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Drosophila Target of Rapamycin Kinase Functions as a Multimer

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ABSTRACT

Target of rapamycin (TOR) is a conserved regulator of cell growth and metabolism that integrates energy, growth factor, and nutrient signals. The 280-kDa TOR protein functions as the catalytic component of two large multiprotein complexes and consists of an N-terminal HEAT-repeat domain and a C-terminal Ser/Thr kinase domain. Here we describe an allelic series of mutations in the Drosophila *Tor* gene and show that combinations of mutations in the HEAT and kinase domains of TOR display the rare genetic phenomenon of intragenic complementation, in which two or more defective proteins assemble to form a functional multimer. We present biochemical evidence that TOR self-associates *in vivo* and show that this multimerization is unaffected by positive or negative signals upstream of TOR. Consistent with multimerization of TOR, recessive mutations in the HEAT and kinase domains can dominantly interfere with wild-type TOR function in cells lacking TSC1 or TSC2. TOR multimerization thus partially accounts for the high apparent molecular weight of TOR complexes and offers novel therapeutic strategies for pathologies stemming from TOR hyperactivity.

THE target of rapamycin (TOR) kinases are required for normal growth and development in a variety of eukaryotic species, and their dysregulation is linked to a number of overgrowth syndromes including tuberous sclerosis and some cancers (reviewed in Fingar and Blenis 2004; Inoki *et al.* 2005; Martin and Hall 2005). In both yeast and mammalian cells TOR has been shown to assemble into at least two distinct multisubunit complexes of 1.5–2.0 mDa (Fang *et al.* 2001; Hara *et al.* 2002; Kim *et al.* 2002; Loewith *et al.* 2002; Wedaman *et al.* 2003). Rapamycin-sensitive outputs of TOR such as protein synthesis and autophagy regulation are mediated by TOR complex 1 (TORC1), which includes TOR in association with the proteins GβL/LST8 and raptor/KOG1. A second complex known as TORC2 contains TOR, GβL, and rictor/AVO3, and functions in a rapamycin-insensitive manner to regulate the actin cytoskeleton (Loewith *et al.* 2002; Wedaman *et al.* 2003; Sarbassov *et al.* 2004). The stoichiometric relationships of these components, the number of unidentified components, and the mechanisms by which they regulate TOR signaling are unclear.

The binding sites of GβL and raptor have been mapped to distinct regions of the 280-kDa TOR polypeptide. The amino-terminal half of TOR consists of a series of tandem HEAT repeats, paired helical structures that align to form a large platform for protein–protein interactions (Andrade and Bork 1995). Multiple sites within the HEAT domain contribute to interactions with raptor (Kim *et al.* 2002), consistent with the widely distributed contacts reported for other HEAT-associated proteins (Groves *et al.* 1999). The TOR kinase domain, of the phosphoinositol kinase-related kinase (PIKK) family, acts as a binding site for GβL as well as for Rheb, a small Ras-related GTPase that mediates regulation of TOR by the tuberous sclerosis complex (TSC) 1 and TSC2 gene products (Kim *et al.* 2003; Long *et al.* 2005). Other defined domains of TOR include a central conserved region of unknown function termed the FAT domain, the FKBP12-rapamycin binding site (FRB) adjacent to the kinase domain, and a highly conserved sequence at the carboxy terminus (FATC) that may couple TOR stability to the redox state of the cell (Dames *et al.* 2005). Mutagenesis and overexpression studies have demonstrated that each of these domains is critical for TOR function. For example, deletions and point mutations in the HEAT, FRB, FATC, and kinase domains have been shown to perturb TOR activity, and the FAT and FRB domains dominantly inhibit TOR signaling when overexpressed (Brown *et al.* 1995; Alarcon *et al.* 1999; Sabatini *et al.* 1999; Vilella-Bach *et al.* 1999; Takahashi *et al.* 2000; Hennig and Neufeld 2002).

In contrast to these targeted mutagenesis studies, few examples of naturally derived or randomly generated mutations in the endogenous *Tor* gene have been described. In other PIKK family members such as ATM and DNA–PK, such mutations have been valuable reagents for structure-function analyses (Blunt *et al.* 1996; Concannon and Gatti 1997). The present study was undertaken to identify loss-of-function point mutations in the Drosophila *Tor* gene, to gain insight into

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functions of and interactions between different domains of TOR. Through this work we have generated a series of *Tor* mutant alleles, including the first reported missense mutations in the TOR HEAT repeats. We demonstrate that particular combinations of *Tor* mutants display intragenic (interallelic) complementation, and we provide genetic and biochemical evidence that this results from assembly of TOR polypeptides with defects in different domains into functional multimers. We discuss the implications of these results for our understanding of TOR function in normal and disease states.

