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Mutations in the L-Arabinose Operon of *Escherichia coli* B/r with Reduced Initiator Function

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Partial reversion mutants derived from a strain containing a strongly polar initiator-defective mutation (*araI1036*) in the L-arabinose operon were found to have several characteristics expected of mutants with reduced initiator function. These reversion mutations are cotransduced with the *ara* region and are probably within the *araI* region. Furthermore, they permit induction of the L-arabinose operon to a level only one-third of the normal wild-type level. These partially functional initiator regions reduce the expression of structural genes in the *cis* position only; they function quite independently of wild-type or defective initiator regions in the *trans* position. These mutants exhibit a two- to threefold *increase* in the rate of expression of *ara* operon genes within one-tenth of a generation after a shift of the growth temperature from 28 to 42°C. This suggests that the temperature optimum for initiation of operon expression is higher for the partial revertant strains than it is for strains containing a wild-type initiator region.

The L-arabinose operon of *Escherichia coli* (Fig. 1) consists of three structural genes, *araB*, *araA*, and *araD*, coding for L-ribulokinase, L-arabinose isomerase, and L-ribulose-5-phosphate-4-epimerase, respectively (9). The expression of these structural genes is controlled by a *cis* acting regulatory region *araOI* (6, 7, 9, 18) and a diffusible product specified by gene *araC* (14, 18). Gene *araC* is controlled independently of the structural genes (21). According to the current model, the product of the *araC* gene may exist in either of two functional states (8). In the absence of inducer (L-arabinose), repressor is the predominant form of the *araC* gene product. It is this form that binds to the operator region to effectively block expression of the operon (8). The addition of inducer is believed to inactivate the repressor and facilitate the formation of the activator form of the *araC* gene product (9, 20). Removal of repressor from the operator, although necessary for *ara* operon expression, is not sufficient: the activator form (*araC* product plus L-arabinose) is required to initiate expression of the *ara* operon of the initiator region (9, 14, 18). In addition to the above regulatory elements specific for the arabinose operon, catabolite activator protein and cyclic adenosine 3',5'-monophosphate (cAMP), as well as ribonucleic acid (RNA) polymerase, are required for initiation of transcription (9, 14).

Evidence for a promoter-like element within the initiator region (*araI*) comes from an analysis of several classes of mutants. (i) In strains that contain deletion mutations extending from *araB* into *araC* (Fig. 1), the remaining *ara* structural gene [e.g., *araA* (isomerase)] cannot be induced, even when the deleted material is replaced in the *trans* position on an episome (18). The region between *araB* and *araC* is therefore essential for *ara* operon induction. (ii) Ara$^+$ revertants of strains containing extensive deletions of the *araC* gene are due to *cis* acting mutations (*araI*^{c}) in the initiator region (7). In these strains *ara* operon induction occurs constitutively at a low level, but may be fully induced by arabinose in the presence of *araC*$^+$ gene product. Presumably these represent configurations of *araI* in which the requirement for activator and inducer has been partially circumvented but which nevertheless can be fully induced. (iii) A series of point mutations, presumably in *araI*, have been identified that lie between genes *araB* and *araC* and exert a strong polar effect upon the expression of adjacent structural genes (6). Since they are not nonsense or frameshift mutations, they are presumed to represent alterations in the *araI* region that block in some way the initiation of operon expression. Two classes of Ara$^+$ revertants have been obtained from these mutants: full revertants and partial revertants. The full revertants are similar to the wild-type parental strains. Strains containing the partial reversion mutations are nonconstitutive and when induced exhibit intermediate levels of *ara* op-

