

TOR2 is required for organization of the actin cytoskeleton in yeast

(signal transduction/rapamycin/TCP-1 chaperonin/phosphatidylinositol kinase/cell growth)

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ABSTRACT The *Saccharomyces cerevisiae* gene *TOR2* encodes a putative phosphatidylinositol kinase that has two essential functions. One function is redundant with *TOR1*, a *TOR2* homolog, and is required for signaling translation initiation and early G_1 progression. The second essential function is unique to *TOR2*. Here we report that loss of the *TOR2*-unique function disrupts polarized distribution of the actin cytoskeleton. A screen for dosage suppressors of a dominant negative *TOR2* allele identified *TCP20/CCT6*, encoding a subunit of the cytosolic chaperonin TCP-1 that is involved in the biogenesis of actin structures. Overexpression of *TCP20* restores growth and polarized distribution of the actin cytoskeleton in a *tor2* mutant. *TCP20* overexpression does not restore growth in a *tor1 tor2* double mutant. We suggest that the unique function of the phosphatidylinositol kinase homolog *TOR2* is required for signaling organization of the actin cytoskeleton during the cell cycle. *TOR2*, via its two functions, may thus integrate temporal and spatial control of cell growth.

Budding yeast undergoes polarized cell growth that is dependent on an asymmetric distribution of the actin cytoskeleton. As *Saccharomyces cerevisiae* initiates a new cell cycle, membrane-localized actin patches concentrate at a previously selected and marked bud site, the secretory apparatus becomes oriented toward this site, and bud emergence begins (1, 2). During maturation of the bud, actin cables become prominent in the mother cell and align toward the bud. The polarized actin distribution is lost during mitosis when the actin patches redistribute to the surface of the mother and the bud. Before cytokinesis, actin patches relocate to the mother–bud neck to target secretion to this region for the formation of the septum. Mutations in genes encoding the small GTPases CDC42, RHO1, RHO3, and RHO4 disrupt polarized growth and, in some cases, the highly asymmetric distribution of actin structures (3–6). However, the nature of the signal(s) received by these Rho-like GTPases and the mechanisms by which they control the actin cytoskeleton during vegetative growth are not known.

In mammalian cells, Rho-like GTPases, including Rho, Rac, and Cdc42Hs, are also involved in the control of the actin cytoskeleton (7). They are thought to be key regulators in signaling pathways linking growth factor receptors to the assembly and organization of the actin cytoskeleton. Using cell-free assays and intact cell systems, it has been shown that Rho-like GTPases interact, physically or functionally, with phosphatidylinositol (PI) kinases. For example, Rho activates PI 3-kinase and PI-4-P 5-kinase (PIP 5-kinase) (8, 9), and Cdc42Hs activates PI 3-kinase (10); PI 3-kinase activates Rac (11); Cdc42Hs, Rho, and Rac physically interact with PI kinases (10, 12, 13). PI kinases or their products, phosphorylated phosphoinositides, have also been implicated in the

control of the actin cytoskeleton. Actin rearrangement in neutrophils and fibroblasts correlates with the synthesis of phosphatidylinositol-3,4,5-triphosphate, and wortmannin, a PI 3-kinase inhibitor, blocks such rearrangement (14–16). In addition, phosphorylated phosphoinositides modulate the interaction of actin-capping proteins with actin *in vitro* (17), and, in permeabilized platelets, activated Rac uncaps actin filament barbed ends through phosphoinositide synthesis (18). This suggests that PI kinases and Rho-like GTPases are part of the mammalian signal transduction pathways that regulate the reorganization of the actin cytoskeleton in response to external stimuli.

TOR1 and *TOR2* are structurally related PI kinase homologs first identified in yeast as the targets of the immunophilin–immunosuppressant complex FKBP–rapamycin (19–22). Treatment with rapamycin or combined deletion of *TOR1* and *TOR2* causes yeast cells to arrest in early G_1 , to undergo a severe reduction in protein synthesis, and to induce several other physiological changes characteristic of starved cells entering stationary phase (G_0) (23). *TOR1* and *TOR2* are presumably part of a novel signaling pathway that activates translation initiation and, thereby, G_1 progression in response to nutrient availability. It remains to be determined that *TOR1* and *TOR2* indeed possess kinase activity.

