Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Mechanisms of cyanobactin biosynthesis

Clarissa Melo Czekster[1,3], Ying Ge[1,3] and James H Naismith [1,2,3]

Cyanobactins are a diverse collection of natural products that originate from short peptides made on a ribosome. The amino acids are modified in a series of transformations catalyzed by multiple enzymes. The patellamide pathway is the most well studied and characterized example. Here we review the structures and mechanisms of the enzymes that cleave peptide bonds, macrocyclise peptides, heterocyclise cysteine (as well as threonine and serine) residues, oxidize five-membered heterocycles and attach prenyl groups. Some enzymes operate by novel mechanisms which is of interest and in addition the enzymes uncouple recognition from catalysis. The normally tight relationship between these factors hinders biotechnology. The cyanobactin pathway may be particularly suitable for exploitation, with progress observed with *in vivo* and *in vitro* approaches.

Addresses
[1] Biomedical Sciences Research Complex, University of St Andrews, North Haugh, St Andrews, Fife KY16 9ST, Scotland, UK
[2] State Key Laboratory of Biotherapy, Sichuan University, China

Corresponding author: Naismith, James H (naismith@st-andrews.ac.uk)
[3] All authors contributed equally to the review.

Current Opinion in Chemical Biology 2016, **35**:80–88

This review comes from a themed issue on **Mechanistic biology**

Edited by **Gregory L Challis** and **Brian Bachmann**

http://dx.doi.org/10.1016/j.cbpa.2016.08.029

Introduction

Despite approximately 3000 human genes having been estimated to be involved in disease states, only a fraction (between 20 and 50%) are thought to be potentially responsive to inhibition by traditional small molecule drugs [1]. Natural products (NPs) have long been successfully employed to bridge the gap between inhibitors and 'undrugable' targets [2]. A particularly promising class of NPs is the ribosomally synthesized and post-translationally modified peptides (RiPPs). These NPs offer the practicality of genetically encoded compound libraries, which can be readily derivatized both enzymatically and chemically giving rise to very diverse molecules.