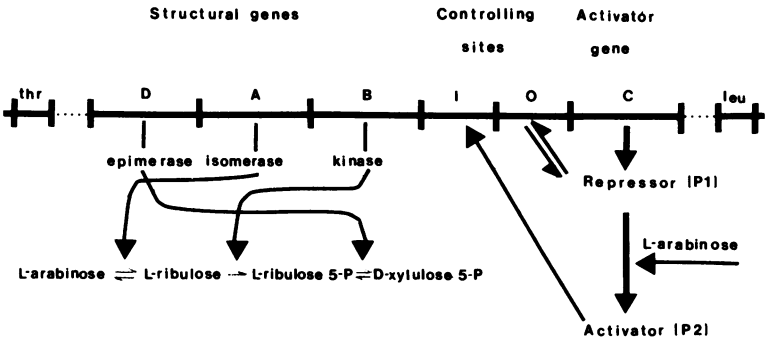

FIG. 1. *L-Arabinose operon of Escherichia coli B/r.*

eron expression. The partial revertant mutations also appear to be closely linked to the original polar mutation (6). These are characteristics expected of mutations with partially functional promoter regions (1, 2, 12, 15). In the analysis that follows, the reduced level of *ara* operon expression exhibited by these mutants will be shown to be a *cis*-dominant effect. Furthermore, the presumed, partially defective initiator regions in these mutants result in an alteration of the temperature optimum for the initiation of *ara* operon expression.

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MATERIALS AND METHODS

Media. All of the media used for the growth of bacteria and bacteriophage P1*bc* have been described previously (4, 6, 18). The following abbreviations are used to designate media components: M, mineral salts; CH, casein hydrolysate; EMB, eosin methylene blue; ara, L-arabinose; glc, glycerol; and fuc, D-fucose.

Bacterial strains. Table 1 lists the strains used. All of the putative *araI* mutants and their revertants were isolated as previously described (6). The designation *araI1036r* will be used to indicate an initiator region in an Ara$^+$ revertant derived from strain ME1098 (*araI1036*).

Strain construction. (i) Double mutants of the type *araA54 araI1036 r*: P1*bc* transducing phage grown on strains of the type *araA$^+$ araI1036 r leu$^+$* was used to transduce IG1051 (*araA54 araC^{c}103 leuB1*) to Leu$^+$. Leu$^+$, Ara$^-$ transductants, identified by replica plating, were tested for the presence of the *araA54* and *araC^{c}103* markers in crosses with SB3103 (F *ara54/araA54*) and SB3114 (F *araC3/araC3*), respectively. The complementation response indicative of the *araA54 araI1036 r* double mutant was positive in crosses with SB3114 (F *araC3/araC3*) on M ara medium and was negative in crosses with SB3103 (F *araA54/araA54*) on M ara medium and with SB3114 (F *araC3/araC3*) on M ara fuc medium. The negative complementation response in crosses with SB3103 (F *araA54/araA54*) on M ara medium indicates the presence of the *araA54* allele; the positive complementation response in crosses with SB3114 (F *araC3/araC3*) on M ara medium indicates a functional *araC* gene. The negative complementation response in crosses with SB3114 (F *araC3/araC3*) on M ara fuc medium indicates that the *araC* gene does not carry the *ara^c* (D-fucose resistant) (4, 18) allele originally present in the transduction recipient, and thus should carry, with high probability, the desired *araI1036 r* mutation which is closely linked to its wild-type *araC* gene. The presence of the *araI1036 r* mutation in the presumed double-mutant transductants was confirmed by growing P1 transducing phage on these clones and then transducing IG1050 (*araΔBIOC768 leuB1*) to Ara$^+$. One or two Ara$^+$ transductants from each strain were assayed for L-arabinose isomerase specific activity after growing at 28 and 42°C. Double-mutant isolates whose Ara$^+$ recombinants exhibited the characteristic effects of temperature on isomerase levels were used for constructing the *leuB1* form of the double mutants and for subsequent merodiploid construction.

(ii) Double mutants of the type *araA54 araI1036 r leuB1*: P1 phage grown on the *araA54 araI1036 r leu$^+$* double-mutant strains were used to transduce strain UP1004 (*thr-1 ara$^+$ leuB1*) to Thr$^+$. These transductants were screened by replica plating and appropriate crosses for the presence of the *araA54*, *araI1036 r*, and *leuB1* mutations. Nonlysogenic derivatives were used in merodiploid construction.

