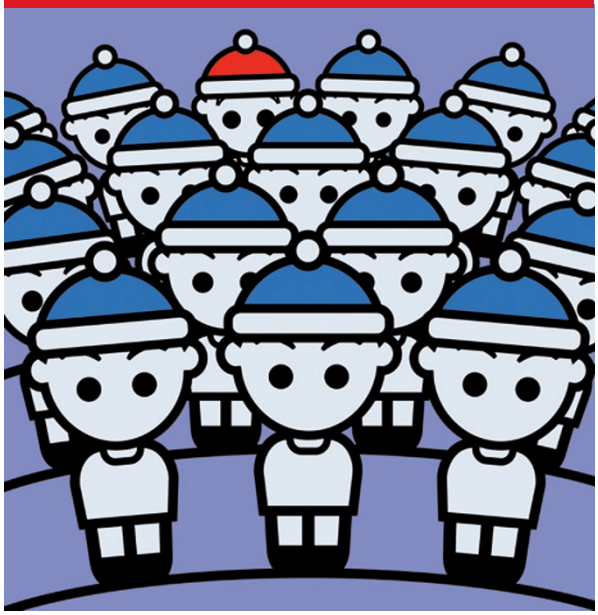
ANTIBIOTICS

Spot the difference



Publishing in *Chemistry & Biology* Truman *et al*. have identified a single amino acid substitution in the active site of the protein Cep15, which is part of the glycopeptide antibiotic chloroeremomycin biosynthetic machinery in *Amycolatopsis orientalis*, that abolishes its catalytic activity. Reversing a point mutation in the *cep15* gene produced a functional enzyme. This finding represents the first time that the function of a bacterial pseudogene has been restored by a point mutation.

Glycopeptides, including teicoplanin and vancomycin, are used to treat patients with multidrug-resistant bacterial infections. Teicoplanin has a glucosaminyl group that results from glycosyl transfer and modification by a COG2120 domain deacetylase. Other glycopeptides, such as chloroeremomycin and balhimycin, lack a glucosaminyl group, although the gene clusters that code for these antibiotics still contain a COG2120 domain deacetylase gene. Truman and colleagues focused on the COG2120 domain protein of the chloroeremomycin cluster, Cep15, to pin down its catalytic function.

Previous reports had assigned either a nucleotidyltransferase function or an *N*-acetylglucosaminyl deacetylase function to Cep15, but Truman *et al*. found that purified Cep15 has neither of these functions and proposed that *cep15* is a non-functional pseudogene. The *cep15* homologue in the teicoplanin cluster was shown to encode a functional deacetylase, whereas the *cep15* homologue in the balhimycin cluster was a pseudogene. Inspection of

> “Reversing a point mutation… produced a functional enzyme.”

sequence alignments revealed that an active-site histidine (His164) had been replaced with an asparagine in the non-functional deacetylases. On the basis of crystal structures of related eukaryotic COG2120 proteins, the same histidine was identified as a ligand for zinc, a cofactor that is essential for catalytic activity. Mutating the *cep15* Asn164 to His164 restored deacetylase activity. This confirmed that the active-site His164 has a pivotal role in the function of these COG2120 enzymes. Pseudogenes are usually removed from streamlined bacterial genomes, so the authors speculate that *cep15* might have an as-yet-unidentified role.

Reactivation of minimally deactivated genes could provide a new source of enzymatic activities for antibiotic engineering.

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ORIGINAL RESEARCH PAPER Truman, A. W. *et al.* The role of Cep15 in the biosynthesis of chloroeremomycin: reactivation of an ancestral catalytic function. *Chem. Biol.* **15**, 476–484 (2008)