When *Escherichia coli* doesn't fit the mold: A pertussis-like toxin with altered specificity

DOI 10.1074/jbc.H117.796094

Chen Chen and Joseph T. Barbieri[1]

From the Department of Microbiology and Immunology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Edited by Henrik G. Dohlman

Bacterial toxins introduce protein modifications such as ADP-ribosylation to manipulate host cell signaling and physiology. Several general mechanisms for toxin function have been established, but the extent to which previously uncharacterized toxins utilize these mechanisms is unknown. A study of an *Escherichia coli* pertussis-like toxin demonstrates that this protein acts on a known toxin substrate but displays distinct and dual chemoselectivity, suggesting this *E. coli* pertussis-like toxin may serve as a unique tool to study G-protein signaling in eukaryotic cells.

Bacterial pathogens utilize a variety of strategies to attack their host. One strategy is the release of protein toxins that catalyze post-translational modifications (PTMs)[2] of host proteins; these PTMs then interfere with a central biological function to elicit a pathological phenotype. For example, protein toxins targeting components of the actin cytoskeleton can modulate motility, toxins targeting components of the ribosome and accessory factors can disrupt protein synthesis, and toxins targeting G-proteins, including monomeric G-proteins and heterotrimeric G proteins, can influence cell growth and metabolism (1). This functional diversity can come even in the context of conserved structural elements, providing motivation to characterize novel toxins with the goal of gaining insights into bacterial function, identifying possible novel mechanisms of pathogenesis and discovering unique tools to dissect eukaryotic signaling pathways. A new study from Littler *et al.* (2) provides a compelling example in these respects, describing the structural and functional characterization of an *Escherichia coli* toxin that acts via an unusual mechanism to cause an unexpected cellular outcome.

The most common PTMs catalyzed by protein toxins are glucosylation, deadenylation, proteolysis, and ADP-ribosylation, in which toxins catalyze the transfer of ADP-ribose from NAD to host proteins (3). Two archetypical examples of ADP-ribosylating toxins act on components of heterotrimeric G-proteins: Cholera toxin ADP-ribosylates $G\alpha_s$, enforcing an activated conformation that alters ion transport and water flow leading to diarrhea (4), whereas pertussis toxin ADP-ribosylates $G\alpha_i$, blocking the ability of $G\alpha_i$ to interact with its associated receptor, resulting in whooping cough and alterations in cell migration behavior (5). In cultured cells, cholera toxin stimulates cell elongation, whereas pertussis toxin stimulates cell clustering, phenotypes that are specific for each toxin and thus can be used to identify toxin action. ADP-ribosylating toxins, including cholera and pertussis toxin, use a conserved "AB" architecture, where A is the modifying enzyme and B binds cell surface receptors and mediates internalization of A. The specific details of the A and B structures, however, can vary. For example, diphtheria toxin is a single chain AB protein that is proteolytically cleaved to create a disulfide-linked N-terminal catalytic domain and a C-terminal translocation receptor-binding domain. Cholera toxin is an AB_5 protein where the catalytic A1 domain is linked to an A2 domain that inserts noncovalently into the channel of a B pentamer. Although the catalytic A domains of the ADP-ribosylating toxins share limited primary amino acid homology, they share overall three-dimensional structure and contain several conserved amino acids, including an active site glutamic acid (6). As a result, scanning genomes for A and B sequences can point not only to uncharacterized toxins, but what the likely structure, and potential function, of the toxin might be.

Extra-intestinal *E. coli*, including the uropathogenic *E. coli* and neonatal meningitis *E. coli*, normally reside in the gut but can damage the host when they invade other systems, such as the urinary tract or nervous system, respectively. Similar to *Vibrio cholera, Bordetella pertussis*, and pathogenic *E. coli* such as enterotogenic *E. coli* and enterohemorrhagic *E. coli*, extra-intestinal *E. coli* are known to encode AB_5 toxins, but whether these toxins are functional and whether their functions follow established mechanisms are unknown.

