Molecular Mechanism of Specific Aminoacyl-tRNA Acetylation by *Salmonella* Typhimurium TacT

The bacterial toxin–antitoxin system contributes to the stress adaptation and persistence of bacteria for survival under environmental stresses and is sometimes involved in bacterial pathogenesis. The Gcn5-related N-acetyltransferase (GNAT) family toxins inhibit cellular protein synthesis by acetylating the α-amino group of the aminoacyl moiety of aminoacyl-tRNAs (aa-tRNAs). While the GNAT toxins from various bacteria have been shown to target different aminoacyl-tRNA species, the molecular basis for their substrate specificities has remained elusive. This report presents the crystal structure of the GNAT toxin TacT from *Salmonella* Typhimurium in complex with acetyl-Gly-tRNA^{Gly}(AcGly-tRNA^{Gly}), revealing the molecular basis of the specific aminoacyl-tRNA acetylation by TacT.

A bacterial toxin–antitoxin (TA) module is a gene pair of a protein toxin that induces cell growth arrest and an antitoxin that counteracts the toxin. Toxins in TA modules target pivotal cellular processes, including DNA replication, transcription, translation, and cell wall synthesis. TA modules have been implicated in bacterial stress adaptation, persistence, and dormancy to survive under various environmental stresses and are sometimes involved in bacterial pathogenesis.

In the last several years, a new type-II TA module, in which the toxin belongs to the Gcn5-related N-acetyltransferase (GNAT) family, has been identified in various bacteria. The GNAT toxin family includes AtaT and AtaT2 from enterohemorrhagic *E. coli* O157:H7 [1-3], ItaT from the *E. coli* HS strain [4, 5], TacT, TacT2, and TacT3 from *Salmonella* Typhimurium and Enteritidis [6, 7], and others. GNAT toxins acetylate the α-amino group of the aminoacyl moiety of aminoacyl-tRNAs (aa-tRNAs), using acetyl-CoA (AcCoA) as the acetyl group donor, thus inhibiting cellular protein synthesis. GNAT toxins from various bacteria reportedly target different aa-tRNA species, and exhibit diverse specificities toward aa-tRNAs.

Among the GNAT toxins, TacT found in *Salmonella* was suggested to be involved in persister formation during the infection of macrophages, which could cause a relapse of the infection after antibiotic treatment [6, 7]. *In vitro*, TacT reportedly acetylated several kinds of isoaccepting aa-tRNAs and inhibited the elongation process of protein synthesis. However, the molecular mechanisms of the preference and selectivity toward aa-tRNAs by TacT had remained elusive.

Since we found that TacT exclusively acetylates Gly-tRNA^{Gly} isoacceptors *in vitro* and *in vivo*, we determined the crystal structure of the TacT in complex with acetyl-Gly-tRNA^{Gly} [**Fig. 1(a)**] [8]. Diffraction data were collected at beamlines BL-17A and BL-1A. In the structure of the TacT:AcGly-tRNA^{Gly} complex, two tRNA^{Gly} molecules bind with one TacT homodimer [**Fig. 1(a)**]. The 3'-acceptor region of each tRNA molecule interacts with the positively charged interfaces between the two molecules of the TacT homodimer [**Fig. 1(b)**], suggesting that TacT dimerization is required for the aa-tRNA binding. The 3'-terminal single-stranded region and the top half of the acceptor stem in tRNA^{Gly} interact with TacT [**Fig. 1(a)**, **(b)**], where the specific sequences in tRNA^{Gly} are recognized by TacT via hydrogen bonds. Among them, the discriminator base U73 forms hydrogen bonds with the side and main chains of Asn79, and the G71 base forms hydrogen bonds with the side chain of Arg78 (**Fig. 2**). Accordingly, U73 and G71 were required for *in vitro* acetylation of Gly-tRNA^{Gly} by TacT. The TacT:AcGly-tRNA^{Gly} structure together with the biochemical analysis has provided the molecular basis for the specific Gly-tRNA^{Gly} acetylation by TacT, where TacT recognizes both the discriminator U73 and G71 of tRNA^{Gly}, a combination that is unique to tRNA^{Gly}, and discriminates tRNA^{Gly} from other tRNAs. Furthermore, the biochemical analysis showed that tRNAs bearing the sequence required for TacT acetylation were not acetylated by TacT *in vitro* when they were charged with cysteine, asparagine, or arginine. Thus, TacT is a Gly-tRNA^{Gly} specific acetyltransferase that recognizes the specific sequence of tRNA^{Gly} as well as aminoacyl moiety of aa-tRNA.

