The Ribosome Functions as a Ribozyme

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protein biosynthesis · ribosomes · ribozymes · RNA structures · translation

Introduction

One of the most exciting developments in biological chemistry in the last two decades has been the discovery that RNA can catalyse chemical reactions. This is no longer the sole preserve of protein-based enzymes, and raises the intriguing possibility that in the early appearance of life on this planet it may have passed through a phase in which RNA served twin roles as both informational and catalytic molecules.

However, RNA has a number of drawbacks as a macromolecular catalyst compared to proteins, the most serious of which is the much more limited range of functional groups that can be brought into play. For example, RNA has to work much harder to modulate the pK_a values of bases if they are to play a role as general acid/bases at neutral pH. The range of chemistry catalysed by RNA in natural ribozymes has been quite limited to date. These have all involved breaking or ligating phosphodiester linkages in RNA by means of transesterification reactions. It was demonstrated that the group I intron ribozyme could be persuaded to exhibit aminoacyl esterase activity,[1] but the rate enhancement achieved was feeble; this is not surprising given the very different stereochemistry of this reaction compared to the normal activity of the ribozyme.

However, the range of reactions that RNA can catalyse has been greatly extended in the laboratory by using RNA enzymes that have been selected from large randomised sequence pools. RNA species have thus been isolated that can bring about cleavage of DNA,[2] RNA polymerisation[3] and ligation,[4] alkyl transfer[5] and acyl transferase activity.[6] Even relatively exotic chemical reactions can be efficiently catalysed by RNA, exemplified by the isolation of a 38-nucleotide (nt) RNA that accelerates a Diels–Alder cycloaddition reaction in trans by over 10^4-fold.[7]

But it now turns out that nature can be quite adventurous too, and that RNA catalysis is of central importance in one of the most important reactions in life. New crystallographic studies of the large subunit of the ribosome at nearly atomic resolution have revealed that peptide bond synthesis is performed by an RNA catalyst, without the direct involvement of protein.

Protein synthesis and the ribosome

Protein synthesis is one of the most fundamental processes occurring in the cell. It requires the repeated formation of peptide bonds between amino acids in a sequence determined by the order of trinucleotide codons in the messenger RNA (mRNA) that carries the base sequence transcribed from the gene. The translation process is accomplished on a large, specialised RNA–protein complex called the ribosome. Transfer RNA (tRNA) molecules function as adaptor species in translation; they "read" the sequence of the mRNA through complementarity of their anticodon loops to individual codons, and carry the appropriate amino acid through acylation of their 3′ terminus.

The bacterial ribosome has a molecular weight of around 2.6 MDa, sedimenting at 70S in the ultracentrifuge. At low magnesium ion concentration it dissociates into two subunits, with a 2:1 mass ratio. The small subunit sediments at 30S, comprising the 16S ribosomal RNA (rRNA) and 21 proteins, while the large subunit sediments at 50S, and comprises the 23S and 5S rRNAs and approximately 31 proteins. In the initiation of translation the small subunit binds to the mRNA before the large subunit, and it is the 30S subunit that is primarily responsible for mediating the decoding of the mRNA sequence. By contrast, the role of the large subunit is the formation of the peptide bonds, generally termed the peptidyl transferase reaction. Thus the small and large subunits play separate roles of information processing and chemistry, respectively.

The ribosome has two main sites at which aminoacyl-tRNAs are bound during the translation process. These are termed the P (peptide) and A (amino acid) sites. The process begins with the binding of *N*-formylmethionine-tRNA (which looks somewhat like a pseudo-peptide) at the P site, and in fact this is the stage at which the 50S subunit binds to form the complete ribosome. A second aminoacyl-tRNA then binds in the A site, with its anticodon bound to the second codon, at which point the chemistry can begin (Figure 1). The nitrogen atom of the α-amino group of the aminoacyl-tRNA bound at the A site attacks the carbon atom of the carbonyl group of the P-site-bound aminoacyl-tRNA^{Met}, forming a peptide bond. The result is a free tRNA bound at the P site, with the nascent peptide attached to the tRNA at the A site. At this point the translocation step occurs; like many steps of translation this requires the participation of a G protein and GTP hydrolysis. The free tRNA diffuses away (via the E site in bacterial ribosomes), and the peptidyl-tRNA translocates into the P site. A new aminoacyl-tRNA binds at the newly vacant A site, and a second cycle of peptide bond synthesis begins. This continues until a termination codon is encountered.

