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Target of rapamycin proteins and their kinase activities are required for meiosis

XIAO-FENG ZHENG* AND STUART L. SCHREIBER†

Howard Hughes Medical Institute, Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

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ABSTRACT **The phosphatidylinositol kinase-related kinases, including Tor1p, Tor2p, FRAP/RAFT, FRP/ATR, ATM, Mec1p, Rad3, and Tel1p, function in signal transduction pathways involved in cell cycle progression and surveillance. The rapamycin-sensitive kinase activities of Tor1p and Tor2p are required for the nutrient-activated protein translation essential for G_1 cell cycle progression in haploid yeast cells. In addition, Tor2p's kinase activity is necessary for its unique rapamycin-insensitive function involved in the assembly of the actin cytoskeleton. In the current study using diploid yeast, we found that the kinase activities of the Tor proteins are also required for two discrete steps during yeast meiosis—the switch between the mitotic and meiotic cell cycles and a later step during meiosis involved in the packaging of resultant haploid cells (spores) into asci. Based on what is known of the mitotic functions of Tor and FRAP proteins, these results likely reflect the requirement for signaling pathways leading to regulated protein translation during meiosis. Mec1p, which is required for meiotic recombination, and the Tor proteins are, therefore, homologous kinases with distinct, yet essential, roles in meiosis.**

Tor1p, Tor2p, and FRAP/RAFT, members of the phosphatidylinositol kinase-related kinases (1), are large molecular weight protein kinases that bind to the immunophilin–immunosuppressant complex, FKBP12–rapamycin. These recently discovered signaling proteins mediate signal transduction from the cell surface to the interior, apparently targeting primarily the protein translational machinery. Thus far, the TOR/FRAP signaling pathway has been shown to regulate the rate of translation of mRNA transcripts encoding proteins involved in G_1 cell cycle progression (for a recent review, see ref. 2). These membrane-to-cytoplasmic signaling events distinguish this pathway from the previously defined cytoplasmic membrane-to-nuclear signal transduction pathways, including the Ras–MAPK, Src–myc, and Jak–Stat pathways. In addition, FRAP mediates a surveillance–feedback signaling pathway that monitors conditions perturbing protein synthesis. Inhibition of protein translation by small RNA ligands such as cycloheximide and anisomycin activates the FRAP/$p70^{S6k}$ pathway, thereby translationally up-regulating ribosomal proteins (3). This bears striking analogy to another surveillance–feedback signaling pathway mediated by another phosphatidylinositol kinase-related kinase, Mec1p, in budding yeast (and possibly by FRP/ATR in humans) (4, 5). MEC1 (also known as ESR1) is a *Saccharomyces cerevisiae* checkpoint gene that monitors DNA damage and the completion of DNA synthesis and that is required for meiosis (ref. 6; for a recent review, see ref. 7).

The amino acid sequences of the C termini of Tor1p, Tor2p, and FRAP are conserved and distantly related to those of the catalytic domains of phosphatidylinositol kinases. In haploid yeast, the kinase activities of Tor1p and Tor2p are required for a common function that confers sensitivity to starvation conditions. Loss of TOR function by gene deletion or by inhibition of the encoded proteins with rapamycin prevents nutrient-activated early events leading to the initiation of protein translation, thereby causing G_0/G_1 cell cycle arrest characteristic of starved cells entering the stationary phase (8). In addition, Tor2p's kinase activity is uniquely involved in an essential function involving the assembly of actin into cytoskeletal structures (9). In this study, we have used diploid budding yeast strains to explore Tor function further. We find that Tor proteins, like their homolog Mec1p, have functions essential for meiosis. The kinase activities of both Tor1p and Tor2p are involved in the switch from the mitotic to the meiotic cell cycle in response to starvation. In addition, these kinase activities are also required for a later step in meiosis. These meiotic functions of the Tor proteins are sensitive to rapamycin, suggesting that they are involved in regulating the rate of translation of specific mRNA transcripts required for the meiotic cell cycle.

MATERIALS AND METHODS

Media, Strains, and Plasmids. One liter of complete synthetic medium used in these studies contains 6.7 g of yeast nitrogen base (Bio 101), 1 g of amino acid dropout powder (Bio 101), and 20 g of dextrose. One liter of yeast extract/peptone/dextrose (YPD) is composed of 20 g of peptone (Difco), 10 g of yeast extract (Difco), and 20 g of dextrose. One liter of presporulation medium (YPA) was prepared by dissolving 20 g of peptone, 10 g of dextrose, and 10 g of potassium acetate. Sporulation medium (SPM) contains 0.3% potassium acetate and 0.02% raffinose (Sigma). Solid media were prepared similarly except they were supplied with 20 g of agar (Difco).

