THE STRINGENT RESPONSE TO INHIBITION OF PEPTIDE CHAIN INITIATION IN *ESCHERICHIA COLI*

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1. Introduction

In most strains of *E. coli* synthesis of the stable RNA species is coordinately depressed if protein synthesis is lowered either by lack of any aminoacid or by inactivation of any aminoacyl-tRNA synthetase [1]. This stringent dependence of RNA accumulation on aminoacid availability or activation was termed the 'stringent response' [2]. Mutant relaxed strains have been obtained [1] in which however this dependence does not occur i.e. stable RNA synthesis is not inhibited in the absence of aminoacids ('relaxed response'). In addition to RNA metabolism, several other cellular activities respond to aminoacid deprivation but only in stringent (*rel*$^+$) strains and not in relaxed (*rel*$^-$) strains (for review see [3]). Namely, in the absence of protein synthesis, unusual guanosine polyphosphates ppGpp and pppGpp accumulate in the nucleotide pool; also the level of polysomes is considerably lowered even though the persisting polysomes are maintained in a state of turnover [4–7].

Protein synthesis in intact bacteria can obviously be arrested by other means than withdrawing an essential aminoacid from the culture medium or inactivating a particular aminoacyl-tRNA synthetase. Thus it can be specifically blocked in the initiation phase by treating the cells with hydroxylamine or trimethoprim which both inhibit the formylation of the initiator *N-formyl*-Met-tRNA$_F$ [8–13].

It has been previously shown that the response of *rel*$^+$ and *rel*$^-$ strains of *E. coli* to treatment by either inhibitor is identical to that to aminoacid deprivation as far as RNA synthesis and guanosine polyphosphate accumulation are concerned [10,11,14]. However, to our knowledge, polysome behaviour has not been studied under these particular conditions of protein synthesis inhibition.

The experimental material presented in this report indicates that polysome metabolism in *rel*$^+$ and *rel*$^-$ strains treated with hydroxylamine differs in many respects from polysome metabolism in aminoacyl-tRNA deprived strains, whereas polysome metabolism in cells treated with trimethoprim does not.

2. Materials and methods

The previously described [15] otherwise isogenic pair of *E. coli* strains 10B6 *rel*$^+$ and 10B6 *rel*$^-$ was used in all experiments. These strains were kindly supplied by Dr A. Muto, Hiroshima, Japan, and Dr G. Edlin, Davis, California. They both are arginine auxotrophs and they harbor a temperature-sensitive valyl-tRNA synthetase (restrictive temperature: 42.5°C). Cells were grown in a minimal Tris–HCl medium supplemented with the following components: 4 mg/ml glucose; 50 μg/ml each of the 20 L-aminoacids; 5 μg/ml vitamin B_1; 40 μg/ml each of adenine–HCl cytosine, guanine–HCl, uracil and thymine. Whenever uracil or proline was labeled, its final concentration was 5–10 μg/ml. Glucose starvation experiments were performed as described before [16] using 0.4 mg/ml glucose.

The method of preparing crude lysates and isolated polysomes has been reported in detail [16]. Briefly, cells were harvested by quick chilling on ice in the presence of 100 μg/ml chloramphenicol to prevent ribosome run-off, and were centrifuged in the cold. Lysates were prepared by the lysozyme–EDTA technique and were layered onto 15–40% RNAase-free-sucrose gradients. After centrifugation for 150

min at 39 000 rev/min in a Beckman SW 41 Ti rotor, gradients were pumped through the continuous-flow cell of a Beckman recording spectrophotometer which monitored the optical density at 260 nm. The proportion of polysomes in the total ribosomal material (polysomes + monosomes + ribosomal subunits) was determined from the absorbancy tracing.

The synthesis of RNA and protein was measured by the incorporation of [^{3}H]uracil (0.15 μCi/ml; 1 Ci/mmol) and [^{14}C]proline (0.04 μCi/ml; 200mCi/mmol) into cold trichloroacetic acid precipitates. The radioactivity was counted using the appropriate double-label setting in a Tri-Carb Packard spectrometer.