(iii) Merodiploids of the type F *araA$^+$ araB$^+$ araI1036/araA54 araB$^+$ araI1036 r*: His$^+$, Leu$^+$ exconjugants were selected from a mating of IG2002 (F *araA$^+$ araB$^+$ araI1036/araA$^+$ araB$^+$ araI1036 his-1*) and strains of the type F$^-$ *araA54 araB$^+$ araI1036 r*

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
UP1004	*thr-1 ara$^+$ leuB1*	Englesberg
SB3103	F *araA54/araA54*	Sheppard
SB3114	F *araC3/araC3*	Englesberg
IG1050	*araΔ BIOC768 leuB1*	Eleuterio
IG1051	*araA54 araC^{c}103 leuB1*	This paper
ME1098	*araI1036 leuB1*	Eleuterio
IG2000	F *araI1036/araI1036*	From ME1098
IG2001	F *araB24/araB24*	This paper
IG2002	F *araI1036/araI1036 his-1*	From IG2000

leuB1. Merodiploids of the desired type were detected by their weak complementation on M ara medium, their fertility for the arabinose region, and their strong Ara$^+$ recombinant response on EMB ara medium. After growth for enzymatic analysis, cell samples were taken, diluted, and plated upon EMB ara to identify Ara$^-$ segregants. Genetic confirmation of both the exogenote and the endogenote within the segregant population was determined by means of crosses with appropriate F′ homogenotes.

(iv) Merodiploids of the type F *araA*$^+$ *araB24 araI*$^+$/*araA54 araB*$^+$ *araI1036 r*: Ara$^+$ exogenotes were selected from a mating of IG2001 (F *araA*$^+$ *araB24 araI*$^+$/*araA*$^+$ *araB24 araI*$^+$) and strains of the *type araA54 araB*$^+$ *araI1036 r*. Genetic confirmation of both the exogenote and the endogenote within the segregant population was determined by means of crosses with appropriate F′ homogenotes.

L-Arabinose isomerase assay. L-Arabinose isomerase activity (EC 5.3.1.4) was determined as previously described (6) except that each sample was frozen and thawed after toluene treatment, and the substrate (L-arabinose) concentration in the reaction mixture was increased to 0.6 mM.

L-Ribulokinase assay. L-Ribulokinase activity (EC 2.7.1.16) was determined by the procedure of Schleif et al. (17).

β-Galactosidase assay. β-Galactosidase activity was determined as described by Pardee, Jacob, and Monod (16) except that each sample was frozen and thawed after treatment with toluene.

Growth of cells for enzymatic assay. Specific activity measurements were made on cultures that had grown for at least four generations under the conditions specified in each table or figure legend. Induction kinetics were determined by taking duplicate 1-ml samples at regular intervals during exponential growth.

RESULTS

Polar *araI* mutants and their revertants. Ara$^+$ revertants of strain ME1098, carrying the putative *araI* defective mutation (*araI1036*), were previously shown to fall into two phenotypic classes: (i) a "full revertant" class that appeared identical to wild type with regard to the utilization of L-arabinose, and (ii) a "partial revertant" class in which expression of the arabinose operon occurred at a rate approximately one-third that of full revertant and wild-type strains (6). The site of ther reversion mutations had previously been found to be cotransduced with the *ara-leu* region by P1 transducing phage. It is not known, however, whether the Ara$^+$ phenotypes of the *araI1036* reversion mutant strains are due to same-site back mutations or closely linked suppressor mutations.

Cell generation times in M ara medium. Partial and full revertant strains, preconditioned in M glc ara medium, were grown for two to three generations in M ara medium at several temperatures ranging from 28 to 42°C. The results (Fig. 2) indicate (i) that partial revertant strains exhibit a generation time twofold greater than full revertants at the optimal growth temperature of 37°C, and (ii) that the generation times of partial revertant strains increase 2.4- to 3.3-fold as the temperature is lowered to 28°C, whereas those of full revertant strains increase only 1.5- to 1.6-fold. This suggests that the rate of L-arabinose utilization by partial revertant strains is both less efficient and more sensitive to temperature fluctuations that that in full revertant and wild-type strains.

