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X4A, X25

PUBLICATION
R. Zhao, E. J. Collins, R.B. Bourret, and R.E. Silversmith. "Structure and Catalytic Mechanism of the *Escherichia coli* Chemotaxis Phosphatase CheZ," *Nature Struct. Biol.* **9**, 570 (2002); P. Matsumura, "Last But Not Least," *Nature Struct. Biol.* **9**, 563 (2002) (Commentary accompanying R. Zhao et al.'s article)

FUNDING
National Institutes of Health
Cancer Research Institute

FOR MORE INFORMATION
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The Last Piece of the Structural Puzzle in Bacterial Chemotaxis

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From the highest mammal to the simplest bacteria, cells respond to extracellular signals with appropriate responses. Signal transduction systems mediate this process and, in both prokaryotes and eukaryotes, phosphoryl groups reversibly attached to proteins provide a fundamental currency of information transfer. Chemotaxis, the signal transduction system that mediates the ability of Escherichia coli to swim towards nutrients and away from toxins, is arguably the best-studied signal transduction system in any organism. The detailed molecular picture of how chemotaxis works involves seven different proteins, six of which have solved atomic structures. The structure of the remaining protein, called CheZ, has long remained a mystery. Scientists at the University of North Carolina at Chapel Hill have determined the structure of CheZ.

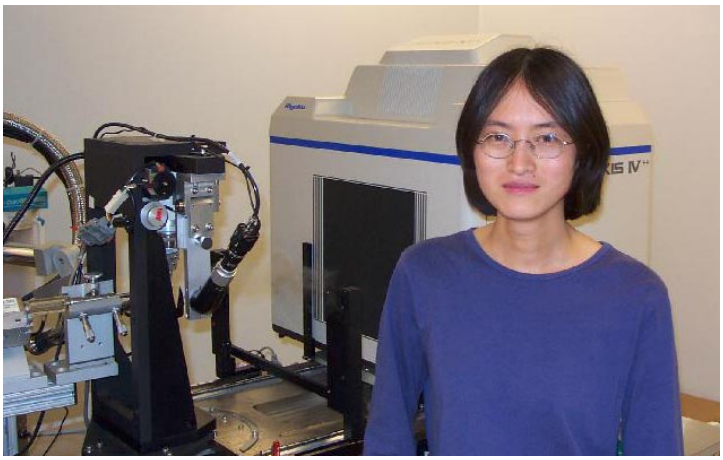
The gut bacterium *Escherichia coli* has the remarkable ability to rotate its flagella either counterclockwise, which results in forward swimming, or clockwise, which causes cell tumbling. The direction of flagellar rotation at any moment is determined by the degree of phosphorylation (addition of a phosphoryl group [$-PO_3^{2-}$]) to the chemotaxis signaling protein CheY, which binds to the base of the flagella. CheY is phosphorylated by the histidine kinase CheA and dephosphorylated by the CheZ phosphatase, the subject of our X-ray crystallographic studies.

Our initial efforts using crystals containing only CheZ were unsuccessful due to the poor diffraction of these crystals. We then shifted strategies and exploited a recent discovery that beryllium fluoride (BeF_3^-) binds to CheY to form a stable replica of phosphorylated CheY, the substrate of CheZ. We obtained crystals containing CheZ and CheY-BeF_3^--Mg^{2+}, and a cryoprotectant of 50% sucrose enabled us to improve the diffraction of these crystals from 8 angstrom (obtained with standard cryoprotectants) to 3.5 angstrom.

The CheZ dimer features an impressively long (105 angstrom) four-helix bundle composed of two helices from each chain, as shown in **Figure 1**. About 130 (of the 214) residues from each chain form an extended amphipathic (having both water-loving and water-repelling groups) alpha helix which folds on itself with a single hairpin turn. Two of these hairpins assemble to form the bundle. The amino-terminal of each chain forms an additional helix, as does the carboxyl-terminal, which interacts with a surface on CheY. The linker region between the carboxyl-terminal helix and the four-helix bundle was not visible in the electron density maps, implying that this region was disordered in the crystal.

For decades, scientists have debated whether CheZ acts as a positive allosteric effector of CheY's own autodephosphorylation activity by binding at a region of CheY away from the active site, or as a traditional phosphatase, which dephosphorylates CheY by binding to its active site. Our results showed two surfaces of interaction between CheY and CheZ. The carboxyl-terminal α-helix of CheZ binds to a surface on CheY that is distant from its active site, but a region of the four-helix bundle of CheZ interacts directly with the active site of CheY.