To study this question, Littler *et al.* (2) queried whole and partial *E. coli* genomes in the NCBI database using known *E. coli* A and B gene sequences and found a group of genes encoding AB_5 toxins related to pertussis toxin that the authors termed *E. coli*-pertussis-like toxins (*Ec*Plt), which were subjected to biological and biochemical characterization. Purified *Ec*Plt elicited a pertussis toxin-like clustering of cultured cells, and the isolated A domain of *Ec*Plt (*Ec*PltA) ADP-ribosylated a 41-kDa host protein with the same molecular weight as pertussis toxin-treated $G\alpha_i$ proteins, suggesting some functional conservation. Introduction of a point mutation to the presumed

The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

[1] Supported in part by National Institutes of Health Grants AI030162 and AI118389. To whom correspondence should be addressed. Tel.: 414-955-8412; Fax: 414-944-6535; E-mail: jtb01@mcw.edu.

[2] The abbreviations used are: PTM, posttranslational modification; *Ec*Plt, *E. coli*-pertussis-like toxin; NAD, nicotinamide adenine dinucleotide; GPCR, G-protein-coupled receptor.

active site glutamic acid (E118D) in *Ec*PltA also reduced activity by 1000-fold, consistent with Glu-118 being the conserved active site residue within this family of toxins.

*Ec*Plt also diverged from pertussis toxin in several ways: A systemic assessment of substrate specificity showed that *Ec*PltA ADP-ribosylated each of the three $G\alpha_i$ isoenzymes, but with slightly different preferences to that of pertussis toxin. Unexpectedly, mass spectrometry analysis showed that *Ec*PltA ADP-ribosylated one of these isoenzymes, $G\alpha_{i3}$, at Lys-345 and Asn-347, rather than at Cys-351 ribosylated by pertussis toxin or indeed any of the Cys residues present in native $G\alpha_{i3}$. Although there are precedents from other protein toxins and the endogenous host transferase to ADP-ribosylate multiple sites within a protein (7), *Ec*Plt is unique in modifying both Lys and Asn amino acids. Mutagenesis studies suggested the two sites could both be necessary for a concerted mechanism of substrate recognition, the details of which will be fascinating to learn. The authors then tested the impact of *Ec*Plt in a cellular assay of forskolin-mediated G protein recruitment to GPCRs, in which mutation of the pertussis toxin modification site Cys-351 prevents pertussis toxin from blocking recruitment. In the case of *Ec*Plt, however, the authors observed only a modest rescue provided by the N347A mutation and no obvious rescue provided by the K345A mutation. These limited effects may reflect the intrinsic differences between agonist activation and forskolin activation or may suggest Lys-345 and Asn-347 modifications affect $G\alpha_i$ action by a different mechanism than Cys-351 modification, possibly uncoupling G-protein signaling through different contacts with the G-protein-coupled receptor.

Finally, Littler *et al.* (2) solved the crystal structure of *Ec*Plt at 2.4 Å (Fig. 1), which demonstrates structural homology, and possibly functional homology, with typhoid toxin (8). Structural comparisons of *Ec*Plt in the inactive oxidized state and the activated reduced state, containing a Cys-41–Cys-192 disulfide bond, further showed how *Ec*Plt is activated via movement of the activation loop to expose the NAD-binding domain. This activation mechanism is different than that of cholera toxin (9) and may provide insight into the less studied activation mechanisms of typhoid toxin and other pertussis-like toxins.

The study from Littler *et al.* (2) provides exciting new information on the molecular and biophysical properties of an understudied subset of AB_5 toxins. In addition to raising questions about the basis of chemoselectivity and revealing new conformational pathways, *Ec*Plt may offer another benefit: Pertussis toxin has proven a useful reagent to dissect not only the molecular basis for the pathogenesis of *Bordetella pertussis*, but also to dissect the basis for GPCR signaling (10). Because *Ec*Plt may have a different basis for uncoupling G-protein signaling, *Ec*Plt may provide a new tool to continue dissection of this important eukaryotic signaling pathway.

