A NOVEL SYSTEM FOR PEPTIDE BOND FORMATION ON NATURAL PRODUCT BIOSYNTHESIS FOUND IN *STREPTOMYCES LIVIDANS* 1326

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Introduction. Non Ribosomal Peptides (NRP) are synthesized by modular systems, which typically include at least two adenilation (A) domains, a condensation (C) domain and a thioesterase or peptide release (T) domain.

Using an evolution-inspired mining approach (1) we found a novel natural product biosynthetic gene cluster in *Streptomyces lividans* 1326. This gene cluster includes a NRP synthetase containing a single A and Reductase (R) domain but no C domains (SLI0883); instead, a homolog of the leucyl/phenylalanyl tRNA protein transferase (L/F transferase; SLI0884) could be annotated.

L/F transferases catalyze the transfer of leucine or phenylalanine from an aminoacyl-tRNA to the N-terminal basic residue of a protein via the N-end rule protein degradation pathway. *In silico* analysis of the A domain suggested its specificity to a basic residue, most likely Arginine. Thus, we predicted that the L/F transferase would attach a Leu or Phe residue, from the cognate aminoacyl-tRNA to the PCP bound Arg residue; afterwards the peptide will be released by the action of the reductase (R domain) upon the thioester group.

Remarkably, this biosynthetic gene cluster is encoded within the plasmid SLP3, which has been linked to metal homeostasis by our group in a recent study (2).

Methods. Evolution-inspired genome mining was used for the identification of the gene cluster (1). Knock-out mutants of SLI0883 and SLI0884 were constructed and confirmed. In order to infer the physiological role of the peptide biosynthetic system we performed transcriptional analysis of the response to copper-induced stress and phenotypical analysis of the Knock-out mutants under stress induced by high concentrations of Cu, Co, Ni, Fe, Mn, and Zn, or general metal depletion.

Results. Transcriptomic analysis showed the involvement of genes associated with SLI0883-4 in copper tolerance, and revealed the presence of metal-associated operator sequences within the gene cluster. Nevertheless, the phenotypic analysis of the K.O. mutants only showed a phenotypical difference on high Ni concentrations.

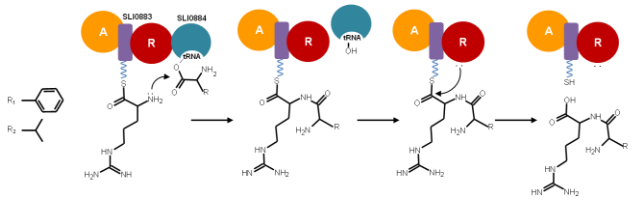

Figure 1. Proposed mechanism of peptide bond formation by the NRPS-tRNA biosynthetic hybrid system.

Conclusions. Genome context and transcriptomic analysis as well as phenotypic characterization of mutants lacking the peptide forming biosynthetic system suggests that this compound is involved in transition metal homeostasis, most likely Ni tolerance.

This is the first time a L/F transferase is found as part of a NRP biosynthetic system, however the same condensation strategy has been previously reported in evolutionary unrelated amynoacyl-tRNA transferases (2, 3 and 4), indicating the convergent recruitment of tRNA-dependent systems to NRP biosynthesis

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