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Differentiation of *arcA*, *arcB*, and *cpxA* Mutant Phenotypes of *Escherichia coli* by Sex Pilus Formation and Enzyme Regulation

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In *Escherichia coli*, mutations in *arcA* (*dye*) or *arcB* anaerobically derepress the synthesis of a multitude of enzymes of aerobic function, and mutations in *arcA* or *cpxA* impair F-pilus formation. It is thought that *arcA* encodes a promoter-recognizing protein, whereas *arcB* and *cpxA* encode sensor proteins which interact with the *arcA* product. In this study we found that anaerobic growth of a wild-type F′ strain decreased the synthesis of both the enzymes and the pilus. Although the two *arcA* mutants examined were both anaerobically derepressed in the enzymes and impaired in aerobic pilus formation as expected, one mutant hyperproduced the pilus anaerobically. The two *arcB* mutants examined showed normal pilus formation when grown aerobically. When grown anaerobically they developed more pili than the wild-type strain did when grown aerobically. When a *cpxA* mutant was examined for synthesis of two aerobic enzymes, normal regulation was found. The available data suggest the following. The *arcA* product anaerobically represses certain genes of aerobic function and activates certain genes related to F function. It appears that the *arcB* product senses the redox or energy state; absence of the gene function shifts the *arcA* product to the nonrepressive form for enzyme synthesis for aerobic pathways. The *cpxA* product, on the other hand, senses the sexual state; absence of the gene function shifts the *arcA* product to the inactive form for F-pilus synthesis.

In *Escherichia coli*, mutations in *arcA* (also referred to as *cpxC*, *dye*, *fexA*, *msp*, *seg*, or *sfrA*) at min 0 of the chromosomal map (2, 9) can cause extensive phenotypic changes. These include impairment of sex pilus synthesis by F^+ or Hfr cells, making them resistant to male-specific bacteriophages which use the pilus for attachment (7, 9), and anaerobic derepression of the synthesis of a set of enzymes that function aerobically, e.g., several primary dehydrogenases, the cytochrome *o* complex, and certain enzymes of the citric acid cycle, the glyoxylate shunt, and the pathway for β-oxidation of fatty acids (6). Since the numerous target genes are under different specific regulation, we proposed the term *arc* modulon to describe the set of operons and regulons under transcriptional control of the *arcA* regulatory gene (6).

In a review of the highly conserved two-component signal transduction systems in bacteria, in which one gene encodes a regulatory protein that recognizes promoters and the other gene encodes a sensor protein that perceives a stimulus and transmits the effect to the regulatory protein, the *arcA* (*dye*) regulatory gene was paired with the *cpxA* sensory gene. The pairing was based on the homology of amino acid sequence with parallel signal transduction systems and on the finding that mutations in both *arcA* and *cpxA* affected the expression of F-plasmid function (8).

Recently we discovered an *arcB* gene (min 69.5). Mutations in this gene gave a profile of enzyme derepression similar to that given by mutations in *arcA*. The ability of a high dose of $arcA^+$ to override the phenotype of an *arcB* mutation suggests that the *arcB* product functions also as a sensor protein for the *arcA* product (4).

In this work we tried to distinguish the phenotypes of *arcA*, *arcB*, and *cpxA* mutants in order to define further the roles of the three genes. Since it is already known that *arcB* mutations derepress the aerobic enzymes and that *cpxA* mutations interfere with the expression of F-plasmid function, we focused instead on the questions of whether *arcB* mutations inhibit F expression and whether a *cpxA* mutation causes enzyme derepression. The strains used are described in Table 1. All cultures were grown at 37°C.

The male-specific phage MS2 produced essentially clear plaques (typically with haloes) on agar seeded with an F′ strain but produced no plaques with an F^- strain (Fig. 1). The isogenic *arcA1* mutant gave barely visible turbid plaques. Although the sex phenotype of this mutant is leaky, its aerobic enzymes were previously shown to be fully derepressed anaerobically (6). The *arcA2* mutant gave no plaques. Both the *arcB1* and the *arcB2* mutants gave haloed plaques which were less clear than those given by the wild-type strain. An F′ *arcB*-deletion strain (ECL593, which acquired the F′ plasmid from K603), however, did give plaques resembling those given by the wild-type strains (data not shown).

Since plaque formation can be influenced by complex factors, including host metabolism, we examined by electron microscopy the presence of sex pili on wild-type and mutant cells grown under aerobic or anaerobic conditions. The pilus was identified by decoration with phage MS2 (Fig. 2).

