CONVERSION OF AMINO ACIDS TO NON-RIBOSOMALLY FORMED PEPTIDES: GENETICS, BIOCHEMISTRY AND STRUCTURAL BIOLOGY OF THE INTERACTION OF ADENYLATING ENZYMES WITH MbtH-LIKE PROTEINS

Björn Boll[1], Dominik A. Herbst[2], Georg Zocher[2], Thilo Stehle[2], Lutz Heide[1]
[1]Pharmaceutical Institute, [2]Interfaculty Institute of Biochemistry
University of Tuebingen, 72076 Tuebingen, Germany
heide@uni-tuebingen.de

Key words: Non-ribosomal peptide biosynthesis; aminocoumarins; MbtH-like proteins

Introduction. The biosynthesis of non-ribosomally formed peptides, such as vancomycin, as well as of aminocoumarin antibiotics, siderophores and glyco-peptidolipids requires the activation of amino acids by adenylate formation. The biosynthetic gene clusters of these bacterial secondary metabolites frequently contain genes for small, so-called MbtH-like proteins of about 70 amino acids. Recently, it was discovered that MbtH-like proteins form complexes with certain adenylating enzymes and that they are required for the activity of those enzymes. However, a considerable number of gene clusters for non-ribosomally formed peptides and related metabolites do not contain *mbtH*-like genes. Even within a single biosynthetic gene cluster, some of the amino acid adenylating enzymes or domains are dependent on MbtH-like proteins, but others are not. The reason for this difference is unknown, and the precise mechanism by which MbtH-like proteins contribute to the adenylation has remained unknown.

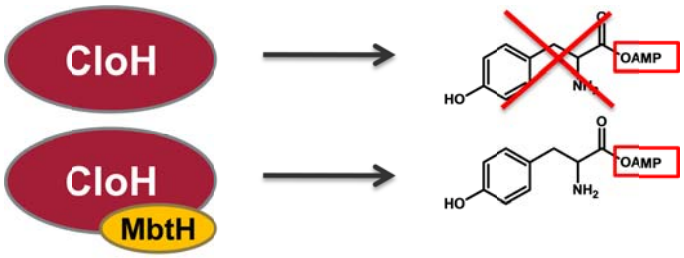

Fig. 1. Tyrosine adenlytion by CloH requires the presence of MbtH-like proteins.

Results. The tyrosine-adenylating enzyme CloH of aminocoumarin antibiotic biosynthesis, and the very similar Pcza361.18 of vancomycin biosynthesis, are dependent on the presence of MbtH-like proteins which represent separate polypeptides. In contrast, SlgN1, a 3-methylaspartate adenylating enzyme involved in the biosynthesis of the hybrid polyketide/NRP antibiotic streptolydigin, contains an MbtH-like domain within the same polypeptide chain. We determined the structure of SlgN1 by X-ray crystallography. Highly conserved tryptophan residues of the MbtH-like domain contribute to its interaction with the adenylation domain. Mutation of S23 of the MbtH-like domain to tyrosine resulted in strongly reduced activity. However, the activity of this S23Y mutant could be restored by addition of an intact MbtH-like protein. This suggests that the interface found in the structure of SlgN1 is the genuine interface between MbtH-like proteins and adenylating enzymes. The MbtH-like domain binds at a site which is distant from the active center and does not make any direct interactions with the substrates. A direct catalytic influence of the MbtH-like domain on the adenylation reaction can therefore be excluded.

RubC1 is a bifunctional enzyme which catalyzes two key reactions in the biosynthesis of the aminocoumarin antibiotic rubradirin. The C-terminal region of RubC1, hereafter termed RubC1b, contains an adenylation and a peptidyl carrier protein domain and activates tyrosine as a precursor of the aminocoumarin moiety. It shows high sequence similarity to the tyrosine adenylating enzymes CloH and Pcza361.18. These two enzymes are dependent on the presence of MbtH-like proteins for their activity. However, the rubradirin gene cluster does not contain an *mbtH*-like gene, and RubC1 shows tyrosine-adenylating activity in the absence of MbtH-like proteins.

We expressed RubC1 as well as RubC1b and investigated them for tyrosine adenylation activity using the ATP/PP_i exchange assay. RubC1b alone was found to be completely inactive, but when an MbtH-like protein was added to RubC1b, a strong tyrosine-adenylating activity was found. In sharp contrast, the RubC1 holoenzyme was able to catalyze the adenylation of tyrosine without addition of MbtH-like proteins. No part of RubC1 shows any sequence similarity to MbtH.

Conclusions. MbtH-like proteins may function as allosteric regulators of adenylating enzymes. However, the effect of MbtH-like proteins on the activity of amino acid-adenylating enzymes is not restricted to proteins with sequence homology to MbtH.

Acknowledgements. This work has been supported by a grant from the Deutsche Forschungsgemeinschaft (SFB766).

References.

1. Baltz, R. H. (2011) Function of MbtH homologs in nonribosomal peptide biosynthesis and applications in secondary metabolite discovery. *J. Ind. Microbiol. Biotechnol*. 38, 1747-1760