Crystal Structure of the Gtr1p<sup>GTP</sup>-Gtr2p<sup>GDP</sup> Protein Complex Reveals Large Structural Rearrangements Triggered by GTP-to-GDP Conversion*  

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The heterodimeric Rag GTPases consisting of RagA (or RagB) and RagC (or RagD) are the key regulator activating the target of rapamycin complex 1 (TORC1) in response to the level of amino acids. The heterodimer between GTP-loaded RagA/B and GDP-loaded RagC/D is the most active form that binds Raptor and leads to the activation of TORC1. Here, we present the crystal structure of Gtr1p<sup>GTP</sup>-Gtr2p<sup>GDP</sup>, the active yeast Rag GTPase heterodimer. The structure reveals that GTP-to-GDP conversion on Gtr2p results in a large conformational transition of this subunit, including a large scale rearrangement of a long segment whose corresponding region in RagA is involved in binding to Raptor. In addition, the two GTPase domains of the heterodimer are brought to contact with each other, but without causing any conformational change of the Gtr1p subunit. These features explain how the nucleotide-bound statuses of the two GTPases subunits switch the Raptor binding affinity on and off.

In eukaryotes, the target of rapamycin complex 1 (TORC1) kinase plays a central role in the regulation of cell growth in response to growth factors, energy levels, and availability of nutrients such as glucose and amino acids (1). The signal transduction through which amino acids activate TORC1 critically depends on the highly conserved Rag GTPases, which are members of the Ras GTPase superfamily (2, 3). Mammalian cells express four members of Rag GTPases: RagA, RagB, RagC, and RagD (4). In yeast, Gtr1p and Gtr2p are the orthologues of RagA/B and RagC/D, respectively (5).

Rag GTPases exist as heterodimers containing a Gtr1p-like Rag GTPase (either RagA or RagB) and a Gtr2p-like Rag GTPase (either RagC or RagD) (4). The function of the heterodimeric complex relies on which guanine nucleotide is bound to each of the Rag GTPases. A combination of GTP-bound RagA/B and GDP-bound RagC/D, which is induced by amino acid abundance, exhibits the highest activity. In mammals, the interaction between the Rag heterodimer and Raptor, a component of mammalian TORC1 (mTORC1), is enhanced when RagC/D is charged with GDP, although the nucleotide-bound state of RagA/B plays a dominant role (3). Through the interaction, mTORC1 is translocated from the cytosol to the lysosomal membranes where the complex is activated by Rheb (6). On the other hand, an inactive heterodimeric complex, whose formation is induced by amino acid deprivation, contains GDP-bound RagA/B and GTP-bound RagC/D. The inactive form is unable to bind Raptor and thus is incapable of activating mTORC1 (2, 3). It remained poorly understood how the nucleotide-bound status of the Rag heterodimer alters the Raptor binding affinity to switch the mTORC1 activity on or off.

Recently, the crystal structure of the Gtr1p-Gtr2p complex (PDB entry: 3R7W), with both subunits bound to the nonhydrolyzable GTP analogue GMPPNP, which represents a partially activated form of the heterodimer, was reported (7). Herein, we report the crystal structure of the Gtr1p<sup>GTP</sup>-Gtr2p<sup>GDP</sup> complex, the most active form of the Gtr1p-Gtr2p complex. Our structure, in comparison with the structure of Gtr1p<sup>GMPPNP</sup>-Gtr2p<sup>GMPPNP</sup>, reveals that Gtr2p undergoes significant structural rearrangements upon GTP-to-GDP conversion on Gtr2p. These changes involve a long segment including switch I, two β-strands, and switch II and accompany positional changes of the two GTPase domains. These observations provide the structural view of how the Rag heterodimeric complex...
serves as a molecular switch in the amino acid-regulated signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Purification of the Gtr1p-Gtr2p Complex**—A two-promoter expression vector containing the coding sequences for full-length Gtr2p and Gtr1p was constructed. The protein complex was produced in the *Escherichia coli* BL21(DE3) RIL strain (Novagen) and purified by Ni\(^{2+}\) affinity. The eluted protein was incubated with tobacco etch virus protease (1:100 ratio, w/w) at 4 °C for 10 h to remove the His\(_{6}\) tag in the presence of 1 mM GTP. The protein was further purified by ion exchange followed by size-exclusion chromatography equilibrated in 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM MgCl\(_2\). The selenomethionine-substituted protein was expressed in the *E. coli* B834 strain (Novagen) grown in a minimal medium supplemented with selenomethionine and purified under the same conditions used for the wild-type protein.

