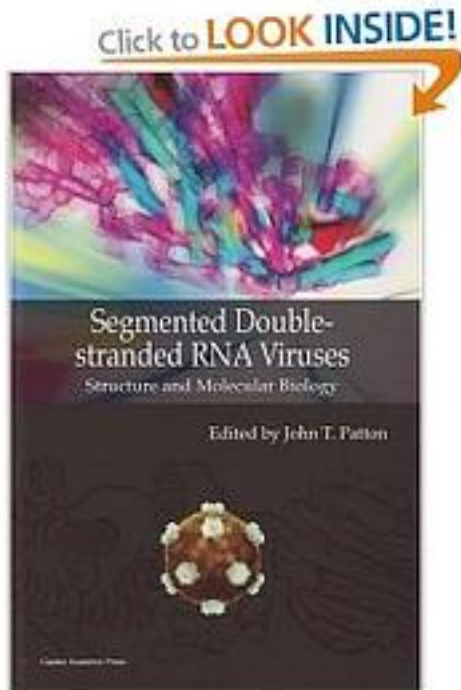


# Segmented Double-stranded RNA Viruses: Structure and Molecular Biology



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The double-stranded (ds)RNA viruses represent a diverse group of viruses that vary widely in host range (humans, animals, plants, fungi, and bacteria), genome segment number (one to twelve), and virion organization (T-number, capsid layers, or turrets). Members of this fascinating group include the rotaviruses, renowned globally as the commonest cause of gastroenteritis in young children, and bluetongue virus, an economically important pathogen of cattle and sheep. In recent years, remarkable

progress has been made in determining, at atomic and subnanometeric levels, the structures of a number of key viral proteins and of the virion capsids of several dsRNA viruses, highlighting the significant parallels in the structure and replicative processes of many of these viruses. By providing unique insights into fundamental aspects of structure-function relationships in virus particles, virus particle assembly, virus-cell interactions, and viral pathogenesis, approaches for the development of novel antiviral strategies and/or agents can be designed.

This timely book brings together all of the key recent research on this disparate group of viruses, providing for the first time a single resource reviewing dsRNA viral structure and molecular biology. Written by well respected and experienced virologists, topics include: the structures of orthoreoviruses, rotavirus, phyto-reoviruses, and bluetongue virus, entry into the bacterial cell, crystal structure of reovirus polymerase  $\lambda 3$ , assembly of the reovirus genome, genomic RNA packaging and replication in the Cystoviridae, and much more. Essential reading for all dsRNA virologists and all other virologists with an interest in molecular and structural biology.

Reviews:

"This is a splendid and very timely book ... The book makes fascinating reading and will be an incentive to many students of natural and biological sciences as well as established virologists who are involved in structure-function studies in this and other viral families. The price is relatively high, but the book is excellent value for money and is highly recommended." from *Microbiology Today* (2008)

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目录: Chapter 1 The Structure of Orthoreoviruses

Kelly A. Dryden, Kevin M. Coombs and Mark Yeager

The orthoreoviruses (reoviruses) are the prototypic members of the virus Reoviridae family, and representative of the turreted members, which comprise about half the genera. Like other members of the family, the reoviruses are non-enveloped and characterized by concentric capsid shells that encapsidate a segmented dsRNA genome. In particular, reovirus has eight structural proteins and ten segments of dsRNA. A series of uncoating steps and conformational changes accompany cell entry and replication. High-resolution structures are known for almost all of the proteins of mammalian reovirus (MRV), which is the best-studied genotype. Electron cryo-microscopy (cryoEM) and X-ray crystallography have provided a wealth of structural information about two specific MRV strains, type 1 Lang (T1L) and type 3 Dearing (T3D). This review describes the icosahedral structures of intact virions, infectious subviral particles that bind to cell surface receptors and core particles, which mediate RNA transcription. The size, shape, stoichiometry, interactions and atomic structures of the constituent proteins are described, as well as their functional properties. Such structural details about the individual proteins and the assembled particles are essential for a complete understanding of how the multiple proteins and genomic segments interact and participate in morphogenesis and infection.

