A Biosynthetic Enzyme Worms Its Way out of a Conserved Mechanism

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Like many promising drug targets, phosphoethanolamine methyltransferase is part of a pathway that is present in a pathogen but not in mammalian hosts. In this issue of *Structure*, Lee and Jez describe a structure of this phospholipid biosynthesis enzyme from a parasitic nematode and reveal a reconfigured active site.

Billions of the world's poorest people are afflicted by pathogenic nematodes, including some horrifying parasites. In communities of the Global North, the main reminder of the worm scourge is the need to give the family dog ivermectin to prevent heartworm. If heartworm parasites mature, the dog will need an arsenical drug not too different from Paul Ehrlich's Salvarsan, the first chemotherapeutic agent. Brutal side effects limit the medical use of arsenical drugs to the direst infections of the Global South, such as the sleeping sickness trypanosome, which cannot be cured any other way. Shared biochemistry makes it tough to target eukaryotic parasites; they are too similar to their eukaryotic hosts. To succeed, we must first identify significant differences and vulnerabilities in the parasite and then, as Ehrlich (1909) said, "wir müssen zielen lernen, chemisch zielen lernen!" ("we must learn to aim, learn to aim chemically!").

In this issue of *Structure*, Lee and Jez (2013) follow Ehrlich's advice in a study of phospholipid biosynthesis in the barber pole worm *Haemonchus contortus*. This gastrointestinal bloodsucker affects ruminant livestock, mainly sheep, and causes substantial economic losses worldwide. *H. contortus* is notoriously prolific and quick to develop resistance to antihelmintic drugs like ivermectin (Gilleard, 2006). New targets and new drugs are needed to keep pace with parasite resistance.

The Kennedy pathway (Figure 1) converts choline (Cho) into phosphatidylcholine (PtdCho), the most abundant phospholipid in eukaryotic membranes (Kent, 1995). An alternative route converts phosphatidylethanolamine to PtdCho using *S*-adenosylmethionine (SAM)-dependent methyltransferase (MT) reactions. Plants, as usual, make it from scratch. Phosphoethanolamine (pEA) is converted to phospho-Cho (pCho) by soluble pEA methyltransferases (PMTs). Plant-like PMTs are required for the growth of the malaria parasite *Plasmodium falciparum* and nematodes (Palavalli, et al., 2006; Witola, et al., 2008). Since the phosphobase methylation pathway is not present in humans, PMT inhibitors have the potential to be selective antiparasite drugs.

Protozoan PMTs perform all three phosphobase methylations using a single MT domain. In malaria PMT (PfPMT), the amine that attacks the electrophilic SAM methyl group is deprotonated by a tyrosine, which is in turn polarized by a histidine (Lee, et al., 2012). Curiously, the histidine may be the more important part of the catalytic dyad. Nematode PMT1 performs the first methylation (pEA to pMME), and PMT2 performs the other two methylations (pMME to pCho) (Lee, et al., 2011). Each PMT contains two MT domains, only one of which is functional. X-ray crystal structures show the same catalytic dyad is present in similar PfPMT and *H. contortus* PMT2 (HcPMT2) active sites. In addition, a single residue may enable HcPMT2 to reject pEA as a substrate. New enzymes evolve by gene duplication and diversification. If the ancestral form is a multispecific (promiscuous) enzyme, only a few mutations may be required for new functions to emerge (Khersonsky and Tawfik, 2010). We generally expect, however, that the

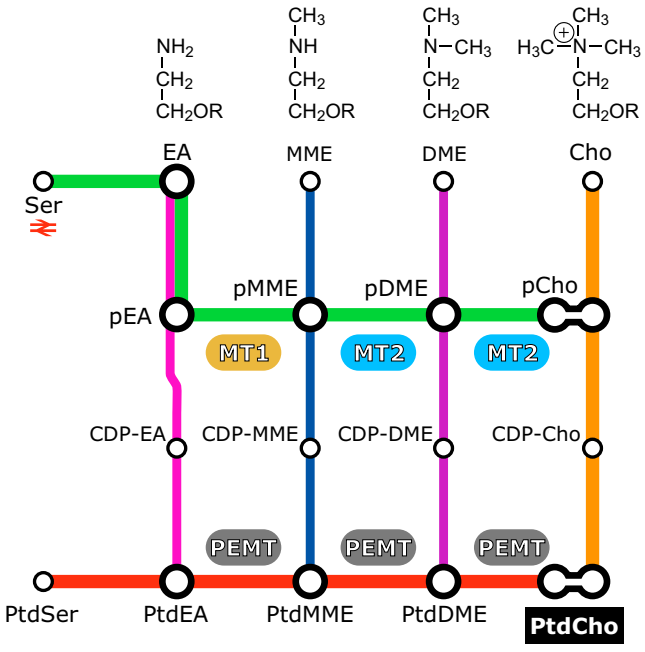

Figure 1. Phosphatidylcholine Biosynthesis in Eukaryotes
The Kennedy pathway (orange line), the primary source of human phosphatidylcholine (PtdCho), requires the essential nutrient choline (Cho). The Bremer-Greenberg pathway (red line) is an alternative route in which a membrane-bound pEA *N*-methyltransferase (PEMT) does all three methylations. Humans do not possess the phosphobase methyltransferase (MT) pathway (green line), which is used by plants, protozoa, and nematodes. The phosphobase methylation pathway relies on part of the Kennedy pathway.

basic active site layout and enzyme mechanism will be conserved.