Strikingly, one residue from CheZ, called glutamine 147, inserts directly into the



Lead author Rui Zhao with a Rigaku generator and Raxis-IV area detector at the x-ray facility at University of Colorado Health Science Center in Denver, where she is currently employed.

CheY active site. Mutagenesis studies showed that this residue was critical for catalytic activity. We now believe that glutamine 147 stimulates the CheY autodephosphorylation reaction by positioning a water molecule in an appropriate position to remove phosphoryl groups, as shown in **Figure 2**. So CheZ activity cannot be classified simply as an allosteric effector or true phosphatase, but instead contains features of both.

Our work provides structural information on the last of the seven proteins involved in *E. coli* chemotaxis. This new description of the CheZ structure and mechanism will enhance our understanding of bacterial chemotaxis.

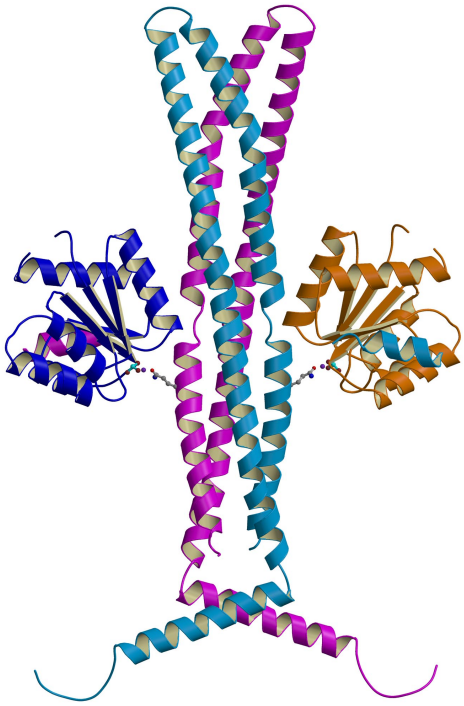


Figure 1. *Ribbon representation of the structures of the chemotaxis protein CheZ and the complex between the chemotaxis protein CheY, beryllium fluoride (BeF_3^-) and magnesium (Mg^{2+}), showing the two chains of the CheZ dimer (light blue and magenta) and two symmetric CheY molecules (orange and dark blue). The active site of CheY is highlighted with ball and stick representations of BeF_3^- (green) and Mg^{2+} (purple). The essential catalytic residue from CheZ, glutamine 147 (gray ball and stick), inserts into the CheY active site.*

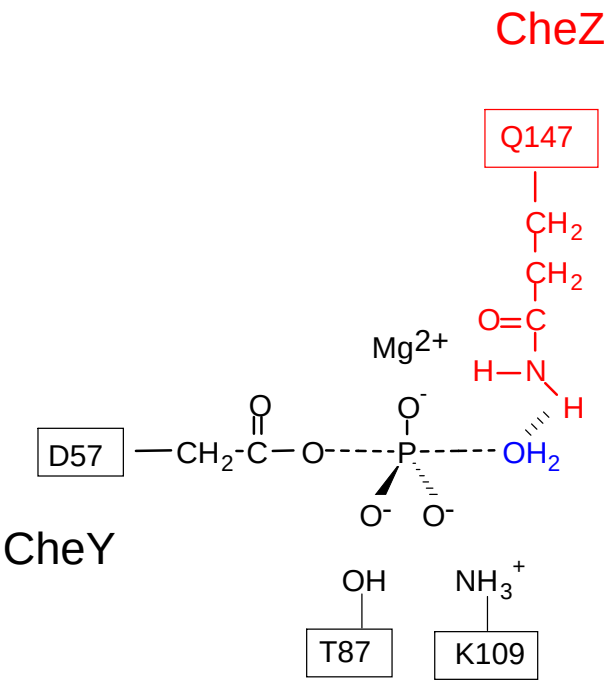


Figure 2. *Model of the transition state in the dephosphorylation of CheY. In the absence of CheZ, CheY (black) catalyzes the reaction with active site residues threonine 87 (T87), lysine 109 (K109) and the bound magnesium ion (Mg^{2+}). In the presence of CheZ (red), the reaction is assisted by the glutamine 147 (Q147) side chain, which may help orient the "attacking" water molecule (blue) through a hydrogen-bonding interaction.*