PRELIMINARY MAPPING OF THE GENETIC LOCUS FOR POTASSIUM TRANSPORT IN ESCHERICHIA COLI

Martin Lubin and David Kessel

Department of Pharmacology
Harvard Medical School
Boston 15, Massachusetts

Received April 14, 1960

Bacteria concentrate potassium from the environment. The intracellular concentration of potassium in Escherichia coli is about the same as the level in mammalian cells, and the intracellular to extracellular distribution ratio, when cells are suspended in a medium very low in potassium, may exceed one thousand.[1, 2]

This report describes some physiologic and genetic properties of a bacterial mutant of E. coli B which is defective in the transport of potassium. A similar mutant has recently been independently isolated by Schultz and Solomon.[3] This and other types of transport-negative mutants[4] were isolated by the penicillin method,[5] as modified by Gorini and Kaufman.[6] The transport-negative mutant (Tr_K^-) grows slowly in media with low concentrations (e.g., 4 gamma/ml) but grows rapidly in high concentrations of potassium (4,000 gamma/ml). Wild-type (Tr_K^+) grows rapidly on high or low potassium (Fig. 1).

The mutant described here was derived from a triply-marked auxotroph which requires histidine, leucine and methionine for growth. The Tr_K^- character spontaneously reverts to wild-type behavior, as judged by growth

on Sodium-A_{HLM} plates*, at relatively high frequency. An overnight culture (10^{10} cells total) grown from a single colony, washed and spread on agar plates containing Sodium-A_{HLM}, was shown to contain 10^2 to 10^3 revert-

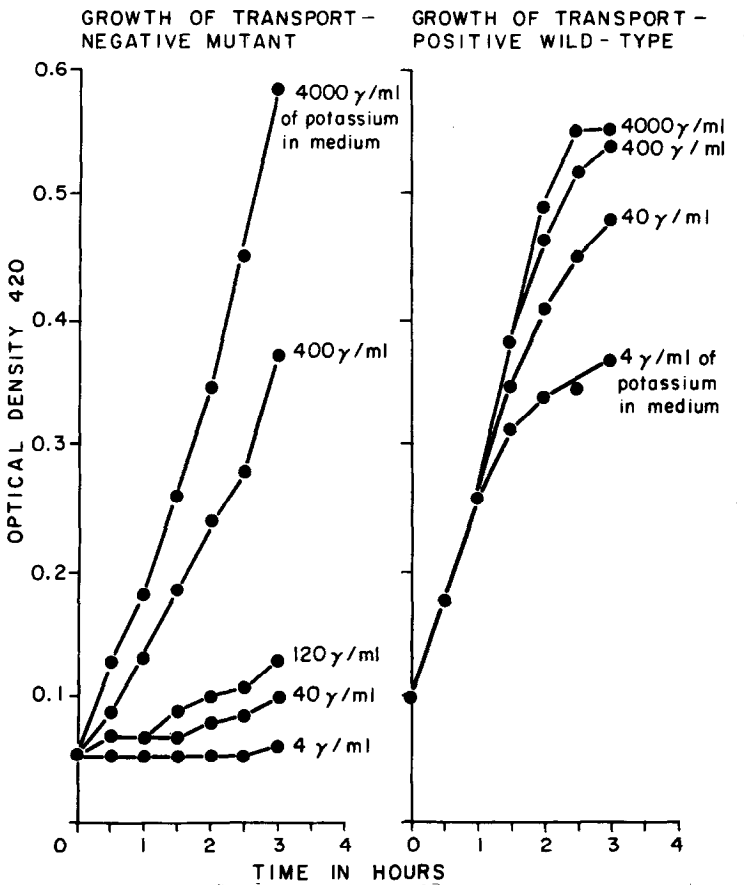

Figure 1. Growth at 37° of mutant Tr_K^- and wild-type Tr_K^+ in mixtures of media A_{HLM} and Sodium-A_{HLM}. Wild-type and mutant cells were grown exponentially, washed, and re-suspended in new medium for the growth curve.

