JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 769–774
0021-9193/88/020769-06$02.00/0

Vol. 170, No. 2

Regulation of Tryptophan Biosynthesis in *Caulobacter crescentus*

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Received 26 May 1987/Accepted 8 October 1987

We present an analysis of the expression of the *trpE* gene and the *trpFBA* operon in the dimorphic bacterium *Caulobacter crescentus*. The catalytic activity of component I of anthranilate synthase, the product of the *trpE* gene, was efficiently inhibited by tryptophan, the end product of the pathway, which suggests that tryptophan biosynthesis is likely controlled at the pathway level in *C. crescentus*. However, *trpFBA* mRNA levels and *trpE* enzyme levels did not vary significantly in wild-type *C. crescentus* in response to the presence of tryptophan in the growth medium or to growth in minimal versus rich medium. This lack of regulation of the *trpE*, *trpF*, *trpB*, and *trpA* genes is consistent with the idea that oligotrophic bacteria, such as *C. crescentus*, do not utilize regulatory mechanisms that greatly alter the biosynthetic capabilities in exponentially growing cells. In contrast, mRNA levels from the 5′-untranslated region and the upstream gene (*usg*) coding region increased dramatically in *C. crescentus trpD* or *hisB* auxotrophs starved for tryptophan or histidine, respectively. Surprisingly, concomitant increases in mRNA levels were not detected from the *trpF*, *trpB*, or *trpA* coding regions downstream in the operon. Thus, severe starvation of *C. crescentus* for amino acids appears to elicit a strong, general transcriptional response that is not observed in bacteria growing exponentially in medium lacking amino acids.

Caulobacter crescentus is a gram-negative, dimorphic bacterium that has been used to study temporal gene expression (4, 12, 15, 16, 20–22). Differentiation of *C. crescentus* is a developmentally programmed sequence of events that occurs during normal exponential growth and is not a response to nutritional deprivation as in other bacteria (17, 20). To fully understand differentiation of *C. crescentus*, it seems important to learn about the structure and regulation of key biosynthetic genes. Because so much is known about tryptophan biosynthesis in other organisms (5, 26–28), we decided to measure the expression of several *trp* genes at the transcriptional and enzyme levels in wild-type or mutant *C. crescentus* cells grown under a variety of culture conditions.

The *C. crescentus trpE*, *trpD*, and *trpC* genes map at a separate chromosomal location from the *trpF*, *trpB*, and *trpA* genes (25). Enzyme assays have been developed for several of the *trp* gene products, and these assays have been used to gauge *trp* gene expression in a variety of organisms (5, 8, 10, 23, 27). In the accompanying paper (19), we show that the *C. crescentus trpF*, *trpB*, and *trpA* genes and a fourth gene upstream of *trpF*, designated *usg*, are arranged into a single operon (Fig. 1). The *usg* gene does not encode a known tryptophan or general aromatic biosynthetic enzyme, but it may be involved in the regulation of the *trpFBA* operon. Because we also identified the promoter for the *trpFBA* operon 30 base pairs upstream of the start of *usg* (19), we could directly analyze transcription of this operon in response to different growth conditions. In this report, we demonstrate that tryptophan biosynthesis in *C. crescentus* is likely regulated at the pathway level by end product inhibition. Although we were unable to detect regulation of tryptophan biosynthesis at the level of gene expression in exponentially growing wild-type *C. crescentus*, our results indicate that a metabolic response occurs in the *C. crescentus trpFBA* operon during starvation for tryptophan or histidine.

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MATERIALS AND METHODS

Materials. Restriction endonucleases and T4 polynucleotide kinase were purchased from New England BioLabs. S1 nuclease and bacterial alkaline phosphatase were from Bethesda Research Laboratories, Inc. Chorismate and L-tryptophan were purchased from Sigma Chemical Co. [γ-^{32}P] ATP (≈5,400 Ci/nmol) was purchased from Amersham Corp.

Bacterial strains and plasmids. *C. crescentus* wild-type strain CB15 and mutant strains SC1361 *hisB*, SC1366 *trpB*, and SC1371 *trpD*, were obtained from B. Ely (25). *Escherichia coli* W3110 prototroph was obtained from C. Yanofsky. DNA fragments used in S1 nuclease mapping experiments were isolated from plasmid pNU14, described in the accompanying paper (19).

S1 nuclease mapping. S1 nuclease mapping was performed as described in the accompanying paper (19). For RNA isolations, *C. crescentus* wild-type strain CB15 was grown exponentially with shaking in PYE (19), M2-sucrose (0.3%), or M2-sucrose plus tryptophan (40 μg/ml) medium at 32°C. For starvation experiments, *C. crescentus* mutant strains SC 1371 *trpD* and SC1361 *hisB* were grown exponentially in PYE plus tryptophan (100 μg/ml) medium at 32°C, collected by low-speed centrifugation, washed with 0.9% (wt/vol) NaCl, and suspended in PYE plus tryptophan, M2-sucrose plus excess tryptophan (100 μg/ml), M2-sucrose plus limiting tryptophan (20 μg/ml), or M2-sucrose medium lacking tryptophan, and incubated with shaking for 40 min at 32°C before RNA isolation.

