Target of rapamycin controls hyphal growth and pathogenicity through FoTIP4 in *Fusarium oxysporum*

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Abstract

*Fusarium oxysporum* is the causal agent of the devastating Fusarium wilt by invading and colonizing the vascular system in various plants, resulting in substantial economic losses worldwide. Target of rapamycin (TOR) is a central regulator that controls intracellular metabolism, cell growth, and stress responses in eukaryotes, but little is known about TOR signalling in *F. oxysporum*. In this study, we identified conserved FoTOR signalling pathway components including FoTORC1 and FoTORC2. Pharmacological assays showed that *F. oxysporum* is hypersensitive to rapamycin in the presence of FoFKBP12 while the deletion mutant strain ΔFofkbp12 is insensitive to rapamycin. Transcriptomic data indicated that FoTOR signalling controls multiple metabolic processes including ribosome biogenesis and cell wall-degrading enzymes (CWDEs). Genetic analysis revealed that FoTOR1 interacting protein 4 (FoTIP4) acts as a new component of FoTOR signalling to regulate hyphal growth and pathogenicity of *F. oxysporum*. Importantly, transcript levels of genes associated with ribosome biogenesis and CWDEs were dramatically downregulated in the ΔFotip4 mutant strain. Electrophoretic mobility shift assays showed that FoTIP4 can bind to the promoters of ribosome biogenesis- and CWDE-related genes to positively regulate the expression of these genes. These results suggest that FoTOR signalling plays central roles in regulating hyphal growth and pathogenicity of *F. oxysporum* and provide new insights into FoTOR1 as a target for controlling and preventing Fusarium wilt in plants.

**KEYWORDS**

FoTOR1 interacting protein 4, *Fusarium oxysporum*, hyphal growth, pathogenicity, target of rapamycin

1 | INTRODUCTION

The fungus *Fusarium oxysporum*, a hemibiotrophic and soilborne species with more than 100 formae speciales, is the causal agent of Fusarium wilt disease, which affects many important agricultural crops worldwide, such as cotton, banana, tomato, and potato (Garcia Bayona et al., 2011; Husaini et al., 2018; Ozbay & Newman, 2004; Ploetz, 2015; Raza et al., 2016; Schmidt et al., 2016), resulting in economic losses. *F. oxysporum* is the major pathogen in various Fusarium wilt diseases and was ranked fifth in a survey of the top 10 fungal...
plant pathogens (Dean et al., 2012), and one of the most difficult plant diseases to control because the spores of *F. oxysporum* can survive in soil for over 10 years (Husaini et al., 2018). The proximity of roots induces the dormant chlamydospores to germinate and initiate infection. Elongated hyphae start to adhere to the plant root surface and penetrate the roots through wounds or root tips. Ultimately, invasive hyphae reach the xylem vessels and proliferate, causing disease (Berrocallobo & Molina, 2008; Di et al., 2003). In the process of phytopathogenic fungi infecting the host, the plant cell wall is a major physical barrier that phytopathogenic fungi must overcome by producing a variety of cell wall-degrading enzymes (CWDEs) that allow fungi to invade host tissues through the degradation of plant cell wall components. The pathogen *F. oxysporum* secretes various CWDEs, such as pectinase, cellulase, and β-glucosidase, to degrade the plant cell wall (Calero-Nieto et al., 2007; Jonkers, 2009; Ospina-Giraldo et al., 2003). As a result, pectin can obstruct the vasculature in plants, thereby preventing water absorption, resulting in plant wilt and death (King et al., 2011); free monosaccharide and oligosaccharides that originate from the plant cell wall are used for fungal growth and reproduction (Gibson et al., 2011).

Target of rapamycin (TOR) is an evolutionarily conserved Ser/Thr protein kinase in eukaryotes. It is well known that the TOR signalling pathway regulates cell growth and proliferation in response to nutrients, energy, and stresses (De Virgilio & Loewith, 2006; Dobrenel et al., 2016; Yuan et al., 2013). Two TOR genes were first identified by screening rapamycin (RAP)-insensitive mutants in budding yeast (Saccharomyces cerevisiae) (Heitman et al., 1991b; Kunz et al., 1993). However, only a single TOR gene has been identified in *Arabidopsis thaliana*, most animals, and humans (Menand et al., 2002; Sabers et al., 1995). The TOR protein contains five conserved regions: HEAT repeats, a FAT domain, an FRB domain, a kinase domain, and an FATC domain (Schmelze & Hall, 2000; Wullschleger et al., 2006). TOR forms two structurally and functionally distinct protein complexes: TOR complex 1 (TORC1) and TORC2 (Loewith et al., 2002). Among eukaryotic species, TORC1 contains the TORC1 regulatory subunits KOG1 (known as RAPTOR in mammals) and Lethal with SEC13 protein 8 (LST8), which regulates cell growth and metabolism in response to nutrient and energy requirements (Wang & Proud, 2009). TORC2 possesses two TORC2-specific subunits, AVO3 (known as RICTOR in mammals) and AVO1 (known as SIN1 in mammals), which controls spatial cell growth and survival by regulating cytoskeletal structure and polarity (De Virgilio & Loewith, 2006; Wullschleger et al., 2006). Despite functional importance, little is known about the TOR signalling pathway in *F. oxysporum*.

In rapidly growing cells, ribosome biogenesis is a major energy-consuming process that accounts for a significant proportion of total transcriptional output (Warner, 1999). The regulation of ribosome biogenesis occurs primarily at the transcriptional level and involves all three nuclear RNA polymerases (ladevaia et al., 2014; Martin et al., 2006). TORC1 positively regulates several steps in ribosome biogenesis, including ribosomal RNA transcription and the synthesis of ribosomal proteins and other components required for ribosome assembly and biogenesis (Ben-Sahra et al., 2013; Chauvin et al., 2014; Tsang et al., 2010). TORC1 interacts directly with Sfp1 and phosphorylates Sfp1 to regulate ribosome biogenesis (Lemplainen et al., 2009). Sfp1 is a transcriptional activator of ribosome biogenesis genes (Fingerman et al., 2003; Marion et al., 2004). Under optimal growth conditions, Sfp1 is phosphorylated by TORC1 and then binds to the promoters of ribosome biogenesis genes to promote their expression in the nucleus. By contrast, nutrient depletion results in its relocalization from the nucleus to the cytoplasm in yeast (Lemplainen et al., 2009). The AGC-family kinase SCH9 (known as S6K in mammals) is another downstream regulatory factor of TORC1. TORC1 phosphorylates and activates SCH9 to regulate ribosome protein synthesis (Chauvin et al., 2014; Iadevaia et al., 2014; Magnuson et al., 2012).

RAP is a macrolide immunosuppressant produced by *Streptomyces hygroscopicus*. It mimics nutrient limitation to arrest cell growth and proliferation. RAP specifically binds to FK506 binding protein of 12 kDa (FKBP12), which interacts with the FRB domain of TOR to form a ternary complex (Heitman et al., 1991b; Loewith et al., 2002). The resulting complex prevents TOR from associating with its scaffold protein RAPTOR and phosphorylating its substrate proteins (Aylett et al., 2016; Hara et al., 2002), which hinders TOR protein activity and results in irreversible arrest of the cell cycle at the G1 phase (Heitman et al., 1991a). RAP can inhibit TORC1, but TORC2 is insensitive to RAP (Loewith et al., 2002). The TORC2-specific subunit RICTOR plays indispensable roles in TORC2 function (Gaubitz et al., 2016; Wullschleger et al., 2005). A recent crosslinking study of TORC2 in budding yeast demonstrated that the C-terminus of RICTOR occupies the FRB domain of TOR kinase, preventing the RAP-FKBP12 complex from binding to the FRB domain of TOR kinase in TORC2, which makes TORC2 insensitive to RAP (Gaubitz et al., 2015). Recent studies have also revealed that treatment with ATP-competitive TOR protein kinase inhibitors, including Torin1, Torin2, Ku-0063794 (KU), and AZD-8055 (AZD), can result in different effects on both TORC1 and TORC2 than RAP treatment (Chresta et al., 2010; Garcia-Martinez et al., 2009), as TOR is directly and specifically targeted by the ATP-binding pocket of the TOR kinase domain, suppressing the functions of both TORC1 and TORC2 complexes (Benjamin et al., 2011).
as a promising target for controlling and preventing Fusarium wilt caused by F. oxysporum in plants.

