Finetuning pristinamycin synthesis in streptomycetes

Sir David Hopwood

The report in this issue by Sezonov et al.[1] will bring joy to the hearts of *Streptomyces* geneticists who have been frustrated by the dearth of quotable examples of the application of their craft to solve practical problems. In a collaboration between a group at the University of Paris-Sud and scientists at the Rhône-Poulenc Rorer company in Paris, genetic engineering has been used to overcome a metabolic bottleneck in the biosynthesis of a valuable antibiotic. Stated so simply, this may sound banal. Not at all. The beauty of the work lies in the way in which many separate strands of biochemical and genetic knowledge and technology have been brought together to achieve this objective.

Pristinamycin, a product of *Streptomyces pristinaespiralis*, is a member of an amazing family of streptomycete antibiotics, each of which consists of two distinctly different chemical classes of components. Both these components are ring-like structures ("macrocyclic lactones") assembled from small building units: In the case of pristinamycin, pristinamycin I (PI) comes from six amino acid residues and pristinamycin II (PII) from a mixture of amino acid and acetate units. PI and PII each inhibit the growth of bacteria without killing them, but together they kill bacteria—including the dreaded *Staphylococcus* and *Streptococcus*—at low concentration. The only problem has been the poor water solubility of pristinamycin; so recently a new, soluble, and therefore injectable, derivative was developed[2]. Its PII component was made by chemical modification of naturally produced PII, but this presented another problem—the natural PII is a mixture of the desired substrate for chemical modification, PIIA, and its unwanted precursor, PIIB. Genetic engineering came to the rescue.

One of the dramatic successes of *Streptomyces* genetics over the past decade has been the cloning and understanding of the complex sets of genes that control the biosynthesis of antibiotics. Each set may consist of several tens of genes, but luckily they have always been found to be clustered closely together, and so can be cloned with comparative ease[3]. The Rhône-Poulenc Rorer group has found at least the majority of those needed to make pristinamycin, including the conversion of PIIB to PIIA. This process occurs by a relatively complex oxidation reaction catalyzed by a heterodimer composed of two proteins, *SnaA* and *SnaB*, using molecular oxygen and reduced flavin mononucleotide[4]. The challenge was to overproduce *SnaA* and *SnaB* and thereby to convert all of the PIIB to PIIA.

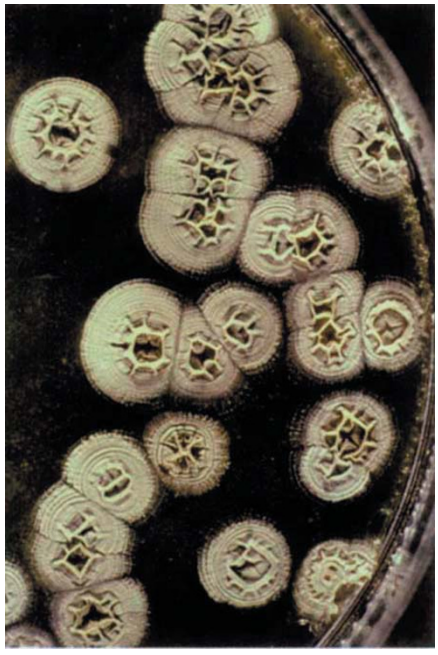

Figure 1. Colonial growth of *Streptomyces pristinaespiralis*, the natural source of pristinamycin.

Because replicating plasmid vectors in *Streptomyces* often depress antibiotic productivity[5], the French group decided to introduce extra copies of the *snaA* and *snaB* genes on an integrating vector that can maintain itself stably at single-copy number in the host chromosome. For this purpose, they used pSAM2, previously studied at the University of Paris-Sud. This plasmid is a member of a fascinating class of *Streptomyces* genetic elements that can exist as conjugative plasmids, but can also integrate into the chromosome by phage-like site-specific recombination events. Integration occurs, not into a dedicated attachment site, like the famous lambda phage of *Escherichia coli*, but into a tRNA gene; in this example, a proline tRNA gene. The advantage is that the elements have a wide host range, because tRNA sequences are highly conserved.

In order to make the engineered strain, the *snaA* and *snaB* genes were cloned in a pSAM2 derivative that could integrate into its tRNA site, but could not excise again because it was disabled in the normal excision function: 100% stability (and a degree of biological containment) was thereby achieved. The *snaA* and *snaB* genes were coexpressed in equimolar amounts from a strong constitutive promoter, *ermE**, another item from the *Streptomyces* genetics toolkit[6]. The result was a strain that made just as much total PII as the starting strain, but all of it was PIIA, and this was achieved "in conditions close to industrial fermentation, including the type of medium, the incubation time, precultural growth, and absence of selective pressure." This last point is of course crucial: It would be unacceptable to have to maintain an antibiotic selection for the vector during large-scale fermentation.

Recently, the goal of using genetic engineering in streptomycetes and their actinomycete relatives to make useful novel compounds has been brought several steps nearer, with the development of the technology to generate libraries of "unnatural natural products"[7,8].

In some ways, cloning for productivity increases is a more complex challenge because the production of metabolites like antibiotics, in contrast to the biosynthesis of a single useful protein, depends on many genes. These include those controlling not only a multistep biosynthetic pathway, but also the components of a complex regulatory network[9]. The present example, and another in which tylosin productivity was improved[5], highlights how a judicious choice of target can provide an opportunity for using some elegant molecular biology to address a productivity bottleneck and thus meet a biotechnological challenge.

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1. Sezonov, G., Blanc, V., Bamas-Jacques, N., Friedmann, A., Pernodet, J.-L., and Guérineau, M. 1997. *Nature Biotechnology* **15**:349–353.
2. Barrière, J.C., Bouanchaud, D.H., Paris, J.M., Rolin, O., Harris, N.V., Smith, C. 1992. *J. Antimicrob. Chemother.* (Suppl. A) **30**:1–8.
3. Vining, L.C. and Stuttard, C. Eds. 1995. *Genetics and Biochemistry of Antibiotic Production*. Butterworth-Heinemann, Newton, MA.
4. Blanc, V. et al. 1995. J. Bacteriol. 177:5206–5214.
5. Baltz, R.H., McHenney, M.A., Cantwell, C.A., Queener, S.W., and Solenberg, P.J. 1997. *Antonie van Leeuwenhoek* **71**:179–187.
6. Bibb, M.J., White, J., Ward, J.M., and Janssen, G.R. 1994. *Mol. Microbiol.* **14**:533–545.
7. McDaniel, R., Ebert-Khosla, S., Hopwood, D.A., and Khosla, C. 1995. *Nature* **375**:549–554.
8. Oliynyk, M., Brown, M.J.B., Cortés, J., Staunton, J., and Leadlay, P.F. 1996. *Chem. Biol.* **3**:833–839.
9. Bibb, M.J. 1996. *Microbiology* **142**:1335–1344.