Genomic organization and alternative splicing of the human and mouse RPTPρ genes

ABSTRACT

We report the first genomic characterization of a RPTP type IIB gene. Alternatively spliced variants may result in different RPTPρ isoforms. Our findings suggest that RPTPρ extracellular and intracellular segments originated as separate modular proteins that fused into a single transmembrane molecule during a later evolutionary period.

INTRODUCTION

Background Protein tyrosine phosphorylation regulates many important cellular functions including signal transduction, growth, differentiation, cell adhesion and axon guidance. The balance between protein tyrosine kinase and phosphatase activity is an integral part of this regulatory mechanism. A large number of protein tyrosine phosphatases have been identified, which fall into the broad categories of cytoplasmic and receptor-like molecules. All receptor-like protein tyrosine phosphatases (RPTPs) contain an extracellular region, a single transmembrane segment and at least one intracellular catalytic domain. They have been subdivided into several classes based on the structure of their extracellular segments (Figure 1). A combination of immunoglobulin-like (Ig) domains and fibronectin type III (FN-III) repeats in the ectodomain defines the type II class of RPTPs. An additional feature of type II RPTPs is a potential proteolytic cleavage site within the membrane-proximal FN-III repeat. Upon cleavage, extracellular N-terminal and predominantly intracellular, membrane bound C-terminal segments are generated, which remain non-covalently associated A subset of the type II class, identified previously as type IIB RPTPs, is characterized by the presence of an N-terminal MAM domain. Currently, four type IIB phosphatases (PTPμ, PTPκ, PCP-2 and RPTPρ) have been reported. The hPCP-2, hPTPκ, and hPTPμ RPTPs are located on human chromosomes 1, 6 and 18, respectively, and hRPTPρ is located on chromosome 20. Several additional human RPTPs (PTPπ, PTPψ, hPTP-J, PTPRO) share very high sequence similarity (>98%) with PCP-2, and are likely to represent the same gene (Unigene database,). There are, in addition, several murine homologues of the four human genes: mPTPκ (Genbank #NM 008983), mPTPμ (#NM008984), mRPTPρ (#AF152556), mRPTPρ -1 and mRPTPρ -2 (# AF162856/7), mRPTPmam4 (#NM 021464), mPTPf (#D88187) and mPTPλ (#U55057). The latter two are likely to be murine homologues of hPCP-2, and mRPTPmam4 is the same gene as mRPTPρ. RPTPρ is the most recently isolated member of the IIB family. Northern blot and in situ hybridization studies have shown that RPTPρ is largely restricted to the central nervous system. Within the CNS, expression is developmentally regulated and, in the mouse, delineates a unique boundary region in the granule cell layer of the cerebellar cortex. Motifs in the RPTPρ extracellular segment (MAM, Ig and FN-III domains) are commonly found in cell adhesion molecules. The two phosphatase domains in the intracellular segment suggest that RPTPρ, like other members of the RPTP family, is involved in signal transduction through protein tyrosine dephosphorylation. The human RPTPρ gene has been mapped to chromosome 20q12-13.1; it is located between anchor markers D20S99 and D20S96, and is flanked by the phospholipase C gamma 1 and splicing factor SRp55-2 genes. The mouse gene maps to a syntenic region at 93 cM on mouse chromosome 2, a region closely linked to Pltp and flanked by the markers, D2Mit22 and D2Mit52. To date, only portions of the human RPTPκ, RPTPμ and PCP-2 genes have been sequenced, however,

the region encompassing the human RPTPρ gene has been sequenced in its entirety (Chromosome 20 sequencing group, Sanger Centre), but it is not, as yet, fully assembled and annotated. The mouse chromosomal region containing the RPTPρ gene has been sequenced (Celera Discovery System), but it is also largely unassembled. In this report, we describe the cloning of the mouse cDNA, the identification of an unusually long 3' UTR, the identification of alternatively spliced exons, and the genomic organization of the human and mouse RPTPρ genes.

CONCLUSION

Conclusions We describe the cloning of the mouse RPTPρ cDNA, the genomic structure and alternative splicing of the mouse and human genes, and the presence of an 8 kb 3'-UTR in human RPTPρ. RPTPρ is the largest RPTP gene characterized to date, extending over more than 1 megabase pairs of genomic DNA. Its considerable length is due, primarily, to expanded introns in the extracellular region. The protein domains of the extracellular segment are encoded by 1 to 3 exons, which form modules that are flanked by phase 1 introns. The majority of introns in the intracellular segment are in phase 0, and are relatively small. These data suggest that the ectodomain and the phosphatase domain arose separately by exon shuffling and duplication and fused at a later evolutionary period. The MAM domain, the region characterizing type IIB phosphatases, possesses a unique genomic structure common to all such domains when located at the N-terminus. The fourth fibronectin repeat in RPTPρ is encoded by three exons, an additional feature found only in type II phosphatases. At least two alternatively spliced exons flank the transmembrane domain, the region showing the greatest variability between the four IIB phosphatases. An additional alternatively spliced exon precedes the catalytic core of the first phosphatase domain. Comparison of the genomic structure of representative members of the RPTP family (types I-V) indicates that the intron/exon organization of both phosphatase domains is highly conserved. There is considerable variation in the length of the 3' UTR in the RPTPs; at 8 kb, the RPTPρ 3' UTR is the longest characterized to date. Our results provide the first characterization of the genomic structure of an RPTP type IIB gene. This information will facilitate future studies of promoter and other regulatory elements responsible for the tissue specificity of gene expression.