2 | RESULTS

2.1 | The TOR signalling pathway is conserved in F. oxysporum

In order to identify evolutionarily conserved TOR signalling pathway components in F. oxysporum, BLASTp analysis of the F. oxysporum f. sp. lycopersici genome database (http://fungi.ensembl.org/Fusarium_oxysporum/Info/Index?db=core) using Schizosaccharomyces pombe TOR signalling pathway components as reference was performed. We found that putative homologous gene sequences encoding key components of TORC1, including TOR, KOG1, and LST8, were present in the genome of F. oxysporum, and putative homologues encoding specific components of TORC2, such as AVO3 and AVO1, were also found in the F. oxysporum genome (Table 1). Interestingly, two TOR gene homologues were detected (FOXG_18412 and FOXG_15946) in the F. oxysporum genome (Lopez-Berges et al., 2010) (Figure 1a). Further analysis revealed that the FoTOR1 (FOXG_18412) protein showed similar conserved domains as in yeast and human, whereas the FATC domain was not detected in the FoTOR2 (FOXG_15946) protein (Figure 1b). Phylogenetic analysis and kinase domain sequence alignment with proteins from other representative organisms indicated that FoTOR1 and FoTOR2 are evolutionarily conserved (Figure 1c,d). Interestingly, there is only one TOR gene in some formae speciales of F. oxysporum (Table S1). We found TOR kinase expansion in eight out of 14 sequenced strains, with six strains containing one copy, seven strains containing two copies, and one strain containing three copies. Phylogenetic analysis showed that the orthologous copies of the TOR kinase (indicated as Core TOR) form a monophyletic group containing two copies, and one strain containing three copies. FoFKBP12 was found (Table 1). Sequence alignment and phylogenetic analysis showed that the orthologous copies of the TOR kinase have different effects on TOR in different species (Chresta et al., 2010; Garcia-Martinez et al., 2009; Montane & Menand, 2013; Xiong et al., 2017). Furthermore, the germination rate and production of spores were reduced upon RAP and Torin1 treatment, and the relative transcript levels of sporulation-related genes were significantly decreased upon FoTOR inhibition in F. oxysporum (Figure S3). In addition, we tested the sensitivity of the ΔFotor2 mutants to RAP and Torin1. The results showed that the growth of the ΔFotor2 mutant was similar to that of the wildtype F. oxysporum strain upon RAP and Torin1 treatment (Figure S4), indicating that TOR inhibitors inhibit mycelial growth of F. oxysporum in a FoTOR2-independent manner. Combined treatment with RAP and Torin1 exerted more obvious growth inhibitory effects than treatment with RAP or Torin1 alone (Figure 2e). The IC50 value of a single drug (RAP 10 nM, Torin1 1 μM) was significantly reduced when F. oxysporum was subjected to combined treatment (Figure 2f), implying that potential synergistic effects can be generated by combining RAP with Torin1. Next, a computer-simulated affected fraction (Fa)–combination index (CI) curve was assessed using CompuSyn software. A synergistic effect (CI < 1) was observed when hyphae were treated with a combination of RAP + Torin1 (Figure 2g). These results suggest that RAP and Torin1 inhibit hyphal growth by simultaneously targeting the TOR signalling pathway in F. oxysporum.

2.2 | TOR inhibitors inhibit mycelial growth of F. oxysporum

RAP is a well-known TOR-specific inhibitor. It specifically binds to FKBP12 to form a gain-of-function complex, which negatively regulates TOR kinase activity (Heitman et al., 1991b). To test whether RAP has an inhibitory effect on TOR in F. oxysporum, we first assayed the sensitivity of wildtype F. oxysporum to RAP. The growth inhibition of F. oxysporum was positively correlated with increasing concentrations of RAP (Figure 2a,c). Consistent with the results of a previous report (Lopez-Berges et al., 2010), RAP inhibited hyphal growth and development in a dose-dependent manner in F. oxysporum. Besides, the second-generation TOR inhibitors Torin1, KU, and AZD were also used for the growth assay. Consistent with the observations in yeast (Atkin et al., 2014), Torin1 but not KU or AZD partially inhibited hyphal growth with a much higher half maximal inhibitory concentration (IC50) value (20 μM) than that of RAP (0.1 μM) (Figure 2b,d), indicating that ATP-competitive inhibitors of TOR kinase have different effects on TOR in different species (Chresta et al., 2010). Inhibition of SpFKBP12 was found (Table 1). Sequence alignment and phylogenetic analysis showed that the inhibitory effect of RAP on TOR in F. oxysporum is mediated by a computer-simulated affected fraction (Fa)–combination index (CI) curve was assessed using CompuSyn software. These results suggest that RAP and Torin1 inhibit hyphal growth by simultaneously targeting the TOR signalling pathway in F. oxysporum.

2.3 | Deletion of FoFKBP12 leads to insensitivity to RAP in F. oxysporum

We found that RAP effectively inhibited hyphal growth at a low concentration (Figure 2). It was previously reported that FKBP12 mediates the inhibitory effects of RAP on TOR (Heitman et al., 1991b). Therefore, we analysed the sequences of FoFKBP12 in the F. oxysporum genome. A single copy of an FKBP12 orthologue (FOXG_08379, named FoFKBP12) encoding a protein with 59% similarity to SpFKBP12 was found (Table 1). Sequence alignment and phylogenetic
analysis showed that FoFKBP12 is evolutionarily conserved among species (Figure 3a,b). Amino acids known to be involved in the formation of the RAP inhibitory ternary complex were conserved in the FoFKBP12 sequence (Figure 3a). In order to test the ability of FoFKBP12 to bind RAP and TOR, we generated FoFKBP12 deletion mutants (Δfokbp12) by a homologous recombination gene deletion strategy (Figure S5a,b). Morphological analyses showed that hyphal growth of the mutant was similar to that of wildtype F. oxysporum biogenesis and CWDEs in Arabidopsis, indicating that FoFKBP12 is dispensable for hyphal growth. The RAP sensitivity test showed that the Δfokbp12 mutant was insensitive to RAP, but the sensitivity to RAP was restored in the complemented strain (Δfokbp12 + FoFKBP12) (Figure 3c). As expected, the wildtype, Δfokbp12, and Δfokbp12 + FoFKBP12 lines showed the same growth phenotypes upon Torin1 treatment (Figure 3c), indicating that Torin1 inhibits mycelial growth of F. oxysporum in an FoFKBP12-independent manner. A previous study showed that Arabidopsis has adapted an evolutionary mutation in the FKBP12 gene, resulting in loss of its ability to bind RAP (Sormani et al., 2007). In order to further confirm the formation of FoFKBP12, FoFKBP12 overexpression Arabidopsis transgenic lines were generated. All FoFKBP12 transgenic lines displayed sensitivity to RAP, which reflected shorter primary root length, smaller cotyledons, and lower fresh weight compared with wildtype Arabidopsis (Figure 3d,e), and this observation was consistent with observations in the ScFKBP12 overexpression line (BP12-2) in Arabidopsis (Ren et al., 2012). We tested the ability of FoFKBP12 to bind RAP in an S. cerevisiae (Y2HGold strain) FKBP12 deletion mutant (Δscfkbp12). The wildtype yeast strain was sensitive to RAP while the Δscfkbp12 mutant was RAP-resistant. FoFKBP12 restored the sensitivity to RAP in the Δscfkbp12 + FoFKBP12 yeast strain (Figure 3f). We analysed the interaction between FoTOR1 and FoFKBP12 by Y2H assays. For each experiment, one pair of Y2H plasmids was cotransformed into the Δscfkbp12 mutant strain. FoFFKBP12 was unable to interact with FoTOR1 without RAP treatment. By contrast, when the medium was supplemented with 1 μg/ml RAP, FoFKBP12 interacted strongly with FoTOR1 (Figure 3g). These results indicate that FoFKBP12 mediates the inhibitory effects of RAP on FoTOR activity in F. oxysporum.