*Minimal medium A, containing glucose, (ref. 7) is high in potassium (4,000 gamma/ml). Medium Sodium-A is identical except for the substitution of sodium for potassium phosphates. Sodium-A, by flame photometric analysis, contained 1 to 2 gamma/ml of potassium, and supports growth of wild-type bacteria because of these traces of potassium. In experiments with potassium added, the sodium concentration of the medium was correspondingly reduced, usually by making appropriate mixtures of medium A and Sodium-A. Medium A_{HLM} and Sodium-A_{HLM} contained 100 gamma/ml of histidine, leucine and methionine. The following abbreviations are used: his = histidine requirement, leu = leucine requirement, met = methionine requirement; his^+, leu^+, and met^+ designate absence of requirement for these amino-acids; lac^+ = ability to ferment lactose. L-amino-acids were used throughout.

ants/10^8 cells. Prolonged growth of the culture in liquid media, especially in one of low potassium content, resulted in favored growth of revertants among the mutant population. Mutant stocks, grown from single colonies, were kept frozen in medium A_{HLM} with 5% glycerol, and were tested for the size of the population of revertants in each experiment.

A number of variations in the composition of the medium was made, without apparent effect on the marked difference in growth rates between Tr_K^+ and Tr_K^- at low levels of potassium. Changing the pH of liquid medium over the range of 6.5 to 7.5, growth on plates at 29° or 22°, or substitution of glycerol for glucose, all gave results similar to those described above.

Reversions, induced by ultraviolet irradiation to either his$^+$ leu met, or his leu$^+$ met, or his leu met$^+$, did not alter the properties of the Tr_K^- marker. Growth of the Tr_K^- mutant on plates was not visibly increased by the addition of any of a variety of amino acids, uridine, or adenine.

Measurements of potassium were made by two methods; the first with K^{42}, the second by flame photometry, with similar results. Suspensions of wild-type and mutant (5×10^8 cells/ml) were incubated at 37° in media containing $K^{42}Cl$. After incubation, cells were collected on Millipore filters (type HA; pore size 0.45 microns), washed briefly with iced Sodium-A and the radioactivity associated with the cells determined.

The kinetics of uptake have not been studied in detail, but timed samples showed that after 20 minutes the period of rapid uptake was complete. With 4,000 gamma/ml of potassium in the medium, cellular content of K^{42} was identical in wild-type and mutant; at 40 gamma/ml, radioactivity in the wild-type was reduced to 35% but counts in the mutant were 25-fold less than in wild-type.

In a typical experiment for measurement of total internal potassium, cells growing exponentially in medium A_{HLM} were centrifuged, washed and

re-incubated in media containing various levels of potassium. After 20 minutes the cells were again sedimented and washed twice with iced Sodium-A. The potassium was extracted with 0.1 M HCl at 100° C and measured on a Baird-Atomic flame photometer (Table I). Other experiments, which confirm the results of Cowie, et al.[1] show that mutant and wild-type cells retain potassium when washed with cold Sodium-A.

MAPPING BY RECOMBINATION

The genetic locus of the Tr_K^+ marker appears to be in the chromosomal region near the marker for leucine biosynthesis. Three types of crosses were performed, using his leu met Tr_K^- str-r as recipient, and either Hfr-Cavalli, Hfr-1 (from the collection of Dr. P. Reeves) or Hfr-Hayes as the donor.**

Because recombination between strain K-12 donors and strain B recipients has not been studied as extensively as that between two K-12 strains, some control experiments were done. Recombination frequencies, expressed as percent of the Hfr population, ranged from .01 to .1 percent, which is expected in crosses between strain K-12 and B. A timing experiment, in which

---

**Hfr-Cavalli transfers markers (in crosses with recipients of strain K-12) in the order T6, lac$^+$, leu$^+$, thr$^+$, met$^+$ ...; Hfr-1 transfer starts at methionine and proceeds to thr$^+$, leu$^+$, lac$^+$ ...; Hfr-Hayes transfers in the order thr$^+$, leu$^+$, lac$^+$, gal$^+$. All are streptomycin-sensitive (str-s), Hfr-Cavalli and Hfr-Hayes grow in minimal medium A, and Hfr-1 requires methionine. The recipient genotype is given in the text above, using a streptomycin-resistant (str-r) derivative of the original Tr_K^- mutant isolated.

Methods were used similar to those described by Wollman and Jacob.[8] For the crosses, medium A supplemented with 0.2 percent Difco yeast extract and 0.2 percent Sheffield tryptic casein hydrolyzate was used.

For selection of leu$^+$ str-r recombinants, plates contained medium A and histidine, methionine and streptomycin (100 gamma/ml). For met$^+$ str-r recombinants, the supplements on selection plates were histidine, leucine and streptomycin. Control platings of donor and recipient were made in each cross. All recombinants scored were tested for the histidine marker, and were his. In scoring for Tr_K^+ or Tr_K^-, each recombinant was dispersed in Sodium-A, and equally sized loopfuls were streaked onto plates, containing either medium A_{HLM} or Sodium-A_{HLM}.

conjugation was interrupted by vigorous pipetting at intervals of 20, 30, 40, 50, and 75 minutes after mixing donor and recipient, was done in the crosses with Hfr-Cavalli and Hfr-1 as donors. In the cross with Hfr-Hayes, samples spaced 2 minutes apart were taken between 10 to 20 minutes.

