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See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus; Luteoviruses; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Geminiviruses; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Potyviruses; Virus Species.

Further Reading

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Glossary

**Polyprotein** An expressed protein comprising two functionally distinct domains.

**Ribosomal frameshift** Translation of a protein from an alternate reading frame.

**Shine–Dalgarno sequence** A consensus sequence (AGGAGG) that helps recruit the ribosome to the mRNA to initiate protein synthesis by aligning it with the start codon.

Introduction

*Leishmania* RNA virus (LRV) is the type species of the genus *Leishmaniavirus* in the family *Totiviridae*. The virus persistently infects the protozoan *Leishmania*. It possesses an icosahedral capsid of approximately 32–33 nm in diameter. The double-stranded (ds) RNA genome of leishmaniaviruses ranges in size from 5.2 to 5.3 kbp in length and encodes two major proteins – the capsid protein and the RNA-dependent RNA polymerase (RdRp). Its capsid protein has a unique endoribonuclease activity that targets the viral genome in a site-specific manner. In addition to primary structure, a hairpin loop upstream of the cleavage site is essential for processing of the viral RNA. The polymerase is expressed as a polyprotein that is processed by a host-encoded cysteine protease. Over time, this ancient virus has elaborated a variety of mechanisms to control copy number that has allowed it to co-evolve with its protozoan host.

History

The first documentation that some strains of *Leishmania* were infected with a virus came in 1974 when virus-like particles (VLPs) were observed in the cytoplasm of *Leishmania hertigi*. However, over a decade would pass before molecular data were reported. Initially, a 6.0 kbp band was visualized on an ethidium bromide-stained gel of...
parasite extracts from *Leishmania braziliensis guyanensis*. A probe generated from cDNA of nucleic acid moiety, termed LR1, failed to hybridize to the parasites genomic DNA confirming its existence as an extrachromosomal entity. Electron microscope examination of sucrose gradient fractions of parasite lysates confirmed the existence of 32 nm VLPs in fractions that contained LR1 RNA (Figure 1).

Additional virus-infected isolates were identified on the basis of RdRp activity. Those isolates exhibiting RdRp activity also harbored a 6 kbp nucleic acid that was determined to be dsRNA. The products generated from polymerase assays showed no hybridization to *Leishmania* genomic DNA, but did hybridize to the 6 kbp dsRNA, thus providing conclusive evidence that the dsRNA served as template. Soon after, electron microscopy correlated the polymerase activity with CsCl2 purified virus particles. To date, 14 leishmaniavirus species comprising both New World and Old World viruses have been identified (Table 1).

Initial studies to characterize the LRV polymerase revealed that the RdRp was associated with viral particles. *In vitro* polymerase assays resulted in the production of genome length RNA as well as a faster migrating RNA species. RNase protection assays confirmed that the genome length was dsRNA and the faster migrating species ssRNA. This evidence supports a dual function for the LRV polymerase as both replicase and transcriptase. The LRV replication cycle appears to undergo a strategy similar to the dsRNA totivirus that infects yeast. LRV replication involves generation of a full-length positive-sense RNA species that is encapsidated and serves as a template for negative-sense RNA synthesis. Positive-sense RNA transcripts are then produced from the parental dsRNA in a conservative manner and extruded into the cytoplasm where they are translated into viral proteins, or encapsidated to serve as template for viral replication.

### Genome Organization

The genome of leishmaniaviruses ranges from 5.2 kbp (Figure 2) for the Old World viruses to 5.3 kbp for New World viruses. Open reading frames (ORFs) are encoded on the positive-sense strand of New World viruses (Figure 3). ORF1 and ORF X encode small ORFs that share no homology to any known protein sequence. These small ORFs correspond to the 5'-untranslated region (UTR), which is 449 nt in length in LRV1-4 showing greater than 90% identity to all known leishmaniaviruses at the nucleotide level. ORF2 encodes the 82 kDa viral capsid protein that has been shown to spontaneously assemble into VLPs when expressed...

