

Compartmentalization of Aspartate Transcarbamylase During Division of *Caulobacter crescentus*

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Introduction

The asymmetric division of *Caulobacter crescentus* produces two morphologically distinct cells; one cell is stalked and sessile, the other flagellated and motile (13). Production of cells of such differing morphologies during division under unstressed physiologic conditions is an unusual feature for a prokaryotic cell division (13). The two morphologic types have different physiologic capabilities (2).

Two outstanding features of the division and differentiation are apparent. One, the portion of the dividing mother cell distal to the stalk produces a flagellum and becomes the motile daughter product due to the ability of predivisional cells to sequester certain macromolecules (10). Two, the sessile cells initiate subsequent asymmetric cell divisions in less time than the motile cells require (13). This difference is the result of the ability to initiate immediate DNA replication in the sessile cell while the DNA of the motile cells does not undergo replication until the morphology of the motile cell has altered to that of a sessile cell (7). This difference in ability to initiate DNA replication seems to account for the increased generation time of the motile cell (3).

The observed failure of the motile cell to replicate its DNA in as short a time as the sessile one is a problem worthy of further investigation. A possible explanation lies in the ability of the predivisional cell to partition macromolecules as is seen in the compartmentalization of flagellin (10), flagellar m-RNAs (4, 11) and methyl-accepting chemotaxis proteins (5). If the partitioning ability of the dividing cells extends to enzymes, then there is a possibility that compartmentalization of one or more enzymes may affect the divisional ability of the motile cell. A partitioning of enzymes involved in the biosynthesis of DNA precursors to the sessile cells could result in a decrease in monomer supply within the motile cells and account for the increased time required for DNA replication in motile cells.

The enzyme aspartate transcarbamylase (ATCase) occupies an important position in the pyrimidine biosynthetic pathway in many organisms (1). This enzyme has been shown in some systems to be subject to a variety of control mechanisms, including transcriptional (1) as well as heterotropic activation and homotropic inactivation (8). In addition, an exquisitely sensitive and very specific method has been developed for the quantification of this enzyme (12). This method has proven accurate even in the presence of high concentrations of other macromolecules (9). The purpose of this research is to investigate populations of the two morphologic types of *Caulobacter crescentus* and to determine if there are differences in the content of ATCase between the motile and sessile forms. If the content of ATCase does differ between the two forms, then to ascertain if the differences could contribute to an explanation of the previously observed contrasting abilities of the two cell types.

Material and Methods

All chemicals were of the highest grade commercially available. Carbamyl phosphate was purified by precipitation from 50% ethanol and stored dessicated at -20°C .

Caulobacter crescentus CB15F (6) is a mutant strain of *Caulobacter* that produces motile and sessile cells that have differing specific gravities and can be separated

on this basis. CB15F was grown in minimal salts medium (13) with 0.2% glucose as the carbon source. Cultures of 25 ml were placed in a New Brunswick Rotator at about 60 rpm for incubation at 30° C. The growth of the cultures was monitored by measuring their absorbance at 560nm.

Cultures as above were harvested in the logarithmic phase of growth. About 8×10^{11} cells were collected in a pellet by centrifuging the cultures in Oak Ridge centrifuge tubes using a Beckman JA-17 rotor at 9,000 rpm for 10 minutes at 0° C then suspended in 25 ml fresh cold medium. Percoll™ (Sigma Chemicals) layered tubes were prepared as follows; 1.5 ml, 1.08 specific gravity, 2.0 ml, 1.07 sp. gr., 2.0 ml, 1.06 sp. gr., 13.5ml, 1.02 sp. gr. and topped with 2.5 ml of the resuspended pellet. The Percoll density layers were prepared by dilution of Percoll with fresh medium. The tubes were spun at 9,000 rpm for 10 min in a Beckman JA-17 rotor at 3° C. The lower, motile fraction was collected at 1.07 sp.gr. and the upper, sessile fraction at 1.06 sp.gr. The absorbance, at 560 nm, of a 1:100 dilution of the collected fraction in fresh medium was measured. The number of cells collected was determined by comparison to a previously prepared standard curve. These diluted samples also were examined using phase contrast microscopy to determine the purity of the separation of the morphologic types.

Similar fractions were pooled, 5 ml of 0.01 M Tris-acetate (pH 8.3) added to each pool, then each pool was sonicated in an ice bath by a Branson Sonifer. The total sonication time was 2.5 min accumulated using a 30 sec. on, 30 sec. off cycle; 10.1 ml of disrupted motile cells and 11.7 ml of sessile were produced. The sonicated material was centrifuged at 12,000 rpm for 10 min. in a Beckman JA-17 rotor at 3° C and the supernatant saved at -20° C for analysis.

Protein analysis was performed as directed in Bio Rad instructions using *Escherichia coli* ATCase as the reference protein standard.

ATCase analysis was according to the method of Pastra-Landis et al. (12)

Results and Discussion

The culture, harvest and specific gravity separation of the CB15F cultures produced approximately 1.7×10^9 motile cells consisting of about 90% motile cells and 10% sessile and 2.5×10^9 sessile cells also at about 90% purity. After the cells were disrupted and cell debris removed by centrifugation these solutions were analyzed for protein content and revealed that on a per cell basis the protein content was quite similar, sessile cells contain 8 picograms/cell and motile, 7 pg/cell.

The ATCase analysis revealed differences between the two populations. The extract of the motile cells revealed that the material which is present and capable of reacting in the ATCase analysis procedure produced about 48 attomoles of carbamyl aspartate per disrupted cell, per minute, under the standard conditions of analysis. The extract of the sessile cells, when analyzed in parallel, revealed the production of 118 amol of CA per cell, per minute. If these figures are corrected for the known imperfections of the purification procedure (90%, 10%, in both cases) then the results are: motile cell extract produced 39 amol/cell/min., and the sessile produced 127 amol/cell/min.

These results do not demonstrate a complete sequestering of the ATCase to the sessile cells, but rather the generation of a difference of about a factor of three between the motile and sessile products content of ATCase. This difference in enzyme content should affect the ability of the cells to produce the intermediate carbamyl aspartate and the later products of the pyrimidine pathway. The possibility of a deficiency of products of the pyrimidine pathway in motile cells, immediately after their formation, might provide a partial explanation of their inability to initiate immediate DNA synthesis.

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