AS component I assays. For preparation of crude extracts, *E. coli* prototroph W3110 was grown to Klett 70 (red filter) at 37°C in 30 ml of Luria-Bertani broth (LB), M2-glucose (0.3%), or M2-glucose plus tryptophan (40 μg/ml) medium, and *C. crescentus* wild-type strain CB15 was grown to Klett 70 at 32°C in 30 ml of PYE, M2-sucrose (0.3%), M2-glucose (0.3%), or M2-glucose plus tryptophan (40 μg/ml) medium. To assay possible induction of *trp* genes by indoleacrylic acid (IAA), *C. crescentus* CB15 and *E. coli* W3110 were

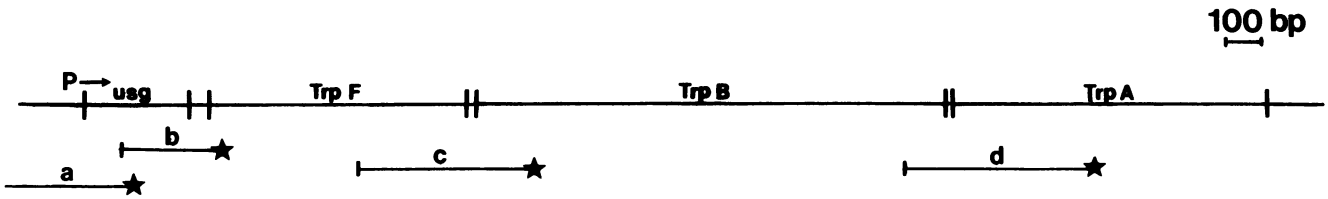

FIG. 1. Structure of the *C. crescentus trpFBA* operon. The reading frames of the *usg*, *trpF* (Trp F), *trpB* (Trp B), and *trpA* (Trp A) genes are indicated. P designates the position of the promoter of the operon. The ^{32}P-labeled ends of noncoding strands of DNA fragments a, b, c, and d, which were used as probes in S1 nuclease mapping experiments, are indicated (★). bp, Base pairs.

grown in 30 ml of M2-glucose medium to Klett 70, centrifuged, washed, suspended in 30 ml of M2-glucose medium containing 10 μg of IAA per ml, and incubated with shaking for 40 min before extract preparation. For starvation experiments, *C. crescentus* mutant strains SC1366 *trpB* and SC1361 *hisB* were grown at 32°C in 60 ml of PYE medium to Klett 70, divided into two 30-ml subcultures, centrifuged, washed, suspended in 30 ml of PYE medium or 30 ml of M2-sucrose medium, and incubated at 32°C with shaking for 40 min before extract preparation.

After growth, cells were collected by centrifugation, washed with 0.9% (wt/vol) NaCl, and the pellets were suspended in 1 ml of 0.1 M Tris hydrochloride (pH 7.8). Cells were subjected to sonic disruption for 60 s on ice and centrifuged at 12,000 × *g* for 5 min, and the supernatant was used as the cell extract in enzyme assays. The assay procedure for AS component I was previously described (8–10). The reaction mixture contained 0.14 mM chorismic acid, 0.01 mM triethanolamine hydrochloride (pH 8.9), 48.0 mM ammonium sulfate, 0.01 mM magnesium acetate, and 1.0 mM dithiothreitol. The reaction was started by adding various amounts of the cell extract to the reaction mixture at 37°C. AS component I activity was monitored by recording the increase in fluorescence at 395 nm (activation wavelength, 323 nm) with a Perkin-Elmer recording spectrophotofluorometer. Specific activity is expressed as nanomoles of anthranilate formed per minute per microgram of total protein. Total protein in the extracts was determined by the Bradford method (3).

For end product inhibition assays, cells were grown in M2-glucose medium and cell extracts were prepared as described above. L-Tryptophan was added to the reaction mixture at the concentrations indicated in Fig. 1 to determine the extent of inhibition of AS component I activity.

RESULTS AND DISCUSSION

Regulation of tryptophan biosynthesis in wild-type *C. crescentus*. In every organism examined so far, AS, which catalyzes the first step in the tryptophan biosynthetic pathway, is inhibited by the end product, tryptophan (5, 26, 27). AS is composed of dissimilar subunits encoded by *trpE* and *trpG* (9, 27, 28), and it has been established that the activity of the *trpE*-encoded (component I) subunit is inhibited by L-tryptophan (8, 10, 27). To avoid potential complications of assaying the activity of a multimer of dissimilar subunits, we simplified our analysis by assaying the ammonium ion-independent component I reaction (8). Figure 2 shows that AS component I is inhibited in extracts of exponentially grown, wild-type *C. crescentus* CB15 by the same concentration range of tryptophan as the analogous *E. coli* enzyme (8). Although the mechanism of this inhibition was not further studied, it seems reasonable to conclude that tryptophan biosynthesis in *C. crescentus* is very likely controlled at the pathway level by mechanisms of end product inhibition found previously in *E. coli* (8–10) and other organisms (5, 26, 27).