MATERIALS AND METHODS

Drosophila strains and culture: Tor^{EP2353}, Tor^{k17004}, $Tor^{\Delta P}$, and *P[Tor^{WT}]* have been previously described (Zhang *et al.* 2000). Tor^{2L1} and Tor^{2L19} were a gift from E. Hafen (Zurich), $Tsc2^{193}$ was a gift from N. Ito (MGH), and $Tsc2^{109}$ was from the Bloomington *Drosophila* Stock Center. Flies were raised at 25° under uncrowded conditions on standard cornmeal/molasses/agar medium.

Mutagenesis: Male *yw* flies isogenic for the second and third chromosomes were fed 25 mm EMS overnight and mated *en masse* to *yw*; *P[w+]/SM6-TM6B* virgin females. Male progeny carrying a mutagenized chromosome in *trans* to the *Tb*-marked *SM6-TM6B* balancer were crossed individually to *yw*; $Tor^{\Delta P}$ *P[w+]/SM6-TM6B* virgin females. From noncomplementing crosses that failed to produce viable Tb^{+} progeny, mutants were recovered as balanced w^{-} flies. To purge second lethal mutations from the *Tor* mutant chromosomes, lines whose viability could not be restored by a 9.4-kb *Tor* rescue construct (Zhang *et al.* 2000) were outcrossed to isogenic *yw* flies; potential recombinant progeny were tested for rescuable failure to complement $Tor^{\Delta P}$. With the exception of Tor^{E161K}, which contains a closely linked second lethal mutation, viability and fertility of each homozygous mutant could be restored by the genomic rescue construct. Tor^{E161K} was classified as a class I mutant on the basis of its pupal lethal phase in heterozygous combination with class III null alleles.

For sequence determination, the *Tor* genomic region was PCR amplified in 4-kb segments from homozygous larvae of each mutant, using a 5:1 ratio of AmpliTaq Gold (Applied Biosystems, Foster City, CA) and Pfu turbo (Stratagene, La Jolla, CA) polymerases. PCR products were sequenced with BigDye terminator v3.1 cycle-sequencing reagents (Applied Biosystems) on an ABI PRISM 3100-Avant genetic analyzer, using primers spaced every 500 bp. Altered nucleotides were confirmed by sequencing products from independent PCR reactions. Assignment of the catalytic and activation loops and residues involved in ATP binding was determined by sequence alignment with PI3Kgamma (Walker *et al.* 1999).

Growth and viability assays: Adult body weight, wing size, cell size, and number were determined as described (Brogiolo *et al.* 2001). Percentage of survival was calculated as the variance from the expected number of nonbalanced progeny.

Immunoprecipitation: A total of 3×10^6 S2 cells per 60-mm plate were transfected with different combinations of plasmids (1 μg each), using the Effectene reagent (QIAGEN, Valencia, CA). After 48 hr, transfected cells were treated with vehicle, rapamycin (20 nM) or insulin (10 μg/ml) for 30 min and extracted in 0.5 ml ice-cold lysis buffer (20 mm HEPES, pH 7.6, 100 mm NaCl, 2 mm EGTA, 2 mm EDTA, 25 mm β-glycerolphosphate, 25 mm NaF, 1 mm Na_3VO_4, 1 mm DTT, 0.1% Triton X-100, and 1mm PMSF). After incubating the cell lysates with 2 μg of anti-HA antibody for 1 hr, 15 μl protein G agarose beads (Amersham, Arlington Heights, IL) were added and rocked at 4° overnight. Immunoprecipitates were washed five times with cold lysis buffer before analysis. Antibodies against HA and FLAG epitopes were from Sigma (St. Louis).

RESULTS

Identification of new point mutations in *Tor*: As an unbiased approach to identify domains and specific residues of the TOR polypeptide critical for its function, we carried out a forward genetic screen for EMS-induced mutations that fail to complement a *Tor* null mutation in Drosophila. From ~7900 mutagenized chromosomes, 7 new *Tor* alleles were identified, each of which resulted in lethality in *trans* to a *Tor* deletion (data not shown). To classify these mutants, we examined the developmental stage at which homozygous and hemizygous mutant animals arrest. This analysis, which also included previously described null and hypomorphic *Tor* alleles from Oldham *et al.* (2000) and Zhang *et al.* (2000), revealed three distinct phenotypic classes, described below.