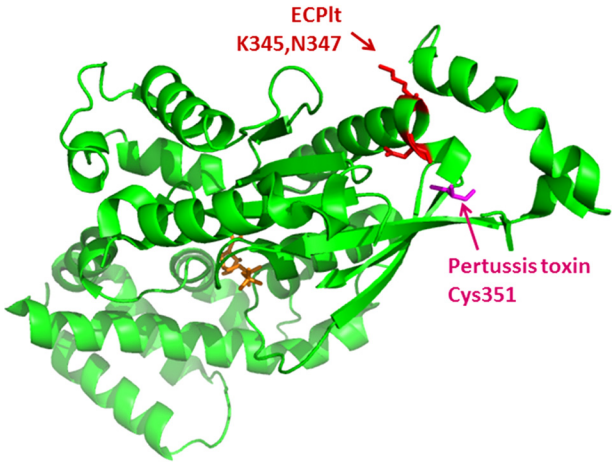

Figure 1. ADP-ribosylation of $G\alpha_i$ by *Ec*Plt and pertussis toxin. *Ec*Plt and pertussis toxin ADP-ribosylate $G\alpha_i$ (Protein Data Bank code 3FFB) on its C-terminal tail, *Ec*Plt at Lys-345 and Asn-347 (*red*), and pertussis toxin at Cys-351 (*pink*), which mediates the interaction with a GPCR. Binding of agonist to the extracellular domain of the GPCR is detected by $G\alpha_i$ initiating a conformational change that exchanges GDP (*orange*) with GTP, leading to intracellular cell signaling.

References

1. Aktories, K., and Barbieri, J. T. (2005) Bacterial cytotoxins: targeting eukaryotic switches. *Nat. Rev. Microbiol.* **3,** 397–410
2. Littler, D. R., Ang, S. Y., Moriel, D. G., Kocan, M., Kleifeld, O., Johnson, M. D., Tran, M. T., Paton, A. W., Paton, J. C., Summers, R., Schrembri, M., Rossjohn, J., and Beddoe, T. T. (2017) Structure–function analyses of a pertussis-like toxin from pathogenic *Escherichia coli* reveal a distinct mechanism of inhibition of trimeric G-proteins. *J. Biol. Chem.* **292,** 15143–15158
3. Simon, N. C., Aktories, K., and Barbieri, J. T. (2014) Novel bacterial ADP-ribosylating toxins: structure and function. *Nat. Rev. Microbiol.* **12,** 599–611
4. Vanden Broeck, D., Horvath, C., and De Wolf, M. J. (2007) *Vibrio cholerae*: cholera toxin. *Int. J. Biochem. Cell Biol.* **39,** 1771–1775
5. West, R. E., Jr., Moss, J., Vaughan, M., Liu, T., and Liu, T. Y. (1985) Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site. *J. Biol. Chem.* **260,** 14428–14430
6. Carroll, S. F., McCloskey, J. A., Crain, P. F., Oppenheimer, N. J., Marschner, T. M., and Collier, R. J. (1985) Photoaffinity labeling of diphtheria toxin fragment A with NAD: structure of the photoproduct at position 148. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 7237–7241
7. Ganesan, A. K., Mende-Mueller, L., Selzer, J., and Barbieri, J. T. (1999) *Pseudomonas aeruginosa* exoenzyme S, a double ADP-ribosyltransferase, resembles vertebrate mono-ADP-ribosyltransferases. *J. Biol. Chem.* **274,** 9503–9508
8. Song, J., Gao, X., and Galán, J. E. (2013) Structure and function of the *Salmonella* Typhi chimaeric A_2B_5 typhoid toxin. *Nature* **499,** 350–354
9. Mekalanos, J. J., Collier, R. J., and Romig, W. R. (1979) Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. *J. Biol. Chem.* **254,** 5855–5861
10. Moss, J., Bruni, P., Hsia, J. A., Tsai, S. C., Watkins, P. A., Halpern, J. L., Burns, D. L., Kanaho, Y., Chang, P. P., and Hewlett, E. L. (1984) Pertussis toxin-catalyzed ADP-ribosylation: effects on the coupling of inhibitory receptors to the adenylate cyclase system. *J. Recept. Res.* **4,** 459–474