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Isolation of "Relaxed" Mutants of *Escherichia coli*

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All previously described mutants showing relaxed control of ribonucleic acid (RNA) synthesis (RC^{rel} strains) have been derived from a common ancestor, *Escherichia coli* strain W6 (L. Alföldi, G. S. Stent, and R. C. Clowes, J. Mol. Biol. **5**:348, 1962), first described by E. Borek, A. Ryan, and I. Rockenbach (J. Bacteriol. **69**:460, 1955). We wish to describe the isolation of independent relaxed mutants and some of their properties.

The method of selection was based on the observation (L. Alföldi et al., Z. Vererbungslehre **94**:285, 1963) that, when an RC^{rel} strain was starved of a required amino acid for 200 min in the presence of glucose and subsequently shifted to the appropriate amino acid-supplemented medium with lactose as the sole energy source, these cells experienced a lag of up to 250 min before exponential growth resumed. On the other hand, strains with stringent control (RC^{str}) subjected to similar treatment resumed growth after a lag of only 40 to 60 min. We reasoned that addition of penicillin during the period from 0 to 120 min after readdition of amino acids in the presence of lactose ought preferentially to kill RC^{str} cells and enrich for relaxed mutants. Reconstruction experiments with mixtures of strain W6 (RC^{rel}) and strain CP78 (RC^{str}), at a ratio of $1:10^6$, showed this approach to be workable.

A derivative of *E. coli* K-12 W677, strain CP78, F^-, RC^{str}, requiring threonine, leucine, histidine, arginine, and thiamine, was used for the selection of relaxed mutants. It was grown in tris(hydroxymethyl)aminomethane (Tris)-glucose medium (G. Edlin and O. Maaløe, J. Mol. Biol. **15**:428, 1966) supplemented with the requirements mentioned above, treated with ultraviolet (UV) light or nitrous acid, and then subjected to five successive rounds of the selective procedure described above; 21 independent mutants were isolated by this method.

Figure 1a shows the uptake of uracil by the parental RC^{str} strain in the presence and absence of a required amino acid. Uracil uptake for the mutants appeared to fall into two broad classes: the "high-relaxed" mutants synthesize as much

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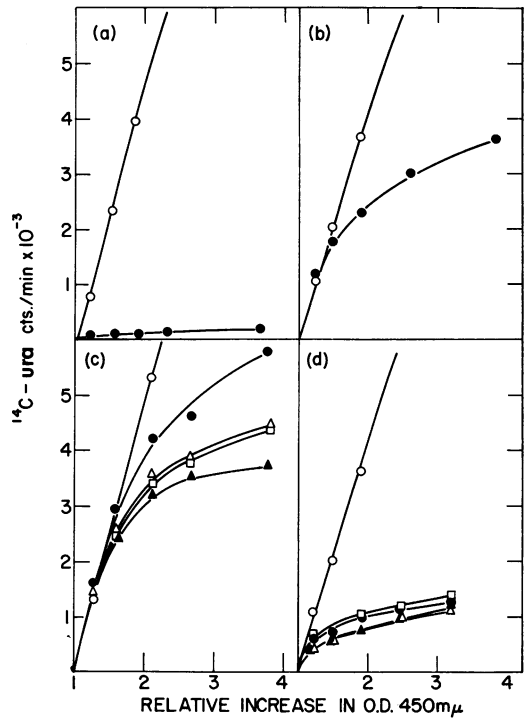

FIG. 1. *Cultures growing exponentially in Tris-glucose medium supplemented with required amino acids were shifted by filtration into Tris-glucose medium with ^{14}C-uracil (5 μg/ml; 0.01 μc/ml), either containing all required amino acids (control) or lacking various amino acids as indicated below. At intervals, 1-ml samples were pipetted into 5% trichloroacetic acid; after 30 min at 5 C, cells were collected on membrane filters, dried, and counted in a Nuclear Chicago scintillation spectrometer. (a) Strain CP78 containing all essential amino acids (○). Strain CP78 starved of threonine (●). (b) Strain W6 containing all essential amino acids (○). Strain W6 starved of methionine (●). (c) Strain CP79 containing all essential amino acids (○); starved of threonine, (●); starved of leucine, (△); starved of arginine, (▲); starved of histidine, (□). (d) Strain CP140, containing all essential amino acids (○); starved of threonine, (●); starved of leucine (△); starved of arginine, (▲); starved of histidine, (□). All the results are displayed in a differential plot showing the acid-precipitable counts per minute incorporated as a function of the relative increase in optical density of the control culture.*