Effect of temperature on the differential rate of *ara* operon expression. The differential rates of expression of the *ara* and *lac* operons of full and partial revertant strains were determined by measuring L-arabinose isomerase and β-galactosidase activities, respectively, over two to three generations of growth under inducing conditions. The results (Table 2 and Fig. 3) demonstrate that the rate of *ara* operon expression in partial revertant strains is approximately one-half that of the wild-type and full revertant strains at 42°C. Whereas full revertant and wild-type strains show no decrease in

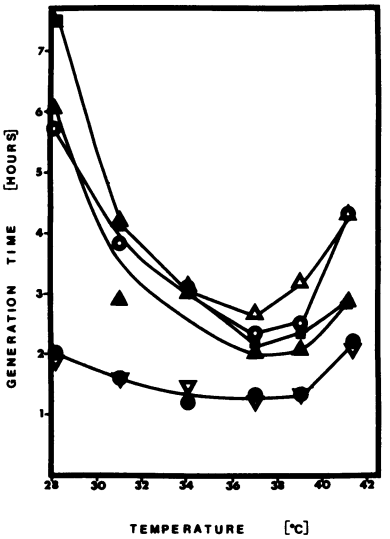

FIG. 2. *Effect of temperature upon utilization of* L-*arabinose for growth in partial and full Ara*$^+$ *revertants of strain ME1098 (araI1036). Generation time is the time required for a twofold increase in culture turbidity (cell number) during the exponential phase of growth. Symbols:* ○, *CN5422 (araI1036 pr1);* △, *CN5424 (araI1036 pr2);* ▲, *CN5430 (araI1036 pr3);* ■, *CN5433 (araI1036 pr4);* ●, *CN5402 (araI1036 fr1);* ▽, *CN5403 (araI1036 fr2) pr, partial revertant; fr, full revertant).*

TABLE 2. *Effect of temperature on the differential rate of operon expression*[a]

Strain	Pertinent genotype	Operon expression[b]		
		28°C	42°C	42°C/28°C
CN5422	*araI1036 prl*	0.20 ± 0.01 (3)	0.49 ± 0.01 (3)	2.5
CN5424	*araI1036 pr2*	0.22 ± 0.02 (3)	0.47 ± 0.02 (3)	2.2
CN5430	*araI1036 pr3*	0.25 ± 0.02 (3)	0.58 ± 0.01 (3)	2.3
CN5433	*araI1036 pr4*	0.23 ± 0.05 (3)	0.54 ± 0.12 (4)	2.3
CN5402	*araI1036 frl*	2.00 ± 0.30 (3)	1.39 ± 0.10 (3)	0.7
CN5403	*araI1036 fr2*	1.76 ± 0.05 (3)	1.35 ± 0.07 (3)	0.8
UP1000	*araI*$^+$	1.01 (1)	0.67 (1)	0.7
UP1003	*araI*$^+$	1.16 (1)	1.11 (1)	1.0
UP1004	*araI*$^+$	1.08 (1)	1.01 (1)	0.9
CN5424	*araI1036 pr2*	3.31 ± 0.1 (2)	3.92 ± 0.04 (2)	1.2

[a] Cells were grown in mineral salts-casein hydrolysate plus inducer medium; samples were taken at periodic intervals over one cell generation.

[b] All results are for the L-arabinose operon (L-arabinose isomerase) except for the final entry in the table, which shows expression of the lactose operon (β-galactosidase) in strain CN5424. The slope of enzyme per milliliter versus culture turbidity at 420 nm was used as a measure of the rate of operon expression. Standard deviations are given, with the number of determinations shown in parentheses.

the rate of *ara* operon expression upon lowering of the temperature to 28°C, partial revertant strains show a 2- to 2.5-fold decrease in rate. The decrease in rate of operon expression in partial revertant strains is specific for the L-arabinose operon, since no such decrease was observed for lactose operon expression in one of these strains (Table 2).

Cells growing exponentially under inducing conditions for the L-arabinose operon were shifted from a growth temperature of 28°C to 42°C. No increase in the rate of L-arabinose production was observed for full revertant strain CN5402 (*araI1036 frl*) after the shift, whereas partial revertant strain CN5424 (*araI1036 pr2*) exhibited an immediate (2 min was required for temperature equilibrium) fourfold increase in *ara* operon expression after the shift to 42°C (Fig. 4).

