNA probes used in Southern blot analysis. Comparative map of human and mouse Purα cDNAs depicting the 5′ UTR from mouse cDNA (1–518 bp), open reading frame (519–1486 bp), and the 3′UTR (1485–1609 bp). The following oligonucleotides were used in the Southern blot analysis of the Purα locus to generate a partial restriction map of the gene. Oligonucleotide a (5′-CGCTAGGGAAGGAGGAGAGA-3′), oligonucleotide b (5′-AAGCTGCAGGATTCGAGCGCAGCATCATGGCG-3′), oligonucleotide c (5′-CGGGTGGACATCCAGAAC-3′), oligonucleotide d (5′-TCCGAACTTGGCCCACAC-3′), and PCR amplified DNA fragment corresponding to e-domain. For amplifications of e-domain, the following primers were used: HPSQ-anti 2a (5′-GTGTGGGCCAAGTTCGGA-3′) and HPRP-2 (5′-GGGTCGACTCTAGATGTGTGTGTGTGTGGGGG-3′). **B:** Structural organization of isolates 7A12 and 15A21. Exons are illustrated by boxes. Within the exons: shaded area, position of the 5′ UTR; open area, open reading frames (ORFs). The stippled area points to the positions of the 3′ UTR of the gene. The partial restriction map shows the position of the restriction enzymes sites. H (*Hin*dIII), X (*Xba*I), P (*Pvu*II), S (*Sma*I), N (*Not*I), E (*Eco*RI). **C:** The approximate size of the Pur locus is represented by the scale shown beneath the diagram. **D:** Nucleotide composition of the Purα genome at the junctions of exon I and exon II. The intron is located within 5′ UTR after 135 bp from the 5′ end of the gene. Exon II contains the rest of the 5′ UTR, the translational start site, the ORF and the 3′ UTR. The gt, ag splice sites of the donor and acceptor sequences of the intron are shown in bold.

after *Xba*I treatment, were grouped into two categories and designated clone 7 and clone 15. Isolates 7A12 and 15A21, from clone 7 and clone 15, respectively, were selected for further restriction enzyme analysis. Using *Bgl*II, *Eco*RI, *Hin*dIII, *Not*I, *Xba*I, *Nar*I, *Pst*I, *Pvu*II, *Sma*I, *Bam*HI, *Kpn*I, and *Xho*I, either alone or in various combinations. Southern blot analysis was performed using either full-length human Purα cDNA, a 218-bp fragment representing the e-domain, or a variety of oligonucleotide DNA sequences (a–d) as probes, as shown in Figure 1A. The results from Southern blot analysis showed that isolate 7A12 lacks the 5′ UTR, while isolate 15A21 contains the complete Purα gene with two exons. The relationship between the two clones and with their restriction enzyme map is shown in Figure 1B. Analysis of the nucleotide composition of each clone confirmed that these clones represent the Purα gene and not the related Purβ. Purα is present in a single copy in the mouse genome, as evidenced by the simple pattern of bands obtained in mouse genomic DNA digestions with