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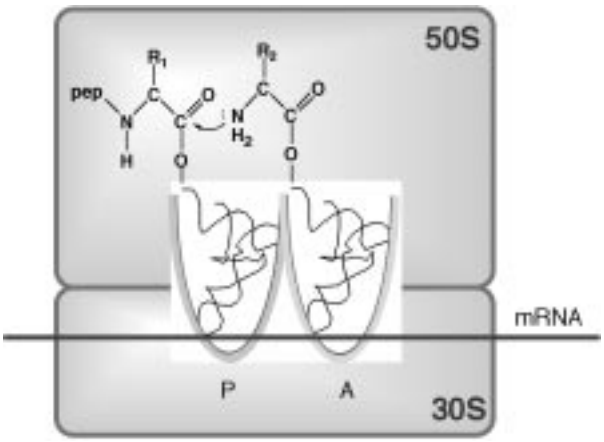

Figure 1. *Schematic representation of protein synthesis on the ribosome. The mRNA is decoded by the tRNA species on the 30S subunit, whereas peptide bond synthesis is catalysed on the 50S subunit. This involves nucleophilic attack by the nitrogen atom of the α-amino group of the aminoacyl-tRNA held at the A site on the carbon atom of the carbonyl group of the peptidoacyl-tRNA bound at the P site.*

The structure of the ribosome

The last three years have seen a determined onslaught on the structure of the ribosome by crystallography (reviewed in ref. [8]). Structures of both subunits[9–11] and the entire ribosome[12] have been solved at different levels of resolution. The most recent chapter in this story has come from Steitz, Moore and colleagues, who have presented the crystal structure of the bacterial 50S subunit at 2.4 Å resolution.[13] The atomic resolution permitted the fitting of 2711 out of the 2923 nt of 23S rRNA; in most cases the sequence could be read from the electron density map with confidence. In addition, the entire 5S rRNA and 27 ribosomal proteins were fitted.

The global shape of the large subunit is approximately that of a hemisphere 250 Å in diameter, with a relatively flat side that forms the interface with the small subunit (Figure 2). There is a deep groove running across this face, which turns out to contain the active site. The edge of the structure contains a number of protuberances, consistent with previous electron microscopic images. The secondary structure of the 23S rRNA is largely in agreement with earlier estimates, and can be considered as being organised in six domains (termed I – VI). However, in the three-dimensional structure these are extensively interconnected, forming a single large entity. The overall structure is largely determined by the RNA, the surface of which is decorated with proteins. Some of these are entirely globular, but some are partly or completely extended and run deep into the RNA core of the ribosome. The RNA contains many interesting features of secondary and tertiary structure, including base triples, pseudoknots and helical junctions. The determination of this structure increases the size of the database of available RNA structures by a significant factor, and mining this information is going to provide a valuable source of insight into RNA conformation for a long time to come.

The peptidyl transferase centre

The peptidyl transferase centre of the ribosome was located[14] by using two cleverly designed inhibitors of translation. These were both based on puromycin, which is an analogue of an aminoacyl-tRNA. One of these inhibitors, the phosphoramidate of the trinucleotide CCdAp and puromycin (CCdA-p-puromycin), uses a phosphate linkage to mimic the tetrahedral intermediate of peptide bond formation, and binds tightly to the ribosome as a result.[15] These compounds were soaked into the ribosome crystals, and the peptidyl transferase centre was thus located in electron density difference maps. It was found to be in the centre of the side facing the 30S subunit, deep within the cleft running across this face (Figure 3). Moreover, a 100 Å tunnel runs from this point to the far side of the 50S subunit, and is the presumed path by which the nascent polypeptide chain exits the ribosome into the cytosol.

The central region of the interface side of the large subunit is largely devoid of protein, and the nearest section of protein was found to be 18 Å away from the peptide analogue bound at the peptidyl transferase centre. The region is entirely composed of tightly packed RNA from domain V of the 23S rRNA. Since there is no way in which any protein could come close to this site, Steitz, Moore and colleagues conclude that peptidyl transfer must be an RNA-catalysed reaction.[14] This is consistent with known genetics on the dispensability of proteins, and with earlier studies of Noller and colleagues[16] who showed that whereas the peptidyl transferase activity of ribosomes is exquisitely sensitive to RNA degradation, it is apparently unaffected by procedures that remove proteins.

