Structural aspects of encapsidation signals in RNA viruses

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Chapter I

General introduction

For a large number of RNA viruses, nucleotide sequences have been identified that are crucial for their successful propagation. These so-called *cis*-acting sequences may regulate replication, transcription, splicing, translation, and virion assembly. Many of these sequences exhibit a particular structure that is specifically recognized by one or more proteins.

A functional RNA-protein interaction relies on the structural integrity of these *cis*-acting elements. Important structural RNA motifs are usually conserved among related viruses. By sequence comparison and structure prediction such conserved structural motifs can be identified within a class of related viruses. The identification of such conserved motifs by itself already hints at a functional relevance of these motifs in the evolution of these viruses.

In this thesis, this principle was applied to viruses belonging to the genus coronaviruses, which include the well-known SARS-coronavirus, and to two genera of plant viruses, the ilarviruses and potexviruses. Special attention was paid to elements that may direct specific encapsidation of the viral RNA genome of these viruses, the so-called packaging signals.

Chapter II reviews the structural aspects of the known packaging signals in positive-strand RNA viruses that are infecting plants and animals.

Combining the power of phylogenetic comparison and solution structure probing on a previously identified packaging region in group IIa coronaviruses resulted in a new model for the encapsidation signal in this group of coronaviruses. Chapter III describes the particular structural features of this model and shows how previous mutagenesis data correspond better with the new model.

A similar packaging signal could not be identified in group I and III coronaviruses or in group IIb SARS-coronavirus at the homologous position of the genome but inspection of their 5' untranslated regions (UTRs) was more promising. Again here, the phylogeny and probing data revealed the presence

of unique conserved structural motifs in group I and IIb coronaviruses, which may be the long-sought packaging signals of these coronaviruses. Chapter IV further argues that the structural organization of *cis*-acting elements in the 5'UTR is group-specific.

Correlation between the lineage of a virus and the structural features of its RNA is also illustrated by a group of plant viruses, the ilarviruses. Chapter V shows that the structural features of the 3' tRNA-like structures are related to the lineage of ilarviruses.

Chapter VI reports further evidence that the 3' tRNA-like structure of *Alfalfa mosaic virus,* a plant virus closely related to ilarviruses, is antagonizing binding of the viral coat protein. To this end the yeast three-hybrid system to measure RNA-protein interactions in vivo was employed.

Analysis of the 5'UTR of another plant virus, *Bamboo mosaic virus*, revealed that its secondary structure is highly similar to the *cis*-acting elements of its co-infecting satellite RNAs. Chapter VII shows that the conserved structure is crucial for genomic RNA accumulation and probably involved in helper-satellite interactions.

Finally, the main findings of this thesis are summarized and discussed in relation to other data in this field in Chapter VIII.