***Cis*-dominant, *trans*-recessive action of partial reversion mutations.** The partial revertant mutant strains derived from strain ME1098 (*araI1036*) could have been derived by two distinct mechanisms. (i) Intragenic reversion: a second mutation could have occurred within the *araI* region permitting partial expression of the *ara* operon. Such a mutation would be *cis* acting and might be analogous to the partially defective promoter mutations in the *lac* operon (1, 5, 12, 15). (ii) Extragenic suppression: a mutation could have occurred within the *araC* gene. The resulting mutant *araC* gene protein might be capable of initiating *ara* operon expression at an *araI1026* mutant initiator. Such a reversion mutation would exert its effect *trans*, via the diffusible *araC* gene product. The first set of merodiploids of the type F *araA*$^+$ *araB*$^+$ *araI1036 C*$^+$/*araA54 araB*$^+$ *araI1036 r araC*$^+$ was constructed to determine whether the partial reversion mutations (*araI1036 pr*) derived from ME1098 (*araI1036*) can act *trans* to suppress the *araI1036* mutation in the *trans* position. Merodiploids were grown for approximately three generations in CH ara liquid medium and then assayed for L-ribulokinase activity. Cell samples were also taken and subjected to progeny testing as described in Materials and Methods. The results presented in Table 2 show that these merodiploids synthesized no L-arabinose isomerase, but did produce levels of L-ribulokinase characteristic of each revertant strain. This indicates that the *araA*$^+$ gene adjacent to the *araI1036* mutation on the exogenote cannot function, whereas the *araB*$^+$ gene linked to the reversion mutation derived from ME1098 (*araI1036*) on the chromosome is active. This must mean that the *araI1036 r* mutation does not act via a diffusible product and is therefore *trans* recessive.

A second set of merodiploids of the type F *araA*$^+$ *araB24 araI*$^+$/*araA54 araB*$^+$ *araI1036 r* was built to demonstrate the independent action of a wild-type and a partially defective initiator region in the same cell. The results (Table 3) indicate that in these merodiploid strains, the isomerase gene (*araA*$^+$) continues to be expressed at the level determined by a wild-type initiator, whereas the ribulokinase gene (*araB*$^+$) is expressed at the level expected of the partially defective initiator regions. These results firmly establish the *trans*-recessive, *cis*-dominant nature of the partial rever-