Yeast strains used in this study are summarized in Table 1. Standard procedures were used for culturing and maintaining strains (10). pGAL1-TOR1 and pGAL1-TOR1 (Asp-2294 → Glu) expression cassettes were excised with *Pst*I from the corresponding pYDF vectors (11) and inserted into the *Pst*I site of pBR315 (*LEU2*) (12). Plasmids expressing pTOR1 (Ser-1972 → Ile), pTOR1 (Ser-1972 → Ile, Asp-2294 → Glu), pTOR2–1 (Ser-1975 → Ile), and pTOR2–1 (Ser-1975 → Ile, Asp-2294 → Glu) were as described (11). To introduce plasmids into SK-1 cells, 5 ml of cells were grown to saturation in YPD, washed three times with sterile doubly distilled H_2O, and resuspended in 0.1 ml of water. The cells were mixed with 0.5

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Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole. YPD, yeast extract/peptone/dextrose; YPA, yeast extract/peptone presporulation medium; SPM, sporulation medium.

*Present address: Division of Molecular Oncology and Departments of Pathology and Medicine, Washington University School of Medicine, CSRBT–1029B, 307 South Euclid Avenue, St. Louis, MO 63110.
†To whom reprint requests should be addressed.

Table 1. Strains used in this study

Strain	Genotype
SK-1(NKY611)	*MAT***a**/α *ura3/ura3 leu2/leu2*
SK-1*TORISR*	*MAT***a**/α *ura3/ura3 leu2/leu2 TOR1/TOR1* (Ser-1972 → Arg)
SK-1*TORISR*	*MAT***a**/α *ura3/ura3 leu2/leu2 TOR2/TOR2* (Ser-1975 → Arg)
BR2495	*MAT***a**/α *his4-260/his4-280 leu2-27/leu2-280 ura3/ura3 arg4-8/ARG4 trp1-1/trp1-289 thr1-1/thr1-4 cyh1OR/CYH10 ade2-1/ade2-1*

μg of plasmid DNA and electroporated using a BTX electroporator (Genetronics, San Diego).

Sporulation Procedures. To induce sporulation, diploid yeast strains BR2495 and SK-1 and their derivatives were grown to saturation in YPD for 24 hr at 30°C with vigorous shaking. The cells were then diluted 1:50 into YPA. BR2495 and SK-1 cells were incubated for an additional 24 and 13.5 hr, respectively, at 30°C. The cells were then pelleted, washed twice with prewarmed sterile distilled water, and resuspended into prewarmed SPM. BR2495 and SK-1 cells were incubated for another 36 and 13.5 hr, respectively, prior to analysis. Asci were counted under the phase-contrast light microscope (Nikon). To test sporulation of SK-1 strains with plasmid-born wild-type or mutant TOR1, the cells were incubated in appropriate complete synthetic medium for 36 hr to saturation, diluted 1:50 into YPA, and incubated for another 13.5 hr at 30°C. The cells were then pelleted, washed twice with prewarmed sterile water, resuspended into prewarmed SPM, and incubated at 30°C for a further 13 hr. Rapamycin or FK506 was added into the culture at the indicated concentrations and times during sporulation from a concentrated stock (50 μM in methanol). Equal volumes of methanol were added to the cultures as controls. To test the effects of overexpression of wild-type or mutant TOR1 and TOR2 proteins on sporulation, yeast cells were grown to saturation for 36 hr in complete synthetic medium, washed twice with sterile distilled water, resuspended into 50 vol of YPA, and incubated for a further 10 hr at 30°C. Galactose (0.1%) was added to induce TOR1 expression for 3.5 additional hours. The cells were washed twice with water, resuspended into SPM containing 0.1% galactose, and incubated for a further 13 hr.

4′,6-Diamidino-2-phenylindole (DAPI) Staining, Dyad Dissection, and Flow Cytometric Analysis. Yeast cells were stained with DAPI (1 μg/ml) for 5 min by mixing 10 μl of culture with 1 μl of DAPI (10 μg/ml). The stained cells were analyzed under the Leitz Laborlux S fluorescence microscope. To dissect the dyads in rapamycin-treated cultures, the cells were incubated with 10 μg of Zymolyase 100T in 100 μl of 1 M sorbitol for 10 min at 37°C, and 20 μl of cells was spread onto a semi-dry YPD/agar plate. Two-spored asci were identified under the phase-contrast microscope (Nikon), picked, and dissected by the Kramer micromanipulator (Kramer Scientific, Elmsford, NY). The dissected ascospores were incubated on YPD plates for 3 days at 30°C to allow germination and the formation of colonies. The germinated cells were picked by toothpicks directly from individual colonies and resuspended into 50% ethanol. The ethanol-fixed cells were pelleted, washed twice with ice-cold PBS, resuspended in propidium iodide staining solution [0.2 M Tris/0.2 M NaCl/0.1 M $MgCl_2$/propidium iodide (50 μg/ml)], and stained for 30 min. The cells were pelleted again, washed twice with PBS, sonicated briefly to disperse any aggregation, and analyzed by Becton Dickinson FACS Analyzer (13).

