a0005

Molecular Biology

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Glossary

g0005 **Apoptosis** A cascade of cellular responses to a stimulus (e.g., virus infection) resulting in cell death.

g0010 **Cytopathic effect** Destructive changes in cell morphology, structure, or metabolic processes resulting from virus infection.

g0015 **Full-length cDNA clone** A copy of an RNA virus genome that has been reverse-transcribed into DNA and placed into a subcloning vector. With positive-sense RNA viruses, infectious genomic RNA is generally directly synthesized from bacteriophage transcription promoters located upstream of the virus sequences.

g0020 **Host cell shut-off** Interruption or cessation of host transcription, translation, or other processes that occurs within virus-infected cells.

g0025 **Interferon** A pro-inflammatory cytokine that is produced after virus infection and stimulates antiviral activities in cells expressing the IFNAR receptor complex.

g0030 **Interferon-stimulated gene (ISG)** A gene whose transcription is increased after interferon signaling. Some ISGs are directly antiviral and can inhibit virus transcription or translation.

g0035 **Positive-sense RNA genome** The genomic material of the virus resembles a cellular messenger RNA and the open reading frame can be translated on host ribosomes.

g0040 **Receptor** Structures (e.g., protein, sulfated polysaccharide, phospholipid) found upon the surface of host cells that interact with virus attachment proteins and mediate attachment of virus particles to cell surfaces. These structures may also mediate subsequent events in cell entry such as viral protein rearrangements leading to membrane fusion.

g0045 **Replicon system** A replication-competent but propagation-incompetent form of a cloned virus. In many replicon systems, the replicon genome can be packaged into a virus-like particle by *trans*-expression of structural proteins.

g0050 **RNA-dependent RNA polymerase** The primary RNA synthesis enzyme for RNA viruses which uses viral genomic RNA as a template for transcription/replication.

Introduction

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p0005 The *Togaviridae* family includes the *Alphavirus* and *Rubivirus* genera. The *Alphavirus* genus is comprised of 29 virus species segregated into five antigenic complexes, most of which are transmitted between vertebrate hosts by mosquito vectors and are capable of replicating in a wide range of hosts including mammals, birds, amphibians, reptiles, and arthropods. In contrast, the sole *Rubivirus* genus member is Rubella virus, which is limited to human hosts and is primarily transmitted by respiratory, congenital, or perinatal routes. Together, the viruses significant worldwide cause human and livestock disease. Alphavirus disease ranges from mild to severe febrile illness, to arthritis/arthralgia and fatal encephalitis, while Rubella virus is an important cause of congenital abnormalities and febrile illness frequently accompanied by arthralgia/arthritis in the developing world. On the molecular level, the togaviruses are relatively uncomplicated, small, enveloped virions with single-stranded, positive-sense RNA genomes encoding two polyproteins, one translated from the genomic RNA and the other from a subgenomic mRNA transcribed during replication. However, their replication is very tightly regulated and intimately associated with host cell metabolic processes. Currently, an important aspect of togavirus research is designed to exploit their molecular biology for therapeutic and gene therapy applications. Here, we summarize major aspects of togavirus structure, genome organization, replicative cycle, and interactions with host cells.

Virion Structure and Genome Organization

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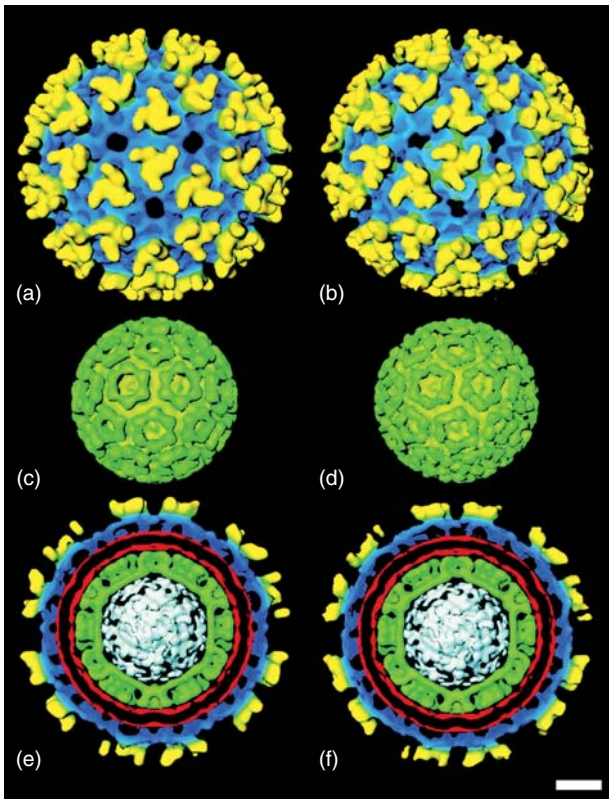
p0010 Togavirus virions are small and enveloped, comprising an icosahedral nucleocapsid composed of 240 capsid (C) protein monomers, cloaked in a lipid envelope studded with membrane-anchored glycoprotein components, E1 and E2. Their tightly packed, regular structure has been revealed by high-resolution reconstruction of the particles by cryoelectron microscopy (**Figure 1**). The envelope glycoproteins of alphaviruses are similarly arranged on the outer surface of particles. E1 and E2 form a stable heterodimer, and three heterodimers interact to form 'spikes' distributed on the virion surface in an icosahedral



