[5] C. Wilson, J. W. Szostak, *Nature* **1995**, *374*, 777 – 782.
[6] H. Suga, P. A. Lohse, J. W. Szostak, *J. Am. Chem. Soc.* **1998**, *120*, 1151 – 1156.
[7] B. Seelig, A. Jäschke, *Chem. Biol.* **1999**, *6*, 167 – 176.
[8] E. Westhof, N. Leontis, *Angew. Chem.* **2000**, *112*, 1651 – 1655; *Angew. Chem. Int. Ed.* **2000**, *39*, 1587 – 1591.
[9] N. Ban, P. Nissen, J. Hansen, M. Capel, P. B. Moore, T. A. Steitz, *Nature* **1999**, *400*, 841 – 848.
[10] W. M. Clemons, Jr., J. L. C. May, B. T. Wimberly, J. P. McCutcheon, M. S. Capel, V. Ramakrishnan, *Nature* **1999**, *400*, 833 – 841.
[11] A. Tocilj, F. Schlunzen, D. Janell, M. Gluhmann, H. A. Hansen, J. Harms, A. Bashan, H. Bartels, I. Agmon, F. Franceschi, A. Yonath, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14 252 – 14 257.
[12] J. H. Cate, M. M. Yusupov, G. Z. Yusupova, T. N. Earnest, H. F. Noller, *Science* **1999**, *285*, 2095 – 2104.
[13] N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905 – 920.
[14] P. Nissen, J. Hansen, N. Ban, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 920 – 930.
[15] M. Welch, J. Chastang, M. Yarus, *Biochemistry* **1995**, *34*, 385 – 390.
[16] H. F. Noller, V. Hoffarth, L. Zimniak, *Science* **1992**, *256*, 1416 – 1419.
[17] B. Zhang, T. R. Cech, *Chem. Biol.* **1998**, *5*, 539 – 553.
[18] A. T. Perrotta, I. Shih, M. D. Been, *Science* **1999**, *286*, 123 – 126.
[19] S. Nakano, D. M. Chadalavada, P. C. Bevilacqua, *Science* **2000**, *287*, 1493 – 1497.
[20] P. Legault, A. Pardi, *J. Am. Chem. Soc.* **1997**, *119*, 6621 – 6628.
[21] G. W. Muth, L. Ortoleva-Donnelly, S. A. Strobel, *Science* **2000**, *289*, 947 – 950.
[22] D. M. Blow, J. J. Birktoft, B. S. Hartley, *Nature* **1969**, *221*, 337 – 340.
[23] F. Michel, J. L. Ferat, *Annu. Rev. Biochem.* **1995**, *64*, 435 – 61.

Discovery of a New Bacterial Polyketide Biosynthetic Pathway

Bradley S. Moore* and Jörn N. Hopke[a]

KEYWORDS:
biosynthesis · chalcone synthase · natural products · polyketides · transferases

Polyketides comprise a large family of structurally diverse natural products that possess broad ranges of biological activities.[1] These therapeutically important agents are synthesized by successive Claisen condensations of extender units derived from (methyl)malonyl-CoA with an acyl-CoA starter unit in a manner reminiscent of fatty acid synthesis. Up until recently, two types of microbial polyketide synthases (PKSs) were widely recognized through molecular genetics.[2] In analogy to the fatty acid synthases (FASs), the distinction between the phylogenetically related type I and the type II PKSs is the organization of their various catalytic sites. Type I describes a system of one or more multifunctional proteins that contain a different active site for each enzyme-catalyzed reaction in polyketide carbon chain assembly and modification. These fall into two subgroups, the modular type I PKSs of bacteria (i.e., 6-deoxyerythronolide B synthase, DEBS) and the iterative type I PKSs of fungi (i.e., 6-methylsalicylic acid synthase, MSAS). In contrast, the type II PKS system is comprised of individual proteins largely carrying one enzymatic activity that is used iteratively in the biosynthesis of bacterial multiaromatic products (i.e., actinorhodin and tetracenomycin).

