G-PROTEIN SIGNALING

A switch saves B_{12} radical status

Vitamin B_{12}–dependent radical enzymes that can undergo irreversible inactivation during catalysis have their own chaperones for the maintenance of catalytic activities. New research defines a mobile loop in the G protein that determines chaperone function and defines a switch III motif akin to that found in classical G proteins.

Tetsuo Toraya

Vitamin B_{12}–dependent enzymes catalyze chemically challenging reactions by using one of two highly reactive species: a primary carbon radical from adenosylcobalamin (AdoCbl) or a super-nucleophilic Co(I) from methylcobalamin (MeCbl). Mammals have only two B_{12} enzymes: AdoCbl-dependent methylmalonyl-CoA mutase (MCM), which catalyzes the reversible isomerization between (*R*)-methylmalonyl-CoA and succinyl-CoA, and MeCbl-dependent methionine synthase, which transfers a methyl group from 5-methyltetrahydrofolate to homocysteine. Dysfunction of these enzymes causes serious diseases, with loss of MCM function causing methylmalonic aciduria and loss of methionine synthase resulting in accompanying homocystinuria[1]. Methylmalonic aciduria can also be caused by mutations in *cblA*, a locus encoding the mitochondrial protein methylmalonic aciduria type A (MMAA), which is implicated in the assembly of AdoCbl into MCM[2]. Previous research showed that the *Methylobacterium extorquens* ortholog of MMAA, MeaB, gates transfer of the AdoCbl cofactor from adenosyltransferase to MCM, protects MCM from oxidative inactivation and reactivates inactivated MCM by cofactor exchange[3]. Now Lofgren *et al.*[4] report structural determinants of this bidirectional signaling between MCM and MeaB, identifying a 'switch III' motif that had previously been defined only in heterotrimeric G proteins.

AdoCbl (**Fig. 1a**) and MeCbl, coenzyme forms of vitamin B_{12}, are organometallic compounds that have fascinated many investigators in various fields with their complicated structures and peculiar functions. AdoCbl-dependent enzymes catalyze intramolecular group-transfer reactions and ribonucleotide reduction using a radical derived from homolysis of the Co-C bond (**Fig. 1b**), whereas MeCbl-dependent enzymes specialize in intermolecular methyl transfer reactions using the Co(I) as a 'super-nucleophile'. Though these highly active species are