f0005 **Figure 1** Structure of New World (VEEV) and Old World (SINV) alphaviruses determined by image reconstructions of electron micrographs. Isosurface view along a threefold axis of VEEV (a) and SINV (b) reconstructions showing outer spike trimers (yellow) and envelope skirt region (blue). Isosurface representations of VEEV (c) and SINV (d) nucleocapsids viewed along a threefold-symmetry axis. Cross-sections through VEEV (e) and SINV (f) perpendicular to the threefold axis and in plane with a vertical fivefold axis showing trimers (yellow), skirt region (blue), virus membrane (red), nucleocapsid (green), and RNA genome (white). Scale bar corresponds to 100 Å. Reproduced from Paredes *et al*. (2001) *Journal of Virology* 75: 9532–9537.

lattice that mirrors the symmetry of the nucleocapsid. The regularity of the spike distribution is determined by the direct hydrophobic interaction of the E2 glycoprotein tail with a pocket on the surface of the C-protein. E2 projects outward from the virion to form the spikes that interact with host-cell attachment receptors, while the E1 glycoprotein, which mediates fusion with host cell membranes, appears to lie parallel to the lipid envelope. The E2 and E1 proteins are glycosylated at one to four positions, depending on the virus strain, during transit through the host-cell secretory apparatus.

p0015 The encapsidated alphavirus genome consists of a nonsegmented, single-stranded, positive-sense RNA molecule of approximately 11–12 kB with a 5′-terminal methylguanylate cap and 3′ polyadenylation, resembling cellular mRNAs (**Figure 2**). The genome is divided into two major regions flanked by the 5′ and 3′ nontranslated regions (NTRs) and divided by an internal NTR: (1) the 5′-terminal two-thirds of the genome encode the four nonstructural proteins (nsPs 1–4); and (2) the 3′ one-third of the genome encodes the three structural proteins (C, precursor E2 [PE2], and precursor E1 [6K/E1]). Overall, the capped and polyadenylated 9.5 kB Rubella virus genome is similarly organized (**Figure 2**), but the 5′-proximal open reading frame (ORF) encodes only two nsPs (P150 and P90) and three structural proteins are encoded by the 3′ ORF (C, E1, and E2).

Infection and Replication Cycle

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p0020 The togavirus genome encodes very few but, therefore, necessarily multifunctional proteins. The replication of these viruses is very tightly regulated and as little as a single nucleotide change can alter susceptibility to cellular defense mechanisms. The following section outlines molecular aspects of togavirus infection and replication pertinent to the virus–cell interaction. Replication processes of viruses of the family *Togaviridae* have been most extensively studied for the alphaviruses, Sindbis virus (SINV) and Semliki Forest virus (SFV), and extrapolated to the other family members.

Virus Attachment, Entry, and Uncoating

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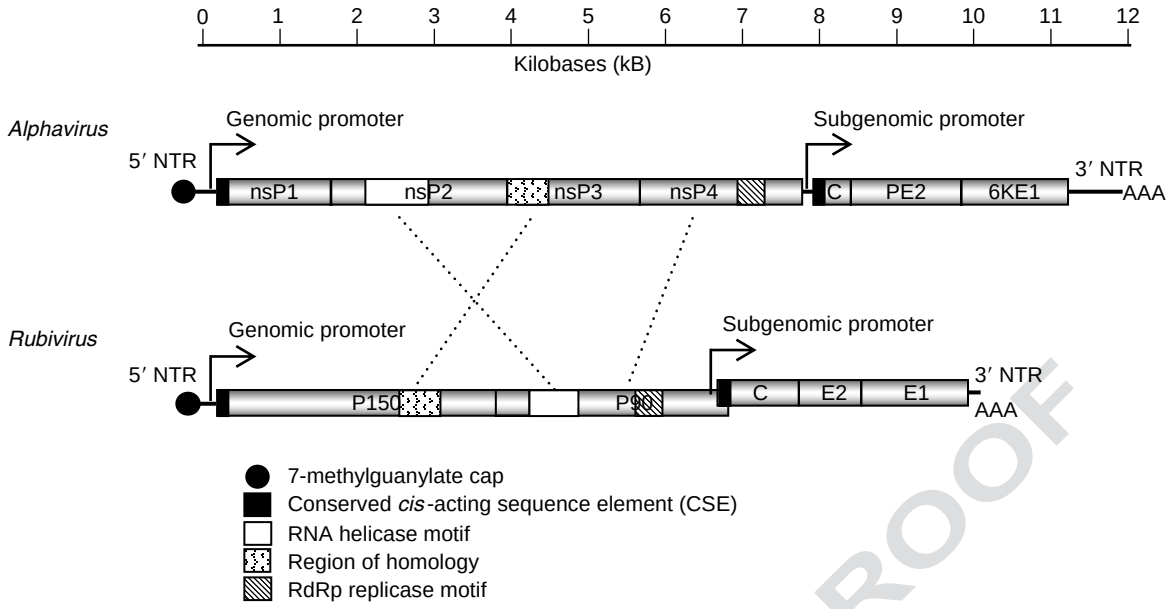
p0025 The entry pathways of Rubella virus have undergone only limited study, but there is some evidence that a glycolipid molecule serves as an attachment receptor on some cell types. The nature of the receptor for naturally circulating, 'wild-type' alphaviruses has been examined more extensively, but remains controversial, as is the case with many arthropod-borne viruses. Typically, arthropod-borne viruses are thought to interact either with a single, evolutionarily conserved molecule expressed on most cells, or to utilize different receptors in different hosts and with cells derived from different tissues. The 67 kD high-affinity laminin receptor (HALR) has been identified as an initial attachment receptor for cell culture-adapted, laboratory strains of SINV and an antigenically related molecule has similarly been identified for Venezuelan equine encephalitis virus (VEEV). In addition, receptor activity for particular cell types has been attributed to several other, as yet uncharacterized, proteins. However, more recent studies have indicated that efficient cell binding by many laboratory strains of alphaviruses is due to interaction with heparan sulfate (HS), a sulfated glycosaminoglycan molecule, and that this phenotype is conferred by positively charged amino acid substitutions in E2 that accompany adaptation to cultured cells. Currently, it is unclear whether or not the 67 kDa HALR or other identified receptor proteins act in concert with HS to bind cell culture-adapted alphavirus strains or if any of these molecules function in the natural replication