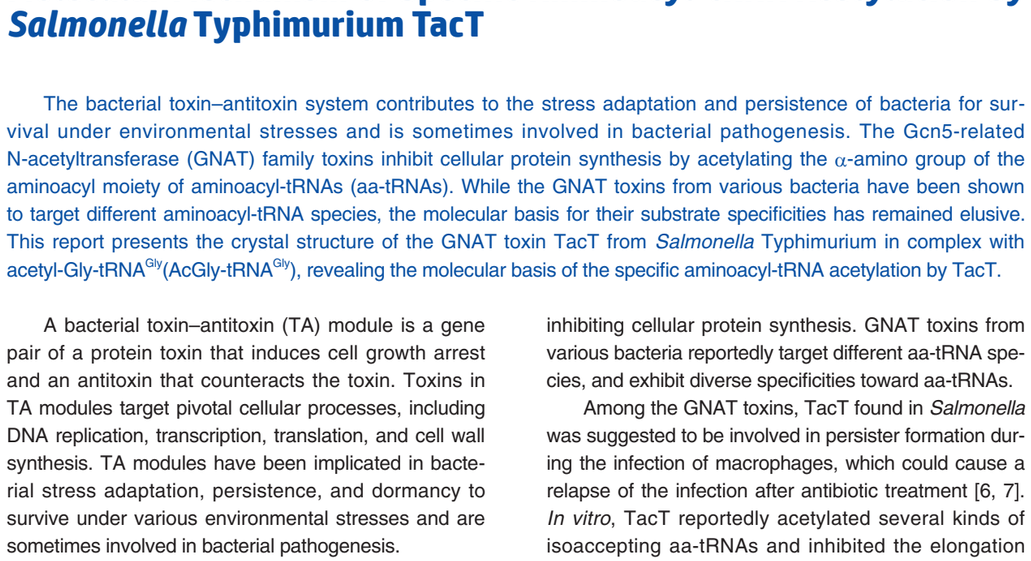

Figure 1: Overall structure of TacT complexed with AcGly-tRNA^{Gly}. **(a)** TacT$_a$ (green), TacT$_b$ (cyan) and AcGly-tRNA^{Gly} molecules (tRNA$_a$ and tRNA$_b$) are shown in ribbon models. **(b)** Electrostatic surface area potentials of the TacT dimer in the TacT:AcGly-tRNA^{Gly} complex.

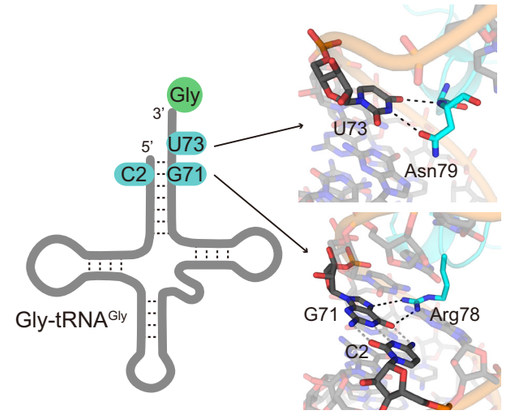

Figure 2: Detailed interactions between TacT and AcGly-tRNA^{Gly}. TacT recognizes the discriminator U73 and G71 in the acceptor of tRNA^{Gly} and discriminates Gly-tRNA^{Gly} from other aminoacyl-tRNAs.

We recently reported the molecular mechanism of aa-tRNA acetylation by enterohemorrhagic *E. coli* AtaT [2], which acetylates several aa-tRNAs charged with hydrophobic and bulky amino acids, such as Trp-tRNA^{Trp}, Phe-tRNA^{Phe}, and Tyr-tRNA^{Tyr}, in addition to Gly-tRNA^{Gly}, *in vivo* and *in vitro*, and specifically recognizes the consecutive G-C pairs in the bottom half of the acceptor stem of substrate aa-tRNAs. AtaT and TacT recognize different sites and sequences in tRNAs for the acetylation of their aminoacyl-tRNA substrates, although they share Gly-tRNA^{Gly} as a target for acetylation.

Understanding the functional mechanism of the toxin in TA modules in pathogenic bacteria is particularly important from pathological and clinical perspectives. Blocking the activity of the GNAT toxin in the persister cells is expected to lead to the resumption of growth and restoration of the antibiotic susceptibility, and thus prevent the recalcitrance and relapse of bacterial infections. Since the *Salmonella* GNAT toxin, TacT, is involved in the dormancy and persistence during macrophage infection, the mechanism of the substrate specificity presented in this study provides advanced structural information for the design of drugs targeting *Salmonella*.

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BEAMLINES

BL-1A and BL-17A

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