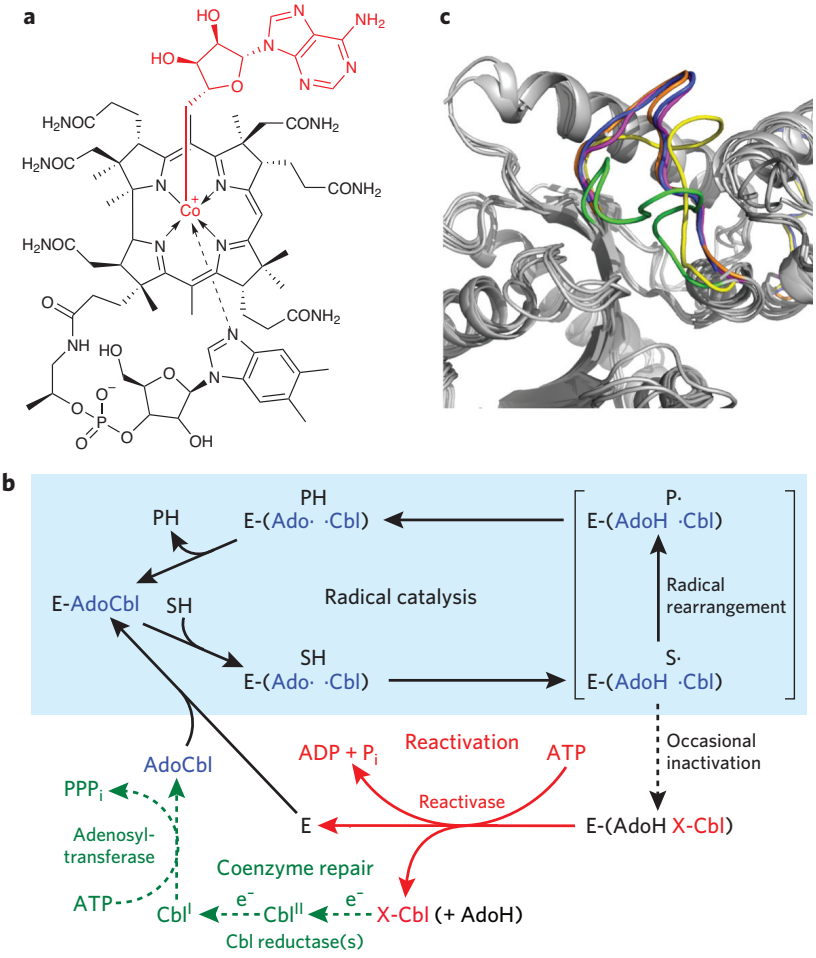

Figure 1 | Chaperones for AdoCbl-dependent radical enzymes. (**a**) AdoCbl or coenzyme B_{12}. The cobalt atom and the adenosyl group are in red. (**b**) Minimal mechanism and coenzyme recycling[5]. The interaction between the apoenzyme (E) and AdoCbl leads to the labilization of the Co-C bond for homolysis. The addition of substrate (SH) shifts the equilibrium in favor of homolysis, and an adenosyl radical (Ado•) abstracts a hydrogen atom from SH, producing a substrate-derived radical (S•) and 5′-deoxyadenosine (AdoH). S• rearranges to the product radical (P•), which then abstracts a hydrogen atom back from AdoH. This leads to the formation of product (PH) and regeneration of AdoCbl. When the holoenzyme undergoes inactivation during catalysis, the coenzyme loses the adenine moiety from its upper axial ligand. In the presence of ATP, reactivase releases the tightly bound, adenine-lacking cobalamin (damaged cofactor; X-Cbl) from the enzyme but not an adenine-containing cobalamin[5,6]. Apoenzyme reconstitutes holoenzyme with intact AdoCbl. X-Cbl is back-converted to AdoCbl by reductive adenosylation catalyzed by cobalamin (Cbl) reductase (or reductases) and adenosyltransferase. (**c**) Comparison of MeaB crystal structures (gray) and the mobile switch III region (colored) (reprinted with permission from Lofgren *et al.*[4]). Apo-MeaB (green), MeaB:2GMP-PNP (blue), MeaB:2GDP (yellow), MeaB:1GDP (magenta), MeaB:2P_i (orange).

necessary for the unusual reactions they catalyze, they are also prone to inactivation owing to the extinction of radical intermediates[5] or the oxidation of Co(I) to Co(II)[1]. How do living organisms address such inactivation?

Studies of microbial B_{12} enzymes have provided critical insights into this question, particularly diol dehydratase, glycerol dehydratase and ethanolamine ammonia-lyase, which catalyze AdoCbl-dependent isomerization reactions accompanying eliminations (**Fig. 1b**)[5]. When these enzymes are inactivated, damaged cofactors are tightly bound to apoenzymes and cannot be displaced by intact AdoCbl. However, inactivated holoenzymes can be rapidly reactivated in permeabilized cells (*in situ*) and *in vitro* by cofactor exchange through a reactivating factor or 'reactivase'[5]. These factors are chaperones with low ATPase activity that form tight complexes with apoenzymes in a nucleotide-dependent manner and release damaged cofactors from inactivated holoenzymes, thus forming apoenzymes that are reconstitutable into active holoenzymes (**Fig. 1b**)[6]. MeCbl regeneration proceeds through a very different pathway, using thioredoxin and thioredoxin reductase to donate an electron to Co(II) and accepting a new methyl group from *S*-adenosylmethionine.