f0010 **Figure 2** Comparison of *Alphavirus* (SINV) and *Rubivirus* (RUB) genome organization. Regions of nucleotide homology and regions encoding homologous amino acid sequence within the nonstructural protein ORF are shown. ORFs are denoted by boxes and nontranslated regions (NTRs) by lines. Note that for SINV the two ORFs are separated by a NTR region, whereas for RUB the two ORFs overlap in different translational frames. Adapted from Dominguez *et al*. (1990) *Virology* 177: 225–238.

cycle of wild-type alphaviruses. Moreover, it has become clear that many noncell culture-adapted alphavirus strains bind only weakly to commonly used cell lines, such as those derived from fibroblasts. Recently, DC-SIGN and L-SIGN, C-type lectins, were found to bind carbohydrate modifications on E1 and/or E2 proteins of both HS-binding and non-HS-binding strains of SINV and VEEV, promoting infection. The interaction of the lectins with virion carbohydrates is greatly enhanced by replication of viruses in mosquito cells, most likely due to the retention of high mannose carbohydrate structures, which are processed to complex carbohydrates in mammalian cells. Therefore, C-type lectins may be important attachment receptors for alphaviruses when transmitted by mosquitoes, and receptor utilization could be determined, at least in part, by the cell type in which alphaviruses replicate. Since DC-SIGN and L-SIGN receptors are expressed by a subset of alphavirus-permissive cells, these results further suggest that infection by alphaviruses may be mediated by different receptors on different cell types.

p0030 Following receptor interactions, which may initiate uncoating-related conformational changes in E1 and E2, alphavirus and Rubella virus virions enter acidified endosomes via a dynamin-dependent process. The decreasing pH in the endosome results in more dramatic conformational changes (threshold of ~pH 6.2 with SFV) in the E2/E1 spike leading to formation of E1 homotrimers and exposure of a putative class II fusogenic domain in E1 that is thought to interact with the host cell membrane, promoting fusion of host and virion lipid bilayers. In mammalian cells, SFV membrane fusion is cholesterol-dependent and sphingolipid-dependent, and inhibitors of endosomal acidification block infection. Endosomal fusion leads to a poorly characterized entry of nucleocapsids into the cytoplasm and to their association with ribosomes, most likely directing final uncoating of the genomic RNA and translation initiation.