While combined disruption of *TOR1* and *TOR2* causes a G_1 arrest, disruption of *TOR1* alone has little or no effect and loss of *TOR2* alone is lethal, with cells arresting throughout the cell cycle (20–22). The lethality of a *TOR2* disruption cannot be suppressed even by overexpression of *TOR1*. This suggests that *TOR2* has at least two essential functions (24). One function is shared with *TOR1*, is rapamycin-sensitive, and is required for translation initiation and G_1 progression, whereas the second essential function is unique to *TOR2*. It is not known what the unique function of *TOR2* might be and how it might be related to the shared G_1 function.

Here we show that loss of *TOR2* alone disrupts the polarized distribution of the actin cytoskeleton during the cell cycle. Overexpression of *TCP20/CCT6* suppresses the lethality of a *TOR2* mutation, but not a *TOR1 TOR2* double mutation, and partially restores polarized organization of the actin cytoskeleton in strains lacking *TOR2*. *TCP20* encodes a subunit of the TCP-1 complex, a chaperonin known to be involved in the folding and assembly of actin structures (25–36). This suggests that the unique function of *TOR2* is involved in the organization of the actin cytoskeleton.

MATERIALS AND METHODS

Strains, Plasmids, and Media. *S. cerevisiae* strains used in this work are listed in Table 1. All strains were isogenic JK9-3da derivatives. Plasmid pJK5 is pSEY68 galp (*amp^r CEN URA3 GAL1* promoter), containing the entire *TOR2* gene under control of the *GAL1* promoter (20). pJK9 is pSEY18

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Abbreviation: PI, phosphatidylinositol.

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Table 1. Strains used in this study

Strain	Genotype
JK9-3da	<i>MATa leu2-3,112 ura3-52 trp1 his4 rme1 HMLa</i>
JK9-3da/ α	<i>MATa/MATα leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1/trp1 his4/his4 rme1/rme1 HMLa/HMLa</i>
MH346	JK9-3da/ α <i>ade2/ade2 tor2::ADE2-3/TOR2</i>
MH349-3d	JK9-3da <i>tor1::LEU2-4</i>
NB17-3d	JK9-3da <i>ade2 his3 HIS4 tor1::HIS3</i>
JK350-18a	JK9-3da <i>ade2 tor2::ADE2-3/pJK5 (pSEYC68galp::TOR2)</i>
SH121	JK9-3da <i>ade2 tor2::ADE2-3/YCplac111::tor2-21^{ts}</i>
SH221	JK9-3da <i>ade2 his3 HIS4 tor1::HIS3 tor2::ADE2-3/YCplac111::tor2-21^{ts}</i>

(*amp^r 2 μ URA3*) containing a 3.0-kb *SalI* restriction fragment encompassing the 3'-region of the *TOR2* gene. pJK10 is a deletion variant of plasmid pJK9 where the internal 1.3-kb *BglII* restriction fragment had been deleted. pJK11 is plasmid pJK9 containing the *tor2^{kin-}* allele, a single nucleotide change converting *TOR2* codon 2,279 from GAC (aspartate) to GCC (alanine). pTOR2kin- (pJK66) is pAS10 containing the *tor2^{kin-}* mutation. pAS10 is pSEYC68 galp (*amp^r CEN URA3 GAL1* promoter) containing amino-terminally 6His-tagged *TOR2* under control of the *GAL1* promoter; amino-terminally 6His-tagged *TOR2* is functionally indistinguishable from untagged *TOR2* (A.S., unpublished work). *YCplac111::tor2-21^{ts}* (*amp^r CEN LEU2*) contains the entire *TOR2* gene and was isolated by hydroxylamine mutagenesis as a temperature-sensitive *TOR2* allele (S. B. Helliwell and N. C. Barbet, unpublished work). pAS26 is *YEplac181 (amp^r 2 μ LEU2)* carrying a 2.8-kb *HindIII-SpeI* genomic DNA insert containing the *TCP20* gene. pAS27 is pSEY18 (*amp^r 2 μ URA3*) carrying a 2.6-kb *SacI* genomic DNA insert containing the *TCP20* gene. The yeast genomic library in a multicopy *LEU2* vector (gift of K. Nasmyth, Institute of Molecular Pathology, Vienna) was in *YEp13* and had an average insert size of 8 kb. The composition of rich medium [yeast extract/peptone/dextrose (YPD)] and synthetic minimal medium (SD, SGal/Gly) complemented with the appropriate nutrients for plasmid maintenance was as described (37). Wild-type strain JK9-3da containing pAS27 was examined for resistance to rapamycin (gift of Sandoz Pharmaceutical) at concentrations of 20 and 200 ng/ml in YPD at 30°C. 5-Fluoroorotic acid (5-FOA) was used to counterselect *URA3* plasmid pJK5 in strain JK350-18a as described (37).

