Peptide binding characteristics of the non-classical class Ib MHC molecule HLA-E assessed by a recombinant random peptide approach

ABSTRACT

HLA-E appears to refold to detectable but reduced levels around a random peptide library in comparison to the results obtained with rat classical class Ia MHC molecules, RT1-A1c and RB1–Au suggesting that this particular molecule may have 'narrow but not exquisite' specificity. A previous report suggests that HLA-E, similar to its mouse counterpart (Qa-1b), can bind synthetic peptides carrying a viral sequence and present them to T lymphocytes.

INTRODUCTION

Term-enhanced Hepadnaviruses are small, enveloped hepatotropic viruses that replicate by reverse transcription of a pregenomic RNA molecule. P binds to the 5' copy of and commences DNA synthesis, using a tyrosine residue near its own amino-terminus as supplementary information. DNA synthesis occurs after 3–4 nucleotides, and then the protein-linked minus-strand DNA is transferred to this copy (the doppelganger) within the 3' terminal duplication. The minus-strand DNA is prepared as a primer by the pregenomic RNA, and its ribonucleaseH activity degrades it during synthesis. In spite of this, the RNAseH activity fails to digest the extreme 5' end of the template ARN, and the fragment is then transferred to direct repeat 2 (DR2), where it serves as a primer for synthesising plus-strand DNA. Unlike the reverse transcriptases of retroviruses that are better understood, the hepadnaviral P protein differs in several ways: it starts DNA synthesis with its amino-terminal domain, cannot be obtained from virions without partial proteolysis or denaturation, and is only active on the endogenous pregenomic RNA that is encapsulated in the viral cores. It is not known how this cleaved out but appears due to the covalent linkage between P and the viral DNA, which is likely to explain why: RNAseH inserts the end of the ribonuclear layer in a RNA:DNA heteroduplex, but it does no damage any DNA or non-hybrid material (in that case the protein P doesn’t cut the part of its own end after it had already been reverse transcriptionally copied into DNA). The absence of RNAseH activity hinders the production of infectious virions or positive polarity DNA. Although not yet measured directly in viral core particles, its presence is suggested by four observations, (i) Sequence alignment of P with known RNsES enzymes shows conservation motifs in P. By modifying their active site residues, the expected RNA:DNA heteroduplex is prevented from being synthesized by blocking mutations in both Hepatitis B Virus (HBV) and duck hepatitic B virus (DHBV) P. The detection of ARNseH activity for the human HEpaticisB virus was confirmed through the expression of an ion called fusion with the maltoses binding protein. Purified DHBV cores were used to generate activity gels, but the apparent molecular weight of RNAseH was lower than that of the native PH, suggesting that P was either cleaved or signaled by a cellular ARNseleH. We attempted to directly detect the RNAseH activity of DHBV P within viral cores using substrates from out of the virus. However, no specific results were obtained despite extensive experiments with different conditions and substrate combinations. Given that P has intrinsic RNAseH activity, we hypothesize that the hepadnaviral ARNs■H activation will be "substrate committed" in a similar way to the template commitment observed for its DNA polymerase activity.

CONCLUSION

Specifically, we describe how the mouse RPTP (mouse phage-tumor-like) cDNA is cloned into the human skeletal muscle, the way in which the mice and humans are genetically expressed through alternative splicing of their respective genes, and the presence of an 8 kb 3'UTR in human "RPTTP" (human minus 2), The largest PCRTA gene known to date, spanning over 1 megabase pairs of genomic DNA, with its considerable length, mostly due to expanded introns in this region). Encoding the protein domains in the extracellular segment consist of modules that are flanked by phase 1 introns, while the majority of intracellular segments are phase 0 and relatively small. These data indicate that the ectodomain originated from exon shuffling and duplication and eventually fused with another phosphatase domain at a later time. The MAM domain, which is the region defining type IIB phosphatases, has a genomic structure that is typical of all these domains when located at the N-terminus. Additionally, three exons encode the fourth fibronectin repeat in RPTP, an extra property present in only type I ATPaSeq. At least two spliced exons flank the transmembrane domain, which is the region of greatest variation between the four IIB phosphatases; another resembling an alternatively arranged exonet precedes the catalytic core of the first ATPase. The genomic structure of representative members of the RPTP family (types I-V) shows that the intron/exon organization of both phosphatase domains is highly conserved. Significant variation exists in the length of their 3' UTRs; the longest known record of a regulated transcriptional region at 8 kb is characterized as 3'UTR, or "under regulation" UTM. We have achieved the first-ever characterization of the genomic structure of an RPTP type IIB gene. This knowledge will assist in future research on the regulatory factors that influence tissue specificity of gene expression.