Genome Translation, Transcription, and Replication

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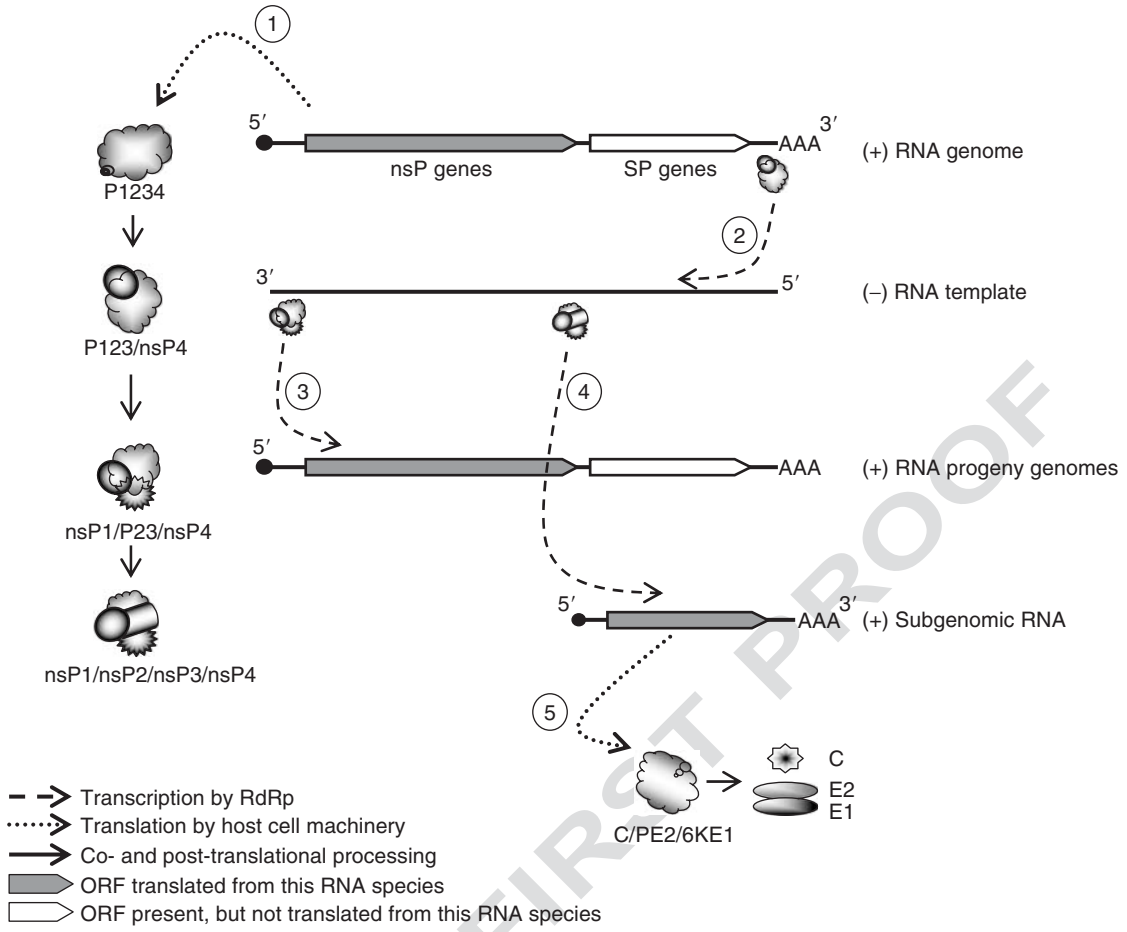
p0035 Togavirus genome transcription and replication occur by a strictly positive-sense strategy and are entirely cytoplasmic (**Figure 3**). The alphavirus replicase complex comprises four nsPs encoded in the 5′-terminal ORF, all of which are essential for viral transcription and replication: (1) nsP1 is a guanine-7-methyltransferase and guanylyltransferase; (2) nsP2 has NTPase, RNA helicase, and RNA triphosphatase activities in its amino-terminal domain, while the carboxy-terminus has highly specific thiol protease activity; (3) nsP3 is a phosphoprotein with unknown function; and (4) nsP4 is the catalytic RNA-dependent RNA polymerase (RdRp). In addition to the final products, some of the processing intermediates have distinct and indispensable functions during the replication process. The 5′-proximal ORF of the Rubella virus genome is also translated as a polyprotein (p200) and cleaved in *cis* by the Rubella virus protease (NS-pro) to form the replication complex comprising P150 and P90. However, the order of its conserved motifs differs from that of the



f0015 **Figure 3** Diagrammatic representation of *Alphavirus* replication in a permissive cell. The replication cycle is depicted as a series of temporally regulated steps: ① Translation and processing of nonstructural polyprotein P1234 or P123; ② Synthesis of complementary, negative-sense RNA by P123/nsP4; ③ Synthesis of progeny genomes by nsP1/P23/nsP4; ④ Synthesis of subgenomic RNAs by nsP1/nsP2/nsP3/nsP4; ⑤Translation and processing of structural polyprotein C/PE2/6KE1. Progeny genomes are then packaged into nucleocapsids and bud at the plasma membrane to release progeny virions.

alphavirus nsP polypeptide (**Figure 2**): P150 contains the putative methyltransferase (N-terminal) and protease sequences (C-terminal), while P90 contains the predicted helicase (N-terminal) and RNA polymerase (C-terminal).

p0040 Once the nsPs have been translated from the infecting, positive-sense RNA genome, full-length RNAs complementary to the genomic sequence (negative-sense) are synthesized, creating partially double-stranded (ds) RNA replicative intermediates. The RdRp complex then switches to preferential synthesis of positive-sense RNAs, which continue throughout the remainder of the infection replication cycle, while negative-sense RNA transcription ceases entirely. Two positive-sense, capped and polyadenylated RNAs are synthesized on the negative-sense template: full-length progeny genomes that are packaged into virions and subgenomic (26S) RNAs that are collinear with the 3′ one-third of the genome and encode the structural protein ORF. The 26S RNA accumulates in the cells to 5–20-fold molar excess over genomic RNA and is efficiently translated into a polyprotein that is co-translationally processed to produce the structural proteins.