Recently, the groups of Horinouchi[3] and Thomashow[4] independently characterized a new polyketide biosynthetic pathway in bacteria for the assembly of small aromatic metabolites. These new bacterial PKSs, which Bangera and Thomashow classify as type III,[4] are members of the chalcone synthase (CHS) and stilbene synthase (STS) superfamily of PKSs previously only found in plants.[5] These enzymes are structurally and mechanistically quite distinct from the type I and type II PKSs and use free CoA thioesters as substrates without the involvement of 4′-phosphopantetheine residues on acyl carrier proteins.

Members of the CHS/STS superfamily of condensing enzymes are relatively modest-sized proteins of 40 – 47 kDa that function as homodimers and typically select a cinnamoyl-CoA starter unit and carry out three successive extensions with malonyl-CoA.[5] Release of the tetraketide followed by cyclization and/or decarboxylation yields a chalcone or a stilbene (Figure 1 a). CHSs appear to be ubiquitous in higher plants and catalyze the first enzymatic reaction to flavonoids, which exhibit a wide range of biochemical, physiological, and ecological activities. The X-ray crystal structure of CHS2 from the legume *Medicago sativa* (alfalfa) was recently determined and provides important structural information on the reaction mechanism of a plant PKS.[6] Several new additions to the CHS/STS superfamily have emerged from plants and deviate from the chalcone and stilbene biosynthetic model by utilizing non-phenylpropanoid starter units, varying the number of condensation reactions, and having different cyclization patterns (e.g. acridone and 2-pyrone synthases).[7–9] Thus, plant enzymes in the CHS/STS superfamily are growing in number and function and

[a] *Prof. Dr. B. S. Moore, Dr. J. N. Hopke*
Division of Medicinal Chemistry
College of Pharmacy
University of Arizona
Tucson, AZ 85721-0207 (USA)
Fax: (+1) 520-626-2466
E-mail: moore@pharmacy.arizona.edu