Chapter 2 Cypovirus

Z. Hong Zhou

The cytoplasmic polyhedrosis viruses (CPVs) form the genus Cypovirus of the family Reoviridae. CPVs are classified into 14 species based on the electrophoretic migration

profiles of their genome segments. Cypovirus is unique among the Reoviridae in that it has only a single capsid shell, which is architecturally similar to the orthoreovirus inner core. Despite lacking protective outer shells, CPV exhibits striking capsid stability and is fully capable of endogenous RNA transcription and processing. The overall folds of CPV proteins are strikingly similar to those of their structural homologs in other reoviruses. However, CPV proteins have insertional domains and unique structures that contribute to their extensive intermolecular interactions. These differences, together with its unparalleled multiple modes of intermolecular complementarity, form the structural basis of the enhanced stability of CPV capsid. The CPV turret protein contains two methylase domains with a highly conserved helix-pair/ $\beta$ -sheet/helix-pair sandwich fold but lacks the  $\beta$ -barrel flap present in orthoreovirus  $\lambda 2$ . The stacking of turret protein functional domains and the presence of constrictions and A spikes along the mRNA release pathway point to a mechanism that uses pores and channels to regulate the highly coordinated steps of RNA transcription, processing, and release.

#### Chapter 3 Rotavirus Structure

Xiaofang Jiang, Sue E. Crawford, Mary K. Estes and B. V. Venkataram Prasad

This chapter describes our current understanding of the rotavirus structure and how structural studies have provided some insights into capsid associated functions including cell entry, antibody neutralization, trypsin-enhanced infectivity and endogenous transcription. In the last two decades several dsRNA viruses have been structurally characterized using cryo-EM and X-ray crystallographic techniques. Despite some distinctive structural features reflecting their host specificities, these studies have revealed several common mechanistic themes particularly in regard to endogenous transcription of the genome. The possible evolutionary implications of the common features among these viruses are also discussed.

#### Chapter 4 Structure and Function of Bluetongue Virus and its Proteins

Polly Roy

The members of Orbivirus genus within the Reoviridae family are arthropod-borne viruses and are responsible for high morbidity and mortality in ruminants. Bluetongue virus (BTV) which causes disease in livestock (sheep, goat, cattle) has been in the forefront of molecular studies for the last three decades and now represents the best understood orbivirus at the molecular and structural levels. BTV, like other members of the family, is a complex non-enveloped virus with seven structural proteins and a RNA genome consisting of 10 variously sized double-stranded (ds) RNA segments. This article will be centred on the molecular dissection of BTV with a view to understanding the role of each protein in the virus replication cycle. Data obtained from studies over a number of years has defined the key players in BTV entry, replication, assembly and exit and has increasingly found roles for host proteins at each stage. Specifically, it has been possible to determine the complex nature of the virion through 3D Cryo-electron microscopy (EM) reconstructions (diameter  $\sim 800$  Å); the atomic structure of proteins and the internal capsid ( $\sim 700$  Å, the first large highly complex structure ever solved); the definition of the virus encoded enzymes required for RNA replication; the ordered assembly of the capsid shell and the protein sequestration required for it; and the role of host proteins in virus entry and virus release. These areas are important in themselves for BTV replication but they also indicate the pathways that related viruses, which includes viruses that are pathogenic to man and animals, might also use providing an informed starting point for intervention or prevention.

#### Chapter 5 Structures of Phytoreoviruses

Matthew L. Baker, Z. Hong Zhou and Wah Chiu

Phytoreoviruses are non-turreted reoviruses that are major agricultural pathogens, particularly in Asia. One member of this family, Rice Dwarf Virus (RDV), has been extensively studied by electron cryomicroscopy and x-ray crystallography. From these analyses, atomic models of the capsid proteins and a plausible model for capsid assembly have been derived. While the structural proteins of RDV share no sequence

similarity to other proteins, their folds and the overall capsid structure are similar to those of other Reoviridae.

