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A Mutation in Escherichia coli that Mimics Diauxie Lag

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SUMMARY

We have described a mutant of E. coli (2S142) which shows a specific inhibition of stable RNA synthesis at 42°. The temperature sensitive lesion differs from the stringent response to amino acid starvation in that the shut off of rRNA synthesis is not associated with an inhibition of protein synthesis. The decay of ppGpp is slow at 42° with little or no pppGpp detectable. This slow decay rate is not observed in the parental strain, D10, or in 2S142 at 30°. Neither 2S142 or D10 are spoT$^-$, nor does the temperature sensitive lesion map near the spoT locus. Thus, the effect of the temperature sensitive lesion on ppGpp metabolism and rRNA synthesis seems to resemble a carbon source downshift (diauxie lag) rather than a stringent response to amino acid starvation.

INTRODUCTION

From evidence accumulated over the past ten years, it seems clear that there exists an inverse relationship between rRNA synthesis and the unusual guanine nucleotides guanosine 5'-diphosphate, 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate, 3'-diphosphate (pppGpp) (1). Elevation of ppGpp levels appears to occur by one of two mechanisms. When stringent (relA$^+$) cells are starved for a required amino acid, the increased ppGpp levels can be accounted for almost entirely by an increase in the rate of synthesis of pppGpp (2) which is rapidly converted to ppGpp. During carbon source downshift (diauxie lag), however, the decay of ppGpp is decreased 4-10 fold while the synthesis of ppGpp changes only slightly (3,4). In addition to the slow decay, a carbon downshift is characterized by the fact that only ppGpp accumulates. No pppGpp is detectable.

We have previously reported the isolation of a temperature sensitive mutant of E. coli which is specifically unable to synthesize stable RNAs at 42° and which accumulates ppGpp at 42° (5,6). Thus, the purpose of the

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present experiments was to investigate further the relationship between ppGpp levels and rRNA synthesis in this mutant.

MATERIALS AND METHODS

The bacterial strains and media used in this study have been described previously (6) with the exception of strain AT2438 (pyrE$^-$, spoT$^+$, rel$^+$) which was obtained from D. Schlessinger. The levels of ppGpp were determined by a modification (6) of the procedure described by Cashel (7). The uptake of [1-^{14}C] glucose was measured essentially as described by Saier et al. (8). Levels of glycolytic intermediates were determined using the fluorometric assays described by Maitra and Estabrook (9).

RESULTS

We have reported that ppGpp levels are elevated in 2S142 but not D10 after 45 min at 42° (5,6). Following the report by Gallant et al. (10) that ppGpp levels may be transiently elevated in some wild type strains following a temperature upshift, we decided to examine ppGpp levels in both strains more closely. Figure 1 shows the time course of ppGpp accumulation in both strains at 42°. Clearly ppGpp levels do not increase significantly in D10 until at least 90 min after the shift, while ppGpp levels increase continuously in 2S142-reaching a plateau of around 700 pmoles/ml/OD_{540} about 2 hours after the shift. Thus, the increase in ppGpp at 42° is specifically associated with the temperature sensitive lesion in 2S142. Furthermore, the increase in ppGpp correlates well with the decrease in rRNA synthesis (Harris and Chaney, manuscript in preparation).

The accumulation of ppGpp is rather slow following the shift to 42° and no pppGpp is detectable at any time in 2S142 (data not shown). These observations are reminiscent of the response of *E. coli* to carbon source downshift (3). Accordingly, the decay rate of ppGpp was measured in both 2S142 and D10 using either tetracycline or chloramphenicol to inhibit ppGpp synthesis. Figure 2B shows the decay in 2S142 at 42°. The addition of either inhibitor causes ppGpp to decay exponentially with a first order decay constant, k_2, of .046 min^{-1} ($t_{\frac{1}{2}}$ = 15 min). The comparable decay rates in 2S142 at 30° and in D10 at either temperature were somewhat more difficult to obtain, however, due to the fact that once the amount of ppGpp reaches

