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Cpr1 cyclophilin and Ess1 parvulin prolyl isomerases interact with the tombusvirus replication protein and inhibit viral replication in yeast model host

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ABSTRACT

To identify host proteins interacting with the membrane-bound replication proteins of tombusviruses, we performed membrane yeast two-hybrid (MYTH) screens based on yeast cDNA libraries. The screens led to the identification of 57 yeast proteins interacting with replication proteins of two tombusviruses. Results from a split ubiquitin assay with 12 full-length yeast proteins and the viral replication proteins suggested that the replication proteins of two tombusviruses interact with a similar set of host proteins. Follow-up experiments with the yeast Cpr1p cyclophilin, which has prolyl isomerase activity that catalyzes *cis–trans* isomerization of peptidyl–prolyl bonds, confirmed that Cpr1p interacted with the viral p33 replication protein in yeast and *in vitro*. Replication of *Tomato bushy stunt virus* replicon RNA increased in *cpr1*Δ yeast, while over-expression of Cpr1p decreased viral replication. We also show that the Ess1p parvulin prolyl isomerase partly complements Cpr1p function as an inhibitor of tombusvirus replication.

Introduction

Viruses are intracellular parasites that rely on the vast resources of the host cells for their replication. The viral replication process requires co-opting of an unknown number of host proteins and the reprogramming of cellular pathways. Another level of complexity in virus–host interactions is the activation of host antiviral responses that target different players and steps in the viral replication process. Not surprisingly, all these processes depend on protein–protein interactions. Therefore, there is a major on-going effort to identify all the host proteins interacting with viral replication proteins.

Tomato bushy stunt virus (TBSV) and the closely related *Cucumber necrosis virus* (CNV) and *Carnation Italian ringspot virus* (CIRV) are tombusviruses with small (+)RNA genomes. Due to the development of yeast (*Saccharomyces cerevisiae*) as a model host, tombusviruses have recently emerged as model viruses to study virus replication, recombination, and virus–host interactions (Jaag et al., 2010; Nagy and Pogany, 2006; Panavas and Nagy, 2003b; Panaviene et al., 2004c; Pogany and Nagy, 2008; White and Nagy, 2004). TBSV codes for two essential replication proteins, p33 and $p92^{pol}$. The auxiliary p33 replication protein is involved in the recruitment of the TBSV (+)RNA to the site of replication, which is the cytosolic surface of peroxisomal membranes (Jonczyk et al., 2007; McCartney et al., 2005; Panavas et al., 2005a; Pogany et al., 2005). The $p92^{pol}$ RNA-dependent RNA polymerase (RdRp) protein, which is the translational readthrough product of the p33 open reading frame, binds to p33 replication protein leading to the assembly of the functional membrane-bound replicase complex (Panavas et al., 2005a; Panaviene et al., 2004c, 2005; Pogany and Nagy, 2008).

Our current knowledge on tombusvirus–host interactions is based on recent genome-wide screens covering 95% of yeast genes that have identified more than 150 host genes affecting TBSV replication or recombination (Jiang et al., 2006; Panavas et al., 2005b; Serviene et al., 2006; Serviene et al., 2005). In addition, proteomics analysis of the highly purified tombusvirus replicase complex identified 6 host proteins in the replicase, in addition to the viral p33 and $p92^{pol}$ replication proteins (Li et al., 2008a, 2009; Serva and Nagy, 2006). These host proteins, such as heat shock protein 70 (hsp70), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Cdc34p Ub-conjugating enzyme, and eEF1A translation elongation factor affect the assembly of the viral replicase complex or regulate viral RNA replication (Li et al., 2008a, 2009; Pogany et al., 2008; Wang and Nagy, 2008; Wang et al., 2009a).

The tombusvirus replication proteins are bound to the peroxisomal membrane or in its absence to the ER membrane in infected cells (Cheng et al., 2005; Jonczyk et al., 2007; McCartney et al., 2005; Pathak et al., 2008). Therefore, many host protein–viral replication protein interactions are expected to occur on membrane surfaces. However, the previous global proteomics approach using the yeast

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Table 1
The name and functions of yeast proteins bound to CNV p33, p92 and CIRV p36.

