Short Communication

Potyviruses differ in their requirement for TOR signalling

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Potyviruses are important plant pathogens that rely on many plant cellular processes for successful infection. TOR (target of rapamycin) signalling is a key eukaryotic energy-signalling pathway controlling many cellular processes such as translation and autophagy. The dependence of potyviruses on active TOR signalling was examined. Arabidopsis lines downregulated for TOR by RNAi were challenged with the potyviruses watermelon mosaic virus (WMV) and turnip mosaic virus (TuMV). WMV accumulation was found to be severely altered while TuMV accumulation was only slightly delayed. In another approach, using AZD-8055, an active site inhibitor of the TOR kinase, WMV infection was found to be severely altered. Moreover, AZD-8055 application can cure WMV infection. In contrast, TuMV infection was not affected by AZD-8055. This suggests that potyviruses have different cellular requirements for active plant TOR signalling.

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Potyviruses are positive ssRNA viruses that represent one of the two largest genera of plant viruses and one of the most threatening agronomically (Gibbs & Ohshima, 2010). At the cellular level, potyvirus infection leads to the formation of nuclear and cytoplasmic inclusions containing viral proteins (Ivanov et al., 2014). Some potyviral VPg protein interacts critically with proteins of the translation initiation complex to promote successful infection (Robaglia & Caranta, 2006). Replication occurs in viral factories resulting from the fusion of cellular and plastidial endomembranes, mediated by the viral 6K2 protein (Grangeon et al., 2012; Wei et al., 2013). The CI and PIPO proteins (Wei et al., 2010) are involved in cell-to-cell movement. Potyviral Hc-pro, which mediates aphid transmission, is also an efficient viral suppressor of the host antiviral RNA silencing (Qu & Morris, 2005). As potyviruses share many structural and biochemical properties and form a phylogenetically homogeneous group of recent radiation (Gibbs & Ohshima, 2010), it is often assumed that they also engage in essentially similar cellular interactions with their hosts.

The target of rapamycin (TOR) protein kinase defines an ancient, conserved, signalling pathway that is involved in controlling cell metabolism, growth and survival in most eukaryotes (Laplante & Sabatini, 2012). Functional studies in plants have demonstrated an important role for TOR in regulating mRNA translation (Deprost et al., 2007; Ren et al., 2011; Schepetilnikov et al., 2013), energy metabolism (Ren et al., 2012), cell cycle progression (Xiong et al., 2013) and autophagy (Liu & Bassham, 2010). In addition, the TOR signalling pathway was recruited to plant-specific functions such as starch synthesis, adaptation to day length and cell wall (Leiber et al., 2010; Moreau et al., 2012; Caldana et al., 2013).

Modulation of mammalian TOR (mTOR) activity by viruses has been described as a way to gain control of the cell translation machinery. Viruses that rely on cap-dependent translation activate mTOR to promote viral protein synthesis (Chuluunbaatar et al., 2010; Kudchodkar et al., 2004, 2006; Moorman & Shenk, 2010). Conversely, viruses that depend on cap-independent IRES-driven translation inactivate mTOR to reduce host cell translation while diverting...
the translation apparatus toward viral RNAs (Gingras et al., 1996). Other processes crucial to viral life cycles have been shown to involve TOR. Hepatitis C virus activates mTOR to promote cell survival and ensure steady-state levels of viral replication, thereby maintaining a state of chronic infection (Mannová & Beretta, 2005; Peng et al., 2010; Shrivastava et al., 2012). Vesicular stomatitis virus circumvents innate host defences by impairing TOR-dependent interferon production (Alain et al., 2010). In plants, cauliflower mosaic virus TAV protein activates TOR to potentiate translation of polycistronic viral RNAs (Schepetilnikov et al., 2011).