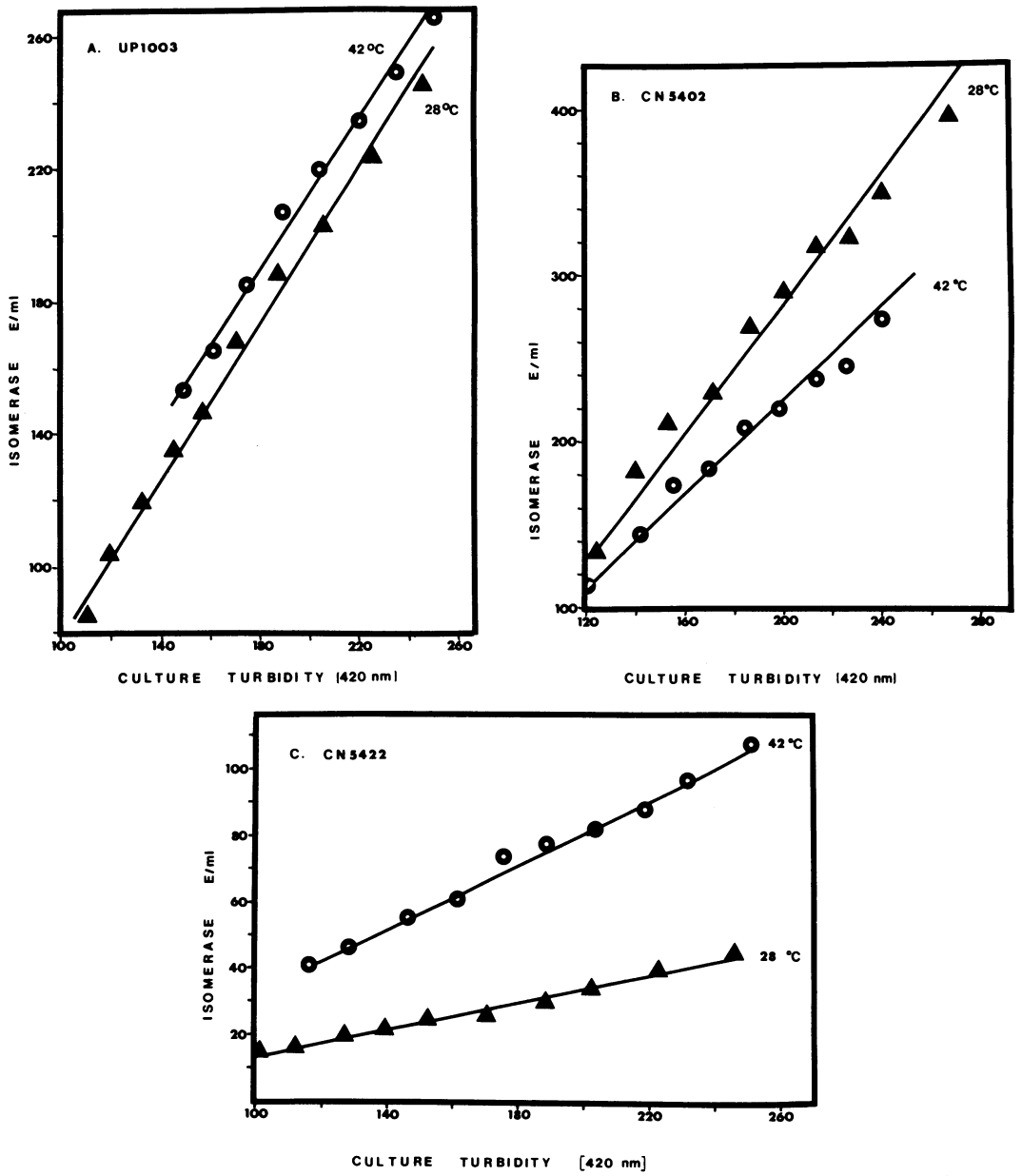

FIG. 3. *Differential rate of* L-*arabinose operon expression at 28 and 42°C. (A) UP1003 (Ara⁺); (B) CN5402 (araI1036 frl); (C) CN5422 (araI1036 prl). Cells growing at 28°C (▲) or 42°C (○) in medium containing mineral salts, casein hydrolysate, and* L-*arabinose were sampled at periodic intervals for* L-*arabinose isomerase activity.*

sion mutations derived from strain ME1098 (*araI1036*).

In haploid strains of the type *araA*⁺ *araB*⁺ *araI1036 r*, the rates of L-arabinose isomerase and L-ribulokinase synthesis are controlled by the same initiator region. Under these conditions the ratios of isomerase to kinase specific activity range between 0.6 and 1.2, as shown in Table 3. In merodiploids of the type F *araA*⁺ *araB24 araI*⁺/*araA*⁻ *araB*⁺ *araI1036 r*, the isomerase and kinase genes are expressed at rates determined by a wild-type and a partially defective initiator, respectively. Merodiploids of this type that contain full reversion mutations derived from strain ME1098 (*araI1036*) have an isomerase/kinase specific activity ratio

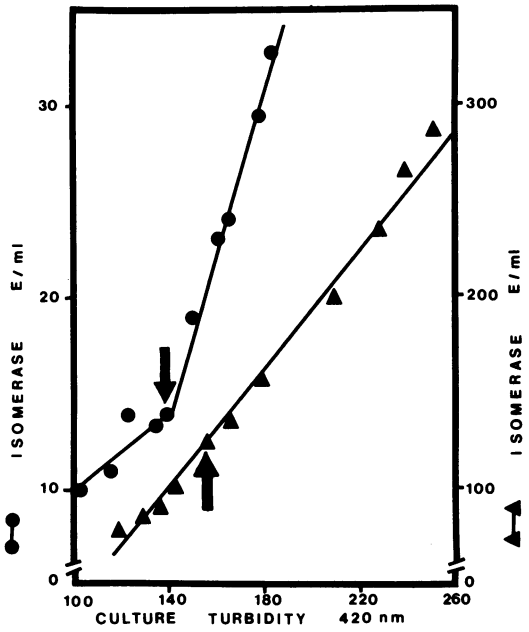

FIG. 4. *Effect of a shift in growth temperature on the differential rate of* L-*arabinose operon expression in full revertant and partial revertant mutant strains derived from strain ME1098 (araI1036). Cells growing at 28°C in medium containing casein hydrolysate and* L-*arabinose were sampled at periodic intervals for* L-*arabinose isomerase activity. The growth temperature was then quickly shifted to 42°C and sampling was continued. The arrow indicates the time of temperature shift. Symbols: ▲, CN5402 (araI1036 fr1); ●, CN5424 (araI1036 pr2).*