TABLE I

Cellular potassium after incubation at various potassium levels

Potassium concentration in medium (gamma/ml)	Potassium in cells after 20 minutes (gamma/10^{12} cells)*	
	Tr_K^+	Tr_K^-
4000	4000	4000
400	4000	2100
40	4000	200
4	3200	<100

*Cell population measured by optical density and viable colony count.

Measurement of the size of the revertant population of Tr_K^+ among the Tr_K^- recipient strain was made in each cross, as an additional control. Because of the relatively high rate of reversion to Tr_K^+, selection was not made for the potassium-transport marker, but for amino acid markers, with subsequent scoring for Tr_K^+ and Tr_K^-.

The timing experiments with Hfr-Cavalli showed injection of the leu^+ marker by 20 minutes. The met^+ marker was clearly injected by 40 minutes although a few met^+ str-r recombinants appeared as early as 20 minutes, and are presumed to reflect inefficient disruption of conjugating pairs. With increasing time, as expected, more leu^+ str-r and met^+ str-r recombinants appeared, with a plateau at about 50 minutes. The injection of leu^+ before met^+ is also expected from the results of K-12 crosses.[8]

With Hfr-1 as donor, a few leu^+ str-r recombinants appeared as early as 20 minutes, more at 30, and a plateau was reached at 40 minutes.

With Hfr-Hayes, early injection of the leu^+ marker occurred.

In the cross with Hfr-Cavalli as donor, scoring of the met^+ str-r recombinants for the leu^+ marker gave surprising results (Table II) which is presumably unrelated to the Tr_K^+ mapping and for which we have no explanation. Recombinants selected for met^+ str-r, as judged by data obtained in crosses with strains of K-12, would be expected to show a distribution of leu^+ to leu, on scoring, of about 2:1 or 1:1. From the data of Table II and similar crosses not tabulated here, the fraction of met^+ str-r recombinants with the leu^+ marker was only 10/88.

TABLE II

Recombinants scored for unselected markers

Donor	Markers selected	Number scored; timed samples pooled	Genetic constitution of recombinants			
			Tr_K^+ transport-positive		Tr_K^- transport-negative	
Hfr-Cavalli	leu^+ str-r	62	met 36	met^+ 8	met 17	met^+ 1
Hfr-Cavalli	met^+ str-r	33	leu 1	leu^+ 1	leu 30	leu^+ 1
Hfr-1	leu^+ str-r	48	34		14	
Hfr-Hayes	leu^+ str-r	60	45		15	

Over two-thirds of the leu^+ recombinants from the crosses with Hfr-Cavalli and Hfr-1 were also Tr_K^+, showing that the potassium-transport marker is in the general region of the chromosome between met^+ and lac^+. The cross with Hfr-Hayes shows early transfer of Tr_K^+. This places the Tr_K^+ marker close to leu^+, but more precise localization has not been tried.

Acknowledgments: The assistance of Miss Sara P. Goolsby, Miss Daisy H. Alioth and Mrs. Michele M. Saint-Paul is gratefully acknowledged. We are indebted to Drs. L. Gorini, H. Amos and A. Garen for helpful discussions. This work was supported by grants from the U. S. Public Health Service (H-1498, RG-6712, Senior Research Fellowship SF-83) and the Muscular Dystrophy Associations of America, Inc.

References

1. D. B. Cowie, R. B. Roberts, and I. Z. Roberts, J. Cell. Comp. Physiol. 34:243 (1949). R. B. Roberts, I. Z. Roberts and D. B. Cowie, ibid, p. 259.

2. G. Lester, J. Bacteriol. 75:426 (1958).

3. S. G. Schultz and A. K. Solomon, Personal communication.

4. M. Lubin, D. H. Kessel, A. Budreau and J. D. Gross. In Press.

5. B. D. Davis, J. Am. Chem. Soc. 70:4267 (1948); J. Lederberg and N. Zinder, ibid, 70:4267 (1948); B. D. Davis, Proc. Natl. Acad. Sci. U. S. 35:1 (1949).

6. L. Gorini and H. Kaufman, Science 131:604 (1960).

7. B. D. Davis and E. S. Mingioli, J. Bacteriol. 60:17 (1950).

8. E. L. Wollman and F. Jacob, La sexualité des bactéries. Masson, Paris, 1959.