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# Packaging in a Yeast Double-Stranded RNA Virus

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**The yeast virus ScV-L1 has only two genes, *cap* and *pol*, which encode the capsid polypeptide and the viral polymerase, respectively. The second gene is translated only as a *cap-pol* fusion protein. This fusion protein is responsible for recognition of a specific small stem and loop region of the viral plus strands, of 19 to 31 bases in length, ensuring packaging specificity. We have used a related virus, ScV-La, which has about 29% codon identity with ScV-L1 in the most conserved region of the *pol* gene, to map the region in *pol* that is responsible for packaging L1. Characterization of a number of chimeric viral proteins that recognize L1 but have the La capsid region delimits the region necessary for recognition of L1 to a 76- to 82-codon portion of *pol*. In addition, we show that overproduction of the La capsid polypeptide results in curing of the ScV-La virus, analogous to the production of plants resistant to RNA viruses by virtue of systemic production of viral coat protein.**

The *Saccharomyces cerevisiae* double-stranded RNA virus ScV-L1 is a simple double-stranded RNA (dsRNA) virus of one essential RNA with two genes, *cap* and *pol*, which encode the capsid polypeptide and the viral RNA-dependent RNA polymerase (RDRP), respectively (4). Its virions consist of a major capsid polypeptide (encoded by *cap*) and a *cap-pol* fusion protein (4, 13). A second dsRNA virus with a genome of very similar size cohabits with ScV-L1 (7). The dsRNA of this virus, ScV-La, is organized in the same fashion. The RDRP region of La is about 29% identical to the same region of L1 (2). In the literature, L1 is synonymous with L-A, and La is synonymous with L-BC (1).

Even though ScV-L1 and ScV-La cohabit in the same yeast cells, neither is capable of packaging the other's RNA (5, 14, 17). The ScV-L1 virus has a satellite virus, ScV-M1, in some strains. M1 encodes a secreted cellular toxin (killer toxin) that kills cells without the immunity function provided by M1. This provides a convenient phenotypic assay for packaging of L1-related RNAs, since M1 has a dual packaging signal (16) that is recognized by the L1 *cap-pol* but not by the La virus. There are a number of in vivo and in vitro assays for packaging in this system. ScV-L1 particles will bind specifically in vitro to the packaging signal, the viral binding site, or VBS (6), with a $K_d$ of about $10^{-9}$ M (16), as detected by gel shift. Overproduction of the packaging signal by an expression vector can interfere with packaging of authentic viral plus strands (12, 16). Production of the *cap* and *cap-pol* fusion proteins via a cDNA expression vector can support ScV-M1 in the absence of ScV-L1 (10, 19). Packaging of cDNA transcripts by endogenous ScV-L1 or by proteins synthesized from cDNA versions of the L1 genome can be detected by purification of viral particles (9, 16). ScV-L1 can be eliminated from cells by overexpression of *cap* and *cap-pol* (18).

We have constructed L1-La chimeric viral genomes and subjected them to a number of the above assays in order to map the region of the L1 genome that is required to package L1 viral RNAs.

**Construction of La-L1 fusions.** The N-terminal portion of L1 *pol* is necessary and sufficient to package L1 RNAs, provided that *cap* and a truncated *cap-pol* are provided (10). The La-L1 fusions constructed retain all (or almost all) of the La *cap*, the La frameshift region, and most of the L1 *pol* region. Four of these are capable of making particles that will package and maintain M1 (Fig. 1).

**Mapping the packaging region in L1.** One test for the function of the fusion constructs requires expressing the *cap* and *cap-pol* fusion proteins from a cDNA expression vector so that ScV-M1 can only be maintained by successful use of these nuclearly produced proteins. A cross is made between a strain defective in nuclear fusion but carrying ScV-L1 and ScV-M1 and a second haploid carrying the expression vector but lacking mitochondria and defective in a gene essential for maintenance of ScV-L1 (*mak10*). Cytoductants that have cytoplasm from the first strain but nuclei from the second are selected. These cytoductants will lose ScV-L1 because of lack of the MAK10 gene product. However, if the *cap* and *cap-pol* gene products translated from mRNA derived from the cDNA expression vector are functional, ScV-M1 will be rescued (10, 19). We scored a number of cloned authentic cytoductants from each cross, showing that fusions containing the L1 *pol* domain starting at least from codon 778 of *cap-pol*, but not from codon 851 or later, were functional in rescuing ScV-M1 (Table 1).