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**Figure 1** An electron micrograph of LRV1-4 particles isolated from a sucrose gradient of *Leishmania* lysate. Reproduced from Cadd TL and Patterson JL (1994) Synthesis of viruslike particles by expression of the putative capsid protein of *Leishmania* RNA virus in a recombinant baculovirus expression system. *Journal of Virology* 68: 358–365, with permission from American Society for Microbiology.

**Table 1** Known leishmaniaviruses and associated sequences

| Viruses  | Host range  | Sequences available                 | Accession numbers  |
|----------|-------------|-------------------------------------|--------------------|
| LRV1-1   | *Leishmania* CUMC1 | Complete genome (1–5284)         | M92355             |
| LRV1-2   | *Leishmania* CUMC3 | Partial 5'-UTR (1–253)          | AF230881           |
| LRV1-3   | *Leishmania* M2904         |                                    |                    |
| LRV1-4   | *Leishmania* M4147         |                                    |                    |
| LRV1-5   | *Leishmania* M1142         |                                    |                    |
| LRV1-6   | *Leishmania* M1176         |                                    |                    |
| LRV1-7   | *Leishmania* BOS12         | Partial 5'-UTR (1–251)          | AF230882           |
| LRV1-8   | *Leishmania* BOS16         | Partial 5'-UTR (1–253)          | AF230883           |
| LRV1-9   | *Leishmania* M6200         | Partial 5'-UTR (1–253)          | AF230884           |
| LRV1-10  | *Leishmania* LC76          | Partial 5'-UTR (1–251)          | AF230885           |
| LRV1-11  | *Leishmania* LH77          | Partial 5'-UTR (1–251)          | AF230886           |
| LRV1-12  | *Leishmania* LC56          |                                    |                    |
| LRV1-13  | *Leishmania* NC            | Partial 5'-UTR/RdRp            | U23810/U39069      |
| LRV2-1   | *Leishmania* SASKH         | Complete genome (1–5241)        | U32108             |
in vitro. The viral polymerase is encoded by ORF3, which contains the conserved six consensus RdRp motifs and overlaps the capsid domain by 71 nt. In vitro data and sequence analysis support the notion that the viral polymerase is expressed by a ribosomal frameshift to yield a capsid–polymerase polyprotein in a manner similar to several other members of the family Totiviridae.

Endoribonuclease Activity

A unique feature of LRV1-4 is that it encodes endoribonuclease activity within its assembled capsid particle. The cleavage event is site specific requiring a minimal essential sequence, and is mediated by both purified wild-type virus particles, as well as in vitro expressed VLPs. Later it was shown that the Old World LRV2-1 virus particles also possessed site-specific endoribonuclease activity.

Mutational analysis of the viral genome suggests that the minimal essential sequence required for site-specific targeting of the capsid endoribonuclease resides within viral nucleotides 249–342 in the 5′-UTR. This region contains conserved secondary structure, which was hypothesized to contain determinants for capsid-mediated cleavage. Nuclease mapping and site-specific mutagenesis identified a hairpin structure 40 nt upstream of the cleavage site that, when eliminated, abrogated cleavage. Reconstituting the hairpin through compensatory mutations allowed for precise cleavage of the substrate. Characterization of endoribonuclease activity associated with baculovirus expressed LRV1-4 VLPs reveals an absolute requirement for divalent cations such as Mg2+ and Ca2+. This requirement is probably for stabilizing substrate secondary structure since electrophoretic mobility shift assay (EMSA) showed that the divalent cation itself changed the substrate RNA mobility. Furthermore, endoribonuclease activity was greatly inhibited by salt concentrations greater than 25 mM, indicating that high concentration of salts may interfere with enzyme-substrate interactions.