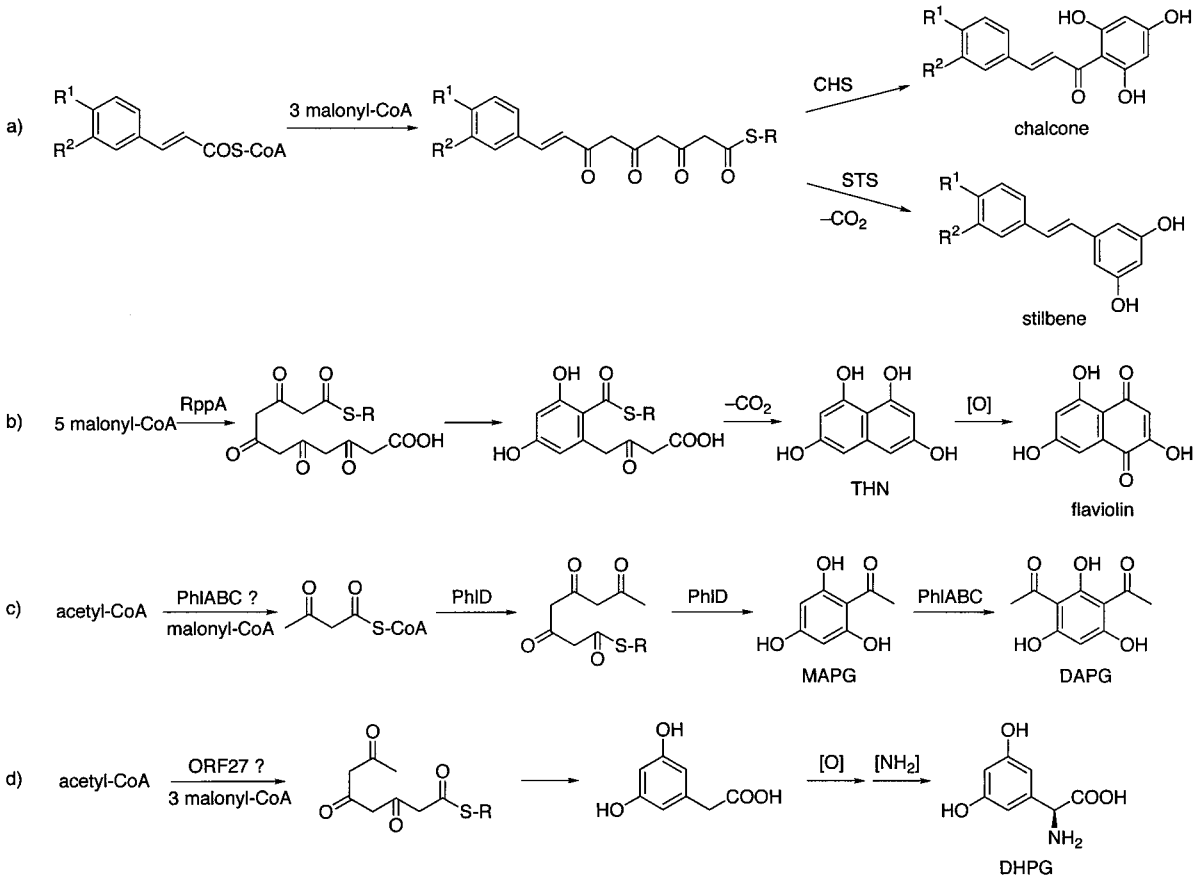

Figure 1. *Proposed biosynthesis of a) chalcone and stilbene in higher plants, b) THN in* Streptomyces griseus, *c) MAPG and DAPG in* Pseudomonas fluorescens, *and d) DHPG in* Amycolatopsis orientalis. *R = coenzyme-A (CoA) or the enzyme active-site cysteine thiol group.*

are not limited in their substrate specificity to plant-specific cinnamoyl-CoA starter units.

The exciting discovery revealed by Funa et al. at the University of Tokyo is the characterization of a CHS-like enzyme in the bacterium *Streptomyces griseus*.[3] The CHS-homologous gene *rppA* confers red-brown pigmentation in *S. griseus*. Incubation of malonyl-CoA with recombinant RppA carrying a poly-histidine tag for convenience in protein purification resulted in the formation of 1,3,6,8-tetrahydroxynaphthalene (THN; Figure 1 b). Air oxidation of THN yielded flaviolin, the precursor to the pigmented melanin polymers. Co-incubation of malonyl-CoA with either *p*-coumaroyl-CoA or acetyl-CoA did not provide alternative products, indicating that RppA is not a CHS and that malonyl-CoA serves as the starter unit. Funa et al. note that this scenario is consistent with the proposed THN biosynthetic pathway in which the final ring closure of the pentaketide may be assisted by the decarboxylation of the carboxy group derived from the starter unit. Amino acid alignment of RppA with CHSs revealed the conserved cysteine residue at position 138 within the active-site motif. This residue was confirmed to function as the active-site cysteine, as a mutant enzyme with a serine replacement at Cys 138 was not enzymatically active. Thus, as in plant CHSs and STSs, RppA Cys 138 functions as the active site of the condensing reaction that covalently binds the starter unit prior to condensation. The in vivo function of *rppA* was further correlated to melanin-like pigments in a *S. griseus rppA* disruption mutant that produced pigmented spores only upon restoration with a plasmid carrying the *rppA* gene. This pigmentation process differs from that in other streptomycetes in which spore pigment polyketides are generated by type II PKSs.[10]

Washington State University researchers, Bangera and Thomashow, independently reported that the bacterial polyketide 2,4-diacetylphloroglucinol (DAPG) is similarly synthesized by a CHS-like protein (Figure 1 c).[4] This broad-spectrum antimicrobial agent is produced by plant-associated fluorescent pseudomonads. The CHS-homologous gene *phlD* is a constituent of the biosynthetic operon *phlACBD* that includes a set of three genes conserved in bacteria and archaea. In vivo expression studies in *Escherichia coli* demonstrated that all four genes are required for the synthesis of both monoacetylphloroglucinol (MAPG) and DAPG. Further expression of individual genes or the incomplete operon indicated that PhlD alone cannot catalyze the synthesis of MAPG, but rather must function collectively with PhlACB. PhlA and PhlC are homologous to cysteine-deficient ketosynthases and to thiolases, respectively, whereas PhlB lacks homology to other known proteins. Bangera and Thomashow speculate that these proteins are responsible for generating the acetoace-

tyl-CoA starter unit for PhlD. Furthermore, the PhlACB proteins collectively convert MAPG to DAPG. In vitro expression studies with recombinant proteins are needed to further investigate this proposed biosynthetic system.

