one such membrane-spanning domain (Olschewski and Becker, 2008).

The approach by Singla et al. (2011) will hopefully trigger the synthesis of many more full-length cell surface receptors, as they are a highly important class of proteins in signal transduction and as drug targets.

REFERENCES

Drew, D., Froderberg, L., Baars, L., and de Gier, J.W. (2003). Biochim. Biophys. Acta *1610*, 3–10.

Johnson, E.C.B., and Kent, S.B.H. (2006). J. Am. Chem. Soc. *128*, 6640–6646.

Lackmann, M., and Boyd, A.W. (2008). Sci. Signal. *1*, re2.

Malakhov, M.P., Mattern, M.R., Malakhova, O.A., Drinker, M., Weeks, S.D., and Butt, T.R. (2004). J. Struct. Funct. Genomics *5*, 75–86.

Mootz, H.D. (2009). ChemBioChem *10*, 2579–2589.

Muir, T.W., Sondhi, D., and Cole, P.A. (1998). Proc. Natl. Acad. Sci. USA *95*, 6705–6710.

Olschewski, D., and Becker, C.F. (2008). Mol. Biosyst. *4*, 733–740.

Shintani, T., Ihara, M., Sakuta, H., Takahashi, H., Watakabe, I., and Noda, M. (2006). Nat. Neurosci. *9*, 761–769.

Singla, N., Himanen, J.P., Muir, T.W., and Nikolov, D.B. (2008). Protein Sci. *17*, 1740–1747.

Singla, N., Erdjument-Bromage, H., Himanen, J.P., Muir, T.W., and Nikolov, D.B. (2011). Chem. Biol. *18*, this issue, 361–371.

Elegant Metabolite Biosynthesis

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Hormaomycin, an NRPS-produced bacterial metabolite involved in microbial signaling, possesses several remarkable structural features. The study by Höfer et al. (2011) employed a range of methodologies to explore and ultimately understand the elaborate biosynthesis of this complex natural product.

Bacteria interact and communicate with other bacteria in a multitude of ways. For example, quorum sensing is used to control density-specific phenotypes that, in turn, enable behaviors such as biofilm formation, virulence, or production of antibiotics and other secondary metabolites. Quite often, small autoinducer molecules such as homoserine lactones, furanosyl borate diesters, and oligopeptides are employed to direct these behaviors by regulating gene expression (Ng and Bassler, 2009) and, in the case of antibiotic production, to activate biosynthetic gene clusters encoding the production of additional metabolites that provide defense for bacteria (El-Sayed et al., 2001). The effectiveness of these latter compounds is in part due to their chemical diversity, as incorporation of unusual functional groups or elements such as halogen atoms can increase both their specificity and biological activity. Recently, it has become possible to directly visualize these types of chemical interactions between bacteria using MALDI imaging mass spectrometry (Yang et al., 2009).

In a remarkable paper published in this issue of *Chemistry and Biology*, Höfer et al. (2011) characterize the biosynthesis of hormaomycin, a highly unusual nonribosomal peptide synthetase (NRPS)-derived molecule from *Streptomyces griseus*. Hormaomycin (also known as takaokamycin) (Omura et al., 1984) induces morphological differentiation and secondary metabolite production in actinobacteria, and is also a potent narrow spectrum antibiotic (MIC 88 pM against coryneform actinobacteria). Thus, hormaomycin is a rare example of a natural product that can both regulate bacterial behaviors and also independently function in a defensive antibiotic role.

Hormaomycin is composed of several unusual residues, making it quite possibly the most structurally unique microbial morphogen yet described. Most dramatically, it possesses two alanine residues with nitro-cyclopropyl groups [(3-Ncp)Ala], two β-methyl phenylalanine residues [(β-Me)Phe], a novel chlorinated pyrrole [5-chloropyrrole 2-carboxylic acid (Chpca)], and a proline unit with a 4-propenyl substituent [4-((Z)-propenyl) proline (4(-Pe)Pro)] (Figure 1). Although the structure of hormaomycin has been confirmed via total synthesis, insights into its biosynthesis have until now been limited to feeding experiments focused on the two unusual (3-Ncp)Ala residues and have revealed their ultimate origin from L-lysine (Brandl et al., 2005). Additional prior work revealed that alternate precursor amino acids could be incorporated into this unit, producing a hormaomycin analog with altered biological properties (activity against the fungus *Candida albicans*) (Zlatopolskiy et al., 2006). One other study identified the pyrrole halogenase from this gene cluster (ultimately HrmQ) and demonstrated that it could be inserted into the chlorobiocin biosynthetic pathway in *Streptomyces roseochromogenes* var. *oscitans*, thereby producing two novel analogs (Heide et al., 2008).