### 2.4 FoTOR1 is a key regulator of ribosome biogenesis and CWDEs in F. oxysporum

The TOR signalling pathway integrates various extracellular and intracellular signals, such as growth factors, nutrients, energy, and other environmental cues, regulating multiple cellular processes, including ribosome biogenesis, protein synthesis, autophagy, and metabolic processes (De Virgilio & Loewith, 2006; Dobrenel et al., 2012). The TOR signalling pathway integrates various extracellular and intracellular signals, such as growth factors, nutrients, energy, and other environmental cues, regulating multiple cellular processes, including ribosome biogenesis, protein synthesis, autophagy, and metabolic processes (De Virgilio & Loewith, 2006; Dobrenel et al., 2012).
FIGURE 1 Structural analysis and functional characterization of FoTORs in Fusarium oxysporum. (a) The genome structure of FoTORs. Red represents exons and white rectangles indicate introns. (b) The conserved functional domains of FoTOR proteins. Conserved domains of FoTOR proteins are compared with those from other organisms. Each value indicates the percentage of identity with the corresponding domain sequences of FoTOR1. The number in parentheses represents the number of amino acids. aa, amino acids. (c) Phylogenetic relationship between the FoTOR proteins and homologues of Saccharomyces cerevisiae (Sc), Homo sapiens (Hs), and Fusarium graminearum (Fg). The phylogenetic tree was generated with MEGA 4.0 using the neighbour-joining method. (d) Comparison of amino acid sequences of the kinase domains of FoTOR proteins with those from other representative organisms, including Sc, Hs, and Fg. (e) Yeast two-hybrid analysis of the interaction between FoTOR1 and FoKOG1 or FoAVO3. Serial dilutions of yeast cells (cells/ml) transformed with the bait and prey constructs were assayed for growth on SD−His−Leu−Trp−Ade medium at 28 °C for 3 days. (f) Mutant generation of FoTOR2 in F. oxysporum. Gel electrophoresis of the TOR2 gene and the Hyg cassette. The TOR2 gene and the Hyg cassette were amplified from wildtype F. oxysporum, ΔFotor2 mutants, and complemented (Com) strains with TOR2 F/R1 and Hyg F/R primers, respectively. Fo, F. oxysporum; Com, complemented. (g) The phenotype of FoTOR2 deletion mutants (ΔFotor2) was similar to that of the wildtype F. oxysporum strain in potato dextrose agar.
To further elucidate the function of the TOR signalling pathway in vegetative growth of *F. oxysporum*, a gene expression profile analysis was performed in *F. oxysporum* upon FoTOR inhibition with RAP treatment. Approximately 61% and 52% of the reads were mapped to the annotated *F. oxysporum* genome and the unigenes, respectively.
FIGURE 3 FoFKBP12 mediates inhibitory effects of RAP on FoTOR activity in Fusarium oxysporum. (a) Comparison of amino acid sequences of FKBP12 from Saccharomyces cerevisiae (Sc), Homo sapiens (Hs), Schizosaccharomyces pombe (Sp), Candida glabrata (Cg), Botrytis cinerea (Bc), Verticillium dahliae (Vd), F. oxysporum (Fo), and Fusarium graminearum (Fg). The black boxes indicate amino acids involved in ternary complex formation. (b) Phylogenetic relationship between FoFKBP12 protein and homologues from other organisms in (a). The phylogenetic tree was generated with MEGA 4.0 using the neighbour-joining method (1,000 bootstrap replicates). (c) Deletion of FoFKBP12 (ΔFoFKBP12) led to resistance to RAP in F. oxysporum. Hyphae of wildtype F. oxysporum, ΔFoFKBP12, and the complemented strain (ΔFoFKBP12 + FoFKBP12) were incubated on medium containing DMSO, RAP, or Torin1 and grown for 5 days. (d) FoFKBP12 overexpression transgenic Arabidopsis lines were sensitive to RAP (5 μM). FoFKBP12, BP12-2, and wildtype seeds were cultured on Murashige & Skoog (MS) plates containing DMSO or RAP for 10 days. Representative seedlings are shown. The experiment was repeated three times. Bar = 1 cm. (e) Fresh weight and root length of FoFKBP12 overexpression transgenic Arabidopsis lines exposed to RAP (5 μM). Each column represents the average of 10 seedlings. The data are presented as the mean ± SD of n = 3 independent experiments. **p < 0.01 compared with wildtype plants (Student’s t test). (f) FoFKBP12 restored the sensitivity to RAP in the yeast FKBP12 mutant (ΔScfkbp12). Strains growth on yeast-peptone-dextrose (YPD) medium with (+) or without (−) 1 μg/ml RAP at 28 °C for 3 days. (g) Yeast two-hybrid analysis of the interaction between FoTOR1 and FoFKBP12. Serial dilutions of yeast ΔScfkbp12 mutant cells (cells/ml) transformed with the FoTOR1-BD and FoFKBP12-AD constructs were assayed for growth on SD–His–Leu–Trp–Ade medium with (+) or without (−) 1 μg/ml RAP at 28 °C for 3 days.
(Figure 4a). A total of 4,237 differentially expressed genes (DEGs) were found between the RAP- and DMSO-treated fungi, of which 1,931 DEGs were downregulated and 2,306 DEGs were upregulated (Figure 4b). To identify the biological functions of these DEGs, gene ontology (GO) enrichment analysis was performed. A total of 334 upregulated GO terms and 156 downregulated GO terms were enriched (Table S2). Among the upregulated genes, the GO terms organism compound metabolic process (GO: 1901564) and organism compound biosynthetic process (GO: 1901566) were highly enriched (Figure 4c, Table S2). Among the downregulated genes, the GO terms transporter activity (GO: 0005215) and transmembrane transporter activity (GO: 0022857) were the most significantly enriched (Figure 4d, Table S2).

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that 55 DEGs, including 47 downregulated and eight upregulated genes, were involved in the "ribosome biogenesis in eukaryotes" KEGG pathway (Figure 4e, Table S3), which was the most enriched pathway among the 96 KEGG pathways detected. Additionally, in the GO category of biological processes, the regulation of ribosome biogenesis, a key signal of cell growth and proliferation, was enriched among the downregulated genes (Figure 4d). Importantly, the genes encoding nucleolar proteins 56 and 58 and U3 small nuclear RNA-associated proteins were significantly downregulated (Table S3). These ribosomal core proteins combine with small nucleolar RNAs to form small nucleolar ribonucleoproteins that play a crucial role in ribosome biogenesis by guiding the processing and modification of preribosomal RNAs. To further confirm these observations, 10 randomly selected genes involved in ribosome biogenesis were selected for quantitative reverse transcription PCR (RT-qPCR) analysis (Figure 4f). They were all downregulated, which was consistent with the RNA-seq data. Additionally, we tested the expression levels of ribosome biogenesis genes in ΔFotor2 and ΔFokkbp12 lines treated with RAP. RAP had no effect on the expression levels of ribosome biogenesis genes in the ΔFokkbp12 mutant, while these genes were downregulated in the ΔFotor2 line treated with RAP (Figure 5a). The RNA-seq results support the previous observations that TOR suppression by RAP inhibits hyphal growth, suggesting that the FoTOR signalling pathway positively regulates ribosome biogenesis and vegetative growth in F. oxysporum.

In the process of host infection, F. oxysporum secretes a large number of CWDEs to degrade plant cell walls, which facilitates invasion and colonization. The mRNA levels of CWDEs, including cellulases, xylanase, and pectinases, were changed upon TOR inhibition (Table S4). Most of the differentially expressed CWDE genes were downregulated (Table 2). Remarkably, most downregulated CWDE genes encode β-glucosidases, which are important components of the cellulase system. β-Glucosidase hydrolyses cellobiose by cleaving the β-(1,4) linkage to generate α-glucose (Olaquyjibe et al., 2016). In addition, xylanase, endoglucanase, and pectate lyase were dramatically downregulated at the mRNA level (Table 2). The expression levels of CWDE genes displayed a consistent downregulation in the wildtype F. oxysporum and ΔFotor2 lines treated with RAP. However, RAP had no effect on the expression levels of CWDE genes in the ΔFokkbp12 mutant (Figure 5b). These results indicate that FoTOR1 plays an important role in the regulation of CWDEs in F. oxysporum.