Growing evidence that TOR signalling is involved in many different viral infection processes prompted us to analyse its role during infection by potyviruses. We first examined the susceptibility of Arabidopsis TOR RNA interference (RNAi) lines, which display constitutively reduced TOR expression levels (Deprost et al., 2007), to watermelon mosaic virus (WMV) (Lecoq & Desbiez, 2008) (Fig. 1a). Although replicating to high titres, WMV infection does not cause visible symptoms on Arabidopsis (Ouibrahim et al., 2014). Plants grown under short days (8 h light/16 h dark cycle) were mechanically inoculated with WMV, and the ability of the virus to infect was determined by anti-coat protein (CP) Double Antibody Sandwich - Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) at 14 days post-inoculation (p.i.) (Fig. 1b). The Col-0 control line proved susceptible to WMV, with viral CP accumulating to a high level in 91 ± 0.2 (SD)% of inoculated plants. In contrast, the TOR RNAi lines were found to be partially resistant to WMV, as viral CP accumulation was observed for only 41 ± 2.3 (SD)% and 62 ± 2.6 SD % of inoculated 65-1 and 35-7 plants, respectively (Fig. 1b). An Reverse-transcription polymerase chain reaction (RT-PCR) assay detecting the viral genomic RNA confirmed the partial resistance phenotype. WMV genomic RNA was detected both locally and systemically in Col-0, whereas it was undetectable in inoculated or systemic leaves for the majority of 65-1 and 35-7 plants, respectively (Fig. 1c). A few TOR RNAi plants sustained local and systemic WMV infection, but virus accumulation was much lower than in Col-0 (Fig. 1c: lines 6, 12, 13). In these plants, viral RNA accumulation was further quantified by quantitative RT-PCR, showing that the amount of WMV RNA was 11 to 20 % (65-1 line) and 7 to 26 % (35-7 line) of that of control Col-0 plants (Fig. 1d). Together, these results demonstrate that WMV is either unable to infect TOR RNAi lines or its ability to mount a systemic infection is reduced.

To independently evaluate the requirement of TOR for WMV accumulation, we used AZD-8055, a recently developed ATP-competitive inhibitor of mTOR that also targets TOR in Arabidopsis (Montané & Menand, 2013). AZD-8055 application was performed as described in the Fig. 2 legend. Dose–response experiments were conducted to test the effect of AZD-8055 on plant growth at a stage corresponding to the virus inoculation stage (Fig. 2a). Based on these data, AZD-8055 at 7.5 μM and 30 μM was used in subsequent virus inoculation experiments. In a first set of experiments, Col-0 plants were mock or AZD-8055 treated 1 day prior to virus inoculation. RT-PCR analysis performed at 10 days p.i. showed that most plants treated by the TOR inhibitor were not infected by WMV while almost all control plants systemically accumulated viral RNA. Indeed, the virus did spread in 92 ± 2.1 SD % of mock treated controls, but only in 17 ± 2.8 SD % and 21 ± 2.6 SD % of plants treated with 7.5 μM and 30 μM of AZD-8055, respectively (Fig. 2b). In order to inhibit TOR at a stage where WMV infection is already systemic, Col-0 plants were mock- or AZD-8055-treated 10 days after virus inoculation. Viral RNA accumulation in whole plant extracts was assessed 6 days later.
AZD-8055 potyvirus, turnip mosaic virus (TuMV).

We also evaluated the impact of TOR inhibition on another infection. An active TOR pathway during both early and systemic escape infection and recover from it, the virus may require the WMV infectious cycle. As TOR deficient plants can

clusion that TOR is a key component involved throughout the majority of AZD treated plants. Collectively, these data are consistent with and complement those obtained by analysing TOR data are consistent with and complement those obtained in the majority of AZD treated plants. Thus, TOR inhibition interferes with the ability of WMV to sustain systemic infection; moreover, treatment with the TOR inhibitor is able to cure the plants of WMV infection. Indeed, even at 10 days p.i., when WMV has spread systematically, the viral RNA became undetectable in 58 ± 0.5 SD % and 88 ± 3.1 SD % of infected plants treated with 7.5 µM and 30 µM of AZD-8055, respectively. Thus, TOR inhibition interferes with the ability of WMV to sustain systemic infection; moreover, treatment with the TOR inhibitor is able to cure the plants of WMV infection. Indeed, even at 10 days p.i., when WMV has spread systematically, the viral RNA became undetectable in the majority of AZD treated plants. Collectively, these data are consistent with and complement those obtained by analysing TOR RNAi lines. They strengthen the conclusion that TOR is a key component involved throughout the WMV infectious cycle. As TOR deficient plants can escape infection and recover from it, the virus may require an active TOR pathway during both early and systemic infection.

