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Novel Mutation to Dominant Fucose Resistance in the L-Arabinose Operon of *Escherichia coli*[1]

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We isolated an unusual mutant in which the arabinose operon carried an *araC* lesion that led to a dominant fucose resistance while retaining recessive constitutivity. All previously characterized C^c mutations have been recessive for both fucose resistance and constitutivity.

Induction of the arabinose operon of *Escherichia coli* normally requires arabinose and the product of the *araC* gene. Thus, the *araC* product is a positive control element (4, 7, 13). D-Fucose, an L-arabinose analogue, antagonizes induction in a wild-type cell, but mutants resistant to fucose can be isolated and are frequently found to be constitutive (C^c), having high levels of arabinose operon enzymes even in the absence of arabinose (1). Both fucose resistance and constitutivity have been found to be recessive to the wild-type allele (C^+) in vivo (4) and in vitro (6). From these and other observations, Englesberg et al. concluded that *araC* protein represses as well as induces (5). Here we report the isolation of a derivative of an $AraC^c$ mutant in which the *araC* gene was further mutated so that its fucose resistance has become dominant to C^+. Nonetheless, the constitutivity evoked by this gene product is still recessive to C^+.

Beginning with an $F'AraC^c$ strain, a derivative possessing a second mutation in the C gene was isolated by requiring a C^c/C^+ diploid strain to grow on minimal-arabinose fucose. An ordinary diploid strain of this structure will not grow on minimal-arabinose fucose medium since the C^c is recessive to C^+. The mutation was isolated on an episome to facilitate transfer between strains. A strain (P113, Table 1) containing an episome carrying a high-level $AraC^c$ mutation ($F'thr^+$ leu^+ *araB145 C6900*) was mutagenized with 20 μg of nitrosoguanidine per ml (1) for 15 min; after several generations of growth in minimal M9-B1-glucose medium (10) supplemented with required amino acids, the cells were mated with strain JTL30 (F^-araC^+) and plated on minimal-arabinose fucose medium (4). Colonies appeared at a frequency of 10^{-5} to 10^{-6}, 10- to 100-fold higher than were obtained when an unmutagenized episome was transferred into the F^-C^+ strain, JTL30. The episomes from 46 fucose-resistant colonies were transferred to another F^-Ara^+ strain (P113), and 32 out of the 46 recipients were dominant fucose resistant, showing that the dominant fucose mutation was episomal. None, however, was dominant constitutive. Several of the episomes were transferred into strain RFS882 (*araΔC*) and all had fully induced levels of L-arabinose isomerase in the absence of inducer, showing that the episomes still carried the high-level constitutive character.

Fucose effects were studied in detail for one dominant fucose-resistant mutant episome, *F'araC6901* (C^{cFd}), and its parental episome, *F'araC6900* (C^c), in isogenic female wild type and *araC* deleted strains (Table 2). The parental females exhibit the expected antagonism of arabinose induction by fucose in the F^-C^+ strain (4) and uninducibility in the $F^-\Delta C$ strain. Both episomes in the $F^-\Delta C$ strain exhibit high-level constitutivity, hyperinducibility by fucose (2), and self-catabolite repression by arabinose (8). The parental C^c episome (*F'araC6900*) in a C^c/C^+ diploid strain leads to isomerase levels reduced far below fully induced levels both in the absence of inducer and in the presence of fucose or arabinose plus fucose, showing that the C^c is recessive to C^+. In contrast, the C^{cFd} mutant episome, *F'araC6901*, gave rise to a low level of arabinose isomerase in the C^c/C^+ diploid strain when no inducer was present and led to fully induced levels in the presence of either fucose or arabinose plus fucose. Thus, the mutation is recessive constitutive and dominant fucose resistant.

Since the arabinose operon on the episome

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TABLE 1. *Bacterial strains*

Strain no.[a]	Genotype	Comments	Source or reference
JTL28	F⁻: *leu thr lac-74 tsx28*	Derived from Pasteur strain RV, P1 transduced to Leu⁻ Ara⁺ Thr⁺ with strain C600 as donor (T6 resistant)	J. Lis[b]
JTL30	F⁻: *leu thr lac-74 tsx28 recA nalA111*	Derived from strain JTL28 by recombination with strain Hfr 111 (recA⁻, *nalA111*) of Joel Kirshbaum.	J. Lis[b]
NMN75	F′: *thr⁺ leu⁺ araB145 C6900/* in NMN96	Spontaneous AraC^c derivative of strain RFS F′145 selected for growth on arabinose-fucose minimal medium.	This work
NMN96	F⁻: *leu araC882 lac-74 tsx28*	Derived from strain JTL28, transduced to Thr⁺ AraΔC with strain RFS882 as donor.	This work
NMN102	F′: *thr⁺ leu⁺ araB145 C6901/* in NMN96	Dominant fucose-resistant (C^{cFd}) derivative of F′ *araC6900*.	This work
P113	F⁻: *metB argG his leu str recA mal xyl mtl*	Derived from JC1553	
RFS882	F⁻: *leu-73 Δ(gal att bio uvrB) lac-74 str lam-830 araC882*	Deletion of most of *araC*	12
RFS F′145	F′: *thr⁺ leu⁺ araB145/ ara-498 thi lac-74*	Highly polar B⁻	12

[a] All strains are *Escherichia coli* K-12.
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TABLE 2. *Effect of* D*-fucose on* L*-arabinose isomerase*[a]

Host strain	Pertinent genotype		Additions to medium			
	Chromosomal genotype	Episomal genotype	None	Arabinose	Fucose	Arabinose + fucose
JTL28	*araC⁺*	None	33	23,700	30	220
NMN96	*araΔC*	None	39	30	27	45
NMN96	*araΔC*	*araC6900* (C^c)	19,900	16,800	54,600	26,300
JTL28	*araC⁺*	*araC6900* (C^c)	610	16,100	**1,420**	**2,080**
NMN96	*araΔC*	*araC6901* (C^{cFd})	22,700	20,300	44,500	26,700
JTL28	*araC⁺*	*araC6901* (C^{cFd})	960	17,300	**41,500**	**27,300**

[a] Cells were grown in 10 ml of M9-B1-glycerol (supplemented with 5×10^{-5} M $MnCl_2$) in 50-ml Erlenmayer flasks at 37 C to a density of 10^8. Arabinose and fucose were added at 0.2% and 0.3%, respectively, as indicated. Threonine or leucine, or both, were added to medium as needed for growth of F⁻ strains. Toluene-treated cells were assayed for L-arabinose isomerase, as described by Schleif (10). Data are presented as monomers isomerase per cell (±10%).

contains a highly polar B⁻ mutation resulting in less than five monomers per cell of isomerase when fully induced, the episomal mutation conferring dominant fucose resistance acts in *trans* and therefore must be a diffusible gene product and is not, for example, in the control region of the operon. Thus, the mutation most likely lies within the C gene itself.

A possible explanation for these results is subunit mixing. Active C protein appears to consist of more than one subunit, since the induction curve with arabinose is cooperative, implying at least a dimeric structure (11). The mutation conferring dominant fucose resistance could form C^{cFd}-C$^+$ hybrid molecules which exist in the repressing conformation in the absence of ligands. Binding of arabinose or fucose would drive these hybrids, but not C^c-C$^+$ hybrids, into the inducing conformation. A well-characterized example of subunit mixing of a regulatory protein occurs in the *lac* system. The *trans* dominance of i^{-d} repressors is presumably due to bad subunits mixing with good to form a repressor that can bind isopropyl-thio-β-D-galactoside but which is unable to bind to the operator and repress (9).

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