Construction of pTOR2kin-. To construct the *TOR2* kinase-dead allele, PCR-based mutagenesis was performed with the mutagenic primers 5'-TTAGGTGCCCGCCACC and 5'-GGTGGCGGGCACCTAA and flanking primers 5'-CTACAACATGTGTGCC and 5'-CAGCACTGACCCTTTTG. A first round of PCR was performed using a mutagenic primer in combination with a flanking primer and *Taq* polymerase (Boehringer Mannheim), the two independent PCR reactions were gel-purified and used in a second round of PCR with only the flanking primers. A 1.3-kb *BglII* fragment encompassing the mutagenized *TOR2* region was excised from the 3.0-kb PCR product and subcloned into the *BglII* site of plasmid pJK10, yielding plasmid pJK11. The mutant DNA was inserted back into *TOR2* on a 3.0-kb *SalI* restriction fragment yielding the full-length *TOR2* kinase-dead allele (plasmid pJK66). The entire 1.3-kb *BglII* region derived by PCR mutagenesis was sequenced to verify that only the desired nucleotide change was introduced.

Genetic Techniques. Yeast plasmid DNA was isolated as described (38). Yeast transformation was performed by the lithium acetate procedure (39). *Escherichia coli* strain DH5 α was used for propagation and isolation of plasmids as described (40).

DNA Manipulations. Restriction enzyme digests and ligations were done by standard methods. All enzymes and buffers were obtained commercially (Boehringer Mannheim). DNA

was sequenced by the dideoxy-chain termination method with the T7 sequencing system (Pharmacia).

Fluorescence Microscopy. Cells were grown to early logarithmic phase, fixed in formaldehyde, and stained with 4',6-diamidino-2-phenylindole (DAPI) or rhodamine phalloidin to visualize DNA and actin, respectively, as described (41).

RESULTS

Isolation of TCP20 as a Dosage Suppressor of a Dominant Negative TOR2 Allele. Catalytically inactive kinases often confer a dominant negative effect because they titrate away an interacting factor. Overexpression of the factor itself or other, downstream components of the pathway can suppress the dominant negative phenotype. To use this strategy to identify components in a pathway with *TOR2*, we constructed a *TOR2* kinase-dead allele (*tor2^{kin-}*) under the control of the inducible *GAL1* promoter. A point mutation was introduced into the sequence encoding the ATP-binding site of the lipid kinase motif of *TOR2*, changing aspartate 2,279 to alanine (see *Materials and Methods*). An equivalent mutation was shown to abolish the enzymatic activity of the VPS34 and p110 PI 3-kinases (42, 43). *tor2^{kin-}* could not provide *TOR2* function as determined by complementation assays; temperature-sensitive *tor2* mutants containing plasmid-borne *tor2^{kin-}* did not grow on galactose medium at nonpermissive temperature, and no viable *tor2* progeny were obtained on galactose medium upon dissection of a heterozygous *tor2/TOR2* diploid (MH346) containing the plasmid-borne *tor2^{kin-}* allele (data not shown). Overexpression of *tor2^{kin-}* in our wild-type strain JK9-3da conferred a slow growth phenotype that was suppressed by overproduction of wild-type *TOR2* (Fig. 1; data not

