Keynote Lecture XIV

How Iron Regulates Virulence in Corynebacterium diphtheriae and Mycobacterium tuberculosis

Randall K Holmes

University of Colorado Health Sciences Center, USA

Randall K Holmes received his MD and PhD in microbiology from New York University in 1968. Following internship and residency at Beth Israel Hospital in Boston, postdoctoral training at the NIH, and a faculty position at the University of Texas Southwestern Medical Center in Dallas, he was appointed in 1976 as the founding chair of the Department of Microbiology and Immunology at the Uniformed Services University of the Health Sciences in Bethesda. Since 1995 he has served as chair of the Department of Microbiology at the University of Colorado Health Sciences Center.

His main research interests are the roles of protein toxins in pathogenesis of bacterial infections. His research on cholera and travelers' diarrhea helped to define the structure, function and immunochemistry of cholera toxin and the related type I and type II heat-labile enterotoxins of Escherichia coli and the use of enterotoxin-like chimeras as potential mucosal vaccines. His research on diphtheria analyzed genetics of phages that encode diphtheria toxin and characterized the diphtheria toxin repressor as the prototype for a novel class of metal-dependent regulatory proteins that control virulence in C. diphtheriae, M. tuberculosis, and several other bacterial pathogens.

He served on the Board of Governors of the American Academy of Microbiology and currently chairs the AAM Awards Committee. He served on the FDA Vaccines and Related Biological Products Advisory Committee, the NIH U.S. Cholera Panel, and the NIH Bacteriology and Mycology 1 Study Section, and he currently chairs the NIH Microbiology and Infectious Diseases Research Committee. He chaired the Microbiology Test Development Committee of the National Board of Medical Examiners and served on the Step I Committee and Composite Committee of the United States Medical Licensing Exam. He currently serves on the Administrative Board of the Council of Academic Societies of the Association of American Medical Colleges.

Diphtheria, which re-emerged as a major epidemic disease in Russia and the Newly Independent States of the former Soviet Union during the 1990s, is a paradigm for pathogenesis of toxin-mediated bacterial diseases. Tuberculosis, which causes more deaths worldwide than any other bacterial infection, is a paradigm for pathogenesis of intracellular bacterial infections. Highly homologous iron-activated regulatory proteins, called the diphtheria toxin repressor (DtxR) in *C. diphtheriae* and the iron-dependent regulator (IdeR) in *M. tuberculosis*, control virulence in these very different pathogens. DtxR and IdeR are prototypes for a large family of metal-dependent global regulators found predominantly in Gram-positive and acid-fast bacteria.

Diphtheria toxin (DT), the principal virulence factor of *C. diphtheriae*, is encoded by certain temperate corynebacteriophages, and all toxinogenic isolates of *C. diphtheriae* are lysogenic for a *tox*$^+$ phage. DT is produced only when *tox*$^+$ strains of *C. diphtheriae* are grown under conditions with limited bioavailability of iron, such as in body fluids or tissues. Production of DT is controlled by the diphtheria toxin repressor (DtxR), a dimeric metalloregulatory protein that uses Fe^{2+} as its co-repressor. When the intracellular concentration of Fe^{2+} is sufficiently high, DtxR binds to the *tox* promoter-operator and inhibits transcription of the *tox* gene, thereby preventing toxin production. Although DtxR was discovered and named because of its role in regulating DT production, DtxR is now known to function as a global regulator of iron-dependent expression of many genes in *C. diphtheriae*. DtxR maintains iron homeostasis by inducing systems for siderophore-dependent iron uptake and assimilation of iron from heme when *C. diphtheriae* is grown under low-iron conditions. We recently

developed a reporter transposon and used it to identify many novel iron-regulated genes in *C. diphtheriae.* Experiments are in progress to determine how DtxR affects each of these novel iron-regulated genes.

The iron-dependent regulator (IdeR) of *M. tuberculosis* is highly homologous with DtxR from *C. diphtheriae,* and both proteins bind to operators with the same 19 bp consensus sequence. DtxR and IdeR can substitute for each other in complementation experiments in *C. diphtheriae* or *M. tuberculosis.* Manabe *et al.* (PNAS 96: 12844-12848, 1999) demonstrated that expression of a constitutively active variant of DtxR in *M. tuberculosis* resulted in attenuation of virulence, and Rodriguez *et al.* (infect. Immun. 70:3371-3381, 2002) used gene microarrays to characterize the iron-dependent transcriptional profile of wild type, *ideR* mutant, and *ideR*-complemented strains of *M. tuberculosis.* Although IdeR is essential for viability of *M. tuberculosis,* DtxR is not essential in *C. diphtheriae.*

In collaboration with Wim Hol and his colleagues, we determined the structure of DtxR and IdeR as inactive apo-repressors (without bound Fe^{2+}), as active holo-repressors (with bound Fe^{2+} or other divalent transition metal ions), and as holo-repressors in complex with double-stranded DNA containing operator sequences recognized by the active repressors. Each monomer of these proteins consists of three domains, an N-terminal DNA-binding domain, a central domain that contains two metal-binding sites and also mediates dimerization of the repressor protein, and a C-terminal SH3-like domain with a metal-binding motif that contributes two ligands for one of the bound metal ions. Binding of appropriate divalent metal ions to the apo-repressor initiates multiple conformational changes that result in expression of the sequence-specific DNA-binding activity of the holo-repressor. Two holo-DtxR or holo-IdeR dimers interact with a cognate operator in DNA to form a "double-dimer" complex in which the two dimers bind on opposite sides of the DNA duplex. Based on this structure and all known DNA recognition sequences for DtxR and IdeR, four partially overlapping copies of a novel 5-base recognition pattern were recently identified within the 19 bp consensus sequence. These structural studies are providing many new insights about the molecular mechanisms by which DtxR and IdeR exert their global effects on iron-dependent gene regulation in *C. diphtheriae* and *M. tuberculosis.* They also provide a starting point for structure-based development of potential therapeutic agents that could act by modulating the activities of these iron-dependent virulence regulators.