Class I: Four of the mutants progressed through larval stages at a reduced rate and eventually arrested as small pupae. This is a weaker phenotype than that of previously described *Tor* mutants, and we designate these as class I mutations (Table 1). Interestingly, we found that each class I mutant results from a missense mutation in the HEAT-repeat domain of TOR (Figure 1A). Three of the four mutations map immediately adjacent to the hydrophobic core residues involved in folding of the HEAT-repeat structure (Figure 1B); in HEAT-repeat-containing proteins with solved crystal structures, analogous residues have been shown to mediate binding to interacting proteins (Chook and Blobel 1999). Although the residues altered by these mutations are well conserved in TOR proteins from a variety of species (Figure 1D), the resulting amino acid substitutions can be found in analogous positions in other HEAT repeats in TOR and other proteins (Perry and Kleckner 2003). Together, these data suggest that class I mutations are unlikely to grossly disrupt the three-dimensional HEAT-repeat structure of TOR, but rather may alter residues involved in specific protein–protein interactions.

Under optimal culture conditions, a low percentage (5–15%) of class I homozygous mutant animals were able to survive to adulthood. These adult escaper flies were about half the size of wild type (Figure 2A) and were developmentally delayed by 2 days at 25°. Interestingly, survival was skewed toward females ~10:1 over males. In addition, both males and females were sterile. Ovaries dissected from class I mutant females displayed arrested development and increased cell death at a previtellogenic stage (17/20 mutant and 5/20 wild-type germaria contained acridine orange positive cells; Figure 2, B–D), consistent with a previously described requirement for nutritional signaling in yolk production and cell survival during oogenesis (Bownes *et al.*

TABLE 1

Phenotypic classification of TOR mutant alleles

Class	Homozygous phenotype	Example
I	Pupal lethal; 5–15% small, sterile adult escapers	
II	Third instar larval arrest	
III	Second instar larval arrest	

Terminal phenotypes are listed for homozygous mutant animals. Images show representative examples of class I (Tor^{A948V}), II (Tor^{G2256D}), and III (Tor^{V2148D}) mutants. Bar, 1 mM.

1988; Drummond-Barbosa and Spradling 2001). Class I mutant males had abnormal testes with a grossly enlarged apical tip (Figure 2F), suggesting an increased number of germline stem cells or early stage spermatogonia. TOR signaling may normally function to promote differentiation or regulate proliferation of these cells.

Class II: One of the new *Tor* mutants was found to develop to the late third instar larval stage and arrest prior to pupation (Table 1). This mutation results in an aspartic acid substitution at a conserved glycine in the catalytic loop of the kinase domain (G2256D; Figure 1, C and D), and is thus likely to impair the catalytic activity of TOR; nonetheless, the relatively normal larval development of Tor^{G2256D} homozygotes indicates this mutant retains some degree of function. Oldham *et al.* (2000) have previously described a mutation in the activation loop of the TOR kinase domain (2L1/P2293L) that displays a similar late larval arrest. We thus designate these two mutants as class II alleles.

Class III: The final two mutants were phenotypically the most severe, arresting as small second instar larvae. Accordingly, one mutation results in a translation stop at codon 528 and the other causes an aspartic acid