In enterobacteria, transcription of the *trp* operon is regulated by the product of the *trpR* gene, which codes for an aporepressor protein, and the corepressor, L-tryptophan (26, 27). IAA, an analog of L-tryptophan, induces *trp* operon expression in *E. coli* by binding to the aporepressor protein and preventing tryptophan from binding (14). To test whether *C. crescentus* is also regulated by repression, we grew a wild-type *C. crescentus* strain and a prototrophic *E. coli* control strain in various media and assayed AS component I activity. *trpE* enzyme activity was 100-fold lower in *E. coli* W3110 grown in rich medium (LB) compared with cells grown in M2-glucose minimal medium lacking tryptophan (Table 1). The addition of 40 μg of tryptophan per ml to the M2-glucose medium caused a sixfold repression of *trpE* enzyme levels compared with bacteria grown in M2-glucose minimal medium without tryptophan. However, AS component I activity in extracts of *E. coli* W3110 grown in

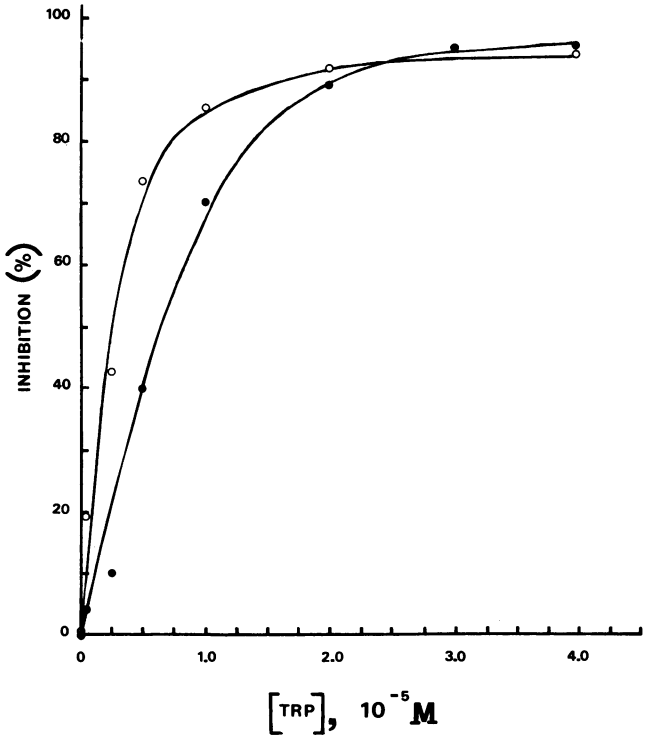

FIG. 2. Inhibition of AS component I specific activity by L-tryptophan (TRP) in *C. crescentus* and *E. coli* cellular extracts. Symbols: ○, extracts from *C. crescentus* wild-type strain CB15; ●, extracts from *E. coli* W3110 prototroph.

TABLE 1. *trpE* enzyme levels in *C. crescentus* and *E. coli* strains grown under different conditions

Strain	Growth condition[a]	Doubling time (min)	AS component I sp act[b]
W3110 (*E. coli* wild type)	LB	43	0.2
	M2-Glc	122	19.7
	M2-Glc-Trp	122	3.6
	M2-Glc-IAA	122	124.1
CB15 (*C. crescentus* wild type)	PYE	86	1.6
	M2-Suc	163	1.8
	M2-Glc	217	2.4
	M2-Glc-Trp	195	2.3
	M2-Glc-IAA	195	2.5
SC1366 (*C. crescentus trpB*)	PYE → PYE[c]	100	2.9
	PYE → M2-Suc		2.4
SC1361 (*C. crescentus hisB*)	PYE → PYE	105	2.9
	PYE → M2-Suc		2.4

[a] LB and PYE, rich media; M2-Suc, minimal medium plus 0.3% sucrose; M2-Glc, minimal medium plus 0.3% glucose; Trp, L-tryptophan added to a final concentration of 40 μg/ml; IAA, IAA added to a final concentration of 10 μg/ml.

[b] Expressed as 10^{-4} nanomoles of chorismate converted to anthranilate per minute per microgram of protein.

[c] Cells were grown to log phase in PYE medium and then centrifuged, washed, and suspended in PYE or M2-Suc medium as indicated for 40 min before harvest.

M2-glucose plus tryptophan medium was still 10-fold higher than the activity in cells grown in LB. The large magnitude of this metabolic regulation of *trpE* expression probably reflects in part the use of M2 minimal medium which is optimized for *C. crescentus*, but not *E. coli*, growth. Therefore, *trpE* enzyme levels in *E. coli* grown in M2-glucose plus tryptophan medium might not be as fully repressed as in certain other minimal media (18). When W3110 cells were grown with 10 μg of IAA per ml, *trpE* expression was dramatically derepressed (about 500-fold) compared with cells grown in LB media.