2.5 | FoTOR1 interacts with FoTIP4 to regulate ribosome biogenesis in F. oxysporum

To further identify new effectors of the FoTOR signalling pathway, we used the Y2H system to screen for interacting proteins of FoTOR1 in a cDNA library of F. oxysporum. The results revealed putative FoTOR1 interacting proteins including FoTIP1/FoFKO1, FoTIP2/FoLST8, and FoTIP3/FoFKBP12. Importantly, a novel FoTOR1 interacting protein 4 (FoTIP4) was detected in F. oxysporum (Figure 5a). Sequence alignment revealed that FoTIP4 is homologous to ScSFP1 with low amino acid sequence similarity (18%). SFP1 is a C2H2-type zinc finger transcription factor that plays an essential role in the control of cell size, regulating the expression of ribosomal proteins and ribosome biogenesis genes in response to nutrient stress, in yeast (Fingerman et al., 2003; Jorgensen et al., 2002; Lempiainen et al., 2009). The gene sequence encoding FoTIP4 (FOXG_00980) is located on chromosome 1 in the F. oxysporum genome. Further Y2H assays verified the interaction between FoTIP4 and FoTOR1 (Figure 5b). Interestingly, FoTIP4 showed interaction with the heat repeat domain of FoTOR1 (Figure 5c,d).

To examine the functions of FoTIP4 in F. oxysporum, FoTIP4 deletion mutant strains (ΔFotip4) were generated (Figure S5c,d). Deletion of FoTIP4 in F. oxysporum led to a considerable defect in hyphal growth and a large reduction in biomass on PDA, and the defect was fully restored in the complemented strain (ΔFotip4 + FoTIP4) (Figure 6a,b). In addition, ΔFotip4 strains were more sensitive to RAP and the protein synthesis inhibitor cycloheximide (CHX) than the wildtype F. oxysporum strain. ΔFotip4 strains displayed a synergistic effect between the FoTIP4 mutation and RAP treatment, but not between the FoTIP4 mutation and treatment with the proteasome inhibitor MG-132. The complemented strain (ΔFotip4 + FoTIP4) showed no detectable changes in sensitivity to RAP and CHX compared with the wildtype F. oxysporum strain (Figures 6a and S7a,b), suggesting that FoTIP4 plays a role in protein synthesis rather than degradation. Furthermore, the number of spores of the ΔFotip4 strain was significantly reduced compared with the wildtype F. oxysporum strain (Figure S8), implying that FoTIP4 is also involved in the regulation of spore development. The transcript levels of ribosome biogenesis genes were significantly decreased in the ΔFotip4 strain compared with the wildtype F. oxysporum strain (Figure 6c). ScSFP1 acts as a transcriptional activator of ribosome biogenesis genes whose promoters contain a ribosomal RNA processing element (RRPE) ([A/T]GAAAATTT) and a polymerase A and C (PAC) box (G[C/A]GATGAG) (Fingerman et al., 2003; Jorgensen et al., 2002). Because the promoter of FoSIK1 (FOXG_12883), which is the key regulator of ribosome biogenesis genes, contains the typical RRPE element and was differentially expressed in our RNA-seq data, we tested whether FoTIP4 can bind to the promoter of FoSIK1
The results showed that FoTIP4 bound to the RRPE element in the promoter of FoSIK1 (Figure 6d). Other ribosome biogenesis genes containing an RRPE regulatory element, such as FOXG_00396 and FOXG_11329, were downregulated in our transcriptomic data (Table S3), indicating that FoTIP4 positively regulates the expression of ribosome biogenesis genes in F. oxysporum. Importantly, reduced FoTOR activity by RAP caused FoTIP4 relocalization to the cytoplasm from the nucleus.
**CWDEs in** The FoTIP4 signalling pathway is a key regulator of the expression of CWDEs through FoTIP4 in *F. oxysporum*. 2.6 | FoTOR signalling regulates the expression of CWDEs through FoTIP4 in *F. oxysporum*

The FoTOR signalling pathway is a key regulator of the expression of CWDEs in *F. oxysporum*, as shown in Table S4. To determine whether FoTIP4 mediates the effects of the FoTOR signalling pathway in the regulation of CWDEs, we examined the pathogenicity of ΔFotip4 mutant strains. In infection assays with potato leaves and tubers, the lesion symptoms of ΔFotip4 mutant strains were reduced by approximately 50% compared with those of the wildtype *F. oxysporum* strain (Figure 7a,b). Additionally, a cellophane penetration assay was performed to verify invasive growth of ΔFotip4 mutant strain (Figure 7d), confirming the conclusions drawn on the basis of our RNA-seq data. EMSAs were performed to detect whether FoTIP4 can bind to CWDE promoters containing the RRPE or PAC box. Our results showed that FoTIP4 bound to the RRPE or the PAC box of the FOXG_01365 and FOXG_13331 promoters (Figure 7e,f, Table S5). Collectively, these results indicate that FoTOR signalling is involved in the regulation of CWDEs through FoTIP4 in *F. oxysporum*.

### DISCUSSION

TOR is an evolutionarily conserved protein kinase that regulates cell growth and metabolism in response to growth factors, hormones, cellular energy status, and nutrient abundance (Ma & Blenis, 2009; Saxton & Sabatini, 2017). In yeast and animals, TOR is engaged in two large complexes: TORC1 and TORC2. TORC1 mediates temporal control of cell growth by activating anabolic processes such as ribosome biogenesis, protein synthesis, transcription, and translation and by inhibiting catabolic processes such as proteolysis and autophagy (Dowling et al., 2010; Iadevaia et al., 2014; Saxton & Sabatini, 2017). TORC2 controls cell proliferation and survival primarily by phosphorylating several members of the AGC (PKA/PKG/PKC) family of protein kinases. The activation of PKC-α by TORC2 regulates the cell shape in a cell type-specific fashion by affecting the actin cytoskeleton (Jacinto et al., 2004). However, little information is known about the TOR signalling pathway in *F. oxysporum*. Based

| Gene ID     | Log$_2$(fold change) | Adjusted $p$ | Annotation                      |
|-------------|----------------------|--------------|--------------------------------|
| FOXG_17421  | −2.2776              | 1.21E−4      | XYNA_FUSO4 Endo-1,4-β-xylanase A |
| FOXG_14550  | −2.1543              | 1.69E−21     | BGLM_NEOFI Probable β-glucosidase M |
| FOXG_09125  | −1.8373              | 5.31E−73     | BGLG_ASPOR Probable β-glucosidase G |
| FOXG_09571  | −1.5896              | 5.64E−14     | BGLF_ASPFC Probable β-glucosidase F |
| FOXG_01306  | −1.5760              | 3.85E−35     | CRF1_ASPFU Probable glycosidase |
| FOXG_16582  | −1.3076              | 1.04E−62     | AGDC_ASPFU Probable α/β-glucosidase |
| FOXG_11081  | −1.2542              | 0.0044       | EGLB_ASPOR Probable endo-β-1,4-glucanase B |
| FOXG_02349  | −1.1044              | 6.70E−30     | BCLA_ASPTN Probable β-glucosidase A |
| FOXG_04597  | −0.9593              | 0.0384       | AGAL2_HYPJE α-galactosidase 2 |
| FOXG_00531  | −0.9080              | 1.29E−90     | GUN_PAEO Endoglucanase |
| FOXG_05950  | −0.9015              | 8.12E−147    | CRF1_ASPFU Probable glycosidase |
| FOXG_05948  | −0.8788              | 0.0033       | PLYB_ASPOR Probable pectate lyase B |
| FOXG_15424  | −0.8675              | 1.59E−09     | ABFC_ASPTN Probable α-L-arabinofuranosidase |
| FOXG_01365  | −0.7566              | 0.0336       | BGLI_NEOFI Probable β-glucosidase I |
| FOXG_10604  | −0.7525              | 3.07E−07     | BGLI_ASPOR Probable β-glucosidase I |
| FOXG_13331  | −0.6406              | 0.0190       | PELF2_ASPTN Probable pectate lyase F-2 |
| FOXG_11735  | −0.6363              | 0.0441       | BGALA_NEOFI Probable β-galactosidase A |
| FOXG_02278  | −0.5686              | 0.0185       | GUB_BACAM β-glucanase |

