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Host transcription factor Rpb11p affects tombusvirus replication and recombination via regulating the accumulation of viral replication proteins

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Abstract

Previous genome-wide screens identified over 100 host genes whose deletion/down-regulation affected tombusvirus replication and 32 host genes that affected tombusvirus RNA recombination in yeast, a model host for replication of *Tomato bushy stunt virus* (TBSV). Down-regulation of several of the identified host genes affected the accumulation levels of p33 and $p92^{pol}$ replication proteins, raising the possibility that these host factors could be involved in the regulation of the amount of viral replication proteins and, thus, they are indirectly involved in TBSV replication and recombination. To test this model, we developed a tightly regulated expression system for recombinant p33 and $p92^{pol}$ replication proteins in yeast. We demonstrate that high accumulation level of p33 facilitated efficient viral RNA replication, while the effect of p33 level on RNA recombination was less pronounced. On the other hand, high level of $p92^{pol}$ accumulation promoted TBSV RNA recombination more efficiently than RNA replication. As predicted, Rpb11p, which is part of the polII complex, affected the accumulation levels of p33 and $p92^{pol}$ as well as altered RNA replication and recombination. An in vitro assay with the tombusvirus replicase further supported that Rpb11p affects TBSV replication and recombination only indirectly, via regulating p33 and $p92^{pol}$ levels. In contrast, the mechanism by which Rpt4p endopeptidase/ATPase and Mps1p threonine/tyrosine kinase affect TBSV recombination is different from that proposed for Rpb11p. We propose a model that the concentration (molecular crowding) of replication proteins within the viral replicase is a factor affecting viral replication and recombination.

Keywords: Tomato bushy stunt virus; Cucumber necrosis virus; RNA replication; RNA recombination; Host factor; Yeast

Introduction

RNA viruses depend on their hosts during their replication in the infected cells to produce viral proteins. Also, the host cells provide membranes and ribonucleotides for RNA synthesis. In addition, RNA viruses actively recruit host proteins to facilitate the replication process (Ahlquist et al., 2003; Buck, 1996; Lai, 1998; Nagy and Pogany, 2006; Noueiry and Ahlquist, 2003; Osman and Buck, 1997; Shi and Lai, 2005). Viral replication proteins and the viral RNA together with host proteins assemble viral replicase complexes on selected host membranes that lead to efficient viral RNA synthesis. Current major research efforts aim at cataloging host proteins that affect virus replication by providing direct functions for the replication process, influencing the amounts of viral replication proteins, or participating in antiviral mechanisms. Accordingly, genome-wide screens of model *Saccharomyces cerevisiae* host for RNA viruses, such as *Brome mosaic virus* (BMV) and *Tomato bushy stunt virus* (TBSV) as well as proteomic analysis of viral replicases identified a large number of host proteins affecting RNA virus replication (Jiang et al., 2006; Kushner et al., 2003; Panavas et al., 2005b; Serva and Nagy, 2006). Altogether, these studies revealed the complex nature of host–virus interactions.

In addition to replication, viruses also undergo RNA recombination, a process that leads to joining of noncontiguous RNA segments (Lai, 1992; Nagy and Simon, 1997; Worobey and Holmes, 1999). RNA recombination is a mechanism that accelerates evolution and adaptation of positive-strand RNA viruses and the emergence of new viral variants (Lai, 1992; Nagy and Simon, 1997; Worobey and Holmes, 1999; Zimmern, 1988). RNA recombination could also increase the fitness of RNA viruses by eliminating deleterious mutations introduced by the error-prone viral RNA-dependent RNA polymerase (RdRp).

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RNA recombinants can often be found in natural infections in plants and animals and interviral recombinants between different viruses and recombinants between host sequences and viruses have also been isolated (Aaziz and Tepfer, 1999; Bonnet et al., 2005; DeStefano et al., 1994; Desvoyes and Scholthof, 2002; Froissart et al., 2005; Gibbs and Weiller, 1999; Greene and Allison, 1994; Moonan et al., 2000; Revers et al., 1996; Tan et al., 2004; Vigne et al., 2005; Worobey and Holmes, 1999). Because RNA recombination can greatly increase viral genome variability, it is a major threat to compromise current antiviral strategies (Keese and Gibbs, 1993; Lai, 1992; Roossinck, 2003; Worobey and Holmes, 1999).

