The mechanistic target of rapamycin complex 1 (mTORC1) is a Ser/Thr protein kinase complex that integrates signals from nutrient availability, energy, and growth factors to regulate cell growth, proliferation, and metabolism (1). Up-regulation of mTORC1 is associated with many diseases such as cancer, type 2 diabetes, and defects in neurodevelopment (1, 2). The mTORC1 complex is a dimer of mTOR/RAPTOR/mLST8 heterotrimeric (3). The mTORC1 kinase activity is tightly regulated by two classes of small GTPases, Rags and RHEB, both of which are necessary for activation (4, 5). In response to the abundance of nutrients, particularly amino acids, active Rag heterodimers bind the RAPTOR subunit of mTORC1 to recruit it to lysosomes (6–8), where mTORC1 can be allosterically stimulated by growth factor-activated RHEB (3, 9–12). Unlike RHEB, which carries a C-terminal farnesylation that weakly associates it to a variety of membranes (13, 14), Rags have no lipid modification. Instead, they associate with the heteromeric Ragulator complex, which has myristoyl and palmitoyl modifications at the N terminus of its LAMTOR1 subunit that localize it to lysosomes (15, 16). Recurrent oncogenic mutations in Rags enhance its association with mTORC1, leading to increased mTORC1 signaling (17–19).

Both Rags and RHEB are members of the Ras-like superfamily of GTPases. However, unlike most members, Rags are obligate heterodimers, with RagA or RagB pairing with RagC or RagD (20). Analysis of the composite genome of *Lokiarchaeum* revealed that Rag GTPases have an archaeal origin closely related to the Arf family of Ras-like GTPases that are involved in vesicular sorting (21), but the mechanistic implication of this similarity was not clear.

Rag heterodimers have four possible nucleotide-binding states but are active for mTORC1 binding only when RagA or RagB is guanosine triphosphate (GTP)-bound and RagC or RagD is guanosine diphosphate (GDP)-bound (6, 7, 22). The GTPase domains communicate so that binding of GTP by one subunit inhibits GTP binding and induces GTP hydrolysis by the other subunit (22). The nucleotide states of Rags are regulated by GTPase-activating proteins (GAPs) such as GATOR1 and folliculin (23–25) and guanine nucleotide exchange factors (GEFs) such as SLC38A9 and Ragulator (26, 27).

To elucidate how human Rags interact with mTORC1 and how RagC mutations activate mTORC1, we determined the structures and dynamics of RagA/RagC complexes in isolation and bound to mTORC1. The structures revealed nucleotide-dependent conformational changes in Rags that are required for mTORC1 binding, enabling us to understand the mechanism by which oncogenic Rag mutations facilitate association with mTORC1.

HDX-MS shows that RagA/RagC protects the α-solenoid of RAPTOR

To map RagA/RagC interactions with the RAPTOR subunit of mTORC1, we carried out hydrogen/deuterium exchange mass spectrometry (HDX-MS). For this we used RagA-Q66L/RagC-T90N-GDP containing mutations that increase mTORC1 association (6, 7, 17, 19). The RagA-Q66L switch II mutation (fig. S1) (28) impairs GTP hydrolysis and is a potent activator of mTOR signaling, with mice bearing this mutation dying within 1 day of postnatal life (29). The RagC-T90N mutation binds only GDP and is the most frequent and potent oncogenic mutation in RagC (17, 19).

RagA/RagC heterodimers were monodisperse (fig. S2A) and formed a 1:1 complex with RAPTOR (fig. S2, B and C). Most RAPTOR peptides that showed decreased HDX upon RagA/RagC binding mapped to a contiguous surface in the region of residues 541 to 678, encompassing three adjacent helical repeats of the RAPTOR α-solenoid (fig. 1A, fig. S3, and table S1). There was also reduced HDX in the insertion before the last two helices of the RAPTOR α-solenoid (peptides 760 to 780 and 805 to 812) and in the WD40 domain itself. On the Rag side of the interface, RagA switch I was protected from HDX by RAPTOR (fig. 1B), whereas RagC switches were not (fig. 1C and table S1). To understand the context of the HDX-MS-measured dynamics, we determined the structures of RagA/RagC heterodimers both free and bound to mTORC1.

