Autoinhibition and Signaling by the Switch II Motif in the G-protein Chaperone of a Radical B$_{12}$ Enzyme*

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MeaB is a G-protein chaperone of methylmalonyl-CoA mutase (MCM). Mutations in the canonical switch II motif disrupt signaling in the MeaB-MCM complex. Conclusions: The switch II loop is autoinhibitory for the intrinsic GTPase activity of MeaB. Significance: Signaling in the MeaB-MCM complex is achieved via nucleotide-dependent conformational coupling between switches II and III.

Background: MeaB is a G-protein chaperone of methylmalonyl-CoA mutase (MCM). Mutations in the canonical switch II motif disrupt signaling in the MeaB-MCM complex. Results: Mutations in the canonical switch II motif disrupt signaling in the MeaB-MCM complex. Conclusion: The switch II loop is autoinhibitory for the intrinsic GTPase activity of MeaB. Significance: Signaling in the MeaB-MCM complex is achieved via nucleotide-dependent conformational coupling between switches II and III.

MeaB is an accessory GTPase protein involved in the assembly, protection, and reactivation of 5′-deoxyadenosyl cobalamin-dependent methylmalonyl-CoA mutase (MCM). Mutations in the human ortholog of MeaB result in methylmalonic aciduria, an inborn error of metabolism. G-proteins typically utilize conserved switch I and II motifs for signaling to effector proteins via conformational changes elicited by nucleotide binding and hydrolysis. Our recent discovery that MeaB utilizes an unusual switch III region for bidirectional signaling with MCM raised questions about the roles of the switch I and II motifs in MeaB. In this study, we addressed the functions of conserved switch II residues by performing alanine-scanning mutagenesis. Our results demonstrate that the GTPase activity of MeaB is autoinhibited by switch II and that this loop is important for coupling nucleotide-sensitive conformational changes in switch III to elicit the multiple chaperone functions of MeaB. Furthermore, we report the structure of MeaB-GDP crystallized in the presence of AlF$_4^{-}$ to form the putative transition state analog, GDP-AlF$_4^{-}$. The resulting crystal structure and its comparison with related G-proteins support the conclusion that the catalytic site of MeaB is incomplete in the absence of the GTPase-activating protein MCM and therefore unable to stabilize the transition state analog. Favoring an inactive conformation in the absence of the client MCM protein might represent a strategy for suppressing the intrinsic GTPase activity of MeaB in which the switch II loop plays an important role.

MeaB from *Methylbacterium extorquens* and its human ortholog CblA play critical roles in the docking of coenzyme-B$_{12}$ (or 5′-deoxyadenosyl cobalamin (AdoCbl)$^2$) into the active site of the client enzyme methylmalonyl-CoA mutase (MCM) (1–4). MCM belongs to the class of AdoCbl-dependent mutases that catalyze 1,2-rearrangement reactions (5). Dietary cobalamin is assimilated into AdoCbl and delivered to MCM in a complex trafficking pathway (6–8). In humans, MCM functions in the mitochondrial catabolism of branched-chain amino acids, odd-chain fatty acids, and cholesterol by converting methylmalonyl-CoA to succinyl-CoA. Mutations in the auxiliary proteins or in MCM itself give rise to methylmalonic acidemia, an inborn error of metabolism that is inherited as an autosomal recessive disease (9–13). Nearly 30 pathogenic mutations have been described in CblA (also known as MMAA) leading to lower AdoCbl levels and consequent impairment of MCM (11).

Our understanding about the function of CblA is derived primarily from biochemical studies on MeaB, which belongs to the G3E family of SIMIBI phosphate-binding loop (P-loop) G-proteins (14). Several members of this family of NTPases serve as metallochaperones. These include CooC, HypB, and UreG, accessory proteins involved in the maturation of nickel-containing enzymes. CooC is a Ni$^{2+}$-binding ATPase that undergoes metal- and nucleotide-dependent dimerization and catalyzes the insertion of nickel into carbon-monoxide dehydrogenase (15). UreG is a GTPase that is needed for the insertion of nickel into urease (16). HypB is activated by GTP-dependent dimerization and is needed for nickel insertion into the [Ni-Fe] hydrogenase (17). Some metallochaperones bind the transition metal and transfer it directly to the target protein, whereas others facilitate metal insertion but do not directly bind the cofactor. MeaB is an example of the latter class as it gates the transfer of AdoCbl from adenosyltransferase (ATR) to MCM (3) but does not itself bind the metal-containing cofactor. GTP hydrolysis by MeaB is required for transfer of AdoCbl from ATR to MCM.

MeaB; MMAA, methylmalonic aciduria type A protein; MCM, methylmalonyl-CoA mutase; ATR, adenosyltransferase; GAP, GTPase-activating protein; GMPPNP, 5′-guanylyl β,γ-imidophosphate; GTPγS, guanosine 5′-3-OTriphosphate; P-loop, phosphate-binding loop; H$_2$OCbl, aquocob(ii)alamin.

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Most G-proteins including the G3E GTPases are predicted to signal via conserved sequence motifs known as switch I and switch II (18–22). Switch I and switch II function as loaded springs that interact with the Mg$^{2+}$ ion in the GTPase site and signal to target proteins by undergoing conformational changes in response to nucleotide binding, hydrolysis, and exchange. In G3E G-proteins, the EXXG peptide defines the minimal consensus sequence for the switch II region (14). The switch I sequence is not strongly conserved with the exception of the nearly ubiquitously present threonine and glutamate residues in Ras-like GTPases (23). The γ-phosphate of GTP makes direct hydrogen bonding contacts with the main-chain amides of the conserved threonine and glycine residues in the switch I and II sequences, respectively. The carboxylate of the switch II glutamate residue forms a water-mediated contact with the Mg$^{2+}$ ion that is also coordinated by the β- and γ-phosphate oxygen atoms. In Ras proteins, the water molecule that serves as a nucleophile in the GTPase reaction is activated via interaction with a conserved glutamine residue in the switch II consensus motif, DXXGQ. In G3E proteins such as HypB, NifH, and SRP, the residue that activates the nucleophilic water is predicted to be a conserved aspartate residue at position 1 in the switch II consensus sequence (24–26). However, the corresponding aspartate in MeaB, Asp-92, is not positioned for catalysis. It is displaced by a conserved glycine residue. In Ras-like GTPases, the EXXGQ peptide defines the minimal consensus sequence for the switch II region (14). The switch I sequence is not strongly conserved with the exception of the nearly ubiquitously present threonine and glutamate residues in Ras-like GTPases (23). The γ-phosphate of GTP makes direct hydrogen bonding contacts with the main-chain amides of the conserved threonine and glycine residues in the switch I and II sequences, respectively. The carboxylate of the switch II glutamate residue forms a water-mediated contact with the Mg$^{2+}$ ion that is also coordinated by the β- and γ-phosphate oxygen atoms. In Ras proteins, the water molecule that serves as a nucleophile in the GTPase reaction is activated via interaction with a conserved glutamine residue in the switch II consensus motif, DXXGQ. In G3E proteins such as HypB, NifH, and SRP, the residue that activates the nucleophilic water is predicted to be a conserved aspartate residue at position 1 in the switch II consensus sequence (24–26). However, the corresponding aspartate in MeaB, Asp-92, is not positioned for catalysis. It is displaced by a conserved glycine residue.