In contrast to the regulation observed for *E. coli*, AS component I activity in *C. crescentus* CB15 cells did not change in response to the presence or absence of tryptophan, the addition of IAA, growth in minimal versus rich medium, or growth in minimal medium containing sucrose instead of glucose as a carbon source (Table 1). Possibly wild-type *C. crescentus* cells growing in minimal medium are not sufficiently limited for tryptophan because of endogenous biosynthesis. On the basis of evolutionary comparisons (7, 28), we expect the tryptophan content of the *trpE* gene product to be very low. Therefore, we reasoned that if *C. crescentus trpE* is regulated, then we might be able to detect increased enzyme activity in a *trpB* auxotroph upon starvation for tryptophan. In a parallel experiment, a *his* auxotroph was starved for histidine to determine whether we could detect a general mechanism that regulates *trpE* enzyme levels. *C. crescentus* SC1366 *trpB* cells and SC1361 *hisB* cells were grown exponentially in PYE medium, centrifuged, washed, and suspended in either PYE or M2-sucrose minimal medium to assay for induction of *trpE* enzyme activity upon starvation for tryptophan or histidine, respectively. AS component I activity remained the same in the *trp* auxotroph and the *his* auxotroph when cells were starved for tryptophan or histidine (Table 1). Thus, *trpE* enzyme levels did not appear to be regulated in *C. crescentus*; however, we cannot completely rule out the possibility that synthesis of the *trpE* gene product was blocked in the latter experiments by starvation for tryptophan or histidine.

Since expression of *C. crescentus trpE* was constitutive under a variety of growth conditions, it was of interest to determine whether the separately located *trpFBA* operon is regulated. Preliminary experiments suggested that levels of tryptophan synthase, which is encoded by *trpA* and *trpB*, were the same in CB15 cells grown with or without tryptophan (J. T. Barrett and B. Ely, personal communication). Because tryptophan synthase levels are very low in *C. crescentus* cellular extracts, we decided to test this result by using S1 nuclease mapping to measure in vivo expression of the *C. crescentus trpFBA* operon. Equal amounts of total RNA extracted from *C. crescentus* cells grown under a variety of conditions were hybridized to an excess of DNA from the internal regions of the *C. crescentus trpFBA* operon (Fig. 1), S1 nuclease-resistant hybrids were resolved by gel electrophoresis, and autoradiographs of gels were quantified by densitometry to determine relative mRNA levels. A similar approach has been used previously to detect and quantify relative changes in mRNA levels (6), but it should be stressed that this kind of experiment does not measure rates of mRNA synthesis.

Results of S1 nuclease mapping experiments using RNA extracted from *C. crescentus* wild-type strain CB15 are shown in Fig. 3A, in which the noncoding strand of a 0.33-kilobase (kb) *Sau*3A-*Sau*3A fragment labeled at its 5′ end was used as a probe (probe a in Fig. 1). This fragment (nucleotide −30 to 300) contains the operon promoter and extends into the coding region of *usg*. When this probe was hybridized to total RNA isolated from CB15 cells grown in PYE (lane 3), M2-sucrose plus tryptophan (lane 4), or M2-sucrose medium (lane 5), an S1 nuclease-resistant dou-

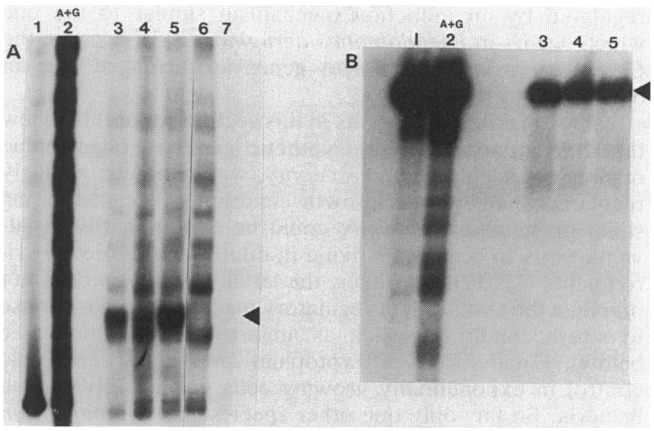

FIG. 3. S1 nuclease analysis of *trpFBA* operon expression in *C. crescentus* wild-type strain CB15. The DNA probes used were the noncoding strand of the 0.33-kb *Sau*3A-*Sau*3A fragment containing the 5′-untranslated and part of the *usg* coding region (see probe a in Fig. 1 and the text) (A) and the noncoding strand of the 0.25-kb *Pst*I-*Bgl*II fragment, containing the *usg-trpF* intergenic region (probe b in Fig. 1) (B). Culture conditions are described in the Materials and Methods. Lane 1, Untreated DNA; lane 2, Maxam-Gilbert A+G sequencing reaction; lane 3, RNA isolated from cells grown in PYE medium; lane 4, RNA isolated from cells grown in M2-sucrose plus tryptophan (40 μg/ml) medium; lane 5, RNA isolated from cells grown in M2-sucrose medium; lane 6, tRNA control; lane 7, control lacking RNA. The arrowheads indicate positions corresponding to transcripts from the *trpFBA* operon (see text).

blet of 149 and 150 nucleotides was observed that corresponds to the start of transcription of the *trpFBA* operon. The intensity of these bands is equivalent in lanes 3 and 4, and the intensity in lane 5 is about two- to threefold greater than that of lanes 3 and 4. Therefore, mRNA transcribed from the region corresponding to the 0.33-kb *Sau*3A-*Sau*3A fragment increased slightly when CB15 cells were grown in minimal medium lacking tryptophan. Results possibly related to this observation are presented in the next section.