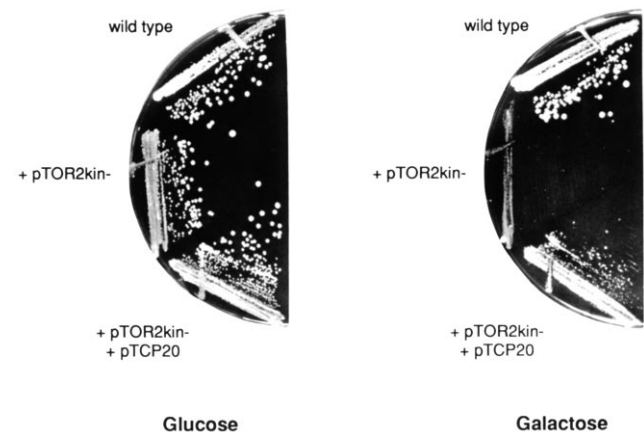


FIG. 1. Overexpression of *tor2^{kin-}* confers a growth defect that is suppressed by overexpression of *TCP20*. Wild-type cells (JK9-3da) transformed either with pTOR2kin- or with pTOR2kin- and pTCP20 (pAS26) were streaked on SD-URA-LEU and SGal/Gly-URA-LEU medium. The *tor2^{kin-}* allele is under control of the *GAL1* promoter and therefore induced on galactose and repressed on glucose.

shown). The above results indicated that *tor2*^{kin-}, as expected, is a dominant-negative allele of *TOR2*.

To identify components that lie in a TOR2 signaling pathway, we screened for high copy number suppressors of *tor2*^{kin-}. A yeast genomic library constructed in a high copy number *LEU2* vector was introduced into JK9-3da/pTOR2kin-, and transformants were selected on SD-URA-LEU (*tor2*^{kin-} repressed) and then screened for growth on SGal/Gly-URA-LEU (*tor2*^{kin-} induced). Out of 7000 transformants screened, 11 transformants were isolated that showed plasmid-dependent suppression of the growth defect conferred by overexpression of *tor2*^{kin-}. Plasmid DNA of these transformants was isolated, analyzed by restriction enzyme digestion, and sequenced from both ends of the insert of the genomic DNA. The obtained DNA sequence information was compared with sequences entered in the European Molecular Biology Laboratory and GenBank data bases using the BLAST program (44). This revealed six different genomic regions among the 11 isolates. The entire sequence of each region was already available, and the open reading frame in each region responsible for the suppression of the growth defect conferred by *tor2*^{kin-} was identified by subcloning and deletion analyses. Three of the suppressors (*MIG1*, *GAL3*, and the *GAL1*, *GAL10*, and *GAL7* cluster) were found to encode proteins of the galactose system, and were thus not analyzed further. Two were open reading frames (*YHR127w* and *YBR057c*) without significant homology to other genes or to each other, and one gene was *TCP20/CCT6* (Fig. 1). *TCP20* is an essential gene required for cytoskeletal function (see below).

***TCP20* Overexpression Restores Growth in a *tor2* Mutant but Not in a *tor1 tor2* Double Mutant.** We examined if *TCP20* overexpression could suppress recessive *tor2* mutations, a temperature-sensitive allele, a promoter alteration, and a null mutation, in addition to *tor2*^{kin-}. First, *tor2*^{ts} strain SH121 was transformed with pAS27 (pSEY18::*TCP20*) and with an empty vector, and was checked for growth at restrictive temperature. Whereas transformants carrying the empty vector were inviable at 37°C, the *tor2*^{ts} cells overexpressing *TCP20* were able to grow at the restrictive temperature, however, not as well as *TOR2* wild-type cells (Fig. 2). Second, pAS26 (YEplac181::*TCP20*) and an empty vector were transformed into strain JK350-18a that contained a genomic disruption of *TOR2* and a plasmid-borne copy of *TOR2* under the control of the *GAL1* promoter (pJK5). The transformants were tested for growth on medium containing glucose as a carbon source

