LEUKEMIA

Free RUNX1

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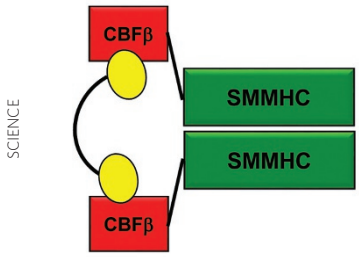

SCIENCE

During hematopoiesis, a heterodimer consisting of core binding factor β (CBFβ) and the transcription factor RUNX1 directly activates the transcription of genes such as *RUNX3* and *CEBPA* that are required for myeloid and lymphoid differentiation. A chromosome 16 inversion found in a subtype of acute myeloid leukemia (AML) cases called inv(16) produces a fusion protein containing CBFβ and smooth muscle myosin heavy chain (CBFβ-SMMHC). This fusion protein binds RUNX1 with high affinity and sequesters it away from its target genes, preventing RUNX1-mediated gene activation. Although chemotherapeutics have been somewhat effective in treating inv(16) clinical cases, there is a strong focus to develop strategies that specifically target the CBFβ-SMHHC fusion protein while sparing wild-type CBFβ activity. Illendula *et al.* used a FRET assay with a Venus-tagged CBFβ-SMMHC and a Cerulean-tagged RUNX1 Runt domain to screen a small-molecule collection for modulators of CBFβ-SMHHC-RUNX1 protein-protein interaction. They identified a lead compound, AI-4-57, that bound specifically to CBFβ and promoted the dissociation of RUNX1 from CBFβ-SMMHC. Considering that the CBFβ-SMMHC fusion protein is oligomeric whereas CBFβ is not, the authors modified their lead compound for potential therapeutic use to produce a bivalent compound, AI-10-49, containing a seven-atom polyethylene glycol-based linker and a trifluoromethoxy substitution that exhibited good *in vivo* pharmacokinetics, selectivity and potency. This compound released RUNX1 from CBFβ-SMMHC selectively, allowing RUNX1 to bind target genes such as *RUNX3* and *CEBPA* and restore gene expression. Mice transplanted with CBFβ-SMMHC leukemic cells exhibited longer survival times when given AI-10-49 with no overt signs of drug toxicity. AI-10-49 treatment reduced the viability and colony-forming ability of human inv(16) leukemia primary cell lines while sparing non-inv(16) and normal bone marrow cells. Overall, these findings validate the disruption of RUNX1-CBFβ-SMMHC protein-protein interactions as a new therapeutic strategy for the treatment of inv(16) AML. *GM*

LIGATION REACTIONS

All about that rate

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Chemoselective ligation reactions, bond-forming reactions that proceed in the presence of diverse, unprotected functional groups, are essential for multiple applications in chemical biology. For reactions that detect or isolate a specific biomolecule, one reagent can be used in large excess. If, however, they are to be used to couple two large molecules, the rate of ligation becomes very important. The high M_w of typical proteins means that, when dissolved, the concentrations are much smaller than for small molecules. High conversions are a prerequisite for effective ligation reactions and, at a typical concentration of 10 μM, this requires a second-order rate constant >1 M^{-1} s^{-1} and ideally >10 M^{-1} s^{-1}. Saito *et al.* have now evaluated the rates and other important factors in the application of eight well-known ligation reactions. They designed substrates that all contain the typical functionalities present in peptides—thiols, carboxylic acids and amine side chains—and vary only in the pendant groups necessary for ligation. Rate constants were measured in 1:1 mixtures of the components at micromolar concentrations; the reactions were followed using the absorbance of a UV tag after separation by HPLC. In addition to the rate, the stability of both the starting materials and the ligated products was evaluated. The highest rate (734 M^{-1} s^{-1}) was observed for a thiol-maleimide ligation, but the stability of some reagents could be an issue. The copper-catalyzed azide-alkyne cycloaddition was reasonably fast (3.4 M^{-1} s^{-1}), though oxidation of thiols and coordination of amines or thiols to copper can pose problems. The strain-promoted version offers a lower rate, but the simple conditions are advantageous. The potassium acyltrifluoroborate ligation offers a good reaction rate (22 M^{-1} s^{-1}) as well as good stability of both starting materials and products. By studying these reactions using realistic substrates and under realistic conditions, Saito *et al.* have produced a primer that should be valuable to those wishing to apply them. *SD*

BIOCATALYSIS

Statins in one step

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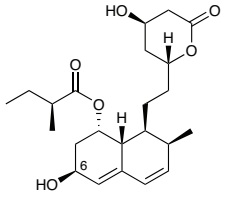

The cholesterol-lowering drug pravastatin is produced from the natural product compactin by insertion of a hydroxyl group at the 6 position. Pravastatin is currently produced in a two-step process in which compactin is collected from *Penicillium citrinum*, a native producer, and then treated with *Streptomyces carbophilus*, which contains a cytochrome P450 enzyme that inserts the hydroxyl group. However, McLean *et al.* envisioned that a redesigned process could improve pravastatin yields. To accomplish this, the authors first tested whether *Penicillium chrysogenum*—already optimized as a bacterial chassis for penicillin production—could be used to make other compounds. Introduction of the compactin biosynthetic pathway led to the facile production of >400 mg l^{-1} compactin in shake flasks, more than 20 times the yield of *P. citrinum*. The authors initially observed the loss of the ester side chain for much of their material, but they identified a single esterase as responsible for cleavage and restored compound production through deletion of the gene. The *S. carbophilus* P450 could not be expressed in an active form in *P. citrinum*, but a proficient P450 catalyst from *Amycolatopsis orientalis* was found. However, the product of the new enzyme had inverted stereochemistry at the 6 position. To fix this, the authors combined structural analysis with error-prone PCR to identify $P450_{Prava}$, containing only five mutations but with an altered 96:4 preference to make the desired stereoisomer. A crystal structure of the mutant suggested that most of the mutated residues changed the shape of the active site to promote substrate binding in the desired orientation. Inclusion of $P450_{Prava}$ in the *P. chrysogenum* strain led to 6 g l^{-1} of pravastatin in batch fermentations, twice the amount of the current industrial process. In addition to offering an improved route to this important drug, this paper highlights the utility of repurposing optimized chassis for biotechnological purposes. *CG*

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