ISOLATION OF RELAXED-CONTROL MUTANTS OF ESCHERICHIA COLI K-12 WHICH ARE SENSITIVE TO GLUCOSE STARVATION

Raymond D. Mosteller and Shing F. Kwan
Department of Biochemistry, University of Southern California
School of Medicine, Los Angeles, California 90033

Received January 19,1976

SUMMARY

Mutants of Escherichia coli K-12 which are sensitive to glucose starvation were isolated by an enrichment procedure using thymine starvation to select for nongrowing cells. Eleven independent isolates were obtained by this method. The mutants are also sensitive to glycerol starvation and to a lesser extent to nitrogen or amino acid starvation. The mutants are more sensitive than the parental strain to inhibitors of protein synthesis but not inhibitors of RNA or DNA synthesis. [^{3}H]-leucine incorporation experiments indicate that protein synthesis is blocked in the mutants during recovery from glucose starvation or chloramphenicol inhibition. Incorporation of [^{3}H]uridine in amino acid-starved cells demonstrates that the mutants are partially relaxed for control of RNA synthesis. Physiological and genetic experiments indicate that these mutants are different from previously isolated relaxed-control mutants.

INTRODUCTION

Synthesis of stable RNA is known to be under the control of amino acid availability in wild-type strains of Escherichia coli (1). Amino acid deprivation causes a marked reduction in accumulation of stable RNA. This control (stringent control) is believed to be mediated by guanosine 3'-diphosphate, 5'-diphosphate, ppGpp, which accumulates during amino acid starvation (2). In vitro, ppGpp inhibits transcription of ribosomal RNA (3) and stimulates synthesis of some messenger RNA's (4). Synthesis of ppGpp occurs during an idling step on ribosomes (5) and requires a ribosome-associated stringent factor (6,7,8). Mutants containing lesions in the relA gene (9,10) are relaxed or partially relaxed for control of RNA synthesis and contain altered stringent factor (11). Two other classes of relaxed mutants (relB and relC) have been isolated (12,13). One of these (relC) contains an altered 50S ribosomal subunit (13). The relC gene maps near rif on the E. coli chromosome. The relB gene has not been completely mapped.

While isolating mutants of E. coli K-12 which are resistant to thymineless death after exhaustion of glucose, we found several spontaneous mutants which are particularly sensitive to carbon source starvation. Further characterization showed that these mutants are affected in protein synthesis and that they exhibit partially relaxed control of stable RNA synthesis similar to that observed in relB, relC and some relA mutants. Physiological and genetic evidence indicates that these newly isolated mutants are different from relA, relB and relC strains. The isolation and partial characterization of these new mutants are described in this report.

METHODS

A thymine auxotroph (strain RM281) of the W3110 strain of E. coli K-12 was used as the parental strain. The relA strain (KL14) was obtained from B. Bachmann. All cultures were grown with vigorous shaking in single strength minimal medium of Vogel and Bonner (14) with citrate omitted (15) and supplemented with 0.2% glucose and 20 μg/ml thymine unless stated otherwise. L-broth contains 1% bacto-tryptone, 0.5% yeast extract, 0.05% NaCl and 0.1% glucose. Enriched medium is minimal medium supplemented with 0.05% casamino acids, 50 μg/ml L-cysteine, 20 μg/ml L-tryptophan, 50 μg/ml each of uridine, adenosine, deoxyadenosine, guanosine, cytidine and 1 μg/ml each of thiamine, pyridoxine·HCl, riboflavin, p-aminobenzoic acid, p-hydroxybenzoic acid, folic acid and 0.1 μg/ml cyanocobalamin. For starvation experiments, cells were washed free of the appropriate nutrient by filtration or by three cycles of centrifugation. Logarithmically growing cultures were used for glucose, glycerol and nitrogen starvation and for chloramphenicol and valine inhibition. Cells were grown in 0.4% glycerol (no glucose) for about ten generations prior to glycerol starvation. Colony-forming units were determined by spreading portions of cultures on 1.3% agar medium containing L-broth and 20 μg/ml thymine. Colonies were counted after 14-16 hrs at 37° C. Viable cells/ml were calculated as described previously (16). Isoleucine starvation was effected by L-valine (200 μg/ml) inhibition. Incorporation of [^{3}H]uridine or [^{3}H]thymine was determined for material insoluble in cold 5% trichloroacetic acid. Incorporation of [^{3}H]L-leucine or [^{3}H]L-alanine was determined for material insoluble in hot (90° C) trichloroacetic acid. Insoluble material was collected on membrane (Millipore) filters and radioactivity determined in a scintillation spectrometer.

