Supplementary material:

The C-terminal portion of p33 containing the RNA-binding region is not an active RNA chaperone in vitro.

To dissect what domains within the p33 replication protein are needed for RNA chaperone activity, first we tested p33C carrying the RPR RNA binding and the S1-S2 protein-protein interaction domains (Fig. S1A) (Panaviene, Baker, and Nagy, 2003; Rajendran and Nagy, 2004; Rajendran and Nagy, 2006). Addition of p33C to the standard p88C RdRp assay with dsDI-72 template revealed slight inhibitory effect on p88C RdRp activity (Fig. S1B), instead of the expected stimulatory effect. P33C also strongly inhibited p88C RdRp activity on dsDI-72(5'Δ69) template (Fig. S1C). Based on these observations, we suggest that p33C does not have an RNA chaperone activity comparable with the full-length p33.

The N-terminal portion of p33 contains an additional RNA-binding region required for RNA chaperone activity.

The lack of RNA chaperone activity by p33C indicated that the N-terminal region in p33 might also have an RNA binding region that is necessary for RNA chaperone activity. Therefore, we tested if the N-terminal segment of p33 could bind to the viral RNA in a gel mobility shift experiment. Indeed, construct p33-N1-82 carrying the N-terminal 82 aa bound to the labeled RNA template in vitro, whereas the shorter version (termed p33-N1-72 with 72aa segment) bound less efficiently (Fig. S1D). These dada suggested that the N-terminal portion of p33

contains ssRNA binding activity. Further experiments have demonstrated that ssRNA-binding by p33-N1-82 is non-specific and much weaker than ssRNA binding by p33C (not shown).

To test if the N-terminal segment of p33 contributes to the RNA chaperone activity of p33, we made construct p33-Δ72-130, which lacked the N-proximal RNA binding region (between positions 72-82) and a transmembrane segment to increase solubility of the recombinant protein in E. coli. When compared with the full-length p33, the purified recombinant p33-Δ72-130 lacked RNA chaperone activity in the S1 nuclease sensitivity assay (Fig. S1E). Thus, the result with p33-Δ72-130 is similar to that obtained with p33C, suggesting that the N-proximal RNA binding region of p33 is likely part of the functional RNA chaperone.

Figure S1.