In addition to gating the transfer of AdoCbl from ATR to MCM, MeaB also functions to protect MCM from inactivation during catalytic turnover and to rescue MCM that is inactivated (2, 3). Thus, in the presence of either GDP or GTP, MeaB slows the rate of MCM inactivation by 30-fold (28), and the exchange of GDP for GTP drives the ejection of inactive cofactor from the active site of MCM. The limited biochemical studies support a role for CblA that is analogous to the better characterized bacterial MeaB in protecting MCM from inactivation and rescuing.

FIGURE 1. Schematic representation of the interactions between GTP and MeaB or HypB. Representative active sites of MeaB-GMPPNP (Protein Data Bank code 4JYC) (a) and HypB-GTP-Y5 (Protein Data Bank code 2HF8) (b) are shown. Based on sequence and structural homology, the residues in MeaB (boxed) corresponding to catalytically important residues in the HypB active site are shown. Residues in red are contributed from the neighboring subunit in the HypB homodimer. The activated water is shown in blue.

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inactive MCM (37). Furthermore, human MCM also exhibits GAP activity with respect to CblA (29).

In this study, we interrogated the role of the switch II motif in mediating the chaperone functions of MeaB and in transmitting the GAP function of MCM. To this end, we used alanine-scanng mutagenesis at positions in switch II that are conserved between MeaB and CblA. We demonstrate that switch II mutations compromise regulation of MeaB while disrupting MCM-dependent GAP activation. We also report the structure of MeaB crystallized in the presence of GDP and AlF$_3^-$. Our results suggest a strategy for switch II-dependent autoinhibition of the intrinsic GTPase activity of MeaB, which exists predominantly in an inactive conformation. Complexation with MCM is predicted to stabilize MeaB in a conformation in which its GTPase function is activated.

EXPERIMENTAL PROCEDURES

Materials—AdoCbl, GMPPNP, ATP, GTP, methylmalonic acid, coenzyme A, and other reagent grade chemicals were purchased from Aldrich. Methylmalonyl-CoA was synthesized using malonyl-CoA synthetase and purified as described previously (38). The HPLC column used to quantify guanosine nucleotides was a μBondapak™ NH$_2$, 10-μm, 125 Å, 3.9 × 300-mm column purchased from Waters.

Construction of Site-specific Mutants—Plasmids encoding the M. extorquens ATR and MCM were generous gifts from Mary E. Lidstrom (University of Washington, Seattle, WA). A synthetic gene encoding Am1 MeaB with 5'-Ncol and 3'-XhoI restriction sites was generated by GenScript Corp. (Piscataway, NJ) to optimize codon usage in Escherichia coli and reduce the GC content. The synthetic MeaB gene was cloned into the pET-21d(+) expression vector (Novagen, Gibbstown, NJ) using the Ncol and XhoI sites. Site-specific mutants of MeaB were generated using a QuikChange kit (Agilent, Gibbstown, NJ) using the NcoI and XhoI sites. Site-specific sequences: E154A, 5'-CGCCGTTGC-3'; E162A, 5'-CGATGTCATTCTGGTGGCAACCGTG-3'; T155A, 5'-CGATGTCATTCTGGTGGCAACCGTG-3'; Q160A/E162A, 5'-CGATGTCATTCTGGTGGCAACCGTGCAACCGCCGTCGGTCAAGAGCGCAACCGCCGTTGGCAGATCT-3'.

Enzyme Purification—Recombinant M. extorquens MeaB, MCM, and ATR were expressed and purified from E. coli BL-21(DE3) as described previously (4, 39). The purified enzymes were flash frozen in liquid nitrogen and stored at −80 °C in 50 mM HEPES buffer, pH 8.0 containing 0.3 M KCl, 10 mM MgCl$_2$, and 10% glycerol (Buffer A).

Crystallization of MeaB—Protein samples were concentrated to 11 mg ml$^{-1}$ in 50 mM HEPES buffer at pH 8.0, 2.5 mM MgCl$_2$, 5 mM GDP-AlF$_3^-$. GDP-AlF$_3^-$ was prepared by mixing GDP, AlCl$_3$, and potassium fluoride at a 1:5.15 ratio and used after gel filtration. The MeaB-GDP crystals were grown by the sitting drop vapor diffusion method at 20 °C by mixing 2 μl of protein solution with 2 μl of reservoir solution, which contained 20% (w/v) PEG 3350 and 0.2 M sodium sulfate. Crystals were cryoprotected for a few minutes before being flash frozen in liquid N$_2$ by transfer to a solution of 20% glycerol, 15% PEG 3350 (w/v), 0.15 M sodium sulfate, 5 mM GDP, and 1 mM AlF$_3^-$ in 25 mM HEPES, pH 7.5. Crystals of MeaB-GDP were of space group P2$_1$ (a = 63.7, b = 78.6, c = 69.6, β = 108.6) with two monomers in the asymmetric unit.