similar to that found in a haploid wild-type strain. Merodiploids containing partial reversion mutations derived from ME1098 (*araI1036*) have ratios five to six times higher than the haploid strains containing initiator regions of the type *araI1036 pr*. This is a much greater difference than can be accounted for on the basis of the threefold difference in the rate of *ara* operon expression specified by haploid strains containing the *araI*$^+$ and *araI1036 pr* initiator regions. It is important to realize that in these merodiploids L-arabinose utilization is limited as a consequence of the reduced kinase specific activity levels specified by the *araI1036 pr* initiator region. This reduced L-arabinose utilization could result in a release from "self catabolite repression" (4, 13) at the *araI*$^+$ initiator region, thus enabling a higher rate of isomerase (*araA*$^+$) expression.

DISCUSSION

Partial Ara$^+$ revertants derived from strain ME1098 (*araI1036*) fulfull two of the essential properties expected of mutants with partially defective initiator regions: (i) the site of the reversion mutation is linked by P1 transduction to the *ara* region, although its exact genetic site has not been located, and (ii) the partial reversion mutant strains specify an intermediate level of *ara* operon expression by a *cis*-dominant, *trans*-recessive mechanism. Furthermore, in these partial revertant strains the rate of *ara* operon expression increases with temperature, an observation that could be accounted for by an increase in the temperature optimum for initiation of operon expression.

The *araI*, region is thought contain the promoter site for the *araBAD* operon (9–11). Strongly polar, promoter-defective point mutants could revert by a same-site reversion to the wild-type nucleotide sequence, or they could revert to a nucleotide sequence that permits an intermediate level of promoter activity. Full and partial revertants may be accounted for by these two mechanisms. The latter could occur by a non-wild-type base-pair substitution at the site of the original mutation or by a base-pair change at a second site within the initiator region (internal suppression).

A promoter region has been defined as the deoxyribonucleic acid (DNA) binding site for RNA polymerase during initiation of transcription (1, 3, 5, 11, 12, 19). In some cases, it may also be the binding site for the action of certain other proteins necessary for the activation of transcription. According to the current model for the regulatory control in the L-arabinose operon (3, 9–11), the frequency of initiation of transcription should depend upon: (i) the affinity of the initiator region (promoter) for RNA polymerase, (ii) the ease of melting of promoter DNA to form an open complex of initiation, (iii) the interaction of *araC* activator with the initiator region, and (iv) the interaction of catabolite activator protein and cAMP with the initiator region and probably with the *araC* activator. The interaction of *araC* activator and catabolite activator protein with the initiator region may facilitate the melting of promoter DNA. Mutations affecting any of these interactions, it is assumed, would alter the frequency of initiation.

In a model developed by Chamberlin (3) for initiation of transcription at a promoter site, the transition between a closed promoter (a complex of RNA polymerase and double-stranded DNA) and an open promoter (a complex of RNA polymerase and single-stranded DNA capable of initiation of transcription) is considered to be a melting event that involves a cooperative unit of seven to eight base pairs. The thermal stability of such a region is related to its adenosine-ribosylthymine content and

TABLE 3. *Induced L-arabinose isomerase and L-ribulokinase specific activity levels in merodiploids*[a]

Strain	Exogenote genotype	Endogenote genotype	L-Arabinose isomerase	L-Ribulokinase	Isomerase/kinase
IG2200	*araI1036*	*araA54 araI1036 pr1*	<1.0	11.0	<0.1
IG2202	*araI1036*	*araA54 araI1036 pr2*	<1.0	16.6	<0.1
IG2204	*araI1036*	*araA54 araI1036 pr3*	<1.0	26.2	<0.1
IG2207	*araI1036*	*araA54 araI1036 pr4*	<1.0	25.1	<0.1
IG2208	*araI1036*	*araA54 araI1036 fr1*	<1.0	139.5	<0.01
IG2210	*araI1036*	*araA54 araI1036 fr2*	<1.0	154.4	<0.01
IG2212	*araI1036*	*araA54 araI*$^+$	<3.4	166.1	<0.1
IG2002	*araI1036*	*araI1036*	<1.0	<1.0	
IG2400	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 pr1*	154.5	21.1	7.3
IG2402	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 pr2*	218.2	29.3	7.4
IG2404	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 pr3*	204.8	30.1	6.8
IG2406	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 pr4*	206.4	32.2	6.4
IG2408	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 fr1*	121.8	104.0	1.2
IG2410	*araA*$^+$ *araB24 araI*$^+$	*araA54 araB*$^+$ *araI1036 fr2*	122.4	110.1	1.1
IG2001	*araA*$^+$ *araB24 araI*$^+$	*araA*$^+$ *araB24 araI*$^+$	261.2	<1.0	>100
UP1027	None	*araA*$^+$ *araB24 araI*$^+$	94.2	<1.0	>100
UP1004	None	*araA*$^+$ *araB*$^+$ *araI*$^+$	66.6	73.9	0.9