The noncoding strand of a 0.25-kb *Pst*I-*Bgl*II fragment labeled at the *Bgl*II site (Fig. 3B) was also used as a probe (probe b in Fig. 1). This fragment extends from base pair 273 to 527 in the sequence, contains the *usg-trpF* intercistronic region, and extends into the *trpF* coding region. As expected, full-length protection of this DNA probe was observed. The levels of mRNA transcribed from this region remained unchanged when CB15 cells were grown in PYE (lane 3), M2-sucrose plus tryptophan (lane 4), or M2-sucrose medium (lane 5). Similarly, when RNA was probed with the noncoding strand of a 0.47-kb *Sst*II-*Eco*RI or 0.50-kb *Sma*I-*Sal*I fragment, which contains the *trpF-trpB* or *trpB-trpA* intercistronic region, respectively (probe c or d in Fig. 1), no change was detected when CB15 cells were grown in PYE, M2-sucrose plus tryptophan, or M2-sucrose medium (data not shown).

Thus, in contrast to the case in enterobacteria, the *C. crescentus trpE* gene and *trpFBA* operon are not strongly regulated at the level of transcription when tryptophan is added to exponentially growing wild-type bacteria. With regard to this conclusion, it should be noted that tryptophan can be taken up by *C. crescentus*, because *trp* auxotrophs grow on minimal medium containing tryptophan. However, the possibility does exist that tryptophan is not transported from the growth medium by wild-type bacteria which are able to synthesize it endogenously. Moreover, it also seems unlikely that the *C. crescentus trpB* and *trpA* genes are regulated by an induction mechanism similar to the one which occurs in *Pseudomonas aeruginosa* (13), because the *C. crescentus trpB* and *trpA* genes are arranged into an operon with *trpF* (19).

Taken together, the results in this section support the view that the expression of biosynthetic genes in oligotrophic organisms, such as *C. crescentus*, would not be strongly regulated under normal growth conditions, because strong shifts in metabolic capacity could be evolutionarily disadvantageous to organisms living in dilute, low-nutrient environments (11, 17). However, the latter hypothesis does not preclude the evolution of regulatory mechanisms in response to stress conditions, such as amino acid starvation (see below). Finally, lack of tryptophan-specific transcriptional control in exponentially growing cells is relatively rare in bacteria. So far, only one other species, *Chromobacterium violaceum*, has been shown to regulate tryptophan biosynthesis solely at the pathway level by feedback inhibition (23).

Transcription of the *trpFBA* operon in *C. crescentus* mutants. Detailed analysis has shown that certain regulatory mechanisms are silent in exponentially growing enterobacteria. Perhaps the best example of this type is the wild-type *Salmonella typhimurium his* operon, which does not respond to the presence of histidine in growth medium (24). In this case, strong relief of *his* operon attenuation manifests itself only during extreme starvation for histidine that can be induced in mutants. To determine whether mRNA levels for the *trpFBA* operon increase when a *C. crescentus trp* auxotroph is starved for tryptophan, RNA was isolated from *C. crescentus* SC1371, which contains a Tn*5* mutation in *trpD*. SC1371 cells were grown in PYE plus tryptophan (100 μg/ml) medium, centrifuged, and washed, and subcultures were suspended in PYE plus tryptophan (100 μg/ml), M2-sucrose plus excess tryptophan (100 μg/ml), M2-sucrose + limiting tryptophan (20 μg/ml), or M2-sucrose medium lacking tryptophan for 40 min before RNA isolation. Equal amounts of total RNA were isolated and hybridized to an excess of noncoding strand DNA corresponding to the probes depicted in Fig. 1, and hybrids were detected with S1 nuclease. Excess (100 μg/ml) and limiting (20 μg/ml) concentrations of tryptophan for strain SC1371 were determined by measuring the doubling times in minimal medium containing various concentrations of tryptophan. A tryptophan concentration of 100 μg/ml gave the maximal doubling time for growth of strain SC1371 in M2-sucrose medium, whereas a tryptophan concentration of 20 μg/ml gave a twofold increase in doubling time.