Mechanistic studies on RNA recombination in cell-free systems based on purified recombinant viral RdRps of BMV, *Cucumber mosaic virus*, *Bovine viral diarrhea virus*, *Hepatitis C virus*, *Turnip crinkle virus* and partially purified *Cucumber necrosis virus* (CNV, a tombuvirus) replicase preparations revealed a replicase-driven template-switching (TS) as the dominant mechanism in RNA recombination (Cheng and Nagy, 2003; Cheng et al., 2005; Kim and Kao, 2001). The TS mechanism is further supported by results obtained with mutants within the viral replication genes that influenced the frequency of recombination and the sites of recombination junctions in vivo (Figlerowicz et al., 1997; Nagy et al., 1995; Panaviene and Nagy, 2003). Moreover, the role of the sequence/structure of the viral RNA in RNA recombination has also been extensively studied (Kim and Kao, 2001; Nagy and Bujarski, 1993, 1996, 1997; Nagy et al., 1998; Wierzchoslawski et al., 2003). Most recombination junctions were found within noncoding regions that include *cis*-acting replication elements, such as promoters for complementary RNA synthesis, subgenomic promoters, replication enhancers and internal replication elements (Nagy and Simon, 1997).

In contrast with our rapidly deepening understanding of the contribution of viral factors to viral RNA replication and recombination, our knowledge on the role of host proteins/factors in viral RNA replication/recombination is poor. A genetically tractable model system, based on a plant tombusvirus and yeast, has been developed to facilitate studies on replication and recombination (Panavas and Nagy, 2003; Pantaleo et al., 2003). In the model system based on TBSV, the viral repRNA is expressed from the galactose/glucose inducible/repressible *GAL1* promoter, whereas the two viral replication proteins, termed p33 and p92^{pol}, are expressed separately from expression plasmids via the constitutive *ADH1* promoter (Panavas and Nagy, 2003; Panaviene et al., 2004). Induction of repRNA expression leads to the assembly of the viral replicase complex consisting of p33, p92^{pol} replication proteins and Ssa1/2p, Tdh2/3p and Pdc1p host proteins on peroxisomal membranes (Panavas et al., 2005a; Serva and Nagy, 2006). Recent systematic screens of the yeast knock out (YKO) library containing 80% and the essential gene library (yTHC) containing ~15% of all the yeast genes, respectively, led to the identification of 126 and 32 genes, which affected tombusviral RNA replication and recombination, respectively (Jiang et al., 2006; Panavas et al., 2005b; Serviene et al., 2005, 2006). More detailed analysis of the mechanism of suppression of viral RNA recombination has been performed with one of the identified genes, namely *XRN1* (Cheng et al., 2006). This work revealed that Xrn1p, which is an evolutionary conserved 5′–3′ exoribonuclease, inhibited viral RNA recombination by rapid and selective degradation of viral RNA recombination intermediates from wt yeast cells (Cheng et al., 2006). The actual roles of the other identified yeast genes in viral RNA recombination are currently unknown.

Previous work has defined that down-regulation of several yeast genes, which affected viral RNA recombination and/or viral RNA replication, also affected the accumulation level of p33 and p92^{pol} replication proteins (Jiang et al., 2006; Panavas et al., 2005b; Serviene et al., 2005, 2006). Therefore, it is conceivable that several of these host genes might have only indirect effect on viral RNA replication/recombination by regulating the amount of viral replication proteins. This model was tested in the current work by developing a tightly regulated expression system for p33, p92^{pol} and the viral replicon (rep)RNA. The results demonstrate that the amount and ratio of p33 and p92^{pol} replication proteins play a significant role in viral RNA replication and recombination, whereas the amount of the replicon RNA was less important. Comparison of these data with another data set obtained via down-regulation of three host protein levels revealed that Rpb11p transcription factor, unlike Rpt4p endopeptidase or Msp1p kinase, affected viral RNA replication and recombination by regulating the accumulation level of p33 and p92^{pol}. Altogether, the data will be very useful for determination of direct or indirect effects of the more than 100 host proteins identified in previous genomic screens for tombusvirus replication and recombination.

Results

Development of tightly regulated expression system for tombusvirus replication proteins and the repRNA in yeast

Systematic screens of 95% of yeast genes for the effect on TBSV RNA replication and recombination have led to the identification of 126 and 32 genes, respectively (Jiang et al., 2006; Panavas et al., 2005b; ; Serviene et al., 2005, 2006). Deletion or down-regulation of several of the identified host genes affected the accumulation levels of p33 and p92^{pol} replication proteins, raising the possibility that these host factors could be involved in the regulation of the amount of viral replication proteins via regulating protein expression and/or degradation and, thus, these factors are indirectly involved in TBSV replication and recombination.

To test the above model, first we had to examine the effects of p33 and p92^{pol} and the repRNA transcript levels on TBSV replication and recombination in yeast. These replication and recombination assays required the development of a tightly regulated expression system for the separate expression of p33 and p92^{pol} and the repRNA transcripts (Fig. 1A). Namely, the p33 open reading frame (ORF) was placed behind the copper-regulated *CUP1* promoter (Mascorro-Gallardo et al., 1996). The p92^{pol} ORF was placed behind the titratable tet promoter (i.e.,