Stringent Response of RNA Synthesis in E. coli Produced by a Temperature Shift-Up

David Patterson and David Gillespie
Department of Biology, Brandeis University
Waltham, Massachusetts 02154

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Summary A temperature shift from 30°C to 44°C transiently inactivates an initiation factor for protein synthesis and secondarily causes a transient depression in RNA synthesis. The depression of RNA synthesis is not found in RC^{rel} strains. In RC^{str} strains the initial rate of RNA synthesis after a shift to 44° is the same whether required amino acids are present or absent

Introduction

We have recently reported that upon elevation of the growth temperature of E. coli A2325, a K12 strain, from 30° to 44°, the rates of both ^{14}C amino acid and ^{3}H-uridine incorporation are markedly but transiently depressed (1). Both f2-RNA promoted binding of ^{35}S-fmet-$tRNA_F^{met}$ to ribosomes and f2-RNA directed cell-free protein synthesis were found to be defective in extracts prepared from 44°-grown cells. The defect was found to be present in the 2M NH_4Cl ribosomal wash proteins. A similar inactivation could be produced in vitro by incubation of the 2M NH_4Cl ribosomal wash proteins for 10 minutes at 43°. Ribosomal wash proteins of 43° grown cells were largely resistant to this inactivation.

This data strongly suggested a coordinated effect of temperature elevation on RNA synthesis and initiation of

protein synthesis. Since a similar type of control is observed in vivo upon withdrawal of a required amino acid from strains of E. coli carrying the RC^{str} allele (2), we decided to test the hypothesis that the coordinated loss of RNA and protein synthetic ability in A2325 upon temperature elevation is related to the control observed in RC^{str} strains of E. coli upon amino acid withdrawal.

Results

Figure 1 shows that when the growth temperature of A2325 is shifted from 30° to 42° or 44° the ability of the cells to incorporate ^{3}H-uridine and ^{14}C-leucine transiently decreases. The effect on RNA synthesis previously has been hypothesized to be a consequence of a primary effect on initiation of protein synthesis (1).

Figure 2 shows that when strains EA7 and EA2 are subjected to temperature elevation from 30° to 44°, both strains show a slight decrease in protein synthetic capacity but only EA7, the RC^{str} strain, shows any decrease in ^{3}H-uridine incorporation. The RC^{rel} strain, EA2, therefore, shows a lack of coordination of RNA and protein synthesis in response to temperature elevation.

If the RNA synthetic control mechanism functional in RC^{str} strains is in fact active upon a growth temperature shift up, then A2325, which does show a "stringent" response to amino acid starvation (Figure 3), should show a marked inability to incorporate uridine, i.e., respond "stringently" to a temperature shift up either in the presence or absence of a required amino acid.

Figure 3 demonstrates that this is in fact the case. Figure 3 part A, shows that A2325 responds stringently to

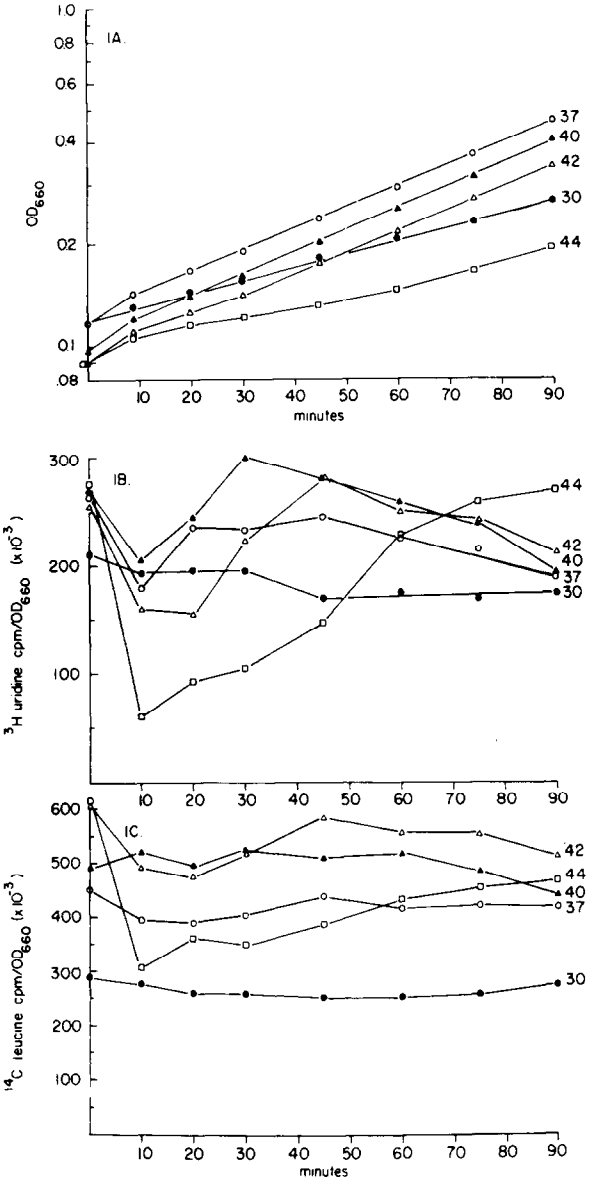

Fig. 1 -- Effect of Temperature Shift-Up on Growth, Protein Synthesis, and RNA Synthesis on A2325