Data Collection and Structure Determination—Diffraction data for MeaB-GDP crystallized in the presence of AlF$_3^-$ were collected at 100 K on beamline GM/CA-CAT 23-ID-B at the Advanced Photon Source, Argonne National Laboratory (Argonne, IL). Data were recorded on a Mar300 detector and processed with XDS (40) to 1.8-Å resolution (Table 1). EPMR (41) was used to determine initial phases for MeaB-GDP-AlF$_3^-$ through molecular replacement using a single monomer of the MeaB-GDP structure (Protein Data Bank code 2QM7 (27)) as a search model. Loops containing residues 62–67, 95–100, 181–186, and 225–231 were removed from the search model to eliminate bias. Initial density allowed for the ligand to be modeled and added. REFMAC (42) of the CCP4 suite (43) was subsequently used for restrained refinement of the model using isotropic individual B-factors to a final R$_{work}$ of 0.180 and R$_{free}$ of 0.219. Model building and modification were performed with Coot (44), and the geometric quality of the models and their agreement with the structure factors were assessed with MolProbity (45). Crystallographic information and refinement statistics are provided in Table 1. Figures were generated with PyMOL (46).

Thermodynamics of GMPPNP Binding—Isothermal titration calorimetry experiments were performed at 10 °C in Buffer A using a 300-μl injection syringe and a 1.43-ml injection cell. Samples were prepared by filtration through a 0.2-μm filter and then degassed under vacuum at 4 °C using a ThermoVac sample degasser. Each titration was performed at least in duplicate. GMPPNP (10-μl injections of 150–400 μM) was added to 10–25 μM MeaB. The data were analyzed using a two-site binding model using the MicroCal Origin program. Values for the dissociation constant at sites 1 and 2 were then compared with the values obtained from the solution of the Gibbs free energy equation: ΔG° = −RTln(K$_J$).

Enzyme Inactivation Assays—Inactivation of MCM during steady-state turnover was monitored by UV-visible spectrophotometry using cob(II)alamin to aquocob(III)alamin (H$_2$OCbl) at 20 °C in 0.1 M potassium phosphate at pH 7.5 containing 10 mM MgCl$_2$. The reactions and sample preparations were performed in the dark to avert spurious H$_2$OCbl formation by photolysis of AdoCbl followed by oxidation. Samples were prepared by the addition of reaction components in the order described below. MCM (25–30 μM)
was reconstituted with an equimolar concentration of AdoCbl. A slight molar excess (35–40 μM) of MeaB was added to the MCM holoenzyme to generate an MCM-MeaB complex. GMPPNP was then added to the reaction mixtures to a final concentration of 1–2 mM. The reaction was initiated by the addition of methylmalonyl-CoA to a final concentration of 4.5–5 mM. The rates of inactivation were determined by plotting the change in absorbance at 351 nm, corresponding to H₂OCbl formation, as a function of time. The kinetic traces were best fit by a single exponential equation: ΔAₜ = ΔA₀ - ΔAᵣe⁻ᵏᵗ where Δₐᵣ is the absorbance at 351 nm as a function of time, Δₐ₀ is the initial absorbance of cob(II)alamin, Δₐᵣ is the reaction phase amplitude for H₂OCbl formation, and k is the observed rate constant for MCM inactivation.

**Assay for Transfer of AdoCbl from ATR to MCM**—The ATP-dependent transfer of AdoCbl from ATR to the MCM-MeaB-GMPPNP complex was performed in the dark at 20 °C and monitored by UV-visible spectroscopy. Two equivalents of AdoCbl were added to 1 eq of ATR in Buffer A to generate holo-ATR. The apo-MCM-MeaB-GMPPNP complex was formed in Buffer A by mixing 40–50 μM MCM with an equimolar concentration of AdoCbl and 50–60 μM MeaB to generate an estimated 40–50 μM complex. GMPPNP was added to a final concentration of 1 mM. Holo-ATR (2:1 AdoCbl:ATR, 40–50 μM ATR) and the MCM-MeaB-GMPPNP complex (40–50 μM) were mixed and incubated for 10 min at 20 °C before addition of ATR to a final concentration of 5 mM. Release/transfer of AdoCbl from ATR was calculated using a ∆ε₅₂₅ = 6.69 mm⁻¹ cm⁻¹. Bound versus free cofactor was separated using an Amicon centrifuge filter (10-kDa cutoff, 20 min, 4 °C, 16,000 × g). The concentration of free AdoCbl in the filtrate was calculated using ∆ε₅₂₅ = 8.0 mm⁻¹ cm⁻¹.

**Release of Cob(II)alamin—MCM** (30–40 μM) was mixed with 45–60 μM wild-type, E154A, T155A, Q160A, S161A, E162A, or Q160A/E162A MeaB in Buffer A at 20 °C under strictly anaerobic conditions such that [MCM]:[MeaB] was 1:1.5. Cob(II)alamin was generated by reduction of H₂OCbl with trim(2-carboxyethyl)phosphine hydrochloride and added to a final concentration equal to that of the MCM-MeaB (wild-type or mutant) complex. The reaction mixture was incubated for 10 min at 20 °C. GMPPNP in anaerobic Buffer A was added to a final concentration of 2 mM. The mixture was then incubated for 20 min at 20 °C. Subsequently, the sample was made aerobic by air oxidation for 2 h and then filtered through a Centricon YM10 filter (10-kDa cutoff) to separate free from bound H₂OCbl. Cob(II)alamin (but not H₂OCbl) is released from MCM and is subsequently oxidized to H₂OCbl, which was quantified using ∆ε₅₂₀ = 26.5 mm⁻¹ cm⁻¹.

**GTPase Activity of MeaB**—The steady-state kinetic parameters for the GTPase activity of wild-type or switch II mutants of MeaB were determined using 20 μM MeaB or 5 μM MeaB in the presence of 10 μM MCM (to generate the MCM-MeaB complex). The reactions were performed in Buffer A containing 25 mM MgCl₂ at 20 °C and initiated by the addition of GTP to a final concentration ranging from 0.01 to 10 mM. The reactions were quenched using 2 M trichloroacetic acid, and the GTPase activity of MeaB alone or the MCM-MeaB complex was determined using an HPLC assay as described previously (4). The concentration dependence for the observed rate of GTP hydrolysis was plotted versus GTP concentration, and the data were fit to a Michaelis-Menten equation to determine values for kₕcat and Kₘ.

**Size Exclusion Chromatography**—Samples were prepared in Buffer A containing 0.5 mM GDP, 80–90 μM wild-type or mutant MeaB ± equimolar MCM, and 0.5 mM AdoCbl in a total volume of 150 μl. The samples were loaded onto a Superdex-200 HR 10/30 column equilibrated with Buffer A. The protein complexes were eluted at 4 °C in the dark to minimize photolysis of AdoCbl.

