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Silencing of *Nicotiana benthamiana* Xrn4p exoribonuclease promotes tombusvirus RNA accumulation and recombination

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ABSTRACT

The cytosolic 5′-to-3′ exoribonuclease Xrn1p plays a major role in recombination and degradation of *Tomato bushy stunt tombusvirus* (TBSV) replicon (rep)RNA in yeast, a model host (Serviene, E., Shapka, N., Cheng, C.P., Panavas, T., Phuangrat, B., Baker, J., and 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.). To test if the plant cytosolic 5′-to-3′ exoribonuclease Xrn4p, similar to the yeast Xrn1p, could also affect TBSV recombination, in this paper, we silenced *XRN4* in *Nicotiana benthamiana*, an experimental host. The accumulation of tombusvirus genomic RNA and repRNA increased by 50% and 220%, respectively, in *XRN4*-silenced *N. benthamiana*. We also observed up to 125-fold increase in the emergence of new recombinants and partly degraded viral RNAs in the silenced plants. Using a cell-free assay based on a yeast extract, which supports authentic replication and recombination of TBSV, we demonstrate that the purified recombinant Xrn1p efficiently inhibited the accumulation of recombinants and partly degraded viral RNAs. Altogether, the data from a plant host and cell-free system confirm a central role for the plant cytosolic 5′-to-3′ exoribonuclease in TBSV replication, recombination and viral RNA degradation.

Introduction

Yeast has emerged as an outstanding model host to study replication of selected plant and animal viruses (Ahlquist et al., 2003; Nagy, 2008; Noueiry and Ahlquist, 2003). Genome-wide and proteomics screens in yeast have led to the identification of more than one hundred host genes affecting *Brome mosaic virus* (BMV) or *Tomato bushy stunt virus* (TBSV) replication and host virus interactions (Jiang et al., 2006; Kushner et al., 2003; Li et al., 2008; Panavas et al., 2005b; Serva and Nagy, 2006; Zhu et al., 2007). Subsequent characterization of individual host genes provided further insights into the functions of these host genes in virus replication (Beckham et al., 2007; Diez et al., 2000; Noueiry et al., 2003; Pathak et al., 2008; Tomita et al., 2003; Wang and Nagy, 2008). In addition, yeast is also useful to study the evolution of RNA viruses (Garcia-Ruiz and Ahlquist, 2006; Panavas and Nagy, 2003). To study the role of host genes in virus evolution, genome-wide and proteomics screens have been performed in yeast leading to the identification of over 30 host genes (Li et al., 2008; Serviene et al., 2006, 2005). Altogether, yeast will likely be advantageous to study pathogenic RNA viruses, which have well known capacity to evolve rapidly to adapt to new environments and/or new hosts (Domingo et al., 2005; Roossinck, 1997, 2003). Indeed, rapid RNA virus evolution, which is based on mutations, recombination and reassortment, is a major threat to all eukaryotic organisms (Chaston and Lidbury, 2001; Lai, 1992; Nagy and Simon, 1997; Roossinck, 1997, 2003; Worobey and Holmes, 1999). It is currently well-documented that the error-prone viral replicase or RNA-dependent RNA polymerase (RdRp) 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 (Cheng and Nagy, 2003; Figlerowicz et al., 1997, 1998; Kim and Kao, 2001; Nagy et al., 1995; Panaviene and Nagy, 2003; Pogany and Nagy, 2008).

One of the identified genes during the genome wide screens was *XRN1*, which codes for a 5′-to-3′ exoribonuclease (Serviene et al., 2006, 2005). Xrn1p is a major component of the RNA degradation pathway in yeast (Johnson, 1997; Sheth and Parker, 2003). *XRN1* affected the stability of viral RNA in yeast, suggesting that it is involved in viral RNA degradation (Cheng et al., 2006). Subsequent works in yeast and in vitro have led to the model that Xrn1p is an inhibitor of viral RNA recombination by affecting the amount of partially-degraded viral RNAs, which serve as substrates for RNA recombination