High-resolution crystal structures of active RagA/RagC heterodimers

HDX-MS identified RagC residues 1 to 34 as highly flexible (fig. S2D). For crystallization, we truncated this region, producing a variant RagA(34–399) that bound RAPTOR the same as full-length RagA/RagC (fig. S2E) and thereby enabled us to determine high-resolution crystal structures of RagA/RagC.

The crystal structure of RagA-Q66L/RagC(34–399)-T90N at 2.6 Å resolution showed a compact arrangement of C-terminal CRD domains that mediate heterodimerization and N-terminal GTPase domains (fig. 1D and table S2). The overall fold of the GTPase domains is similar to other Ras-like GTPases, with conserved loops (G1 to G5 motifs) that engage bound nucleotides, as well as regions that change conformation depending on whether GTP or GDP is bound, known as switches (30). RagA is bound to GTP and has Mg²⁺ associated with the GTP γ-phosphate, whereas RagC is bound to GDP and has no bound Mg²⁺ (fig. 1, E and F). Whereas RagA has switch I, interswitch, and switch II ordered (fig. 1D and fig. S1), the RagC-T90N-GDP GTPase domain has no density for all of switch I and interswitch strand β2 (residues 84 to 105) and all of switch II (116 to 130). The C-terminal CRDs have a roadblock fold consisting of a central five-stranded antiparallel β sheet sandwiched between two α-helical layers (31).
The whole complex has a pseudo-twofold symmetry, with the two GTPase domains close to each other and their switches on opposite faces of the complex (Fig. 1D).

**The cryo-EM structure of mTORC1 bound to RagA/RagC**

To understand how active RagA/RagC interacts with intact mTORC1, we used cryo-electron microscopy (cryo-EM). In order to stabilize mTORC1 bound to RagA-Q66LGTP/RagC-T90NGDP, we used chemical cross-linking and expressed the RagA/RagC heterodimer with RagC fused to another mTORC1-binding protein, PRAS40, which is largely disordered (fig. S4 and table S3). Using this mTORC1-RagA/RagC complex (fig. S5A), we generated a final reconstruction of mTORC1 at 4.1 Å resolution (figs. S5 and S6A and table S4).

This reconstruction showed extra density adjacent to the \( \alpha \)-solenoid region of RAPTOR (fig. S6A) that HDX-MS identified as the RagA/RagC binding site. The TOS motif from PRAS40 (fused to RagC) contacts a groove between the RAPTOR N-terminal conserved (RNC) and the \( \alpha \)-solenoid of RAPTOR, as also observed by HDX-MS (fig. S4, C to E).

Focused classification with signal subtraction (32) showed that about 9.5% of particles were bound to RagA/RagC, corresponding to 90,809 particles, and reconstruction of the mTORC1-RagA-Q66LGTP/RagC-T90NGDP complex at 5.5 Å resolution revealed density for the RagA/RagC into which we could readily fit our high-resolution RagA/RagC crystal structure (Fig. 2 and fig. S6, B to D). RagA/RagC interacts with the convex surface of the RAPTOR \( \alpha \)-solenoid (Fig. 3A). The GTPase-containing ends of the horseshoe-shaped RagA/RagC heterodimer are closest to the RAPTOR \( \alpha \)-solenoid, with the CRDs pointing away from RAPTOR (Fig. 2, B and C). The RagA GTPase domain makes much more extensive RAPTOR contacts than RagC, and the interface agrees with our HDX-MS analysis (Fig. 3, A and B). The overall conformation of mTORC1 bound to RagA/RagC heterodimers is nearly identical to the conformation of apo-mTORC1 (3).

Three helices from RAPTOR (\( \alpha \)24, \( \alpha \)26, and \( \alpha \)29) in the region of residues 546 to 650 of the RAPTOR \( \alpha \)-solenoid make an extensive network of interactions with switch I and interswitch strand \( b \)2 of RagA-Q66LGTP (Fig. 3A). The GTPase-containing ends of the horseshoe-shaped RagA/RagC heterodimer are closest to the RAPTOR \( \alpha \)-solenoid, with the CRDs pointing away from RAPTOR (Fig. 2, B and C). The RagA GTPase domain makes much more extensive RAPTOR contacts than RagC, and the interface agrees with our HDX-MS analysis (Fig. 3, A and B). The overall conformation of mTORC1 bound to RagA/RagC heterodimers is nearly identical to the conformation of apo-mTORC1 (3).