The crystal structures of two reactivases revealed that the α subunits have a structural feature common to the ATPase domains of actin superfamily proteins including Hsp70 chaperones, whereas the β subunits have similar folds to the β subunits of cognate dehydratases[7]. As expected from the structures, subunit swapping actually occurs upon enzyme–chaperone complex formation[8], and a modeled structure suggests that induced steric repulsion between the subunits in the complex results in the release of damaged cofactors from inactivated holoenzymes[7,8]. However, the detailed nucleotide-switch mechanism by which the chaperones perform their biological functions was unknown. Similarly, it was unclear how these results would translate to MMAA and MeaB, which belong to the G3E family of phosphate-binding loop (P-loop) GTPases[9] and are thus structurally distinct.

Lofgren *et al.*[4] now investigate a new switch element of the G-protein chaperone in their report of crystal structures of MeaB in the apo form in the absence of phosphate and in the GMP-PNP (nonhydrolyzable analog of GTP)-bound form. By comparing these and previously reported structures in the apo form in the presence of phosphate and in the GDP-bound form[10], they identified a switch III region (in analogy to the corresponding region at the same position in heterotrimeric G-protein α subunit) that adopts substantially different conformations in the different structures and may be linked to the identity of the bound nucleotides in the GTP-binding site (**Fig. 1c**). To examine the role of this conformational flexibility in signaling, the authors introduced mutations into the switch III residues that are conserved in MeaB orthologs. Spectroscopic, kinetic and thermodynamic analysis of the mutants indicated that the switch III mutations suppressed the GTPase-activating function of MCM toward MeaB, rendered AdoCbl transfer from adenosyltransferase to the MCM–MeaB complex GTP independent and lowered protection against cofactor oxidation during MCM turnover and ejection of cob(II)alamin from MCM. From these results, the authors concluded that the switch III region of MeaB is required for bidirectional signaling between MeaB and MCM. Mutations corresponding to two patient mutations in human MMAA—also located within the loop—led to similar results, suggesting the importance of the switch III loop in the function of MMAA and in methylmalonic aciduria more generally.

The conclusion of Lofgren *et al.*[4] that the switch III region is important for MeaB signaling may be applicable to its mammalian and other bacterial orthologs, a possibility that should be tested. It will also be interesting to determine whether the GTPase chaperones and the reactivases are convergent in their 'gating'[3] or 'gatekeeping'[2] function for AdoCbl loading in addition to their similar reactivation activities, as suggested by their ability to discriminate between intact and damaged cofactors[3,5–8]. Finally, what happens after signaling? Further crystal structures of the MCM–MeaB and MCM–MMAA complexes in nucleotide-bound forms are eagerly awaited to define the next steps.

Tetsuo Toraya is at the Graduate School of Natural Science and Technology, Okayama University, Tsushima-naka, Kita-ku, Okayama, Japan.
e-mail: toraya@cc.okayama-u.ac.jp

Published online 21 July 2013
doi:10.1038/nchembio.1314

References

1. Froese, D.S. & Gravel, R.A. *Expert Rev. Mol. Med.* **12**, e37 (2010).
2. Froese, D.S. *et al. J. Biol. Chem.* **285**, 38204–38213 (2010).
3. Padovani, D. & Banerjee, R. *Proc. Natl. Acad. Sci. USA* **106**, 21567–21572 (2009).
4. Lofgren, M. *et al. Nat. Chem. Biol.* doi:10.1038/nchembio.1298 (21 July 2013)
5. Toraya, T. *Chem. Rev.* **103**, 2095–2127 (2003).
6. Mori, K. & Toraya, T. *Biochemistry* **38**, 13170–13178 (1999).
7. Shibata, N. *et al. Structure* **13**, 1745–1754 (2005).
8. Mori, K. *et al. FEBS J.* **277**, 4931–4943 (2010).
9. Leipe, D.D. *et al. J. Mol. Biol.* **317**, 41–72 (2002).
10. Hubbard, P.A. *et al. J. Biol. Chem.* **282**, 31308–31316 (2007).

Competing financial interests

The author declares no competing financial interests.