We also evaluated the impact of TOR inhibition on another potyvirus, turnip mosaic virus (TuMV). TOR RNAi lines were mechanically inoculated with a GFP-tagged strain of TuMV (Beauchemin et al., 2005) and the progress of virus infection was analysed by monitoring GFP fluorescence every day for a 10 day period. At 3 days p.i., all Col-0 plants exhibited fluorescent GFP foci in inoculated leaves, while only a few 65-1 and 35-7 plants showed GFP-positive signals (five and four plants among a group of ten, respectively). By 5 days p.i., GFP was visible in the inoculated leaves of all plants, but the number and size of fluorescent spots were reduced in the TOR RNAi lines compared to Col-0 (Fig. 3a). At 7 days p.i., clear GFP signals were observed in the systemic leaves of all Col-0 plants, but only in half of the TOR RNAi lines. By 10 days p.i., GFP was produced systemically in all plants. Thus, TuMV-GFP is able to systemically infect the TOR RNAi lines although with a delay in infection compared to WT. DAS-ELISAs performed 14 days after inoculation with the non-tagged TuMV strain CDN1 confirmed the efficient systemic virus infection of the TOR RNAi lines (Fig. 3b). The effect of TOR inhibitors on TuMV infection was then evaluated. In this case, TuMV-GFP (Grangeon et al., 2012) was inoculated by agroinfiltration of a fully expanded leaf 5 days before treatment with 30 µM of AZD-8055. Either systemic leaves or all leaves were treated. GFP fluorescence of systemic leaves was recorded 10 days after treatment. Although this experiment did not give a quantitative view of virus accumulation, the strong fluorescence observed in young systemic leaves shows that all plants were susceptible to TuMV infection (Fig. 3c). TuMV, like other potyviruses, encodes Hc-Pro, an efficient viral silencing inhibitor (Kasschau et al., 2003). The susceptibility of AZ-8055 treated plants to TuMV indicates that a potential reversal of RNAi, in TOR RNAi lines, is probably not the cause of their susceptibility.

Two main scenarios can be considered to provide an explanation of the mechanism underlying WMV resistance in TOR-deficient conditions. TOR might be involved, directly or indirectly, in an essential step of the virus life cycle, so that inactivation of TOR would impair viral multiplication.
Functional features of plant TOR support the idea that it may be required for efficient viral RNA translation. Indeed, global translational defects evidenced by low levels of polysomes, soluble protein and rRNA accumulation have been observed upon TOR inhibition (Deprost et al., 2007; Ren et al., 2011). TOR also interacts with eIF3c and possibly associates with ribosomal subunits (Schepetilnikov et al., 2011). These results suggest a mechanistic link between TOR and the translation initiation machinery, which plays a critical role in plant–potyvirus interactions (Le Gall et al., 2011).

Indeed, several animal and plant viruses were found to hijack the TOR pathway to reroute host translational functions (Chuluunbaatar et al., 2010; Gingras et al., 1996; Kudchodkar et al., 2004, 2006; Moorman & Shenk, 2010; O’Shea et al., 2005; Schepetilnikov et al., 2011; Yu et al., 2005).

The second scenario involves a TOR-dependent antiviral defence mechanism. In Arabidopsis, downregulation of TOR has been associated with constitutive autophagy (Liu & Bassham, 2010). This suggests that TOR deficiency could lead to active autophagic elimination of viral components, in line with the well-known role of autophagy in immune defence against invading pathogens, including many viruses (Shoji-Kawata & Levine, 2009). However, we also found that, in contrast to WMV, downregulation of TOR did not have such a strong impact on the accumulation of TuMV. This suggests that TuMV interacts differently with components of the TOR pathway. Although potyviruses form a homogeneous group, they can differ in cellular aspects of their infection process. For example, in Arabidopsis, TuMV cannot multiply in mutants of the isoform of eukaryotic Initiation Factor 4E [eIF(iso)4E] (Robaglia & Caranta, 2006), while WMV replicates efficiently in both the eIF(iso)4E and eIF4E mutants (J. L. Gallois). This suggests that WMV can use both eIF4E isoforms, or none of them, while TuMV is strictly dependent on a functional eIF(iso)4E. Interestingly, these two viruses also belong to distinct phylogenetic groups among potyviruses. WMV belongs to the Beet Common Mosaic Virus (BCMV) supergroup, and TuMV is related to...
to the Potato Virus Y (PVY) supergroup (Gibbs & Ohshima, 2010). Several subcellular components were found to be involved in successful potyvirus infection. These comprise the endomembranes and organelles (Grangeon et al. 2012; Wei et al., 2013), the cytoskeleton (Haikonen et al., 2013) and post-translational modification complexes (Pérez et al., 2013). All these cellular components have also been functionally connected to the TOR pathway, and potyvirus species or subgroups may engage specific interactions with them.

Overall, we have found that the TOR pathway plays a role in both establishment and maintenance of systemic WMV infection in Arabidopsis, and that inhibition of TOR activity is sufficient to cure plants of WMV infection. Given the agronomic importance of potyviruses, the investigation of their cellular links with the TOR pathway may offer new breeding or therapeutic avenues using gene titration of their cellular links with the TOR pathway may engage specific interactions with them.


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