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Nonribosomal peptides for iron acquisition: pyochelin biosynthesis as a case study

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Microbes synthesize small, iron-chelating molecules known as siderophores to acquire iron from the environment. One way siderophores are generated is by nonribosomal peptide synthetases (NRPSs). The bioactive peptides generated by NRPS enzymes have unique chemical features, which are incorporated by accessory and tailoring domains or proteins. The first part of this review summarizes recent progress in NRPS structural biology. The second part uses the biosynthesis of pyochelin, a siderophore from *Pseudomonas aeruginosa*, as a case study to examine enzymatic methods for generating the observed diversity in NRPS-derived natural products.

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Iron uptake: siderophores

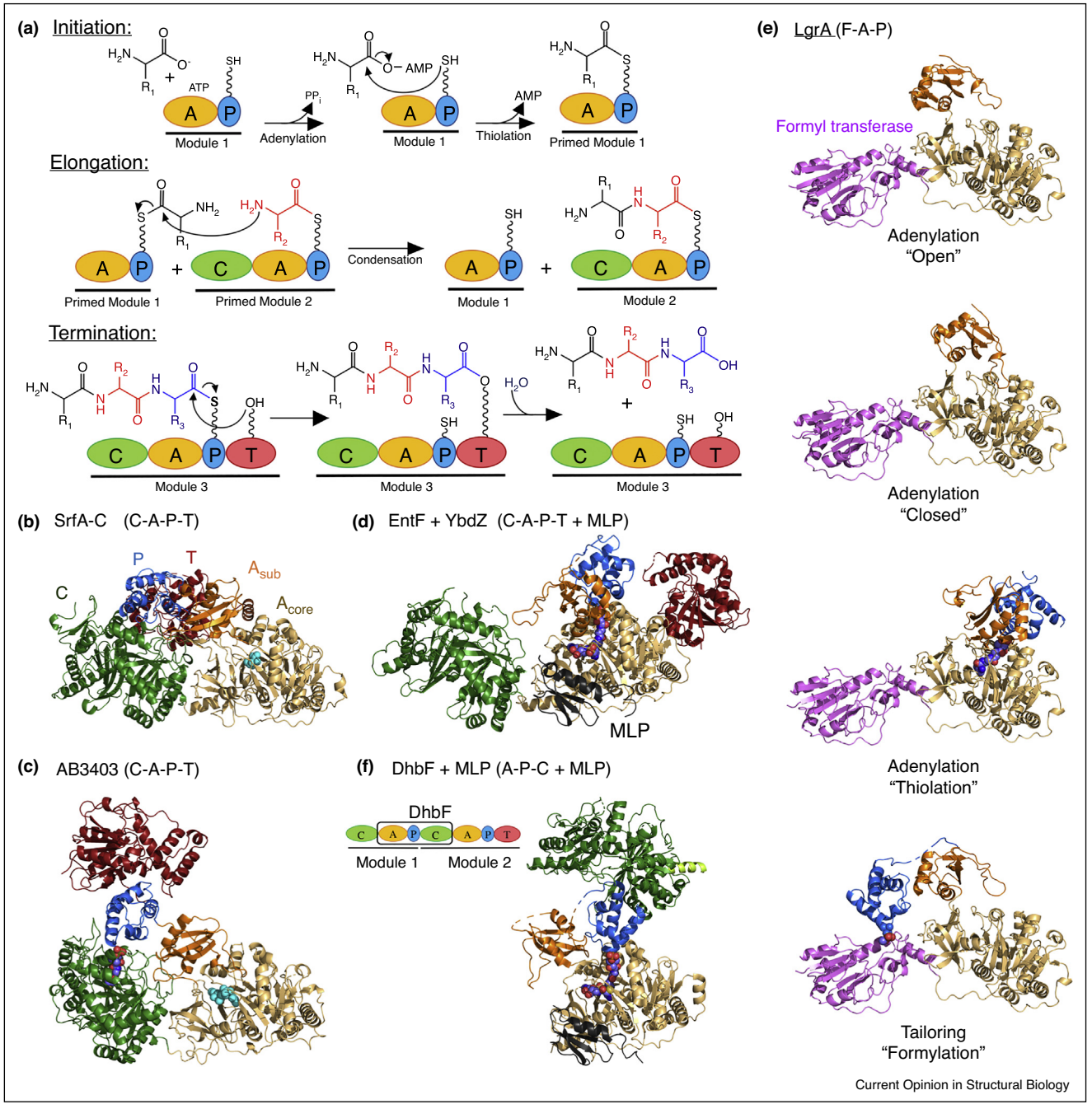
Iron is an essential nutrient that is needed by microbes to perform critical biological processes necessary for survival [1]. Due to the paucity of free iron in aerobic environments, microbes have developed intricate systems to acquire iron from their surroundings [1]. One such system is the biosynthesis of small molecules known as siderophores that have a high affinity for ferric iron [2]. When iron availability is low, microbes synthesize and secrete siderophores, and selectively reimport the iron-loaded form from the surrounding environment. Siderophore biosynthesis in bacteria is accomplished by nonribosomal peptide synthetase (NRPS) enzymes, polyketide synthase (PKS) enzymes, and/or by NRPS independent siderophore (NIS) synthetase enzymes [3–5]. Typically these enzymes do not have human homologues and knockdown experiments of siderophore biosynthesis have shown partial or complete suppression of pathogenicity for many different bacteria, making these enzymes attractive drug targets [6]. This review will provide an overview of the structural biology of NRPS enzymes and then focus on the structural and catalytic aspects of the enzymes involved in the biosynthesis of pyochelin, a siderophore from *Pseudomonas aeruginosa*, highlighting the involved accessory and tailoring activities that generate the unique chemical structure of pyochelin.