Not so here. The surprise is that HcPMT1 has jettisoned the catalytic dyad entirely. The pEA phosphate, not histidine, activates the tyrosine general base by way of a water molecule. The tyrosine is provided by a different part of the MT domain, and it approaches the phosphobase amine from an orthogonal direction, relative to the substrates. Moreover, the switch to substrate-assisted catalysis clearly alters local electrostatics. If this doesn't amount to a genuinely new mechanism for carbon-nitrogen bond formation—the enzymological data that might settle the question are not in hand—it comes right up to that edge. At a minimum, the versatile MT domain is even more plastic than previously realized (Liscombe, et al., 2012).

Those who wish to inhibit phosphobase methylations in parasitic nematodes now know to look for more than one inhibitor. If binding site rigidity correlates with substrate specificity, it may be easier to find high-affinity ligands for HcPMT1 than the promiscuous PMTs.

Like all good science, the work by Lee and Jez (2013) raises questions without obvious answers, such as: "why muck with a perfectly good active site"? The HcPMT1 pEA k_{cat}/K_M is not tremendously higher than in promiscuous PMTs (Lee, et al., 2011). Tyrosine-to-phenylalanine mutants are nowhere near dead (Lee, et al., 2012; Lee and Jez, 2013); maybe it is enough to point an amine at the business end of SAM? If catalytic rationalizations are unsatisfying, we are left to ponder fanciful regulatory schemes. In that context, it may be relevant that both HcPMTs contain a "vestigial" MT domain that cannot bind SAM. Like a modern city built on its predecessor, it can be difficult to tell if the derelict twin is just a structural support or if it contains useful hidden infrastructure.

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REFERENCES

Ehrlich, P. (1909). Chem. Ber. *42*, 17–47.

Gilleard, J.S. (2006). Int. J. Parasitol. *36*, 1227–1239.

Kent, C. (1995). Annu. Rev. Biochem. *64*, 315–343.

Khersonsky, O., and Tawfik, D.S. (2010). Annu. Rev. Biochem. *79*, 471–505.

Lee, S.G., and Jez, J.M. (2013). Structure *21*, this issue, 1778–1787.

Lee, S.G., Haakenson, W., McCarter, J.P., Williams, D.J., Hresko, M.C., and Jez, J.M. (2011). J. Biol. Chem. *286*, 38060–38068.

Lee, S.G., Kim, Y., Alpert, T.D., Nagata, A., and Jez, J.M. (2012). J. Biol. Chem. *287*, 1426–1434.

Liscombe, D.K., Louie, G.V., and Noel, J.P. (2012). Nat. Prod. Rep. *29*, 1238–1250.

Palavalli, L.H., Brendza, K.M., Haakenson, W., Cahoon, R.E., McLaird, M., Hicks, L.M., McCarter, J.P., Williams, D.J., Hresko, M.C., and Jez, J.M. (2006). Biochemistry *45*, 6056–6065.

Witola, W.H., El Bissati, K., Pessi, G., Xie, C., Roepe, P.D., and Mamoun, C.B. (2008). J. Biol. Chem. *283*, 27636–27643.

The Architecture of Restriction Enzymes

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In this issue of *Structure*, Lyumkis and colleagues describe a high resolution structure of a polymerized form of the SgrAI restriction enzyme, which shows that it forms a helical assembly with four enzyme molecules per turn of the helix. The DNA is arranged on the periphery of the protein helix pointing away from the helical axis.

Classically, a restriction endonuclease (REase) or restriction enzyme is defined by the nature of its DNA recognition sequence and where the enzyme cleaves DNA containing the recognition sequence (Roberts et al., 2003). The most widely used REases recognize short palindromic sequences and cleave at the sequence to generate blunt or sticky ends suitable for further manipulations. These simple REases are in the Type IIP class, and their structures showed a simple homodimer, with each monomer recognizing half of the recognition sequence (see REBASE for links to structures; Roberts et al., 2010). This perception of REases as homodimers dominates textbook descriptions and the common way of thinking about REases.

However, since the first atomic structures, REases have been found to show a very large number of different architectures. REases have been found as monomers, homo-dimers and hetero-dimers, hetero-trimers, homo-tetramers and hetero-tetramers, hetero-pentamers, and even tetradecamers (see REBASE for links to structures; Roberts et al., 2010). These complexes may not even be the most active form of the REase. Many REases show optimal activity only when two or more copies of the recognition sequence are present, indicating that higher order complexes form when the enzymes bind to DNA (e.g., Smith et al., 2013). Imaging techniques have shown many examples of loops of DNA being formed by these higher order REase structures (e.g., Shlyakhtenko et al., 2007).

Nancy Horton and her colleagues have been studying the SgrAI REase from