The fusions varied from being 26 to 47% as efficient as the wild type in rescuing ScV-M1. As expected from these values, unlike cytoductants with the wild-type L1 cDNA, the fusion cDNA cytoductants continue to slowly lose ScV-M1. The vector control does not rescue ScV-M1 (Table 1), eliminating the possibility of packaging of M1 by residual ScV-L1 particles. In addition, growth of the cytoductants on nonselective medium (resulting in loss of the cDNA expression plasmid) results in loss of ScV-M1 from all of the killer cytoductants, while growth on selective medium (retaining the expression plasmid) results in retention of ScV-M1 in at least 10% of the cytoductants through another 20 generations. Other tests confirm that the La-L1 fusion cDNA particles package the M1 VBS with low efficiency (see below). Thus, the region of the L1 *cap-pol* protein that is necessary for recognizing the L1 VBS lies beyond residue 778. Since a C-terminal limit of the packaging domain has been established as residue 859 (10), this limits the region necessary to 82 amino acids.

As expected, none of the fusions was capable of eliminating (curing) ScV-L1 (Table 1). We were unable to detect in vitro

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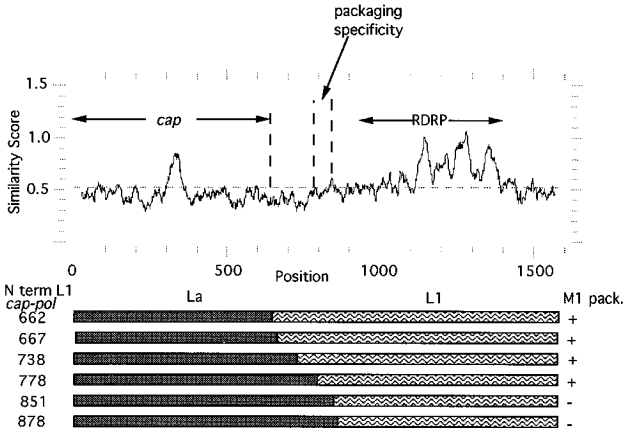


FIG. 1. La-L1 chimeras. The portions of the La and L1 dsRNA genomes incorporated into each construct are shown in the bar diagrams. Above is a PLOTSIM quantification of a GAP alignment (3, 11) of L1 and La *cap-pol*, showing peaks of similarity in the RDRP region and one peak of similarity in *cap*. In the lower diagram, the N-terminal amino acid of the L1 *pol* region of each fusion protein is shown, as is the result of the M1 packaging experiment (see Table 1).