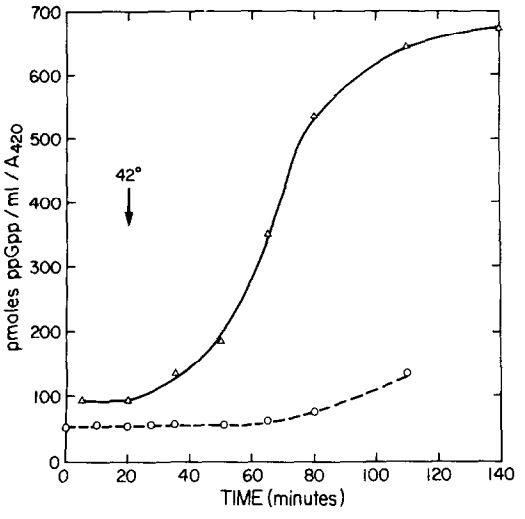

Figure 1. Accumulation of ppGpp in D10 and 2S142 at 42°. 2S142 and D10 were grown in low phosphate medium (5) containing all 20 amino acids at 50 μg/ml and 100 μc/ml of [^{32}P]Pi. When the cultures had grown to 0.5 x 10^8 cells/ml, the [^{32}P]Pi was added. At 1 x 10^8 cells/ml, we started to remove aliquots for ppGpp determination. The cultures were shifted to 42° and further aliquots removed at the indicated times. Levels of ppGpp were determined as described previously (5).

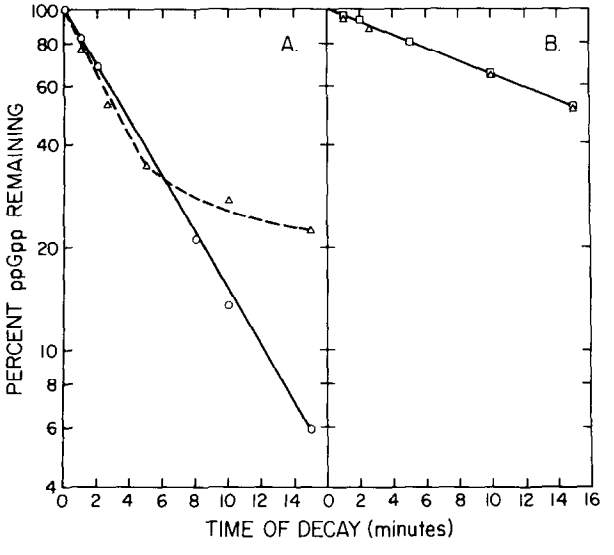

Figure 2. Decay of ppGpp in 2S142 at 30° and 42°. 2S142 was cultured in low phosphate medium as described in Figure 1. The carbon source used was either 0.2% glucose alone (as in Figure 1) or 0.02% glucose + 1% sodium succinate (for the glucose to succinate downshift). [^{32}P]Pi was added to the cultures at 0.5 x 10^8 cells/ml. ppGpp levels were determined following the addition of 100 μg/ml of chloramphenicol or tetracycline. The ppGpp values are

a basal level of 40-50 pmoles/ml/OD_{540}, there is little or no subsequent decay in either of these strains. Therefore, in 2S142 at 30°, for example, only about 50% of the ppGpp present is degraded in the presence of either tetracycline or chloramphenicol. The excess ppGpp that does decay, however, appears to decay with a t ½ of 3 min (Figure 2A, Δ--Δ).

D10, on the other hand, has only basal levels of ppGpp at either 30° or 42°. However, we were able to obtain good decay rates in both strains by measuring the decay following a glucose to succinate downshift. This technique gives a 5 to 10 fold increase in ppGpp levels and thus makes it much easier to measure the decay rates accurately. Kari et al (11) have reported that if the decay is determined following the simultaneous addition of glucose and chloramphenicol, the rate obtained is characteristic of the normal decay rate in glucose alone and not the slower decay characteristic of a carbon source downshift. Certainly our data is consistent with this observation. For example, the t ½ for decay of ppGpp in CP76 (met^-, $relA^+$, $spoT^+$) following the glucose to succinate downshift was 20 sec or less which is exactly what one observes following chloramphenicol addition to amino acid starved cells. As seen in Figure 2A (o——o), the decay rate obtained using this technique in 2S142 at 30° was virtually identical to the value obtained previously by simply adding chloramphenicol or tetracycline to growing cells. In D10 ppGpp appeared to decay with a t ½ of 3 min at 30° and 6.5 min at 42° (Figure 3A and 3B, o——o).