Gene[1]	FR[2]	Ver[3]	Gene function[4]
ANB1	6/1/0	+	Translation elongation factor eIF-5A, previously thought to function in translation initiation; similar to and functionally redundant with Hyp2p
AQY1	3/0/0	+	Spore-specific water channel that mediates the transport of water across cell membranes
ARC15	0/0/1	nd	Subunit of the ARP2/3 complex, which is required for the motility and integrity of cortical actin patches
ARF1	1/0/0	+	ADP-ribosylation factor, GTPase of the Ras superfamily involved in regulation of coated vesicle formation in intracellular trafficking within the Golgi; functionally interchangeable with Arf2p
BGL2	0/0/2	nd	Endo-beta-1,3-glucanase, major protein of the cell wall, involved in cell wall maintenance
CCW12	2/0/0	–	Cell wall mannoprotein
COF1	1/0/0	+	Cofilin, promotes actin filament depolarization in a pH-dependent manner; binds both actin monomers and filaments and severs filaments; thought to be regulated by phosphorylation; ubiquitous and essential in eukaryotes
CPR1	2/0/0	+	Cytoplasmic peptidyl-prolyl cis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues
EGD2	13/3/0	+	Alpha subunit of the heteromeric nascent polypeptide-associated complex (NAC) involved in protein sorting and translocation, associated with cytoplasmic ribosomes
FAA3	0/1/0	nd	Long chain fatty acyl-CoA synthetase, has a preference for C16 and C18 fatty acids
FBA1	1/0/0	–	Fructose 1,6-bisphosphate aldolase, required for glycolysis and gluconeogenesis; catalyzes conversion of fructose 1,6 bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P
FIP1	1/0/0	+	Subunit of cleavage polyadenylation factor (CPF), interacts directly with poly(A) polymerase (Pap1p) to regulate its activity
FRE8	1/0/0	nd	Protein with sequence similarity to iron/copper reductases, involved in iron homeostasis
GND1	1/0/0	–	6-phosphogluconate dehydrogenase, catalyzes an NADPH regenerating reaction in the pentose phosphate pathway
HST1	0/1/0	nd	NAD(+)-dependent histone deacetylase
HTA2	2/0/0	+	Histone H2A, core histone protein required for chromatin assembly and chromosome function; one of two nearly identical (see also HTA1) subtypes
ICY2	1/0/0	+	Protein of unknown function; mobilized into polysomes upon a shift from a fermentable to nonfermentable carbon source
KIN4	0/1/0	nd	Serine/threonine protein kinase that inhibits the mitotic exit network (MEN) when the spindle position checkpoint is activated
MFalpha1	3/0/0	nd	Mating pheromone alpha-factor, pheromone-dependent signal transduction
PGK1	1/0/0	nd	3-phosphoglycerate kinase, catalyzes transfer of high-energy phosphoryl groups from the acyl phosphate of 1,3-bisphosphoglycerate to ADP to produce ATP
PKH2	0/0/1	nd	Serine/threonine protein kinase involved in sphingolipid-mediated signaling pathway that controls endocytosis; redundant with Pkh1p
RHR2	1/0/1	+	Constitutively expressed isoform of DL-glycerol-3-phosphatase; involved in glycerol biosynthesis, induced in response to both anaerobic and osmotic stress
RNR4	1/0/0	+	Ribonucleotide-diphosphate reductase (RNR), small subunit; the RNR complex catalyzes the rate-limiting step in dNTP synthesis
RPL4A	0/1/0	nd	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl4Bp and has similarity to E. coli L4 and rat L4 ribosomal proteins
RPL9A	1/0/0	+	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl9Bp and has similarity to E. coli L6 and rat L9 ribosomal proteins
RPL11A	0/0/1	nd	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl11Bp; involved in ribosomal assembly
RPL13A	1/0/0	+	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Bp; not essential for viability; has similarity to rat L13 ribosomal protein
RPL14A	0/1/0	nd	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl14Bp and has similarity to rat L14 ribosomal protein