TABLE 1. *Isolation and characteristics of newly isolated RC^{rel} mutants*

Strain	Derived from	Mutagen	Selection procedure[a]	^{14}C-uracil uptake during arginine starvation[b]	Number of transductants[c]	
					Stringent	Relaxed
CP 78	W677			3.1		
CP 79	CP 78	UV	a—thr	66	4	1
CP 80	CP 78	UV	a—leu	55	27	9
CP 81	CP 78	UV	a—his	48	27	8
CP 82	CP 78	UV	a—arg	61	19	8
CP 83	CP 78	UV	a—thr leu his arg	67	16	4
CP 84	CP 78	UV	b—thr	54	31	7
CP 85	CP 78	UV	b—thr	60	25	5
CP 86	CP 78	UV	b—thr	54	26	8
CP 87	CP 78	UV	b—thr	56	26	8
CP 88	CP 78	UV	b—leu	32	6	1
CP 89	CP 78	UV	b—leu	10	6	1
CP 90	CP 78	UV	b—leu	31	21	12
CP 91	CP 78	UV	b—leu	54	24	13
CP 92	CP 78	UV	b—leu	47	22	6
CP 93	CP 78	UV	b—leu	57	16	14
CP 96	CP 78	UV	b—his	60	19	9
CP 97	CP 78	UV	b—arg	31	18	7
CP 138	CP 78	HNO_2	b—thr	51	20	6
CP 139	CP 78	HNO_2	b—thr	56	18	7
CP 140	CP 78	HNO_2	b—leu	19	29	3
CP 141	CP 78	HNO_2	b—leu	13	5	3
CP 99	AB325			3.2		
CP 100	CP 99	UV	a—arg, his	54		
CP 107	15TAU			4.9		
CP 142	CP 107	UV	a—arg	68		
CP 143	CP 107	UV	a—arg	71		
CP 144	CP 107	UV	a—arg	69		
W6					20	7

[a] Two procedures were used in the selection of relaxed mutants. (a) mutagen-treated cultures, grown in Tris-0.2% glucose medium plus the required amino acids, were collected on a membrane filter, resuspended in fresh medium lacking the amino acid(s) indicated, and incubated at 37 C for 3 hr. They were again collected on a membrane filter and resuspended in Tris-0.2% lactose medium plus all required amino acids and 2,000 units (per ml) of penicillin G. After 120 min, penicillinase (a gift from Leo Pharmaceutical Products) was added. A portion of the culture was added to fresh Tris-glucose medium to prepare an overnight culture for the succeeding (identical) round of treatment. The entire cycle was repeated five times. Colonies were picked after the final round of selection and tested for relaxedness. In general, 10 to 60% of the colonies isolated after this procedure were relaxed (as measured by ^{14}C-uracil uptake). (b) Mutagen-treated cultures were grown overnight in Tris-0.2% glucose medium containing an excess (50 μg/ml) of all amino acids required by the strain except the one(s) indicated, which was present in growth-limiting concentrations (2 μg/ml). Next morning the culture was centrifuged, washed once with buffer, and resuspended in Tris-lactose medium containing all required amino acids and penicillin for 120 min. After penicillinase treatment, a portion of the culture was inoculated into fresh Tris-glucose medium limiting in one amino acid and grown overnight. The entire cycle was repeated five times.

[b] Uracil uptake was measured as described in the legend of Fig. 1. The results are expressed as incorporation into acid-precipitable material of the amino acid-starved culture relative to the incorporation into the fully supplemented culture, after one generation of growth.

[c] Phage 363 stocks were prepared on the relaxed strains shown in the first column. *E. coli* AT 13-18 (*arg A*$^-$ *RC*^{str}), obtained from R. Lavallé, was used as recipient; *arg*$^+$ recombinants were selected and scored for the state of the *RC* gene by uracil uptake or by a microscope technique developed by R. E. MacDonald (*personal communication*). The results are expressed as the number of stringent or relaxed *arg*$^+$ transductants.

RNA during amino acid starvation as does the classical RC^{rel} strain (compare Fig. 1b and 1c), whereas, in the "low-relaxed" mutants there is an increase of only about 20% in RNA content after one generation (Fig. 1d).

Table 1 gives a résumé of the characteristics of 25 independently selected relaxed mutants. Mutants could be selected by starving for one or another of the required amino acids during the selection procedure. Relaxed mutants were also obtained for *E. coli* K-12, AB325, F^-, RC^{str}, requiring histidine, arginine, serine, and thiamine (obtained from G. Stent), and from an *E. coli* 15T strain, requiring thymine, uracil, and arginine (S. S. Cohen and H. D. Barner, Proc. Natl. Acad. Sci. U.S. **40**:885, 1954; O. Maaløe and P. C. Hanawalt, J. Mol. Biol. **3**:144, 1961).

The classical *RC* locus in strain W6 is cotransducible with the *argA* locus (R. Lavallé, *personal communication*). Transduction with bacteriophage 363 (obtained from R. Lavallé) showed the same close linkage between the *argA* locus and the mutated sites of all of our relaxed strains (Table 1). In addition, the transductants showed the same "degree of relaxedness" as did the donor strains on which the transducing phage stocks were prepared; thus, the "degree of relaxedness" is a reflection of the particular mutation in the *RC* gene. No stringent phenotype was produced by complementation when an F′ carrying the RC^{rel} (W6) gene was introduced into a "high-relaxed" strain (CP 79) or into "low-relaxed" strains (CP 88 and CP 141; Fiil, *in preparation*).

These mutations thus appear to be located in the same *RC* gene.

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