RESULTS

Tor Proteins Are Involved in the Initiation of Meiosis in Response to Starvation. We have tested the effects of rapamycin on diploid yeast strains deprived of nutrients. In contrast to haploid cells, diploid cells undergo sporulation, a meiotic differentiation process. BR2495 (MAT**a**/α) is a diploid yeast strain that has low sporulation efficiency, with 20% of the total cells forming spores after 36 hr in SPM (Fig. 1*a*). When incubated with rapamycin, however, there is a dramatic increase in sporulation with more than 80% cells forming spores (Fig. 1*a*). FK506, a structural analog of rapamycin, does not

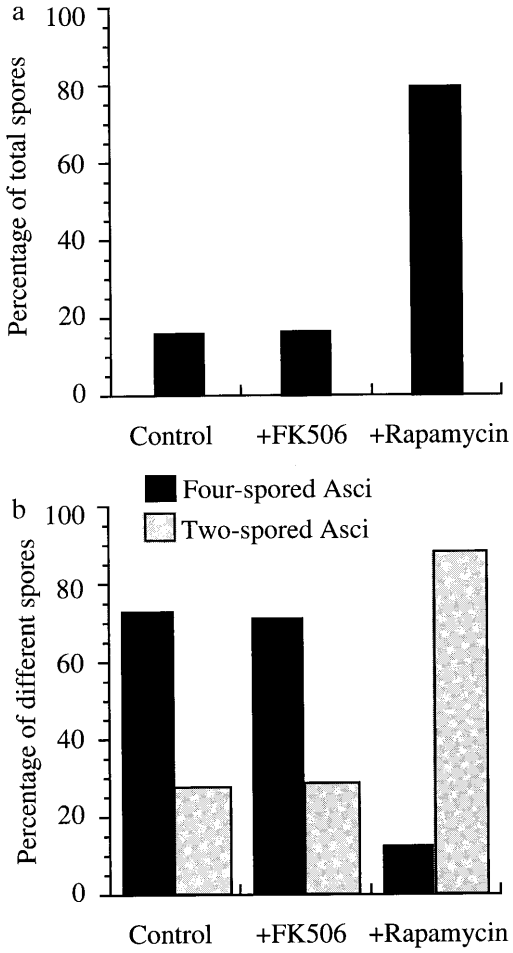

FIG. 1. (*a*) Rapamycin causes an increase in sporulation efficiency in BR2495 cells. Diploid yeast BR2495 cells were grown to saturation in YPD medium for 24 hr at 30°C with vigorous shaking. These cells were added to YPA medium at a 1:50 dilution and incubated for a further 13.5 hr at 30°C. The cells were then pelleted, washed twice with sterile water, resuspended into SPM with or without rapamycin or FK506, and incubated for another 36 hr. Different asci were counted under the phase-contrast light microscope. The results from three different experiments were averaged (n = 250). Control, methanol (solvent for both FK506 and rapamycin); FK506, 100 nM final concentration; rapamycin, 100 nM final concentration. (*b*) Increased dyad population in the presence of rapmycin. The same samples were counted for proportions of different ascospores. The percentage of single-spored asci (monads) was very low (<1%) and omitted from the results. A small percentage of three-spored asci (triads) were counted as four-spored asci (tetrads) in this analysis. The results show an average of three different experiments (n = 250).

exhibit a similar effect (Fig. 1*a*). These observations suggest that BR2495 has an intrinsic defect in the starvation response pathway(s) involving TOR and that this defect inhibits sporulation.

To test directly whether Tor proteins are involved in the early response to nutrient deprivation, we switched to a different laboratory diploid yeast strain, SK-1. SK-1 cells sporulate efficiently (>95% of the cells) under laboratory conditions and are often considered "wild type" in studies of meiosis. We argued that if the TOR pathway is involved directly in controlling the starvation response, as does the RAS1–cAMP pathway (for a recent review, see ref. 14), inhibition of Tor1p and Tor2p should result in sporulation even in rich culture conditions. We added rapamycin to a saturated SK-1 cell culture in YPD medium. After a 12-hr incubation at 30°C, nearly 60% of the cells sporulated (Fig. 2*a*). In contrast, the untreated cells or the FK506-treated cells remained unsporulated (Fig. 2*a*). Thus, like the loss of function *RAS* mutations *ras*$^-$, inhibition of Tor proteins by rapamycin alone is sufficient for SK-1 cells to sporulate even in the presence of rich carbon and nitrogen sources. Interestingly, adding rapamycin to logarthmic-phase SK-1 or BR2495 cells is not sufficient to induce sporulation, despite the fact that rapamycin does cause G_1 cell cycle arrest in these cells under these conditions. These results suggest that an additional signal or signals is needed for the decision to switch from mitosis into meiosis in exponentially growing cells and that this signal is provided under conditions of saturation growth.