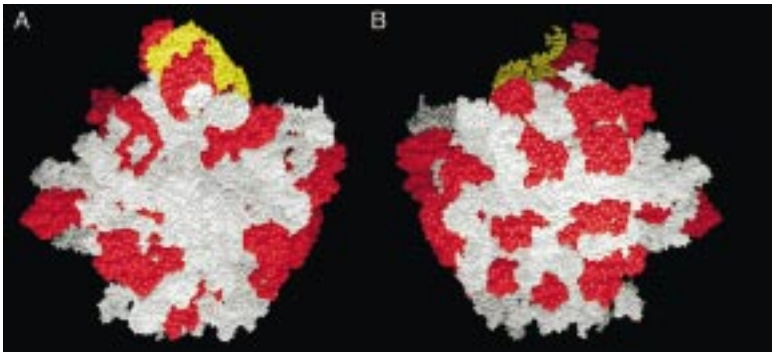

Figure 2. *The structure of the 50S subunit of the ribosome at 2.4 Å resolution. The 23S rRNA is coloured grey, and the 5S rRNA yellow. The proteins are coloured red. A) The view onto the side that interfaces with the 30S ribosomal subunit. Note that the centre is devoid of protein. B) The view onto the reverse side that forms the outer surface of the 50S ribosomal subunit. (These images were prepared using the deposited coordinates of Steitz and co-workers,[13] PDB code 1FFK.)*

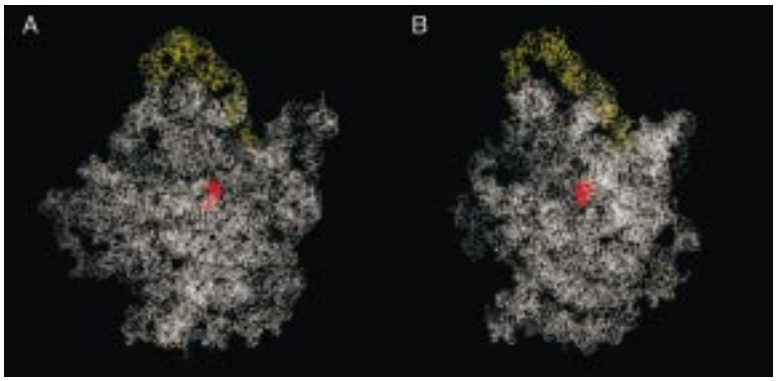

Figure 3. *Location of the peptidyl transferase centre on the 50S subunit of the ribosome. The 23S and 5S rRNA are shown in grey and yellow, respectively, with the active-site nucleotides highlighted in red. The proteins have been removed from these images for clarity. A) View looking down on the face that interfaces with the 30S ribosomal subunit. B) View rotated 90° about the vertical axis, showing the deep cleft that runs across the interface. (These images were prepared using the deposited coordinates of Steitz and co-workers,[13] PDB code 1FFK.)*

So if the peptidyl transferase centre is not an enzyme, what is it? The inhibitor CCdA-p-puromycin was bound to a site in the RNA derived by the folding together of two regions within domain V. Interestingly, the RNA sequence in this region is similar to that of an RNA selected by Zhang and Cech for its ability to catalyse peptide bond synthesis.[17] The closest nucleobase to the nascent peptide bond was found to be adenine 2486 (corresponding to A 2451 in the conventional numbering of *Escherichia coli* 23S rRNA). Perhaps surprisingly, the atom N3 of this base was located 3 Å from the oxygen atom of the phosphoramide moiety (analogous to the carbonyl oxygen atom of the nascent peptide linkage), and 4 Å from the nitrogen atom of the amide group (Figure 4A). No other titratable group was found closer than 5 Å, and thus this was essentially the only candidate for a functional group that might participate in the reaction as a general base.

At first sight, nucleobases appear to be rather poor raw material for this kind of chemistry, because their pK_a value is generally so low. This is one of the problems RNA always faces in performing the role of an enzyme, compared to a protein which has much more promising functional groups. However, in principle the environment could alter the pK_a value to bring it closer to neutrality, that is, comparable to the pH at which the ribosome functions. There is evidence for the perturbation of the pK_a value of a critical cytosine in the hepatitis delta virus ribozyme,[18, 19] and NMR spectroscopic data indicate that an adenine base in the lead-dependent ribozyme (leadzyme) has a pK_a value close to neutrality.[20]

So is there experimental evidence in favour of a substantially altered pK_a value of A 2486 in the 23S rRNA of the 50S subunit? Nissen et al.[14] point out that the N–O distance in the crystal structure indicates that the adenine N3 atom is protonated at the pH of the crystal (5.8), implying a pK_a of >6 for this nitrogen atom. There is also evidence for a perturbed pK_a of A 2486 in solution, derived by measuring the reactivity to methylation