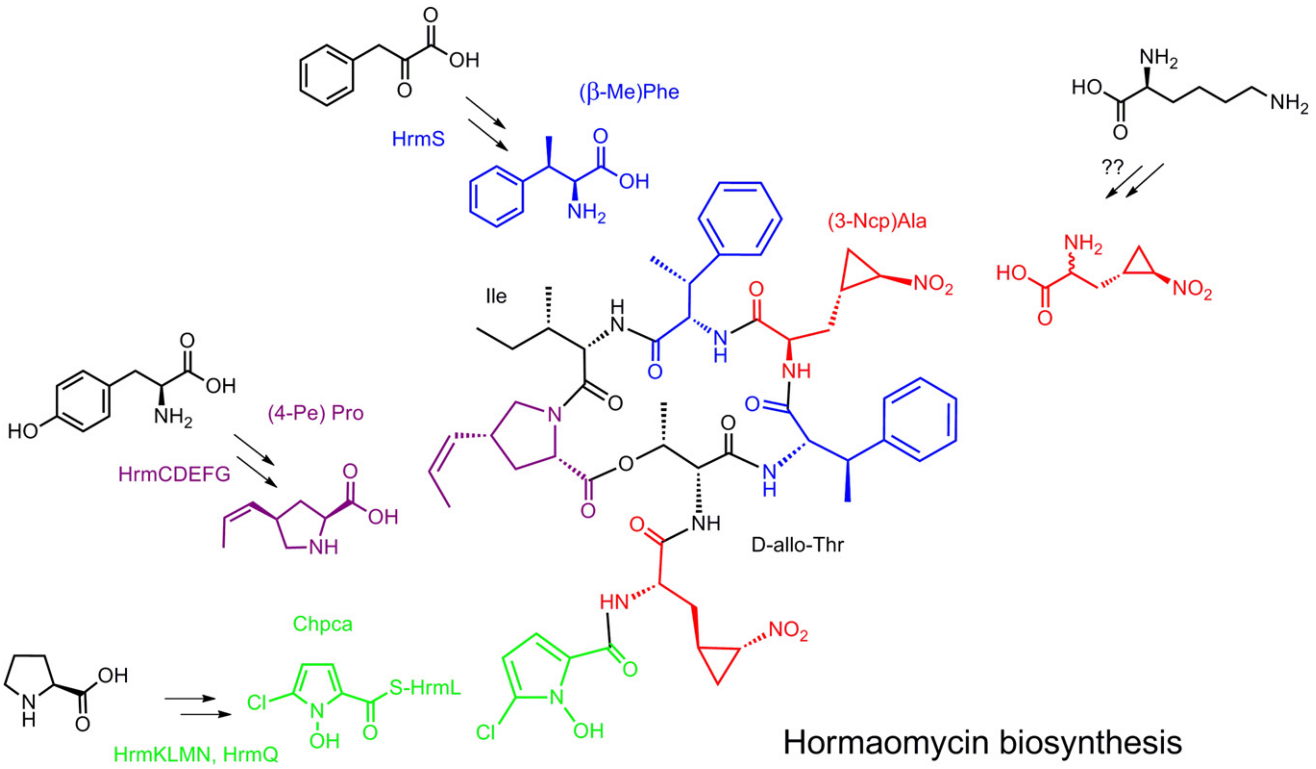

Figure 1. Biosynthetic Origin of Hormaomycin Residues as Determined by Höfer et al.
(β-Me)Phe: β-methyl phenylalanine (blue); (3-Ncp)Ala: 3-(*trans*-2′-nitrocyclopropyl)alanine (red); Chpca: 5-chloropyrrole 2-carboxylic acid (green); 4(-Pe)Pro: 4-((Z)-propenyl) proline (purple). Putative enzyme functions are shown with each predicted biosynthetic reaction.