**RESULTS**

**Characterization of a New MeaB-GDP Crystal Structure**—MeaB crystallized in the presence of GDP-AlF₄⁻, a potential transition state mimic, in the P₂₁ space group and a unit cell similar to that reported for MeaB crystallized in the presence of GDP (Table 1). However, AlF₄⁻ was not observed in any of the more than 10 structures that were solved. Furthermore, despite the presence of Mg²⁺ in the crystallization solution, the cation was not observed in the new MeaB-GDP crystal structure (Fig. 2a), which appeared to be very similar to that of MeaB-GDP published previously (27). However, closer inspection revealed two significant differences as described below.

Superposition of the new MeaB-GDP and previously determined MeaB-GDP structures yielded root mean square deviation values of 2.31 Å for the Ca atoms of all residues. Superimposition of the MeaB dimers and alignment with respect to the nucleotides revealed no differences in the nucleotide binding
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sites between the new MeaB-GDP and previously reported MeaB-GDP structures. However, Arg-108 in the previously reported MeaB-GDP structure (Fig. 2b, yellow) is solvent-exposed and facing away from the active site cavity, whereas it is swung in and engages in a salt bridge contact with the carboxylate of Glu-154 in the new structure of MeaB-GDP (Fig. 2b, blue). To assess whether the observed structural differences were due to slightly different crystallization conditions, the structure of MeaB-GDP was redetermined under the conditions used to obtain the new MeaB-GDP structure crystallized in the presence of AlF$_4^-$). The new and published (Protein Data Bank code 2QM7) structures of MeaB-GDP were identical.

Additional differences between the new MeaB-GDP and previously determined MeaB-GDP structures are observed in the switch III region (residues 178–188) and its interaction with conserved residues Gln-160 and Glu-162 in switch II (Fig. 2c). In the new structure of MeaB-GDP reported here, the side chain of Gln-160 (Fig. 2c, blue) is swung $\sim 25^\circ$ from its position in the previously reported MeaB-GDP structure (yellow). The electron density for the side chain of Lys-188 in the new MeaB-GDP structure allows it to be modeled in two configurations. In one configuration, Lys-188 contacts switch II via an ionic interaction with the carboxylate of Glu-162 and a single hydrogen bond with the carbonyl oxygen of Gly-159. In the other configuration, the interactions between Lys-188 and switch II are disrupted. In contrast, hydrogen bond contacts between Lys-188 of switch III and Glu-162 or any other part of switch II are not observed in the previous MeaB-GDP structure (Fig. 2c, yellow). The contacts formed between switch II and switch III in the MeaB-GDP structure reported here are also distinct from those captured in the structure of MeaB-GMPPNP in which the side chain of Lys-188 interacts with the carboxylate of Glu-162 and the amide side chain of Gln-160 (Fig. 2c, gray).

The new MeaB-GDP and previously reported MeaB-GDP structures also differ in the interaction of the switch III region with a flexible loop from the adjacent monomer extending between residues 224 and 232. In one of the monomers of the new MeaB-GDP structure, the backbone carbonyl oxygen of Glu-183 is hydrogen-bonded to Ser-230 (Fig. 2d, blue). In contrast, the conformation of switch III in the previously solved MeaB-GDP structure precludes Glu-183 from contacting the 224–232 loop (Fig. 2d, yellow). The only contact formed between switch III and the 224–232 loop in the previously reported MeaB-GDP structure is a single hydrogen bond between the side chain of His-224 and the carboxylate residue Asp-182. At the same subunit interface of the new MeaB-GDP structure, Asp-182 is disordered. In the opposing subunit interface of the MeaB-GDP structure reported here, Asp-182 does not engage in a hydrogen bond interaction with the 224–232 loop and is displaced $\sim 9$ Å compared with its position at the corresponding subunit interface in the previously reported MeaB-GDP structure. The relative orientations of the switch I, II, and III loops in the new MeaB-GDP structure are shown in Fig. 3.

**FIGURE 2.** Comparison of the MeaB-GDP (Protein Data Bank code 4LC1), MeaB-GDP (Protein Data Bank code 2QM7), and MeaB-GMPPNP structures. a, the overall structure of MeaB-GDP (Protein Data Bank code 4LC1) in which the individual subunits are shown in light and dark gray, respectively, and GDP is shown in sphere representation. Switch motifs I, II, and III are colored red, blue, and cyan, respectively. b, comparison of the orientations and interactions of switch I and II residues in the structures of MeaB-GDP (Protein Data Bank code 4LC1) and MeaB-GDP (Protein Data Bank code 2QM7). GDP and select amino acid residues are shown in stick representation. The switch I and II regions from the new and previously determined MeaB-GDP structures are shown in blue and yellow, respectively. The dashed blue lines represent hydrogen bonding or ionic interactions in the new MeaB-GDP structure. c, comparison of the interactions between switch II and switch III regions in the structures of MeaB-GDP (Protein Data Bank code 4LC1), MeaB-GDP (Protein Data Bank code 2QM7) (yellow), and MeaB-GMPPNP (Protein Data Bank code 4JYC). The dashed lines represent hydrogen bonding interactions between the switch II and III regions in structures of MeaB-GDP (Protein Data Bank code 4LC1) (blue sticks; blue dashes), MeaB-GDP (Protein Data Bank code 2QM7) (yellow sticks), and MeaB-GMPPNP (gray sticks; gray dashes). Lys-188 in the new MeaB-GDP structure is shown in two conformations based on the electron density, d, a close-up of the interactions of switch III residues with the flexible loop on the adjacent subunit (224–232) in the superimposed MeaB-GDP (Protein Data Bank code 4LC1) (blue dashes) and MeaB-GDP (Protein Data Bank code 2QM7) (yellow dashes) structures. The switch II and 224–232 loops are shaded blue and yellow in the MeaB-GDP and MeaB-GDP (Protein Data Bank code 2QM7) structures, respectively. e, electron density for neighboring active sites in the MeaB-GDP structure (Protein Data Bank code 4LC1). Waters are shown as red spheres, and GDP and residues Glu-154 and Arg-108 are shown in yellow.
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TABLE 2
Thermodynamic data for GMPPNP binding to MeaB

| Enzyme       | Site 1 | Site 2 |
|--------------|--------|--------|
| Wild type    | $K_m$  | $K_a$  |
| E154A        | 1.3 ± 0.3 | 3.7 ± 0.5 |
| Q160A        | 0.7 ± 0.1 | 3.3 ± 0.6 |
| S161A        | 1.1 ± 0.3 | 3.9 ± 0.8 |
| E162A        | 0.04 ± 0.02 | 0.6 ± 0.1 |
| Q160A/E162A  | 0.16 ± 0.01 | 0.48 ± 0.07 |

* Determined previously (4).