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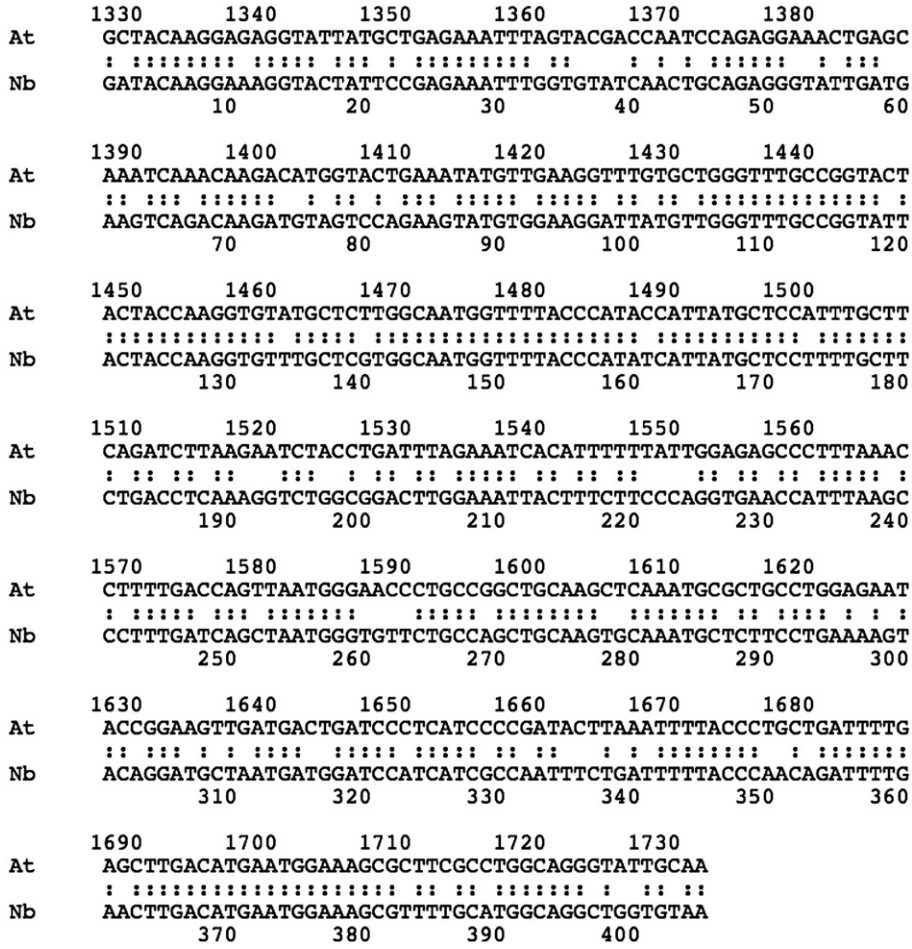

Fig. 1. Partial sequence of the *NbXRN4* cDNA. Comparison of the cloned 405 bp segment of the *NbXRN4* sequence with the *AtXRN4* shows 76% identity.

(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 infecting plants. They replicate rapidly in infected cells by assembling the viral replicase, containing p33 and $p92^{pol}$ viral replication proteins and host factors (Li et al., 2008; Nagy and Pogany, 2006; Nagy and Pogany, 2008; Serva and Nagy, 2006; White and Nagy, 2004). A small TBSV replicon (rep)RNA replicates efficiently in yeast co-expressing p33 and $p92^{pol}$ replication proteins (Panavas and Nagy, 2003; Panaviene et al., 2004). Also, the yeast-based replication of the repRNA 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.

To test whether the role of cytosolic Xrn4p 5′-to-3′ exoribonuclease of plants is comparable to the yeast Xrn1p in suppression of TBSV recombination, we silenced *XRN4* in *N. benthamiana*, an experimental host. Infection of the silenced plants with tombusvirus genomic and repRNAs led to increased accumulation of the viral RNAs, including novel recombinant RNAs and partially degraded viral RNAs. Altogether, the effect of *XRN4* knockdown in *N. benthamiana* was similar to the effect observed in *xrn1*Δ yeast, suggesting that these cytosolic 5′-to-3′ exoribonucleases play comparable roles in tombusvirus replication, recombination and viral RNA degradation in a plant and yeast hosts.

Results

Partial cloning of NbXRN4

The yeast cytosolic Xrn1p 5′-to-3′ exoribonuclease is a host inhibitor of tombusviral RNA recombination (Cheng et al., 2006) that was identified during genome-wide screens in yeast model host (Serviene et al., 2006; Serviene et al., 2005). We wanted to learn if the analogous cytosolic 5′-to-3′ exoribonuclease Xrn4p from *N. benthamiana* could affect the accumulation and recombination of tombusvirus RNA in a plant host via down-regulation of *NbXRN4* expression using gene silencing. To reduce the level of *XRN4* mRNA in *N. benthamiana*, we chose to use a gene silencing approach, which requires sequence information about the targeted gene (Dinesh-Kumar et al., 2003). Since the genome of *N. benthamiana* is only partially sequenced, we used *Solanum lycopersicum* sequence to design primers to RT-PCR-amplify a segment of *NbXRN4* gene, which is analogous with the yeast *XRN1* based on works with *Arabidopsis* model plant (Kastenmayer and Green, 2000; Souret et al., 2004). As shown in Fig. 1, we have determined the sequence of a 405 bp long region of *NbXRN4*, which showed 76% identity with the *AtXRN4* sequence. The high sequence identity between *NbXRN4* and *AtXRN4* gives high confidence that we cloned a segment of the *NbXRN4* gene. This was further supported by successful amplification of the *NbXRN4* sequence with a second set of RT-PCR primers covering a segment that did not overlap with the 405 bp long region in the *NbXRN4* gene (Fig. 2A).