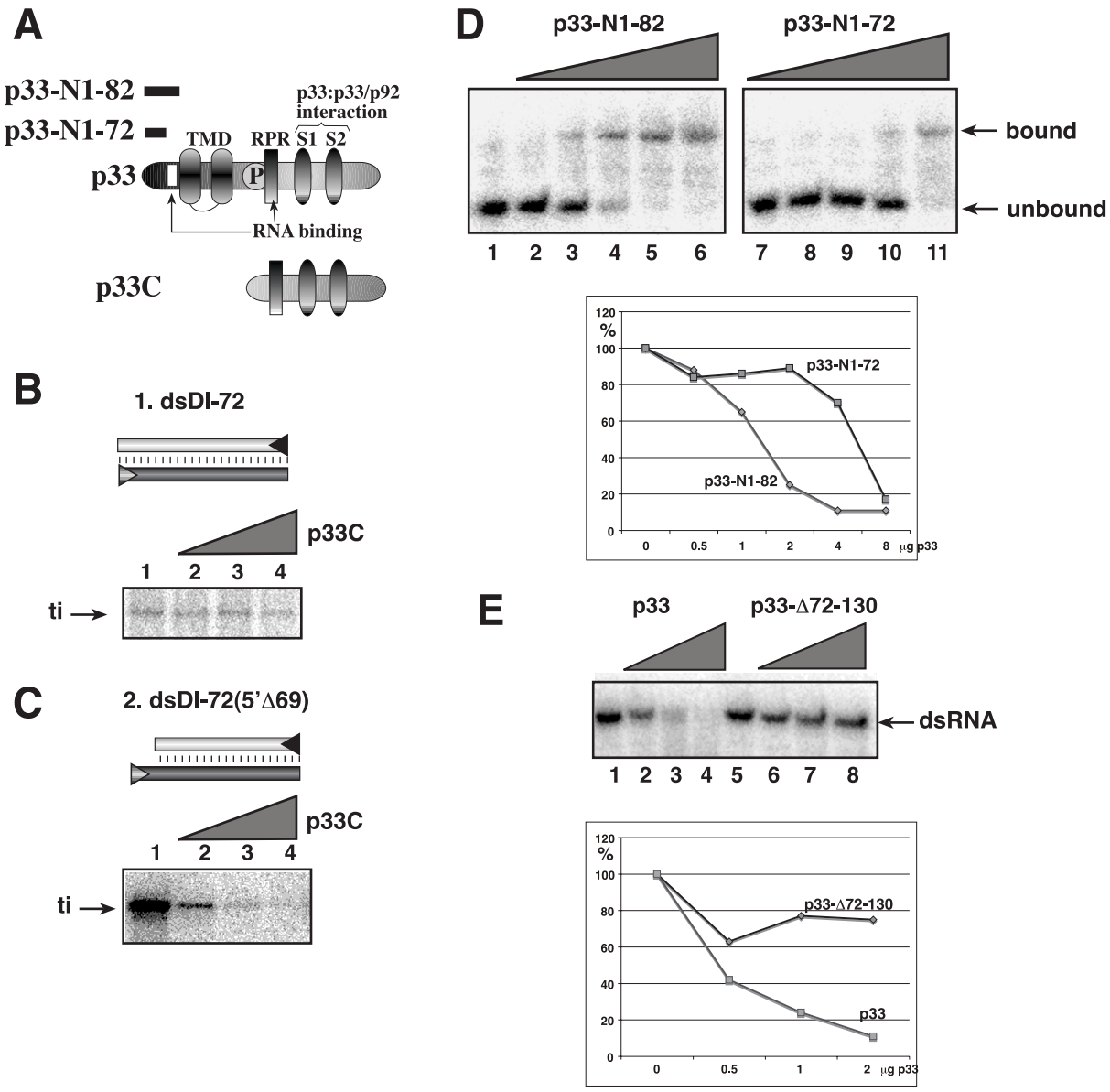

Fig. S1. The lack of RNA chaperone activity of p33 mutant missing the N-terminus in vitro. (**A**) Schematic presentation of CNV p33 and mutants with known functional domains. The only previously known function of the N-terminal sequence was its involvement in the localization of p33 to the peroxisomal membrane (including the trans-membrane domains, TMDs). The C-terminal sequence carries the Arginine-proline-rich (RPR) domain involved in RNA binding and

the S1 and S2 subdomains involved in p33:p33/p92 interaction. (**B**) Representative denaturing gels of radiolabeled RNA products synthesized by *in vitro trans*cription with p88C RdRp in the presence of 0, 0.5, 1 and 2 μg of purified recombinant p33C are shown. The samples were treated with S1 nuclease. See Fig. 5B for further details. (**D**) Gel-mobility shift assay that shows RNA binding by the truncated p33 variants. The ^{32}P-labeled probe (20 ng) was used with 0, 0.5, 1, 2, 4 and 8 μg of purified recombinant p33-N1-82 and p33-N1-72. The free RNA and the shifted band representing the RNA-protein complex are marked with arrows. The amount of free RNA left in the samples was quantified and shown as % of the total probe in the absence of protein (lanes 1 and 7). (**E**) Representative denaturing gels of ^{32}P-labeled dsRI RNA template that remained after the S1 nuclease treatment in the presence of 0, 0.5, 1 and 2 μg of purified recombinant p33 mutant lacking the second RNA binding sequence are shown. The samples were phenol/chloroform extracted and precipitated prior to gel-analysis. Note that we used a p33 mutant with increased solubility in *E. coli* due to the missing hydrophobic TMD domain.

Materials and methods

RNA gel mobility shift assay: RI(-)probe of DI-72 RNA was labeled with [^{32}P]UTP using T7 RNA polymerase. Approximately 20 ng of labeled RNA was incubated with MBP-p33 mutant for 20 min at room temperature in the RdRp buffer (Rajendran and Nagy, 2003; Rajendran, Pogany, and Nagy, 2002). The samples were analyzed by electrophoresis on native 5% acrylamide gels, run at 200 V for 20 min at 4°C in Tris-acetate-EDTA (TAE) buffer. Analysis was as described above.

REFERENCES

Panaviene, Z., Baker, J. M., and Nagy, P. D. (2003). The overlapping RNA-binding domains of p33 and p92 replicase proteins are essential for tombusvirus replication. *Virology* **308**(1)**,** 191-205.

Rajendran, K. S., and Nagy, P. D. (2003). Characterization of the RNA-binding domains in the replicase proteins of tomato bushy stunt virus. *J Virol* **77**(17)**,** 9244-58.

Rajendran, K. S., and Nagy, P. D. (2004). Interaction between the replicase proteins of Tomato bushy stunt virus in vitro and in vivo. *Virology* **326**(2)**,** 250-61.

Rajendran, K. S., and Nagy, P. D. (2006). Kinetics and functional studies on interaction between the replicase proteins of Tomato Bushy Stunt Virus: requirement of p33:p92 interaction for replicase assembly. *Virology* **345**(1)**,** 270-9.

Rajendran, K. S., Pogany, J., and Nagy, P. D. (2002). Comparison of turnip crinkle virus RNA-dependent RNA polymerase preparations expressed in Escherichia coli or derived from infected plants. *J Virol* **76**(4)**,** 1707-17.