JOURNAL OF BACTERIOLOGY, Oct. 1982, p. 521–523
0021-9193/82/100521-03$02.00/0

Vol. 152, No. 1

Regulation of D-Arabinose Utilization in *Escherichia Coli* K-12

A. CHRISTOPHER SKJOLD†* AND DAVID H. EZEKIEL‡
Albert Einstein Medical Center, Philadelphia, Pennsylvania 19141

Received 27 January 1982/Accepted 24 May 1982

Studies involving λ phage transduction of the D-arabinose utilization gene (*dar*$^+$) in *Escherichia coli* K-12 indicated the product of this gene to be a *trans*-dominant activator. An apparent anomaly regarding this hypothesis exists in that a diploid recessive lysogen (λ *dar*$^-$/*dar*$^-$) can spontaneously become capable of growth on D-arabinose.

After prolonged exposure to D-arabinose, mutants of *Escherichia coli* K-12 can be selected which have acquired the ability to grow on this sugar (3). It is believed that a single spontaneous mutation occurs in the regulator gene of the L-fucose utilization system, allowing this system to recognize D-arabinose (or its metabolite) as the inducer for the synthesis of the L-fucose utilization enzymes (4). Since these enzymes have substrate specificity for D-arabinose and its metabolites, they can catabolize D-arabinose into dihydroxyacetone phosphate and glycolaldehyde, compounds which are both apparently usable by the cell for energy (4). In an earlier work, we demonstrated that the D-arabinose regulatory gene could be cotransduced by a λ phage carrying the L-fucose utilization genes (7). If such transductants exist in the λ *dar*$^+$/*dar*$^-$ state, then certain regulatory mechanisms are suggested. In this report we examine the genetic aspects of these transductants and propose a model for the D-arabinose (and presumably L-fucose) utilization regulatory mechanism.

Strain DE13, a λ *argA dar*$^+$ transductant, was grown in L-broth (5) modified by the addition of 1.0% NaCl and the elimination of glucose (Table 1 lists all strains used). At exponential phase, cells were removed from the culture and cured of λ by heat-pulse (6) or spontaneous methods. Cured strains were tested for arginine requirements on minimal medium (1), as previously described (7), and for D-arabinose utilization on eosin-methylene blue base agar containing 1.0% D-arabinose. Plates were incubated for 1 to 2 days at 32°C. In each case of an authentic λ cure, conversion of *dar*$^+$ to *dar*$^-$ and *argA*$^+$ to *argA*$^-$ was found to occur as well. We have previously demonstrated that this strain contains the entire copy of the infecting transducing phage and has not been simply converted to *dar*$^+$ by a genetic recombinational event (7). Thus, the transducing phage must originally have integrated into the host chromosome in a single-site recombinational event, and the transduced chromosome must exist as a partial heterozygote, λ *dar*$^+$/*dar*$^-$. This situation suggests that regulation of D-arabinose utilization in *E. coli* K-12 occurs by the action of a *trans*-dominant activator produced by the *dar*$^+$ gene. We arrived at this premise by the following reasoning. (i) A repressor as the sole mode of regulation is ruled out because if it existed in the *dar*$^-$ host, it would continue to repress the operon in the diploid condition. The idea of an activator system entering with the phage is much more consistent with the data presented. (ii) Regulation is dominant because the introduction of phage *dar*$^+$ overrides the host *dar*$^-$ condition. Since the phage *dar*$^+$ gene is adjacent to a fragmented operon, the *dar*$^+$ gene product must migrate through the host cytoplasm to the host *fuc*$^+$ operon. Thus, the activation is of the *trans* type of regulation and must involve a diffusable activator substance. The utilization of L-arabinose is also regulated by an activator substance, but it is speculated that repression also plays a part in the overall regulator mechanism (2). We have no evidence in our system that a repressor mechanism exists, but if so, it seems that its action would necessarily be recessive to that of the activator.

Strain NF161 was grown in modified L-broth, containing 0.2% maltose, to late exponential phase. Cells from this culture were transduced with λ *fucA argA dar*$^-$ by cross-streaking on agar selection plates (7) containing solid minimal medium with 0.2% D-arabinose as the only carbon-energy source. Plates were incubated for 3 to 4 days at 33°C. Since no *dar*$^+$ genes existed in the host or the transducing phage, we were surprised to find the frequent, but very slow, appearance of anomalous Dar$^+$ transductants. Such transductants could be derived directly on

† Present address: Ames Division, Miles Laboratories, Inc., Elkhart, IN 46515.
‡ 6526 N. 12th St., Philadelphia, PA 19126.