where the *GAL1* promoter is repressed. Again, *tor2* cells carrying an empty vector did not grow, whereas cells overexpressing *TCP20* were able to form colonies (data not shown). Finally, to determine if *TCP20* overexpression could suppress a *TOR2* null allele, the above strain JK350-18a containing a *TOR2* disruption, *TCP20* in high copy (pAS26), and plasmid-borne *TOR2* (pJK5) was streaked on 5-FOA medium (see *Materials and Methods*) to clear the plasmid-borne copy of *TOR2*. JK350-18a overexpressing *TCP20* was unable to grow in the presence of 5-FOA (data not shown). Thus, *TCP20* overexpression could suppress the growth defect caused by a *tor2*^{ts} mutation and by a severe reduction in the amount of TOR2, but could not restore growth in the complete absence of TOR2.

Previous results have indicated that TOR2 has two functions: one function is redundant with TOR1 and the other function is unique to TOR2 (20, 22, 45). The observation that *TCP20* overexpression suppressed the growth defect of a *TOR1 tor2* strain, where TOR1 still provides the shared function, suggested that *TCP20* is related to the TOR2-unique function. To investigate this further, we examined if overexpression of *TCP20* could suppress either *tor2*^{kin-} or a recessive *tor2*^{ts} mutation in cells that lacked *TOR1*. Whereas *TCP20* overexpression was able to rescue *tor2*^{ts} cells and to suppress *tor2*^{kin-} in the presence of TOR1 (*TOR1 tor2*^{ts} and *TOR1 TOR2/tor2*^{kin-} strains SH121 and JK9-3da/pTOR2kin-, respectively), as described above, it was unable to do so in the absence of TOR1 (*tor1 tor2*^{ts} and *tor1 TOR2/tor2*^{kin-} strains SH221 and NB17-3d/pTOR2kin-, respectively) (Fig. 2 and data not shown). In addition, overexpression of *TCP20* could not suppress the dominant negative effect of a *tor1*^{kin-} construct (A.S., J.K., S. B. Helliwell, unpublished work) (45). Overexpression of *TCP20* also did not suppress the slight growth defect of a *TOR1* deletion and did not confer resistance to rapamycin (data not shown). These findings indicated that *TCP20* overexpression suppresses the loss of the TOR2-unique function but not the loss of the TOR1-shared function, and suggest that *TCP20* is related to the TOR2-unique function.

Cells Lacking *TOR2* Are Defective in the Organization of the Actin Cytoskeleton. *TCP20*, recently renamed *CCT6*, is a member of the *TCP1* gene family and encodes a subunit of the cytosolic TCP-1 chaperonin complex (25, 26). This chaperonin has been implicated specifically in the folding of actin and tubulin. Mammalian TCP-1 complexes have been shown to bind *in vitro* to denatured or newly synthesized actin and tubulin monomers and to release them upon ATP-hydrolysis in an altered, assembly competent conformation (27–31). In yeast, eight members of the *TCP1/CCT* gene family have been identified and the five examined by disruption analysis have all been shown to be essential (25, 26, 32–36). Conditional alleles of these genes all exhibit a disorganization of the actin cytoskeleton and disruption of microtubule-mediated processes such as nuclear segregation, supporting the role of the TCP-1 complex in the function of actin and tubulins.

The suggestion that *TCP20* is functionally related to TOR2 (see above) led us to examine if the lack of *TOR2* caused a defect in the organization of the actin cytoskeleton. Wild-type (JK9-3da) and *tor2*^{ts} (SH121) cells were grown at permissive temperature (30°C) and then shifted to restrictive temperature (37°C) for 6 h. Cells were fixed and stained with phalloidin (see *Materials and Methods*). Whereas wild-type cells displayed the normal polarized distribution of actin at the various stages of the cell cycle, cells lacking *TOR2* were increased in size and exhibited a randomized distribution of actin in all phases of the cell cycle in essentially all cells (Fig. 3). Unbudded cells just prior to entering S phase were not able to assemble (or maintain) the actin cap at the previously selected bud site. Most (>90%) of small- and middle-sized budded cells showed a random distribution of actin patches in both the mother and