[a] Steady-state levels of arabinose operon enzymes were determined after two to three generations of growth in mineral salts-casein hydrolysate plus 2.6×10^{-2} M L-arabinose, at 37°C, as described in Materials and Methods.

can be described quantitatively by a value T_m, the temperature at which half the binary complexes of RNA polymerase and DNA are in the open state. Transcription at a temperature below T_m is assumed to be quite slow for illustrative purposes. It has been calculated that a promoter mutation in which one adenosine-ribosylthymine base pair is replaced by a guanine-cytosine base pair would have the overall effect of raising the T_m by 10 to 20°C, which might exceed the physiological temperature range, thus effectively closing the promoter (3). Mutation to the "cold-sensitive" partial revertant phenotype may have raised the T_m of the *ara* initiator (promoter) region, resulting in a marked reduction in initiator of transcription at 28°C. Alternatively, the partially defective initiator region could raise the temperature optimum of some other event associated with the initiation of *ara* operon expression. It may well be that a "cold-sensitive" phenotype with regard to operon expression will be characteristic of partially defective promoters, just as "heat sensitivity" is often used to characterize mutationally altered proteins.

The "cold-sensitive" phenotype of the partial revertant mutants was evident at two distinct, but related, physiological levels. First, the generation times of partial revertant strains increased much more rapidly than those of full revertant strains or wild-type strains when the growth temperature was decreased from 37°C to 28°C. This indicates that partial revertant strains utilize L-arabinose with lower relative efficiency at 28°C than do full revertant strains or wild-type strains. Second, the rate of *ara* operon expression in partial revertant strains, unlike that in full revertant strains, immediately increased when the growth temperature was raised from 28 to 42°C. Such a result would be consistent with a base-pair mutation that has altered the T_m of a promoter region for initiation of transcription.

Merodiploid analysis was used to establish the *cis*-acting nature of the partial reversion mutations derived from strain ME1098 (*araI1036*). In a merodiploid of the type F *araA*$^+$ *araB*$^+$ *araI1036*/*araA*$^-$ *araB*$^+$ *araI1036 r*, the *araA*$^+$ gene adjacent to the *araI1036* mutation was not expressed when an *araI1036 r* mutation was placed in the *trans* position. This indicates that the *araI1036 r* mutation does not specify a diffusible product that can initiate transcription of the *ara* operon at an *araI1036* initiator region in the *trans* position. This formally demonstrates the *trans*-recessive nature of the *araI1036 pr* mutations.

A second test, with a merodiploid of type F *araA*$^+$ *araB24 araI*$^+$/*araA54 araB*$^+$ *I1036 r* showed that the *araA*$^+$ gene, whose expression was directed by *araI*$^+$, produced wild-type levels of L-arabinose isomerase, whereas the *araB*$^+$ gene, under the control of the *araI1036 r* initiator, produced levels of L-ribulokinase characteristic of each *araI1036* revertant. This indicates that each of the two *ara* operons in this merodiploid are under the independent control of their distinct initiator regions. This

formally demonstrates the *cis*-dominant nature of the partially defective initiator regions.

The partial revertants described in this study represent an additional class of alterations of the *araI* region that result in reduced expression of the *ara* operon. Current investigations are oriented toward obtaining and characterizing mutants that will facilitate the identification of regions in *araI* responsible for interacting with RNA polymerase, *araC* gene protein, and presumably catabolite activator protein.

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