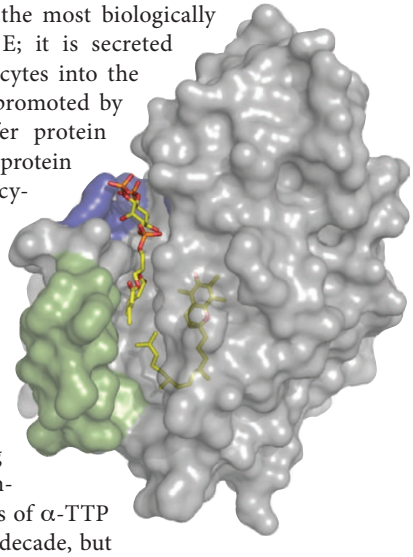
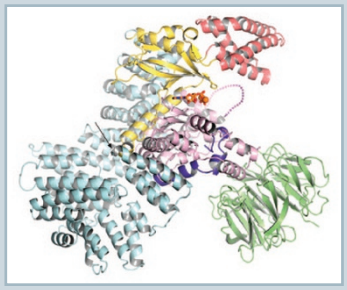
## Getting your vitamins—out



α-Tocopherol (α-toc) is the most biologically active form of vitamin E; it is secreted from mammalian hepatocytes into the plasma. This process is promoted by the α-tocopherol transfer protein (α-TTP), a lipid-binding protein thought to transfer endocytosed α-toc to the plasma membrane; a transporter would then secrete it into circulation. Mutations in α-TTP can result in ataxia with vitamin E deficiency (AVED), a genetic disorder characterized by low levels of circulating vitamin E and neurodegeneration. Crystal structures of α-TTP have been available for a decade, but its precise function in α-toc transport, as well as why some of the AVED mutations are deleterious, remained unclear. Now Arai and colleagues have used those mutants to obtain further insights into α-TTP function. First, they found that the disease-related R59W mutant could bind α-toc and mediate its intermembrane transfer between liposomes, but it could not facilitate α-toc secretion by hepatoma cells. In addition, the wild-type protein could bind two forms of phosphatidylinositol bisphosphate (PI(3,4)P2 and PI(4,5)P2, or PIPs) *in vitro*, both of which are concentrated in the plasma membrane, whereas the R59W mutant could not. The authors then solved the crystal structures of α-TTP in complex with α-toc and either one of the PIPs. The structures showed that the negatively charged head of the PIPs was bound to a positively charged cleft in α-TTP. Arg59 and other arginine residues mutated in AVED line the cleft and mediate contacts with PIPs. The new α-TTP structures also revealed that PIPs are bound close to the α-toc binding site, a hydrophobic groove with a lid. Interestingly, this lid appears in a more open conformation in the new structures, compared to a previous α-TTP–α-toc structure. This change in lid conformation suggests that PIP binding to α-TTP could induce the opening of the α-toc pocket and hence promote its transfer. In fact, the authors saw an increase in α-toc transfer by wild-type α-TTP when the acceptor liposome membrane contained PIPs; conversely, the presence of PIPs in the donor membrane inhibited α-toc transfer. In contrast, addition of PIPs had no effect on intermembrane transfer of α-toc by the R59W mutant. The authors propose that α-TTP loaded with α-toc interacts with PIPs in the plasma membrane; this would result in the opening of the α-toc binding-pocket lid and α-toc transfer to the plasma membrane. Thus, binding to PIPs would have a dual role in α-TTP function: targeting the protein to the plasma membrane and stimulating α-toc release. The new crystal structures would represent a transient intermediate form, in which both α-toc and PIP are bound. (*Science* http://dx.doi.org/10.1126/science.1233508, published online 18 April 2013) *IC*

## mTOR: restricted access



Mammalian target of rapamycin (mTOR) is an atypical protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family and is involved in controlling cell growth in response to environmental cues. It is a shared component of two distinct complexes, mTORC1 and mTORC2, that integrate signals from various cellular pathways and exhibit differential sensitivity to rapamycin. Though it was previously established that a complex between rapamycin and the protein FKBP12 inhibits mTOR by interacting with its FRB domain, the molecular mechanism involved has remained unclear. Now, Pavletich and colleagues provide unprecedented mechanistic insights into the activity and regulation of this key metabolic enzyme by solving the crystal structure of a 1,500-amino-acid fragment of mTOR comprising the FAT, FRB, kinase and FATC domains bound to mLST8, a component of mTORC1 and mTORC2. The mTOR–mLST8 complex exhibits a compact shape—with mLST8 and the FRB domain protruding from the kinase domain—and displays a well-ordered activation loop stabilized by FATC-domain interactions. Cocrystals of the same complex with $Mg^{2+}$ and ADP or with the ATP transition-state mimic $MgF_3^-$ reveal a catalytic-cleft conformation consistent with intrinsic activity in the absence of other mTORC components. Comparison with CDK2, also bound to $MgF_3^-$, indicates that mTOR uses a canonical protein-kinase catalytic mechanism rather than a lipid-kinase mechanism proposed for the primordial PI3K Vps34. The new structure also indicates that access to mTOR's catalytic cleft is partially occluded by the FRB domain, whereas a model constructed by overlaying the structure of the isolated FRB domain bound to rapamycin–FKBP12 suggests that a near-complete occlusion of the catalytic cleft is the basis for rapamycin-mediated inhibition. Several previously identified hyper-activating mutations map to elements seen to limit active site access, and this suggests that active site restriction is a key component of mTOR regulation. However, binding of the mTOR substrate S6K to the FRB domain is also shown to facilitate the phosphorylation of S6K by mTOR, a result indicating a function of the FRB domain in recruiting substrates to the active site. Given the central role of mTOR in regulating cell growth, and that its deregulation is often observed in disease states such as cancer and type 2 diabetes, mTOR represents a promising therapeutic target. By reporting the structures of mTOR–mLST8 bound to selective (Torin2 and PP242) or dual-specificity (PI-103) inhibitors, Pavletich and colleagues also provide a useful structural framework for understanding the potency and specificity of ATP-competitive compounds toward mTOR. (*Nature* **497**, 217–223, 2013) *SL*

*Written by Inês Chen & Stéphane Larochelle*