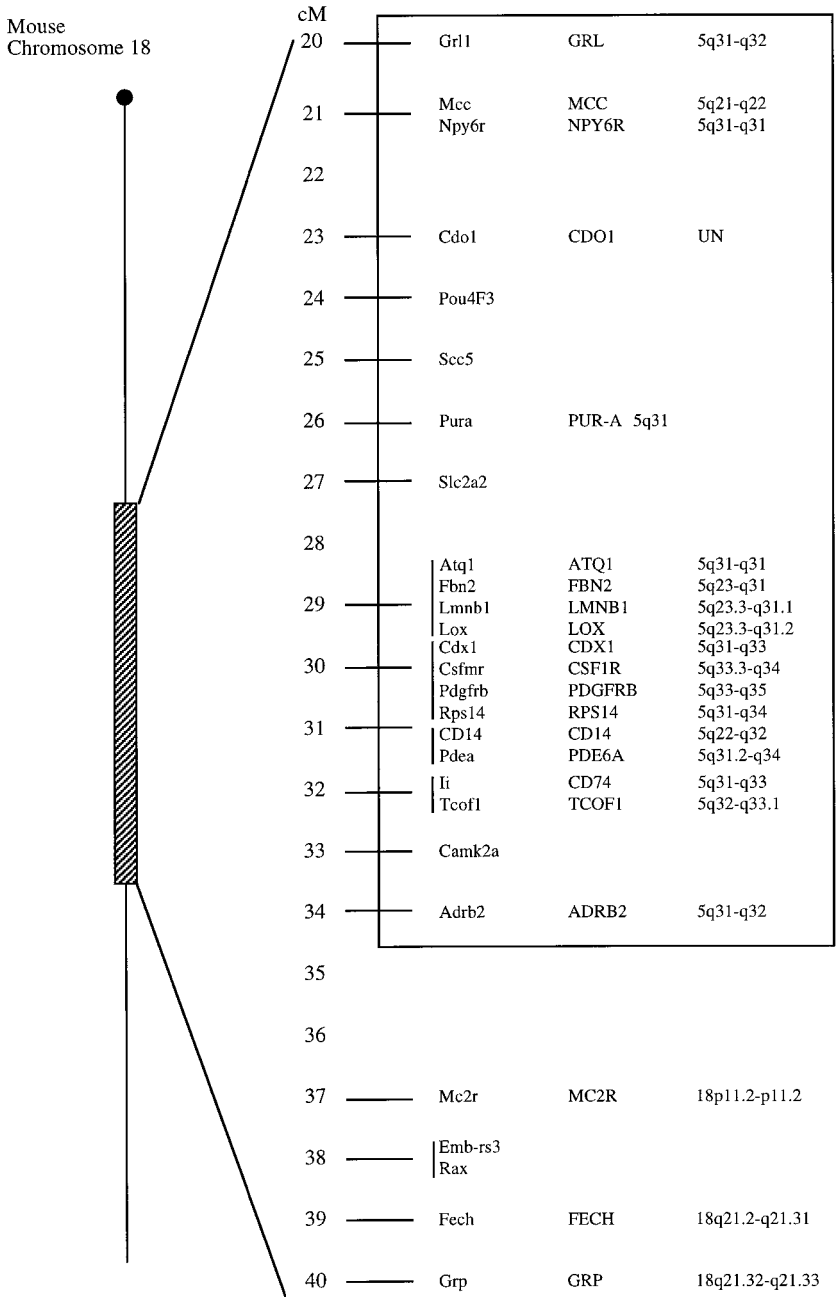

Fig. 2. Chromosomal localization of Purα and homology relationships between mouse and human chromosomes. Mouse chromosome 18 is represented by the vertical line on the left, and the box on the right represents homology group (H.G.) 53. The likely location of Purα and of other mouse, rat, and human genes with H.G. 53 is shown in the left and center columns in this box, flanked most proximally by the gene encoding the glucocorticoid receptor (*Grl1*, on mouse chromosome 18 at 20 centiMorgans (cM)) and most distally by the adrenergic receptor β2 gene (*Adrb2*, at 34 cM). Human gene locations are shown by chromosome regions in the far right column. For other abbreviations, see http://www.informatics.jax.org/searches/markerform.shtml.

several enzymes and Southern blot hybridization (data not shown). Figure 1C illustrates the approximate size of the Purα locus, highlighting restriction enzyme sites, and the position of the exons. The first exon of Purα contains the first 388 bp of the 5′ UTR and the second exon has the rest of the 5′ UTR, the translational start site, and the complete open reading frame followed by 239 bp of the 3′ UTR as present in the mouse cDNA. The intron present in the mouse Purα gene is approximately 6 kb long and the splice junction fulfills the GT-AG role as shown in Figure 1D.

The human Purα gene is located on chromosome 5q31 [Ma et al., 1995], a region that is syntenic to mouse chromosomes 11 and 18 (http://www.ncbi.nlm.gov.Omim/Homology/human5.html). Therefore, it is likely that the mouse homologue would map to one or the other of these chromosomes. A search of several mouse strains using PCR primers flanking a (CA)10 dinucleotide repeat located in the 3′ UTR of the mouse cDNA did not display a useful polymorphism (data not shown). However, the LEW and LER inbred rat strains differ at this locus, as defined by these primers flanking the dinucleotide repeat. We designed and used the following primers, which flank the repeats to amplify a polymorphic site in the rat genome: mPur 1360 forward primer (5′-AAGAGAAACAGAGGGAGAAGCGGG-3′), mPur 1585 reverse primer (5′- GCGGCCGCTCTTTACAGTTATATTTTCTC-3′). An F2 cross of LEW and LER which had already been typed for numerous microsatellite markers on all chromosomes, was screened for this polymorphism using the standard microsatellite technique [Butterfield et al., 1998]. In this study, DNA samples from 51 rats were scored for Purα alleles. The Purα gene mapped unequivocally to rat chromosome 18, which is highly homologous to mouse chromosome 18 (http://www.informatics.jax.org; http://ratmap.gen.gu.se). For mapping Purα in mice, we used 19 mice from the original panel of ES-derived lines that segregated the wild type and neodisrupted Purα alleles and are distinguishable by Southern blot analysis. Testing of five primers from each of the two chromosomes (11 and 18) revealed linkage of Purα to markers D18Mit35 and D18Mit51 on mouse chromosome 18 (Fig. 2). The map location of Purα in both rodent species is approximately 5 cM from the glucocorticoid receptor (http://www.informatics.jax.org; http://ratmap.gen.gu.se). This location is also concordant with the mapping of the glucocorticoid receptor to chromosome 5q in the human genome. Thus, our results establish that Purα is a new member of a known, tightly conserved homology group (HG53) (http://www.ncbi.nlm.gov.Omim/Homology/human5.html). This map location should facilitate the study of the possible genetic role of this gene in transcriptional processes and other cellular functions including replication.

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