RESULTS

Isolation of Mutants. Overnight (glucose-limited) cultures of strain RM281 were subjected to three cycles of thymine starvation in glucose-minimal medium. Portions of each culture were spread on L-broth agar medium and incubated for 24 hours at 37°C. Single colony isolates were tested for resistance to thymine starvation following growth in limiting

TABLE 1

EFFECT OF THYMINE OR GLUCOSE STARVATION

Bacterial strain	Colony-forming units per ml ($\times 10^{-7}$)				Viable cells per ml ($\times 10^{-7}$)
	Thymine starved (4 hrs)		Glucose starved		Glucose starved
	glucose limited culture	logarithmic culture	2 hrs	24 hrs	2 hrs
parent					
RM281	0.0005	0.0001	1.0	2.90	1.50
mutants					
SK370	5.9	0.0100	0.010	0.60	0.80
SK371	3.2	0.0005	0.010	0.010	0.80
SK372	1.6	0.0030	0.010	0.20	1.00
SK373	13.2	0.040	0.003	0.070	1.60
SK374	11.0	0.032	0.003	0.030	3.50
SK375	0.6	0.003	0.007	0.20	1.50

Cultures containing 10^7 colony-forming units (or viable cells) per ml were starved as indicated and assayed as described in methods.

(0.1%) glucose. Among twenty-four colonies tested, six colonies were found which are resistant to thymine starvation (Table 1). The resistant behavior of these strains does not change after repeated single colony isolations. Logarithmically growing cultures of these strains are also less sensitive than strain RM281 to thymine starvation (Table 1).

Resistance of the mutant strains to thymine starvation was found to be related to their sensitivity to glucose starvation. The sensitivity can be detected as a decrease in colony-forming ability (Table 1) or as a lag in growth (data not shown) following glucose starvation. The colony-forming ability in cultures of five of the strains increases ten to sixty

TABLE 2

EFFECT OF GLYCEROL, NITROGEN OR ISOLEUCINE STARVATION

Bacterial strain	Colony-forming units per ml ($\times 10^{-7}$)					
	Glycerol starved		Nitrogen starved		Isoleucine starved	
	3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
parent RM281	1.6	1.6	1.2	1.2	2	2
mutants						
SK370	0.030	0.30	0.940	0.550	0.290	0.160
SK371	0.001	0.001	0.750	0.25	0.230	0.150
SK372	0.020	0.90	1.30	0.20	0.130	0.007
SK373	0.020	0.30	1.0	0.50	1.0	0.040
SK374	0.006	0.070	0.50	0.20	0.60	0.007
SK375	0.020	0.50	0.170	0.450	0.230	0.010

Cultures containing 10^7 colony-forming units per ml were starved as indicated and assayed as described in methods.

fold after twenty-four hours of starvation (Table 1). Recovery is probably not due to cell growth or division since the turbidity and total cell concentration of the cultures does not increase during starvation (data not shown). This suggestion is supported by the fact that the actual viability of cells remains fairly constant during starvation (Table 1).

The colony-forming ability of each of these strains is also very sensitive to glycerol starvation but less sensitive to nitrogen starvation or valine inhibition (Table 2). These results indicate that some process which is necessary for normal growth is blocked by a prior period of nutrient starvation, particularly carbon starvation.

Effect of glucose starvation on protein synthesis. In order to determine which aspects of cell growth are affected by glucose starvation,