RNA from strain SC1371 was hybridized to the noncoding strand of the 0.33-kb *Sau*3A-*Sau*3A fragment that contains the *trpFBA* operon promoter (probe a in Fig. 1) (Fig. 4A). When SC1371 cells were shifted from growth in PYE plus tryptophan to M2-sucrose plus excess tryptophan (lane 4), or M2-sucrose plus limiting tryptophan (lane 5), RNA levels increased about 10- or 3-fold, respectively, compared with cells grown in PYE plus tryptophan (lane 3). By contrast, when SC1371 cells were starved for tryptophan by a shift from growth in PYE plus tryptophan to M2-sucrose medium lacking tryptophan, mRNA levels increased dramatically by at least 100-fold (lane 6). RNA from strain SC1371 was also hybridized to the noncoding strand of the 0.25-kb *Pst*I-*Bgl*II fragment (probe b in Fig. 1), which contains the *usg-trpF* intercistronic region and extends into the *trpF* coding region (Fig. 4B). Surprisingly, mRNA levels corresponding to this

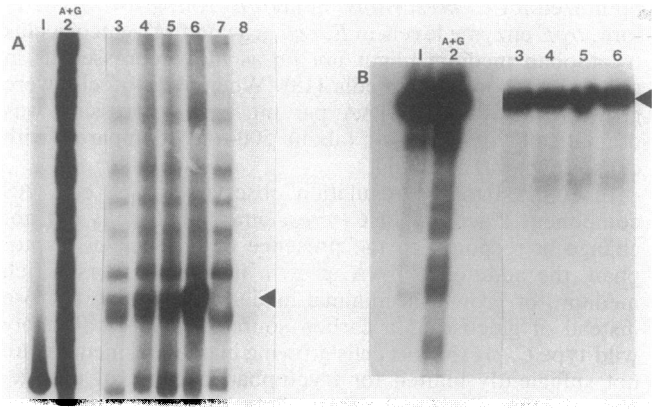

FIG. 4. S1 nuclease analysis of *trpFBA* operon expression in *C. crescentus trp* auxotroph strain SC1371. The DNA probes used were the noncoding strand of the 0.33-kb *Sau*3A-*Sau*3A fragment containing the 5'-untranslated and part of the *usg* coding region (probe a in Fig. 1) (A) and the noncoding strand of the 0.25-kb *Pst*I-*Bgl*II fragment containing the *usg-trpF* intergenic region (probe b in Fig. 1) (B). Downshifts are described in Materials and Methods. Lane 1, Untreated DNA; lane 2, Maxam-Gilbert A+G sequencing reaction; lane 3, RNA isolated from cells grown in PYE plus tryptophan (100 μg/ml); lane 4, RNA isolated from cells grown in PYE plus tryptophan and suspended in M2-sucrose plus excess tryptophan (100 μg/ml); lane 5, RNA isolated from cells grown in PYE plus tryptophan and suspended in M2-sucrose plus limiting tryptophan (20 μg/ml); lane 6, RNA isolated from cells grown in PYE plus tryptophan and suspended in M2-sucrose medium; lane 7, tRNA control; lane 8, control lacking RNA. The arrowheads indicate positions corresponding to transcripts from the *trpFBA* operon (see text).

region of the *trpFBA* transcript did not change when SC1371 cells were shifted from growth in PYE plus tryptophan (lane 3) to M2-sucrose plus excess tryptophan (lane 4), M2-sucrose plus limiting tryptophan (lane 5), or M2-sucrose medium lacking tryptophan (lane 6). mRNA levels corresponding to the *trpF-trpB* and the *trpB-trpA* intergenic regions of the *trpFBA* transcript (probes c and d, respectively, in Fig. 1) also did not change in analogous downshift experiments (data not shown).

To determine whether the increase in mRNA levels transcribed from the 5′-untranslated and *usg* coding regions of the *C. crescentus trpFBA* operon is tryptophan specific, RNA was isolated from *C. crescentus his* auxotroph SC1361 *hisB*, which was grown with or without histidine. Except for their amino acid auxotrophies, strains SC1371 *trpD* and SC1361 *hisB* are essentially isogenic. In this experiment, parallel cultures of SC1371 and SC1361 were grown exponentially in PYE medium, centrifuged, washed, and suspended in M2-sucrose minimal medium for 40 min to induce starvation for tryptophan and histidine, respectively. DNA from the 0.33-kb *Sau*3A-*Sau*3A fragment that contains the operon promoter (probe a in Fig. 1) was used as the hybridization probe. The amount of RNA corresponding to the 5′-untranslated and *usg* coding regions of the *trpFBA* operon increased significantly when strain SC1361 was starved for histidine (Fig. 5). The intensity of the S1 nuclease-resistant doublet in lane 4 (SC1361 *hisB* cells shifted to M2-sucrose) is at least 80-fold greater than that of the doublet in lane 3 (SC1361 *hisB* cells grown in PYE). As expected from the results presented above, RNA levels from this region increased when strain SC1371 *trpD* was shifted from growth in PYE to M2-sucrose minimal medium (lanes 5 and 6); however, in this particular experiment, the increase was at least 800-fold. In addition, we consistently observed that the mRNA levels detected in these hybridization experiments were at least threefold higher in SC1361 *hisB* in PYE (lane 3) than SC1371 *trpD* in PYE (lane 5).

