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RELAXED CONTROL OF RNA SYNTHESIS DURING NUTRITIONAL SHIFTDOWNS OF A *hisU* MUTANT OF *SALMONELLA TYPHIMURIUM**

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SUMMARY

Among the classes of histidine regulatory mutants isolated in *Salmonella typhimurium*, three of these mutants (*hisT*, *hisW* and *hisU*) exhibit pleiotrophic effects on the regulation of expression of other amino acid biosynthetic operons (1, 2). While the regulatory patterns of the *hisT* mutants are explained by the defective tRNA pseudouridylate synthetase (3), the exact function of the *hisW* and *hisU* loci are not as clearly defined, although both mutants exhibit reductions in the relative amino acid acceptance activity of several tRNA's (4). In studies of tRNA synthesis and processing in one such *hisU* mutant (*hisU1820*), we unexpectedly observed continued RNA synthesis during nutritional (carbon and energy source) transitions. It was also shown that this relaxed control of stable RNA formation is independent of the *relA* gene product.

One of the more intriguing observations on the function of the *hisU* gene is that the disparity between the tRNA content of *hisU* and *hisU*$^+$ was most apparent if the cultures were rapidly quenched with TCA prior to harvesting (4). In contrast, when these cells were slow-chilled and harvested, virtually no difference in the tRNA content of *hisU* mutant and normal strains was observed (5). This observation was interpreted to indicate that the *hisU* mutant is altered in a posttranscriptional function. We sought to effect physiological conditions that would mimic the effect of rapid quenching of the cultures by subjecting the *hisU* and normal strains to growth rate transitions. In contrast to the immediate and essentially total cessation of stable RNA accumulation in the *hisU*$^+$ strain, the *hisU* mutant exhibited continued and substantial RNA synthesis during nutritional shiftdowns, suggesting a novel function of the *hisU* gene.

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Table 1

Strain	Genotype	Source
TA799	*hisU*, *relA*$^+$, *ara9*	P. E. Hartman
JC100	*hisU*$^+$, *relA*$^+$, *ara9*	P22/LT-2X TA799
JC109	*hisU*, *relA*$^+$, *ara9*, *leu*	EMS mutagenesis of TA799
JC108	*hisU*$^+$, *relA*$^+$, *ara9*, *leu*	EMS mutagenesis of JC100
TA471	*hisU*$^+$, *relA*$^+$, *hisΔO-H2253*, *hisT1504*	M. Levinthal/R. G. Martin
TA705	*hisU*$^+$, *relA*, *hisΔO-H2253*, *hisT1504*	M. Levinthal/R. G. Martin

EXPERIMENTAL

Organisms and growth media and methods. The *Salmonella typhimurium* strains used, their relevant genotypes and sources are given in Table 1. The minimal medium used was the minimal salts medium of Vogel and Bonner (6) except in the nutritional shift studies, for which the basal salts solution described by Fraenkel and Neidhardt (7) was used. Glucose was added at a concentration of 0.5% in excess carbon and energy source medium and at a concentration of 0.025% in the glucose to succinate shiftdown studies. The enriched medium was 0.8% Difco nutrient broth in minimal medium supplemented with 0.2% glucose. L-leucine was provided at a concentration of 100 µg/ml. Growth was measured (8), and incorporation of [^{3}H]uridine into the TCA insoluble fraction was determined (9) by previously described methods.

RESULTS

Figure 1 shows that upon shifting cultures previously grown in enriched medium to minimal-succinate medium, the *hisU* mutant strain exhibited an immediate and substantial incorporation of [^{3}H]uridine into RNA and that this accumulation of RNA continued for at least 60 minutes. This observation is in distinct contrast to that of the normal strain, which showed no accumulation of RNA during a comparable period in minimal-succinate medium (Figure 2). Furthermore, even in a less severe glucose to succinate transition in which the cells were allowed to exhaust the supply of glucose, the *hisU* mutant showed RNA synthesis in significant excess of that of the normal strain (Figure 2).

The possibility that this *hisU* mutant possesses a relaxed phenotype similar to that exhibited by well characterized *relA* mutants was examined. It is well established that *relA* strains fail to resume growth for as long as 6 hours following a severe nutritional shiftdown (10). We therefore examined the question of the growth response of this *hisU* strain during such a growth

