

Regulation of antibiotic resistance

Two Component Regulators and Peptide/polymyxin resistance

As described in a separate section, cationic antimicrobial peptides are ubiquitous in nature as components of innate immune defences. A number of labs including ours are designing improved variants as a therapeutic alternative to conventional antibiotics. We and others recently demonstrated that peptide susceptibility is controlled by two 2-component regulators PhoPQ (3,4), and PmrAB (5). In addition, PhoQ in particular controls virulence both in neutropenic mice (4) and in the chronic rat lung model (Levesque, personal communication) and our unpublished work implicates a role in motility and biofilm development. These two component regulators comprise transmembrane sensors with histidine kinase activity (PhoQ, PmrB), which transfer a phosphate and thus activate the cognate regulators PhoP, and PmrA respectively, and these components are evolutionarily related to each other and to a series of 3 other two component regulators, one of which, PmrSR, we recently demonstrated to have a role in intrinsic resistance to polymyxin B and swimming motility. The natural signal to which these regulators respond is low Mg^{2+} and Ca^{2+} in the medium, and they appear to have a variety of downstream effector genes (5), including prominently an LPS modification operon that arabinosaminylates the LPS, blocking peptide self-promoted uptake across the outer membrane. *Pseudomonas* from the CF lung has the characteristic LPS modification associated with constitutive expression of resistance, even though CF lungs are not Mg^{2+}/Ca^{2+} deficient (3). We have utilized the bioinformatic programs Weblogo and Prophecy to predict the DNA binding sites for PhoP and PmrA and confirmed most of the instances in which these appear through studying the regulator-dependent expression of the genes downstream from these motifs, as well as performing gel shift experiments to confirm binding to DNA fragments containing these motifs. The results were somewhat surprising and indicate that there are relatively few genes directly regulated by PhoP and PmrB even though microarray experiments have indicated hundreds of genes in the low Mg^{2+} dependent regulon, many of which are directly dependent on these transcription factors. Thus we have proposed and are testing the hypothesis that these involve a regulatory cascade emanating from these transcription factors.

In addition we have demonstrated (5) that peptides, e.g. bovine indolicidin, can themselves induce resistance through activation of the PmrAB operon and others independently of the self-regulatory PmrAB system. Conversely some peptides are very weak inducers of this resistance-controlling operon, making them better candidates for use as therapeutics in CF. Thus we are investigating the basis for and mechanism of peptide-mediated induction of peptide resistance.

Adaptive resistance

One poorly defined area of antibiotic resistance is adaptive resistance, which describes the transient, reversible resistance that occurs in response to an antimicrobial agent. It is particularly prominent in the case of *Pseudomonas aeruginosa* in which the susceptibility of this organism to antibiotics rarely reflects in vitro susceptibility. We are attempting to understand this phenomenon using microarrays and screening of our lux fusion library, and are applying this to the study of ciprofloxacin, aminoglycosides and indolicidin adaptive resistance.

As mentioned an important tool is offered by microarray analyses that reveal global changes in gene expression in response to environmental changes and thus are well suited to providing a detailed picture of bacterial responses to antibiotic treatment. These responses are represented by patterns of co-regulated genes termed gene expression signatures, which provide insight into the mechanism of action of antibiotics as well as the general physiological responses of bacteria to antibiotic-related stresses. The complexity of such profiles is challenging the notion that antibiotics act on single targets and are consistent with the concept that there are multiple targets coupled with common stress responses. A more detailed knowledge of how known antibiotics act and induce adaptive resistance should reveal new strategies for antimicrobial drug discovery.

References: **1.** Hancock REW. 2001. *Lancet Infect. Dis.* 1:156-64; **2.** Boman HG. *J Intern Med*, 2003. 254: 197-215; **3.** Ernst RK, et al. 1999. *Science* 286:1561-5; **4.** Macfarlane ELA, Hancock, REW, et al. 1999. *Molec. Microbiol.* 34:305-316; **5.** McPhee JB, Hancock REW. 2003. *Molec. Microbiol.* 50:205-219; **6.** Brazas, M, Hancock REW. 2005. *Antimicrob. Agents Chemother.* 49: 3222-3227.