Six additional bacterial CHS homologues, or type III PKSs, of unknown functions have been deposited in genetic databases and suggest that this new polyketide biosynthetic pathway may be widespread in bacteria. The biosynthetic gene cluster for the vancomycin group antibiotic chloroeremomycin contains a CHS homologue (ORF27)[11] that is presumably involved in the assembly of the 3,5-dihydroxyphenylglycine (DHPG) residue that Williams and co-workers demonstrated in 1982 to be acetate-derived (Figure 1 d).[12] Expression of a CHS-like gene is regulated by the phytochrome gene *ppr* in the purple photosynthetic bacterium *Rhodospirillum centenum*.[13] The photoactive yellow protein domain of Ppr contains the chromophore 4-coumarate that functions in plant CHSs as the CoA thioester starter unit. Although the function of the *R. centenum* type III PKS has not yet been characterized, it is tempting to speculate that it may operate as a bona fide CHS, as this bacterium has the rare ability to produce the phenylpropanoid *p*-coumarate. Genome sequencing of the bacteria *Streptomyces coelicolor*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, and *Deinococcus radiodurans* identified further CHS/STS homologues of unknown functions.

The eight CHS-homologous bacterial proteins in the database are clearly members of the growing CHS/STS superfamily of condensing enzymes and belong to a third class of bacterial PKSs. These proteins are phylogenetically unrelated to all other microbial PKSs and FASs, including those that are functionally equivalent (RppA and the fungal type I PKS WA are collectively THN synthases) or from the same bacterial species (*P. fluorescens* contains both type I (PltC) and type III (PhlD) PKSs, while *S. coelicolor* has both type II (ActI and WhiE) and type III PKSs) (Figure 2). The structural and functional similarities of the bacterial type III PKSs with the plant CHSs imply a common evolutionary origin that may have evolved through horizontal gene transfer between plant-associated bacteria and higher plants. These suggestions are supported by distance matrix calculations and maximum likelihood analysis (Figure 2).

On the basis of these studies, the occurrence of bacterial type III PKSs appears widespread. In that regard, Funa et al. speculate that THN may be an intermediate in the biosynthesis of a small group of prenylated naphthoquinones produced by several *Streptomyces* spp. strains.[3] Such members include the monoterpene-substituted naphthoquinones naphterpene[14] and furaquinocin A,[15] the sesquiterpene-substituted naphthoquinones marinone and neomarinone,[16] and the dimethylallyl/monoter-