Although the GTPase domains form most of the interface with RAPTOR, there are some contacts with the CRD domains. These involve the C terminus of RagA helix \( \alpha \)8 with Thr680 in RAPTOR helix \( \alpha \)31. Although the GTPase domains form most of the interface with RAPTOR, there are some contacts with the CRD domains. These involve the C terminus of RagA helix \( \alpha \)8 with Thr680 in RAPTOR helix \( \alpha \)31. Although the GTPase domains form most of the interface with RAPTOR, there are some contacts with the CRD domains. These involve the C terminus of RagA helix \( \alpha \)8 with Thr680 in RAPTOR helix \( \alpha \)31.
engage two adjacent structural elements of
RAPTOR, helix α29 (Thr639, Asn643, Met646) and the end of the long, mostly disordered insertion after α31.

Mutations of RAPTOR residues in helices α26 (Trp593-Cys594 or Arg597-Asp598) and α29 (Thr639, Asn643, Met646) that contact the RagA-Q66L<sub>GTP</sub> switch I/Interswitch greatly reduce binding to RagA/RagC (Fig. 3C). Previously, RagA mutations were identified that prevent RAPTOR interaction (33), and they map to the interface with RAPTOR in our structure. We also determined the cryo-EM structure of mTORC1-RagA-Q66L<sub>GTP</sub>/RagC-T90N<sub>GDP</sub> where RagA/RagC was not covalently fused to PRAS40. Note that in both cryo-EM structures, the RagA/RagC heterodimer interacts with RAPTOR in the same manner (figs. S7 and S8).

Cancer-associated mutations in RagA/RagC affect communication between GTPase domains

Cancer-associated mutations in RagC increase mTORC1 binding (17–19), and we wanted to gain insights into the structural basis for this effect. The mutations cluster in various nucleotide-sensing elements of RagC: the P-loop (e.g., Ser<sup>125</sup>), switch I (e.g., Thr<sup>90</sup>), Interswitch (e.g., Trp<sup>115</sup>, Asp<sup>116</sup>), and switch II (e.g., Pro<sup>118</sup>) (fig. S1). The RagC-T90N mutant had switch regions disordered (Fig. 1D). To see whether this disorder is specific for the T90N mutation, we also determined a crystal structure of RagC-S75N<sub>GDP</sub> at 2.5 Å resolution (table S2). The RagC-S75N mutation in the P-loop impairs GTP binding by eliminating the interaction of Ser<sup>125</sup> with Mg<sup>2+</sup>. The structures of RagC-S75N<sub>GDP</sub> and RagC-T90N<sub>GDP</sub> are very similar, except that RagC-S75N<sub>GDP</sub> has helix α2 of switch I (residues 86 to 93) ordered in one of the two heterodimers in the crystal asymmetric unit (Fig. 4A); this finding suggests that S75N destabilizes but does not completely disorder switch I. Hence, T90N causes a greater perturbation in switch I than does S75N, consistent with the more potent phenotype of the T90N mutation in cells (17). The structure of the isolated wild-type RagC GTPase domain bound to a GTP analog [PDB ID 3LLU (34)] shows a completely ordered switch I and helix α2 that closely superimposes with this helix in RagC-S75N<sub>GDP</sub>. In the RagC-S75N<sub>GDP</sub> structure, OG1 of the Thr<sup>90</sup> side chain is close to the O2’ of the bound nucleotide (3.7 Å), so it is likely that the larger T90N substitution leads to disorder of helix α2.

RagA/RagC GTPase domain contacts can be grouped into three sets (Fig. 4B). One set is at the center of the interface where the G5 motifs of the two domains meet, with RagA-Trp<sup>165</sup> near the equivalent of this residue in RagC-Tyr<sup>221</sup> (Fig. 1E and fig. S1). The second set involves interactions between RagA switch I helix α2 and the RagC loop immediately following the G5 motif. In particular, there is a water-mediated interaction between RagA-Arg<sup>34</sup> and the side chain of RagC-Asp<sup>222</sup> (Fig. 4B). The third set consists of interactions between RagC switch I helix α2 and the RagA

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**Fig. 2. Architecture of the mTORC1-RagA/RagC complex.**