TABLE 3
Kinetic parameters for GTP hydrolysis by MeaB mutants

| MCM          | $k_{cat}$   | $K_m$   | -Fold GAP activation |
|--------------|------------|---------|----------------------|
| Wild type    | 0.044 (μM) | 0.20 (μM) | 96 |
| E154A        | 0.35 (μM)  | 0.23 (μM) | 1.3 |
| Q160A        | 0.45 (μM)  | 0.49 (μM) | 37 |
| S161A        | 0.11 (μM)  | 0.73 (μM) | 3.5 |
| E162A        | 0.41 (μM)  | 0.71 (μM) | 95 |
| Q160A/E162A  | 0.20 (μM)  | 0.36 (μM) | 100 |

GMPPNP Binding to Switch II MeaB Mutants—The MeaB homodimer binds nucleotides with negative cooperativity (Table 2). GMPPNP binds to sites 1 and 2 with $K_D$ values of 0.8 ± 0.5 and 9.5 ± 1.9 μM, respectively. The $K_m$ values for GMPPNP binding range from 0.16 ± 0.01 to 1.3 ± 0.3 μM at site 1 and from 0.48 ± 0.07 to 9.6 ± 1.4 μM at site 2. Overall, the switch II mutations do not have a substantial impact on GMP-PNP binding with the exception of the E162A and Q160A/E162A mutants in which the affinity for the nucleotide increases ~5–20-fold. GMPPNP binds to each switch II mutant with negative cooperativity.

GTPase Activity of Switch II Mutants—The intrinsic and GAP-stimulated GTPase activities of the single and double switch II mutants were compared with wild-type MeaB (Table 3). Surprisingly, the E154A, Q160A, and Q160A/E162A mutants exhibited ~5–10-fold activation of the intrinsic GTPase activity compared with wild-type MeaB. The GAP function of MCM was suppressed in the E154A, Q160A, and Q160A/E162A mutants compared with the ~100-fold rate enhancement observed with wild-type MeaB. The S161A mutation had a negligible impact on the intrinsic GTPase rate, which was activated in the presence of MCM. The E162A mutation led to a modest reduction in the intrinsic and GAP-stimulated rates of GTP hydrolysis but resulted in the same ~5-fold GAP activation as seen for wild-type MeaB. The T155A mutant exhibited a modest 2.5-fold increase in the intrinsic GTPase activity and a mild impairment in GAP activation (37- versus 100-fold for wild-type MeaB). The Michaelis-Menten analysis yielded a $K_{in}$ value of 0.414 mM for the wild-type MeaB–MCM complex, which was ~2-fold higher than the $K_{in}$ value for wild-type MeaB alone (Table 3). Compared with wild-type MeaB, the switch II mutations led to a ~0.5–3.5-fold change in the $K_{in}$ value for GTP in isolated MeaB and an ~0.5–1.7-fold change in the MCM-MeaB complex.

Switch II Mutations Affect the Rate of Oxidative Inactivation of MCM—In the presence of wild-type apo-MeaB, the rate of oxidative inactivation of MCM is 8.8 × 10⁻³ min⁻¹ (Fig. 4a). Addition of GMPPNP or GDP to the wild-type MCM-MeaB complex decreases the inactivation rate nearly 23-fold (3.9 × 10⁻⁴ min⁻¹) (2). The 23-fold effect of wild-type MeaB-GMPPNP on MCM inactivation is slightly larger than the 15-fold effect that we have reported previously and may be due to buffer differences (2). Although the Q160A (2.8 × 10⁻⁴ min⁻¹) and E162A (2.8 × 10⁻⁴ min⁻¹) mutants do not appreciably impact the rate of MCM inactivation, E154A (2.0 × 10⁻⁴ min⁻¹) and Q160A/E162A (2.1 × 10⁻⁴ min⁻¹) lead to slightly enhanced protection. The S161A (4.4 × 10⁻² min⁻¹) and
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**FIGURE 4. Impact of switch II mutations on the chaperone functions of MeaB.**

*a* time-dependent inactivation of MCM in complex with wild-type apo-MeaB, wild-type MeaB-GMPPNP, or mutant MeaB-GMPPNP. The increase in absorbance corresponding to formation of H₂OCbl via oxidation of the intermediate cob(II)alamin formed during MCM turnover was monitored as described under "Experimental Procedures." The data presented here are representative of ≥2 experiments and were fit to a single exponential equation described under "Experimental Procedures." *b*, ATP-dependent AdoCbl release or transfer from holo-ATR to the MCM-MeaB complex (containing wild-type or switch II mutant MeaB) in the presence of GMPPNP. The data represent the AdoCbl equivalents that were either released into solution from ATR or transferred to MCM and are the average of ≥2 independent experiments performed as described under "Experimental Procedures." *c*, displacement of cob(II)alamin from MCM-cob(II)alamin-MeaB (wild type or switch II mutants) following addition of GMPPNP under anaerobic conditions as described under "Experimental Procedures." The data represent the average of ≥2 independent experiments ± S.D. (error bars).

T155A (6.8 × 10⁻³ min⁻¹) mutations exhibit 11- and 17-fold higher rates of inactivation, respectively, than wild-type MeaB.

**Impaired GTPase-dependent AdoCbl Transfer from ATR to MCM by Switch II Mutants**—The *M. extorquens* ATR binds 2 eq of AdoCbl per homotrimer, and binding of ATP to the vacant site initiates the transfer of a single equivalent of AdoCbl to the MCM-MeaB complex (3). In the presence of GMPPNP, AdoCbl transfer to the wild-type MCM-MeaB complex is blocked. Instead, 1 eq of the cofactor is released from ATR into solution (Fig. 4b). With GMPPNP bound to the MCM-E154A MeaB mutant complex, AdoCbl transfer becomes modestly uncoupled from nucleotide hydrolysis (0.82 eq released and 0.18 eq transferred). Cofactor transfer from ATR to the complexes of MCM and T155A, Q160A, S161A, or E162A MeaB in the presence of GMPPNP (0.46–0.52 eq of AdoCbl is transferred) is more significantly impaired. The Q160A/E162A double mutation severely impacts GTPase gating of AdoCbl transfer from ATR, and 0.9 eq of the cofactor was transferred instead of being released into solution in the presence of GMPPNP.