Nonribosomal peptide synthetases (NRPS): chemical logic of peptide chain formation

NRPS enzymes are large multidomain and multifunctional enzymes that display a chemical logic in which each module is responsible for the addition of a single amino acid to a growing peptide chain, including non-proteinogenic amino acids and hydroxy acids. An NRPS module consists of a condensation (C) domain, an adenylation (A) domain, and a peptidyl carrier protein (P) domain, except for the first initiation module, which lacks a condensation domain. The P-domain acts as a tethering system for the growing peptide chain, and must be post-translationally modified with coenzyme A on a conserved serine residue thereby generating a 4′phosphopantetheinyl (Ppant) swinging arm. A-domains have been named the 'gate keepers' of the NRPS assembly line as they selectively activate and incorporate the appropriate amino acids into the growing peptide chain [7]. The A-domains activate an amino acid by catalyzing the formation of an aminoacyl-AMP, using ATP and releasing inorganic pyrophosphate (Figure 1a). The Ppant thiol of the P-domain performs a nucleophilic attack on the carboxyl group of the aminoacyl-AMP, eliminating AMP and generating an aminoacyl-thioester bond, thereby priming the module. Once primed, the thioester tether on the P-domain transfers its amino acid cargo to subsequent domains, similar to a mechanical crane.

Peptide bond formation, or elongation, is catalyzed between the amino acids of two primed modules within the C-domain of the downstream module. The upstream thioester bond is broken as the peptide bond is formed, thereby transferring the elongating peptide to the downstream module. This process is repeated with each subsequent module until the full chain is made. Chain elongation is terminated by a thioesterase (T) domain in the terminal module [8]. The peptide is transferred from the thioester of the P-domain to a conserved serine residue of the T-domain, generating an amino ester, and allowing for hydrolysis and release of the mature peptide [8].

Figure 1



NRPS assembly line and crystal structures highlighting intra-domain and inter-domain contacts and movements. **(a)** NRPS natural product biosynthesis takes place in three phases: initiation, elongation, and termination. Adenylation and subsequent thiolation of the amino acid onto the Ppant tether prime a module. A condensation reaction is performed between amino acids tethered to primed modules to generate a peptide bond, thereby elongating the peptide chain. Termination and release of the mature peptide occurs by hydrolysis within a terminal thioesterase domain after transfer of the product to a conserved serine. Throughout this review: yellow = adenylation (A); blue = peptidyl carrier domain (P); green = condensation (C); red = thioesterase (T). **(b)** The first crystal structure of a complete NRPS module, SrfA-C (PDB: 2VSQ). Note that the A_{core}-domains (light yellow) are aligned in parts (b) – (f) of this figure to highlight domain movements. The substrate leucine (cyan spheres) is in the A_{core}-domain. The A_{sub}-domains are in dark yellow. **(c)** The full module of AB3403 (PDB: 4ZXI) with the Ppant tether (purple carbon and red oxygen spheres) in the closed C-domain ready to accept an upstream peptide. The substrates AMP and Gly (cyan spheres) are in the A_{core}-domain active site. **(d)** A full-module crystal structure of EntF with its MLP, YbdZ (black), bound to the A_{core} (PDB: 5JA1). The mechanistic-based inhibitor, Ser-AVS, is covalently attached to the Ppant tether (spheres) which is trapped in the A_{core}-domain. **(e)** Domain movements of the A_{sub}-domain and P-domain are highlighted by the multi-domain structures of LgrA. These snap-shots show the movement of the A_{sub}-domain in 'open' and 'closed' (PDB: 5ES5) conformations during adenylation, and the movement of the A_{sub}-domain and P-domain during thioester