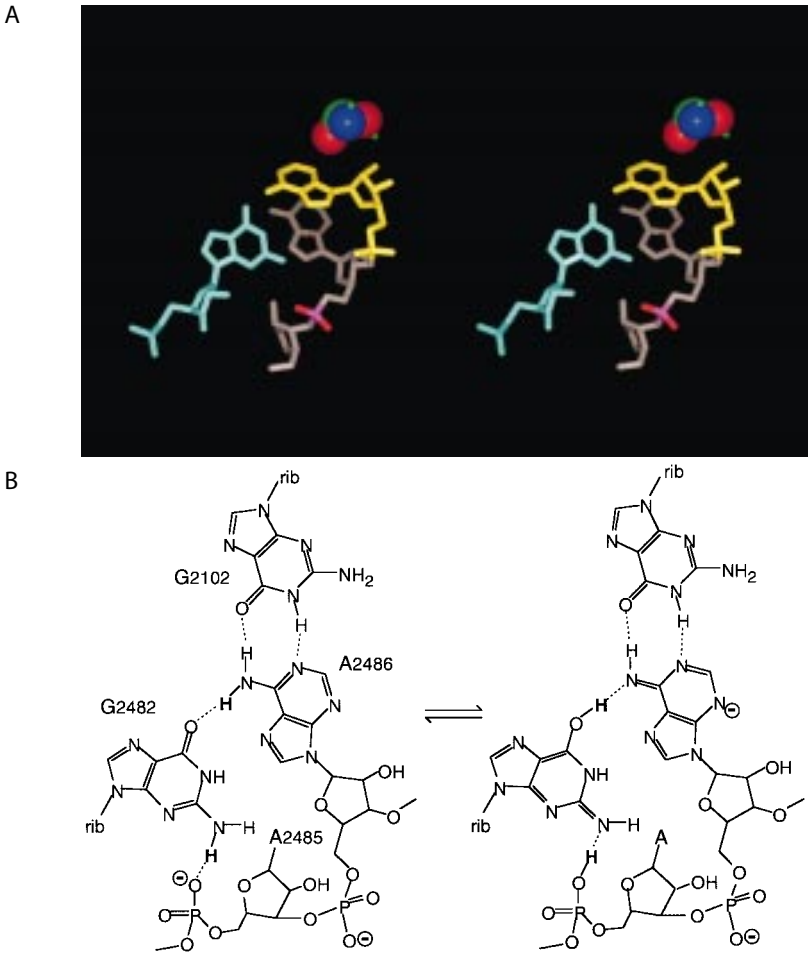

Figure 4. *The active site of the ribosome. A) Stereo view of the position of the nascent peptide, and the proposed active-site nucleotides. The tetrahedral carbon centre (actually a phosphate group in the inhibitor used to determine the structure) is shown in space-filling representation. The nearest nucleobase is adenine 2486 (yellow), which is hydrogen-bonded to guanine 2482 (blue). G2482 is hydrogen-bonded to the buried phosphate group (purple/red) of A2485. (Image prepared from the deposited coordinates of Steitz and co-workers,[14] PDB code 1FFZ.) B) A mechanism to explain an elevated pK_a value of atom N3 of adenine 2486. The proposed charge relay mechanism[14] involves shuttling protons from atom N2 of G2482 to A2485, and from atom N6 of A2486 to O6 of G2482, forming imino tautomers of the two bases. The result is that the negative charge of the buried phosphate group of A2485 is relayed to atom N3 of A2486, thereby raising its pK_a value. rib = ribose.*

by dimethyl sulfate as a function of pH. In an accompanying paper, Strobel and coworkers[21] show that the pK_a value of this adenine must be around neutrality, although they cannot distinguish between protonation of N1 and N3 in these experiments.

Steitz, Moore and co-workers suggest a mechanism by which the pK_a value of the N3 atom of A2486 might be raised in the environment of the ribosome.[14] They note that A2486 is hydrogen-bonded to the guanine base of G2482. In addition, G2482 forms a hydrogen bond to the buried phosphate group of A2485. They suggest that the proton on atom N2 of G2482 (i.e. the exocyclic amine group) could be transferred onto the phosphate group, leading to tautomeric shifts of both A2486 and G2482 to imino forms, with the result that the negative charge ends up being localised on the N3 atom of A2486 (Figure 4B). In essence, they propose that the pK_a value of this nitrogen atom is raised by a charge relay mechanism from the phosphate group via the intervening guanine base, rendering it able to serve as a general acid/base functional group in catalysis at cellular pH. A nearby tightly bound potassium ion may also play a role in this.