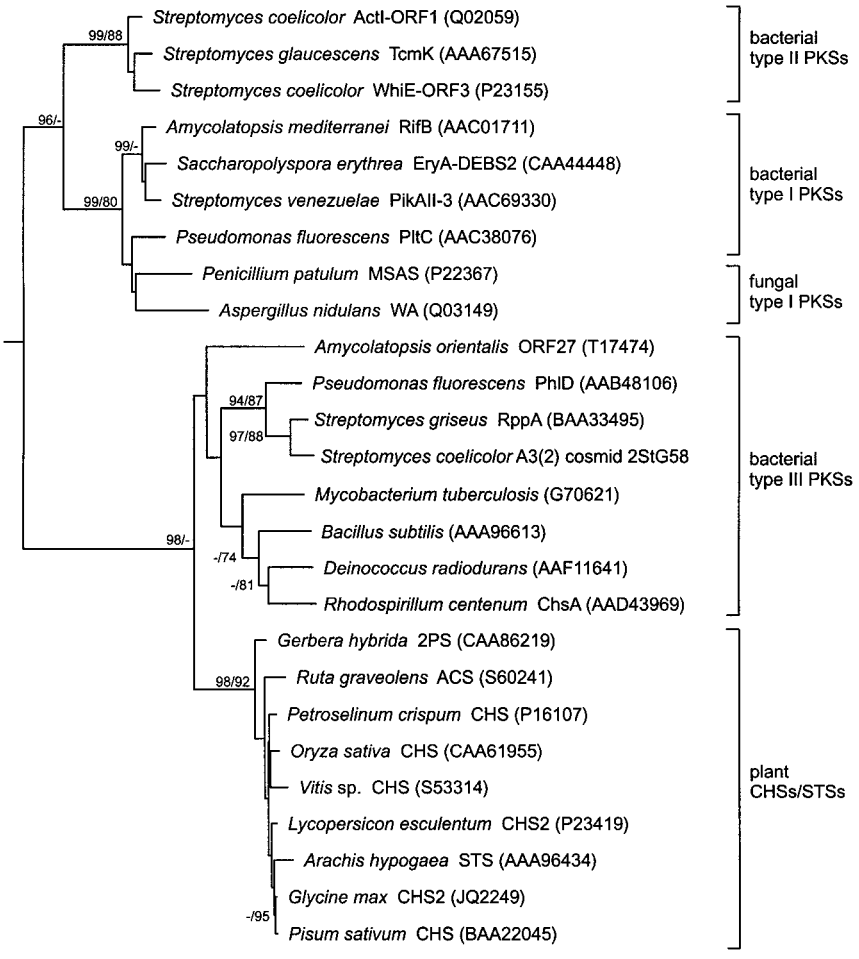

Figure 2. *Phylogenetic tree of bacterial type III PKSs in relation to ketosynthase domains from other types of PKSs. Sequences were retrieved from GenBank (accession numbers given in parentheses) and aligned with ClustalX. (The* S. coelicolor *sequence from cosmid 2StG58 was rather retrieved from http://sanger.ac.uk/Projects/S_coelicolor/.) The tree was constructed by applying maximum likelihood (Puzzle 4.0 and ProtMl programs) and distance matrix methods (ProtDist from the Phylip package) and using the PAM and JJT models. The tree was rooted with a bacterial FAS sequence as outgroup (not shown). Numbers at branching points are quartet puzzling/ProtDist bootstrap support values. Only values > 70% are shown. Different groups of PKSs form monophyletic clusters as shown by strong bootstrap support values. Bacterial type III PKSs and plant CHSs/STSs appear to be of common ancestry. In contrast to the above maximum likelihood tree, the distance matrix/neighbor-joining tree (data not shown) places the* A. orientalis *ORF27,* P. fluorescens *PhlD,* S. griseus *RppA and* S. coelicolor *sequences as sister taxa to the plant CHSs/STSs.*

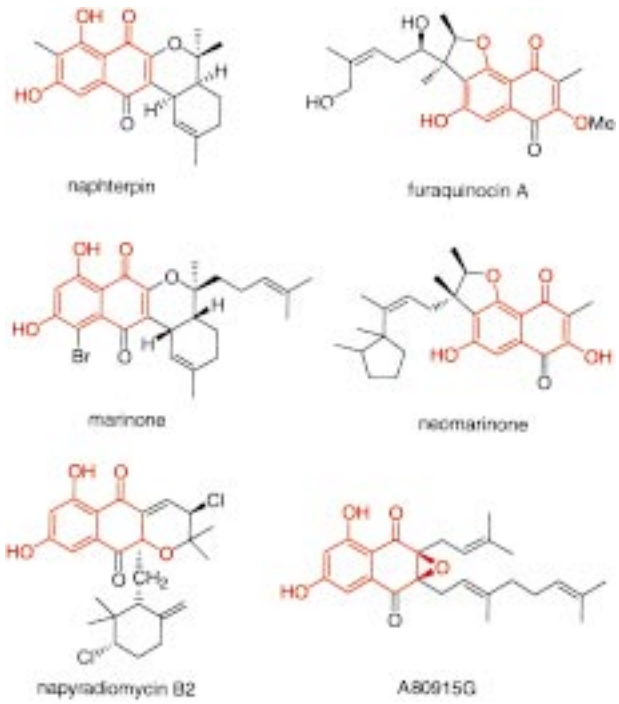

Figure 3. *Structures of prenylated naphthoquinones from* Streptomyces *spp. The postulated THN-derived units are depicted in red.*

pene-disubstituted naphthoquinones belonging to the napyradiomycin family (Figure 3).[17] Feeding experiments support this claim, as the naphthoquinone core in naphterpene and furaquinocin A is derived from a symmetrical naphthalene unit that is acetate-derived.[14, 15]

The seminal work by the groups of Horinouchi and Thomashow in the functional characterization of the bacterial type III PKSs RppA and PhlD, respectively, will undoubtedly heighten interest in this new group of condensing enzymes in bacteria. The occurrence and functional breadth of the type III PKSs will definitely be subjects of future communications.