#### Chapter 6 The Yeast dsRNA Virus L-A Resembles Mammalian dsRNA Virus Cores

Reed B. Wickner, Jinghua Tang, Nora A. Gardner and John E. Johnson

The L-A dsRNA virus of the yeast *Saccharomyces cerevisiae* has a single 4.6 kb genomic segment that encodes its major coat protein, Gag (76 kDa) and a Gag-Pol fusion protein (180 kDa) formed by a -1 ribosomal frameshift. L-A can support the replication and encapsidation in separate viral particles of any of several satellite dsRNAs, called M dsRNAs, each of which encodes a secreted protein toxin (the killer toxin) and immunity to that toxin. L-A and M are transmitted from cell to cell by the cytoplasmic mixing that occurs in the process of mating. Neither is naturally released from the cell or enters cells by other mechanisms, but the high frequency of yeast mating in nature results in the wide distribution of these viruses in natural isolates. Moreover, the structural and functional similarities with dsRNA viruses of mammals has made it useful to consider these entities as viruses. The common structure of L-A dsRNA virions and the cores of dsRNA viruses of higher eukaryotes may reflect similarities in their replication cycles, specifically, the fact that both transcription and replication reactions occur within the virion requiring movement of the RNA template during this process. The L-A major coat protein has an unique mRNA decapping activity essential for expression of the capless viral messages.

#### Chapter 7 Dissecting the Assembly Pathway of Bacterial dsRNA Viruses: Infectious Nucleocapsids Produced by Self-Assembly

Minna M. Poranen, Roman Tuma and Dennis H. Bamford

The virions of double-stranded RNA bacteriophages of the family Cystoviridae possess a triple-layered structure surrounding the tri-segmented genome. Genome-containing double-shelled nucleocapsids are formed by a self-assembly process that can be accomplished in vitro using purified proteins and RNA constituents. The assembly process involves formation of empty particles, which package single-stranded genomic precursor RNA molecules. Subsequently, the precursor RNA molecules are replicated into the double-stranded form within the particle. Finally, the genome-containing particles acquire the nucleocapsid surface shell. Two viral enzymes, the packaging NTPase P4 and the polymerase P2, are part of the assembly process. The assembly of the inner protein capsid involves tetramerization of the major capsid protein P1 and subsequent interaction with the enzymatic components (P2 and P4). The in vitro reconstituted  $\Phi 6$  nucleocapsids can penetrate the host cell plasma membrane and initiate a productive infection cycle.

#### Chapter 8 Infectious Bursal Disease Virus (IBDV): A Segmented Double-Stranded RNA Virus With a T=13 Capsid That Lacks a T=1 Core.

José R. Castón, José F. Rodríguez and José L. Carrascosa

Infectious Bursal Disease Virus (IBDV) is the best-characterized member of the family Birnaviridae. These viruses have bipartite dsRNA genomes enclosed in single-layered icosahedral capsids with T=13I geometry. IBDV shares functional strategies and structural features with many other icosahedral dsRNA viruses, except that it lacks the T=1 (or pseudo T=2) core common to the Reoviridae, Cystoviridae, and Totiviridae. Interestingly, the IBDV capsid protein exhibits structural domains that show homology to those of the capsid proteins of some positive-sense single-stranded RNA viruses, such as the nodaviruses and tetraviruses, as well as the T=13 capsid shell protein of the Reoviridae. The T=13 shell of the IBDV capsid is formed by trimers of VP2, a protein generated by removal of the C-terminal domain from its precursor, pVP2. The trimming of pVP2 is performed on immature particles as part of the maturation process. The other major structural protein, VP3, is a multifunctional component lying under the T=13 shell that influences the inherent structural polymorphism of pVP2. The virus-encoded RNA-dependent RNA polymerase, VP1, is incorporated into the capsid through its association with VP3. VP3 also interacts extensively with the viral dsRNA

genome, an organization that probably contributes to virion stability. The role of pVP2 and VP3 specific sequences in capsid assembly has been studied using heterologous expression systems. Such analyses have shown that the formation of the T=13 capsid involves the interaction of an amphiphilic  $\alpha$ -helix located at the C-terminal domain of pVP2 with a specific sequence at the C-terminus of VP3. X-ray crystallography has provided additional insight into the structure and interaction of VP2, the basic building block of IBDV particle.

