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Analysis of genes for the β-ketoadipate pathway revealed mechanisms underlying evolutionary divergence of controls governing biodegradation in bacteria. Transcriptional regulators that respond to muconate in Acinetobacter calcoaceticus and Pseudomonas putida diverged recently from a common ancestor. This divergence produced the A. calcoaceticus catM repressor gene and the P. putida activator catR gene. Thus a single ancestor gave rise to one gene that exercises negative control and another gene that exerts positive control over transcription.

Independently transcribed genes for related physiological functions are clustered in the A. calcoaceticus chromosome, and the evolutionary basis for selection of this supraoperonic clustering is unknown. Advances in the genetics of this organism will make it possible to explore the genetic and physiological consequences of engineered transpositions which alter the structure of supraoperonic clusters. The genetic procedures also allow systematic genetic analysis of pobR, a newly discovered regulatory gene which activates transcription of pobA. This study should reveal amino acid residues that contribute to the function of the

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BLOCK 19: Abstract continued...
regulatory gene product and also should indicate the contribution of DNA sequence to acquisition of specific mutation in the pobA-pobR region.

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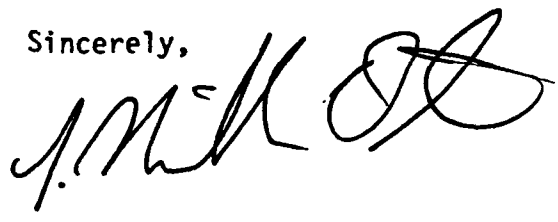

Dr. L. Nicholas Ornston 21256-LS
Yale University
Department of Biology
New Haven, CT 06511

CONTROL OF BIODEGRADATION IN BACTERIA

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L. NICHOLAS ORNSTON

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CONTROL OF BIODEGRADATION IN BACTERIA

As DNA sequences become available, it is increasingly evident that the catabolic pathways of bacteria evolved by the genetic stitching together of genes encoding enzymes with different catalytic activities. Genetic combinations that permitted growth were selected and refined by further mutation so that they became physiologically effective. Part of the refinement was the acquisition of transcriptional controls that assured that the structural genes were expressed only when their function was demanded. Past research supported by the ARO allowed elucidation of diverse sets of transcriptional controls that have evolved in otherwise closely related bacteria. The present research program has increased insight into the mechanisms underlying these controls and allowed development of procedures that will allow investigation of the basis for their selection in the natural environment.

Our research program has focused upon the β-ketoadipate pathway, a widely distributed bacterial system for utilization of aromatic acids. The pathway is a universal trait in fluorescent Pseudomonas species, and expression of its enzymes is governed by induction. One gene controlling induction is catR which neighbors catB and is transcribed divergently from the catBC operon in Pseudomonas putida. The CatR protein responds to the inducer muconate and activates transcription of the catBC operon.

As judged by ribosomal RNA homology, Acinetobacter calcoaceticus is closely related to Pseudomonas putida, and regulation of the cat genes in the two species is similar in some respects. For example, the cat structural genes of Acinetobacter are induced in response to muconate, and the regulatory gene governing induction is transcribed divergently from catB. Indeed the regulatory proteins controlled by muconate in Acinetobacter and Pseudomonas exhibit 40% identity in their aligned amino acid sequences. It therefore is remarkable that the proteins exhibit directly opposed modes of action: whereas the Pseudomonas catR gene encodes a transcriptional activator, the product of the Acinetobacter catM gene is a repressor.

The basis for selection of positive control in Pseudomonas and negative control in Acinetobacter is unknown. One possibility might be that negative control is favored for governing expression of the relatively AT rich DNA of Acinetobacter, but this interpretation is difficult to defend because we now know that another regulatory gene, pobA, encodes a transcriptional activator in Acinetobacter. Through genetic engineering, it should be possible to determine if the Pseudomonas catRBC system is physiologically effective in Acinetobacter, and we intend to explore this possibility.

The organization and control of catabolic genes in Acinetobacter and in Pseudomonas differ in other respects. We now know that the Acinetobacter genes are tightly linked in supraoperonic clusters. We have determined the complete DNA sequence of the 16 kbp of DNA containing the 11 structural genes for enzymes that convert benzoate to citric acid cycle intermediates in Acinetobacter. The genes are organized in four tightly linked operons. We also have characterized a 20 kbp DNA segment containing twelve structural genes associated with the metabolism of shikimate, quinate and p-hydroxybenzoate to citric acid cycle intermediates in Acinetobacter. We have sequenced the portion of this region containing the pcaIJFDBCHG operon and another portion containing the pobA gene. Divergently transcribed from pobA is its transcriptional activator, pobR. Downstream from pobR is pobS, a gene that governs pobA by repression. Thus a single structural gene, pobA, is subject to separately directed activation and repression.

We have developed methods for direct selection of Acinetobacter mutant strains in which expression of pobA is prevented. The genetic properties of Acinetobacter make it relatively easily to localize and to sequence mutations, so we have initiated an analysis of spontaneous mutants with dysfunctions in pobA and pobR. We expect the results to give insight into structure-function relationships in the products of the genes and further anticipate that the study will increase our understanding of the nature of spontaneous mutation. The system is particularly attractive because it will allow the study of unstable mutants. Such strains occur frequently but, for technical reasons, are rare components of most genetic investigations.

The genetics of Acinetobacter also allows ready selection of engineered variants in which genes have been transferred from one supraoperonic cluster to another supraoperonic cluster in the same chromosome. We intend to design strains carrying such rearrangements so that we may analyze the physiological and genetic consequences of disruptions in supraoperonic clusters.

Publications supported by the Army Research Office

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Scientific Personnel Supported by the Project

Christopher Hartnett, Graduate Student, Ph.D. received 1989

Leslie Gregg-Jolly, Graduate Student, Ph.D. received 1990

Gail Hartnett, Graduate Student

L. Nicholas Ornston, Professor