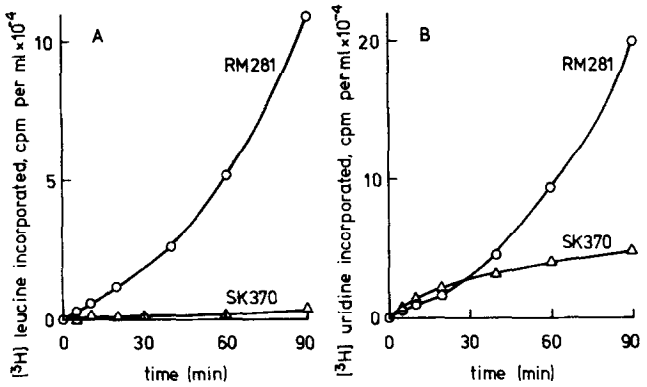

Figure 1. Recovery from Glucose Starvation. Cultures of strains RM281 and SK370 (1 x 10^8 cells/ml) were starved for glucose for 2 hrs and then labeled with (A) [^{3}H]leucine (1 μCi/ml, 25 Ci/mole) or (B) [^{3}H]uridine (1 μCi/ml, 25 Ci/mole) in minimal medium containing 0.5% glucose.

we examined incorportion of [^{3}H]leucine, [^{3}H]uridine, and [^{3}H]thymine in strains RM281 and SK370 during recovery from glucose starvation. The results indicate that [^{3}H]leucine incorporation is almost totally blocked in mutant SK370 but not in the parental strain (Fig. 1A). [^{3}H]Uridine incorporation is similar in both strains for about 20 to 30 minutes after which incorporation is markedly reduced in the mutant (Fig. 1B), probably as a result of blocked protein synthesis. [^{3}H]Thymine incorporation is very similar in both strains during recovery (data not shown). These results suggest that the starvation-sensitive component in mutant SK370 is involved in protein synthesis but not RNA or DNA synthesis.

Sensitivity to inhibitors of protein synthesis. Growth of the parental and mutant strains were compared on minimal-agar medium containing various concentrations of several metabolic inhibitors. The parental and mutant strains exhibit similar sensitivities to rifampicin, nalidixic acid, hydroxyurea, fluorouracil, sodium azide and dinitrophenol (data not shown). However, all six mutants are more sensitive than the parental strain to inhibitors of protein synthesis (chloramphenicol, sulfacetamide,

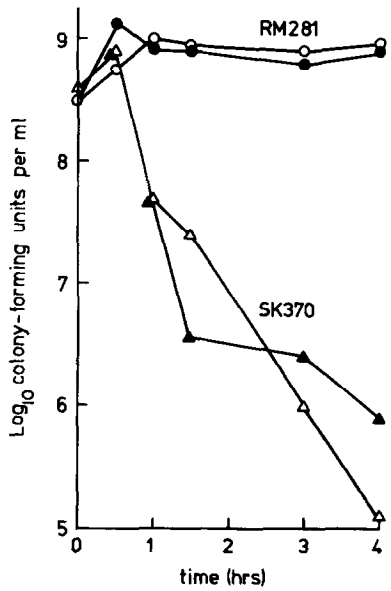

Figure 2. Chloramphenicol Inhibition. Cultures of strains RM281 and SK370 (4×10^8 cells/ml) growing in minimal medium (open symbols) or enriched medium (closed symbols) were treated with 20 μg/ml of chloramphenicol for the times indicated and assayed as described in methods.

erythromycin, kasugamycin). The colony-forming ability of cultures of mutant SK370, but not strain RM281, is reduced drastically by chloramphenicol inhibition in the presence or absence of amino acids, nucleosides and vitamins (Fig. 2). This resembles the effect of glucose starvation on colony-forming ability (Table 1). During recovery from two hours of chloramphenicol inhibition, [^{3}H]leucine incorporation but not [^{3}H]uridine or [^{3}H]thymine incorporation is specifically blocked. These results are very similar to those obtained for recovery from glucose starvation (see Fig. 1). Using the thymine-starvation enrichment procedure and sulfacetamide sensitivity as a screening test, we have isolated five more independent mutants which exhibit properties similar to the previously isolated mutants.

Relaxed control of RNA synthesis. [^{3}H]uridine incorporation experiments during glucose starvation and chloramphenicol inhibition suggested

