

NEW STRATEGIES TO TREAT AND PREVENT VIRAL INFECTION REVEALED FROM STUDIES OF MECHANISMS OF RIBAVIRIN RESISTANCE

Craig E. Cameron

Pennsylvania State University, University Park, PA

Replication of the genomes of all RNA viruses is catalyzed by the virus-encoded RNA-dependent RNA polymerase (RdRp). During each round of replication, the RdRp makes a few errors that lead to a population of genetically distinct viral variants. The quasispecies nature of RNA viruses is responsible for immune escape and the rapid development of antiviral drug resistance. Is it possible that a more fundamental role exists for the quasispecies in RNA virus biology? To address this question, we have used a ribavirin-based selection/screen to identify polioviruses with reduced or enhanced sensitivity to this drug. These mutant viruses encode RdRps with higher or lower incorporation fidelity than the wild-type enzyme. We have used these viruses to demonstrate that RdRp fidelity is optimized, is a determinant of viral virulence, and is a target for attenuation and vaccine development. In addition, studies with these mutant viruses lead us to suggest that classical RNA viruses obey population genetics theory. We have exploited our panel of RdRps to investigate the mechanistic basis for incorporation fidelity. We have shown that a conformational-change step that occurs at the active site during the nucleotide-addition cycle is responsible for incorporation fidelity. This conformational-change step appears to be controlled by a network of remote-site and active-site amino acid residues that exhibit correlated motions on the nanosecond timescale. Changes in the dynamics of this network appear to be communicated to the active site by a single amino acid residue that is located in a conserved structural motif, termed D, which is present in all RdRps and reverse transcriptases. Amino acid substitutions of this motif-D residue increase RdRp fidelity *in vitro*, reduce replication speed in cell culture and attenuate the virus in a very permissive animal model. We will discuss the possibility that polymerase dynamics represents a novel target for antiviral drug development and for development of attenuated viruses that can be used as vaccine strains.