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Posttranslational Peptide-Modification Enzymes in Action: Key Roles for Leaders and Glutamate

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In this issue of *Cell Chemical Biology*, Ortega et al. (2016) determine the structure of another lantibiotic dehydratase with a tRNA^{Glu}-dependent mechanism of modification. Moreover, they identify a common recognition motif involved in leader peptide binding in a number of different peptide-modification enzymes. These findings open up new mining possibilities and allow novel approaches in peptide engineering.

Ribosomally produced and posttranslationally modified peptides (RiPPs) form a group of compounds that has gained increased attention over the last several years (Arnison et al., 2013). These peptides display a great chemical diversity and reflect Nature's optimization strategy that separates substrate recognition (based on leader peptide binding) from catalysis to produce a large variety of bioactive molecules employing a modest number of promiscuous enzymes. Understanding the mechanism and substrate requirement of these enzymes is of particular interest not only because they are of fundamental biochemical interest as they display an array of unique activities, but because the knowledge gained from them can open possible uses in host/substrate combinations different from the natural one, thus increasing the diversity of the RiPPs we have available to us.

Lantibiotics are RiPPs that exhibit antimicrobial activity (Knerr and van der Donk, 2012). They contain several lanthionine rings that originate from the addition of a thiol group from a cysteine to a dehydroamino acid. Both the dehydration and cyclization steps are performed by either one or two different enzymes. In type II lantibiotics, a single multifunctional enzyme (LanM) can first activate the serine and threonine residues via phosphorylation and then dehydrate and finally catalyze the formation of thioether bridges. Conversely, in type I lantibiotics the modifications are carried out by two separate

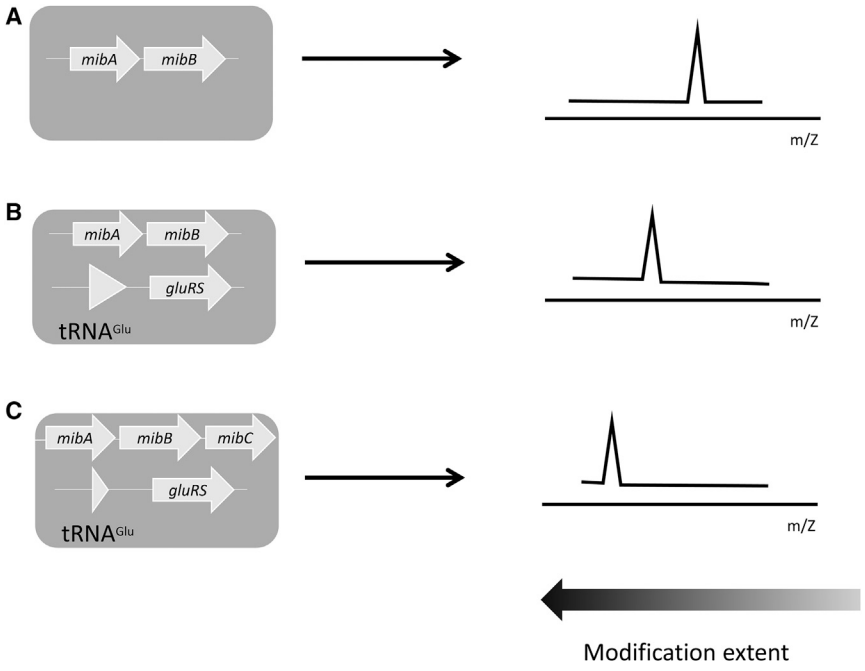

Figure 1. Schematic Representation of the Modification Extent of Microbisporicin
Schematic representation of the modification extent of microbisporicin in *Escherichia coli* (A) inducing the structural gene (*mibA*) and the dehydratase (*mibB*), (B) including the tRNA^{Glu} and tRNA-synthetase, and (C) adding the cyclase gene *mibC*.

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enzymes, i.e., a dehydratase, LanB, which has no significant homology to LanM, and a cyclase generally termed LanC that shares some homology with the type II enzymes. Recently, the structure of the lantibiotic dehydratase NisB was reported (Ortega et al., 2015). NisB has a domain responsible for the glutamylation of serines and threonines, unlike the intermediate phosphorylation observed with LanM enzymes, and an elimination domain that breaks the aminoacyl-prepeptide intermediate to install the dehydroamino acid. The glutamyl residue is provided by a glutamyl-tRNA, which is an unprecedented mechanism. This $tRNA^{Glu}$-dependent dehydration has now also been observed in microbisporicin (Ortega et al., 2016) and in the thiopeptide thiomuracin from a different RiPP class (Hudson et al., 2015). This suggests a possible general dehydration mechanism of type I lantibiotic dehydratases, shared by other homologs even when the glutamylation and elimination domains are split as in the case of thiomuracin modification enzymes. In both cases, i.e., microbisporicin and thiomuracin, a clear preference for $tRNA^{Glu}$ has been observed. Thus, the heterologous expression of the enzyme together with the host $tRNA^{Glu}$ results in no or only partial modification of the substrate, even though the native leader peptide is attached. The addition of the native $tRNA^{Glu}$ to the system substantially increases the modification extent (Figures 1A and 1B).

