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Tombusvirus RNA replication depends on the TOR pathway in yeast and plants

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ABSTRACT

Similar to other (+)RNA viruses, tomato bushy stunt virus (TBSV) utilizes metabolites, lipids, membranes, and co-opted host factors during replication. The coordination of cell metabolism and growth with environmental cues is performed by the target of rapamycin (TOR) kinase in eukaryotic cells. In this paper, we find that TBSV replication partially inhibits TOR activity, likely due to recruitment of glycolytic enzymes to the viral replication compartment, which results in reduced ATP levels in the cytosol. Complete inhibition of TOR activity with rapamycin in yeast or AZD8055 inhibitor in plants reduces tombusvirus replication. We find that high glucose concentration, which stimulates TOR activity, enhanced tombusvirus replication in yeast. Depletion of yeast Sch9 or plant S6K1 kinase, a downstream effector of TOR, also inhibited tombusvirus replication in yeast and plant or the assembly of the viral replicase in vitro. Altogether, the TOR pathway is crucial for TBSV to replicate efficiently in hosts.

1. Introduction

Viruses are obligate intracellular parasites that depend on the host cell to supply the energy, ribonucleotides, lipids, and other necessary materials for their replication. RNA virus replication requires the recruitment of many cellular proteins, remodeling and proliferation of intracellular membranes, and retargeting of trafficking vesicles (den Boon and Ahlquist, 2010; Fernandez de Castro et al., 2016; Nagy and Pogany, 2012; Paul and Bartenschlager, 2015; Wang, 2015). Similar to other (+)RNA viruses, tomato bushy stunt virus (TBSV) assembles numerous membrane-bound viral replicase complexes (VRCs) to replicate the viral RNAs in infected cells. During the replication process, TBSV utilizes various subcellular membranes, lipids, metabolites and co-opted host factors (Nagy, 2016; Nagy and Pogany, 2012; Nagy et al., 2014). TBSV can replicate in the model host yeast (*Saccharomyces cerevisiae*) using two viral replication proteins, p33 and $p92^{pol}$ (Nagy and Pogany, 2006; Panavas and Nagy, 2003; Panaviene et al., 2004). Our multiple genome-wide screens of yeast and global proteomic approaches revealed that TBSV replication is affected by over 500 host proteins (Jiang et al., 2006; Li et al., 2008; Mendu et al., 2010; Panavas et al., 2005; Serviene et al., 2006, 2005; Shah Nawaz-ul-Rehman et al., 2012). Moreover, several co-opted host factors, such as heat shock protein 70 (Hsp70), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), eukaryotic elongation factor 1A (eEF1A), eEF1Bγ, DEAD-box RNA helicases, and the ESCRT (endosomal sorting complexes required for transport) family proteins (Barajas et al., 2009a; Li et al., 2010; Nagy, 2008; Sasvari et al., 2011), are recruited into VRCs through the interactions with p33 and $p92^{pol}$. These co-opted host proteins are required for VRC assembly or participate in viral RNA synthesis (Li et al., 2010; Nagy and Pogany, 2011; Pogany et al., 2008). TBSV also needs sterols and phospholipids to create numerous vesicle-like structures in peroxisomal boundary membranes (Barajas et al., 2014; Chuang et al., 2014; Sharma et al., 2010, 2011; Xu and Nagy, 2015). Such dramatic changes in cellular metabolism caused by robust TBSV replication likely affect cellular energy and metabolic homeostasis.

In general, nutrients, such as glucose and amino acids, are essential to promote cell growth in all organisms. Host cell demands nutrients to synthesize RNA, DNA, proteins and lipids. Production of proteins, lipids and nucleotides is affected by cellular metabolism, the balance between anabolism and catabolism pathways. The coordination of cell metabolism and growth with environmental cues is performed by the target of rapamycin (TOR) kinase in eukaryotic cells (Ben-Sahra and Manning, 2017; Betz and Hall, 2013; Shimobayashi and Hall, 2014; Weisman, 2016; Wullschleger et al., 2006). TOR pathway regulates protein synthesis, lipid and nucleotide synthesis, glucose homeostasis, autophagy and various signaling for cell growth and proliferation. TOR (TOR1 and TOR2 in yeast) is a serine/threonine protein kinase and forms the core of TORC1 complex, which phosphorylates downstream effectors.