Note: Log$_2$(fold change) and adjusted $p$ values are from the RNA-seq analysis. The table lists representative genes encoding cell wall-degrading enzymes, as identified by RNA-seq.
on the recently released genome database of *F. oxysporum*, we identified putative components of the TOR signalling pathway, including TORC1 and TORC2 (Table 1). We found two TOR gene homologues (*FOXG_18412* and *FOXG_15946*) in the *F. oxysporum* genome. A previous study showed that high transcript levels of *FoTOR1* (*FOXG_18412*) and very low transcript levels of *FoTOR2* (*FOXG_15946*) were detected after growth in minimal liquid medium containing 25 mM NH₄NO₃, and the *FoTOR2* protein contains a truncated N-terminal HEAT repeat domain compared with the *FoTOR1* protein (Lopez-Berges et al., 2010). *FoTOR2* encodes a truncated TOR protein; the FATC domain was not detected in *FoTOR2* (Figure 1). Furthermore, deletion of *FoTOR1* may be lethal, while deletion of *FoTOR2* has no effect on hyphal growth in *F. oxysporum*. Phylogenetic analysis showed that *FoTOR1* protein was clustered as the core TOR group, while *FoTOR2* protein was clustered into the TOR paralogues group; these results were consistent with a previous study (DeIulio et al., 2018). KOG1/RAPTOR functions as a scaffold coupling TOR to substrates in *S. cerevisiae* and animals (González & Hall, 2017). Y2H assays demonstrated that *FoTOR1*, rather than *FoTOR2*, interacts with *FoKOG1*. Interestingly, neither *FoTOR1* nor *FoTOR2* interacted with *FoAVO3* in our Y2H assays, implying that there may be no functional TORC2 in *F. oxysporum*. Additional studies are required to determine the presence of a functional TORC2 complex in *F. oxysporum*. Collectively, the results from the present study indicate that *FoTOR1* is a main regulator of the *FoTOR* signalling pathway in *F. oxysporum*, whereas the function of the *FoTOR2* protein remains unclear.

In *S. cerevisiae* and animals, the TOR signalling pathway has been studied in detail (Cornu et al., 2013; De Virgilio & Loewith, 2006; Saxton & Sabatini, 2017; Wullschleger et al., 2006). Several specific drugs have been developed to control TOR activity, such as RAP, Torin1, KU, and AZD (Chresta et al., 2010; Garcia-Martinez et al., 2009; Heitman et al., 1991a). We applied TOR-specific inhibitors to detect *FoTOR* activity in *F. oxysporum*. RAP and Torin1 can effectively inhibit mycelial growth of *F. oxysporum* in a dose-dependent manner (Figure 2). In this study, we found that the *FoFKBP12* deletion mutant was resistant to RAP (Figure 3). Resistance of FKBP12 deletion mutants to RAP has been reported in other fungi, including *B. cinerea*, *F. graminearum*, *Fusarium fujikuroi*, and *Mucor circinelloides* (Bastidas et al., 2012; Melendez et al., 2009; Teichert et al., 2006; Yu et al., 2014). Consistent with the *FgFKBP12* deletion mutant of *F. graminearum* (Yu et al., 2014), the *FoFKBP12* deletion did not affect mycelial...
growth and development. Similar to the interaction between FgFKBP12 and FgTOR, RAP is required for the interaction between FoFKBP12 and FoTOR1, but FoFKBP12 was unable to interact with FoTOR2 with or without RAP, further indicating that FoTOR1 is an essential component of functional TOR complexes in F. oxysporum.
FIGURE 7 FoTIP4 regulates the pathogenicity of Fusarium oxysporum. (a) The pathogenicity of F. oxysporum was significantly reduced when FoTIP4 lost its function. Spores of wildtype F. oxysporum and ΔFotip4 were point-inoculated on the surface of potato leaves and tubers grown for 4 days. Representative leaves and tubers are shown. Each strain was inoculated on 10 potato leaves or tubers every time, and the experiment was repeated three times. (b) Lesion diameter of potato leaves and tubers. Each column represents the average of 10 potato leaves or tubers. The data are presented as the mean ± SD of n = 3 independent experiments. (c) Effect of ΔFotip4 mutation on penetration of cellophane membranes. Fungal colonies of wildtype F. oxysporum and ΔFotip4 were grown for 4 days at 28 °C on top of cellophane membranes on potato dextrose agar plates (Before), and then the cellophane with the fungal colony was removed and plates were incubated for 2 days to determine the presence of mycelial growth on the plate (After). (d) Relative transcript levels of cell wall-degrading enzyme (CWDE) genes FOXG_17421 (endo-1,4-β-xylanase A), FOXG_01365 (β-glucosidase I), FOXG_14550 (β-glucosidase), and FOXG_13331 (pectate lyase) in wildtype F. oxysporum and ΔFotip4 strains. The data are presented as the mean ± SD of n = 3 independent experiments. **p < 0.01 compared with F. oxysporum (Student’s t test). (e) The electrophoretic mobility shift assay showed that FoTIP4 binds to the promoter of the β-glucosidase gene (FOXG_01365) containing the PAC box. (f) FoTIP4 binds to the promoter of the pectate lyase gene (FOXG_13331) containing the RRPE and PAC boxes. The symbols – and + represent absence and presence, respectively.
RAP can specifically inhibit FoTOR activity at a low concentration in *F. oxysporum*; therefore, RAP was employed to elucidate the function of FoTOR. RNA-seq analysis showed that FoTOR inhibition results in changes in many metabolic processes (Figure 4), which further indicates that FoTOR plays a key role in growth and development. The ribosome content of a cell potentially imposes an upper limit on the rate of protein synthesis it can sustain. Ribosomal biosynthesis and protein synthesis are very energy-intensive processes; TORC1 regulates ribosome content and protein synthesis by responding to the cellular energy status (Chauvin et al., 2014; Iadevaia et al., 2014). Application of RAP mimics nutrient limitation and inhibited FoTORC1 activity, which decreases the mRNA expression levels of ribosome biogenesis genes (Figure 4). Moreover, CWDEs were significantly downregulated upon FoTOR inhibition, showing that FoTOR plays roles in the regulation of the pathogenicity of *F. oxysporum*. Similarly, TOR inhibition reduces mycelial growth and pathogenicity in other fungi, such as *B. cinerea*, *V. dahliae*, and *F. graminearum* (Li et al., 2019; Xiong et al., 2019; Yu et al., 2014).

In this study, we identified a novel FoTOR1 interacting protein, FoTIP4, in a yeast library screening (Figure 5). It is well known that KOG1, as the recruitment protein of TORC1, can recruit substrates to TOR. We found that FoTIP4 interacts with FoTOR1 rather than FoKOG1, implying that FoTIP4 is a new component of FoTORC1. BLASTp analysis showed that FoTIP4 is a unique transcription factor in vascular fungal pathogens. However, there is no homologue of FoTIP4 in the examined mammals, plants, and exogenous fungi such as *Phytophthora infestans*, implying that TIP4 may contribute to mycelial growth and pathogenicity in vascular fungal pathogens. As the homologue of FoTIP4, Sfp1 is phosphorylated by TORC1 and positively regulates the expression of ribosome biogenesis genes in yeast (Albert et al., 2019; Lempiainen et al., 2009). We found that FoTIP4 is a new component of the FoTOR signalling pathway and mediates FoTOR signalling to regulate the expression of ribosome biogenesis and CWDE-associated genes as well as pathogenicity in *F. oxysporum* (Figures 6 and 7). Consistent with this, we observed that the FoTIP4 deletion mutant strains (ΔFoTIP4) displayed considerable defects in hyphal growth and pathogenicity. In addition, EMSAs showed that FoTIP4 can bind to the promoters of ribosome biogenesis and CWDE-related genes to positively regulate the expression of these genes. In summary, our results provide new insights into FoTOR signalling in the vascular fungus *F. oxysporum*. FoTOR1 may serve as a promising target for controlling and preventing Fusarium wilt caused by *F. oxysporum* in plants.

