Molecular mechanisms of RNA virus replication

Enteroviruses are a subfamily of small, pathogenic, icosahedral viruses. They have been associated with more than 20 clinically recognized syndromes, including poliomyelitis and polioencephalitis, respiratory illnesses, aseptic meningitis, pleurodynia, gastroenteritis, hepatitis, and acute hemorrhagic conjunctivitis. One of the most extensively studied members of this family is poliovirus, infamous as the etiologic agent of paralytic poliomyelitis. Although wild-type poliovirus infections have been eradicated from the Western Hemisphere, the virus still circulates in Third World countries. Importantly, poliovirus is a model for a number of enteroviruses that cause more than 2,000 cases of neonatal sepsis a year in the United States, such as those produced by echoviruses. Because several aspects of the poliovirus replication strategy are likely to be shared by other enteroviruses, the study of poliovirus RNA replication and identification of viral and cellular factors involved in the process may help in designing therapeutic drugs for pathogenic picornaviruses. Furthermore, as the poliovirus eradication campaign progresses to its conclusion it would be important to discover novel approaches to control viral replication in case of re-emergence of the virus. Importantly, the study of poliovirus RNA synthesis uncovered a novel mechanism of initiation of nucleic acid synthesis that permits the amplification of a single initial molecule of RNA into thousands of RNA progeny in only a few hours. The elucidation of this process is likely to contribute to our general understanding of viral RNA replication. Finally, the identification of cellular factors involved in viral RNA replication may shed light on the role of these factors in normal cellular processes.

To examine the molecular events that lead to virus replication we have focused on a ribonucleoprotein complex that forms at the 5'-end of the viral RNA. This complex is composed of the first 100 nucleotides of the genomic RNA, a viral-encoded protein, and a ribosomal-associated cellular factor. We have started to define the structural details and multiple functions function of this complex. Correct formation of this complex is essential for the synthesis of both genome and antigenome viral RNA and also participates in modulating translation. To analyze the function of this complex, we have developed multiple approaches ranging from biochemistry to cell biology, from classic virus genetics to structural biology. This approaches employed both cell free system as well as tissue culture experiments. We have discovered that microinjection of viral RNA into Xenopus laevis oocytes initiates a complete and authentic viral replication cycle, which yields a high level of infectious viruses, but only if polioviral RNA is co-injected with factors present in HeLa cells. Two additional mammalian cell factors are required for viral replication in oocytes, one involved in initiation of translation, and the other in RNA synthesis. Thus, microinjection in oocytes can be used essentially as an in vitro system in which to identify and further analyze the function of viral and cellular factors, and biochemically dissect mechanisms of poliovirus synthesis.

Analyzing the precise role of the RNA cloverleaf element in plus-strand synthesis has been hindered by its role in minus-strand synthesis, as mutations disrupting the structure and/or functions on the cloverleaf disrupt minus-strand RNA synthesis. To overcome this limitation, we have recently developed a novel approach to analyze the cis-acting elements within the cloverleaf and their multiple roles during virus replication. Poliovirus replicons were engineered to contain two tandem cloverleaf structures. Mutagenesis analysis showed that each of these elements is able to promote initiation of the minus-strand RNA. In contrast, only the distal 5'-most cloverleaf could support plus-strand initiation. This experimental setup was exploited to define the critical features of the cloverleaf structure required for plus-strand RNA initiation. These studies reveal initiation of genomic RNA synthesis requires all the same features that for initiation of antigenome replication, including the binding sites for the viral polymerase precursor 3CD and the host factor PCBP located within the cloverleaf structure. Specifically required to initiate plus-strand RNA synthesis is the stem a sequences within the cloverleaf structure. Strikingly, these studies revealed that the viral 2C protein binds to the cloverleaf and is directly involved in plus-strand RNA synthesis. These results define the determinants of genomic RNA initiation in poliovirus.