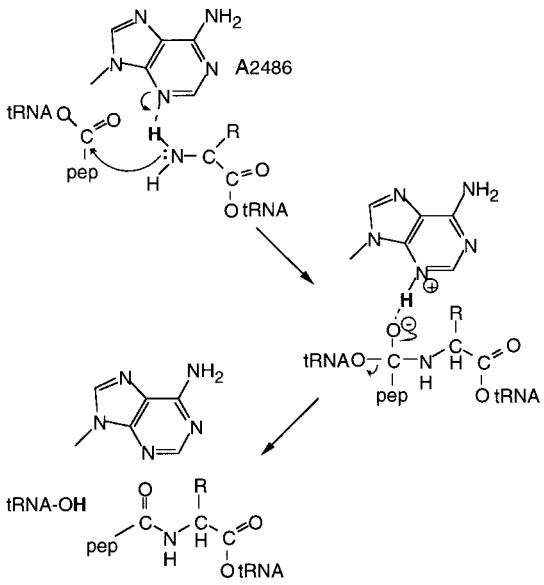

Figure 5. *The catalytic mechanism of peptide bond synthesis proposed by Steitz, Moore and colleagues.[14] A2486 plays a key role, as a general base to increase the nucleophilicity of the attacking nitrogen atom, in the stabilisation of the oxyanion transition state, and in the departure of the leaving group. pep = nascent peptide chain.*

The mechanism of peptide bond synthesis

The formation of the peptide bond requires attack of the α-amino group of the A-site-bound amino acid on the terminal carbonyl carbon atom of the peptide in the P site. It is proposed[14] that a proton of the amino group is transferred to the N3 atom of A2486, thereby increasing its reactivity as a better nucleophile (Figure 5). In addition, it is suggested that the resulting tetrahedral oxyanion is stabilised by accepting a hydrogen bond from the transiently protonated N3 atom of A2486. On resolution of the tetrahedral intermediate, the proton is finally transferred to the 3′ oxygen atom of the liberated tRNA in the P site, which can then diffuse away, via the E site of the ribosome. The mechanism should probably be regarded as a working model at present, and will undoubtedly be subjected to intense experimental scrutiny and refinement in the next period.

A number of features of the proposed mechanism of peptidyl transferase bear comparison with protein enzymes, and in particular with the serine proteases. Enzymes like α-chymotrypsin essentially carry out the opposite of peptide bond formation, and the peptidyl transfer can be compared to the reverse of the first half of this reaction. In addition, the serine proteases use a charge relay system (Asp^{102} – His^{57} – Ser^{195}) to increase the reactivity of a serine hydroxy group in the nucleophilic attack on the carbonyl group of the peptide bond.[22] The RNA and protein enzymes use a number of common features in their catalytic strategies, including orientation of reacting groups, acid/base catalysis and transition state stabilisation in the active site, and modulation of functional group pK_a values. Despite the very different character of the two biopolymers, RNA and protein may have arrived at rather similar mechanisms to carry out related tasks.

Conclusion

The most important conclusion of the new study of the 50S subunit can be simply stated—the ribosome is a ribozyme! This is an extremely significant finding for a number of reasons. It places RNA catalysis centre stage in cell function, carrying out one of the most important reactions in the processes of life. No longer is RNA catalysis the sole preserve of a few slimy things found in ponds and the like, but it is playing a key role in all cells. Moreover, the demonstration that peptidyl transfer is catalysed by RNA markedly extends the range of chemistry that ribozymes carry out in nature. Previously all the known ribozymes carried out variations on the theme of phosphoryl transesterification reactions, but nucleophilic attack on an sp^2 carbon atom is a significant new departure in ribozyme chemistry. The ribosome may afford us a glimpse into the postulated RNA world that existed before proteins provided the chemical muscle of the cell. Thus the ribosome may be a kind of molecular fossil sitting inside us all. This poses the question of whether there are other ribozymes carrying out important functions in the cell that are hitherto unsuspected. An obvious contender for this would be the spliceosome. The chemical similarity of mRNA splicing and the group II intron ribozyme[23] suggests that the former could well be RNA-catalysed, and no enzyme has yet been assigned to this role. It would take a brave man to bet against this. The ribosome has provided a huge shot in the arm for RNA chemistry, and the field is now set to march on to further glories.

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[1] J. A. Piccirilli, T. S. McConnell, A. J. Zaug, H. F. Noller, T. R. Cech, *Science* **1992**, *256*, 1420 – 1424.
[2] D. L. Robertson, G. F. Joyce, *Nature* **1990**, *344*, 467 – 468.
[3] E. H. Ekland, D. P. Bartel, *Nature* **1996**, *382*, 373 – 376.
[4] E. H. Ekland, J. W. Szostak, D. P. Bartel, *Science* **1995**, *269*, 364 – 370.

[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

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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

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