### EXPERIMENTAL PROCEDURES

#### 4.1 Fungal strains and culture conditions

The *F. oxysporum* strain was isolated from Chongqing local potato with dry rot (Fusarium wilt) and was verified through sequencing of the internal transcribed spacer (ITS). The wildtype *F. oxysporum* strain, deletion mutants, and complemented strains were routinely cultured on PDA at 27 °C. For extraction of genomic DNA and conidia production, hyphae were incubated in potato dextrose broth (PDB) at 27 °C with shaking at 160 rpm (Lopez-Berges et al., 2010).

#### 4.2 Construction of vectors for gene deletion and complementation

The primers used to amplify the flanking sequences or coding sequence of each gene are listed in Table S6. Gene deletion and complementation of *F. oxysporum* were carried out as described previously (Luo et al., 2016). Agrobacterium *tumefaciens* AGL-1 was used to transform the conidia of *F. oxysporum*. *A. tumefaciens*-mediated transformation was performed as described previously (Maruthachalam et al., 2011). Randomly selected transformants were transferred to fresh PDA with hygromycin.

#### 4.3 Construction and screening of a Y2H library

Total RNAs were extracted from mycelium of *F. oxysporum* using the RNAprep Kit (TIANGEN). The first-strand cDNA was obtained using 1–2 μg of total RNA as template. This first-strand cDNA was then used as template for low-cycle (c.20) long-distance PCR (LD-PCR) amplification to generate 3–6 μg double-stranded cDNA (dscDNA). These dscDNAs were purified using CHROMA SPIN TE-400 columns (Takara) to obtain DNA for library construction. The cDNA library for Y2H screening was fused with the GAL4 activation domain of the pGAD7 vector as prey using the Matchmaker Gold Yeast Two-Hybrid System (Clontech). The FoTOR1 (FOXG_18412) gene was fused with the GAL4 DNA-binding domain in pGBKT7 to ensure that there was no autoactivation and toxicity, and the FoTOR1 fusion protein was used as bait to identify interacting proteins. Co-transformation of the library cDNA (prey) and the plasmid pGBK7-FoTOR1 (bait) into Y2HGold yeast strain allowed interaction between prey and bait.

#### 4.4 Yeast two-hybrid assay

In order to construct plasmids for Y2H analyses, the coding sequences of genes were amplified from the cDNA of *F. oxysporum*. The genes were inserted into the yeast GAL4 binding domain vector pGBK7 or the GAL4 activation domain vector pGAD7 (Clontech). Pairs of Y2H plasmids were co-transformed into *S. cerevisiae* Y2HGold following the PEG/LiAc transformation protocol (Clontech). Transformants were grown at 28 °C for 5 days on synthetic medium lacking leucine and tryptophan (SD–Leu–Trp). Then yeast colonies were transferred to synthetic medium lacking histidine, leucine, tryptophan, and adenine (SD–His–Leu–Trp–Ade) containing 40 μg/ml X-a-Gal and 200 ng/ml aureobasidin A as
described in the manual (Clontech). The pair of plasmids pGBK7-53 and pGAD7-T was used as a positive control. The pair of plasmids pGBK7-Lam and pGAD7-T was used as a negative control. Three independent experiments were performed.

### 4.5 Fluorescence microscopy

The full-length FoTIP4 encoding sequence was subcloned downstream of the FoTIP4 promoter in Gateway entry vector p8GWN to generate Pfotip4::FoTIP4-eGFP. These recombinant constructs were transformed into pEarleyGate303 through LR recombination reactions (Ren et al., 2011). The plasmids were introduced into A. tumefaciens AGL-1 and the conidia of *F. oxysporum* were transformed as described previously. Transformants were transferred to PDA containing hygromycin for further analysis. Green fluorescent protein (GFP) and 4′,6-diamidino-2-phenylindole (DAPI) fluorescence were observed using a confocal laser scanning microscope (Olympus; Fluoview FV1200).

### 4.6 Electrophoretic mobility shift assay

The full-length FoTIP4 coding sequence was inserted in the vector pCold TF (Takara) and expressed in *Escherichia coli* BL21 (DE3) by incubation with 0.5 mM IPTG for 5 hr at 16 °C. The recombinant protein was purified with a His60 Ni Gravity Column (Takara) according to the manufacturer’s instructions. The probes containing an RRPE or PAC box derived from FoSIK1 (FOXG_12883), pectate lyase gene (FOXG_13331), and β-glucosidase gene (FOXG_01365) promoters were labelled with biotin using the EMSA Probe Biotin Labeling Kit (Beyotime). The same unlabelled DNA fragment was used as a competitor, while the RRPE or PAC box within a probe changed into AAAAAAAA was used as a negative control. The EMSA was performed using the electrophoretic mobility shift assay kit (Beyotime) according to the manufacturer’s instructions.

### 4.7 Transcriptome sequencing and analysis

Hyphae of *F. oxysporum* were grown for 4 days in PDB at 27 °C with shaking at 160 rpm, treated with 1 μM RAP or DMSO (as a control), and incubated for 12 hr. Total RNA of *F. oxysporum* mycelium was isolated using the RNAprep Pure Plant Kit (TIANGEN). For each treatment, three independent biological replicates were performed. An Illumina HiSeq 2000 platform was used to sequence the cDNA library, and 100-bp paired-end reads were generated. The clean reads were mapped to the *F. oxysporum* reference genome using TopHat2 software. Cufflinks and Cuffdiff were used to assemble the mapped reads and identify DEGs, respectively. GO enrichment (corrected *p* value < 0.05) of the DEGs was performed using GOseq software. The KEGG pathway enrichment of DEGs (corrected *p* value < 0.05) was obtained using KOBAS software (Mao et al., 2005).

### 4.8 RT-qPCR

Total RNA of *F. oxysporum* mycelium treated for 12 hr in PDB containing DMSO or RAP (1 μM) was isolated using the RNAprep Pure Plant Kit (TIANGEN). Relative transcript levels were assayed by one-step real-time PCR analysis using the CFX96 real-time PCR system (Bio-Rad). Real-time PCR primers were designed using Primer Premier v. 5.0 (details are presented in Table S6). *FoElF1α* was used as an internal control. The data are presented as the mean ± SD of three independent experiments.

### 4.9 Combination index value measurement

CI values were used to quantitatively measure the interaction between Torin1 and RAP. The interaction is categorized as synergism (CI < 1), additive effects (CI = 1), or antagonism (CI > 1) (Chou, 2006). Hyphae of *F. oxysporum* were incubated on PDA containing different concentrations of Torin1 or RAP or a combination of Torin1 and RAP for 6 days at 27 °C. Colony diameter was measured to calculate growth inhibition. Experiments were repeated three times. *Fa* represents the fraction of colony diameter affected by the reagent. The *Fa* values, which were calculated using CompuSyn software, indicate growth inhibition.