p0045 Alphavirus genome replication and transcription are tightly regulated temporally by sequential processing of the nsPs, which alters replicase composition and RdRp activity. For some alphaviruses (e.g., SFV), the nsPs are translated as P1234, while others (e.g., SINV) translate the nsPs as either P1234 or P123 polyproteins, depending upon read-through of an opal termination codon. The nascent P1234 polyprotein is partially processed by autocatalytic nsP2 protease-mediated cleavage at the 3/4 junction and assembled into the primary P123/nsP4 RdRp complex. This short-lived P123/nsP4 primarily synthesizes genomic, negative-sense RNA replicative intermediates. Membrane-bound P123 is cleaved relatively slowly in *cis* at the 1/2 junction, perhaps to enable formation of protein–protein interactions prior to proteolytic processing, and to form the nsP1–P23–nsP4 replicase that preferentially synthesizes positive-sense

RNA. After the aminoterminus of nsP2 has been released, rapid autocatalytic cleavage of P23 produces a mature, stable RdRp complex (nsP1–nsP2–nsP3–nsP4), which transcribes only positive-sense RNA. Evidence suggests that nsP1–P23–nsP4 and the mature replicase may be biased, respectively, toward synthesis of genome-length or subgenomic mRNA, providing further temporal regulation of the replication cycle. Late in infection, when free nsP2 concentrations are high, cleavage at the 2/3 junctions is favored. P1234 polyproteins are rapidly processed into short-lived P12 and P34 precursors, thereby precluding assembly of new replication complexes and terminating negative-strand synthesis. *Cis*-cleavage of the P12 precursor gives rise to nsP1, which targets to the plasma membrane, and nsP2. Interestingly, much of nsP2 is transported to the nucleus, suggesting a function beyond the replication and transcription of viral RNAs, as discussed below. P34 is cleaved in *trans* by free nsP2, yielding nsP3, which aggregates in the cytoplasm, and nsP4, which is rapidly degraded by the ubiquitin pathway.

p0050 Specificity of togavirus RNA replication by the RdRp replicase is achieved via differential recognition of *cis*-acting conserved sequence elements (CSEs) in the termini of the viral genome and negative-sense genome template. These CSEs are conserved in all alphaviruses, as well as in Rubella virus. A 19-nucleotide (nt) CSE in the 3′ NTR immediately upstream of the poly(A) tail, is the core promoter for synthesis of genome-length, negative-sense RNA replicative intermediates, and probably interacts with the 5′ NTR via translation initiation factors to initiate replication and/or translation. The complement of the 5′ NTR in the minus-strand RNA serves as a promoter for the synthesis of positive-sense genomes. A third *cis*-acting element that is essential for replication, the 51-nt CSE, is also found near the 5′ end of the genomic RNA within the nsP1 gene. The primary sequence and two-stem-loop secondary structure of this CSE are highly conserved among alphaviruses and serve as replication and translation enhancers. Finally, a 24-nt CSE is found upstream of and including the start of the subgenomic RNA, the complement of which in the negative-strand forms the 26S subgenomic promoter and translation enhancer element.

p0055 Togavirus RNA replication occurs on cytoplasmic surfaces of endosome-derived vesicles or cytopathic vacuoles (CPVs). Expression of the nsP polyprotein (particularly nsP1) actively modifies intracellular membranes to create these compartments and to mediate membrane association of the replicase via membrane phosphatidylserine and other anionic phospholipids. The surfaces of CPVs have small invaginations or 'spherules', in which the nsPs and nascent RNA are sequestered, creating a microenvironment for RNA replication, synthesis of structural proteins, and assembly of nucleocapsids. The formation of CPVs in togavirus-infected cells may be linked to stress-induced autophagic mechanisms intended for the targeted degradation of cellular proteins and organelles, but exploited by the virus.

Structural Protein Maturation, Genome Packaging, and Egress of Virions

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p0060 The subgenomic mRNAs of alphaviruses and of Rubella virus are translated as polyproteins; however, processing of the polyprotein differs between the two. With alphaviruses, the capsid protein is cleaved autoproteolytically from the E2 precursor (known as PE2 or P62) in the cytoplasm and the remaining polyprotein is translocated into the ER lumen, where N-linked oligosaccharide addition occurs. The 6K hydrophobic protein, which is primarily located in the ER membrane, is then cleaved from the polyprotein by the host signalase. With rubella virus, the capsid-E2 and E2–E1 cleavages are all completed by signalase. Furthermore, Rubella virus lacks the 6K protein and mature E2 is produced in the ER. Alphavirus PE2 and E1 proteins form heterodimers in the ER that are anchored by their transmembrane domains and involve intra- and intermolecular disulfide linkages and associations with host chaperone proteins such as Bip and calnexin/calreticulin. The PE2/E1 heterodimers are routed to the cytoplasmic membrane and PE2 is cleaved into mature E2 by a host furin-like protease as a late event, in a structure between the *trans* Golgi network and cell surface. The cleaved E3 fragment is lost from some (e.g., SINV), but not all (e.g., SFV) virus particles. PE2-containing virions appear to bud normally from vertebrate cells, but are often defective in conformational rearrangements associated with cell fusion, supporting the hypothesis that the presence of PE2 stabilizes the glycoprotein heterodimer during low pH exposure in the secretory pathway.