In most experiments, hydroxylamine and trimethoprim (2,4-diamino-5-[3,4,5-trimethoxybenzyl]-pyrimidine) were added to the culture medium at the final concentration of respectively 16.5 μg/ml ($5\cdot10^{-4}$ M) and 25 μg/ml ($8.6\cdot10^{-5}$ M). As shown previously [9,11], at a concentration of 10^{-3} M hydroxylamine stops the synthesis of DNA, RNA and protein while at lower concentrations it preferentially inhibits protein synthesis in its initiation phase.

3. Results and discussion

The effects of hydroxylamine and trimethoprim on protein and net RNA synthesis in strains 10B6 *rel*$^+$ and 10B6 *rel*$^-$ are shown in fig.1. In both strains, proline incorporation (diagrams c and d) is almost totally inhibited in the presence of hydroxylamine and it is reduced to no more than 10% of the control in the presence of trimethoprim showing that either drug is a strong inhibitor of protein synthesis. Uracil incorporation in strain 10B6 *rel*$^+$ is reduced to nearly zero under treatment by either inhibitor (diagram b), whereas in strain 10B6 *rel*$^-$ under hydroxylamine treatment it is reduced after 30 min to only about 80% and, in the same strain under trimethoprim treatment, to only about 70% of the control (diagram a). These results are in agreement with previous reports [10,11]. They indicate mainly that, as far as RNA synthesis is concerned, hydroxylamine as well as trimethoprim treatment mimics aminoacid deficiency in *rel*$^+$ and *rel*$^-$ strains. In other similar experiments not shown here, the effects of higher concentrations of inhibitor on protein and net RNA synthesis have been tested in both strains. No significant change in the effects of trimethoprim is observed after a 20 min treatment within the range 25–100 μg/ml. On the contrary, a higher concentration of hydroxylamine induces a higher inhibition of RNA synthesis namely in the 10B6 *rel*$^-$ cells. Therefore the aforesaid low concentration of 16.5 μg/ml has been used through all the experiments described here.

The effects of hydroxylamine and trimethoprim

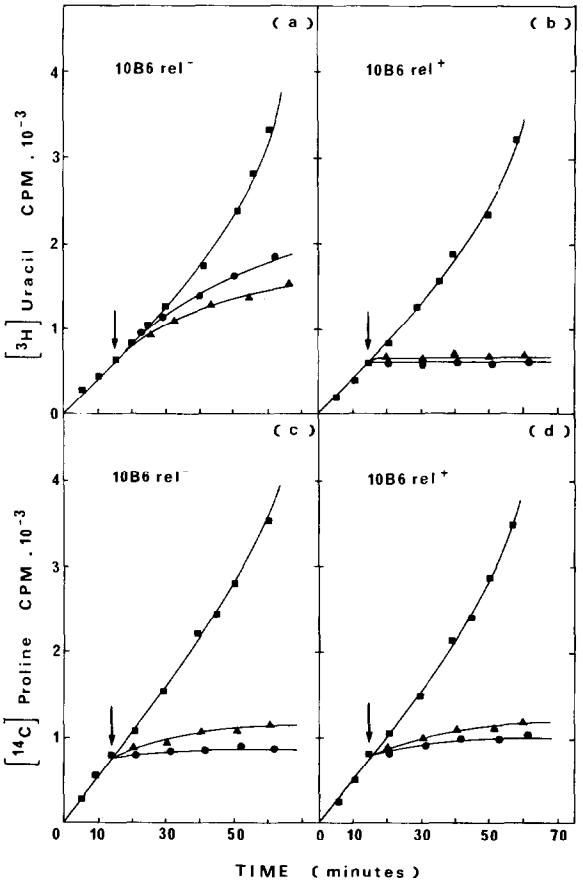

Fig.1. Effects of hydroxylamine and trimethoprim on protein and RNA synthesis in strains 10B6 *rel*$^+$ and 10B6 *rel*$^-$. Cells were grown at 30°C for 15 min in the presence of labeled uracil and proline. Then either hydroxylamine (●) or trimethoprim (▲) was added (arrow) into a portion of the culture. The rest of the culture was maintained at 30°C in the absence of any inhibitor and was used as a control (■). Aliquots (0.4 ml) were taken after the indicated times, submitted to cold trichloracetic precipitation and their radioactivity was counted.