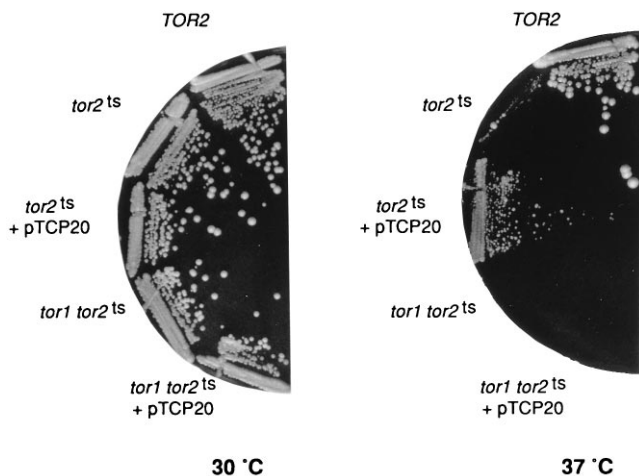


FIG. 2. Overexpression of *TCP20* suppresses the growth defect of a *tor2*^{ts} mutation but not of a *tor1 tor2*^{ts} double mutation. Wild-type *TOR2* (JK9-3da), *tor2*^{ts} (SH121), and *tor1 tor2*^{ts} cells (SH221) carrying an empty vector or pTCP20 (pAS27) were streaked on YPD and incubated at 30°C or 37°C. *tor2*^{ts} and *tor1 tor2*^{ts} cells carrying an empty vector are not viable at 37°C.

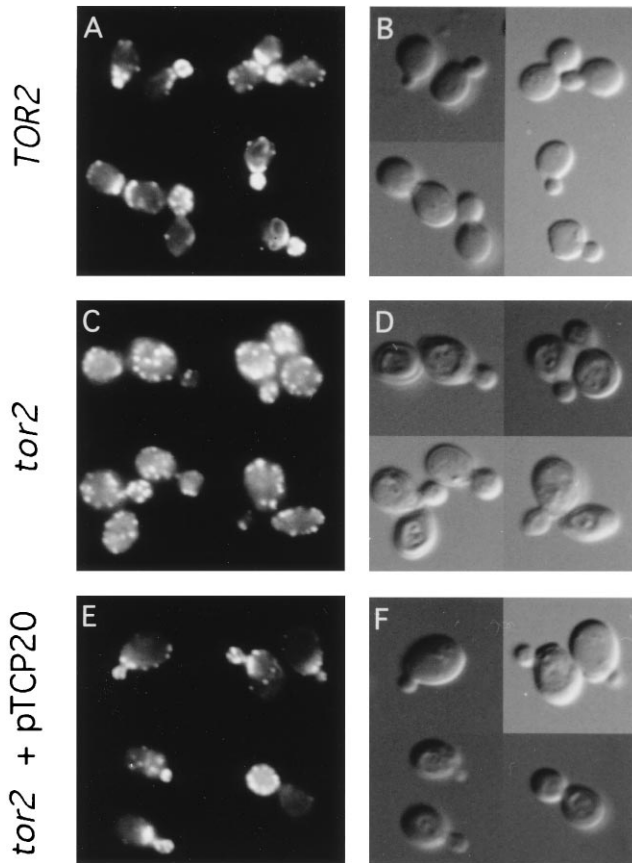


FIG. 3. Overexpression of *TCP20* suppresses the actin organization defect of *tor2^{ts}* cells. Logarithmic cultures of wild-type *TOR2* (JK9-3da) (A and B) and *tor2^{ts}* (SH121) cells carrying an empty vector (C and D) or p*TCP20* (pAS27) (E and F) were grown at 30°C, shifted to 37°C for 6 h, fixed, stained with rhodamine phalloidin, and observed by fluorescence (A, C, and E) and Nomarski (B, D, and F) microscopy. *tor2^{ts}* cells carrying an empty vector have a random actin distribution.

the daughter cell, with no concentration of patches in the daughter cell. Furthermore, no actin cables could be detected in the mother cell. Large-budded cells, in all cases, failed to display a relocation of actin patches to the mother–bud neck before cytokinesis. The same result was obtained with cells that carried the *TOR2* gene under control of the *GAL1* promoter (strain JK350-18a) and that were shifted to glucose for 20 h. Thus, cells lacking only *TOR2* were severely defective in the organization of the actin cytoskeleton. Cells lacking only *TOR1* (strain MH349-3d) displayed a completely normal, cell cycle-dependent, polarized distribution of actin (data not shown). We could not examine the cell cycle-dependent distribution of actin in cells lacking both *TOR1* and *TOR2* because such a strain arrests in *G*₁.

