The Study at the Molecular Level of the New Zealand Isolate of Lucerne Transient Streak Sobemovirus and its Satellite RNA.

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SUMMARY

Lucerne transient streak sobemovirus (LTSV) is a small icosohedral plus-sense RNA plant virus that naturally infects lucerne. It is a member of the Sobemovirus group of plant viruses and is found as three separate isolates; Australian, New Zealand (-NZ) and Canadian. Four Sobemoviruses, including LTSV, can be associated with a specific viroid-like satellite RNA (virusoid). These virusoids possess sequences capable of forming a hammerhead structure which has previously been shown to direct site-specific self-cleavage *in vitro*. This self-cleavage reaction is believed to function *in vivo* by processing plus and minus oligomeric virusoid RNAs into monomeric forms as part of the rolling-circle replication mechanism.

The work presented in this thesis describes the determination and analysis of the complete sequence of the genomic RNA of LTSV-NZ and the development and kinetic characterisation of a ribozyme self-cleavage system based on the plus-sense hammerhead sequence of the virusoid of LTSV-NZ (vLTSV-NZ).

The Complete Sequence of the Genomic RNA of LTSV-NZ

The complete sequence of the 4,275 nucleotide long genomic RNA of LTSV-NZ was determined and a genome organisation proposed based on the identification of all large open reading frames (ORFs). This organisation is compared to that previously published for Southern bean mosaic sobemovirus, cowpea strain (SBMV-C).

A high degree of sequence and organisational similarity was observed between the two viruses except at the 5'-termini. In this region, LTSV-NZ differed significantly from SBMV-C in possessing two small ORFs rather than one. The organisation shown in LTSV-NZ appears to be shared by an unpublished account of the genome organisation of Velvet tobacco mottle sobemovirus (VTMoV).

Protein functions are proposed for the deduced amino acid sequences from the two largest ORFs on the basis of similarities to those of SBMV-C and the identification of amino acid sequence motifs associated with specific protein functions. Domains identified were for

the coat protein, RNA-dependent RNA polymerase (RdRp) and serine protease. The viral protein genome linked (VPg) domain was tentatively identified as a region containing a high proportion of basic residues. No evidence was found for the NTP-binding domain previously proposed for SBMV-C. Possible functions for the products of the remaining ORFs are discussed. A translation strategy for the virus is proposed which involves a combination of ribosome scanning for two subgenomic RNAs, and a combination of leaky-scanning and a frame shifting event for the genomic RNA.

The evolutionary relationship between LTSV-NZ, SBMV-C and VTMoV was proposed on the basis of comparisons of the genome organisations and alignments of the RdRp and serine protease domains. The inferred phylogenetic tree confirmed a monophyletic relationship for these viruses. The most parsimonious interpretation of this result suggests that an ancestral Sobemovirus genome organisation was more similar to those of LTSV-NZ and VTMoV than to that of SBMV-C. The possible origins of a completely overlapping ORF found in both LTSV and SBMV-C is discussed on the basis of observed nucleotide biases in this region.

A Ribozyme System Based on the Plus vLTSV Hammerhead Structure

The ribozyme system, consisting of two RNAs, a 13-mer ribozyme (Rib.) and a 41-mer substrate (Sub.), were prepared using T7 RNA polymerase directed transcription of synthetic DNA oligonucleotide templates. Both RNAs, when incubated together in the presence of Mg^{2+}, were shown to direct self-cleavage at the expected site. Multiple turnover for this cleavage reaction was demonstrated when the Rib. RNA was incubated in the presence of a molar excess of Sub. RNA. Substitution of all residues in the Rib. RNA with deoxyribonucleotides was shown to abolish self-cleavage.

The energy of activation (E_a) and Michaelis-Menten kinetic parameters (K_m, V_{max} and k_{cat}) for the ribozyme system were determined by fitting the appropriate kinetic data to their respective equations. The values for these parameters were compared to published values for other hammerhead ribozyme systems. The vLTSV ribozyme system was found to possess a relatively high K_m, average V_{max} and k_{cat} and low $\frac{k_{cat}}{K_m}$ (specificity constant) values. The optimum reaction temperature for the vLTSV ribozyme system was found to be 50°C. The values of these findings are interpreted as being due to the presence of competing non-productive structures in the Sub. RNA.