**Rescue of MCM by Switch II Mutants**—Our previous studies have shown that MeaB promotes expulsion of cob(II)alamin that has become uncoupled from 5'-deoxyadenosine during turnover in MCM (3). Here, we examined the role of switch II in exerting this chaperone function of MeaB. In the presence of stoichiometric cob(II)alamin (with respect to MCM) and an excess of each switch II mutant of MeaB, cob(II)alamin is bound to MCM. Following addition of an excess of GMPPNP to the wild-type MeaB-MCM-cob(II)alamin complex, ~97% of the inactive cob(II)alamin is detected in the filtrate, indicating that the inactive cofactor was ejected from the MCM active site (Fig. 4c). In contrast, in the presence of the MeaB mutant E154A, T155A, Q160A, or S161A, ~55–65% of cob(II)alamin remained associated with MCM. In the MCM-MeaB complex harboring the E162A single or the Q160A/E162A double mutation, ~80% of cob(II)alamin remained bound to MCM following addition of GMPPNP.

**DISCUSSION**

Although the roles of switch motifs in the catalytic and signaling mechanisms of many G-protein have been studied quite extensively (14, 20, 21, 47, 48), their role in cofactor delivery is not as well characterized. Unlike the structures of CooC and NifH, the crystal structures of MeaB obtained in the presence of Pₐ, GMPPNP, and GDP (crystallized in the presence and absence of AlF₃) have failed to capture a catalytically active GTPase conformation. This is also reflected in the absence of Mg²⁺ in the active site of all available MeaB crystal structures, raising questions about the possible significance of these inactive conformations to MeaB function. In this study, we examined the role of the conserved switch II residues in suppressing the intrinsic GTPase activity of MeaB in the absence of MCM.

In HypB and other G3E GTases, the general base that activates water is a conserved aspartate residue (Asp-69 in HypB; Fig. 1b) positioned N-terminally to the switch I sequence (26). The structure of HypB-GTPγS shows Asp-69 positioned ~5–6 Å from the χ-phosphinomethyl group of GTPγS and interacting with water molecules, one of which presumably serves as the nucleophile. The residue corresponding to Asp-69 in HypB is Asp-92 in MeaB. The MeaB structures containing GDP (crystallized in the presence of AlF₃ or GMPPNP) demonstrate that hydrophobic side chains of Val-156 and Val-158 in switch II impede access of Asp-92 to the nucleotide (Fig. 5, a–c). We speculate that the interaction between Glu-154 and Arg-108 is important for maintaining an autoinhibitory conformation in MeaB that is alleviated in the MCM-MeaB complex. In all MeaB structures with the exception of MeaB-GDP (Protein Data Bank code 2QM7), Glu-154 interacts with neighboring residues, e.g. Lys-68 from the P-loop and Arg-108 in switch I (Fig. 5, a and c). Some or all of these interactions are expected to be disrupted if Glu-154 serves as a Mg²⁺ ligand in the MCM-MeaB complex. The postulated dual role of Glu-154 in autoinhibition and GAP activation is consistent with the ~10-fold increase in the intrinsic GTPase activity of the E154A mutant and its relative insensitivity to the GAP activity of MCM.

The similar effect of the E154A, Q160A, and Q160A/E162A mutations on the intrinsic and GAP-stimulated GTPase activities is surprising (Table 3). The modest impact of the E162A point mutation on the intrinsic and GAP-stimulated GTPase activities suggests that the kinetic phenotype of the Q160A/E162A double mutant results primarily from the alanine substitution of Gln-160. Gln-160 is not positioned near the active
site in any of the MeaB structures, is not conserved in other G3E GTPases (e.g., CooC, NifH, and HypB), and is not predicted to directly participate in catalysis. Rather, Gln-160 is highly mobile and engages in various interactions with residues in the switch II and switch III loops and with a conserved threonine (Thr-163) immediately C-terminal to switch II (Fig. 6). Thus, Gln-160 forms direct or water-mediated hydrogen bonds with Glu-161, Glu-162, Thr-163, or Lys-188. However, within individual subunits of the apo-MeaB (Fig. 6c) and previously reported MeaB-GDP (Fig. 6d) structures, Gln-160 is not engaged in hydrogen bonding interactions. Hence, although our kinetic data suggest that Gln-160 is important for autoinhibition, the structures of MeaB do not provide insights into a possible mechanism. The inability of the S161A and E162A mutations to mirror the Q160A phenotype suggests that the individual disruption of their interaction with Gln-160 is insufficient to disrupt the autoinhibited conformation of MeaB.

The switch II residues Thr-155, Ser-161, and Glu-162 are not conserved among G3E GTPases. Furthermore, obvious roles for Thr-155, Ser-161, and Glu-162 in GTP binding or in the GTPase reaction are not apparent from the MeaB structures that are available. The T155A and S161A mutations result in modest perturbations of the GTPase activity of isolated MeaB and in the MCM-MeaB complex (Table 3). These data indicate that neither Thr-155 nor Ser-161 plays a very important role in autoinhibition. In contrast, E162A enhances the affinity of iso-
lated MeaB for GTP at sites 1 and 2 by 20- and 15-fold, respectively (Table 2). Thus, the effect of the E162A substitution on autoinhibition of the intrinsic GTPase activity might be exerted via enhanced affinity for and stabilization of the substrate, GTP.

