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13. ABSTRACT *(Maximum 200 words)*

The *Acinetobacter calcoaceticus pca-qui-pob* supraoperonic gene cluster encodes bacterial enzymes that metabolize aromatic and hydroaromatic compounds in the environment. Our investigation is directed to understanding how mutation, gene rearrangement and selection contributed to evolution of the transcriptional controls exercised over genes in the cluster. The complete nucleotide sequence of the 18 kbp gene cluster has been determined, and genetic manipulations have been used to explore mechanisms contributing to expression of the genes. The results reveal that structural gene expression is governed by complex interactions between the products of different regulatory genes some of which share common ancestry. Additional controls appear to be exercised by compartmentation of some catabolic enzymes outside the inner cell membrane. Recombination appears to have made a major contribution to the evolution of existing control mechanisms, and their maintenance may be influence by continuing recombination. Contributions of recombination to mutation and repair are under investigation as are specific molecular mechanisms underlying transcriptional controls.

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EVOLUTION OF REGULATORY GENES GOVERNING BIODEGRADATION IN ACINETOBACTER CALCOACETICUS

FINAL REPORT

L. N. Ornston

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The *Acinetobacter calcoaceticus pca-qui-pob* supraoperonic gene cluster encodes bacterial enzymes that metabolize aromatic and hydroaromatic compounds in the environment. Our investigation is directed to understanding how mutation, gene rearrangement and selection contributed to evolution of the transcriptional controls exercised over genes in the cluster. The complete nucleotide sequence of the 18 kbp gene cluster has been determined, and genetic manipulations have been used to explore mechanisms contributing to expression of the genes. Several surprises have emerged from this investigation.

1. The *catIJF* and *pcaIJF* genes, separated by about 270 kbp of DNA in the *Acinetobacter calcoaceticus* chromosome, exhibit 99% nucleotide sequence identity extending over 2.2 kbp. Earlier evidence had indicated that *catIJF* could serve as template for repair of mutations in *pcaIJF*. This process has been explored in greater detail. The repair has been shown to be mediated by gene conversion, and *pcaIJF* can mediate repair of mutations in *catIJF*. This process of ongoing genetic exchange may contribute to conservation of codon usage patterns which make *catIJF* and *pcaIJF* unusual among *A. calcoaceticus* genes. Nucleotide tracts extending up to 881 base pairs were shown to be transferred by during repair (Kowalchuk et al.,1994,1995), and the process was shown to depend upon *recA* (Gregg-Jolly and Ornston,1994).

2. The ease with which *recA* mutations can be introduced into the *A. calcoaceticus* chromosome (Gregg-Jolly and Ornston,1994) makes it possible to explore interaction of mutant and wild type alleles in the absence of recombination. Thus it was possible to demonstrate that *pobR*, the transcriptional activator of *pobA* (the structural gene for *p*-hydroxybenzoate hydroxylase), participates in regulation of its own synthesis. The molecular basis for transcriptional controls in the *pobR-pobA* intergenic region was explored (DiMarco and Ornston,1994). Downstream from *pobR* and expressed in the same transcript is *pobS*, an apparent repressor of *pobA* expression. It is not clear why a transcriptional repressor should be expressed directly downstream from a transcriptional activator. The physiological functions of *pobS* are still under examination.

3. Possibilities for introduction of a *recA* null mutation into the *A. calcoaceticus* chromosome also made it possible to explore contributions of specified DNA fragments to quinate and shikimate catabolism. The *quiA* gene encodes a transmembrane oxidase that converts quinate to dehyddroquinate and shikimate to dehydroshikimate. This gene and other genes associated with the catabolism of quinate and shikimate to protocatechuate lie between *pcaG* and *pobS* in the *A. calcoaceticus* chromosome (Elsemore and Ornston,1994).

4. Conversion of dehydroquinate to dehydroshikimate is a necessary step in the biosynthesis of aromatic amino acids. In enteric bacteria, the biosynthetic gene is encoded by *aroD*. Catabolic conversion of dehydroquinate to dehydroshikimate is mediated by the enzyme encoded by *quiB*, and an *A. calcoaceticus* DNA fragment containing *quiB* was identified on the basis of its ability to complement a null mutation in *E. coli aroD*. Nucleotide sequence analysis revealed that *A. calcoaceticus quiB* resembles the biosynthetic *aroD* of other organisms and is unlike the genes with the catabolic *quiB* function. Therefore gene rearrangement appears to have placed a biosynthetic gene in a catabolic cluster during the evolution of *A. calcoaceticus*.

5. Further characterization of genes in the *qui* region revealed *quiX* which encodes a porin-like protein. Inroduction of null mutations into the gene do not prevent growth with quinate or shikimate, but the possibility that the protein contributes to growth in the presence of low substrate concentrations has not been explored.

6. Nucleotide sequence similarities suggest a transport function for *pcaK*, another gene within the *pca-qui-pob* cluster. The physiological contribution of *A. calcoaceticus pcaK* is under investigation.