TABLE 1. Strains used[a]

Strain	Relevant genotype	Source or reference
E. coli K-12		
10	HfrH	M. Gottesman
NF161	λ^s *argA*	N. Fiil
NF161	(λ *c*I857)	Infection with λ *c*I857, selection on λ *c*I60
AT706	HfrH λ^- *fucA*	A. L. Taylor
DE4	HfrH Δ(*gal-bio*) *fucC dar*$^+$ λ^r (λ *c*I857 in *fucB*)	7
DE13	NF161 (λ *c*I857 *fucA dar*$^+$ *argA*)	7
DE6	HfrH λ^s λ^- Δ(*gal-bio*)	7
DE29	NF161 (λ *c*I857 *fucA argA dar*$^-$)	This work
DE36	DE29, spontaneous Dar$^+$	This work
Phage		
λ *c*I857 *fucA argA dar*$^+$		Heat lysate of DE4
λ *c*I857 *fucA argA dar*$^-$		Heat lysate of DE29
λ *c*I857		7
λ *c*I60		E. Signer
λ *c*I90 *c*17		M. Gottesman
λ *vir*		M. Gottesman

[a] Except for λ *c*I60, λ *c*I90 *c*17 and λ *vir*, all phages and prophages referred to in the text carry the *c*I857 temperature-sensitive *c*I mutation. Also, all λ *dar* phage carry the markers *argA* and *fucA*.

D-arabinose minimal medium or indirectly by selecting for *argA*$^+$ or *fuc*$^+$ and then testing for Dar$^+$. DE29, a *dar*$^-$ *argA*$^+$ isolate obtained from a cross of NF161 and λ *fucA dar*$^-$ *argA*, was analyzed for polylysogeny (6) and found to be singly lysogenic. After conversion to Dar$^+$, however, isolated strains tested polylysogenic. One strain, DE36, was selected for further study. Oddly, high-frequency transducing lysates from DE36 could readily transduce *argA*, but transduced Dar$^+$ only at the much slower rate characteristic of DE29 lysates. In fact, there seemed to be little if any difference between the transductional characteristics of these two lysates. It appears that lysogenization with λ *dar*$^-$ is necessary for this phenomenon to occur, since λ^- and λ lysogenic control strains [NF161 and NF161(λ)] convert to Dar$^+$ only at much lower frequencies over longer periods of time. To test whether conversion to the anomalous Dar$^+$ condition is a permanent one, strain DE36, grown in modified L-broth, was allowed to spontaneously revert to Dar$^-$, using eosin-methylene blue–D-arabinose medium plates to indicate nonfermentors. Dar$^-$ clones were tested for number of prophage after the method of Shimada et al. (6). Resistance to λ *c*I60 was scored as a prophage content of one; resistance to λ *c*I90 *c*17 was scored as a prophage content of two or more. λ *vir* was used as a control each time to check that tested strains had not become λ resistant. Dar$^-$ clones were found to contain λ but were not polylysogenic. These revertants appeared identical in every way to DE29 and retained their ability to re-revert to Dar$^+$ just as before. Conversely, heat curing DE36 resulted in strains that were λ^s Dar$^-$ and equally divided as to *argA*$^+$ and *argA*$^-$. Revertants of these types could no longer spontaneously convert to Dar$^+$. Since the test for polylysogeny is essentially a titration of the concentration of cellular repressor, a positive test does not necessarily mean an increased number of λ genomes within the host chromosome, but could also indicate an increased rate of λ repressor protein synthesis. In any event, the change in λ repressor status seems to be related to the concomitant conversion to D-arabinose utilization since both situations remain very closely coupled in both forward and backward transitions. The heat-pulse curing studies indicate a fairly close physical linkage between the two markers.

The phenomenom described above is quite an unusual genetic event and is not easily accounted for by traditional genetic explanations (i.e., various types of λ escape synthesis). One possible clue to the nature of the effecting mechanism is that, upon curing or excising λ *dar*$^-$ from the host chromosome of DE36, both the cured host and the excised transducing phage were shown to undergo complete reversion to their parental phenotypes. These data argue against a mutational type of event and suggest something more transitory in nature such as a gene dosage mech-

anism resulting perhaps from a duplication of λ *dar*⁻ DNA. A sufficient quantity of *dar*⁻ gene product generated in this fashion may be capable of turning on the D-arabinose (L-fucose) utilization genes.

We thank R. P. Mortlock and D. J. LeBlanc for many valuable discussions and R. J. Tyhach and M. T. Skarstedt for critical review of this manuscript. The secretarial assistance of S. Darsie and P. Burmeister is gratefully acknowledged.

This work was supported in part by National Science Foundation grant GB-8492 and in part by Public Health Service grant CA-11094 from the National Institutes of Health.

LITERATURE CITED

1. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. **60**:17–28.
2. **Englesberg, E., C. Squires, and F. Meronk, Jr.** 1969. The L-arabinose operon in *Escherichia coli* B/r: a genetic demonstration of two functional states of the product of a regulator gene. Proc. Natl. Acad. Sci. U.S.A. **62**:1100–1107.
3. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: origin of a D-ribulokinase activity in *Escherichia coli*. J. Bacteriol. **106**:82–89.
4. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106**:90–96.
5. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
6. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. **63**:483–503.
7. **Skjold, A. C., and D. H. Ezekiel.** 1982. Analysis of lambda insertions in the fucose utilization region of *Escherichia coli* K-12: use of λ *fuc* and λ *argA* transducing bacteriophages to partially order the fucose utilization genes. J. Bacteriol. **152**:120–125.