E. coli A2325 cells in early lag growth in 3XPATHB$_1$ (3) at 30° were shifted to the appropriate temperature. At the times shown, 1.0 ml samples were added to 0.05 ml pre-warmed medium supplemented with 5 μC ^{3}H-uridine plus 0.5 μC ^{14}C-leucine. After 10 minutes of incubation, excess ice cold 5% TCA was added, and the precipitates were collected and washed on nitrocellulose filter, dried and counted. For more experimental details, see reference 3.

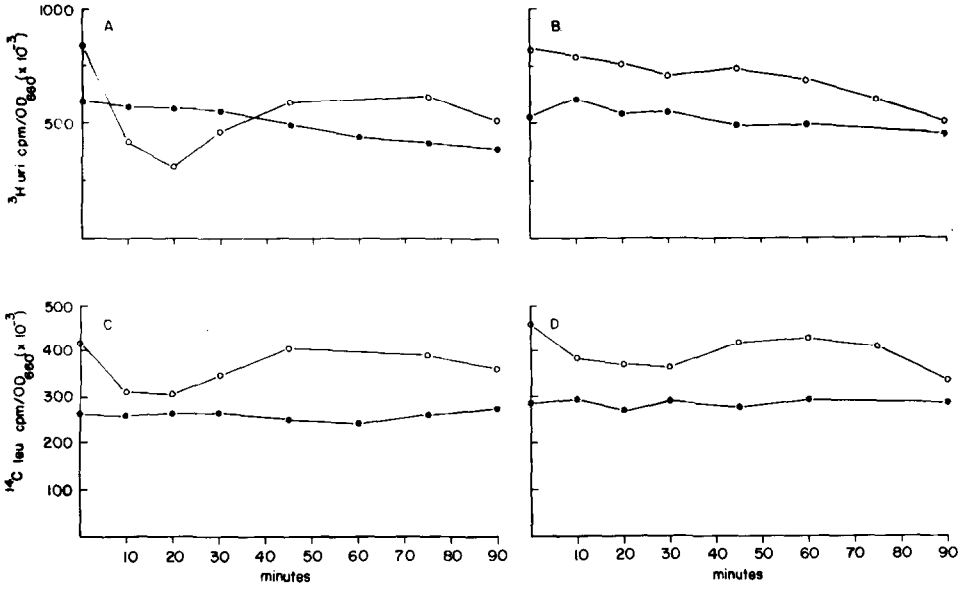

Fig. 2 -- Effect of a Shift of Growth Temperature from 30° to 44° on Strains EA7 and EA2

See Legend, Figure 1 for experimental details. Closed circles = 30°, opened circles = 44°. Strains EA7 and EA2 were obtained from Dr. E. Signer. EA7 is met⁻ biotin⁻ RC^{str} and is descended from the "archtype" stringent strain 58-161 while EA2 is a met⁻ bio⁺ RC^{rel} mutant of 58-161 (4,5). For growth of strains EA2 and EA7, 3xPATHB1 was supplemented with methionine, 60 µg/ml, and biotin, 3 µg/ml. Fig. 2A -- C = EA7; Fig. 2B -- D = EA2.

amino acid (histidine) starvation at 30°. Part B shows that when A2325 is grown at 30° and subjected to the stringency assay at 44°, the cells are essentially unable to incorporate ^{3}H-uridine either in the presence or absence of histidine for at least 40 minutes after addition of isotope and amino acid. Parts C and D show that EA7 and EA2 show, in this assay system, the expected stringent and relaxed responses to amino acid starvation.

We should point out that if the data in Figure 3 are re-plotted as ^{3}H-cpm vs relative OD_{660} increase in the fully supplemented culture (as in references 2,4,and 5), the lag in