In this new work, Höfer et al. (2011) first conduct a series of isotope-labeled precursor studies to gain insight into the metabolic building blocks for hormaomycin and then use the resulting knowledge of adenylation domain specificity coupled with the previously characterized halogenase to efficiently clone and sequence the 48.4 Kb biosynthetic gene cluster. A detailed bioinformatic analysis of the hormaomycin gene cluster provided critical insights into putative mechanisms involved in constructing this unique molecule. For example, the pathway is quite specific for the starter unit, 5-chloropyrrole, and provision of pyrrole-2-carboxylate to cultures enhanced hormaomycin yields 4 fold. The products of five genes, *hrmKLMNQ*, are proposed to form the Chpca group (green structure in Figure 1) from proline, based on homology with related genes involved in chlorinated pyrrole formation. To investigate the origin of the (β-Me)Phe moieties (blue), feeding studies first demonstrated phenylalanine incorporation and S-adenosyl methionine (SAM)-dependent methylation. By homology reasoning, HrmS was identified as the putative methyltransferase and likely adds the methyl group to the α-keto acid phenyllactate; lack of an aminotransferase in the gene cluster suggests use of an aminotransferase from primary metabolism. Precursor feedings with *p*-fluoro-Phe led to the production of novel hormaomycin analogs that lacked one or both of the β-methyl groups of the two β-methyl-Phe residues, suggesting that this fluorinated analog functions as a selective inhibitor of the methyltransferase. Next, the authors' analysis suggested that tyrosine is incorporated and modified by HrmC-G to produce the (4-Pe)Pro residue (purple). HrmF is suspected to be involved in the dioxygenase cleavage of dihydroxyphenylalanine (DOPA), itself produced by action of HrmE on tyrosine. Expression of HrmF and biochemical characterization demonstrated its robust catalytic efficiency in cleaving DOPA to a dihydropyrrole intermediate. Subsequent transformations are less certain but at some stage involve a unique terminal methylation by SAM, a result also indicated by ^{13}C-labeled precursor feeding studies. The genes encoding the biosynthetic proteins forming the intriguing (3-Ncp)Ala groups (red) remain unknown but, by process of elimination, appear to involve *hrmIJ*, two genes with no characterized homologs that are the subject of continuing biochemical study by the Piel laboratory. What is clear, and clearly exciting, is that the formation of the cyclopropyl rings in these two residues occurs by a process unlike any currently described.

This study by Höfer et al. (2011) elegantly examines the mechanisms involved in creating chemical diversity in an exceptionally complex natural product, hormaomycin. The article not only explores how these compounds are made, but an analysis of the gene cluster also uncovered intriguing evolutionary insights into the pathway. The hormaomycin gene cluster possesses regions with very high sequence identity shared between adenylation domains, with the only differences occurring within the amino acid specificity pockets. This suggests that recombinatorial swapping of short DNA regions encoding substrate specificity is sufficient to

create new chemical diversity in NRPS-derived natural products, and thus entire domain or module exchanges are not necessarily required for pathway evolution. Furthermore, some hormaomycin biosynthetic enzymes are promiscuous, allowing for the precursor-directed biosynthesis of analogs with varied biological activity and demonstrating the promise of this pathway to engineer new bioactive molecules.

In summary, hormaomycin is a fascinatingly complex bacterial signaling molecule and antibiotic. A wonderful interplay of isotope-labeled precursor feeding experiments, analytical chemistry, biosynthetic gene cluster identification, bioinformatics, protein expression, and biochemical characterization were used to unravel this puzzle and make its biosynthesis comprehensible. The confluence of these approaches provided keen insights of various methods by which to produce analog structures and engineer the pathway, ultimately harnessing bacterial communication to make valuable contributions to pharmaceutical biotechnology.

REFERENCES

Brandl, M., Kozhushkov, S.I., Zlatopolskiy, B.D., Alvermann, P., Geers, B., Zeeck, A., and de Meijere, A. (2005). European Journal of Organic Chemistry *54*, 854–863.

El-Sayed, A.K., Hothersall, J., and Thomas, C.M. (2001). Microbiology *147*, 2127–2139.

Heide, L., Westrich, L., Anderle, C., Gust, B., Kammerer, B., and Piel, J. (2008). ChemBioChem *9*, 1992–1999.

Höfer, I., Crüsemann, M., Radzom, M., Geers, B., Flachshaar, D., Cai, X., Zeeck, A., and Piel, J. (2011). Chem. Biol. *18*, this issue, 381–391.

Ng, W.-L., and Bassler, B.L. (2009). Annu. Rev. Genet. *43*, 197–222.

Omura, S., Mamada, H., Wang, N.J., Imamura, N., Oiwa, R., Iwai, Y., and Muto, N. (1984). J. Antibiot. (Tokyo) *37*, 700–705.

Yang, Y.-L., Xu, Y., Straight, P., and Dorrestein, P.C. (2009). Nat. Chem. Biol. *5*, 885–887.

Zlatopolskiy, B.D., Radzom, M., Zeeck, A., and de Meijere, A. (2006). European Journal of Organic Chemistry *11*, 1525–1534.