[1] D. O'Hagan, *The Polyketide Metabolites*, Horwood, Chichester, UK, **1991**.
[2] D. A. Hopwood, *Chem. Rev.* **1997**, *97*, 2465 – 2497.
[3] N. Funa, Y. Ohnishi, I. Fujii, M. Shibuya, Y. Ebizuka, S. Horinouchi, *Nature* **1999**, *400*, 897 – 899.
[4] M. G. Bangera, L. S. Thomashow, *J. Bacteriol.* **1999**, *181*, 3155 – 3163.
[5] J. Schröder in *Comprehensive Natural Products Chemistry, Vol.* 1 (Eds.: D. Barton, K. Nakanishi, O. Meth-Cohn), Elsevier, Amsterdam, **1999**, pp. 749 – 771.
[6] J.-L. Ferrer, J. M. Jez, M. E. Bowman, R. A. Dixon, J. P. Noel, *Nat. Struct. Biol.* **1999**, *6*, 775 – 784; J. M. Jez, J.-L. Ferrer, M. E. Bowman, R. A. Dixon, J. P. Noel, *Biochemistry* **2000**, *39*, 890 – 902.
[7] K. T. Junghanns, R. E. Kneusel, A. Baumert, W. Maier, D. Gröger, U. Matern, *Plant Mol. Biol.* **1995**, *27*, 681 – 692.
[8] S. Eckermann, G. Schröder, J. Schmidt, D. Strack, R. A. Edrada, Y. Helariutta, P. Elomaa, M. Kotilainen, I. Kilpeläinen, P. Proksch, T. H. Teeri, J. Schröder, *Nature* **1998**, *396*, 387 – 390.
[9] T. Akiyama, M. Shibuya, H. M. Liu, Y. Ebizuka, *Eur. J. Biochem.* **1999**, *263*, 834 – 839.
[10] T.-W. Yu, Y. Shen, R. McDaniel, H. G. Floss, C. Khosla, D. A. Hopwood, B. S. Moore, *J. Am. Chem. Soc.* **1998**, *120*, 7749 – 7759.
[11] A. M. A. van Wageningen, P. N. Kirkpatrick, D. H. Williams, B. R. Harris, J. K. Kershaw, N. J. Lennard, M. Jones, S. J. M. Jones, P. J. Solenberg, *Chem. Biol.* **1998**, *5*, 155 – 162.
[12] S. J. Hammond, M. P. Williamson, D. H. Williams, L. D. Boeck, G. G. Marconi, *J. Chem. Soc. Chem. Commun.* **1982**, 344 – 346.
[13] Z. Jiang, L. R. Swem, B. G. Rushing, S. Devanathan, G. Tollin, C. E. Bauer, *Science* **1999**, *285*, 406 – 409.
[14] K. Shin-ya, K. Furihata, Y. Hayakawa, H. Seto, *Tetrahedron Lett.* **1990**, *31*, 6025 – 6026, and references therein.
[15] S. Funayama, M. Ishibashi, K. Komiyama, S. Omura, *J. Org. Chem.* **1990**, *55*, 1132 – 1133, and references therein.
[16] I. H. Hardt, P. R. Jensen, W. Fenical, *Tetrahedron Lett.* **2000**, *41*, 2073 – 2076, and references therein.
[17] D. S. Fukuda, J. S. Mynderse, P. J. Baker, D. M. Berry, L. D. Boeck, R. C. Yao, F. P. Mertz, W. M. Nakatsukasa, J. Mabe, J. Ott, F. T. Counter, P. W. Ensminger, N. E. Allen, W. E. Alborn, Jr., J. N. Hobbs, Jr., *J. Antibiot.* **1990**, *43*, 623 – 633, and references therein.