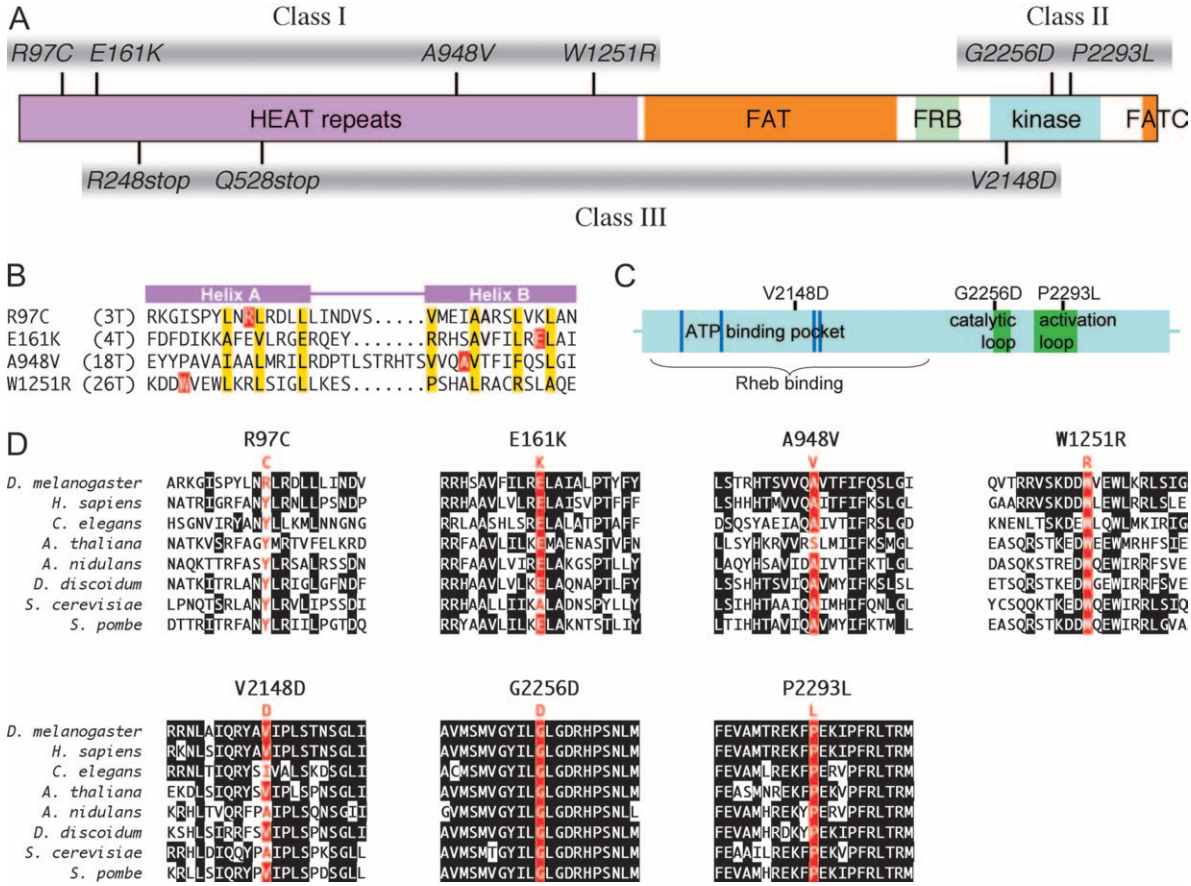

Figure 1.—Graphical representation of *Tor* mutant alleles. (A) Mutations are shown relative to defined structural and functional domains of the TOR polypeptide and are classified according to phenotypic severity and genetic interactions (see text). (B) Alignment of the four HEAT repeats carrying class I mutations. The two α-helices of each repeat are indicated above the alignments. The seven hydrophobic core residues of each repeat (Andrade *et al.* 2001) are highlighted in yellow, and mutated residues are highlighted in red. HEAT-repeat numbering (in parentheses) is from Perry and Kleckner (2003). (C) Mutations in the TOR kinase domain (residues 2065–2351) map to the catalytic and activation loops (green), and to the hydrophobic region of the ATP-binding pocket (residues predicted to interact with ATP are represented by dark blue stripes). The 153 amino acid Rheb-binding domain (Long *et al.* 2005) is indicated. (D) Multi-species sequence alignment of the region surrounding each TOR substitution mutation. Solid boxes indicate identical residues. $Tor^{R248stop}$ and Tor^{P2293L} are from Oldham *et al.* (2000), who refers to these alleles as *2L19* and *2L1*, respectively.