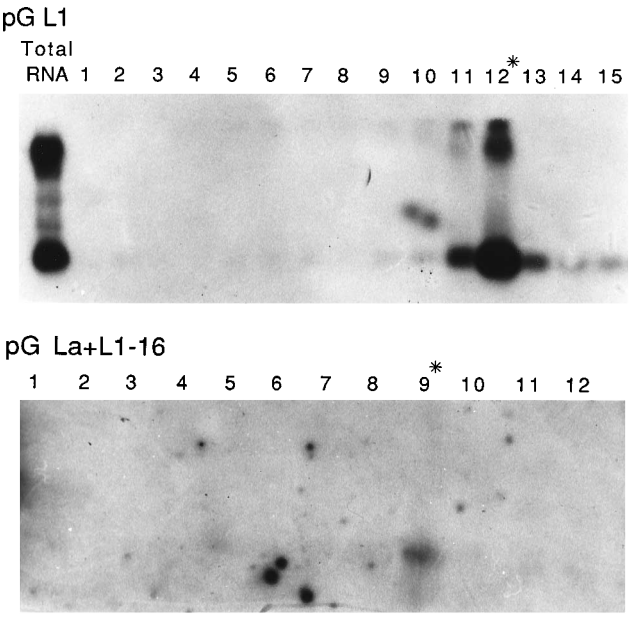


FIG. 2. In vivo packaging of the M1 VBS. Yeast strain T1#41-1 was transformed with two plasmids, one expressing the L1 or L1-La cDNA and the other expressing the M1 encapsidation signal from the AS fragment. Viral particles were purified by CsCl gradients, and Northern (RNA blot) hybridizations were performed on RNA extracted from gradient fractions. The upper panel shows the result with plasmids pGL1 and pMacAS, and the lower panel shows the result with pGLa+L1-16 and pMacAS. Note that the two gradients were of equal volume, but fewer fractions of larger volume were taken in the experiment shown in the lower panel, and 10 times as much of each fraction in the lower panel was extracted for RNA and used for the Northern analysis. The lower panel was exposed 30 times longer than the upper panel, so that the total RNA control in the lower panel is overexposed (and excised). Particle peak fractions are indicated by asterisks. The most prominent band of RNA in fractions 12 (pGL1) and 9 (pGLa+L1-16) is the correct size for the AS transcript, as determined with DNA and RNA markers (not shown).

binding to the L1 VBS by viral particles synthesized by fusion constructs (Table 1). We were able to detect packaging of synthetic M1 VBS-containing sequences in CsCl-purified particles. This assay for function of La-L1 cDNAs requires the production of the *cap* and *cap-pol* proteins from one cDNA expression vector and production of synthetic L1 or M1 plus strands from a second expression vector in a strain lacking autonomous ScV-L1 particles. The second construct utilized the *Acc*I-*Sph*I (AS) fragment of M1, a region of 133 bases with two VBS sequences (16). The wild-type L1 cDNA expression vector results in easily detectable packaging of the AS transcript, but the fusions package this transcript less efficiently in vivo. One such experiment is shown in Fig. 2. The rescue of ScV-M1 is a much more sensitive test of function, since any rescue is automatically amplified by the subsequent replication of the packaged RNA.

**Analysis of point mutants.** Within the N-terminal region of the *pol* reading frame, there are several motifs in common between La and L1 (Fig. 3). Since the sequence SPRRK (starting at 854) had been suggested as an integral portion of the RNA-binding domain responsible for packaging (10), we altered it to the La sequence. The resulting mutant, L1-m2, is

TABLE 1. Function of L1-La chimeras and mutants

| Designation | Amino acids[a] | % of cytoductants with killer phenotype[b] | No. of cytoductants tested[c] | Cure of L1 | Cure of La | M1 VBS[d] | |
|---|---|---|---|---|---|---|---|
| | | | | | | In vivo packaging | In vitro binding |
| L1 wt | | 98 | 106 | + | − | ++ | + |
| L1-10 | | 76 | 13 | − | − | + | + |
| L1-m2 | | 100 | 67 | − | − | ++ | ND |
| Vector | | 1.5 | 133 | − | − | − | − |
| La+L1-1 | 878 | 5.8 | 102 | − | + | ND | ND |
| La+L1-2 | 851 | 6.2 | 65 | − | + | − | ND |
| La+L1-3 | 778 | 26 | 109 | − | + | +/− | ND |
| La+L1-4 | 738 | 21 | 116 | − | + | ND | ND |
| La+L1-16 | 667 | 47 | 110 | − | −/+ | +/− | − |
| La+L1-23 | 662 | 30 | 33 | − | −/+ | ND | ND |

[a] In the chimeras, the amino-terminal L1 residue of the fusion is indicated. Construct La+L1-1 had bases 16 to 2665 of La and 2633 to 4579 of L1; construct La+L1-2 had bases 16 to 2608 of La and 2555 to 4579 of L1; construct La+L1-3 had bases 16 to 2380 of La and 2333 to 4579 of L1; construct La+L1-4 had bases 16 to 2227 of La and 2204 to 4579 of L1; construct La+L1-16 had bases 16 to 2076 of La and 1995 to 4579 of L1; and construct La+L1-23 had bases 16 to 2020 of La and 1985 to 4579 of L1.

[b] In this assay, a *kar1 MAK10* [*LIM1*] strain is mated with a rho$^-$ *mak10*(pGL1a) strain carrying a plasmid from which a chimeric La-L1 mRNA is transcribed.

[c] Number of cytoductants of correct genotype tested for killer phenotype.

[d] −, not detectable; +/−, detectable; +, easily detectable; ++, very detectable. ND, not determined.

