Chemistry & Biology
Previews

Better Chemistry through Regulation

Justin R. Nodwell[1,2,*] and Leslie Cuthbertson[1,2]
[1]Michael DeGroote Institute for Infectious Diseases Research
[2]Department of Biochemistry and Biomedical Sciences
McMaster University, Hamilton, Ontario L8N 3Z5, Canada
*Correspondence: nodwellj@mcmaster.ca
DOI 10.1016/j.chembiol.2011.12.001

Finding ways to increase the biosynthesis of medically important microbial secondary metabolites is a challenge of microbial chemical biology. Lechner et al. (in this issue of *Chemistry & Biology*) show that transcriptional regulation can be manipulated to selectively increase the production of a desired metabolite.

Without actinomycetes, medicine would not be what it is today. As a part of their ''secondary'' or nonessential metabolism actinomycetes produce small molecules that have an extraordinary range of biological activities. These metabolites are used as anticancer agents, immune suppressants, and most famously, as antibiotics. The majority of secondary metabolites are polyketides, nonribosomally derived peptides, or combinations thereof. Their biosynthetic pathways often require dozens of enzymes and are encoded in discrete genomic islands. The genes for the core synthases can be easily identified by sequence homology. Genome sequencing has revealed that actinomycete genomes encode many more secondary metabolites than originally anticipated based on prior screening efforts—some harbor close to 40 distinct clusters (Nett et al., 2009). In the laboratory, many of these molecules are produced at very low levels and finding ways to activate their synthesis is an important area of ongoing research.

Most secondary metabolite biosynthetic gene clusters encode regulatory proteins that control the transcription of the biosynthetic and/or self-resistance genes. Their regulation can be very complex: the biosynthetic gene cluster for pristinamycin IA and IIA for example, a massive brute weighing in at 210 kbp, encodes seven DNA binding transcription factors (Mast et al., 2011). In addition to pathway specific regulators, pleiotropically acting regulators, typically encoded outside biosynthetic clusters, control multiple metabolites. It is likely that a regulatory network consisting of both pleiotropic and pathway-specific regulators explains the feeble production of so many secondary metabolites under laboratory conditions.

Manipulating the regulation of secondary metabolism is an attractive means of enhancing the yields of poorly expressed molecules. Overexpressing pathway-specific regulators has been used to enhance yields of known molecules (Stutzman-Engwall et al., 1992) and to activate ''silent'' gene clusters and identify new molecules (Laureti et al., 2011). Secondary metabolite yields have also been enhanced through the expression of pleiotropic regulators (McKenzie et al., 2010). Technologies that take advantage of regulatory mechanisms can complement other strategies that have been developed for small molecule discovery (Gomez-Escribano and Bibb, 2011; Hosaka et al., 2009; Komatsu et al., 2010).

Lechner et al. (2011 [in this issue of *Chemistry & Biology*]) report a new twist on the use of regulatory genes to manipulate secondary metabolism—the use of a pathway-specific regulator to manipulate the structure of a secondary metabolite. Many biosynthetic pathways produce mixtures of related molecules rather than a single species. These analogs can have different biochemical activities but their shared chemical properties can make purification of individual species a substantial challenge. Salinosporamide A is a covalent inhibitor of the 20S proteasome that is in clinical trials for cancer therapy. It is a chlorinated molecule produced by the actinomycete *Salinispora tropica* along with several related but unchlorinated molecules (Figure 1). Chlorination is required for covalent inhibition of the 20S proteasome by salinosporamide A and nonhalogenated derivatives are not clinically useful as proteasome inhibitors. Biosynthesis of salinosporamide A requires the precursor chloroethylmalonyl-CoA, produced by a subpathway of eight enzymes encoded in the *sal* gene cluster (Eustáquio et al., 2009). It is this precursor that ensures chlorination of the final biosynthetic product. In contrast, other salinosporamide derivatives are synthesized from precursors drawn directly from primary metabolism (Figure 1).

The *sal* biosynthetic gene cluster encodes three putative transcription factors including the LuxR-like protein SalR2. Lechner and colleagues show that SalR2 binds and activates the promoters of *salL*, *salM*, and *salN* in addition to exerting an autoregulatory effect on its own promoter. SalL, SalM, and SalN are required for three of the earliest steps in chloroethylmalonyl-CoA biosynthesis. Deletion of *salR2* eliminates the biosynthesis of chloroethylmalonyl-CoA and hence salinosporamide A, but has no impact on nonhalogenated salinosporamide derivatives. By placing *salR2* under the control of a constitutive promoter, the authors were able to double the yields of salinosporamide A without affecting production of other nonhalogenated species. Most pathway specific activators are thought to regulate entire biosynthetic pathways rather than precursor subpathways. The data presented by Lechner et al. (2011) clearly shows that subpathways can be regulated independently of the core synthase genes.