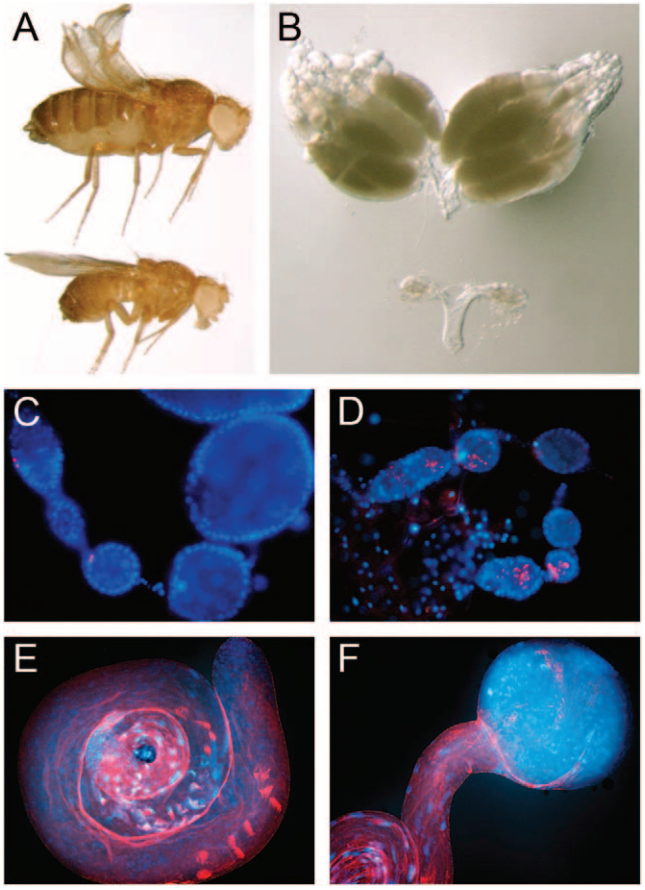

Figure 2.—Defective growth and gonadogenesis in *Tor* class I homozygous escaper adults. (A) Control (top; Tor^{A948V}/CyO) and class I homozygous mutant (bottom; Tor^{A948V}/Tor^{A948V}) adult females. (B) Brightfield image of ovaries from control (top) and Tor^{A948V} homozygotes (bottom). (C and D) Ovarioles from control (C) and Tor^{A948V} homozygous (D) mutant females, dissected and stained unfixed with acridine orange (apoptotic cells, red) and Hoechst 33342 (DNA, blue), are shown. (E and F) Testes from control (E) and Tor^{A948V} homozygous (C) males, stained for tubulin (red) and DNA (blue), are shown.

substitution at a conserved valine in the hydrophobic region of the ATP-binding pocket of the kinase domain (V2148D). In side-by-side comparison of these mutants with two previously described null alleles, $Tor^{2L19/R248stop}$ and $Tor^{\Delta P}$ (Oldham *et al.* 2000; Zhang *et al.* 2000), we were unable to distinguish any differences in growth rate or final size at arrest. We conclude that these mutants result in complete loss of function and categorize this group of four mutants as class III.

TOR behaves genetically and biochemically as a multimer: We next examined the phenotype of heteroallelic (*trans*-heterozygous) combinations within and between different classes of *Tor* mutations. As expected, the phenotypic severity of class I and class II alleles was slightly increased in heteroallelic combination with a class III (null) allele, whereas flies carrying two different alleles from within the same class displayed similar phenotypes and survival rates as either homoallelic mutant (Figure 3 and data not shown). Surprisingly, heteroallelic combinations of any of the four class I alleles with either class II allele resulted in an apparent restoration of wild-type TOR function. Class I/class II *trans*-heterozygotes developed to adulthood in nearly normal numbers, and the adult animals were fully fertile and of approximately normal size and weight (Figure 3A). The developmental delay and skewed male:female ratio observed in class I mutants was also rescued in the heteroallelic animals (data not shown). Such intragenic (interallelic) complementation is rare, but can result from self-dimerization of proteins with mutations in distinct functional domains, resulting in an active enzyme (Catcheside and Overton 1958). As class I mutations map to the HEAT-repeat domain and class II mutants to the TOR kinase domain, these results are consistent with TOR functioning as a dimer or multimer.

To investigate the possibility of TOR multimerization, we coexpressed full-length Drosophila TOR tagged with either FLAG or HA epitopes, and tested for co-immunoprecipitation of differentially tagged TOR molecules. In extracts from S2 cells coexpressing FLAG–TOR and HA–TOR, immunoprecipitation using anti-HA antibodies resulted in coprecipitation of FLAG–TOR (Figure 3C). This interaction was specific, as no FLAG–TOR was detected in HA immunoprecipitates in the absence of HA–TOR expression. Reciprocally, immunoprecipitation of FLAG–TOR from these cells allowed specific coprecipitation of HA–TOR. TOR–TOR association was not affected by treatment with rapamycin or insulin (Figure 3C) or by Rheb overexpression (data not shown), indicating that TOR multimerization is likely constitutive. Consistent with these observations, Wedaman *et al.* (2003) reported that the apparent size-by-gradient centrifugation of TOR complexes in yeast is unaltered by rapamycin or starvation for carbon or nitrogen. We also found that association was not disrupted by class I (A948V) or class II (G2256D and P2293L) mutations (data not shown), consistent with the intragenic complementation of these alleles. We thus conclude that TOR self-associates to form stable complexes containing two or more TOR molecules. Furthermore, the genetic complementation data indicate that this form of TOR is functional.

***Tor* point mutants behave as strong dominant suppressors of *Tsc2*:** One characteristic of homo-multimeric proteins is their susceptibility to dominant-interfering effects of mutant subunits (Sheppard 1994). Each of the *Tor* mutations described here is fully recessive to wild type (data not shown), suggesting that either assembly of these mutants into a TOR complex does not dominantly inhibit the function of wild-type TOR or the activity of the remaining functional TOR complexes is sufficient for normal growth and development. It was shown previously that heterozygous disruption of *Tor* can partially suppress the lethal effects of *Tsc1* or *Tsc2*