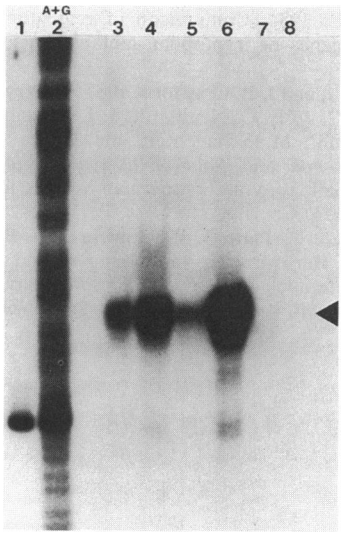

FIG. 5. S1 nuclease analysis of *trpFBA* operon expression in a *C. crescentus trp* auxotroph and a *C. crescentus his* auxotroph. The DNA probe was the noncoding strand of the 0.33-kb *Sau*3A-*Sau*3A fragment containing the 5′-untranslated and part of the *usg* coding region (probe a in Fig. 1). Downshifts are described in Materials and Methods. Lane 1, Untreated DNA; lane 2, Maxam-Gilbert A+G sequencing reaction; lane 3, RNA isolated from strain SC1361 *hisB* grown in PYE medium; lane 4, RNA isolated from strain SC1361 *hisB* shifted from growth in PYE to M2-sucrose medium; lane 5, RNA isolated from strain SC1371 *trpD* grown in PYE; lane 6, RNA isolated from strain SC1317 *trpD* shifted from growth in PYE to M2-sucrose medium; lane 7, tRNA control; lane 8, control lacking RNA. The arrowhead indicates positions corresponding to transcripts from the *trpFBA* operon (see text).

Several explanations are possible for the results shown in Fig. 4 and 5. The amount of promoter-distal *trpFBA* transcript indeed may have increased in the *trp* auxotroph upon starvation for tryptophan; however, this region of the transcript may be much less stable than the promoter-proximal region. Since we measured levels of mRNA rather than rates of mRNA synthesis, we would not have detected a rapidly degraded 3′ end of the *trpFBA* transcript. If this is the correct explanation, then an RNA secondary structure, such as the extremely stable putative one between *usg* and *trpF* (19), may protect the 5′ region of the transcript from degradation. A precedent for such a mechanism is found in *Rhodopseudomonas capsulata*, which is evolutionarily related to *C. crescentus*. The 3′ portion of the *R. capsulata rxcA* transcript, encoding the B870 light-harvesting and reaction center proteins, is rapidly degraded to give rise to two slowly decaying mRNAs, which both encode only the light-harvesting polypeptides. The unstable 3′ portion of the mRNA is delimited by two alternative stem-loop structures, which may act as barriers to 3′ exoribonucleases and thereby protect the upstream portion of the mRNA (1). Additional experiments will be needed in which the rates of synthesis of portions of the *trpFBA* transcript are measured to determine if differential mRNA instability underlies our observations.

The presence of a transcriptional terminator downstream from the *usg* coding region is another feasible explanation for the observed increase in only the 5′-untranslated and *usg* portions of the *trpFBA* transcript. The extremely stable RNA secondary structure between *usg* and *trpF* is a candidate for such a transcription terminator (19). According to this notion, the mRNA level for the *usg* gene would increase when cells are starved for tryptophan, whereas the mRNA level for the *trpF*, *trpB*, and *trpA* genes would remain the same. This hypothesis suggests that the *usg* gene product is required during starvation, perhaps to increase translation of transcripts from biosynthetic genes, such as *trpF*, *trpB*, and *trpA*. In this regard, it is interesting that when a *his* auxotroph was starved for histidine, the level of mRNA from the 5′-untranslated and *usg* coding regions of the *trpFBA* transcript again increased (Fig. 5). Therefore, there appears to be a significant nonspecific component to this starvation response. Even though the relative increase in transcript level was greater for tryptophan than histidine starvation (Fig. 5), it is not possible to tell whether this increase has a tryptophan-specific component or whether tryptophan starvation simply induces more nonspecific signal. It is also important to point out that there seems to be a strong global transcription response to amino acid starvation in *C. crescentus*, irrespective of whether the entire *trpFBA* or only the *usg* transcript increases. The possibility of such a global response to amino acid starvation needs to be taken into account in the interpretation of previous experiments in which a *C. crescentus* strain containing a *cys* transcriptional fusion was starved for cysteine (2).

ACKNOWLEDGMENTS

We thank B. Ely, and B. Nichols for providing bacterial strains, B. Ely, P. Schoenlein, and I. Crawford for helpful discussions, and M. Johnson and L. Taylor for help with preparation of the manuscript.

This work was supported in part by National Science Foundation grant DMB-8417005 and grant 83-58 from the Illinois Division of the American Cancer Society.