(A) Schematic representation of mTORC1 components (mTOR, RAPTOR, and mLST8). (B) Overall cryo-EM-based model of the mTORC1-RagA/RagC complex. The two ordered regions of the PRAS40 moiety of the fusion construct are also shown. (C) Three views of the mTORC1-RagA/RagC complex show RagA/RagC sitting on top of the RAPTOR α-solenoid, with the GTPase domains making most of the interactions.
Comparison of the CRDs from the free RagA/RagC with those in the mTORC1-bound RagA/RagC shows a small shift in the orientation of the two CRDs so that in the mTORC1-bound form, the top surface of the CRDs that embraces the GTPase domains is more splayed (fig. S9 and movie S1). Comparison of the CRD dimer from the free RagA/RagC with the CRD dimer bound to Ragulator (35) also shows shifts between the two CRDs (fig. S9). Together these results indicate that the interface between the CRD domains has some flexibility. This could be exploited by interactors such as Ragulator to exert changes on the GTPase domains via the CRDs, which could contribute to the established role of Ragulator as a GEF for RagC (27).

Rearrangements within the RagA/RagC heterodimer when bound to mTORC1
Comparing the crystal structure of free RagA-Q66LGTP/RagC-T90NGDP and the cryo-EM structure of RagA/RagC bound to mTORC1 reveals a shift in the interface between the GTPase domains by ~7 Å (Fig. 4C and movie S1). This creates a more open space between the two GTPase domains, which in the free RagA/RagC would be kept closer by interactions involving switch I helix α2. This might explain why the oncogenic RagC-T90N mutation, which has helix α2 disordered, binds more easily to RAPTOR, because the RagC helix α2/RagA interactions are already disrupted before the heterodimer binds to RAPTOR. A similar structural change could occur in the RagC-L91P mutant associated with follicular lymphomas (17). Residue Lys84 in the α1-α2 loop of RagC switch I forms salt links with residues Asp290 and Asp294 of helix α8 in the CRD (Fig. 4A). The lymphoma-associated mutation RagC-K84T (17) would likely disrupt this interaction, which could facilitate RagC rearrangement relative to RagA as seen in the complex with mTORC1.

Structural basis for relaying nucleotide binding to the CRDs
Although only the RagAGTP/RagCGDP state binds RAPTOR, the reverse, inactive state, RagAGDP/RagCGTP, is essential for terminating mTORC1 activation. Furthermore, some Rag interactors such as galectin-8 preferentially associate with this state (36). Therefore, a structural understanding of both states is important. In the active heterodimer, nucleotide-sensitive elements in the GTPase domain of the RagA/RagC complex are already disordered, binds more easily to RAPTOR, because the RagC helix α2/RagA interactions are already disrupted before the heterodimer binds to RAPTOR. A similar structural change could occur in the RagC-L91P mutant associated with follicular lymphomas (17). Residue Lys84 in the α1-α2 loop of RagC switch I forms salt links with residues Asp290 and Asp294 of helix α8 in the CRD (Fig. 4A). The lymphoma-associated mutation RagC-K84T (17) would likely disrupt this interaction, which could facilitate RagC rearrangement relative to RagA as seen in the complex with mTORC1.

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Comparison of the CRDs from the free RagA/RagC with those in the mTORC1-bound RagA/RagC shows a small shift in the orientation of the two CRDs so that in the mTORC1-bound form, the top surface of the CRDs that embraces the GTPase domains is more splayed (fig. S9 and movie S1). Comparison of the CRD dimer from the free RagA/RagC with the CRD dimer bound to Ragulator (35) also shows shifts between the two CRDs (fig. S9). Together these results indicate that the interface between the CRD domains has some flexibility. This could be exploited by interactors such as Ragulator to exert changes on the GTPase domains via the CRDs, which could contribute to the established role of Ragulator as a GEF for RagC (27).