Table 1
Proportion of persisting polysomes in cells under various conditions of protein synthesis inhibition

	Control	Hydroxylamine	Trimethoprim	Temperature-shift
Strains 10B6 *rel*$^+$	46(100)	19(41)	21(46)	17(37)
Strains 10B6 *rel*$^-$	56(100)	26(46)	35(63)	56(100)

Cells were grown at 30°C in the exponential phase (control) then subjected to either inhibitor treatment for 15 min or shifted to the restrictive temperature of 42.5°C for the same time period. Polysomes were extracted and their proportion (in %) in the total ribosomal material was determined (see Materials and methods). The percent of polysomes relative to the control is given in brackets. Mean values from 3–6 experiments are expressed.

on polysome level in strains 10B6 *rel*$^+$ and 10B6 *rel*$^-$ are shown in table 1. It is clear that trimethoprim treatment results in a higher reduction of polysome level in *rel*$^+$ cells as compared to *rel*$^-$ cells while hydroxylamine treatment induces the same high reduction of polysome level in both strains. The level of persisting polysomes in strain 10B6 *rel*$^+$ in the presence of either inhibitor is similar to that observed under valyl-tRNA deprivation at 42.5°C [6,16]. In contrast, the level of persisting polysomes in hydroxylamine treated 10B6 *rel*$^-$ cells is surprisingly low as compared to that in cells shifted to 42.5°C. Since maintenance of polysome level under aminoacid starvation or aminoacyl-tRNA deprivation accompanies specifically the relaxed response [4,6,17–20], it thus appears that trimethoprim but not hydroxylamine can be used for characterizing *rel*$^+$ and *rel*$^-$ strains.

In order to check whether persisting polysomes are stable structures, rifampicin, an inhibitor of RNA synthesis [21], has been added to 10B6 *rel*$^+$ and 10B6 *rel*$^-$ cultures previously treated with either hydroxylamine or trimethoprim. In all cases, polysomes are totally degraded into monosomes within 15 min of rifampicin treatment. This result suggests that persisting polysomes undergo continual turnover rather than being 'frozen' structures.

Therefore a study has been made of the process of polysome reassembly in cells treated with hydroxylamine or trimethoprim in which polysomes had been previously converted to monosomes by glucose starvation.

Table 2 shows that in 10B6 *rel*$^-$ cells polysome reassembly is nearly complete whatever the inhibitor present in the culture medium, and it is similar to that measured in valyl-tRNA deprived cells. In 10B6 *rel*$^+$ cells, polysome reassembly is markedly inhibited in trimethoprim treated cells as well as in cells shifted to 42.5°C, whereas it is only slightly prevented in the presence of hydroxylamine. Thus, here again, the effects of hydroxylamine on polysome metabolism are different from those observed in aminoacyl-tRNA deprived cells. This might be due to

Table 2
Proportion of reassembled polysomes in cells under various conditions of protein synthesis inhibition

	Control	Hydroxylamine	Trimethoprim	Temperature-shift
Strains 10B6 *rel*$^+$	52(100)	44(85)	23(44)	18(35)
Strains 10B6 *rel*$^-$	63(100)	61(97)	59(94)	66(105)

Cells were grown at 30°C with a limiting amount of glucose then subjected to glucose starvation for 90 min. At zero time, glucose was re-added to the medium and the cells were either maintained at 30°C (control) or shifted to 42.5°C or treated with either inhibitor. After 15 min, reassembled polysomes were extracted and their proportion (in %) was determined. The percent of polysomes relative to the control is given in brackets. Mean values from 2–4 experiments are expressed.

undetermined side effects of this inhibitor.

In conclusion, our results indicate that *rel*$^+$ and *rel*$^-$ strains of *E. coli* in the presence of trimethoprim, but not in the presence of hydroxylamine, behave essentially like aminoacid starved auxotrophic cells or aminoacyl-tRNA deprived cells as far as both RNA synthesis and polysome metabolism are concerned. Consequently trimethoprim can apparently serve as a tool for distinguishing *rel*$^+$ from *rel*$^-$ bacterial strains even if these are prototrophic.

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