tween enzymes made from RNA and those made from DNA in terms of scope of activity and existence of favored sequences for particular catalytic activities. In addition, for both RNA and DNA enzymes we have specific functional models to test (e.g., Figure 1). Thus, the time is ripe for earnest application of X-ray crystallography and NMR spectroscopy to understand DNA enzyme structure and activity.

Beyond their implications for DNA enzyme function, the new results can be considered in the larger context of nucleic acid enzyme research [14]. With respect to the in vitro activities of (deoxy)ribozymes, we are in a transitional period in the development of this field. Ever-increasing attention is being devoted to applying nucleic acid enzymes as widely as possible for particular tasks such as RNA cleavage, rather than identifying qualitatively "new" activities and immediately moving on to other studies. For example, in addition to RNA cleavage, various labs are using RNA and DNA enzymes to prepare reagents for subsequent biophysical experiments [15], to sense metal ions in solution [16, 17], to sense bioorganic molecules [18], to address chemical reactions of importance for prebiotic RNA world chemistry [19], and to explore reactions related to those of modern biochemistry [20]. I view these developments as encouraging signs that the nucleic acid enzyme field is maturing. Hopefully, the next two decades will reveal even more exciting progress in understanding nucleic acid enzymes—both natural and artificial—and applying them to important theoretical and practical problems.

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Selected Reading

1. Breaker, R.R., and Joyce, G.F. (1994). Chem. Biol. *1*, 223–229.
2. Emilsson, G.M., and Breaker, R.R. (2002). Cell. Mol. Life Sci. *59*, 596–607.
3. Santoro, S.W., and Joyce, G.F. (1997). Proc. Natl. Acad. Sci. USA *94*, 4262–4266.
4. Pyle, A.M., Chu, V.T., Jankowsky, E., and Boudvillain, M. (2000). Methods Enzymol. *317*, 140–146.
5. Joyce, G.F. (2001). Methods Enzymol. *341*, 503–517.
6. Cruz, R.P.G., Withers, J.B., and Li, Y. (2004). Chem. Biol. *11*, this issue, 57–67.
7. Faulhammer, D., and Famulok, M. (1996). Angew. Chem. Int. Ed. Engl. *35*, 2837–2841.
8. Faulhammer, D., and Famulok, M. (1997). J. Mol. Biol. *269*, 188–202.
9. Li, J., Zheng, W., Kwon, A.H., and Lu, Y. (2000). Nucleic Acids Res. *28*, 481–488.
10. Santoro, S.W., and Joyce, G.F. (1998). Biochemistry *37*, 13330–13342.
11. Salehi-Ashtiani, K., and Szostak, J.W. (2001). Nature *414*, 82–84.
12. Doherty, E.A., and Doudna, J.A. (2000). Annu. Rev. Biochem. *69*, 597–615.
13. Nowakowski, J., Shim, P.J., Prasad, G.S., Stout, C.D., and Joyce, G.F. (1999). Nat. Struct. Biol. *6*, 151–156.
14. DeRose, V.J. (2002). Chem. Biol. *9*, 961–969.
15. Murakami, H., Kourouklis, D., and Suga, H. (2003). Chem. Biol. *10*, 1077–1084.
16. Liu, J., and Lu, Y. (2003). J. Am. Chem. Soc. *125*, 6642–6643.
17. Mei, S.H., Liu, Z., Brennan, J.D., and Li, Y. (2003). J. Am. Chem. Soc. *125*, 412–420.
18. Robertson, M.P., and Ellington, A.D. (2000). Nucleic Acids Res. *28*, 1751–1759.
19. Johnston, W.K., Unrau, P.J., Lawrence, M.S., Glasner, M.E., and Bartel, D.P. (2001). Science *292*, 1319–1325.
20. Coppins, R.L., and Silverman, S.K. (2004). Nat. Struct. Mol. Biol. *11*, in press.

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Aureolic Acids: Similar Antibiotics with Different Biosynthetic Gene Clusters

In this issue of *Chemistry & Biology*, Méndez and colleagues describe the sequence and organization of the chromomycin gene cluster [20]. Unexpectedly, the arrangement is starkly different from the mithramycin biosynthetic cluster, despite similarity in the individual genes and the near identical structures of the two antibiotic aureolic acids.