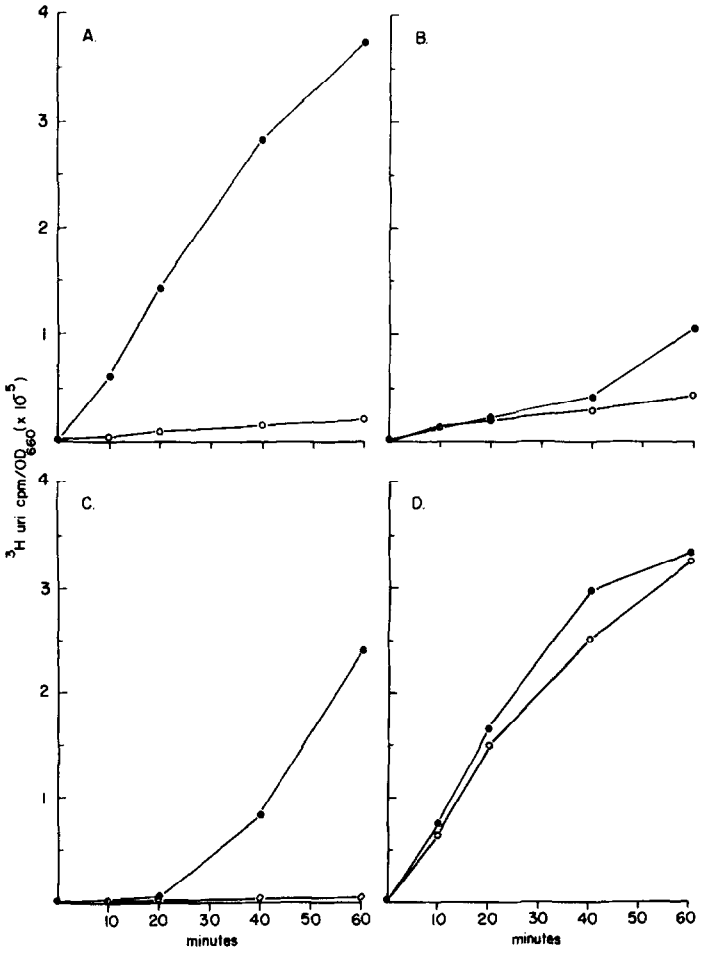

Fig. 3 -- Assay for Stringent Control of RNA Synthesis

Cells in early log growth in 3xPATHB$_1$ medium supplemented appropriately were harvested by centrifugation, washed 1x in 3xPATHB1, minus amino acids and glucose, and resuspended in 3xPATHB$_1$, lacking histidine in the case of A2325 or methionine in the case of EA2 or EA7. The cultures were split into 2 equal aliquots and shaken at 30° for 10 minutes. At t = 0, each culture received ^{3}H-uridine, 1 µC/ml, plus ^{3}H-uridine, 5 µg/ml. One culture of each pair simultaneously received the appropriate amino acid at 60 µg/ml. At the times shown, 0.5 ml aliquots were added to excess ice cold 5% TCA and OD$_{660}$ was determined. Samples were processed as described in legend to Figure 1. Open symbols = -his, closed symbols = +his. A = A2325, 30°; B = A2325, 44°; C = EA7, 30°, D = EA2, 30°.

Part C, Figure 3 is eliminated, and the differences between the two curves in part B is decreased. The interpretation of the data is the same.

Discussion

These data, we feel, lead to the hypothesis that the effect of a temperature shift up on a RC^{str} strain in the conditions described is analagous to the effect of amino acid deprivation with respect to RNA synthesis. We have presented evidence previously that the effect of such a shift is most probably to inactivate (at least partially) some component of the initiation machinery of protein synthesis (1). These data support the hypothesis of Lengyel that it is the initiation step of protein synthesis which is involved in cellular control of RNA synthesis (6). Further evidence for this hypothesis comes from Figure 3, part C, which shows that after starvation for methionine, EA7, RC^{str}, shows a lag in RNA synthesis even after re-addition of methionine. This could be explained on the basis that starvation for methionine, which is necessary for initiation of most proteins in E. coli, specifically inactivates the initiation machinery in a way in which deprivation of other amino acids does not.

Finally, we should point out that one characteristic of the stringent response is that inhibition of RNA synthesis by amino acid starvation can be overcome by addition of chloramphenicol to the starved culture (7). We have previously shown that $T^{s}68b$, a temperature sensitive mutant of A2325 which is apparently defective in protein chain elongation at 44^{o}, does incorporate large amounts of ^{3}H-uridine after a shift to 44^{o}, thus showing "relaxed" synthesis of RNA under conditions of inhibition of protein synthesis (8).

Thus we feel that the described system strongly supports the hypothesis that control of RNA synthesis and protein synthesis are coordinated through some step in the initiation

of protein synthesis. The experimental system described above offers the advantages of experimental simplicity and the existence of at least one mutant, T^s68b, which alters the standard control relationships. Finally, the ability to demonstrate some type of *in vitro* defect which appears to be correlated with the *in vivo* control defect, offers the possibility of determining experimentally the nature of the molecules responsible for the effects observed *in vivo*.

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Present Addresses: For D.P.; Department of Biophysics, University of Colorado, Medical Center, Denver, Colorado, 80220. For D.G.; National Institutes of Health, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224.

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