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Expression of the *Arabidopsis Xrn4p* 5′–3′ exoribonuclease facilitates degradation of tombusvirus RNA and promotes rapid emergence of viral variants in plants

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Abstract

Rapid RNA virus evolution is a major problem due to the devastating diseases caused by human, animal and plant-pathogenic RNA viruses. A previous genome-wide screen for host factors affecting recombination in *Tomato bushy stunt tombusvirus* (TBSV), a small monopartite plant virus, identified Xrn1p 5′–3′ exoribonuclease of yeast, a model host, whose absence led to increased appearance of recombinants [Serviene, E., Shapka, N., Cheng, C.P., Panavas, T., Phuangrat, B., Baker, J., Nagy, P.D., (2005). Genome-wide screen identifies host genes affecting viral RNA recombination. Proc. Natl. Acad. Sci. U. S. A. 102 (30), 10545–10550]. In this paper, we tested if over-expression of Xrn1p in yeast or expression of the analogous Xrn4p cytoplasmic 5′–3′ exoribonuclease, which has similar function in RNA degradation in *Arabidopsis* as Xrn1p in yeast, in *Nicotiana benthamiana* could affect the accumulation of tombusvirus RNA. We show that over-expression of Xrn1p led to almost complete degradation of TBSV RNA replicons in yeast, suggesting that Xrn1p is involved in TBSV degradation. Infection of *N. benthamiana* expressing AtXrn4p with *Cucumber necrosis tombusvirus* (CNV) led to enhanced viral RNA degradation, suggesting that the yeast and the plant cytoplasmic 5′–3′ exoribonuclease play similar roles. We also observed rapid emergence of novel CNV genomic RNA variants formed via deletions of 5′ terminal sequences in *N. benthamiana* expressing AtXrn4p. Three of the newly emerging 5′ truncated CNV variants were infectious in *N. benthamiana* protoplasts, whereas one CNV variant caused novel symptoms and moved systemically in *N. benthamiana* plants. Altogether, this paper establishes that a single plant gene can contribute to the emergence of novel viral variants.
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Keywords: Exoribonuclease; Virus replication; RNA degradation; Virus evolution; Cucumber necrosis virus; Yeast; Tomato bushy stunt virus

Introduction

Human, animal and plant-pathogenic RNA viruses are well known for their capacity to evolve rapidly to adapt to new environments and/or new hosts (Domingo et al., 2005; Roossinck, 1997, 2003). Due to rapid RNA virus evolution, which is based on mutations, recombination and reassortment (Chaston and Lidbury, 2001; Lai, 1992; Nagy and Simon, 1997; Roossinck, 1997, 2003; Worobey and Holmes, 1999), it is a major challenge to develop long-lasting antiviral methods. Current models propose a central role for the error-prone viral replicase or RNA-dependent RNA polymerase (RdRp) that introduces mutations at high frequencies (Chaston and Lidbury, 2001; Contreras et al., 2002; Domingo et al., 2005; Garcia-Arriaza et al., 2004; Quinones-Kochs et al., 2001; Roossinck, 1997; Steinhauer et al., 1992) and drives efficient RNA recombination events (Figlerowicz et al., 1997; Figlerowicz et al., 1998; Nagy et al., 1995; Panaviene and Nagy, 2003). Accordingly, high mutations rate (Domingo et al., 2005; Roossinck, 2003; Simmonds, 2004; Steinhauer et al., 1992) and template-switching type of recombination events were demonstrated both in vivo and in vitro for several RNA viruses (Cheng and Nagy, 2003; Kim and Kao, 2001; Nagy et al., 1998).

In addition to the viral replicase and other viral factors, the host could also play significant role in RNA virus evolution

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(Grenfell et al., 2004; Holmes, 2004; Honig et al., 2004; Huang and Tsai, 1998; Manzin et al., 2000; Ohshima et al., 2002; Sala and Wain-Hobson, 2000; Tagariello et al., 2004). Dissecting the role of host genes in virus evolution, however, turns out to be a major challenge. The use of yeast as a model host for virus evolution has recently yielded new insights into the role of individual host genes in viral RNA recombination. A systematic, genome wide screen of ~95% of all yeast genes, based on single gene knock out library of yeast (YKO) and the essential gene library (yTHC), for recombination of tombusvirus RNA has led to the identification of 32 host genes, whose modifications (deletions or down-regulation) either suppressed or promoted viral recombination in vivo (Serviene et al., 2006, 2005). One of the identified genes was *XRN1*, coding for a 5′–3′ exoribonuclease, which is a major component of the RNA degradation pathway in yeast (Johnson, 1997; Sheth and Parker, 2003). Interestingly, *XRN1* affected the stability of viral RNA in yeast, suggesting that it is involved in viral RNA degradation (Cheng et al., 2006). These observations promoted a model that Xrn1p might be directly involved in viral RNA recombination by affecting the rate of tombusvirus RNA degradation, thus also affecting the amount of partially-degraded viral RNAs, which serve as substrates for RNA recombination (Cheng et al., 2006). These works suggested a close relationship between viral RNA degradation and RNA recombination (Cheng et al., 2006; Serviene et al., 2005).