Processing of a Viral Polyprotein by a Cellular Protease

The inability to observe a capsid–polymerase fusion protein in vivo is explained by a proteolytic cleavage event that separates the two domains. Host cell extract from either virus infected or uninfected Leishmania strains produced a cleavage event in an in vitro expressed capsid–polymerase polyprotein. In both cases, a specific cleavage event was observed to yield both 95 and 82 kDa fragments, which matched well the predicted sizes of the individual polymerase and capsid products, respectively. The presence of specific proteolytic cleavage activities in both virus-infected and -uninfected parasite extracts indicates that the protease is host-encoded. It has been recently demonstrated that the protease responsible for cleavage is a unique trypsin-like cysteine protease. The purified protease is related to the cysteine proteases of Leishmania mexicana found to be important for survival within macrophages. They are part of a large array of cathepsin L-like proteases that are differentially expressed. That processing of the Gag-Pol polyprotein is linked to an essential gene for parasite survival suggests that it plays a role in ensuring maintenance the LRV infection. The role of the proteolytic processing in generating functional viral proteins and/or its role in the virus replication has yet to be deciphered.

It is hypothesized that excessive production of viral proteins may adversely affect host macromolecule synthesis potentially leading to cell death, and ultimately a selective pressure to eliminate the virus. Targeting a fusion protein is an effective approach to maintain a low copy number. It is believed that the LRV replication cycle

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**Figure 2**  New World parasite strains containing a dsRNA moiety. Equal concentrations of total RNA from late-log phase parasites were incubated in the presence (+) or absence (−) of mung bean nuclease/DNase then resolved on a 1% agarose gel. The gel was stained with ethidium bromide then visualized on a UV transilluminator. Note the presence of dsRNA at approximately 5.2 kbp.

**Figure 3**  Schematic representation of genome organization of New World and Old World leishmaniaviruses. Reproduced from Scheffter S, Widmer G, and Patterson JL (1994) Complete sequence of Leishmania RNA virus 1-4 and identification of conserved sequences. 199: 479–483, with permission from Elsevier.
is similar to the yeast LA virus. From gag-pol overlapping reading frames, capsid protein is produced primarily until a ribosomal frameshifting event occurs. The Gag-Pol fusion proteins dimerize, enabling the binding of positive-sense viral RNA, which primes Gag polymerization and genome packaging. It can be envisioned that by cleaving the fusion protein at the junction of the Gag-Pol domains, packaging would ultimately be aborted. This scenario would require tight regulation of protease activity, since indiscriminate proteolysis would lead to loss of virus.

**Mechanisms of Viral Persistence**

Expression of proteins in *Leishmania*, as well as other trypanosomes, requires a highly conserved 39 nt splice leader. Early studies have shown that LRV1-4 transcripts do not possess the leader sequence and also lack a cap structure. Therefore, LRV1-4 must translate its proteins by a cap-independent mechanism. Computer sequence analysis of LRV1-4 clones has revealed that the 5′-UTR contains extensive secondary structure similar to those that promote internal initiation in poliovirus. In fact, it has been demonstrated that the 5′-UTR can function as a cis-element in promoting ribosomal entry. These observations, in addition to its similarity to the internal initiation sites of picornaviruses, suggest that the 5′-UTR functions as an internal ribosome entry site (IRES) in LRV1-4.

If the 5′-UTR of LRV1-4 functions as an IRES, how does one reconcile the observed removal of the UTR with its presumed role as a cis-acting internal initiation element? The conundrum may be explained by the hypothesis that the endoribonuclease activity plays a regulatory role in maintaining a persistent infection. Phylogenetic evidence has confirmed that LRV1-4 is an ancient virus that co-evolved with its protozoan host. Since the parasite’s reproductive cycle is predominately asexual, and there is no evidence horizontal transfer between parasites, viral persistence is dependent on maintaining a copy number compatible with host survival. Excessive production of viral proteins may adversely affect host macromolecule synthesis potentially leading to cell death, and ultimately, a selective pressure to eliminate the virus. Cleavage of the LRV1-4 transcripts could alter the functionality of the message by affecting translation, RNA stability, RNA packaging, and/or viral replication. These events are not exclusive as LRV1-4 is likely to employ a variety of regulatory mechanisms to maintain a persistent infection. It has been demonstrated that cleavage of LRV1-4 RNA correlates with the growth of the parasite. In addition, it has been shown that overexpression of capsid protein results in a suppression of LRV replication. These observations support a tightly regulated model of viral translation in which viral transcripts are being translated efficiently by cap-independent mechanisms involving the 5′-UTR. As the molar concentration of VLPs increases, endoribonuclease cleavage increases, thus maintaining a low-level viral persistence by decreasing efficiency of viral translation.