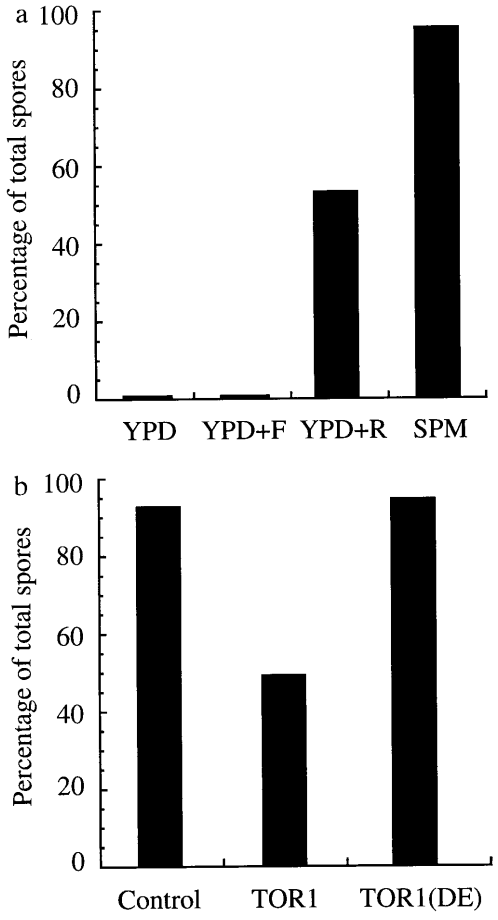

FIG. 2. (*a*) Rapamycin induces SK-1 cells to sporulate in saturated YPD culture. Diploid SK-1 cells were incubated in YPD for 24 hr at 30°C with vigorous shaking. The cells reached saturation and arrested in G_1. The cell culture was equally divided into three aliquots. Each aliquot was incubated with methanol (control), 100 nM FK506 (YPD + FK506), or 100 nM rapamycin (YPD + rapamycin). As a positive control, the same saturated SK-1 cell culture in YPD was diluted 1:50 into YPA and incubated for 13.5 hr. The cells were washed with sterile water, resuspended into SPM, and incubated for another 13 hr. The proportion of total sporulated cells was calculated as in Fig. 1*a* (average of three experiments, $n = 250$). (*b*) Overexpression of plasmid-borne wild-type, but not the kinase-inactive mutant TOR1, partially blocks sporulation. The diploid SK-1 cells carrying pGAL1 alone, pGAL1-TOR1, or pGAL1-TOR1 (Asp-2294 → Glu) were incubated in complete synthetic medium-Leu-Glu for 36 hr at 30°C with vigorous shaking, washed twice in sterile water, and resuspended into the 50 vol of YPA for another 10 hr. Galactose (0.1%) was added to the culture. The cells were incubated for another 3.5 hr during protein induction, then washed twice with sterile water, resuspended in SPM containing 0.1% galactose, and incubated for 13 hr. The samples were analyzed as in *a* (average of three experiments, $n = 250$).

We have also introduced extrachromosomal copies of wild-type *TOR1* under the control of the GAL1 promoter for inducible overexpression by galactose. A TOR1 kinase-inactive mutant, TOR1 (Asp-2294 → Glu), was used as a negative control because it lacks the rapamycin-sensitive G_1 function (11). Moderate overexpression of wild-type TOR1 prior to switching to sporulation medium reduced the sporulation efficiency from more than 95% to 48% (Fig. 2*b*). In contrast, cells with the control vector alone or expressing the kinase-negative mutant TOR1 (Asp-2294 → Glu) sporulated at a level comparable to the wild-type strain (Fig. 2*b*). Thus, inhibition of Tor proteins appears necessary for the initiation of sporulation in response to starvation. Thus, these results indicate that the rapamycin-sensitive kinase function of Tor proteins is required for the switch from mitotic growth to the differentiative meiotic cell cycle.

The Kinase Function of Tor Is Also Required for Tetrad Formation. Approximately 20% BR2495 cells form asci after incubation in SPM for 36 hr, of which 73% asci are four-spored (tetrad) and 26% are two-spored (dyad) (Fig. 1*b*). Rapamycin causes a dramatic increase in the total asci to 80% (Fig. 1*a*). However, to our surprise, the majority of the rapamycin-treated asci are dyads (86%) and only a small percentage are normal tetrads (14%) (Fig. 1*b*). FK506 did not have a significant effect on the sporulation efficiency ("early meiotic function") nor the distribution of different asci ("late meiotic function"). Although rapamycin did not improve the sporulation efficiency of the SK-1 cells, it did alter the spore distribution dramatically. After rapamycin treatment, 80% of the asci are two-spored in comparison to only 20% for the control (Fig. 3*a*). These dyad ascospores were dissected and induced to grow vegetatively. Their growth demonstrated that the dyads are viable; flow cytometry revealed that they are haploid (1N DNA content). Nuclear staining with DAPI also revealed that the cells had undergone two rounds of chromosome segregation, forming four nuclei, yet only two nuclei were successfully enclosed within the two ascospore cell walls (data not shown). These observations suggest that TOR is required for the packaging of the four nuclei within the asci during late meiosis. The sensitivity of SK-1 to rapamycin for tetrad formation is comparable to that of G_1 cell cycle arrest in haploid cells. The inhibition of tetrad formation increases with increasing rapamycin concentration. Rapamycin at 100 nM causes maximal inhibition of tetrad formation (Fig. 3*b*). Higher drug concentrations (up to 10 μM) do not appear to cause further abnormality. FK506 does not significantly perturb spore distribution even at very high concentration (up to 10 μM, Fig. 3*b*).