The aureolic acids, chromomycin A3, mithramycin, olivomycin, UCH9, and durhamycin A, are a family of aromatic polyketides that share an identical tricyclic core (Figure 1) [1]. The aureolic acids are neoplastic antibiotics that act against gram-positive bacteria and also stop the proliferation of tumor cells [2]. In the presence of Mg^{2+}, these compounds inhibit replication and transcription processes by interacting with G-C-rich regions in the minor groove of DNA [3]. Mithramycin (plicamycin), the only clinically used aureolic acid, is currently in limited use for the treatment of some testicular cancers [4], Paget's Bone Disease [5], and the treatment of hypercalciuria that results from certain tumors [6].

The biosynthetic pathway of mithramycin, a representative member of the aureolic acids, was ultimately elucidated by genetic studies of the producing organism, *Streptomyces argillaceus* [1]. Sequencing of the mithramycin biosynthetic gene cluster indicated that ten acetate units are converted into a 20 carbon chain by the combined action of an acyl carrier protein, a ketosynthase, and a chain length factor in a mechanism that is

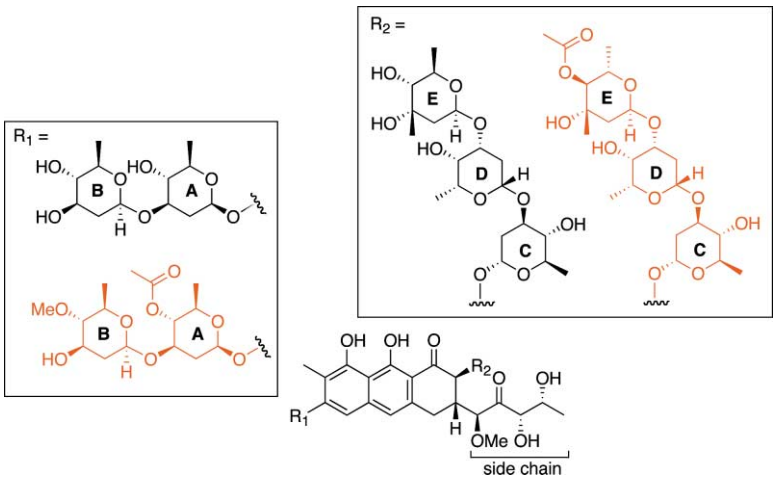

Figure 1. Structures of the Chromomycin and Mithramycin Products

Black, mithramycin; red, chromomycin.

typical of aromatic (type II) polyketide biosynthesis [7]. A ketoreductase, aromatase, and two cyclases convert this linear chain to a tetracyclic intermediate that is subsequently hydroxylated, methylated, and glycosylated [1]. In the penultimate step, the unsaturated carbonyl of one of the rings is subjected to a Bayer-Villager oxidation that converts the 6-membered ring to a 7-membered lactone, which then hydrolyzes to generate the final tricyclic ring structure [8]. In the final step, one of the keto groups of the newly opened fourth ring is reduced to a hydroxyl group to yield mithramycin [9].

The aglycone of mithramycin is identical to chromomycin, with the only structural differences between the two compounds residing in the sugars that decorate the polyketide. Specifically, the saccharides of chromomycin are derivatized with acetyl groups, a methoxy group, and the stereochemistry of saccharide E is altered (Figure 1). Subtle structural differences can modulate the biological activity of an antibiotic; in this case, the acetyl groups appear to enhance the binding of chromomycin to short DNA sequences in vitro [10]. However, despite the improved DNA binding relative to mithramycin, the clinical use of chromomycin (also known as toyomycin or aburamycin) is limited due to toxic side effects such as necrosis and renal toxicity [11].

Here, Méndez and coworkers report the sequence of the chromomycin gene cluster (*Streptomyces griseus*), the second member of the aureolic acid family to be sequenced. [20] Given the similarity between the chemical structures of mithramycin and chromomycin, it was expected that the two clusters would be nearly identical. However, although there is high homology between individual mithramycin and chromomycin genes, the arrangement of the genes in the chromomycin cluster is organized in a substantially different way. For example, in the mithramycin cluster, the genes that encode the proteins for polyketide biosynthesis are clustered together. In the chromomycin system, the homologs of these genes are scattered throughout the cluster and are interspersed with genes involved in regulation, resistance, and sugar biosynthesis.

