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Cloning and Characterization of a Gene, *argR*, that Participates in Arginine Regulation in *Pseudomonas aeruginosa* PAO1

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To investigate the presence of trans-acting factors for arginine network, gel retardation assays were performed with crude extracts of *P. aeruginosa*, and an arginine-inducible DNA-protein complex was detected that binds specifically to the promoter regions of two arginine biosynthetic operons, *car* and *argF*. Employing an *argF::lacZ* transcriptional fusion, a mutant defective in arginine regulation was isolated by Tn5 mutagenesis and arginine-hydroxamate selection. This mutant was unable to utilize arginine or ornithine as a carbon source and was impaired in formation of the DNA-binding protein identified in cell-free extracts of the parent strain. Cloning and gel retardation experiments localized the *argR* gene on a 1.3 kb fragment. Determination of the nucleotide sequence revealed that the *argR* gene encodes a polypeptide of 329 amino acids with a molecular mass of 36.8 kDa. A search of the GeneBank protein database indicates that ArgR has significant homology to members of the AraC/XylS family. The *argR* gene was inactivated by gene replacement. The resulting strain was unable to utilize arginine or ornithine as source of carbon, and enzymes necessary of aerobic catabolism and biosynthesis of arginine were not regulated as is not case with the parent strain. The combined nucleotide sequence analysis indicated that *argR* is a member of arginine/ornithine transport operon as the terminal sixth genes. S1 and fusion experiments established that this operon is transcribed from a promoter upstream of the first gene in the operon, *aotJ*. This promoter was shown to be inducible by arginine under a variety of growth conditions but to a lesser extent with more effective carbon and nitrogen sources. The transport operon is followed by an arginine utilization operon (*aru*). The *aru* operon was also shown to be expressed from a promoter upstream of the first gene in the operon, *aruC* (encoding acetylornithine aminotransferase). This promoter was shown to be controlled by arginine in a similar manner to that shown for *aot* operon. The ArgR protein was over-produced and purified to homogeneity. Results of SDS-PAGE and gel filtration indicated that the native ArgR protein is a dimer of identical 37-kDa subunits. Gel retardation experiments with *carA*, *argF*, *aotJ*, and *aruC* operators yield dissociation constants in the range of 10^{-11} to 10^{-12} M, and these constants were not changed by addition of L-arginine in the reaction buffer. A combination of DNaseI, premethylation interference, depurination footprinting experiments revealed that ArgR binds to a region of 45 to 46 bps that overlaps the promoter sequence for *car* and *argF*, and is upstream of the -35 region for *aotJ* and *aruC*. Strong ArgR-nucleobase contacts also identified in a conserved TGTCGC sequence in the binding site.

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