**Crystallographic data and refinement statistics**

| Description                        | Value          |
|------------------------------------|----------------|
| Data collection                    |                |
| Space group                        | C22\(_2\)       |
| Cell dimensions (Å)                | a = 90.7, b = 148.9, c = 117.8 |
| Wavelength (Å)                     | 0.97928 (peak) |
| Resolution (Å)                     | 30.0–3.1       |
| R\(_{free}\)/R\(_{work}\)          | 9.3 (34.7)*    |
| I/\(I_0\)                          | 20.5 (3.0)     |
| Completeness (%)                   | 99.2 (98.3)    |
| Redundancy                         | 8.4            |

**Overall Structure of the Gtr1p\(^{GTP}\)-Gtr2p\(^{GDP}\) Complex**—The Gtr1p-Gtr2p complexprepared from *E. coli* was crystallized without the addition of any nucleotides or nucleotide analogues. The HPLC analysis revealed that the purified complex contained 40% GDP and 60% GTP (data not shown), indicating that about 40% of the GTP molecules bound to the GTPases were converted into GDP during protein purification. The crystal structure of the Gtr1p-Gtr2p complex was determined, and the final model was refined to a resolution of 3.1 Å. The structure revealed that Gtr1p and Gtr2p were bound to GDP and GTP, respectively, thus forming the Gtr1p\(^{GTP}\)-Gtr2p\(^{GDP}\) complex (Fig. 1A). Each subunit is composed of two physically discernable domains: an N-terminal GTPase domain and a C-terminal dimerization domain, designated hereafter as the G domain and the C domain, respectively. The C domains of the two subunits are structurally very similar and primarily responsible for the heterodimerization through the intermolecular packing of the central α-helices cradled by the intermolecular β-sheet. These interactions are related by a pseudo two-fold symmetry and cause the nucleotide-binding sites of the two G domains to face each other in an antiparallel mode. The G domain of Gtr1p\(^{GTP}\) contains six β-strands and six α-helices including a short 310-helix (αG2) inserted in the switch I region (Fig. 1B). The number of secondary structural elements and the folding topology are similar to those of other Ras-related GTPases. An Mg\(^{2+}\) ion is present at the nucleotide-binding site of Gtr1p, and the γ-phosphate of GTP is grasped by the two switch regions through hydrogen bonds, as has been observed with other GTPases. In a sharp contrast, the G domain of Gtr2p\(^{GDP}\) is composed of five β-strands and five α-helices (Fig. 1B), and its nucleotide-binding site lacks a bound Mg\(^{2+}\) ion (Fig. 1C) as a result of a large conformational transition, which is described below in detail. This finding is consistent with a previous observation that Mg\(^{2+}\) is not required for GDP binding to RagC (4). Biochemical studies suggested that the absence of Mg\(^{2+}\) ion can increase the rate of GDP dissociation up to 1000-fold (11, 12). Apparently, the GDP binding affinity of Gtr2p is less tight than the GTP binding affinity of Gtr1p, as supported by the relatively weak electron density map and high B-factor values for GDP. Furthermore, the occupancy of GDP is ~40% lower than that of GTP, which is consistent with the result of HPLC analysis. The loss of several interactions mediated by Mg\(^{2+}\) at the nucleotide-binding site is presumably responsible for the comparatively weak GDP-Gtr2p interaction.