#### Chapter 9 Structural Basis of Mammalian Orthoreovirus Cell Attachment

Pierre Schelling, Jacquelyn A. Campbell, Thilo Stehle and Terence S. Dermody

Mammalian orthoreoviruses are nonenveloped viruses that display strain-specific disease phenotypes following infection of newborn mice. In several cases, these differences in disease expression segregate genetically with the viral attachment protein,  $\sigma 1$ . The homotrimeric  $\sigma 1$  protein is an elongated fiber with an N-terminal tail topped with a C-terminal head. A domain in the tail of serotype 3  $\sigma 1$  binds to  $\alpha$ -linked sialic acid, whereas the head domain binds to junctional adhesion molecule-A (JAM-A). The crystal structure of a C-terminal region of  $\sigma 1$  has significantly advanced our understanding of several important properties of this protein, such as its multimeric state, its conformational dynamics, and its interaction with receptors sialic acid and JAM-A. The analysis of repetitive motifs in the crystallized fragment also has served to approximate key parameters of the full-length protein, such as its overall structure and flexibility. Furthermore, numerous structural and functional relationships between  $\sigma$  and the adenovirus attachment protein, fiber, have suggested an evolutionary link in the receptor-binding strategies of these two viruses. The existence of such a link is also supported by structural and functional similarities shared by the  $\sigma 1$  receptor JAM-A and the coxsackievirus and adenovirus receptor (CAR). Both JAM-A and CAR contain two immunoglobulin-like domains, form homodimers at regions of cell-cell contact, and contain sequences in their cytoplasmic tails that anchor the proteins to the actin cytoskeleton. It thus appears that the strategy for attachment and entry of reovirus is less similar to other members of the Reoviridae family and more similar to that of adenovirus.

#### Chapter 10 Structure and Functions of the Orthoreovirus $\sigma 3$ Protein

Leslie A. Schiff

Protein  $\sigma 3$  serves a number of distinct roles in the orthoreovirus life cycle. First, as a major structural protein that forms the outermost layer of the reovirus particle, it imparts significant environmental stability to virions. Virion  $\sigma 3$  also plays a critical role as a determinant of cell entry, as it must be degraded from particles during their activation for membrane penetration. In addition,  $\sigma 3$  is one of the most abundant proteins synthesized in reovirus-infected cells. Early in infection  $\sigma 3$  is thought to serve a regulatory function through its capacity to bind dsRNA and interfere with dsRNA-activated innate immune pathways. Both the structural and regulatory functions are influenced by the capacity of  $\sigma 3$  to interact with its structural partner,  $\mu 1$ . High-resolution structures have been determined for both the 365-residue  $\sigma 3$  protein and  $\sigma 3$  in complex with its capsid partner  $\mu 1$ . This structural data has provided a framework around which new and existing biochemical and biological information can be analyzed to provide new insight into the molecular details of the reovirus life cycle.

#### Chapter 11 Rotavirus Cell Entry

Philip R. Dormitzer

Rotavirus cell entry is mediated by a series of molecular interactions and rearrangements. While we now have an initial map of the rotavirus entry pathway, this map is far from complete. Some steps in rotavirus entry, such as proteolytic priming, initial attachment, and virion uncoating, correspond to well-understood steps in the entry pathways of other viruses, allowing extrapolation from data specific to rotavirus. However, the central event in rotavirus entry, the passage of a large non-enveloped particle through a lipid bilayer, though also a necessary step in some other viral entry

pathways, is not well understood for any non-enveloped virus. Structural data indicate that the rotavirus outer capsid protein, VP4, undergoes a fold-back rearrangement associated with a two-to-three fold reorganization of the protruding portion of the VP4 spike. While the function of this molecular event has not been determined experimentally, its resemblance to the fusogenic rearrangements of enveloped virus fusion proteins suggests a shared element between the entry mechanisms of enveloped viruses and a non-enveloped virus.