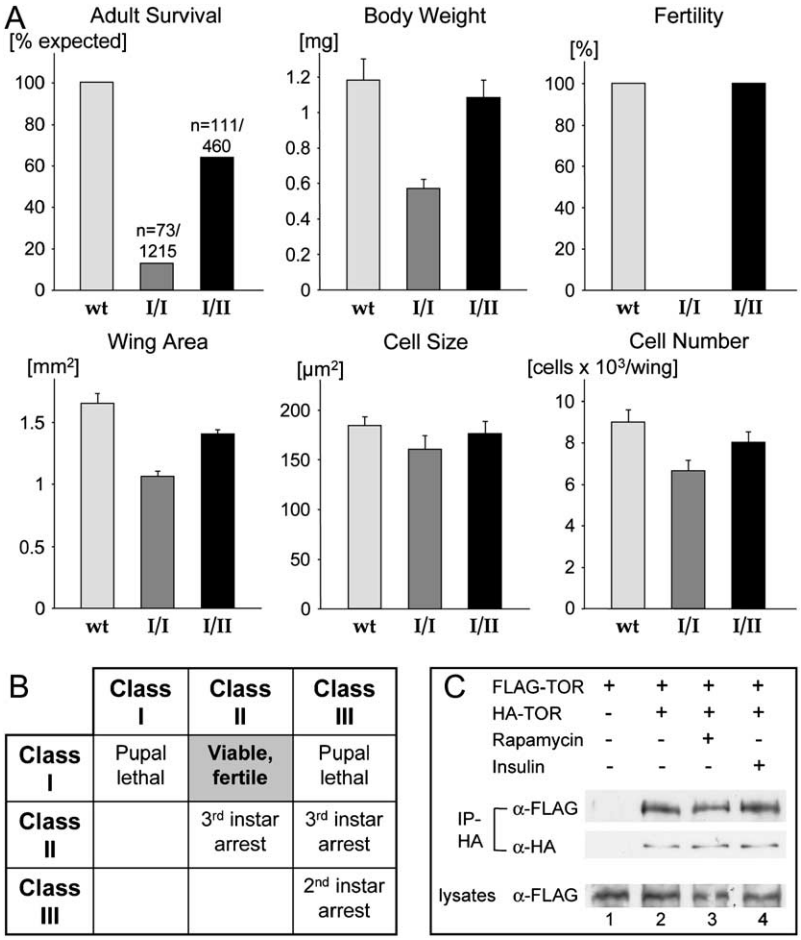

FIGURE 3.—Genetic and physical self-interaction of TOR proteins. (A) Survival, growth and fertility parameters for control (wt), class I homoallelic (I/I), and class I/class II heteroallelic (I/II) mutants. Data shown are for Tor^{A948V} (class I) and Tor^{G2256D} (class II). Error bars indicate standard deviation from the mean. (B) Summary of complementation tests between class I, II, and III *Tor* mutants. Crosses of any of the four class I alleles with either of the two class II alleles result in viable, fertile progeny. (C) TOR–TOR interaction in Drosophila S2 cells. Drosophila S2 cells were transfected with FLAG–TOR (lane 1) or FLAG–TOR plus HA–TOR (lanes 2–4) and treated with rapamycin or insulin as indicated. A small fraction of the cell lysate was probed with anti-FLAG to evaluate FLAG–TOR protein level (bottom gel). The remaining lysate was immunoprecipitated (IP) with anti-HA antibody. The HA–IP product was probed with anti-FLAG (top gel) and anti-HA (middle gel). FLAG–TOR was detected in the HA–IP product in the presence (lane 2), but not in the absence (lane 1), of HA–TOR, indicating a specific interaction between FLAG–TOR and HA–TOR. This interaction was not appreciably affected by rapamycin (lane 3) or insulin treatment (lane 4).

loss-of-function mutations (GAO *et al.* 2002; RADIMERSKI *et al.* 2002), indicating that TOR activity may be limiting under conditions of increased signaling in *TSC* mutant cells. We repeated these genetic interaction tests using each class of *Tor* allele. Surprisingly, this analysis revealed that the degree of *TSC* suppression did not correlate with the phenotypic severity of the *Tor* mutation. In particular, the lethality of *Tsc2*-homozygous animals was suppressed to a significantly greater degree by any of the four hypomorphic class I *Tor* mutations than by the null class III alleles (Figure 4A). One of the two class II *Tor* alleles (P2293L) also behaved as a strong suppressor in this assay. Similar interactions were observed with *Tsc1* (data not shown). The observation that *TSC* mutant phenotypes are suppressed more strongly by class I and II *Tor* alleles than by null *Tor* alleles argues that under conditions of high signaling input, specific *Tor* point mutants can act in a dominant-negative manner to inhibit the function of wild-type TOR, again consistent with TOR functioning as a multimer.