These decay rates were further verified by measuring the decay in both strains following methionine starvation. One observes a slight (30-40%) elevation of ppGpp levels during methionine starvation. As seen in Figure 3

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expressed as a percent of the value obtained before the addition of chloramphenicol or tetracycline and are plotted on a semilog scale.
Figure 2A: Decay at 30°. Δ--Δ, decay in exponentially growing cells following the addition of 100 µg/ml chloramphenicol; o--o, decay 20 min after a glucose to succinate downshift following the addition of 100 µg/ml of chloramphenicol + 0.2% glucose. Figure 2B: Decay after 45 min at 42°. Δ—Δ, decay following the addition of 100 µg/ml chloramphenicol; □ —□, decay following the addition of 100 µg/ml tetracycline.

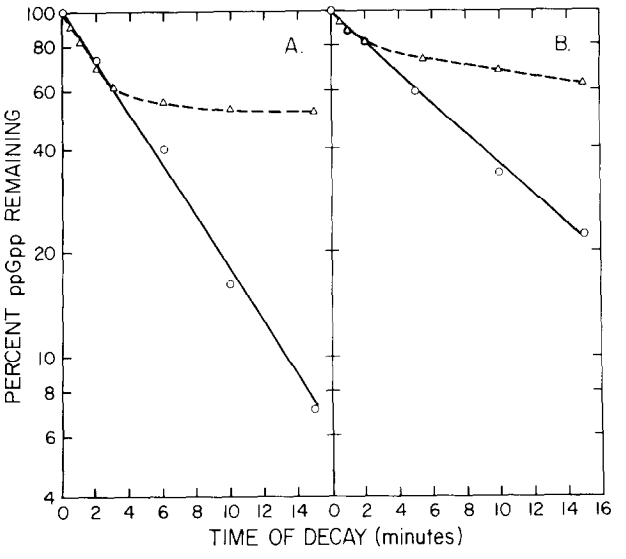

Figure 3. Decay of ppGpp in D10 at 30° and 42°. The experimental conditions were essentially the same as in Figure 2 except that, in some cases, the cultures were grown in limiting (1 µg/ml) methionine. Figure 3A: Decay at 30°; Δ--Δ, decay 30 min after methionine starvation following the addition of 100 µg/ml chloramphenicol; o___o, decay 20 min after a glucose to succinate downshift following the addition of 100 µg/ml chloramphenicol + 0.2% glucose. Figure 3B: Decay after approximately 45 min at 42°; Δ--Δ, decay 30 min after methionine starvation; o___o, decay 20 min after a glucose to succinate downshift.

(Δ--Δ), the decay rates in D10 are identical to those obtained following the carbon source downshift. Similar results were obtained with 2S142 (data not shown). It seems safe to conclude, therefore, that the temperature sensitive lesion in 2S142 does lead to a markedly reduced rate of ppGpp decay at 42°.

The fact that 2S142 behaves as if it were undergoing a carbon source downshift at 42° could, of course, have several trivial explanations. For example, a temperature sensitive lesion in the uptake and/or utilization of glucose would cause an apparent carbon source starvation. Accordingly, we measured the uptake of ^{14}C-glucose at 30° and 42°. The data are shown in Figure 4. Clearly glucose uptake is not restricted at 42°. The levels of glycolytic intermediates were also measured at both temperatures-and during a glucose to succinate downshift. As seen in Table I, there is little significant variation between the levels of glycolytic intermediates at 30° and 42°.

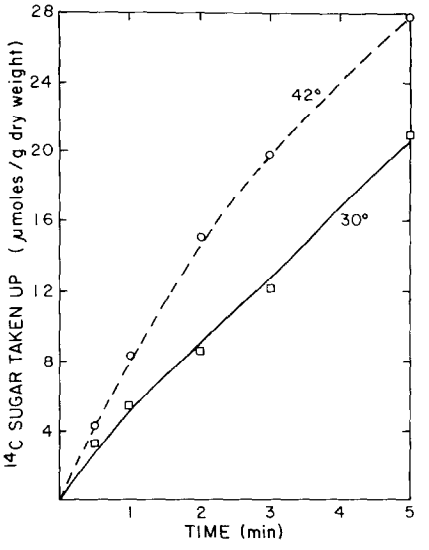

Figure 4. Uptake of [1-^{14}C] glucose in 2S142 at 30° and 42°. Cells were grown at 30° in minimal salts + glucose to a cell density of 5-8 x 10^7 cells/ml. At that point the culture was split into 2 aliquots with one being incubated at 30° and the other at 42°. After 45 min, both cultures were collected by filtration and resuspended in minimal salts medium without a carbon source at 10^8 cells/ml. The uptake of [1-^{14}C] glucose was determined as described in Materials and Methods.