#### Chapter 12 Entry of a Segmented dsRNA Virus Into the Bacterial Cell

Minna M. Poranen and Dennis H. Bamford

The entry pathway of bacterial dsRNA viruses of the family Cystoviridae is a multi-step process, which does not resemble the entry mechanisms employed by other bacteriophages, rather having features common with viruses infecting animal cells. As the virion passes the multi-layered cell envelope of its gram-negative host the structural layers of the virion are removed in a stepwise manner. After adsorption to the receptor the entry is initiated by fusion between the virus membrane and the host outer membrane, followed by peptidoglycan penetration and finally by endocytic-like internalisation of the viral particle. Spike protein P3, fusion protein P6, lytic enzyme P5, and NC surface shell protein P8 are exposed one by one to perform their role and to direct the viral core to its destination, the host cell cytosol. The energetic state of the host cell plays an important role during the entry of dsRNA phage  $\phi 6$ .

#### Chapter 13 Crystal Structure of Reovirus Polymerase $\lambda 3$

Yizhi Jane Tao and Stephen C. Harrison

The reovirus polymerase and those of other viruses belonging to the Reoviridae function within the confines of a protein capsid to transcribe the tightly packed dsRNA genome segments. The crystal structures of the reovirus polymerase  $\lambda 3$  and its initiation and elongation complexes have been determined by X-ray crystallography. The  $\lambda 3$  structure shows a fingers-palm-thumb core, similar to those of other viral polymerases, surrounded by major N- and C-terminal elaborations, which create a cage-like structure, with four channels leading to the catalytic site. These four channels serve as the inlet and outlet for the template, substrate, dsRNA product, and mRNA transcript during replication and transcription. A 5' cap binding site has been found on the surface of  $\lambda 3$ , suggesting that  $\lambda 3$  may employ a template retention mechanism by which attachment of the 5' end of the plus-sense strand facilitates insertion of the 3' end of the minus-sense strand into the template channel.

#### Chapter 14 Structure-Function Insights Into the RNA-Dependent RNA Polymerase of the dsRNA Bacteriophage $\Phi 6$

Minni R. L. Koivunen, L. Peter Sarin and Dennis H. Bamford

RNA-dependent RNA polymerases (RdRPs) are critical components in the life cycle of double-stranded RNA (dsRNA) viruses. However, it is not fully understood how these important enzymes function during viral replication. Expression and characterization of the purified recombinant RdRP of bacteriophage  $\Phi 6$ , a member of the Cystoviridae family, is the first direct demonstration of RdRP activity catalyzed by a single protein from a dsRNA virus. The recombinant  $\Phi 6$  RdRP is highly active in vitro, possesses RNA replication and transcription activities, and is capable of using both homologous and heterologous RNA molecules as templates. The crystal structure of the  $\Phi 6$  polymerase, solved in complex with a number of ligands, provides insights towards understanding the mechanism of primer-independent initiation of RNA-dependent RNA polymerization. Furthermore, the purified  $\Phi 6$  RdRP displays processive elongation in vitro and self-assembles along with polymerase complex proteins into subviral particles that are fully functional in vitro and in vivo. This chapter will review what is currently known about the structure and function of the  $\Phi 6$  RdRP with a special emphasis on the mechanism of primer-independent initiation of  $\Phi 6$  viral RNA synthesis.