Two additional findings further support this notion. First, we tested genetic interactions between *Tsc2* and two *P*-element-induced *Tor* alleles with phenotypes intermediate between class I and II (OLDHAM *et al.* 2000; ZHANG *et al.* 2000). The P alleles, which affect signaling by reducing *Tor* expression, were unable to rescue *Tsc2* mutants to viability (data not shown), indicating that the rescue by class I and II alleles is not due to a particular, intermediate level of TOR activity. Second, we found that class I and class II point mutants were capable of partially rescuing *Tsc2* mutants even in the presence of an additional, transgenic copy of wild-type *Tor* (Figure 4B). These animals, which contained two wild-type copies and one mutant copy of *Tor*, survived to the late pupal stage. In contrast, the partial suppression of *Tsc2* by class III null *Tor* alleles was completely blocked in the presence of the wild-type *Tor* transgene (Figure 4B). Thus class I and II *Tor* alleles, which are recessive to wild type under normal conditions, behave as dominant-inhibiting alleles in *Tsc2* mutant animals.

Interestingly, the *Tor* mutations that strongly suppress *Tsc2* map to domains of TOR with roles in substrate interactions: the class I mutations disrupt the HEAT domain required for association with raptor, which plays an important role in substrate docking and presentation (HARA *et al.* 2002), and the Tor^{P2293L} mutation affects the activation loop, which has been shown to be critical for substrate specificity in the related PI3-kinases (PIROLA *et al.* 2001). As these mutations confer stronger dominant-negative phenotypes than the more severe mutations

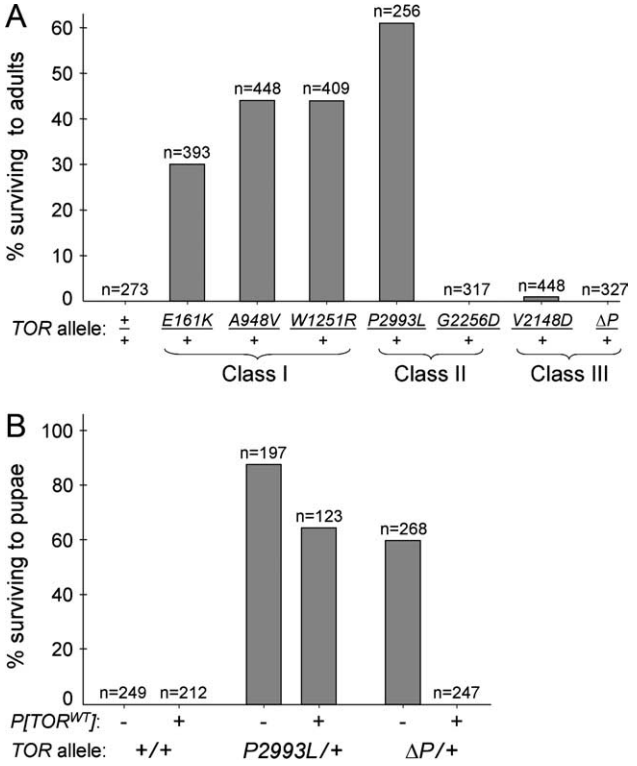

FIGURE 4.—*Tor* point mutants dominantly suppress *Tsc2* lethality. (A) Shown is the percentage of viability of $Tsc2^{109}/Tsc2^{193}$ adults heterozygous for the indicated *Tor* alleles. Note that *Tsc2* lethality is suppressed to a greater extent by the class I and class II point mutants than by the class III null alleles. (B) Class II but not class III *Tor* alleles can dominantly suppress *Tsc2* lethality even in the presence of two copies of wild-type *Tor*. One copy of a *Tor* genomic rescue construct ($P[Tor^{WT}]$) blocks the ability of $Tor^{\Delta P}$ (class III) but not Tor^{P2993L} (class II) heterozygotes to rescue $Tsc2^{109}/Tsc2^{193}$ animals to pupal viability. *n*, number of animals scored. Genotypes: (A) $Tor*/+$; $Tsc2^{109}/Tsc2^{193}$, (B) $Tor*/+$; $Tsc2^{109}/Tsc2^{193}$, and $Tor*/+$; $Tsc2^{109}$ $P[Tor^{WT}]/Tsc2^{193}$.

that disrupt the catalytic loop or ATP-binding pocket of TOR, we speculate that substrate interactions may involve cooperation of two or more TOR proteins, whereas a single kinase domain may be sufficient for catalytic activity.