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state, we tried but did not succeed in obtaining diffracting crystals for this heterodimer. As an alternative strategy, we used HDX-MS to examine differences in conformation between the active (RagA-Q66L<sub>GTP</sub>/RagC-T90N<sub>GDP</sub>) and reverse (RagA-T21N<sub>GDP</sub>/RagC-Q120L<sub>GTP</sub>) states (Fig. 5D and table S5). Overall, both Rags showed less HDX throughout the GTPase domains when GTP-bound compared with GDP-bound, indicating a more compact domain when bound to GTP. Furthermore, upon GTP binding to RagA, there is a distinct protection in its CRD domain (e.g., in CRD helix α8, β7/β8 and the hinge that are engaged with the GTPase domain), suggesting communication of the nucleotide state to the CRD (Fig. 5D and movie S2). Residues making up a pocket on the surface of the RagA CRD that accommodate the interswitch in GTP-bound RagA have increased exchange in the reverse state, which we attribute to retraction of the RagA interswitch, exposing the RagA CRD pocket. Consistent with this, the GDP-bound RagA interswitch has increased HDX (displayed on the RagA<sub>GTP</sub> crystal structure in Fig. 5D and on a RagA<sub>GDP</sub> model in movie S2). For RagC, there is less change in HDX in the CRD upon GTP binding.

Comparing the yeast Gtr1<sub>GTP</sub>/Gtr2<sub>GDP</sub> structure (PDB ID 4ARZ) with human RagA<sub>GTP</sub>/RagC<sub>GDP</sub> indicates very large conformational differences, both in switches and in the relative orientations of GTPase domains, with the Gtr2 GTPase domain rotated about 36° relative to the RagC GTPase domain (fig. S11A). The arrangement of the Gtr1/2 GTPase domains is not compatible with binding to mTORC1 in the same manner as RagA/RagC (fig. S11B). This suggests that the Gtr1/2 GTPase domains may reorient in order to bind Kog1, the yeast homolog of RAPTOR. The very different conformation of the Gtr2<sub>GTP</sub> switch I region and the extreme orientation of the Gtr2 GTPase domain relative to RagC may reflect a fundamental difference between RagC and Gtr2. This could explain why RagA-Q66L can complement a Gtr1-deficient strain, whereas neither wild-type RagC nor a GDP-bound mutant RagC could complement Gtr2 deficiency in yeast (38). Despite these differences, the binding site of Gtrs on Kog1 maps to a similar region on the Kog1 α-solenoid (39).

Given the role of the Rag heterodimers in recruiting mTORC1 to lysosomes, the constitutive association of yeast TORC1 with the vacuole is surprising (5). A recent report elegantly showed that upon glucose starvation, yeast TORC1 forms inactive, vacuole-associated helical tubes named TOROIDs and that the TOROID formation is antagonized by active Rags (Gtr1<sub>GTP</sub>/Gtr2<sub>GDP</sub>) in cells (40). Fitting our RagA/RagC heterodimers into the cryo-EM reconstruction of the tubes, in accordance with the arrangement present in our mTORC1-RagA/RagC complex, suggests that the Gtr1/2 binding would not be compatible with the TORC1 arrangement in the TORIODs (fig. S11C). This might mean that Gtr1/2 binding could directly regulate assembly or disassembly of the tubes to activate TORC1. Further work is needed to test this structure-based proposal.

**Fig. 4. Interactions between GTPase domains in the RagA/RagC heterodimer.** (A) Comparison of RagA-Q66L<sub>GTP</sub>/RagC-T90N<sub>GDP</sub> with RagA-Q66L<sub>GTP</sub>/RagC-S75N<sub>GDP</sub>, illustrating ordering of helix α2 in switch I of RagC-S75N. Superposition was on the RagA subunit. (B) Three sets of interactions between RagA<sub>GTP</sub> and RagC<sub>GDP</sub> GTPase domains. (C) A change in the orientation of RagA/RagC GTPase domains in free RagA/RagC relative to mTORC1 bound to RagA/RagC. Superposition was on the RagA subunit. The view is similar to (B).

**Implications for yeast TORC1 signaling**

RagA/RagC binding causes no conformational change in mTORC1, which suggests that the role of the Ragulator/Rags complex is to localize mTORC1 to lysosomes where it can be allosterically activated by RHEB. Rag/RAPTOR interaction requires a GTP-loaded RagA, so that RagA switch I and interswitch are ordered, because they make most of the interactions with RAPTOR. A reverse state of Rags with GDP-loaded RagA and GTP-loaded RagC does not bind RAPTOR as well (6, 7), because...
RagA<sub>GDP</sub> would have the switch regions disordered, whereas RagC<sub>GTP</sub> could not interact with RAPTOR, because RagC residues analogous to RAPTOR-binding residues of RagA are not conserved (fig. S12).