RPL30	1/0/0	+	Protein component of the large (60S) ribosomal subunit, has similarity to rat L30 ribosomal protein
RPL32	1/0/0	–	Protein component of the large (60S) ribosomal subunit, has similarity to rat L32 ribosomal protein
RPL35A	1/0/0	+	Protein component of the large (60S) ribosomal subunit, identical to Rpl35Bp and has similarity to rat L35 ribosomal protein
RPP2B	2/0/0	nd	Ribosomal protein P2 beta, a component of the ribosomal stalk, which is involved in the interaction between translational elongation factors and the ribosome; regulates the accumulation of Rpp1Ap and Rpp1Bp in the cytoplasm
RPS2	1/0/0	nd	Protein component of the small (40S) subunit, essential for control of translational accuracy; phosphorylation by C-terminal domain kinase I (CTDK-I) enhances translational accuracy; similar to E. coli S5 and rat S2 ribosomal proteins
RPS3	0/1/0	nd	Protein component of the small (40S) ribosomal subunit, has apurinic/apyrimidinic (AP) endonuclease activity
RPS4A	0/1/0	nd	Protein component of the small (40S) ribosomal subunit; mutation affects 20S pre-rRNA processing; identical to Rps4Bp and has similarity to rat S4 ribosomal protein
RPS12	2/0/0	+	Protein component of the small (40S) ribosomal subunit; has similarity to rat ribosomal protein S12
RPS15	0/1/0	nd	Protein component of the small (40S) ribosomal subunit; has similarity to E. coli S19 and rat S15 ribosomal proteins
RPS30B	2/0/1	+	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps30Ap and has similarity to rat S30 ribosomal protein
SAM37	1/0/0	+	Component of the Sorting and Assembly Machinery (SAM or TOB complex) of the mitochondrial outer membrane, which binds precursors of beta-barrel proteins and facilitates their outer membrane insertion; contributes to SAM complex stability
SCS2	1/0/0	+	Integral ER membrane protein that regulates phospholipid metabolism, disruption causes inositol tauxotrophy above 34 degrees C, VAP homolog
SGT2	1/0/0	–	Glutamine-rich cytoplasmic protein that contains tetratricopeptide (TPR) repeats, which often mediate protein-protein interactions; has similarity to human SGT, which is a cochaperone that negatively regulates Hsp70
SHO1	1/0/0	+	Transmembrane osmosensor, participates in activation of both the Cdc42p- and MAP kinase-dependent filamentous growth pathway and the high-osmolarity glycerol response pathway
SPC2	2/0/0	+	Subunit of signal peptidase complex, which catalyzes cleavage of N-terminal signal sequences of proteins targeted to the secretory pathway; homologous to mammalian SPC25
SSN8	1/0/0	nd	Cyclin-like component of the RNA polymerase II holoenzyme, involved in phosphorylation of the RNA polymerase II C-terminal domain
SSS1	3/0/0	+	Subunit of the Sec61p translocation complex that forms a channel for passage of secretory proteins through the endoplasmic reticulum membrane
STM1	1/0/0	–	Protein required for optimal translation under nutrient stress; perturbs association of Yef3p with ribosome's; involved in TOR signaling; binds G4 quadruplex and purine motif triplex nucleic acid
SUR7	1/0/0	nd	Plasma membrane protein that localizes to furrow-like invaginations, membrane sphingolipid content are altered in mutants
TDH2	1/0/0	+	Glyceraldehyde-3-phosphate dehydrogenase, involved in glycolysis and gluconeogenesis
TDH3	1/0/0	nd	Glyceraldehyde-3-phosphate dehydrogenase, involved in glycolysis and gluconeogenesis
TEF1*	6/0/2	+	Translational elongation factor EF-1 alpha; also encoded by TEF2; functions in the binding reaction of aminoacyl-tRNA (AA-tRNA) to ribosomes
TMA19	1/0/0	+	Protein that associates with ribosome; homolog of translationally controlled tumor protein; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and relocates to the mitochondrial outer surface upon oxidative stress

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