Alternatively, cleavage of the 5′-UTR may represent removal of translation-attenuating cis-elements. Other viral systems, such as coronaviruses, have translation-attenuating elements which are selected for during persistent infection. The extensive secondary structure present in the LRV1-4 UTR may function in the same respect. Cleavage of the LRV1-4 5′-UTR exposes a cryptic protein-binding site on transcripts that permits complexing with *Leishmania* cytoplasmic proteins. Although the translation machinery of *Leishmania* has not been well characterized, the speculation is that these factors are accessory proteins which may facilitate translation. Analysis of LRV1-4 UTR sequences has identified a pyrimidine-rich region complementary to a pyrimidine-rich sequence of *Leishmania* 18S rRNA. It is postulated that binding of ribosomal RNA would facilitate translation analogous to the Shine–Dalgarno sequence in prokaryotes. In this case, the cleavage of the 5′-UTR would be removing translation attenuation factors and noninitiating AUGs to allow for efficient viral gene expression through the binding of translation accessory proteins as well as rRNA to cleaved transcripts.

Another manner in which the virus may interact with its host is through the cleavage of host RNA. Sequence analysis of *Leishmania* rRNA and the endoribonuclease cleavage site of both New World and Old World viruses have identified several regions of the rRNA sequence that possess similarity to the cleavage site. If the hypothesis were validated, cleavage of rRNA would likely have an effect on host translation machinery. Since the translation of host genes is predicated upon the presence of a splice-leader sequence, host genes may have an advantage over viral transcripts in recruiting translation machinery. LRV has adapted by allowing for efficient internal initiation via an IRES; the endoribonuclease may have evolved to provide an additional role in promoting viral gene expression by decreasing translation of cellular mRNAs.

It is entirely possible that the cleavage event benefits the virus in multiple ways. When virus copy number is low, translation is allowed to proceed via a cap-independent mechanism using the LRV IRES. As copy number increases, transcripts are cleaved in addition to *Leishmania* rRNA and a slower more regulated form of translation occurs. Cryptic sites are exposed on the cleaved transcript and bind host translation accessory proteins. The short 5′-cleaved fragment also binds the cleaved transcript blocking assembly of translation accessory proteins, thus decreasing the
rate of viral protein synthesis. The result is a tightly regulated persistent infection compatible with parasite survival.

**Leishmaniavirus: A Model**

Recent advances in studies on leishmaniavirus have permitted the creation of a model for the LRV-replication cycle (Figure 4). As described above, there are many opportunities for regulation that have potentially aided in maintaining a persistent infection as the virus co-evolved with its host. Beginning with a mature particle containing dsRNA and viral transcriptase, dsRNA is transcribed in a conservative fashion and the resultant transcript is extruded from capsid. At a low molar abundance of assembled particles, viral proteins are translated by a cap-independent mechanism utilizing the LRV IRES located within the 5'-UTR. As the number of viral particles increases, the endoribonuclease activity of the LRV capsid cleaves the viral transcripts slowing the rate of translation. The cleaved 5'-UTR and the 3'-cleaved product compete for host proteins, which may be essential for translation of viral transcripts lacking a cap structure and now an IRES. Translation results in the production of a large number of functional viral proteins. Full-length viral RNA then presumably binds to a yet-to-be-determined binding site on the RdRp ensuring encapsidation of the genome. Replication follows through an unknown mechanism, thus reconstituting the dsRNA genome.

**Figure 4** A model of LRV replication. Reproduced from MacBeth KJ and Patterson JL (1998) Overview of the leishmaniavirus endoribonuclease and functions of other endoribonucleases affecting viral gene expression. *Journal of Experimental Zoology* 282(1–2): 254–260 (review), with permission from Research Trends.

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See also: Giardiaviruses; Totiviruses; Yeast L-A Virus.