7. Procedures for selection of null mutants in *pob* and *pca* genes were developed and applied to a detailed examination of mutations in *pcaH,G*, genes encoding the homologous protein subunits of protocatechuate 3,4-dioxygenase. This investigation (Gerischer and Ornston,1995) revealed regions of DNA in which secondary structures formed between slipped DNA strands may predispose specific nucleotides to genetic alteration. Mutants isolated in this study facilitated understanding of a promoter region directly upstream of the *pca* operon and led to identification of *pcaU*, a divergently transcribed activator of the *pca* operon. The *pcaU* and *pobR* transcriptional activators are closely related members of a sparsely represented gene family. Despite their similarity, the activators are highly selective in the genes that they control. Exploration of *pcaU* and the mechanisms by which it exerts control are continuing.

8. Analysis of spontaneous *pcaH,G* mutants (Gerischer and Ornston,1995) revealed an insertion sequence, IS1236, which was responsible for about 10% of the sequenced mutations. Insertion of this genetic element in *pcaH,G* was somewhat imprecise in the sense that the length of repetition of flanking nucleotides varied considerably. Remarkably, IS1236 accounts for 5 out of 6 mutations isolated thus far in *pobR*, and in every case the insertion is flanked by a precise 3 base pair repetition. Thus both the frequency and the mode of insertion appear to be influenced by the target DNA. Investigation of IS1236 continues.

7. A longstanding investigation was brought to conclusion with publication of the nucleotide sequence of the *catR,BCA* region from the *Pseudomonas putida* (Houghton et al.,1995). This work is of particular interest because it suggests that nucleotide sequences may have been exchanged between *catC* and *catA* after transposition introduced the latter gene directly downstream from *catA*. Possible participants in the slippage of DNA strands that would be required for intergenic sequence exchange emerged from identication of REP (Repetitive Extragenic Palindrome)-like genetic elements at different locations in the *catR,BCA* cluster from two different fluorescent *Pseudomonas* strains.

Our present investigations are directed towards further characterization of transcriptional controls exercised over genes in the *pca-qui-pob* supraoperonic cluster. Transcription-terminating omega elements and *lacZ* reporter genes have been introduced at different loci within the cluster, and these constructs will give a indication of the sites where transcriptional control is exercised. More specific information will be obtained by identifying sites of transcriptional initiation.

In an effort to analyse the physiological basis for supraoperonic clustering, the *catA* gene has been transposed from its normal locus within the *ben-cat* cluster to a position within *pobA*. The strain containing the transposition grows with benzoate (a physiological process requiring expression of *catA*) only if *p*-hydroxybenzoate (which elicits expression of the *pobA* transcript is present). In principle, selection for growth with benzoate in the absence of *p*-hydroxybenzoate should yield mutants expressing *pobA-catA* constitutively, but such mutants have not been obtained. This failure may be a reflection of the extremely tight *pobA* transcriptional controls which are under further investigation. As part of this study, an alternative approach (selection for elevated expression of *folA* after it had been placed downstream from *pobA* on a plasmid) led to heightened *pobA* expression in *E. coli* (Fernandez et al., submitted for publication).

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Personnel

David A. Elsemore, Ph.D., 1995

Anthony A. DiMarco

George A. Kowalchuk, Ph.D., 1994

Ulrike Gerischer

Nicholas Ornston

Inventions

None

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on Reverse Side.)

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SECTION I – SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR / SUBCONTRACTOR (If "None," so state) None

a. NAME(S) OF INVENTOR(S) (Last, First, MI)	b. TITLE OF INVENTION(S)	c. DISCLOSURE NO., PATENT APPLICATION SERIAL NO. OR PATENT NO.	d. ELECTION TO FILE PATENT APPLICATIONS				e. CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER	
			(1) United States		(2) Foreign			
			(a) Yes	(b) No	(a) Yes	(b) No	(1) Yes	(2) No

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR / SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) Name of Inventor (Last, First, MI)	(2) (a) Name of Inventor (Last, First, MI)	(1) Title of Invention	(2) Foreign Countries of Patent Application
(b) Name of Employer	(b) Name of Employer		
(c) Address of Employer (Include ZIP Code)	(c) Address of Employer (Include ZIP Code)		

SECTION II – SUBCONTRACTS (Containing a "Patent Rights" clause)

6. SUBCONTRACTS AWARDED BY CONTRACTOR / SUBCONTRACTOR (If "None," so state) None

a. NAME OF SUBCONTRACTOR(S)	b. ADDRESS (Include ZIP Code)	c. SUBCONTRACT NO.(S)	d. DFAR "PATENT RIGHTS"		e. DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S)	f. SUBCONTRACT DATES (YYMMDD)	
			(1) Clause Number	(2) Date (YYMM)		(1) Award	(2) Estimated Completion

SECTION III – CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR / SUBCONTRACTOR (Not required if [ ] Small Business or [ ] Non-Profit organization.) (X appropriate box)

a. NAME OF AUTHORIZED CONTRACTOR / SUBCONTRACTOR OFFICIAL (Last, First, MI)	c. I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.	
b. TITLE ~~Suzanne K. Polmar, Ph.D. Director~~ Grant and Contract Administration	d. SIGNATURE	e. DATE SIGNED Feb 1, 1991

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