### 4.10 Pathogen inoculation and cellophane invasion assays

Pathogen inoculation was performed by point inoculation on the surface of potato leaves and tubers with conidia of wildtype *F. oxysporum, ΔFotip4* mutants, and the complemented strain (*ΔFotip4 + Fotip4*) (10^7 conidia/ml) as described previously (Thatcher et al., 2009). Inoculated leaves and tubers were cultured on moist filter paper at 27 °C in a short daylight condition for 4 days. Each strain was inoculated on at least 10 potato leaves or tubers every time. The cellophane invasion assay was performed as described previously (Prados Rosales & Di, 2008). Each experiment was repeated at least three times.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the National Natural Science Foundation of China (nos. 32002105, U1804231, 31801911, and 31972469), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (34-IUA-02), local financial funds of the National Agricultural Science & Technology Center (NASC), Chengdu (NASC2020AR08 and NASC2021KR03), and the National Key R&D program of China (2017YFE0115500).
AUTHOR CONTRIBUTIONS
M.R. and L.L. designed the experiments. L.L., T.Z., and Y.S. performed the experiments. L.L., T.Z., Y.S., and X.L. analysed the data. L.L., T.Z., R.D., and M.R. wrote the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
Albert, B., Tomassetti, S., Gloor, Y., Dilg, D., Mattarocci, S., Kubik, S. et al. (2019) Sfp1 regulates transcriptional networks driving cell growth and division through multiple promoter-binding modes. Genes & Development, 33, 288–293.
Atkin, J., Halova, L., Ferguson, J., Hitchin, J.R., Lichawska-Cieslar, A., Jordan, A.M. et al. (2014) Tor1-mediated TOR kinase inhibition reduces Wee1 levels and advances mitotic commitment in fission yeast and HeLa cells. Journal of Cell Science, 127, 1346–1356.
Aylett, C.H.S., Sauer, E., Imseng, S., Boehringer, D., Hall, M.N., Ban, N. et al. (2016) Architecture of human mTOR complex 1. Science, 351, 48–52.
Bastidas, R.J., Shertz, C.A., Lee, S.C., Heitman, J. & Cardenas, M.E. (2012) Rapamycin exerts antifungal activity in vitro and in vivo against Mucor circinelloides via FKB12-dependent inhibition of TOR. Eukaryotic Cell, 11, 270–281.
Benjamin, D., Colombi, M., Moroni, C. & Hall. M.N. (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nature Reviews Drug Discovery, 10, 868–880.
Ben-Sahra, I., Howell, J.J., Asara, J.M. & Manning, B.D. (2013) Stimulation of de novo pyrimidine synthesis by growth signalling through mTOR and S6K1. Science, 339, 1323–1328.
Berrocillobo, M. & Molina, A. (2008) Arabidopsis defense response against Fusarium oxysporum. Trends in Plant Science, 13, 145–150.
Calero-Nieto, F., Di Pietro, A., Roncero, M.I. & Hera, C. (2007) Role of the transcriptional activator xlnR of Fusarium oxysporum in regulation of xylanase genes and virulence. Molecular Plant-Microbe Interactions, 20, 977–985.
Chauvin, C., Koka, V., Nouschi, A., Mieulet, V., Hoareau-Aveilla, C., Dreazen, A. et al. (2014) Ribosomal protein S6 kinase activity controls the ribosome biogenesis transcriptional program. Oncogene, 33, 474–483.
Chou, T.C. (2006) Theoretical basis, experimental design, and computerized simulation of synergy and antagonism in drug combination studies. Pharmacological Reviews, 58, 621–681.
Chresta, C.M., Davies, B.R., Hickson, I., Harding, T., Cosulich, S., Critchlow, S.E. et al. (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Research, 70, 288–298.
Conru, M., Albert, V. & Hall, M.N. (2013) mTOR in aging, metabolism, and cancer. Current Opinion in Genetics & Development, 23, 53–62.
De Virgilio, C. & Loewith, R. (2006) Cell growth control: little eukaryotes make big contributions. Oncogene, 25, 6392–6415.
Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D. et al. (2012) The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology, 13, 804.
Delulio, G.A., Guo, L., Zhang, Y., Goldberg, J.M., Kstler, H.C. & Ma, L.J. (2018) Kinome expansion in the Fusarium oxysporum species complex driven by accessory chromosomes. mSphere, 3, e00231-18.
Di, P.A., Madrid, M.Z., Delgado-Jarana, J. & Mig, R. (2003) Fusarium oxysporum: exploring the molecular arsenal of a vascular wilt fungus. Molecular Plant Pathology, 4, 315–325.
Dobrenel, T., Caldana, C., Hanso, J., Robaglia, C., Vincentz, M., Veit, B. et al. (2016) TOR signaling and nutrient sensing. Plant Biology, 67, 261–285.
Dowling, R.J.O., Topisirovic, I., Alain, T., Bidinosti, M., Fonseca, B.D., Petroulakis, E. et al. (2010) mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science, 328, 1172–1176.
Fingerman, I., Nagaraj, V., Norris, D. & Vereshon, A.K. (2003) Sfp1 plays a key role in yeast ribosome biogenesis. Eukaryotic Cell, 2, 1061–1068.
Garcia Bayona, L., Grajales, A., Cardenas, M.E., Sierra, R., Lozano, G., Garavito, M.F. et al. (2011) Isolation and characterization of two strains of Fusarium oxysporum causing potato dry rot in Solanum tuberosum in Colombia. Revista Iberoamericana de Micología, 28, 166–172.
García-Martínez, J., Moran, J., Clarke, R., Gray, A., Cosulich, S., Chresta, C. et al. (2009) Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). The Biochemical Journal, 421, 29–42.
Gaibitz, C., Oliveira, T., Prouteau, M., Leitner, A., Karuppasamy, M., Konstantinidou, G. et al. (2015) Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. Molecular Cell, 58, 977–988.
Gaibitz, C., Prouteau, M., Kusmider, B. & Loewith, R. (2016) TORC2 structure and function. Trends in Biochemical Sciences, 41, 532–545.
Gibson, D.M., King, B.C., Hayes, M.L. & Bergstrom, G.C. (2011) Plant pathogens as a source of diverse enzymes for lignocellulose digestion. Current Opinion in Microbiology, 14, 264–270.
González, A. & Hall, M.N. (2017) Nutrient sensing and TOR signaling in yeast and mammals. EMBO Journal, 36, 397–408.
Hara, K., Maruki, Y., Long, X., Yoshino, K.-I., Oshiro, N., Hidayat, S. et al. (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell, 110, 177–189.
Heitman, J., Movva, N.R. & Hall, M.N. (1991a) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science, 253, 905–909.
Heitman, J., Movva, N.R., Hiestand, P.C. & Hall, M.N. (1991b) FK-506-binding protein Proline rotamase is a target for the immunosuppressive agent FK-506 in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America, 88, 1948–1952.
Husaini, A.M., Sakina, A. & Cambay, S.R. (2018) Host–pathogen interaction in Fusarium oxysporum infections: where do we stand? Molecular Plant-Microbe Interactions, 31, 889–898.
Iadevaia, V., Liu, R. & Proud, C.G. (2014) mTORC1 signaling controls multiple steps in ribosome biogenesis. Seminars in Cell & Developmental Biology, 36, 113–120.
Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Rüegg, M.A., Hall, A. et al. (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nature Cell Biology, 6, 1122–1128.
Jonkers, W. (2009) The role of the F-Box protein Frlp1 in pathogenicity of Fusarium oxysporum. PhD thesis, Swammerdam Institute for Life Sciences. Amsterdam, Netherlands. https://hdl.handle.net/11245/1.307564
Jorgensen, P., Nishikawa, J.L., Breitkreutz, B.-J. & Tyers, M. (2002) Systematic identification of pathways that couple cell growth and division in yeast. Science, 297, 395–400.
King, B.C., Waxman, K.D., Nenni, N.V., Walker, L.P., Bergstrom, G.C. & Gibson, D.M. (2011) Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. Biotechnology for Biofuels, 4, 4.
Kos-Braun, I.C. & Kos, M. (2017) Post-transcriptional regulation of ribosome biogenesis in yeast. Microbial Cell, 4, 179–181.
Kunz, J., Henriques, R., Schneider, U., Deuter-Reinhard, M., Movva, N.R. & Hall, M.N. (1993) Target of rapamycin in yeast, TOR2, is an
essential phosphatidylinositol kinase homolog required for G1 progression. Cell, 73, 585–596.