**Basis for the Different Intrinsic GTPase Activities of Gtr1p and Gtr2p**—The observed GTP binding to Gtr1p and GDP binding to Gtr2p in our crystal structure reflect the previous observations that purified RagA does not exhibit an intrinsic GTPase activity (13) and that RagC has a basal GTPase activity, converting ~40% of GTP to GDP within 4 h (4). The extremely
low GTPase activity has been also observed with the Arf subfamily members and Rab3A (14–16). Interestingly, Gtr1p shares similar features with the Arf subfamily members, as well as Rab3A. First, like the Arf subfamily members, Gtr1p does not have a conserved tyrosine residue present in the switch I region of other small GTPases such as the Ras, Rho, and Ran subfamily members, which possess a catalytic activity (Fig. 1D). This tyrosine residue provides a hydrogen bond to the $\gamma$-phosphate group of GTP and is believed to stabilize the transition state generated during GTP hydrolysis (17). The corresponding residue in Gtr1p is Leu-38, and it does not interact with GTP (Fig. 1D). Second, as observed for Rab3A, Ser-15 on the P-loop of Gtr1p provides a hydrogen bond to the $\gamma$-phosphate, whereas the corresponding residue glycine in most of the Ras subfamily members does not. The equivalent interaction mediated by Ser-31 at the nucleotide-binding pocket of Rab3A (Fig. 1D) was proposed to impose a stereochemical constraint against GTP hydrolysis (15). In supported of this, mutation of Ser-31 of Rab3A into glycine increased the GTPase activity by severalfold (18). These observations indicate that Gtr1p is locked in the GTP-bound form, unless GTP hydrolysis on this subunit is assisted by a GTPase-activating protein.

The nucleotide-binding pocket of Gtr2p is distinct from that of Gtr1p in that the P-loop of Gtr2p contains a conserved Arg-18, whereas the corresponding residue in Gtr1p is substituted with Ser-15. In the Gtr1pGTP/Gtr2pGDP structure, the side chain of Arg-18 in Gtr2p is close to the $\gamma$-phosphate group of the bound GDP. The residues interacting with the bound GDP are shown as a stick model. The $\gamma$-phosphate of GTP is stabilized by the guanidine group of Arg-18, which is reminiscent of the catalytic residue Arg-48 in the P-loop of human guanylate-binding protein 1, whose side chain is reoriented by homodimerization to stabilize the transition state (19). These observations imply that the difference...
in the intrinsic GTPase activities of Gtr1p and Gtr2p arises mainly from the amino acid sequence difference in the P-loop of the two proteins; the P-loop of Gtr1p contains an inhibitory residue (Ser-15), whereas that of Gtr2p instead contains a residue that stabilizes the transition state (Arg-18).

Conformational Transition of the Gtr2p G Domain Triggered by GTP Hydrolysis—The structure of the G domain in the Gtr2p<sub>GDP</sub> subunit is strikingly different from the typical GTPases in the GDP-bound state and also from the G domain of Gtr2p bound to GMPPNP. The difference is a consequence of a considerable conformational rearrangement of a long segment composed of residues 28–70 triggered by GTP-to-GDP conversion. The segment encompasses switch I, the βG2 and βG3 strands, and switch II (Fig. 2A). The switch I segment is moved far away from the bound GDP and restructured to become the C-terminal part of the helix αG1. In particular, the Thr-44 residue, which coordinates an Mg<sup>2+</sup> ion in Gtr2p<sub>GMPPNP</sub>, relocates its position by more than 34 Å. This relocation of the switch I segment is accompanied by the detachment of βG2 from the central β-sheet and its transformation into a loop segment. The strand βG3 is reconstituted by different residues: residues 53–59 in GMPPNP-bound Gtr2p and residues 58–64 in GDP-bound Gtr2p (Fig. 2B). Notably, superposition of βG3 in the two different nucleotide-bound states shows that the chemical properties of the side chains are conserved (Fig. 2B), and therefore the interactions between βG3 and βG1 in the two states are similar (data not shown). The switch II region also undergoes a prominent conformational change (Fig. 2A), especially at the N-terminal portion. The side chain of Glu-62 coordinating the bound Mg<sup>2+</sup> ion in Gtr2p<sub>GMPPNP</sub> undergoes an ∼16 Å movement in Gtr2p<sub>GDP</sub> (Fig. 2A). The relocated switch II region becomes flexible, as manifested by the poorly defined electron densities of this region.

Large Domain Movement Triggered by GTP Hydrolysis—The conformational transition of the Gtr2p G domain is accompanied with large changes in the relative orientation of the G domains, whereas the C domains of the two subunits do not undergo a notable change as these domains in the Gtr1p<sub>GTP</sub>-Gtr2p<sub>GDP</sub> and Gtr1p<sub>GMPPNP</sub>-Gtr2p<sub>GMPPNP</sub> complexes are well superposable with r.m.s. deviations of 1.20 Å for their 216 Ca atoms. As shown in Fig. 3A, the G domain of Gtr2p exhibits an ∼28° rotational movement relative to its C domain. The G domain of Gtr1p also undergoes a rigid-body movement, but it is a relatively small ∼6° rotation relative to its C domain. The two relocated G domains are in contact with each other and constitute 13% of the binding interface between the two subunits, whereas the two domains are separated from each other in the Gtr1p<sub>GMPPNP</sub>-Gtr2p<sub>GDP</sub> structure. Significantly, the interdomain contact does not alter the conformation of the G domain of Gtr1p<sub>GTP</sub> because these domains in the two structures are almost identically superposable with an r.m.s. devia-