p0065 Budding of particles occurs through an interaction between C and the cytoplasmic tail of E2; however, it is unclear at which point in the secretory pathway this interaction occurs and whether the interaction is between C monomers, oligomers, or RNA-containing preformed nucleocapsids. With alphaviruses, RNA packaging is directed by an RNA secondary structure in either the nsP1 or nsP2 genes, depending upon the virus, leading to selective packaging of the genome over the subgenome, which is in molar excess. In the accepted model, final budding occurs at the cytoplasmic membrane at sites enriched in E2/E1 heterodimers and is driven by E2 tail-C interactions that force an extrusion of the host lipid bilayer, envelopment of the particle, and release.

Infectious Clone Technology

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p0070 Knowledge of the genome organization and replication cycle of the togaviruses have enabled the construction of

full genome-length cDNA clones from which infectious RNA molecules can be transcribed *in vitro* and transfected into permissive cells to initiate productive virus replication. This procedure allows easy introduction of specific mutations into the virus genome followed by generation of genetically homogeneous virus populations encoding the mutation(s). Indeed, much of the work described in this article was performed using virus mutants generated from cDNA clones. In addition, 'double-subgenomic promoter viruses' (DP-virus) have been created in which the subgenomic promoter is reiterated immediately upstream of the authentic subgenomic promoter or at the 3′ end of the E1 coding region to drive expression of genes used as reporters of infection or vaccine vectors (e.g., GFP, luciferase, or immunogens from infectious microorganisms) or to test the effect of protein/RNA expression in the context of a togaviral infection (e.g., antiviral or apoptosis-inhibiting proteins, or interfering RNAs). Propagation-defective virus-like particles or 'replicon' systems have also been developed in which the structural protein genes are deleted and replaced with a heterologous protein gene. With alphaviruses, replicon genomes can be packaged to form replicon particles by expressing the structural proteins in *trans*. Typically, the replicon genome RNA is co-electroporated with two 'helper' RNAs, which are replicated and transcribed by the nsPs provided by the replicon genome and separately encode the capsid and PE2/6K/E1 proteins. Use of these bipartite helper systems minimizes the potential for generation of propagation-competent progeny. In contrast, Rubella virus exhibits a dependence of expression on the nsP ORF from the same nucleic acid as the structural proteins, limiting this approach. Replicon particles can infect a single cell and express the heterologous protein at high levels from the subgenomic promoter but, in the absence of structural protein expression, no progeny virions are produced. Therefore, these vectors allow detailed examination of the interaction of the virus with single cells and can also be used as vaccines with little possibility that adverse effects associated with propagating vial vectors will occur in vaccinated hosts. The results of numerous studies have indicated that togavirus DP-viruses and replicons will likely become important vector systems for the delivery of heterologous gene products to cells *in vitro* and *in vivo*.

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Togavirus–Host Cell Interactions

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The togavirus–cell interaction can be viewed as a conflict between the cell and the virus: the virus must replicate to propagate itself, generally leading to cytopathic effects (CPEs), while the host cell attempts to suppress virus replication in order to avoid CPE or to save the greater organism from death (in which case the individual cell may be sacrificed). In the following section, these interactions are addressed as a continuum from poorly restrained virus replication leading to rapid CPE on one extreme, to host-cell-circumscribed virus replication and prolonged cell survival on the other.

Cell Viability and CPE

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Historically, alphaviruses (e.g., prototypic SINV) have been considered to be strongly cytopathic, causing rapid killing of cultured vertebrate cells that are highly permissive of their replication. In contrast, alphavirus replication in mosquito cells is most often associated with persistent infection and limited cell death. Rubella virus is also cytopathic for vertebrate cells at high infection multiplicities, but can develop persistent infection at low multiplicity and does not infect mosquito cells. Only recently has the complex interaction between the virus infection cycle and cell death begun to be unraveled. Infection of many vertebrate cell lines with alphaviruses or rubella virus results in morphological changes (e.g., chromatin condensation, nuclear fragmentation, and formation of membrane-enclosed apoptotic bodies), as well as molecular changes (e.g., caspase activation and DNA fragmentation), associated with programmed cell death/apoptosis. Expression of SINV structural glycoproteins, particularly the transmembrane domains, can cause apoptosis in rat AT-3 prostatic adenocarcinoma cells. Furthermore, mutation from glutamine to histidine at position 55 of the SINV E2 glycoprotein has been associated with increased apoptosis in cultured cells and neurons in mice. However, although accumulation of viral structural proteins accelerates CPE development, apoptosis also occurs in replicon-infected cells in the absence of structural protein synthesis, linked to replication and nsP2 activities. Complicating this picture further is that interaction of high concentrations of UV-inactivated SINV particles with cell surfaces can initiate cell death pathways in cells of some lines, suggesting that viral replication/gene expression is not always required for killing. Finally, evidence also exists for alphavirus-induced necrotic cell death (i.e., not involving active cellular processes) in subpopulations of infected neurons *in vivo*. Therefore, the stimulus for CPE caused by togaviruses may depend upon the virus genotype, dose, the infected cell type, and/or the infection context. At the molecular level, multiple pathways, including mitochondrial cytochrome *c* release, TNF-α death receptor signaling, sphingomyelinase activation and production of ceramide, and redox-stress and inflammatory-stress pathway activation have been associated with