To test directly whether rapamycin prevents tetrad formation as a result of inhibiting Tor1p and Tor2p, we introduced an arginine substitution by homologous recombination at the conserved residue, Ser-1972 in the TOR1 locus or Ser-1975 in the TOR2 locus of the SK-1 strain. These substitutions have been shown previously to disrupt the ability of FKBP12-rapamycin to bind and inhibit Tor1p and Tor2p (11). Both mutations conferred virtually complete sporulation resistance to low concentrations of rapamycin (<1 nM) (data not shown;

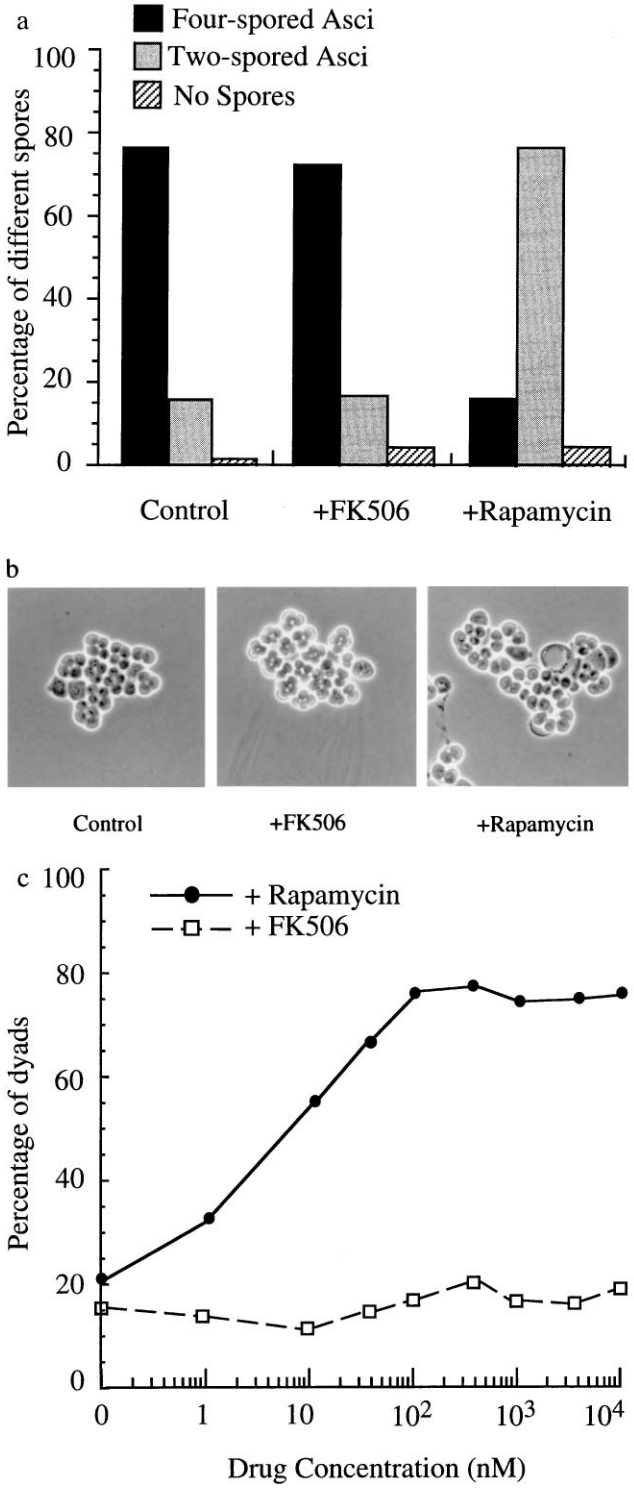

FIG. 3. (*a*) Rapamycin causes unusually high proportion of two-spored asci. Diploid SK-1 cells were incubated in YPD for 24 hr at 30°C with vigorous shaking and then diluted 1:50 into YPA. After 13.5 hr of further incubation, the cells were washed twice with sterile water and resuspended into SPM supplemented with methanol (control), 100 nM FK506 (+ FK506), or 100 nM rapamycin (+ rapamycin) for another 13 hr. Proportions of the different asci were counted (n = 250). The results shown were averaged from three different experiments. (*b*) The control and FK506- and rapamycin-treated spores under the light microscope (×1,000). (*c*) Dosage effects of FK506 and rapamycin on sporulation. Diploid SK-1 cells were cultured in YPD and grown to saturation density for 24 hr at 30°C with vigorous shaking. These cells were then diluted 1:50 into YPA. After 13.5 hr of further incubation, the cells were washed twice with sterile water and resuspended into SPM supplemented with 0, 1, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 nM FK506 or rapamycin, respectively. After incubation for another 13 hr, the proportion of two-spored asci was counted (n = 250). The results shown were averaged from three different experiments.

