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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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

Streptomyces griseus for a number of years, and, in this issue of *Structure*, show that the normally dimeric REase forms large polymeric cylindrical structures when DNA is present (Lyumkis et al., 2013). The initial atomic structure of SgrAI showed a common homodimeric structure bound to a single copy of the recognition sequence (Dunten et al., 2008). This sort of single-site substrate does not give full enzyme activity; full activity was only achieved in the presence of at least two copies of the recognition sequence. This led to a suggestion that SgrAI might form a tetramer on DNA (Dunten et al., 2008), as observed for several other REases (see REBASE for links to structures; Roberts et al., 2010). This would lead to loops of DNA on multi-site DNA substrates as long as the sites were sufficiently well separated to allow the inherently stiff DNA duplex to bend back on itself.

The current work reveals an extraordinary oligomerization of the enzyme when short DNA duplexes are present (Lyumkis et al., 2013). A range of oligomeric forms are observed, with each enzyme dimer interacting with neighboring enzymes to form a cylinder with enzymes spiralling around the cylinder axis in a helical manner. Four enzymes are present in each turn of the helix, and each enzyme has a bound DNA duplex, both ends of which project away from the cylinder. The ends of neighboring duplexes are close together, but in an orientation that makes it difficult to imagine them as capable of being directly linked by the addition of a few more base pairs. However, if the recognition sites were well spaced on a long piece of DNA, then the observed enzyme cylinder would have multiple loops of DNA projecting from it (Figure 1).

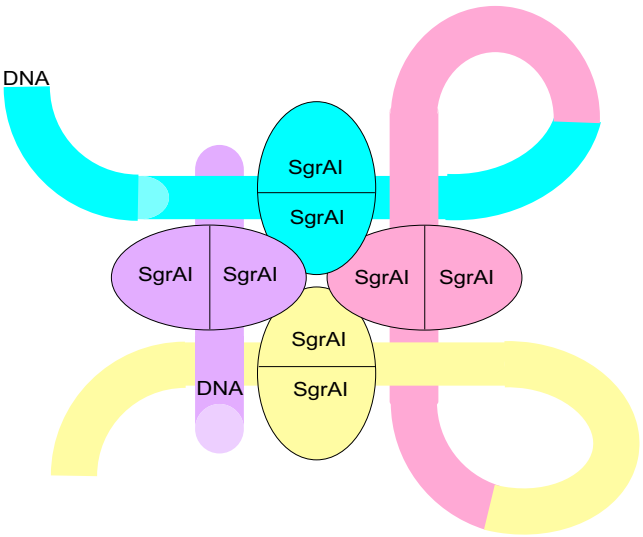

Figure 1. A Schematic View down the Helical Axis of the SgrAI-DNA Assembly
Each SgrAI dimer is shown as a shaded oval, and there are four enzymes in each helical turn of the assembly. The DNA segments bound to each enzyme are shown in the same color as the enzyme. The DNA has been extended between the yellow and pink enzymes, and the pink and cyan enzymes to join together to form loops. Other linkages of the DNA between the enzymes are possible and only limited by the flexibility of the DNA duplex and the distance between SgrAI recognition sites on the DNA.

What is the meaning of this unusual assembly? Of course, it might just be a bizarre artifact due to experimental conditions, but this would seem unlikely as the enzyme shows, like many other REases, a robust requirement for at least two recognition sites for optimal DNA cleavage activity. The requirement for two sites for optimal cleavage would suggest that a tetramer would be sufficient. The larger oligomers observed might, therefore, just be an evolutionary accident and not essential for activity. This would be plausible if the oligomers had no detrimental effect on the wellbeing of the host cell. However, if we assume that the assembly is neither an artifact nor an evolutionary accident, then this REase can not only cleave DNA but can also compact DNA into a small volume. This compaction may have some use for the host organism. It has long been speculated that the DNA fragments produced by REases in vivo can be used as substrates for recombination (see e.g., Maldonado-Contreras et al., 2013). Having a large number of such fragments in close proximity, and perhaps also in close proximity to recombination machinery, in the cell might be advantageous in evolutionary terms.

Lastly the structure revealed here indicates that the thousands of known REases, of which hundreds can be simply purchased, are likely to show a wide range of unusual and unexpected structural features (Roberts et al., 2010). They are not just the simple tools of molecular biology, but also marvelous architectural structures.

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