Since the TCP-1 complex has also been implicated in the biogenesis and function of microtubules, we assessed if loss of *TOR2* also caused a defect in microtubule function similar to that observed in *tcp* mutants (32–36). We examined if a *tor2* mutant, like *tcp* mutants, had a defect in nuclear segregation by staining cells with the DNA-specific stain DAPI (see *Materials and Methods*). Both in wild-type cells (strain JK9-3da) and in *tor2* cells (strains SH121 and JK350-18a) grown at the nonpermissive condition for the mutant cells, the DNA in large-budded cells had divided and segregated into the mother and daughter cells or was localized in the mother–bud neck. Neither in a wild-type strain nor in the *tor2* strains did we find significant numbers of mononucleate large-budded cells or multi- or anucleate unbudded cells as observed with *tcp* mutants. From this we conclude

that nuclear segregation and thus microtubules function is not affected upon loss of *TOR2*.

***TCP20* Overexpression can Partially Restore Polarized Organization of the Actin Cytoskeleton in *tor2* Cells.** We next asked if *TCP20* overexpression restored organization of the actin cytoskeleton in cells lacking *TOR2*. The high copy number plasmid containing *TCP20* (pAS27) was introduced into *tor2^{ts}* strain SH121, and transformants were grown at 30°C, shifted to 37°C for 6 h, and fixed and stained with phalloidin. In comparison to *tor2^{ts}* cells carrying an empty vector, which behaved as described above, *tor2^{ts}* cells transformed with *TCP20* exhibited less severe defects in the organization of the actin cytoskeleton (Fig. 3). Approximately 35% of the cells with small buds showed a wild-type distribution of actin; actin patches were concentrated in the bud, and actin cables were visible in the mother cells. A small percentage of large-budded cells overexpressing *TCP20* exhibited the normal relocation of actin to the mother–bud neck. Cells with middle-sized buds, however, appeared unchanged and still displayed a random distribution of actin patches in both the mother and daughter cell. Thus, *TCP20* overexpression weakly suppressed both the growth defect and the actin distribution defect of a *tor2* mutant. The correlation between the extent of suppression of the actin and growth defects and the fact that *TCP20* affects actin function suggest that *TCP20* might suppress the growth defect of a *tor2* mutant by restoring actin function.

DISCUSSION

We have shown that loss of the PI-kinase homolog *TOR2* results in disruption of the asymmetric distribution of the yeast actin cytoskeleton during the cell cycle. In a *tor2* mutant, actin patches are not concentrated in the bud but are randomly distributed throughout the mother and the daughter cell, and no actin cables are visible in the mother cell. We isolated *TCP20*, which encodes a subunit of the eukaryotic cytosolic TCP-1 chaperonin, as a dosage suppressor of a dominant negative *TOR2* allele (*tor2^{kin-}*). Both in mammalian and yeast cells, the TCP-1 complex has been implicated specifically in the folding and biogenesis of the cytoskeletal proteins actin and tubulin (25–36). The mammalian TCP-1 complex associates with and facilitates the folding of newly synthesized actin monomers *in vitro*, and yeast mutants defective in genes encoding subunits of the TCP-1 complex have disorganized actin structures. Furthermore, overexpression of *TCPI* or *ANC2*, encoding two other subunits of the TCP-1 complex, suppresses *act1-1* and *act1-4*, mutant alleles of the actin gene that prevent actin cable formation (33, 35). Overproduction of *TCP20* restores actin organization and viability in a *tor2* mutant.