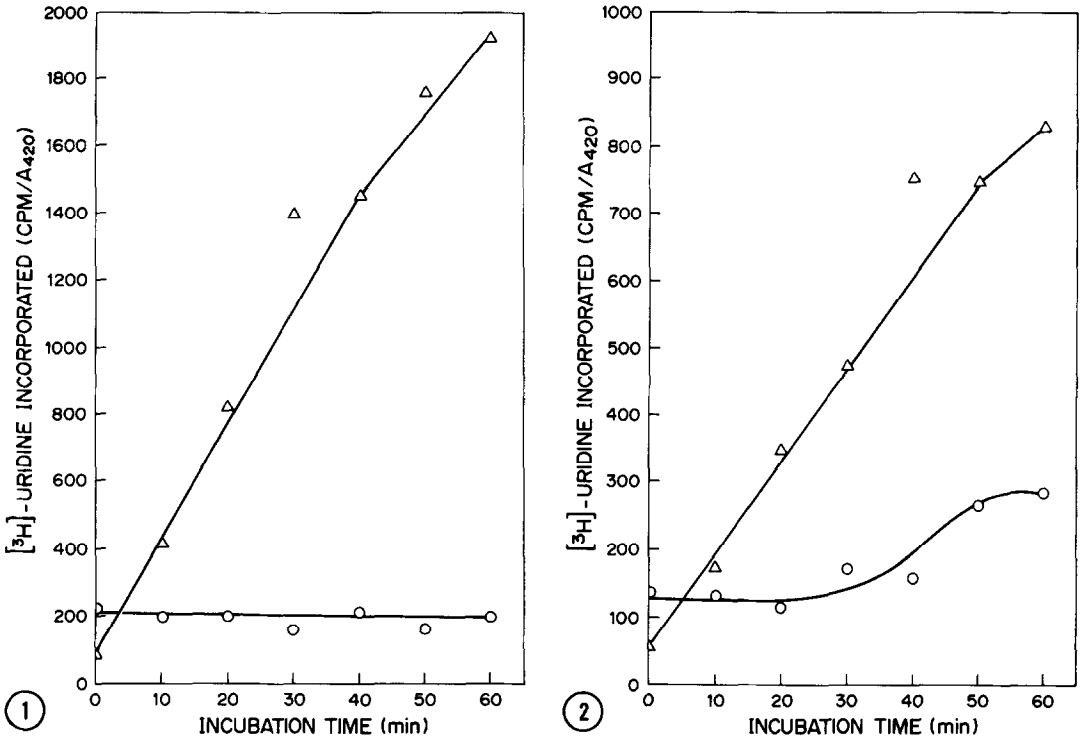

Figure 1. RNA accumulation following growth rate transition from nutrient broth to minimal-succinate medium.
Cultures grown exponentially in nutrient broth medium were collected by centrifugation, washed twice with minimal medium and resuspended in minimal-succinate medium. At zero time, [^{3}H]uridine (0.1 µCi/ml as the final concentration) was added to each culture and the [^{3}H]uridine incorporation into the TCA-insoluble fraction was determined by use of a liquid scintillation spectrometer. The results are expressed as counts/min/A_{420} for strains JC100 (0) and TA799 (Δ).

Figure 2. RNA synthesis during a carbon and energy source transition from glucose to succinate.
The cells initially grown in minimal medium supplemented with 0.025% glucose and 0.5% succinate were allowed to exhaust the supply of glucose. At zero time, [^{3}H]uridine was added to each culture and RNA accumulation was measured as described in Figure 1 for strains JC100 (0) and TA799 (Δ).

media transition. The results shown in Figure 3 indicate that the *hisU* exhibited a growth recovery comparable to that of the isogenic normal strain following such a severe nutritional transition. Conversely, a *relA* derivative of *S. typhimurium* originally isolated by Martin (11) was unable to resume growth for at least 3 hours following an identical growth media transition

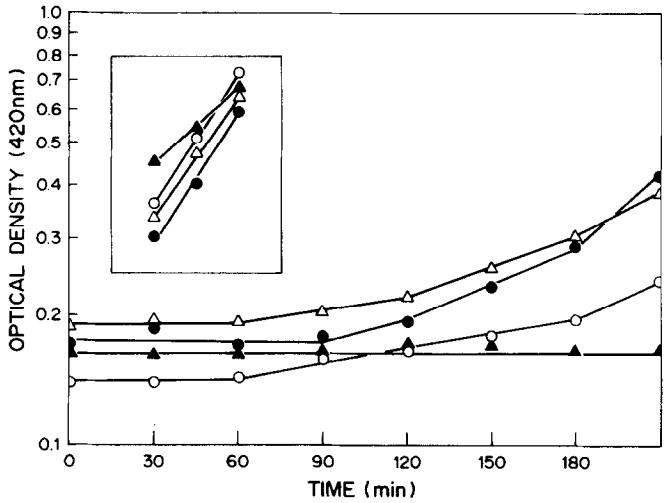

Figure 3. Resumption of growth following a severe nutritional shiftdown. Growth was measured at A_{420} for each culture before (see the insert) and after a shift from nutrient broth-glucose to minimal-succinate medium. The symbols designate growth of strains JC100 (○), TA799 (●), TA471 (△), and TA705 (▲).