![Figures 2 and 3](https://example.com/figures.png)
tion of 0.64 Å for 181 aligned Cα atoms. This observation indicates that the nucleotide binding status of one subunit neither dictates GTP or GDP binding to the other subunit nor changes the intrinsic GTPase activity of the other subunit. Another notable feature is that the domain movement in the Gtr2pGDP subunit places the Ile-214 residue in the C domain into a hydrophobic pocket in the G domain, which is newly created by the hydrophobic residues Tyr-172, Phe-175, Ser-176, and Val-179 from gG6 and the residues Val-29, Asn-32, Met-33, and Leu-36 from gG1 (Fig. 3B). It is also noted that the surface area buried between the two domains of Gtr2p is increased from ~564 to 1056 Å² by the domain movement, indicating that the interdomain interaction of Gtr2pGDP is more extensive than that of Gtr2pGTP (Fig. 3B). Thus, the C domain plays an important role in the conformational transition by stabilizing the relocated segment in the G domain. Those hydrophobic residues in Gtr2p and the corresponding residues in Gtr1p are all highly conserved (data not shown), suggesting that a very similar rotational G domain movement in Gtr1p is likely to occur upon GTP-to-GDP conversion by this subunit.

Implications and Concluding Remarks—Raptor is known to selectively bind to the RagA/B subunit of the RagA/B-RagC/D heterodimer. Furthermore, the Raptor binding is largely dependent on the nucleotide binding status of RagA/B rather than that of RagC/D (2, 3). Based on the high sequence similarity between the RagA family and the RagC family, we generated a model structure of GDP-bound Gtr1p by using the structure of Gtr2pGDP as a template in homology modeling (20). As expected, the model structure of Gtr1pGDP is very similar to the structure of Gtr2pGDP. This model clearly shows that the residues of Gtr1p, whose corresponding residues in RagA were shown to be involved in the interaction with Raptor (7), are located along the segment that undergoes the conformational transition (Fig. 4A). Conceivably, the RagA family members undergo a considerable conformational transition upon GTP hydrolysis, as observed for Gtr2p, and the significant structural difference between the GTP-bound and GDP-bound forms of this subunit is the main structural basis responsible for the association or dissociation of Raptor from the RagA/B-RagC/D heterodimer. It is also known that RagAGTP-RagCGDP is less active than RagA<sup>GTP</sup>-RagC<sup>GTP</sup> (3), implying that the RagC/D subunit is involved in the interaction between the RagA/B subunit and Raptor. Because the conformation of RagA/B<sup>GTP</sup> will not be affected by the nucleotide-bound status of RagC/D according to the structural comparison of the two forms of the Gtr1p-Gtr2p heterodimer, RagC/D bound to GDP is likely to interact with Raptor directly and contribute to the Raptor binding to RagA/B<sup>GTP</sup>. The crystal structures of the two forms of the Gtr1p-Gtr2p heterodimers indicate that the Raptor-interacting surface of RagA/B<sup>GTP</sup> would be close to and form a continuous surface with the RagC/D subunit in the RagA/B<sup>GTP</sup>-RagC/D<sup>GDP</sup> heterodimer (Fig. 4B) because of the G domain movements. Probably, RagC/D<sup>GDP</sup> at this orientation is able to interact with Raptor. The affinity of RagC/D<sup>GDP</sup> for Raptor, however, should be weak because the RagA/B-RagC/D heterodimer is inactive when the RagA/B subunit is in the GDP-bound form.

In conclusion, the prominent conformational transition observed in this work provides a structural basis for how the Rag GTPase heterodimers serve as a molecular switch through interacting with Raptor. Our structure in conjunction with the structure of Gtr1p<sup>GMPNPN</sup>-Gtr2p<sup>GMPNPN</sup> provides an important framework to address the existing questions and to devise experimental approaches to fully elucidate the molecular mechanisms underlying the regulation of Rag GTPases and the activation of mTORC1 in the amino acid-regulated signaling pathway.

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