#### Chapter 15 Structure and Function of P4, a dsRNA Virus Packaging Motor

Erika J. Mancini and Roman Tuma

This review focuses on the biochemical, biophysical, and structural characterization of P4, the protein that forms the packaging motor of the Cystoviridae family of dsRNA bacteriophages. P4 exists as a hexameric ring that is positioned atop the 5-fold vertices of the procapsid shell. The ATPase activity of P4 is coupled to translocation of the viral genomic precursors into the confines of a preformed procapsid. Recently determined structures of P4 from bacteriophage  $\Phi 12$  in complex with nucleotide diphosphates and triphosphates together with the essential ion  $Mg^{2+}$  have captured snapshots of the packaging motor in different states along the catalytic pathway. Surprisingly, the structure of P4 shows a close similarity to RecA-like hexameric motors including the ubiquitous family of hexameric helicases. A model for the mechanism of action of P4 and other hexameric molecular motors has been proposed, which is the first model based on high-resolution structures captured along the catalytic pathway. The model was confirmed by biochemical data. While genomic packaging by P4 in the Cystoviridae family of dsRNA bacteriophages may share some features with dsDNA packaging by terminases of tailed bacteriophages, the implications of the present work for the mechanism of packaging in other dsRNA viruses are not obvious, but cannot be excluded.

Chapter 16 Structure and Function of the Rotavirus NSP2 Octamer, An Essential Component of the Viroplasm

Zenobia F. Taraporewala, Mukesh Kumar, B.V. Venkataram Prasad and John T. Patton

Viruses in the family Reoviridae produce cytoplasmic inclusion bodies (viroplasms) that serve as sites of genome packaging, replication and early stages of virion assembly. To support the complex events that occur in the viroplasm, the resident viral proteins must be dynamic and capable of multiple protein-protein and protein-RNA interactions. In rotavirus-infected cells, nucleation of viroplasms involves two nonstructural proteins, NSP2 and NSP5. NSP2 is a single-stranded (ss) RNA-binding protein with helix-destabilizing, NTPase and RTPase activities. The protein self-assembles into doughnut-shaped octamers made up of two stacked tetramers. Electropositive grooves that span the surface of the octamer competitively bind two ligands, ssRNA and NSP5, providing a mechanism of modulating the activity of NSP2 between two functions. The NSP2 monomer contains a histidine-triad (HIT)-like fold that is shared by a large ubiquitous class of cellular nucleotide-binding proteins, one not previously seen in a viral protein. The HIT-like motif of the monomer is contained within a deep cleft that functions as the catalytic site for the hydrolytic activities of the protein. In this chapter, we review the current information on the structure of the NSP2 octamer, its catalytic activities, and the nature of its interactions with NTPs, ssRNA and other viral proteins. Based on these properties, we discuss potential roles for NSP2 in virus replication. Finally, the features of NSP2 are contrasted with the inclusion-forming proteins of other members of the Reoviridae.

Chapter 17 Function and Structure of Rotavirus NSP3

Michelle M. Becker, Stefan T. Arold, Stephen .K. Burley, Rahul C. Deo, Caroline M. Groft, Damien Vitour and Didier Poncet

Viruses cannot carry out the functions of life unless they are inside a host cell where they hijack cellular processes, controlling and diverting those processes to complete their own life tasks, often at the cell's expense. Examining how viruses short circuit the safety controls in place to regulate cellular events that are finely tuned to respond to changes in the environment can reveal concepts in both viral and cellular biology. Rotaviruses encode a protein, NSP3, known to intercept one cellular function, that of translation, such that capped, but not polyadenylated, viral mRNAs are preferentially translated in the presence of cellular mRNAs optimized for translation by the cellular machinery. The generalities of these interactions have been determined through molecular biology, but the specifics necessary for NSP3 to carry out the misdirection of the components of the translation apparatus are revealed only through detailed

structural analysis. In this chapter, the interactions of NSP3 with eukaryotic initiation factor 4G and with the 3' end of viral RNA, as well as the contacts required to form NSP3 homodimers are discussed. In addition, a novel NSP3-interacting protein is presented and this protein, dubbed RoXaN (Rotavirus: X protein associated with NSP3), has no known function, however the potential contact between NSP3 and RoXaN bears intriguing similarities to interactions between other cellular proteins involved in cytoskeletal rearrangements through paxillin. All of this indicates that a single viral protein may have multiple functions during the viral life cycle through interactions with various cellular proteins and pathways, and these are revealed in intricate detail through structural studies.