togavirus-induced cell death, further supporting the idea that induction of particular mechanisms may be dependent upon multiple virus and host cell factors.

s0050 ## Host-Protein Synthesis Shut-off

p0085 Togavirus replication in highly permissive vertebrate cells devastates cellular macromolecular synthesis, arresting both cellular transcription and translation by independent mechanisms. Transcriptional downregulation of cellular mRNAs and rRNAs can be directly mediated by the mature SINV nsP2 protein even in the absence of viral replication and is critically involved in the production of CPE. This activity is determined by integrity of the nsP2 carboxy-terminal domain, not by helicase or protease activity and can be greatly reduced by point mutations in this region. Moreover, the degree of nsP2-mediated pro-apoptotic stimulus appears to vary considerably between different togaviruses. Nonstructural protein-mediated inhibition of cellular transcription in highly permissive cells, such as fibroblast cell lines, dramatically suppresses the cell's ability to generate an antiviral stress response and is likely beneficial for togavirus replication and dissemination. However, infection with the noncytopathic nsP2 mutants described above restores the cell's response. In contrast, infection of dendritic cells and macrophages, which may be less permissive to infection than fibroblasts or may express a different ensemble of antiviral response mediators, produces a vigorous stress response after virus exposure and large quantities of new mRNAs are synthesized.

p0090 Translation of cellular mRNAs is also dramatically inhibited within hours of togavirus infection in highly permissive cells. This process of host-protein synthesis 'shut-off' occurs independently of transcriptional arrest. The degree to which translational shut-off occurs is inextricably linked with the extent of viral RNA replication, such that incomplete translation inhibition is associated with permissivity and mutations in viral replicase complex proteins, particularly nsP2. As a cellular response, translational inhibition is a stress-induced defense mechanism, able to recognize viral infection, amino acid starvation, iron deficiency, and accumulation of misfolded proteins in the endoplasmic reticulum (ER) via the activation of stress kinases. The activity of four distinct stress kinases converges to phosphorylate eukaryotic translation initiation factor (eIF) 2α, inhibiting GTP–eIF2–tRNAi-Met ternary complex formation and globally suppressing translation initiation. However, the expression of specific stress-inducible cellular proteins continues under conditions of phosphorylated eIF2α and generalized shut-off. If the stress response fails to clear the virus and restore homeostasis, the effects of prolonged shut-off become detrimental to the infected cell, leading to CPE and apoptotic cell death. Phosphorylation of eIF2α during togavirus infection is triggered by the presence of dsRNA primarily through activation of the dsRNA-dependent protein kinase (PKR). In some situations, eIF2α phosphorylation appears to be largely responsible for translation inhibition, since the overexpression of a nonphosphorylatable eIF2α mutant abrogates shut-off despite efficient viral replication. However, the arrest of host protein synthesis has also been shown to occur independently of PKR activity and in the apparent absence of eIF2α phosphorylation, by an unknown mechanism.

p0095 In response to this cellular defense mechanism, togaviruses have evolved degrees of tolerance to translational shut-off, enabling them to circumvent this block and redirect the cell's inoperative translational apparatus to the synthesis of viral proteins. Indeed, once shut-off has occurred, virtually the only proteins synthesized by the cell are virus-encoded structural proteins, expressed from the subgenomic mRNA. This implies that host protein synthesis shut-off confers an advantage to the virus, allowing usurpation of cellular translation machinery and potentially dampening the host cell's antiviral stress response. Translation enhancer elements located in the 5′ termini of SINV and SFV 26S (and, very likely, Rubella virus) mRNAs, particularly the highly stable RNA hairpin downstream of the AUG start codon, facilitate the continued expression of alphavirus structural proteins during the translation inhibition imposed by eIF2α phosphorylation. SFV infection has been shown to induce the transient formation of stress granules containing cellular TIA-1/R proteins, which sequester cellular mRNAs, but disassemble in proximity to the viral replicase. The temporal correlation between eIF2α phosphorylation, stress granule assembly and localized disassembly, and the transition from cellular to viral protein synthesis suggest that these may be important processes in generalized shut-off of protein synthesis and avoidance of the shut-off by the 26S mRNA. It is likely that the togaviruses also evade and/or antagonize PKR/phospho-eIF2α-independent translation arrest. Notably, the 26S mRNA also has a low requirement for the translation complex scaffolding protein, eIF4G, although integrity of eIF4G is not known to be affected by togavirus replication.