Recent studies have shown that similar aromatic polyketide [12], streptomycin [13], and penicillin [14] gene clusters or portions of clusters are found in otherwise distantly related organisms. These observations suggest that horizontal gene transfer of secondary metabolic pathways occurs among distantly related species [15, 16]. In other words, the biosynthetic apparatus for an antibiotic (or other metabolite) can be transferred to another organism, presumably conferring some selective advantage for the recipient species. However, if either mithramycin or chromomycin was acquired by horizontal gene transfer, the differences in the two clusters suggest that substantial gene reorganization must have taken place. The driving force for such reorganization remains unclear.

Aside from any evolutionary implications, this study reveals that a specific arrangement of genes does not necessarily affect the resulting protein machinery and production of the antibiotic. This information is important for the emerging field of "combinatorial biosynthesis," in which biosynthetic pathways are modified by deleting, adding, or replacing genes to produce a modified metabolite [17–19]. Modification of a biosynthetic gene cluster may lead to the development of analogs that retain biological activity while exhibiting mitigated side effects. The authors used combinatorial biosynthesis in this study and in previously reported work to investigate the role of the aliphatic side chain in aureolic acid activity (Figure 1). By deleting a reductase that acts on this side chain, mithramycin and chromomycin analogs with modified side chains were produced that exhibited improved (in the case of mithramycin [9]) or reduced (chromomycin) activity with respect to the original antibiotic.

In the context of combinatorial biosynthesis, a practice in which gene clusters are genetically altered, it would be particularly useful to establish whether there are any rules regarding the organization of clusters. A preliminary analysis of the mithramycin and chromomycin clusters suggests that a dramatic rearrangement in the order of the genes still results in successful expression of the aureolic acid antibiotic. As sequencing efforts of metabolic pathways continue, other examples of rearranged clusters may emerge that will provide further insight into how antibiotic biosynthetic gene clusters are arranged.

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Selected Reading

1. Rohr, J., Méndez, C., and Salas, J.A. (1999). Bioorg. Chem. *27*, 41–54.
2. Yarbro, J.W. (1972). Proc. Chemother. Conf. Mithramycin (Mithracin): Develop. Appl. Symp. Ther. Testicular Neoplasms, 8–12.
3. Gause, G.F. (1975). In Antibiotics III: Mechanism of Action of Antimicrobial Antitumor Agents, J.W. Corcoran and F.E. Hahn, eds. (Berlin: Springer-Verlag), pp. 197–202.
4. American Cancer Society, http://www.cancer.org/docroot/CDG/content/CDG_plicamycin.html?internal=1
5. Elias, E.G., and Evans, J.T. (1972). J. Bone Joint Surg. Am. *54A*, 1730–1736.
6. Robins, P.R., and Jowsey, J. (1973). J. Lab. Clin. Med. *82*, 576–586.
7. Staunton, J., and Weissman, K.J. (2001). Nat. Prod. Rep. *18*, 380–416.
8. Prado, L., Fernandez, E., Weissbach, U., Blanco, G., Quiros, L.M., Brana, A.F., Méndez, C., Rohr, J., and Salas, J.A. (1999). Chem. Biol. *6*, 19–30.
9. Remsing, L.L., Gonzalez, A.M., Nur-e-Alam, M., Fernandez-Lozano, M.J., Brana, A.F., Rix, U., Oliveira, M.A., Méndez, C., Salas, J.A., and Rohr, J. (2003). J. Am. Chem. Soc. *125*, 5745–5753.
10. Banville, D.L., Keniry, M.A., and Shafer, R.H. (1990). Biochemistry *29*, 9294–9304.
11. Reynolds, R.D., Fisher, J.I., Jensen, P.A., Pajak, T.F., and Bateman, J.R. (1976). Cancer Treat. Rep. *60*, 1251–1255.
12. Metsa-Ketela, M., Halo, L., Munukka, E., Hakala, J., Mantsala, P., and Ylihonko, K. (2002). Appl. Environ. Microbiol. *68*, 4472–4479.
13. Egan, S., Wiener, P., Kallifidas, D., and Wellington, E.M.H. (2001). Antonie Van Leeuwenhoek *79*, 127–133.
14. Buades, C., and Moya, A. (1996). J. Mol. Evol. *42*, 537–542.
15. Lawrence, J.G., and Hendrickson, H. (2003). Mol. Microbiol. *50*, 739–749.
16. Walton, J.D. (2000). Fungal Genet. Biol. *30*, 167–171.
17. Tsoi, C.J., and Khosla, C. (1995). Chem. Biol. *2*, 355–362.
18. Rohr, J. (1995). Int. Ed. Engl. *34*, 881–885.
19. Leadlay, P.F. (1997). Curr. Opin. Chem. Biol. *1*, 162–168.
20. Menéndez, N., Nur-e-Alam, M., Braña, A.F., Rohr, J., Salas, J.A., and Méndez, C. (2004). Chem. Biol. *11*, this issue, 21–32.