LITERATURE CITED

1. **Belasco, J. G., J. T. Beatty, C. W. Adams, A. von Gabain, and S. N. Cohen.** 1985. Differential expression of photosynthesis genes in *R. capsulata* results from segmental differences in stability within the polycistronic *rxcA* transcript. Cell **40:** 171–181.
2. **Bellofatto, V., L. Shapiro, and D. A. Hodgson.** 1984. Generation of a Tn5 promoter probe and its use in the study of gene expression in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. USA **81:**1035–1039.
3. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Bryan, R., R. Champer, S. Gomes, B. Ely, and L. Shapiro.** 1987. Separation of temporal control and trans-acting modulation of flagellin and chemotaxis genes in *Caulobacter*. Mol. Gen. Genet. **206:**300–306.
5. **Crawford, I. P.** 1980. Comparative studies on the regulation of tryptophan synthesis. Crit. Rev. Biochem. **8:**175–189.
6. **Hall, C. V., and C. Yanofsky.** 1982. Regulation of tryptophanyl-tRNA synthetase formation. J. Bacteriol. **151:**918–923.
7. **Henner, D. J., L. Band, and H. Shimotsu.** 1984. Nucleotide sequence of the *Bacillus subtilis* tryptophan operon. Gene **34:**169–177.
8. **Ito, J., E. C. Cox, and C. Yanofsky.** 1969. Anthranilate synthetase, an enzyme specified by the tryptophan operon of *Escherichia coli*: purification and characterization of component I. J. Bacteriol. **97:**725–733.
9. **Ito, J., and C. Yanofsky.** 1966. The nature of the anthranilic acid synthetase complex of *Escherichia coli*. J. Biol. Chem. **241:** 4112–4114.
10. **Ito, J., and C. Yanofsky.** 1969. Anthranilate synthetase, an enzyme specified by the tryptophan operon of *Escherichia coli*: comparative studies on the complex and the subunits. J. Bacteriol. **97:**734–742.
11. **Kruznetsov, S. I., G. A. Dubinina, and N. A. Lapteva.** 1979. Biology of oligotrophic bacteria. Annu. Rev. Microbiol. **33:** 377–387.
12. **Lagenaur, C., and N. Agabian.** 1978. *Caulobacter* flagellar organelle: synthesis, compartmentation, and assembly. J. Bacteriol. **135:**1062–1069.
13. **Manch, J. N., and I. P. Crawford.** 1982. Genetic evidence for a positive-acting regulatory factor mediating induction in the tryptophan pathway of *Pseudomonas aeruginosa*. J. Mol. Biol. **156:**67–77.
14. **Morse, D. E., R. D. Mosteller, and C. Yanofsky.** 1969. Dynamics of synthesis, translation, and degradation of *trp* operon messenger RNA in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **34:**725–739.
15. **O'Neill, E. A., and R. A. Bender.** 1987. Periodic synthesis of phospholipids during the *Caulobacter crescentus* cell cycle. J. Bacteriol. **169:**2618–2623.
16. **Osley, M. A., M. Sheffery, and A. Newton.** 1977. Regulation of flagellin synthesis in the cell cycle of *Caulobacter*: dependence on DNA replication. Cell **12:**393–400.
17. **Poindexter, J. S.** 1981. The caulobacters: ubiquitous unusual bacteria. Microbiol. Rev. **45:**123–179.
18. **Rose, J. K., and C. Yanofsky.** 1972. Metabolic regulation of the tryptophan operon of *Escherichia coli*: repressor-independent regulation of transcription initiation frequency. J. Mol. Biol. **69:** 103–118.
19. **Ross, C. M., and M. E. Winkler.** 1988. Structure of the *Caulobacter crescentus trpFBA* operon. J. Bacteriol. **170:**757–768.
20. **Shapiro, L., N. Agabian-Keshishian, and I. Bendis.** 1971. Bacterial differentiation. Science **173:**884–892.
21. **Shaw, P., S. L. Gomes, K. Sweeney, B. Ely, and L. Shapiro.** 1983. Methylation involved in chemotaxis is regulated during *Caulobacter* differentiation. Proc. Natl. Acad. Sci. USA **80:** 5261–5265.
22. **Sheffery, M., and A. Newton.** 1981. Regulation of periodic protein synthesis in the cell cycle: control of initiation and termination of flagellar gene expression. Cell **24:**49–57.
23. **Wegman, J., and I. P. Crawford.** 1968. Tryptophan synthetic pathway and its regulation in *Chromobacterium violaceum*. J. Bacteriol. **95:**2325–2335.
24. **Winkler, M. E.** 1987. Biosynthesis of histidine, p. 395–411. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
25. **Winkler, M. E., P. V. Schoenlein, C. M. Ross, J. T. Barrett, and B. Ely.** 1984. Genetic and physical analyses of *Caulobacter crescentus trp* genes. J. Bacteriol. **160:**279–287.
26. **Yanofsky, C.** 1984. Comparison of regulatory and structural regions of genes of tryptophan metabolism. Mol. Biol. Evol. **1:**143–161.
27. **Yanofsky, C., and I. P. Crawford.** 1987. The tryptophan operon, p. 1453–1472. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
28. **Yanofsky, C., T. Platt, I. P. Crawford, B. P. Nichols, G. E. Christie, H. Horowitz, M. Van Cleemput, and A. M. Wu.** 1981. The complete nucleotide sequence of the tryptophan operon of *Escherichia coli*. Nucleic Acids Res. **9:**6647–6668.