The Interferon-Mediated Antiviral Response s0055

p0100 Antiviral activity of interferon-alpha/beta (IFN-α/β) is a critical determinant of the outcome of togavirus infection both *in vitro* and *in vivo*. In mice, the absence of this response results in greatly increased susceptibility to alphavirus infection and disease. In cell culture, IFN-α/β pretreatment profoundly blocks virus replication and protects cells from CPE induced by all togaviruses tested. However, some alphaviruses (e.g., VEEV) appear to be more resistant to the effects of exogenously added IFN-α/β than others (e.g., SINV). All togaviruses tested also stimulate the production of IFN-α/β from cultured cells to

varying degrees. Presumably, IFN-α/β induction results from triggering of cellular pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), PKR and/or cytoplasmic RNA helicases such as RIG-I or MDA-5 by 'pathogen-associated molecular patterns' (PAMPs). In addition to IFN-α/β induction, these receptors stimulate a general inflammatory response in infected cells by activating the NF-κB pathway (which is activated after SINV infection). Historically, cytoplasmic dsRNA, produced as a component of the togavirus replicative cycle has been considered the primary PAMP; however, UV-inactivated preparations of some alphaviruses (e.g., SFV) can elicit inflammatory responses in certain cultured cells suggesting multiple pathways of pathogen detection and response. Secreted IFN-α/β signals through its cognate receptor on infected and uninfected cells to upregulate expression of many IFN-stimulated genes (ISGs), producing antiviral proteins, some of which directly inhibit togavirus replicative processes. In less permissive vertebrate cells, induced IFN-α/β can act upon the infected cell to suppress togavirus replication, prevent overt CPE, and promote a persistent infection.

p0105 Several studies have evaluated the individual and combined contribution of the two best-characterized IFN-α/β-inducible antiviral pathways, the PKR pathway (described above), and the coupled 2–5A synthetase/RNase L pathway, to the control of alphavirus replication. The latter pathway is composed of the interferon-inducible 2′–5′ oligoadenylate synthetase (OAS) family of dsRNA-dependent enzymes and dormant, cytosolic RNase L. dsRNA-activated 2′–5′ OAS synthesizes 2′–5′ linked oligoadenylates that specifically bind and activate RNase L which then cleaves diverse RNA substrates, thus inhibiting cellular and viral protein synthesis. As described above, constitutively expressed and IFN-α/β-inducible PKR is activated by dsRNA binding to phosphorylate eIF2α, causing a decline in cap-dependent translation of viral and cellular mRNAs. Infection of mice with targeted disruption of the PKR gene, the RNase L gene or both, revealed that PKR, but not RNase L, is involved in the early control of SINV replication *in vivo* and in primary cell cultures. Surprisingly, IFN-α/β-mediated antiviral responses against SINV are largely intact in the absence of PKR and/or RNase L, confirming the existence of 'alternative' IFN-α/β-induced pathway(s) capable of curtailing togavirus replication. Gene transcription and *de novo* protein synthesis are required for this activity. Although the proteins involved have yet to be characterized, one such pathway involves a novel inhibition of cap-dependent translation of infecting viral genomes in the absence of eIF2α phosphorylation. In addition, the interferon-inducible proteins MxA, ISG15, and the zinc-finger antiviral protein (Zap) have some inhibitory effects upon alphavirus replication. Considering the potent inhibitory effects of IFN-α/β toward numerous togaviruses, the expression of additional mechanisms to evade or antagonize the IFN-α/β response is likely critical for togavirus virulence, although convincing evidence of their existence has yet to be presented.

Conclusions and Perspectives

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p0110 Research into the molecular biology of togaviruses has two primary goals: (1) to understand the relationship of virus interactions with single cells to replication and disease pathogenesis in natural hosts and, subsequently, to identify targets for therapeutic intervention; and (2) to manipulate virus–cell interactions to capitalize upon the potential for use of these viruses as tools for gene therapy and vaccination. In the examples described above, the dissection of virus attachment, entry, translation, replication, effects upon host cells, and host cell responses to infection all have identified vulnerabilities through which virus infection may be curtailed and many laboratories are currently developing antiviral and/or disease-ameliorating strategies. Furthermore, research into virus stimulation of CPE or stress responses has led to an improved understanding of how to, for example, maximize CPE (important for the development of tumor-destroying vectors) or minimize host antiviral activity (important for expression of immunogens by vaccine vectors). Some important issues that remain to be addressed include the full elucidation of the complex relationship of virus replication to development of CPE, the nature of antiviral stress response mediators capable of blocking virus replication, and the mechanisms through which togaviruses antagonize and/or evade these responses.

See also: Togaviridae (00627); Togaviruses - others (00629).

Further Reading

Griffin DE (2001) In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 4th edn., p. 917. Philadelphia: Lippincott. Au2

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Strauss JH and Strauss EG (1994) The alphaviruses: Gene expression, replication and evolution. *Microbiology Reviews* 58: 491.