ANTIBACTERIALS

Clamping down on *Mtb*

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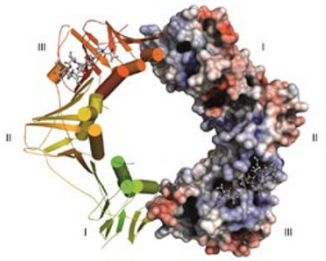

SCIENCE

Natural products derived from bacteria have served as a rich source for antibacterial lead compounds. For instance, griselimycin (GM) is a cyclic peptide isolated from *Streptomyces* strains that has antibacterial activity against *Mycobacterium* species, such as the tuberculosis-causing *Mycobacterium tuberculosis* (*Mtb*), including strains that are resistant to known drugs. Given the need to identify therapeutics for *Mtb* infection, Kling *et al.* focused on generating GM analogs with improved potency, metabolic stability and safety profile. They first defined a total synthesis route to GM and, in this process, identified cyclohexyl-griselimycin (CGM) as having similar activity against a range of *Mtb* strains *in vitro*, including strains that are mono-resistant to first- and second-line antituberculosis drugs. CGM had good ADME (drug-like) properties and was bactericidal in both acute and chronic tuberculosis (TB) mouse models of infection. Treatment with a combination of CGM, rifampicin and pyrazinamide was more effective in the acute model than the commonly used drug combination of rifampicin, pyrazinamide and isoniazid, suggesting a novel mechanism of action for CGM. To determine the target of CGM, the authors first explored a *Streptomyces* GM biosynthetic gene cluster and found a homolog of *dnaN*, which encodes the sliding clamp of DNA polymerase. Overexpressing *dnaN* increased resistance to GM but not to any other anti-TB drug. Likewise, GM-resistant strains isolated from *Mycobacterim smegmatis* and from an *Mtb in vivo* infection model had amplified DnaN expression. *In vitro* binding assays confirmed binding of GM and CGM to DnaN from *M. smegmatis*, *Mtb* and *E. coli*, and co-crystal structures helped define the binding sites. The authors also observed induction of the SOS response, indicative of DNA strand breaks in GM-exposed *M. smegmatis*, suggesting that clamp action in replication is inhibited by GM in cells. Even though resistance occurred with CGM, this was accompanied by considerable fitness costs, suggesting that targeting *dnaN* would be an effective TB treatment. *MB*

PROTEIN REGULATION

Inteins under wraps

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Inteins are self-splicing elements that excise themselves from translated proteins to leave behind the extein, or mature protein. The majority of functionally relevant residues are thought to be internal to the intein, though a few residues in the extein sequence directly bordering the intein have been shown to influence function. Some inteins are known to be conditionally regulated, but most documented cases have focused on intein-specific mechanisms, and it is not known whether exteins could have a larger role in regulating splicing. To investigate this question, Topilina *et al.* considered the RadA/RecA family of recombinases, in which the intein is inserted in one of five points—two newly reported in this work—either at monomer-monomer interfaces or within the ATP-binding site. Comparison of the intein from the hyperthermophile *Pyrococcus horikoshii* RadA in its native site and within an engineered MBP-GFP construct demonstrated that the MBP-GFP product formed very efficiently, whereas the native construct showed no splicing at 25 °C even after several days. Splicing activity could be increased either by raising the temperature or by adding an ionic liquid and detergent; splicing was also coincident with changes in protein structure, as monitored by CD, but not in ATPase activity, suggesting that the flexibility of the extein was moderating intein splicing. A model of the unspliced RadA pointed to several charged residues in the extein in close proximity physically, but not sequentially, to functionally important intein residues; mutation of these residues, but not charged residues elsewhere in the sequence, led to substantial increases in splicing. These combined results led to a model in which the extein-intein interaction at low temperatures prevents intein splicing, while temperatures akin to those in the organism's native environment release these contacts to promote splicing and RadA activation. The authors speculate that this regulation may help prevent ATP consumption under stressful conditions. *CG*

LIPID METABOLISM

Working out SCD's kinks

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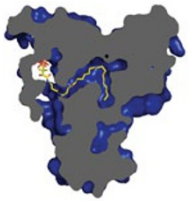

NSMB

Stearoyl-CoA desaturase (SCD) is a metabolic enzyme embedded in endoplasmic reticulum membranes that acts on saturated fatty acyl–CoAs to generate the early precursor to unsaturated cellular lipids. SCD activity is key to maintaining the balance between levels of saturated and monounsaturated lipids, and preclinical studies have shown that a reduction in SCD activity can improve lipid metabolic profiles. As such, drug-based inhibition of SCD activity could be indicated for the treatment of metabolic conditions such as obesity and diabetes. To provide further insight into SCD's mechanism of action—and potentially pave the way to the rational design of specific inhibitors—Wang *et al.* and Bai *et al.* simultaneously reported the crystal structures of human and mouse SCD1, respectively, bound to its substrate stearoyl–coenzyme A. The structures reveal an overall fold that Wang *et al.* describe as mushroom-like in which four transmembrane helices form a V-shaped stem, with the rest of the polypeptide forming a cytoplasmic cap. The studies highlight two key features of SCD1: a catalytic center where two zinc ions (likely to be iron in the native enzyme) are found to be coordinated mainly by histidine residues, and a tunnel lined with hydrophobic residues that contains the acyl tail of the stearoyl-CoA molecule. This hydrophobic tunnel displays a sharp kink in the vicinity of the two catalytic metals that is also located precisely where a *cis* double bond is introduced by SCD1 in the substrate acyl chain. The observed enzyme-substrates interactions and consequent substrate orientation also suggest a basis for both regioselectivity and stereoselectivity of the reaction, as the bond subject to desaturation is forced to adopt the *cis* conformation within the tunnel. Bai *et al.* additionally suggest a path for substrate exit and how cytochrome b5—the electron donor in the reaction—likely interacts with SCD1 during catalysis. These new insights should provide context for the further development of SCD1 inhibitors and potentially the treatment of metabolic disease. *SL*

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