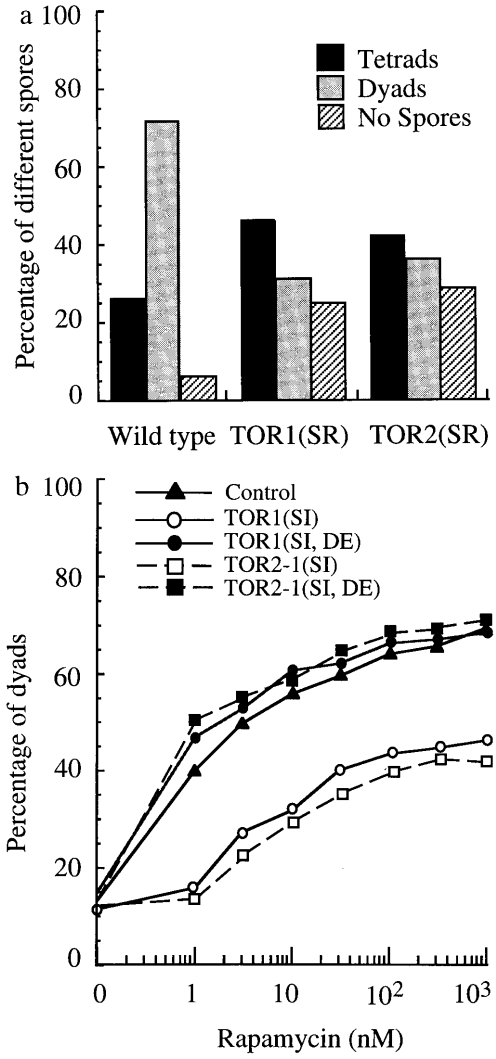

FIG. 4. (a) TOR1 (Ser-1972 → Arg) or TOR2 (Ser-1975 → Arg) renders dominant rapamycin resistance during meiosis. Wild-type diploid SK-1 [**a**/α, *TOR1/TOR1, TOR2/TOR2*] or SK-1 with chromosomal rapamycin-resistant mutations Ser-1972 → Arg in TOR1 [**a**/α, *TOR1/TOR1* (Ser-1972 → Arg), *TOR2/TOR2*] or Ser-1975 → Arg in TOR2 [**a**/α, *TOR1/TOR1, TOR2/TOR2* (Ser-1975 → Arg)] cells were incubated in YPD for 24 hr at 30°C with vigorous shaking. These cells were then diluted 1:50 into YPA. After 13.5 hr of further incubation, the cells were washed twice with sterile water and resuspended into SPM supplemented with 100 nM rapamycin. The samples were analyzed as in Fig. 3*a*. (*b*) The kinase activities of Tor proteins are required for rapamycin-sensitive meiotic functions. Diploid SK-1 strains containing a control plasmid, a plasmid expressing *TOR1* (Ser-1972 → Ile), *TOR1* (Ser-1972 → Ile, Asp-2294 → Glu), *TOR2–1* (Ser-1972 → Ile), and *TOR2–1* (Ser-1972 → Ile, Asp-2294 → Glu), respectively, were incubated in selection medium for 36 hr to saturation, diluted 1:50 into YPA for another 13 hr before being resuspended into SPM supplemented with different concentrations of rapamycin for a further 13.5 hr. The results represent an average of three different experiments (n = 250).

see Fig. 4*b* for the related Ile mutant). However, their resistance to rapamycin decreases slightly with the increase of rapamycin concentration. For example, 45% of the TOR1 (Ser-1972 → Arg) strain [*MAT***a**/α, *TOR1/TOR1*(Ser-1972 → Arg)] formed tetrads and 28% dyads in 100 nM rapamycin

(Fig. 4*a*). A Ser → Ile mutation in either TOR1 or TOR2 also causes similar rapamycin resistance (Fig. 4*b*). We have previously found that FKBP12-rapamycin can bind slightly to TOR1 (Ser-1972 → Arg) when added at much higher concentration (data not shown), and we feel this is the likely cause of the decreased rapamycin resistance at higher rapamycin concentrations. Thus, these results indicate that Tor1p and Tor2p are the direct targets of rapamycin responsible for the inhibition of tetrad formation. It is interesting to note that the Ser-1972 → Arg in the TOR1 locus or Ser-1975 → Arg in the TOR2 locus has sharply increased the percentage of cells that do not initiate sporulation. In comparison to only about 5% wild-type SK-1 cells unable to sporulate, more than 25% of the mutant cells are not able to sporulate (Fig. 4*a*). The Ser → Ile mutations render even higher percentage of cells unable to sporulate (40%) (data not shown).