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Unraveling the Pathway of Lipoic Acid Biosynthesis

Lipoic acid is almost universally required for aerobic metabolism. However, the mechanism for its synthesis and incorporation into proteins has remained elusive. A groundbreaking study published in the December issue of *Chemistry & Biology* [20] uncovers critical features of the lipoic acid biosynthetic pathway.

Learning about the pyruvate dehydrogenase complex (PDC) has evolved into a rite of passage for every beginning biochemist. Few have been spared the analogy of the lipoyl group acting as a crane in a construction yard, methodically removing intermediates from one subunit of the complex and successively delivering them to the active sites of others. Pyruvate and coenzyme A (CoA) enter the complex, while CO_2 and acetyl-CoA exit. In the process, NAD^+ is reduced to NADH. Other very worthy cofactors, such as thiamin diphosphate (TDP) and flavin adenine dinucleotide (FAD), participate in the reaction; however, the lipoyl group and its associated subunit (E2) serve as the core of this gigantic factory. The lipoyl group makes repeat performances in several other multienzyme complexes that are involved in primary and secondary metabolism. These include the α-ketoglutarate dehydrogenase complex (KGDC) of the citric acid cycle, the branched-chain 2-oxo acid dehydrogenase complex (BCDC), which is important in the metabolism of several of the branched-chain amino acids, and the glycine cleavage system (GCS), which degrades glycine to CO_2 and ammonia, while using the α carbon of the amino acid to generate N^5,N^{10}-methylene-tetrahydrofolate. Again, all of this takes place with concomitant reduction of NAD^+ to NADH [1].

The Biosynthesis of Lipoic Acid

Given the importance of the lipoyl group in central metabolism, it should not be surprising that many organisms elaborate more than one pathway to incorporate it into those complexes that require it. In the cell, very little lipoate exists as the free acid; almost all is tethered to the ϵ-amino group of a conserved lysine residue on lipoyl-accepting domains of target complexes. Pioneering studies from John Cronan's laboratory indicate that *E. coli* maintain at least two pathways for attaching the lipoyl group to these target lysine residues (Figure 1) [2]. Lipoic acid that the organism obtains from the medium is first activated by ATP, and then transferred and appended with concomitant release of AMP. In *E. coli*, both steps are catalyzed by a lipoate-protein ligase, which is designated LplA [3]. LplA also will use octanoic acid as a substrate, albeit with reduced efficiency [4]. Alternatively, the lipoyl group can be synthesized endogenously as an offshoot of fatty acid biosynthesis. The exact details of this pathway have not been completely illuminated; however, the major players have been identified and are currently being characterized. LipB is a lipoyl (octanoyl)-transferase; it can transfer either a lipoyl or octanoyl group from a bacterial type II acyl carrier protein (ACP) to lipoyl-accepting domains [4, 5]. LipA catalyzes sulfur insertion into the octanoyl group, forming the lipoyl appendage [6]. Evidence for similar endogenous pathways in several eukaryotes has also surfaced, providing rationale for the previously unexplained