

**Molecular mechanisms of programmed ribosomal frameshifting and
cap-independent translation of *Dianthovirus***

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Summary

Chapter I

The genomic RNAs of positive-strand RNA viruses are often polycistronic. Therefore, these viruses must have some strategies to translate the downstream open reading frame (ORF). Programmed -1 ribosomal frameshifting (-1 PRF) is one viral translation strategy to express overlapping genes in positive-strand RNA viruses. *Red clover necrotic mosaic virus* (RCNMV) uses this strategy to express its replicase component protein p88, which is encoded in RNA1. Previous studies identified the two *cis*-acting RNA elements, which are located adjacent to the slippage site in RCNMV RNA1 and are required for -1 PRF. In addition to these elements, it was suggested that the third *cis*-acting RNA element required for -1 PRF could exist in the 3' untranslated region (UTR) of RCNMV RNA1. In this study, I used a cell-free translation system to map the *cis*-acting RNA elements required for -1 PRF. The results show that a small stem-loop structure adjacent to the cap-independent translation element in the 3' UTR of RCNMV RNA1 is required for -1 PRF. Site-directed mutagenesis experiments suggested that this stem-loop regulates -1 PRF via base-pairing with complementary sequences in a bulged stem-loop adjacent to the shifty site. The existence of RNA elements responsible for -1 PRF and the cap-independent translation of replicase proteins in the 3' UTR of RNA1 might be important for switching translation to replication and for regulating the

ratio of p88 to p27. Finally, I present a model for regulation of translation and replication in RCNMV RNA1. In this model, p27 interacts with its template RNA1 except for the 3' UTR. The production of sufficient amounts of p27 allows 5'BulgeSL to access SLCsSL by an unknown mechanism. The formation of base-pairings between 5'BulgeSL and SLCsSL facilitates the translation of p88 via -1 PRF. p88 interacts with the 3' UTR of its template RNA1. The interaction of p88 or the formation of the 480-kDa replicase complex disrupts the structures of the 3' RNA elements required for both cap-independent translation and -1 PRF, causing a switch from translation to replication of RCNMV RNA1.

Chapter II

Viruses employ an alternative translation mechanism to exploit cellular resources at the expense of host mRNAs and to allow preferential translation. Plant RNA viruses often lack both a 5' cap and a 3' poly(A) tail in their genomic RNAs. Instead, cap-independent translation enhancer elements (CITEs) located in the 3' untranslated region (UTR) mediate their translation. Although eukaryotic translation initiation factors (eIFs) or ribosomes have been shown to bind to the 3' CITEs, our knowledge is still limited for the mechanism, especially for cellular factors. In this study, I used *Red clover necrotic mosaic virus* (RCNMV) as a model virus for studying viral cap-independent translation mechanism. The genome of RCNMV consists of two RNA molecules, RNA1 and RNA2. Both genomic RNAs lack a 5' cap and a 3' poly(A) tail, and they initiate translation in cap-independent manner. RNA1 has an RNA element named 3'TE-DR1 in the 3' UTR that can replace a cap structure, and is essential for cap-independent

translation. In addition, RNA1 has an adenine-rich sequence (ARS) upstream of 3'TE-DR1 in the 3' UTR. PABP directly binds to ARS and the binding is required for recruiting 40S ribosomes to facilitate translation. On the other hand, RNA2 does not possess an RNA element like 3'TE-DR1 and ARS, and translation from RNA2 is coupled to RNA2 replication. Precise mechanisms of cap-independent translation of RCNMV remain elucidated. Here, I screened *Arabidopsis thaliana* mutants to identify which eIF4F/eIFiso4F components promote the cap-independent translation of RCNMV genomic RNAs. I found that RCNMV requires all eIF4F/eIFiso4F component genes for infection. Using *Arabidopsis* protoplasts, I show that eIF4E, eIF4G, and eIFiso4G2 are required for RCNMV replication. Luciferase assay in *Arabidopsis* protoplasts revealed that eIF4E and eIF4G are required for 3'TE-DR1-mediated translation of RNA1. I confirmed the requirement of eIF4E and eIF4G for the cap-independent translation of RNA1 using *in vitro* translation assays with recombinant eIF4F/eIFiso4F. Furthermore, to investigate the requirement of eIF4F/eIFiso4F for the cap-independent translation of RNA2 independently of that of RNA1, RNA2 and replicase-expressing plasmids were transfected into *Arabidopsis* protoplasts. I found that eIFiso4E and eIFiso4G1 are required for translation of RNA2. These results show that the requirements of eIF4F/eIFiso4F for cap-independent translation differ between RNA1 and RNA2. Such differential preferences of eIFs between viral genomic RNAs might contribute to regulating viral gene expression during RCNMV infection in host plants.