A *tor2* mutant arrests growth throughout the cell cycle within three to five generations, whereas a *tor1 tor2* double mutant arrests growth in *G*₁ within one generation, and *TOR1* overexpression cannot suppress a *TOR2* deficiency (20, 22, 23). Thus, *TOR2* has two essential functions, one of which is *TOR2*-unique and the other is redundant with *TOR1*. Previous studies have demonstrated that a *tor1 tor2* double mutant arrests growth in *G*₁ because of a defect in translation initiation (23). Three related observations presented here suggest that a *tor2* single mutant is inviable because of a defect in the actin cytoskeleton. First, a *tor2* mutant displays a defect in the cell cycle-dependent distribution of the actin cytoskeleton. Second, overexpression of *TCP20*, previously shown to affect actin function, suppresses the lethality of a *TOR2* disruption. Third, *TCP20* overexpression restores actin cytoskeleton distribution in a *tor2* mutant. Additional observations suggest that the essential, actin-related function of *TOR2* may be the *TOR2*-unique function. First, the *TOR2*-unique function is essential,

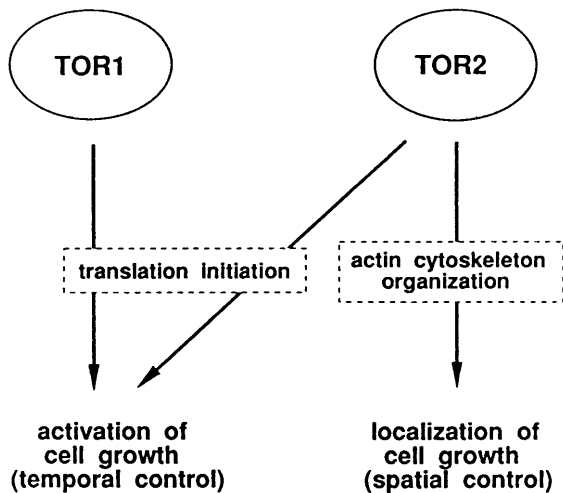


FIG. 4. Model of TOR2 unique and redundant functions controlling cell growth.

as the actin-related function appears to be. Second, cells disrupted only in *TOR1* show a completely normal polarized distribution of actin throughout the cell cycle; it is not possible to determine if a *tor1 tor2* double mutant has a defect in the cell cycle-dependent distribution of actin because these mutants arrest in G_1 . Third, overexpression of *TCP20* does not suppress the lethality of a *TOR1 TOR2* double mutation nor the slight growth defect conferred by a *TOR1* disruption alone. Fourth, *TCP20* overexpression does not confer resistance to rapamycin which is proposed to inhibit only the TOR1-shared function of TOR2 (45). Thus, it appears that the essential TOR2-unique function is to control the organization of the actin cytoskeleton. Finally, because *TCP20* was isolated as dosage suppressor of the kinase-dead *tor2^{kin-}* allele, we conclude that the presumed PI kinase activity of TOR2 is required for the function of TOR2 in the organization of the actin cytoskeleton. Zheng *et al.* (45) have suggested previously that an intact kinase domain is required for the unique function of TOR2.

How might TOR2 control organization of the actin cytoskeleton? Because PI kinases and Rho-like GTPases have previously been linked in controlling the actin cytoskeleton (see Introduction), the simplest model is that TOR2 is part of a signaling pathway involving small GTPases. This pathway might control the interactions between actin and the actin binding proteins, such as profilin, cofilin, fimbrin, capping protein, and verprolin (46, 47), which are responsible for the function and organization of the actin cytoskeleton. Increased amounts of *TCP20* might suppress a defect in this signaling pathway by compensating for an altered or missing interaction between actin and one of its binding proteins. It is unlikely that *TCP20* is suppressing by restoring TOR2 function directly because *TCP20* suppresses several different *TOR2* alleles including a promoter alteration.

Why should one protein, such as TOR2, control both translation initiation and organization of the actin cytoskeleton? This would provide a means of integrating temporal and spatial control of cell growth (Fig. 4). TOR2 might both activate synthesis of new material when nutrients are available and, via organization of the actin cytoskeleton, orient the newly synthesized material toward the appropriate site of growth, thus ensuring that growth is temporally and spatially coordinated.

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