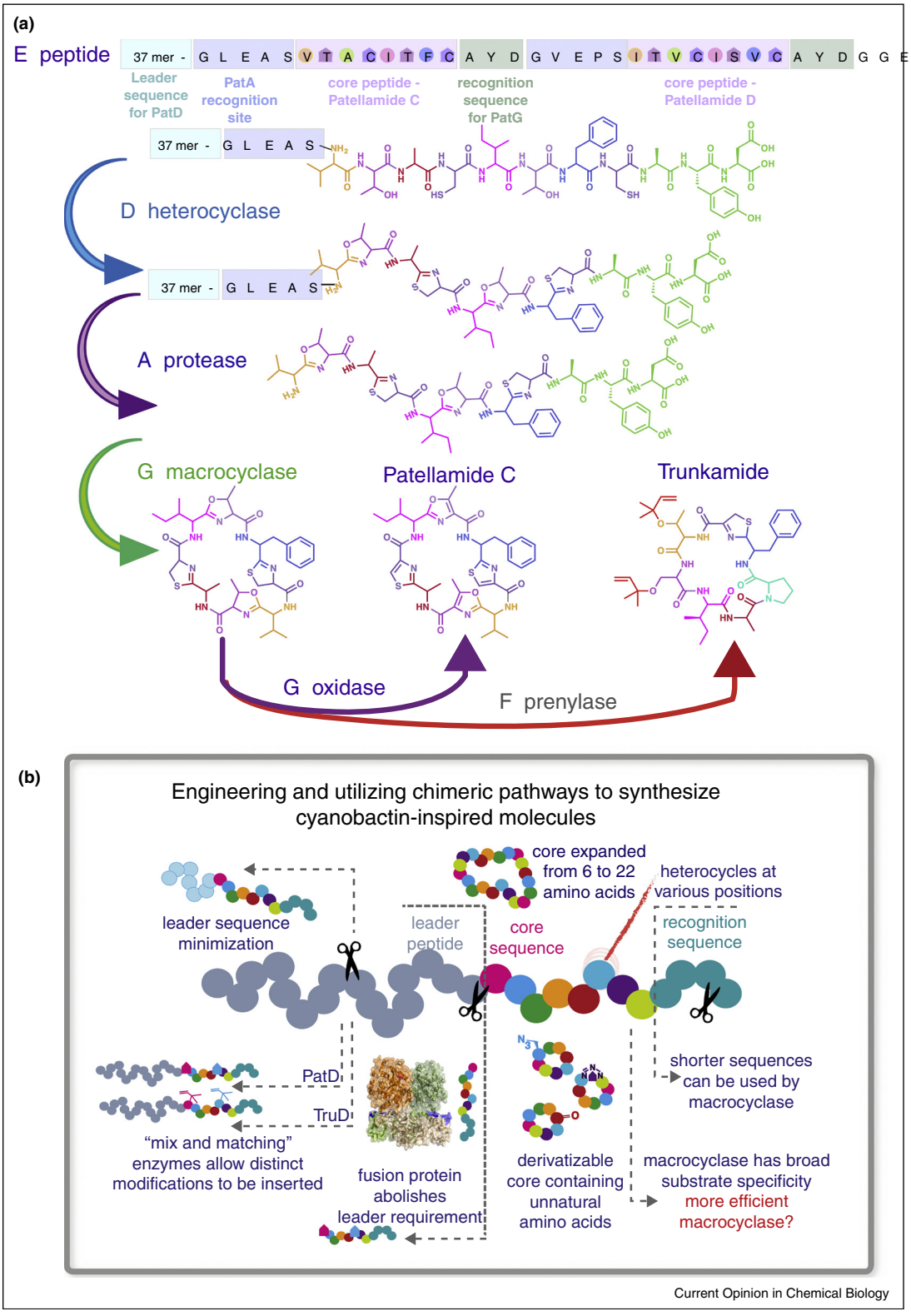
Cyanobactins are RiPPs which in general, although not always, contain a macrocyclic ring — forming a peptide bond between the N-terminal amine and another amino acid next to a recognition sequence. They can also contain heterocyclized (cyclodehydratized) serine, threonine and/or cysteine residues yielding (methyl)oxazoline and thiazoline rings or isoprenoid amino acids derivatives [3,4]. Cyanobactins are encoded as a precursor peptide ('E' in Figure 1a) which possesses a leader sequence recognized by the cyclodehydratase proteins responsible for heterocycle formation ('D' in Figure 1a). Once heterocyclized, the precursor peptide is cleaved by a serine protease ('A' in Figure 1a) to contain solely the core natural product sequence followed by a short recognition sequence. The core peptide sequence is then macrocyclized by another serine protease working 'in reverse', that is catalyzing peptide bond formation to yield the cyclic peptide ('G' in Figure 1a). The cyclic peptide can then be further post-translationally modified by prenylation ('F' in Figure 1a) and/or oxidation to include thiazoles and oxazoles (Oxidase domain of the 'G' protein in Figure 1a).

This pathway, albeit extremely promising in terms of generating RiPPs diversity, has several defects from a technology view that should be improved to make the production of diverse cyclic peptides in large scale routine. Several approaches have been used to trim the precursor peptide by eliminating the requirement of a long peptide leader (which is discarded during processing) [5••], to introduce unnatural amino acids, to process long sequences in the core peptide [6•,7••], to devise chimeric pathways utilizing enzymes from different pathways and organisms and to produce cyanobactins *in vitro* in a one-pot reaction [7••,8]. Figure 1b schematically illustrates the progress made so far and highlights targets for further improvement. Our understanding of the catalytic and chemical mechanisms of the enzymes involved in cyanobactin biosynthesis has greatly increased in the past few years. In this review article we discuss the major discoveries and current proposed mechanisms for the enzymes involved in cyanobactin biosynthesis.

Heterocyclase

Peptide heterocycles reduce polarity, add chemical diversity and conformational rigidity to peptides. They are common in both linear and macrocyclic bioactive natural products [9,10]. Many cyanobactins, such as patellamides [11–15], trunkamides [16,17] and microcyclamides [18–20] contain thiazol(in)e or oxazol(in)e rings within their cyclic backbones. Phylogenetic studies [21–23] showed that clusters capable of biosynthesizing cyanobactins that bear heterocycles encode a YcaO domain-containing heterocyclase (cyclodehydratase), denoted in each pathway

Figure 1



Exploring and improving the biosynthesis of cyanobactins. **(a)** Natural products are synthesized as a precursor peptide ('E' in Figure 1a), which is sequentially processed by the action of cyclodehydratase proteins responsible for heterocycle formation ('D' in Figure 1a), a serine protease ('A' in Figure 1a), a macrocyclase ('G' in Figure 1a), and finally modified by prenylation ('F' in Figure 1a) and/or oxidation (oxidase domain of the 'G' protein in Figure 1a). **(b)** There is considerable potential for engineering cyanobactins.