To test whether Tor's kinase activity is necessary for this meiotic function, we introduced extrachromosomal copies of the rapamycin-resistant mutant, *TOR1* (Ser-1972 → Ile), or a kinase-dead rapamycin-resistant double mutant, *TOR1* (Ser-1972 → Ile, Asp-2294 → Glu) into the SK-1 strain. When treated with rapamycin, both endogenous, wild-type TOR gene products are inhibited, leaving TOR1 (Ser-1972 → Ile) as the only TOR gene to provide the rapamycin-sensitive functions (11, 15). TOR1 (Ser-1972 → Ile) provided rapamycin-resistance (Fig. 4*b*), allowing formation of tetrads in the presence of rapamycin. However, the addition of the second kinase-inactivating mutation (Asp-2276 → Glu) completely prevented the Ser-1972 → Ile mutation from conferring resistance (Fig. 4*b*). Similar results were obtained with TOR2 (Fig. 4*b*). Thus, the kinase domains of Tor1p and Tor2p are required for their rapamycin-sensitive late meiotic function.

To map the stage of the late meiotic function, we added rapamycin into a synchronized SK-1 cell culture at increasing times after induction of sporulation. After resuspended into SPM, SK-1 cells quickly enter the meiotic cell cycle synchronously. Premeiotic DNA replication starts within 30 min and is complete at around 3 hr, which is followed by the pachytene stage, where homologous recombination occurs. Thereafter, two meiotic cell divisions occur between 6 and 8 hr. Spore-packaging completes the entire sporulation process at about 10 hr (refs. 16 and 17 and data not shown). The final proportions of dyads were counted as an indicator of rapamycin inhibition. The rapamycin-treated cells initiated and finished their premeiotic DNA replication on schedule as shown by flow cytometric analysis (data not shown), indicating that rapamycin does not interfere with sporulation initiation or premeiotic DNA replication. Rapamycin does not appear to perturb the timing of the entire sporulation process, since the time it takes for visible ascospores to appear is approximately the same for both the control and rapamycin cultures. High proportions of two-spored asci are produced when rapamycin is added between 0 and 3 hr. However, the effect of rapamycin drops sharply between 3 and 4 hr, and rapamycin is no longer effective when added after 4 hr. Thus, there appears to be a critical window between 3 and 4 hr when the kinase activities of Tor1p and Tor2p are required for efficient tetrad formation, apparently at the pachytene stage of meiosis (Fig. 5).

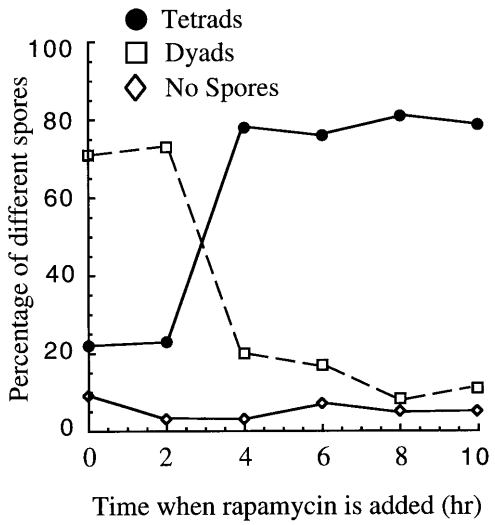

FIG. 5. Timing of the late rapamycin-sensitive step during late meiosis. Diploid SK-1 cells were incubated in YPD for 24 hr at 30°C with vigorous shaking. These cells were then diluted 1:50 into YPA. After 13.5 hr of further incubation, they were washed twice with sterile water and resuspended into SPM (0 hr), and then 100 nM rapamycin was added to various culture aliquots at 0, 2, 4, 6, 8, and 10 hr, and the proportion of different asci in each sample was analyzed as in Fig. 3*a*.