DISCUSSION

Intragenic complementation denotes the unusual genetic behavior whereby individuals carrying two different mutant alleles within the same gene display the wild-type phenotype. First described nearly 50 years ago (CATCHESIDE and OVERTON 1958), it was shown that this phenomenon can result from the formation of a functional multimeric protein composed of partially defective monomers that mutually compensate for each other. Alternatively, a gene may display intragenic complementation if it encodes a polypeptide with two independent functions. Although a kinase-independent function of TOR has been proposed (ERBAY and CHEN 2001), this mechanism is unlikely to explain the observed complementation between the HEAT- and kinase-domain alleles reported here, as the function of these domains is intertwined: the HEAT repeats play a critical role in recruiting substrates for phosphorylation by the kinase domain. Indeed, small deletions and site-directed mutations in the HEAT repeats have been shown to disrupt phosphorylation of TOR substrates (SABATINI *et al.* 1999; HENTGES *et al.* 2001). With our biochemical data showing direct physical self-association of TOR, these genetic results thus strongly indicate that TOR is capable of multimerizing *in vivo*. While this manuscript was in preparation, Hall and colleagues reported co-immunoprecipitation experiments showing that both yeast TORC2 and human mTOR also exist in an oligomeric state (WULLSCHLEGER *et al.* 2005). Thus, multimerization appears to be a conserved feature of TOR. In agreement with our genetic data indicating that the multimeric form of TOR is functional, these authors were able to detect TOR kinase activity only in a high molecular weight form of TORC2, presumably representing dimeric TORC2.

The finding that TOR is capable of functioning as a multimer has several important implications for TOR signaling. The calculated molecular mass of the known components of mammalian TORC1 and TORC2 each totals ~500 kDa, considerably less than the 1500–2000 kDa size of TOR complexes observed by gel filtration. Although it is likely that additional components await identification, it is notable that the minimal complex of TOR, GβL, and raptor are sufficient to recapitulate nutrient-sensitive regulation of TOR (KIM *et al.* 2003). Thus the ultimate mass of TOR complexes may reflect only a limited number of additional unknown subunits, with the remainder contributed by multimerization of TOR and possibly other TORC components. Multimerization of TOR is likely to influence both its regulation by upstream signals and its interactions with downstream targets. The large interaction surface afforded by two or more TOR molecules may contribute to the remarkable ability of TOR to regulate multiple cellular processes and to respond to multiple cues. Indeed, our results suggest that substrate interactions are sensitive to cooperative interactions between TOR molecules. In addition, the close proximity of two or more TOR molecules within a complex is likely to facilitate intermolecular autophosphorylation. Although the consequences of TOR autophosphorylation are unknown, inter- and intramolecular autophosphorylation is likely to occur at distinct sites and to have differing effects on TOR signaling. In this regard, we note that mutual *trans*phosphorylation of ATM dimers has recently been shown to play a critical role in regulating the activity of this PIKK-family kinase (BAKKENIST and KASTAN 2003).

The findings presented here also have implications for the role of TOR in human disease. A number of pathological conditions stem from abnormally high levels of TOR signaling, including tuberous sclerosis (caused by mutations in the *TSC1* and *TSC2* genes),

Cowden's disease (caused by mutations in *PTEN*), and some forms of cardiac hypertrophies and cancers (Inoki *et al.* 2005). The observation that recessive point mutations in *Tor* can dominantly interfere with wild-type TOR function in *Tsc1* and *Tsc2* mutant but not wild-type flies suggests that it may be possible to exploit properties of TOR multimerization for therapeutic purposes. For example, retroviral delivery of genes encoding mutant alleles of *Tor* may provide a means to reduce TOR signaling specifically in cells with inappropriately high TOR activity, with minimal effects on normal cells. We note that the high sensitivity of *PTEN* mutant cells to low concentrations of rapamycin (Neshat *et al.* 2001) may reflect a similar dominant inhibition of TOR under conditions of elevated TOR signaling. Our results also suggest that small molecules or peptides that interfere with TOR multimerization may provide an alternative to rapamycin as novel inhibitors of TOR signaling.

This work raises a number of questions concerning the mechanisms and role of TOR multimerization. It will be important to identify the domains of TOR required for multimerization, to determine whether TOR multimerization is direct or is mediated by other components within the complex, and whether such components are also present in multiple copies. In this regard, it is interesting to note that raptor contains a sequence motif related to dimeric caspases (Ginalski *et al.* 2004). It is also unclear whether TOR multimerizes within TORC1, TORC2, or both. The failure to observe physical interactions between TOR1 and TOR2 in yeast (Wedaman *et al.* 2003), which can each assemble into TORC1, is consistent with TOR multimerization being a unique feature of TORC2, which contains TOR2 but not TOR1. Finally, it will be interesting to determine whether multimerization is a feature common to other members of the PIKK family of kinases and whether multimerization is subject to regulation. The PIKK ATM has recently been shown to convert from an inactive dimeric state to an active monomer in response DNA damage. In contrast, our results indicate that the multimeric form of TOR is active and that its multimerization is likely to be constitutive.

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