We have speculated that switches II and III communicate for bidirectional signal transmission between MeaB and MCM (28). Lys-188 in switch III and Glu-162 and Gln-160 in switch II might be important for relaying information about nucleotide identity and hydrolysis between these switch motifs (Fig. 2c). A mutation in CblA corresponding to the Lys-188 residue in MeaB is pathogenic (49). We have shown that mutation of Lys-188 has pleiotropic consequences including loss of regulated GTPase-dependent AdoCbl loading into MCM and impaired repair of inactive MCM. In this study, we demonstrate that single mutations of the interacting partner residues Gln-160 and Glu-162 partially uncouple cofactor docking in MCM from GTP hydrolysis in MeaB (Fig. 4b) and ejection of inactive cofactor from MCM (Fig. 4c). The Q160A/E162A double mutant is more substantially impaired than either single mutant and more closely resembles the phenotype of the K188A/E mutants (28). Similar perturbations in MeaB function, albeit of varying magnitude, are also observed with other switch II mutants, consistent with a role for the entire switch II motif in signaling between MeaB and MCM via switch III.

G-proteins are engaged in a diverse array of regulatory processes (22, 50, 51). They are among most common and ancient regulatory proteins in nature (14). An increasing body of evidence demonstrates significant variations in the mechanisms of and factors used for GTP hydrolysis and signal transduction that are not readily predicted by sequence and structural homology (52). This complexity is also evident within the G3E metallochaperone family in that MeaB, which possesses an atypical active site, is stabilized in an autoinhibitory conformation via its switch II motif in the absence of the GAP protein MCM.