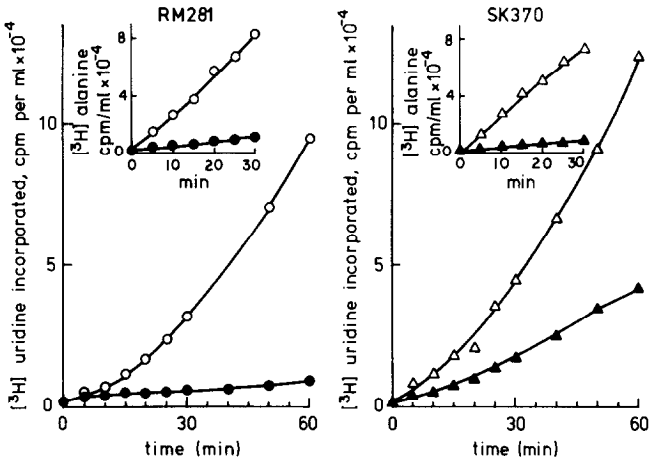

Figure 3. Valine Inhibition of RNA Synthesis and Protein Synthesis. Cultures of strains RM281 and SK370 (1 x 10^8 cells/ml) growing in minimal medium were labeled with [^{3}H]uridine (1 μCi/ml, 25 Ci/mole) or [^{3}H]alanine (1 μCi/ml, 25 Ci/mole) in the absence (open symbols) and presence (closed symbols) of 200 μg/ml of L-valine.

that mutant SK370 may be relaxed for control of RNA synthesis. Therefore, we tested six of the mutants (SK370-SK375) for [^{3}H]uridine incorporation during isoleucine starvation (valine inhibition). All six mutants exhibited relaxed control but not as "relaxed" as a strain (KL14) which contains the classical relA-1 mutation. Some of these data are presented in Figure 3. Transduction experiments with bacteriophage P1 have confirmed that the relaxed property and the sulfacetamide sensitivity of our mutants do not cotransduce with the argA locus and therefore are not altered in the relA gene which codes for stringent factor. Further, resistance to sulfacetamide can be transferred to these mutants by Hfr strains KL16-99 and PK191 (17), indicating that these mutations are not in the relC locus described by Friesen et al. (13). The mutants described here do not exhibit a lag in RNA synthesis during amino acid starvation (Fig. 3) as described for relB mutants (12) and are not sensitive to elevated temperature as described for some relB mutants (12). Therefore, we conclude that these mutants are probably a new class of relaxed mutants which are par-

ticularly sensitive to carbon source starvation. Experiments are in progress to complete the genetic mapping and to determine whether a ribosomal component is altered in these mutants.

ACKNOWLEDGEMENTS

This work was supported by a Comprehensive Cancer Center Grant (CA 14089) to the University of Southern California/LAC Medical Center from the National Cancer Institute (USPHS). We are grateful to Sharon Vonderohe for typing the manuscript.

REFERENCES

1. Neidhardt, F. (1966) Bacteriol. Rev. 30, 701-719.
2. Cashel, M. and Gallant, J. (1969) Nature 221, 838-341.
3. Reiness, G., Yang, H-L., Zubay, G. and Cashel, M. (1975), Proc. Natl. Acad. Sci., USA 72, 2881-2885.
4. Aboud, M. and Pastan, I. (1975) J. Biol. Chem. 250, 2189-2195.
5. Haseltine, W., Block, R., Gilbert, W. and Weber, K. (1972) Nature 238, 381-385.
6. Haseltine, W. A. and Block, R. (1973) Proc. Natl. Acad. Sci., USA 70, 1564-1568.
7. Cochran, J. W. and Byrne, R. W. (1974) J. Biol. Chem. 249, 353-360.
8. Block, R. and Haseltine, W. A. (1975) J. Biol. Chem. 250, 1212-1217.
9. Stent, G. S. and Brenner, S. (1961) Proc. Natl. Acad. Sci. USA 47, 2005-2014.
10. Fiil, N. and Friesen, J. (1968) J. Bacteriol. 95, 729-731.
11. Block, R. and Haseltine, W. A. (1973) J. Mol. Biol. 77, 625-629.
12. Lavallé, R. (1965) Bull. Soc. Chim. Biol. 47, 1567-1570.
13. Friesen, J. D., Fiil, N. P., Parker, J. M. and Haseltine, W. A. (1974) Proc. Natl. Acad. Sci. USA 71, 3465-3469.
14. Vogel, H. and Bonner, D. M. (1956) J. Biol. Chem. 218, 97-106.
15. Berkowitz, D., Hushon, J. M., Whitfield, H. S. Jr. (1968) J. Bacteriol. 96, 215-220.
16. Nakayama, H. and Couch, J. L. (1973) J. Bacteriol. 114, 228-232.
17. Low, K. B. (1972) Bacteriol. Rev. 36, 587-607.