DISCUSSION

In haploid yeast, sufficient nutrient supplies trigger the TOR-signaling pathway leading to, among others, eIF4E-dependent translation initiation. The cells pass through G_1 and progress into S phase, where DNA synthesis occurs. Starvation or treatment with rapamycin causes inhibition of the kinase activities of Tor proteins and inhibition of translation initiation for proteins required for entry into and progression through the G_1 phase of the cell cycle. As a result, cells enter a quiescent state (G_0) (8). In diploid yeast, starvation induces a cell cycle switch from the proliferative (mitotic) state to a sporulative (meiotic) state. Our results with diploid yeast cells show that Tor proteins have an integral role in the nutrient-sensing mechanism that regulates this switch. Although active Tor proteins (Ser-1972/1975 mutants resulting from homologous recombination or wild-type protein overexpressed by using a galactose-inducible promoter) prevent the cells from exiting the mitotic cell cycle, inhibition of Tor proteins induces entry into meiosis. However, more than one pathway is likely to be required for the initiation of meiosis since inhibiting Tor proteins in cells in the logarithmic phase of growth is not sufficient to induce sporulation. Another pathway thought to link the absence of nutrients to quiescence (haploid cells) and

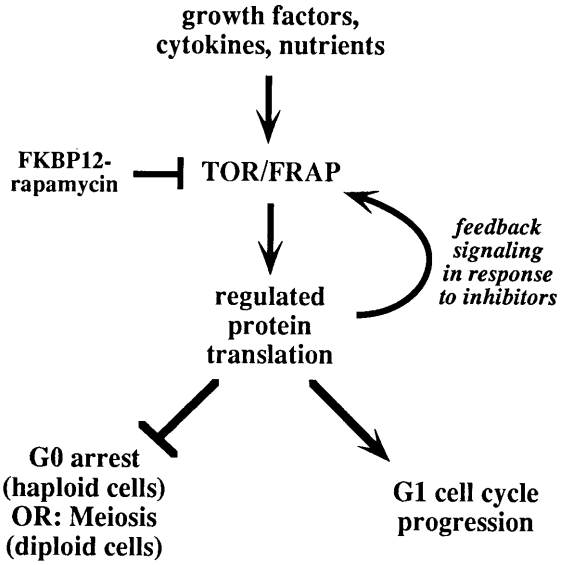

FIG. 6. Outline of TOR/FRAP pathways. Extracellular signals pass through the Tor and FRAP proteins, suppressing quiescence or meiosis and promoting cell cycle progression. The pathway can also be activated with cell-permeable RNA ligands that inhibit protein synthesis, a process reminiscent of the activation of the Mec1p-mediated pathway by cell-permeable DNA ligands or by inhibitors of DNA synthesis (2).

meiosis (diploid cells) in budding yeast is mediated by the Ras protein (14). In both pathways, inactive forms of the proteins (Ras and Tor proteins) lead to an exit from the proliferative state. The relationship between these pathways remains to be detemined, although Tor proteins do not appear to be acting upstream of Ras since rapamycin causes G_1 arrest of cells containing a hyperactive *RAS2* allele (8).

Tor and FRAP proteins appear to function in surveillance pathways that monitor changes in the extracellular environment (nutrients, growth factors, or cytokines) and regulate the levels of synthesis of proteins necessary for cell cycle exit or progression (refs. 2 and 8, and Fig. 6). In addition, intracellular circumstances that perturb protein synthesis can also activate the FRAP-$p70^{S6K}$ pathway to enhance the efficiency of protein synthesis (refs. 2 and 3 and Fig. 6). Tor proteins' kinase activities are required for the initiation of protein synthesis necessary for G_0 exit and G_1 cell cycle progression or possibly, in analogy to FRAP, for overcoming toxic conditions that interfere with protein synthesis. In contrast, inhibiting the kinase activities of Tor proteins is a prerequisite for the quiescent state or sporulation under starvation conditions.

As noted above, Ser-1972 → Arg in TOR1 or the Ser-1975 → Arg in TOR2 significantly reduces the ability of yeast cells to undergo meiosis. Since overexpression of TOR1(Ser-1972 → Ile) in haploid cells appears to cause overreplication of nuclear DNA (data not shown and ref. 11), these mutations may cause a gain of function in Tor proteins, possibly by activating their kinase activities. Sporulation of diploid yeast cells should provide a sensitive assay for the function of mutant Tor proteins.

We have also shown that the kinase activities of Tor proteins are required for a "late meiotic function" necessary for tetrad formation. Inhibiting this function results in a significant increase in the number of ascospores containing two, rather than four cells with 1n DNA content. These asci show four (presumed) nuclei that are stained with DAPI, yet only two are contained within spore cell walls. This function is also likely to involve control of protein translation since it is sensitive to rapamycin. The synthesis of specific proteins in late meiosis (apparently the pachytene stage) may be required for steps in meiotic cell division or spore packaging leading to tetrad formation. Further experiments are needed to reveal the molecular targets controlling these processes. Possible candidate meiotic genes are those known to be expressed during the pachytene stage and required for the later stages of sporulation (16). Although FRAP is expressed in at least low-to-moderate levels in all human tissues examined, like the human Mec1p homolog FRP/ATR (4, 5), it is highly expressed in testis (18). Therefore, the meiotic functions of Tor1p, Tor2p, and Mec1p may be conserved in humans as well. Understanding these functions and the surveillance and other pathways mediated by phosphatidylinositol kinase-related kinases is an important goal in signal transduction research.

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