SalR2 is an atypical response regulator lacking the conserved residues required for phosphorylation. How the expression and activity of atypical response regulators like SalR2 are regulated remains an open question. It has been suggested that the activities of similar transcription factors are regulated by the final products

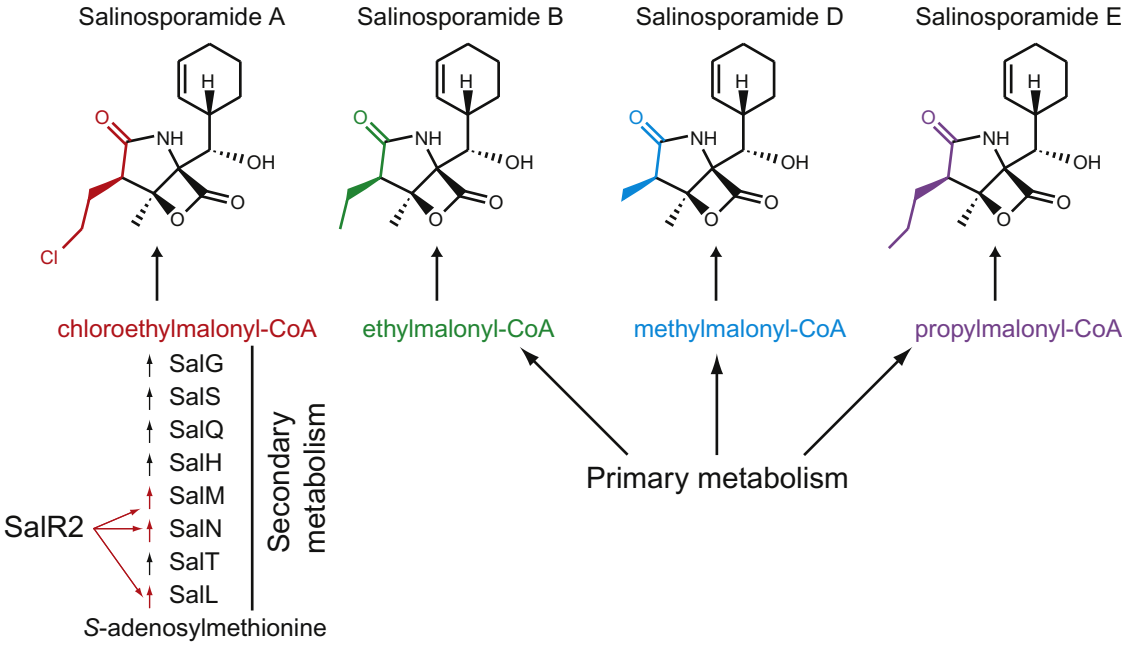

Figure 1. SalR2 Regulates a Precursor Subpathway Required for Salinosporamide A Biosynthesis
The precursors required for the biosynthesis of other salinosporamide derivatives are derived directly from primary metabolism.

of the biosynthetic gene clusters in which they are encoded. However, very high concentrations of small molecule were required to achieve this biochemical effect and it is unclear whether this is biological significant or widespread (Wang et al., 2009). If correct, however, it could be consistent with an additional layer in the biosynthetic regulation of salinosporamides and other secondary metabolites and could be utilized to further manipulate secondary metabolite production by preventing feedback inhibition.

The *Salinispora* are not widely studied and the biotechnological resources available for their genetic manipulation are limited. Lechner et al. (2011) have adapted a promoter for use in *S. tropica*, which has broad implications in other related organisms. Marine actinomycetes like *Salinispora* are recognized for their production of novel secondary metabolites (Imhoff et al., 2011) and genetic tools like the ones developed here will be crucial for further study of the biosynthesis and regulation of secondary metabolism in these organisms.

The work presented by Lechner et al. (2011) illuminates another example of the complex regulatory backdrop to secondary metabolism and underscores how much there is yet to learn. Although polyketide and nonribosomal peptide production are fairly well understood in biochemical terms, we know surprisingly little about how and why their expression and biosynthesis are regulated. We do not understand how secondary metabolism is integrated into primary metabolism, if there is a preferable order to the expression of biosynthetic enzymes, whether these enzymes assemble into multiprotein complexes or how biosynthetic and self-resistance proteins interact. Indeed, our understanding of why actinomycetes synthesize such a large array of secondary metabolites is limited. Knowledge of the regulatory mechanisms governing secondary metabolite biosynthesis and resistance could yield new ideas for enhancing synthesis, manipulating the structures of known molecules, and for discovering new ones.

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