Cloning and sequence of the rat retinoblastoma (Rb) gene cDNA

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Received November 4, 1992; Revised and Accepted November 23, 1992

GenBank accession no. L07126

The retinoblastoma (RB) susceptibility gene serves as a model for the study of tumor suppressor genes. The human and mouse RB genes have been thoroughly characterized (1, 2). The human gene is approximately 200 kb, contains 27 exons, and produces a message of 4.7 kb in humans and 4.6 kb in mice. Inactivation of the human retinoblastoma susceptibility gene, as a result of deletion or mutation, has been associated with the development of a variety of human cancers (3–6). Oncogenic mutations in this gene consistently appear to be recessive, loss of function mutations that result in an absent or non-functional protein. Most of the inactivating mutations that have been reported occur in conserved regions of the gene, that codes for critical functional domains such as phosphorylation sites and DNA binding regions of the pRB protein (4, 6). In order to understand the involvement of the RB tumor suppressor gene in rat carcinogenesis, we have undertaken to isolate and sequence the rat RB cDNA.

About 1×10^6 phage from a rat cDNA library in λ gt10 were screened with a human retinoblastoma cDNA probe (1). One positive clone (R961) was identified and DNA was purified. The 2.1 kb cDNA fragment was then subcloned in the bacterial sequencing vector, pUC19 (7). Subclone fragments were then sequenced in both directions using M13 universal and reverse sequencing primers and aligned based on the method of Staden using the GCG's Fragment Assembly Programs (8). The complete nucleotide sequence of 2113 bp is available from GenBank (Accession No. L07126). The rat R961 RB cDNA sequence is homologous to the 3' region of mouse and human Rb genes. Homology between rat and mouse (2) is more than 92%, and between rat and human is about 81% (1). We observed a short polyA stretch at the 3' end of R961. A consensus polyadenylation sequence (AATAAA) was also found within 18 nt of the start of the polyA stretch. An open reading frame was found to start from nt 2 and ended at nt 349 (Figure 1). Only 4 amino acids did not match with the human sequence, whereas 9 amino acids did not match with mouse peptide. The deduced amino acid sequence from this cDNA sequence is about 97% homologous with that of the human RB protein (pRB) sequence at the carboxy terminus. Human pRB with a deletion of this carboxy-terminus domain fails to interact with E2F transcription factor (9). This region of pRB may also be a target for phosphorylation by the CDC2 kinase (10). Thus the carboxy-

terminus domain has a definite role in pRB-mediated repression of E2 transcription which might also explain why extensive homology has been maintained among the peptide sequences of rat and two other (human and mouse) known RB proteins.

ACKNOWLEDGEMENTS

This work was supported by grant CA 43199, and Center Programs CA 13343 and ES 00260 from the NIH.

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Mouse	KISEGLPTPT	KMTPRSRLV	SIGESFGTSE	KFQKINQMC	846
Rat	KISEGLPTPT	KMTPRSRLV	SIGESFGTSE	KFQKINQMC	40
Human	KISEGLPTPT	KMTPRSRLV	SIGESFGTSE	KFQKINQMC	853
Mouse	NSDRVLKRSA	EGGNPFKPLK	RFDIEGSD	EADGSKHLP	886
Rat	NSDRVLKRSA	EGGNPFKPLK	RFDIEGSD	EADGSKHLP	80
Human	NSDRVLKRSA	EGGNPFKPLK	RFDIEGSD	EADGSKHLP	893
Mouse	ESKFQOKLAE	MTSTRTRMQK	QNDSDMS	NKEEK*	921
Rat	ESKFQOKLAE	MTSTRTRMQK	QNDSDMS	NKEEK*	115
Human	ESKFQOKLAE	MTSTRTRMQK	QNDSDMS	NKEEK*	928

Figure 1. Comparison of deduced amino acid sequence of rat RB protein with that of mouse and human sequences. The single letter amino acid sequences of the Rb proteins are presented. Shading reflects non-conservation of amino acids among the sequences. * = Termination codon.

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