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662 .......YSWRCPRRVDRTGGQCFSRVNVIEPSHGPRPTRYILQEPGTYP 704
            :  .. | :|...|::.|... .. : . ..   | : || .
664 QYAILTPSGTTTDIRSGRGTNQSYRRGRTSTGYRIGVEDDEDL.DIGTVK 712
          .         .         .         .         .
705 AWIRFRNRVQAVSRQ..KATHFLFDIVPAAVISDFTTSDTSSFAYKSHTY 752
   :.:  ..:.|...  .|||.|   :.|. |.: ...|..: .:. :..
713 YIVPLYLNGDNVAQNCLEATHVL...IKACSIANRIVDDGEGHCFTQQGL 759

          .         .   D     .         .         .
753 AAN.........VTALRFSDTYALYVQTDTNMTILSPAARRQASATYSQ 792
    |..          |.|:|:::  |.||: . |:| .|  .  |.:||.|.
760 AQQWIFHRGEMIFVKAVRIGQLNAYYVDYK.NVTNYSLKTAAQVGATISN 808
          .         .         .         .         .
793 ..VAGFCYNTPTVMDSL.ANILDVDRNIRPKHFKGLRLYTSS.KVTAQHH 838
      ||. | ... ..| ||. |. : ||..   ...: ... ::|. ||
809 NLRHGFVDNQQDAYTRLVANYSDTRKWIRDNFTYNYNMEKEKYRITQYHH 858

          .       PSRK.        .         .
839 THLRPDELVEAAAKVSPRRKYYLMCVVELLANLQVDLEAA 878
    ||:| .:|.       |.||.  : . |
859 THVRLKDLF.......PSRKIVKLEGYEA.......... 880
```

FIG. 3. Alignment by GAP (3, 11) of the L1 *cap-pol* (top line) with the La *cap-pol* (bottom line) in the region involved in packaging specificity. The region of the L1 *cap-pol* proposed by others as being required for packaging (10, 15) is italicized. The region that we have identified as being required for packaging is underlined. Two point mutations of L1 analyzed are shown, with the substituted amino acids shown above the L1 *cap-pol* sequence. Identities are indicated by vertical lines, and similarities are indicated by double dots.

fully functional in rescue of ScV-M1 and packaging of the AS fragment in vivo (Table 1), but it does not cure ScV-L1. Another mutant, L1-m10, which is altered by substitution of an aspartic residue for a conserved alanine at position 775 (within the ALYV motif), is slightly affected in rescue of ScV-M1 but not in in vitro binding to the L1 VBS and shows slightly lower in vivo packaging of the AS fragment (Table 1). These mutants are therefore consistent with somewhat narrower limits for the L1 packaging domain (residues 778 to 853, or 76 amino acids).

**Elimination of ScV-La.** It is possible to eliminate ScV-L1 particles from cells by overproduction of the L1 *cap-pol* fusion from cDNA expression vectors, while overproduction of *cap* by itself has no effect (18). The situation with La appears to be quite different, since all of the fusion constructs that have an intact *cap* gene cure ScV-La. This is shown in Fig. 4 and summarized in Table 1. Constructs 16 and 23 are lacking 16 and 33 amino acids, respectively, from the C terminus of the La *cap* protein and fail to efficiently cure ScV-La. One construct that does not make viral particles (pGLa+L1-15) does not cure ScV-La. Another plasmid, producing only the La *cap* without any L1 sequences, does cure ScV-La (data not shown). We conclude that expression of the La *cap* gene is adequate to eliminate ScV-La. This is analogous to the resistance conferred on plant hosts of RNA viruses by overexpression of their capsid polypeptides (8).

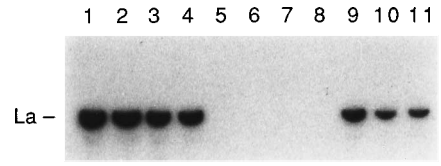


FIG. 4. Curing of ScV-La by expression of chimeric La-L1 cDNAs. Total RNA isolated from transformants of strain T1#41-1 with yeast expression vector pG1 or its derivatives was used for Northern blots with an La-specific probe (antisense nucleotides 2237 to 2724). Lane 1, pG1; lane 2, pGL1; lane 3, pGL1-m2; lane 4, pGL1-m10; lane 5, pGLa+L1-1; lane 6, pGLa+L1-2; lane 7, pGLa+L1-3; lane 8, pGLa+L1-4; lane 9, pGLa+L1-15; lane 10, pGLa+L1-16; lane 11, pGLa+L1-23. The same amount of RNA was used in each lane, as determined by ethidium bromide staining.

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