Tombusviruses are small RNA viruses of plants, which replicate rapidly in infected cells with the help of the viral replicase, containing p33 and p92^{pol} viral replication proteins and host factors (Nagy and Pogany, 2006; Serva and Nagy, 2006; White and Nagy, 2004). A small tombusvirus replicon (rep)RNA replicates as efficiently in yeast, a model host, co-expressing p33 and p92^{pol} replication proteins as in plant hosts (Panavas and Nagy, 2003; Panaviene et al., 2004). In addition, replication of the repRNA in yeast requires the same cis-acting RNA elements and viral protein factors as in host plants (Panavas and Nagy, 2003; Panaviene et al., 2004; Rajendran and Nagy, 2006), suggesting that host factors utilized by tombusviruses in yeast could be similar to host factors used in *Nicotiana benthamiana* and other host plants. Based on this assumption, we have tested the role of the *Arabidopsis* AtXrn4p protein, which is known to have similar function in cytoplasmic RNA degradation in *Arabidopsis* as Xrn1p has in yeast (Kastenmayer and Green, 2000; Souret et al., 2004). Indeed, expression of AtXrn4p in yeast complemented the deficiency of *xrn1Δ* yeast (Kastenmayer and Green, 2000), confirming that AtXrn4p is functionally analogous with the yeast Xrn1p. In this work, we found that expression of AtXrn4p in *N. benthamiana* infected with *Cucumber necrosis tombusvirus* (CNV) stimulated degradation of the CNV RNA. In addition, we also detected the rapid emergence of new CNV RNA variants with various 5′ deletions. Interestingly, several of the newly generated tombusvirus variants were infectious in *N. benthamiana* protoplasts and one mutant was infectious in *N. benthamiana* plant. The latter mutant invaded the plants systemically and caused mild symptoms on uninoculated leaves. Altogether, this paper establishes that a single plant gene can contribute to the emergence of novel viral variants, which could impact on virus evolution.

Results

Rationale

A systematic genome-wide screen for the effect of host genes on viral RNA recombination led to the identification of Xrn1p 5′–3′ exoribonuclease, among other host proteins, as a major host factor affecting viral RNA recombination (Serviene et al., 2006, 2005). Accumulation of tombusvirus recombinants was increased in *xrn1Δ* yeast, suggesting that Xrn1p is a suppressor of tombusvirus recombination (Cheng et al., 2006). The above works in yeast also supported an important role for Xrn1p in degradation of tombusvirus RNA. Encouraged by these observations, we wanted to learn if over-expression of the yeast Xrn1p in yeast or expression of the analogous Xrn4p in *N. benthamiana* could affect the accumulation and/or evolution of tombusvirus RNA in yeast and in plants.

Over-expression of Xrn1p in yeast leads to rapid degradation of the tombusvirus replicon RNA

To test the effect of Xrn1p on tombusvirus accumulation and evolution, we over-expressed the yeast Xrn1p in yeast cells co-expressing p33/p92^{pol} tombusvirus replication proteins and the DI-72 replicon (rep)RNA from yeast expression plasmids. The control yeast expressing only p33/p92^{pol} proteins from the *ADH1* constitutive promoter and DI-72 RNA from the galactose inducible *GAL1* promoter (Fig. 1A) supported efficient replication of the repRNA, based on Northern blot analysis of total RNA extract prepared from yeast (Fig. 1B, lanes 1–2). On the contrary, over-expression of Xrn1p inhibited the accumulation of the DI-72 repRNA by ~98% (Fig. 1B, lanes 3–4). Similar to the full-length repRNA, the partial degradation products of the repRNA were barely detectable in the Xrn1p over-expression strain. We also observed similar pattern with a second repRNA, denoted DI-AU-FP (Fig. 1A). DI-AU-FP repRNA contains an artificial AU-rich segment positioned between region I (RI) and RII, which facilitates the generation and accumulation of recombinant (rec)RNAs (Fig. 1C, lanes 1–2) (Serviene et al., 2005; Shapka and Nagy, 2004). Both the original DI-AU-FP repRNA and the recRNAs accumulate to easily detectable levels in the wt yeast, whereas yeast over-expressing Xrn1p showed 82% reduction in repRNA and 99% reduction in the recRNA (Fig. 1C, lanes 3–4). These observations are in agreement with previous findings, which are based on complementation data, that Xrn1p suppresses the generation of recRNAs and that Xrn1p reduces the accumulation of partial degradation products of the repRNAs in yeast (Cheng et al., 2006). The current over-expression data support that excess amount of Xrn1p reduces the accumulation of the repRNA. This suggests that the repRNA has an Xrn1p-sensitive stage during its accumulation in yeast.

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