Lempiäinen, H., Uotila, A., Urban, J., Dohnal, I., Ammerer, G., Loewith, R., et al. (2009) Sfp1 interaction with TORC1 and Mss6 reveals feedback regulation on TOR signaling. Molecular Cell, 33, 704–716.

Li, L., Zhu, T., Song, Y., Luo, X., Feng, L., Li, Z., and Li, H. (2016) Functional characterization of target of rapamycin signaling in Verticillium dahliae. Frontiers in Microbiology, 10, 501.

Loewith, R., Jacinto, E., Wullschlegler, S., Lorberg, A., Crespo, J.L., Bonenfant, D., et al. (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Molecular Cell, 10, 457–468.

Lopez-Berges, M.S., Rispal, N., Prados-Rosas, R.C., and Di Pietro, A. (2010) A nitrogen response pathway regulates virulence functions in Fusarium oxysporum via the protein kinase TOR and the bZIP protein MeaB. The Plant Cell, 22, 2459–2475.

Luo, X., Mao, H., Wei, Y., Cai, J., Xie, C., and Sui, A., et al. (2016) The fungal-specific transcription factor Vdpf influences conidia production, melanized microsclerotia formation, and pathogenicity in Verticillium dahliae. Molecular Plant Pathology, 17, 1364–1381.

Ma, X.M. and Blenis, J. (2009) Molecular mechanisms of mTOR-mediated translational control. Nature Reviews Molecular Cell Biology, 10, 307–318.

Magnuson, B., Ekin, B., and Finger, D.C. (2012) Regulation and function of ribosomal protein S6 kinase (56K) within mTOR signalling networks. The Biochemical Journal, 441, 1–21.

Mao, X., Cai, T., Olyarchuk, J.G., and Wei, L. (2005) Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics, 21, 3787–3793.

Marion, R.M., Regev, A., Segal, E., Barash, Y., Koller, D., Friedman, N., et al. (2004) Sfp1 is a stress- and nutrient- sensitive regulator of ribosomal protein gene expression. Proceedings of the National Academy of Sciences of the United States of America, 101, 14315–14322.

Martin, D., Powers, T., and Hall, M. (2006) Regulation of ribosome biogenesis: where is TOR? Cell Metabolism, 4, 259–260.

Maruthachalam, K., Klosterman, S.J., Kang, S., Hayes, R.J., and Subbarao, K.V. (2011) Identification of pathogenicity-related genes in the vascular wilt fungus Verticillium dahliae by Agrobacterium tumefaciens-mediated T-DNA insertional mutagenesis. Molecular Biotechnology, 49, 209.

Melendez, H.G., Billon-Grand, G., Fevre, M., and Mey, G. (2009) Role of the Botrytis cinerea FKBP12 ortholog in pathogenic development and in sulfur regulation. Fungal Genetics and Biology, 46, 308–320.

Menand, B., Desnos, T., Nussaume, L., Berger, F., Bouchez, D., Meyer, C., et al. (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proceedings of the National Academy of Sciences of the United States of America, 99, 6422–6427.

Montane, M.H. and Menand, B. (2013) ATP-competitive mTOR kinase inhibitors delay plant growth by triggering early differentiation of meristematic cells but no developmental patterning change. Journal of Experimental Botany, 64, 4361–4374.

Olajuyigbe, F.M., Nlekerem, C.M., and Ogunyewo, O.A. (2016) Production of rapamycin and its effect on tomato development in Arabidopsis. Current Genetics, 44, 49–57.

Ozbay, N. and Newman, S.E. (2004) Fusarium crown and root rot of tomato and control methods. Plant Pathology Journal, 3, 9–18.

Ploetz, R.C. (2015) Fusarium wilt of banana. Phytopathology, 105, 1512–1521.

Prados Rosales, R.C., and Di, P.A. (2008) Vegetative hyphal fusion is not essential for plant infection by Fusarium oxysporum. Eukaryotic Cell, 7, 162.

Raza, W., Ling, N., Zhang, R., Huang, Q., Xu, Y., and Shen, Q. (2016) Success evaluation of the biological control of Fusarium wilts of cucumber, banana, and tomato since 2000 and future research strategies. Critical Reviews in Biotechnology, 37, 202–212.

Ren, M., Qiu, S., Venglata, P., Xiang, D., Feng, L., Selvaraj, G., et al. (2011) Target of rapamycin regulates development and ribosomal RNA expression through kinase domain in Arabidopsis. Plant Physiology, 155, 1367–1382.

Ren, M., Venglata, P., Qiu, S., Feng, L., Cao, Y., Wang, E., et al. (2012) Target of rapamycin signaling regulates metabolism, growth, and life span in Arabidopsis. The Plant Cell, 24, 4850–4874.

Sabers, C.J., Martin, M.M., Brunn, G.J., Williams, J.M., Dumont, F.J., Wiederrecht, G., et al. (1995) Isolation of a protein target of the FKBP12- rapamycin complex in mammalian cells. Journal of Biological Chemistry, 270, 815–822.

Saxton, R.A. and Sabatini, D.M. (2017) mTOR signaling in growth, metabolism, and disease. Cell, 168, 960–976.

Schmelzle, T. and Hall, M.N. (2000) TOR, a central controller of cell growth. Cell, 103, 253–262.

Schmidt, S.M., Lukasiewicz, J., Farrer, R., Van, D.P., Bertoldo, C., and Rep, M. (2016) Comparative genomics of Fusarium oxysporum f. sp. melonis reveals the secreted protein recognized by the Fom-2 resistance gene in melon. New Phytologist, 209, 307–318.

Sormani, R., Yao, L., Menand, B., Ennard, N., Lecampion, C., Meyer, C., et al. (2007) Saccharomyces cerevisiae FKBP12 binds Arabidopsis thaliana TOR and its expression in plants leads to rapamycin susceptibility. BMC Plant Biology, 7, 26.

Teichert, S., Wottawa, M., Schonig, B., and Tudzynski, B. (2006) Role of the Fusarium fujikuroi TOR kinase in nitrogen regulation and secondary metabolism. Eukaryotic Cell, 5, 1807–1819.

 Thatcher, L.F., Manners, J.M., and Kazan, K. (2009) Fusarium oxysporum hijacks COI1-mediated jasmonate signaling to promote disease development in Arabidopsis. The Plant Journal, 58, 927–939.

 Tsang, C.K., Liu, H., and Zheng, X.F. (2010) mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes. Cell Cycle, 9, 953–957.

 Wang, X. and Proud, C.G. (2009) Nutrient control of TORC1, a cell-cycle regulator. Trends in Cell Biology, 19, 260–267.

 Warner, J.R. (1999) The economics of ribosome biosynthesis in yeast. Trends in Biochemical Sciences, 24, 437–440.

 Wullschlegler, S., Loewith, R. and Hall, M.N. (2006) TOR signaling in growth and metabolism. Cell, 124, 471–484.

 Wullschlegler, S., Loewith, R., Oppliger, W., and Hall, M.N. (2005) Molecular organization of target of rapamycin complex 2. Journal of Biological Chemistry, 280, 30697–30704.

 Xiong, F., Liu, M., Zhuo, F., Yin, H., Deng, K., Feng, S. et al. (2019) Host-induced gene silencing of BcTOR in Botrytis cinerea enhances plant resistance to grey mould. Molecular Plant Pathology, 20, 1722–1739.

 Xiong, F., Zhang, R., Meng, Z., Deng, K., Que, Y., Zhuo, F. et al. (2017) Brassinosteroid insensitive 2 (BIN2) acts as a downstream effector of the Target of Rapamycin (TOR) signaling pathway to regulate phototrophic growth in Arabidopsis. New Phytologist, 213, 233–249.

 Yu, F., Gu, Q., Yun, Y., Yin, Y., Xu, J.R., Shim, W.B. et al. (2014) The TOR signaling pathway regulates vegetative development and virulence in Fusarium graminearum. New Phytologist, 203, 219–232.

 Yuan, H.X., Xiong, Y. and Guan, K.L. (2013) Nutrient sensing, metabolism, and cell growth control. Molecular Cell, 49, 379–387.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.