The structures suggest how the nucleotide-bound state of one GTPase domain is communicated both between subunits to the paired GTPase domain and within a subunit to its CRD (Figs. 4 and 5 and movie S2). First, consistent with communication between GTPase domains (22), both RagA and RagC have helix α2 (in switch I) contacting the paired GTPase domain and filling the space between them (Fig. 4, A and B). Second, there are several sets of interactions between the GTPase and CRD domains within a subunit that HDX suggests are dynamic and nucleotide-dependent, including switch I and the interswitch. The interswitch of Rag GTPases apparently undergoes a nucleotide-dependent register shift of strand β3 relative to β1 that could be part of a mechanism to transmit nucleotide-binding information from the GTPase domain to the CRD (Fig. 5). This is analogous to conformational changes that accompany transition from the GDP- to GTP-bound states of Arf family GTPases (Fig. 5C) (41, 42) and is consistent with the evolutionary relationship of the Rags to the Arf family (21). In Arfs, this interswitch toggle between retracted and protruded conformations coordinates membrane binding with GTP loading (Fig. 5B). In Rags, the interswitch toggle could be part of a mechanism that rotates one GTPase domain via a CRD fulcrum relative to the other GTPase domain. This would change RagA/RagC GTPase domain contacts, making it less favorable for the heterodimer to accommodate GTP in both GTPase domains at the same time, as kinetically observed (22).

mTORC1 activity is intricately regulated in a signal- and location-specific manner. Membrane compartments act as signaling platforms that serve to colocalize mTORC1 with its activating G protein RHEB, which is targeted transiently to most endomembranes by farnesylation (13). The lysosomal activity of mTORC1 in amino acid signaling is achieved through its dynamic interface with the Rags-Ragulator lysosomal scaffold (8, 43). Rags couple mTORC1 to lysosomes by binding to RAPTOR with their GTPase domains and to Ragulator with their CRDs. Because of large allosteric changes in mTOR that are incompatible with a mixed mTOR dimer, two RHEB molecules bind mTORC1 cooperatively (3). In contrast, two soluble RagA/RagC heterodimers bind independently to mTORC1, because RagA/RagC binding does not introduce conformational changes in mTORC1.

We propose an organization of active mTORC1 on membranes based on our structure of the mTORC1-RagA/RagC complex, the previously published structure of the mTORC1-RHEB complex (3), and the crystal structure of Ragulator bound to RagA/RagC CRDs (35, 44) (Fig. 6). In this model, RagA/RagC, associated with membranes through Ragulator (via the lipidated N terminus of LAMTOR1), and RHEB, associated with membranes through a C-terminal farnesylation, can be bound at the same time to mTORC1, yet still allow the mTOR active sites to face the cytosol. The RHEB-binding surface of mTORC1 would be near a RHEB-containing membrane, and the first ordered residue of the LAMTOR1
subunit of Ragulator (residue 96) would be about 105 Å from the membrane surface, which suggests that the 95 flexible N-terminal LAMTOR1 residues could easily reach the membrane. Further structural and kinetic analysis of mTORC1 complexes on membranes will be essential to fully appreciate the roles of structural dynamics of mTORC1 with its regulators and the roles of membranes in regulation of mTORC1.

REFERENCES AND NOTES
28. See supplementary materials.

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Architecture of human Rag GTPase heterodimers and their complex with mTORC1

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Mastering regulation

The mechanistic target of rapamycin complex 1 (mTORC1) is known as the master kinase, acknowledging its key role in integrating multiple signals to regulate cell growth. When nutrients are abundant, heterodimers of Rag, a class of small guanosine triphosphatase, bind to mTORC1 and recruit it to the lysosome. Here, other signaling pathways converge on the mTORC1 complex. Anandapadamanaban et al. determined cryo-electron microscopy and crystal structures of a RagA/RagC heterodimer. The structures, together with dynamic studies, explain the nucleotide states required for binding to mTORC1 and support a mechanism for conformational communication between the RagA and RagC subunits in the heterodimer. RagA/RagC binding causes no conformational change in mTORC1, which is consistent with the idea that mTORC1 must sense additional growth regulators before it is activated.

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