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REFERENCES

1. Korotkova, N., and Lidstrom, M. E. (2004) MeaB is a component of the methylmalonyl-CoA mutase complex required for protection of the enzyme from inactivation. J. Biol. Chem. 279, 13652–13658
2. Padovani, D., and Banerjee, R. (2006) Assembly and protection of the radical enzyme, methylmalonyl-CoA mutase, by its chaperone. Biochemistry 45, 9300–9306
3. Padovani, D., and Banerjee, R. (2009) A G-protein editor gates coenzyme B₃₂ loading and is corrupted in methylmalonic aciduria. Proc. Natl. Acad. Sci. U.S.A. 106, 21567–21572
4. Padovani, D., Labun'ska, T., and Banerjee, R. (2006) Energetics of interaction between the G-protein chaperone, MeaB, and B12-dependent methylmalonyl-CoA mutase. J. Biol. Chem. 281, 17838–17844
5. Banerjee, R. (2003) Radical carbon skeleton rearrangements: catalysis by coenzyme B₁₂-dependent mutases. J. Biol. Chem. 278, 939–945
6. Banerjee, R. (2006) B₁₂ trafficking in mammals: a for coenzyme escort service. ACS Chem. Biol. 1, 149–159
7. Banerjee, R., Gherasim, C., and Padovani, D. (2009) The tinker, tailor, soldier in intracellular B₁₂ trafficking. Curr. Opin. Chem. Biol. 13, 484–491
8. Gherasim, C., Lofgren, M., and Banerjee, R. (2013) Navigating the B₁₂ road: assimilation, delivery, and disorders of cobalamin. J. Biol. Chem. 288, 13186–13193
9. Leal, N. A., Olteanu, H., Banerjee, R., and Bobik, T. A. (2004) Human ATP: cob(Ⅱ)alamin adenosyltransferase and its interaction with methionine synthase reductase. J. Biol. Chem. 279, 47536–47542
10. Dobson, C. M., Wai, T., Leclerc, D., Kadir, H., Narang, M., Lerner-Ellis, J. P., Hudson, T. J., Rosenblatt, D. S., and Gravel, R. A. (2002) Identification of the gene responsible for the cblB complementation group of vitamin B₁₂-dependent methylmalonic aciduria. Hum. Mol. Genet. 11, 3361–3369
11. Dobson, C. M., Wai, T., Leclerc, D., Wilson, A., Wu, X., Doré, C., Hudson, T., Rosenblatt, D. S., and Gravel, R. A. (2002) Identification of the gene responsible for the cblA complementation group of vitamin B₁₂-responsive methylmalonic acidemia based on analysis of prokaryotic gene arrangements. Proc. Natl. Acad. Sci. U.S.A. 99, 15554–15559
12. Ledley, F. D., Lumetta, M., Nguyen, P. N., Kolhouse, J. F., and Allen, R. H. (1988) Molecular cloning of L-methylmalonyl-CoA mutase: gene transfer and analysis of mut cell lines. Proc. Natl. Acad. Sci. U.S.A. 85, 3518–3521
13. Leal, N. A., Park, S. D., Kima, P. E., and Bobik, T. A. (2003) Identification of the human and bovine ATP: cob(Ⅱ)alamin adenosyltransferase cDNAs based on complementation of a bacterial mutant. J. Biol. Chem. 278, 9227–9234
14. Leipe, D. D., Wolf, Y. I., Koonin, E. V., and Aravind, L. (2002) Classification and evolution of P-loop GTPases and related ATPases. J. Mol. Biol. 317, 41–72
15. Jeon, W. B., Cheng, J., and Ludden, P. W. (2001) Purification and characterization of membrane-associated CooC protein and its functional role in the insertion of nickel into carbon monoxide dehydrogenase from Rhodospirillum rubrum. J. Biol. Chem. 276, 38602–38609
16. Zambelli, B., Stola, M., Musiani, F., De Vriendt, K., Samyn, B., Devreese, B., Van Beeumen, J., Turano, P., Dikiy, A., Bryant, D. A., and Ciurli, S. (2005) UreG, a chaperone in the urease assembly process, is an intrinsically unstructured GTPase that specifically binds Zn²⁺. J. Biol. Chem. 280, 4684–4695
17. Rey, L., Imperial, J., Palacios, J. M., and Ruiz-Aragues, T. (1994) Purification of Rhizobium leguminosarum HypB, a nickel-binding protein required for hydrogenase synthesis. J. Bacteriol. 176, 6066–6073
18. Milburn, M. V., Tong, L., deVos, A. M., Brünger, A., Yamaizumi, Z., Nishimura, S., and Kim, S. H. (1990) Molecular switch for signal transduction: structural differences between active and inactive forms of proton-coupled rac proteins. Science 247, 939–945
19. Stouten, P. F., Sander, C., Wittinghofer, A., and Valencia, A. (1993) How does the switch II region of G-domains work? FEBS Lett. 320, 1–6
20. Sprang, S. R. (1997) G protein mechanisms: insights from structural analysis. Annu. Rev. Biochem. 66, 639–678
21. Vetter, I. R., and Wittinghofer, A. (2001) The guanine nucleotide-binding switch in three dimensions. Science 294, 1299–1304
22. Wittinghofer, A., and Vetter, I. R. (2011) Structure-function relationships of the G domain, a canonical switch motif. Annu. Rev. Biochem. 80, 943–971
23. Schefzek, K., Ahmadian, M. K., Kabsch, W., Wiesmüller, L., Lautwein, A., Schmitz, F., and Wittinghofer, A. (1997) The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science 277, 333–338
24. Egea, P. F., Shan, S. O., Napetschnig, J., Savage, D. F., Walter, P., and Stout, R. M. (2004) Substrate twinning activates the signal recognition particle and its receptor. Nature 427, 215–221
25. Lanzilotta, W. N., Fisher, K., and Seefeldt, L. C. (1997) Evidence for electron transfer-dependent formation of a nitrogenase iron protein–molybdenum–iron protein tight complex. The role of aspartate 39. J. Biol. Chem. 272, 4157–4165
26. Gasper, R., Scrima, A., and Wittinghofer, A. (2006) Structural insights into HypB, a GTP-binding protein that regulates metal binding. J. Biol. Chem. 281, 27492–27502
27. Hubbard, P. A., Padovani, D., Labun’ska, T., Mahlstedt, S. A., Banerjee, R., and Drennan, C. L. (2007) Crystal structure and mutagenesis of the metallochaperone MeaB: insight into the causes of methylmalonic aciduria. J. Biol. Chem. 282, 31308–31316
28. Lofgren, M., Padovani, D., Koutmos, M., and Banerjee, R. (2013) A switch III motif relays signaling between a B$_{12}$ enzyme and its G-protein chaperone. Nat. Chem. Biol. 9, 535–539
29. Froese, D. S., Kochan, G., Muniz, J. R., Wu, X., Gileadi, C., Ugocchukwu, E., Krysztosfinska, E., Gravel, R. A., Oppermann, U., and Yue, W. W. (2010) Structures of the human GTPase MMAA and vitamin B$_{12}$-dependent methylmalonyl-CoA mutase and insight into their complex formation. J. Biol. Chem. 285, 38204–38213
30. Chan, K. H., Li, T., Wong, C. O., and Wong, K. B. (2012) Structural basis for GTP-dependent dimerization of hydrogenase maturation factor HypB. PLoS One 7, e30547
31. Li, Q., and Cerione, R. A. (1997) Communication between switch II and switch III of the transducin $\alpha$ subunit is essential for target activation. J. Biol. Chem. 272, 21673–21676
32. Warner, D. R., Gejman, P. V., Collins, R. M., and Weinstein, L. S. (1997) A novel mutation adjacent to the switch III domain of Gs$\alpha$ in a patient with pseudohypoparathyroidism. Mol. Endocrinol. 11, 1718–1727
33. Natochin, M., Gasimov, K. G., and Artemyev, N. O. (2002) A GPR-protein interaction surface of Gi$\alpha$; implications for the mechanism of GDP-release inhibition. Biochemistry 41, 258–265
34. Day, P. W., Tesmer, J. J., Sterne-Marr, R., Freeman, L. C., Benovic, J. L., and Wedegaertner, P. B. (2004) Characterization of the GRK2 binding site of G$\alpha$. J. Biol. Chem. 279, 53643–53652
35. Scrima, A., Thomas, C., Deaconescu, D., and Wittinghofer, A. (2008) The Rap-RapGAP complex: GTP hydrolysis without catalytic glutamine and arginine residues. EMBO J. 27, 1145–1153
36. Seewald, M. I., Körner, C., Wittinghofer, A., and Vetter, I. R. (2002) RanGAP mediates GTP hydrolysis without an arginine finger. Nature 415, 662–666
37. Takahashi-Ijíuez, T., García-Arellano, H., Trujillo-Roldán, M. A., and Flores, M. E. (2011) Protection and reactivation of human methylmalonyl-CoA mutase by MMAA protein. Biochem. Biophys. Res. Commun. 404, 443–447
38. Padovani, D., and Banerjee, R. (2006) Alternative pathways for radical dissipation in an active site mutant of B$_{12}$-dependent methylmalonyl-CoA mutase. Biochemistry 45, 2951–2959
39. Lofgren, M., and Banerjee, R. (2011) Loss of allostery and coenzyme B$_{12}$ delivery by a pathogenic mutation in adenosyltransferase. Biochemistry 50, 5790–5798
40. Kabsch, W. (2010) XDS. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132
41. Kissinger, C. R., Gehlhaar, D. K., and Fogel, D. B. (1999) Rapid automated molecular replacement by evolutionary search. Acta Crystallogr. D Biol. Crystallogr. 55, 484–491
42. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. D Biol. Crystallogr. 53, 240–255
43. Collaborative Computational Project, Number 4 (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. 50, 760–763
44. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132
45. Davis, I. W., Leaver-Fay, A., Chen, V. B., Block, J. N., Kapral, G. J., Wang, X., Murray, L. W., Arendall, W. B., 3rd, Snoeyink, J., Richardson, J. S., and Richardson, D. C. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 35, W375–W383
46. DeLano, W. L. (2010) The PyMOL Molecular Graphics System, version 1.3r1, Schrödinger, LLC, New York
47. Wittinghofer, A. (1998) Signal transduction via Ras. Biol. Chem. 379, 933–937
48. Chen, Y., Yoo, B., Lee, J. B., Weng, G., and Iyengar, R. (2001) The signal transfer regions of G$\alpha$. J. Biol. Chem. 276, 45751–45754
49. Dempsey-Nunez, L., Illson, M. L., Kent, J., Huang, Q., Brenner, A., Watkins, D., Gilfix, B. M., Wittwer, C. T., and Rosenblatt, D. S. (2012) High resolution melting analysis of the MMAA gene in patients with cblA and in those with undiagnosed methylmalonic aciduria. Mol. Genet. Metab. 107, 363–367
50. Bohm, A., Gaudet, R., and Sigler, P. B. (1997) Structural aspects of heterotrimeric G-protein signaling. Curr. Opin. Biotechnol. 8, 480–487
51. Wittinghofer, F. (1998) Ras signalling. Caught in the act of the switch-on. Nature 394, 317, 319–320
52. Anand, B., Majumdar, S., and Prakash, B. (2013) Structural basis unifying diverse GTP hydrolysis mechanisms. Biochemistry 52, 1122–1130