Certainly if the temperature sensitive lesion had been in an enzyme which directly affected the metabolism of glucose, one would have expected to see a build up of the intermediates preceding that enzyme and a decrease of those intermediates which followed it. No such pattern was observed. Since most glucose is metabolized via the glycolytic pathway, it appears that the metabolism of glucose is not significantly altered at 42°.

DISCUSSION

In a number of respects, the mutation of 2S142 seems to mimic a carbon source downshift. The extent of the temperature sensitivity is dependent on the carbon source used (manuscript in preparation). Furthermore, the available data suggest that the observed accumulation of ppGpp at 42° is due to a decreased rate of ppGpp degradation. Of course, the strongest evidence is the direct comparison between the decay rates at 30° and 42° (Figure 2).

The lack of any detectable pppGpp also seems to suggest that the effect

Table I. Levels of Glycolytic Intermediates in 2S142 at 30° and 42°

Glycolytic Intermediates	mμmoles/ml/OD_{420}		
	30° [a]	42° [a]	Glu → Succ [b]
Glucose-6-phosphate	2.41	2.08	0.35
Fructose-6-phosphate	0.53	.36	0.14
Fructose-1,6-diphosphate	9.77	6.93	0.18
Dihydroxyacetone-phosphate	1.66	1.12	0.01
Glyceraldehyde-3-phosphate	0.26	.96	0.04
1,3 Diphosphoglyceric Acid	0.06	0.07	0.06
3-Phosphoglyceric acid	1.11	0.91	0.01
Phosphoenolypyruvate	0.10	0.10	<.02
Pyruvic acid	1.40	1.30	0.65

[a] 150ml cultures of 2S142 were grown in minimal salts medium + glucose at 30° to a cell density of 1.0 x 10^8 cells/ml. At this point the culture was split into two aliquots. One culture was incubated at 30° and the other at 42°. After 45 min each culture was rapidly filtered through a .45μ millipore filter. The phosphorylated metabolites were extracted with 2-3mls of 5% perchloric acid and assayed as described by Maitra and Estabrook (9).

[b] This assay was carried out as described above except that the carbon source used was 0.02% glucose + 1% sodium succinate and the growth was at 30° only. The cells were harvested 20 min after exhaustion of glucose.

is primarily due to decreased ppGpp turnover rather than increased pppGpp synthesis. The slow decay rate observed in 2S142 at 42° is virtually identical with either tetracycline or chloramphenicol-and thus appears to be independent of the drug used. We cannot eliminate the possibility that the rate of synthesis of pppGpp is also affected at the restrictive temperature. However, the five fold decrease in the rate of ppGpp degradation is certainly sufficient, in itself, to account for the observed accumulation of ppGpp.

The observated decay rates for D10 and for 2S142 at 30° ($t_{\frac{1}{2}}$ = 3-6 min) are similar to those reported by Laffler and Gallant (12) for $spoT^-$ strains which do not accumulate pppGpp under most conditions. However, we have carried out a series of experiments in which P1 lysates of either D10 or 2S142 were used to infect AT2538 ($pyrE^-$, $spoT^+$, rel^+). In each case, over 50 $pyrE^+$

transductants were selected and none of them were spoT$^+$ (data not shown). Since the spoT and pyrE genes show a 70% frequency of cotransduction (12), it appears that neither D10 or 2S142 are spoT$^-$.

The exact nature of the temperature sensitive lesion in 2S142 remains to be determined. The experiments reported in this paper suggest that the temperature sensitive lesion mimics carbon source downshift, but is not caused by a defect in glucose uptake and/or utilization. We feel that this mutant may prove very useful in studying the mechanism by which E. coli controls ppGpp levels (and rRNA synthesis) during changes in the growth rate.

ACKNOWLEDGEMENTS

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