The crystal structure of NisB contained the substrate prenisin, showing a recognition motif for the nisin leader peptide. The structure of the dehydratase MibB from the microbisporicin biosynthesis machinery without substrate attached is also available. This motif identified in NisB is also present in MibB (Ortega et al., 2016). Moreover, it is present as well in other RiPP PTM enzymes that install quite different modifications, such as MccB from the microcin J25 gene cluster, the cyclodehydratase LynD from cyanobactins, the type II cyclodehydratase CylM, or the dehydrogenase cofactor PqqD (Figure 2) (Burkhart et al., 2015; Ortega and van der Donk, 2016).

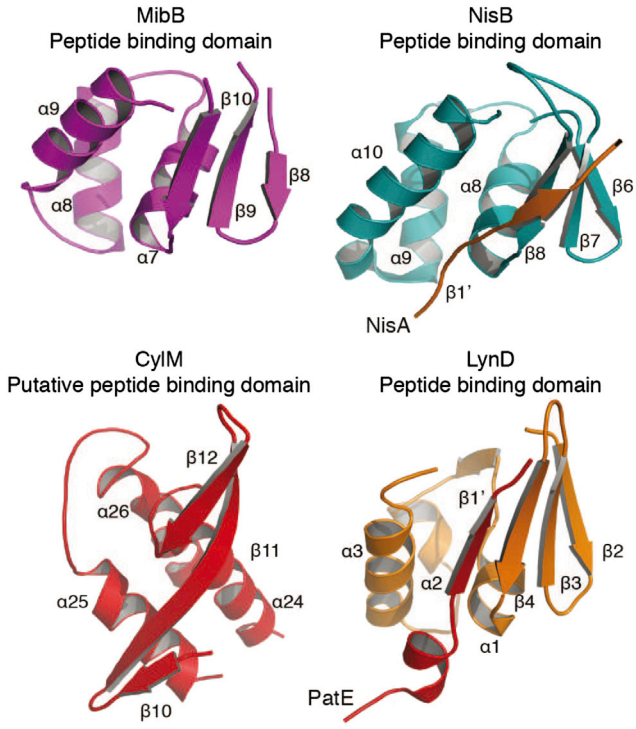

Figure 2. Structural Representation of the Leader Recognition Motif
Structural representation of the leader recognition motif formed by three-stranded antiparallel β sheets followed by a winged helix-turn-helix motif of MibB (magenta), NisB (cyan), CylM (red), and LynD (orange). Figure reproduced from Ortega et al., 2016, with permission.

Remarkably, the presence of the cyclase MibC is required for the full modification of microbisporicin (Figure 1C), stressing the importance of the correct interaction and alternating modification by the dehydratase and cyclase for complete biosynthesis (Lubelski et al., 2009; Ortega et al., 2016). Considering that for the maturation of thiomuracin, the oxazole rings must be present before dehydration and the influence of core peptide binding to the PTM enzyme in haloduracin (Burkhart et al., 2015; Thibodeaux et al., 2015), the role of the core peptide as an additional recognition motif is essential but not yet fully understood.

These data provide an explanation for some of the observed difficulties in the heterologous expression of lantibiotics and indicate the importance of the native $tRNA^{Glu}$ for complete dehydration. This knowledge will further facilitate the use of well-known and easily modifiable strains for heterologous expression. The identification of a common leader recognition motif in diverse PTM enzymes can in the future facilitate the in silico identification of novel RiPP clusters, even if they might belong to yet unidentified RiPP classes. Additionally, it opens new venues for peptide engineering, both in vitro and in vivo, using such enzymes in various combinations. However, it still remains a challenge to elucidate how the PTM enzymes dynamically interact with each other and with the substrate during modification in the bacterial cell, where strict compartmentalization is not possible. Will the leader be bound at the same cleft during the whole processive modification of the prepeptide or will it slide, possibly by a pulling force? Is there a hopping or scooting mode of the modification enzymes during the sequential modifications? The information discussed here paves the way to a better understanding of these intriguing processive enzymes and will enable the development of even more highly valuable bioactive compounds.

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