TOR is evolutionarily conserved from yeasts to plants and humans

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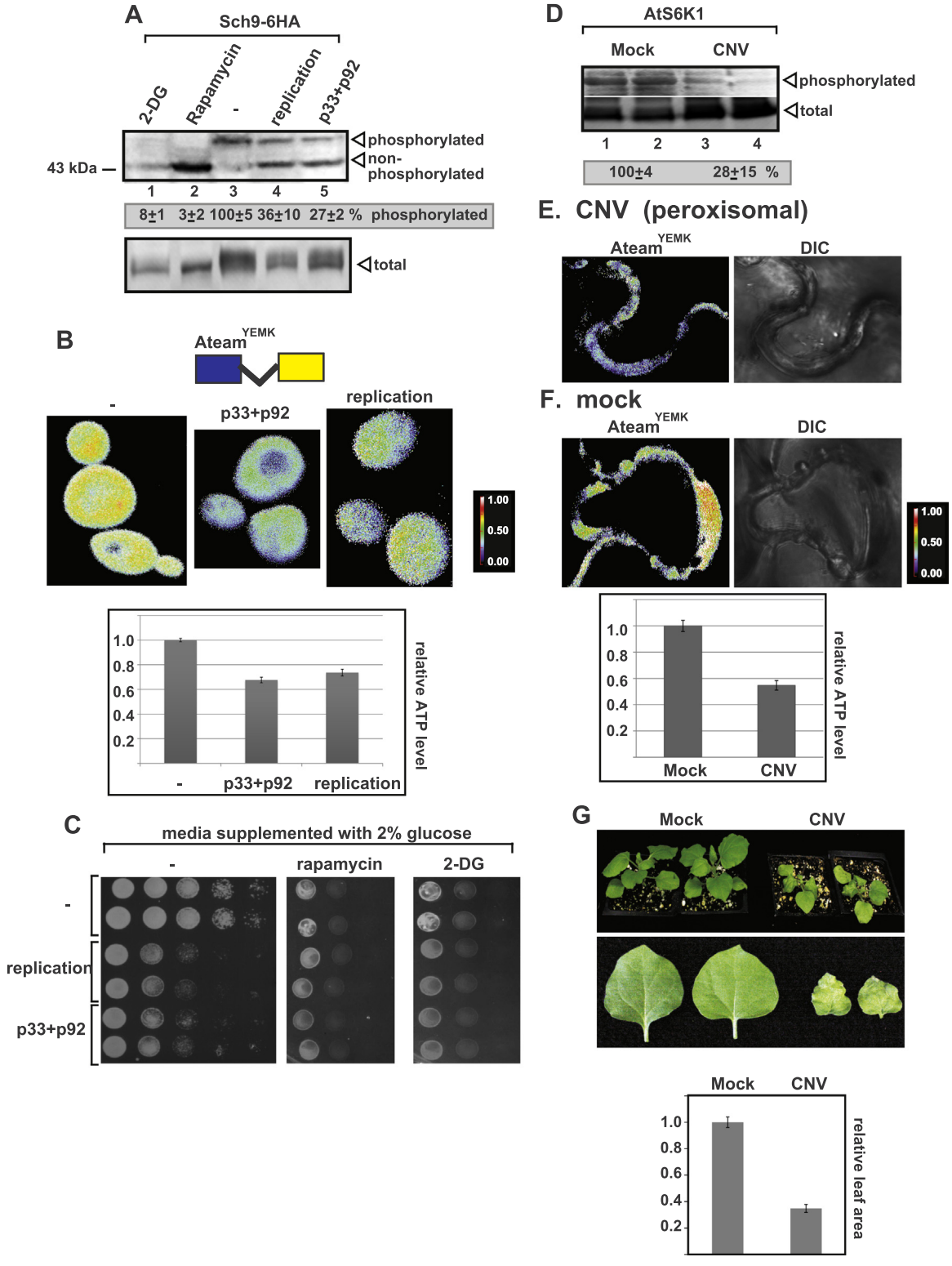
(Baena-Gonzalez and Sheen, 2008; Ben-Sahra and Manning, 2017; Betz and Hall, 2013; Laplante and Sabatini, 2012; Xiong and Sheen, 2014). TOR kinase builds two distinct TOR protein complexes, TORC1 and TORC2 in yeast and human (Foster and Fingar, 2010; Loewith et al., 2002). TORC1 is sensitive to rapamycin treatment through FKBP12-rapamycin binding to TORC1 (Loewith et al., 2002). In response to nutrients, TORC1 promotes anabolic processes, including gene transcription, protein translation, and ribosome biogenesis, and inhibits catabolic processes, such as autophagy (Ben-Sahra and Manning, 2017; Shimobayashi and Hall, 2014, 2016; Wullschleger et al., 2006). Depletion of glucose, amino acids, or rapamycin treatment inhibit TORC1 activity, resulting in a rapid dephosphorylation of human and plant S6Ks and yeast Sch9 kinase (Ben-Sahra and Manning, 2017; Ma and Blenis, 2009; Urban et al., 2007; Xiong and Sheen, 2012). As a downstream regulator of TORC1, S6K1 (Sch9p in yeast) kinase controls cell size, cell cycle, ribosome biosynthesis, and protein translation (Goranov



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