#### Chapter 18 Analyses of Rotavirus NSP4 Genetic Groups, Structure, and Function Judith M. Ball, Rebecca D. Parr and Clarence E. Schutt

NSP4 is a nonstructural, multi-functional glycoprotein with roles in RV morphogenesis, pathogenesis, and intracellular signaling events. Although traditionally classified as an integral ER glycoprotein, new data show NSP4 localizes to multiple intracellular sites outside of the ER. Novel NSP4-protein and -lipid interactions have been unveiled, which contribute to RV pathogenesis, and NSP4 structure and intracellular transport. Complimentary to these reports, several new functions have been disclosed, primarily through the use of silencing mRNA techniques. NSP4 contributes to viroplasm formation, distribution of viral proteins in infected cells, and regulation of viral transcription. As one of several virulence factors, NSP4 induces diarrhea by promoting a signaling event at the cell surface resulting in chloride secretion. NSP4 sequences are divided into distinct genetic groups that vary both in size and intracellular interactions. The crystal structure of NSP4 residues 95-137 discloses an extended, helical domain that folds as a coiled-coil and encompasses several binding sites. With the C-terminal structure in place, dissection of the many links between NSP4 structure-function and the complex interplay between NSP4 and host cell proteins are ongoing. This chapter summarizes NSP4 genetic variations and groupings, roles in rotavirus replication and pathogenesis, and the potential impact of NSP4 structure on function.

#### Chapter 19 The Infectious Reovirus RNA - Reverse Genetics System: The Assembly of the Reovirus Genome

Michael R. Roner and Wolfgang K. Joklik

Viruses of the Reoviridae family, which encompasses nine genera and more than 200 members, possess genomes that comprise ten or twelve double-stranded (ds) RNA segments. Using reovirus as a model, we have developed a functional reverse genetics system for these viruses, by means of which alterations introduced artificially into their genomes are transferred to the genomes of viable infectious virus particles. The system consists of (a) the plus strands of nine wild type reovirus genome segments; (b) ssRNA transcripts generated from a genetically modified cDNA that replace the wild type tenth genome segment; and (c) a cell line transformed to express the protein normally encoded by the tenth genome segment. With this system, we have generated ST3 reoviruses with an engineered S2 genome segment, an M1 genome segment, and both S2 and M1 genome segments. Both the S2 and M1 engineered genes contain a functional CAT gene. The viruses are stable, replicate in cells that have been transformed to express the S2 gene product protein  $\sigma_2$ , the M1 gene product protein  $\mu_2$  or both, and express high levels of CAT activity. This technology, which we are extending to the orbiviruses and rotavirus, provides a powerful system for basic studies of dsRNA virus replication and modification.

#### Chapter 20 Genomic RNA Packaging and Replication in the Cystoviridae Leonard Mindich

The Cystoviridae, bacteriophages with genomes of 3 dsRNA segments, produce empty procapsid structures that are capable of packaging transcripts of their genomic segments, synthesizing minus strands to form dsRNA and finally transcribing the genome and secreting the plus strands from the complex. All of these reactions can be



carried out in vitro using particles assembled from proteins whose synthesis is directed by cDNA copies of the viral genomes and RNA transcribed from cDNA copies of the genomic segments. Packaging is dependent upon the specific binding of pac sequences near the 5' ends of the transcripts to sites on the outside of the procapsids. Packaging is serially dependent in that the transcript of S is packaged first and then that of M and finally the transcript of the L segment. The in vitro packaging and replication system has facilitated studies of the rules of packaging, replication and recombination in this virus family. It has also led to the development of powerful reverse genetics technology resulting in the effective manipulation of the structure and content of the viral genomes.

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