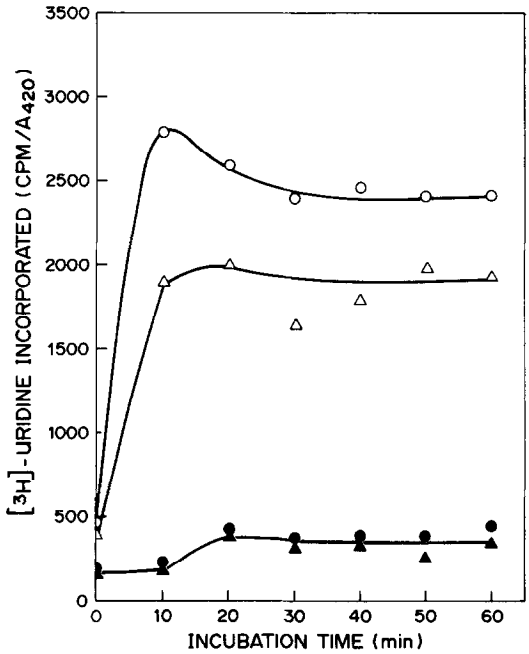

Figure 4. RNA accumulation during leucine restriction. Cultures grown exponentially in leucine-supplemented minimal medium were collected by centrifugation, washed with minimal medium, and resuspended in both leucine-supplemented minimal-glucose minimal medium and minimal-glucose medium. The incorporation of [^{3}H]uridine into RNA was determined as described in Figure 1 over a 60-minute incubation period. The symbols designate RNA accumulation in strains JC100 (○) and TA799 (△) in leucine-supplemented medium and that of JC100 (●) and TA799 (▲) in minimal medium.

(Figure 3), also (10). A further indication that the alteration in control of RNA synthesis in this *hisU* is independent of the *relA* gene product is provided in Figure 4, in which it is shown that RNA synthesis is strictly dependent upon leucine supplementation of leucine auxotrophs of both the *hisU* and the normal strains.

DISCUSSION

One of the classes of *hisU* mutants (*hisU1206*) has been shown to possess an alteration in RNA processing activity (12); however, efforts to demonstrate a comparable alteration in *hisU1820* have been unsuccessful (13). This difference might be explained by the indication that these two mutations are genetically separable (13, 14).

While studies of RNA synthesis have not been reported for strains possessing the *hisU1206* mutation, the results of the present report clearly indicate that a strain containing the *hisU1820* mutation exhibits a relaxed phenotype during nutritional transitions, but apparently retains the amino acid (aminoacylated-tRNA-ribosome)-mediated *relA* gene product pattern of control of RNA synthesis. Given that the relaxed phenotype of this *hisU* mutant appears somewhat dissimilar to any previously described in *Escherichia coli* (15), it is noteworthy that the *spoT* function has been associated with control of RNA synthesis during carbon and energy source transitions (16). The *spoT* gene product apparently functions in the degradation of ppGpp; thus RNA synthesis is halted during nutritional transitions owing to a decreased rate of degradation rather than synthesis of ppGpp (17). In addition, the *relX* gene product is presumed to have a role in gearing ppGpp formation to carbon and energy metabolism in *E. coli* (15). The *relX* locus is located at 59.4 minutes and near the *relA* locus (59.2 minutes) on the *E. coli* genetic map (18); the *relA* locus occupies a similar position (at 61 minutes) on the *S. typhimurium* genetic map (19). It is significant that the *hisU1820* mutation is located at 80 minutes (13, 19), clearly apart from the known *relA* and *relX* loci. Importantly, owing to the virtual coincidence in genetic location of *hisU* (at 80 min.) on

the *S. typhimurium* chromosome and *spoT* (at 81 min.) on the *E. coli* chromosome (18, 19), the possibility that *hisU* is the opposite allele of the hitherto undiscovered *spoT* mutation in *S. typhimurium* would appear to merit consideration. Accordingly, analyses of the patterns of pppGpp and ppGpp formation under various growth conditions are presently being conducted to assess the validity of this suggestion.

Moreover, we have recently reported that the levels of expression of the *ilvEDA* operon and the *ilvC* gene are considerably reduced in this *hisU* mutant during growth in minimal-glucose (partially repressed) medium, suggesting a deficiency of a positive control element (20). Thus, it would seem reasonable to accommodate the evidence reported herein into a model for regulation of expression of the *ilv* genes. In this respect, while the altered regulation of synthesis of the *ilv* gene products could be explained by a *hisU*-mediated tRNA processing defect, a role for ppGpp as a positive effector in setting the ratio of *ilv* gene expression to the overall ratio of stable RNA/protein would appear to be an efficient regulatory process.

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