The percentage of aerobically or anaerobically grown cells

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TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype	Derivation	Source or reference
JC411[a]	F⁻ *arcB6 cpxB1 argG6 fhuA2 gal-6 galP63*? *hisG1 lacY1 leuB6 mtlA-2 malT1*(λ^r) *rfbD1*? *rpsL104 supE44 tonA2 xyl-7 metB1*		B. J. Bachmann
JC411-1	F⁻ *arcB*$^+$ *zgi*::Tn*10 cpxB1* (otherwise as in JC411)	*arcB*$^+$ transduced from ECL592 to JC411 by linked Tn*10*[b]	This study
AE1010	Hfr *arcB6 cpxB1 thyA* (otherwise as in JC411)		7
AE1019[a]	Hfr *arcB6 cpxA2 cpxB1 met*B$^+$ (otherwise as in AE1010)		7
AE1019-1	Hfr *arcB*$^+$ *zgi*::Tn*10 cpxA2 cpxB1* (otherwise as in AE1019)	*arcB*$^+$ transduced from ECL592 to AE1019 by the linked Tn*10*[b]	This study
K603	*ara-14 galK2 galT22 leuB6 mtl-1 lacY1 supE44 xyl-5 thi-1 thr-1 trpE63* (F1-10 [F1:: Tn*10*])		B. J. Bachmann
71-18	Δ(*lac-proAB*)*XIII supE44 thiA* (F′ *proA*$^+$*B*$^+$ *lacI*^{q} Δ*lacZM15*)		11
ECL547	F⁻ *sdh*$^+$ Φ(*sdh-lac*) *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 flb-5301 ptsF25* Δ*frd-101*		6
ECL584	F⁻ *arcA*$^+$ near *zjj*::Tn*10* (otherwise as in ECL547)		6
ECL585	F⁻ *arcA1 zjj*::Tn*10* (otherwise as in ECL547)		6
ECL589	F⁻ *arcA2* (otherwise as in ECL547)		This study
ECL591	F⁻ *arcB2* (otherwise as in ECL547)		This study
ECL592	F⁻ *arcB*$^+$ near *zgi*::Tn*10* (otherwise as in ECL547)		4
ECL593	F⁻ Δ(*zgi*::Tn*10-glnF*) (otherwise as in ECL547)		4
ECL594	F⁻ *arcB1 zgi*::Tn*10* (otherwise as in ECL547)		4
ECL599	F⁻ (otherwise as in 71-18)	Curing F′ of strain 71-18	This study
ECL617	F′ *arcA1 zjj*::Tn*10* (otherwise as in 71-18)	*arcA1* transduced from ECL585 to 71-18 by the linked Tn*10*	This study
ECL618	F′ *arcA2 zjj*::Tn*10* (otherwise as in 71-18)	*arcA2* transduced from ECL589 to 71-18 by the linked Tn*10*[c]	This study
ECL619	F′ *arcB1 zgi*::Tn*10* (otherwise as in 71-18)	*arcB1* transduced from ECL594 to 71-18 by the linked Tn*10*	This study
ECL620	F′ *arcB2 zgi*::Tn*10* (otherwise as in 71-18)	*arcB2* transduced from ECL591 to 71-18 by the linked Tn*10*[d]	This study

[a] Strains JC411 and AE1019 were sensitive to growth inhibition by toluidine blue. Introduction of *arcB*$^+$, but not *arcA*$^+$, into these dye-sensitive strains conferred dye resistance and restored the anaerobic repressibility of two indicator enzymes, aconitase and L-lactate dehydrogenase. Since AE1019 was derived from JC411, it is inferred that both strains harbored the same mutation, which we designate *arcB6*.

[b] Strain ECL592 was the Tn*10* donor; Tc^r transductants were selected on LB agar containing 10 μg of tetracycline per ml (3) and scored on tryptone agar containing 0.2 mg of toluidine blue per ml.

[c] Tn*10* was originally from strain ECL584.

[d] Tn*10* was originally from strain ECL592.

TABLE 2. F-pilus production in parent and mutant strains[a]

Strain	Genotype	% of cells with F pili		No. of F pili/cell (mean ± SE)	
		$+O_2$	$-O_2$	$+O_2$	$-O_2$
71-18	F′ *arcA*$^+$ *arcB*$^+$	80	34	1.0 ± 0.1	0.56 ± 0.13
ECL599	F$^-$ *arcA*$^+$ *arcB*$^+$	0	0	0	0
ECL617	F′ *arcA1* *arcB*$^+$	18	80	0.24 ± 0.12	1.7 ± 0.23
ECL618	F′ *arcA2* *arcB*$^+$	0	0	0	0
ECL619	F′ *arcA*$^+$ *arcB1*	66	78	1.2 ± 0.12	1.9 ± 0.25
ECL620	F′ *arcA*$^+$ *arcB2*	64	72	0.84 ± 0.10	1.5 ± 0.18

[a] Cells were grown on glucose minimal medium (5) aerobically ($+O_2$) or anaerobically ($-O_2$) and examined according the procedure given in the legend to Fig. 2. For each strain 50 cells were randomly examined.

TABLE 3. Aconitase and L-lactate dehydrogenase in various strains

Strain	Genotype	Sp act (nmol/min per mg of protein)[a]	
		Aconitase	L-Lactate dehydrogenase
ECL547	*arcB*$^+$	16	0.6
ECL594	*arcB1*	94	7.6
JC411-1	*arcB*$^+$ *cpxB1*	28	2.7
JC411	*arcB6* *cpxB1*	140	17.0
AE1019-1	*arcB*$^+$ *cpxA2* *cpxB1*	20	2.0
AE1019	*arcB6* *cpxA2* *cpxB1*	75	47.0

[a] Cells were grown anaerobically on 0.5% casein acid hydrolysate–20 mM pyruvate plus 20 mM L-lactate minimal medium (5).

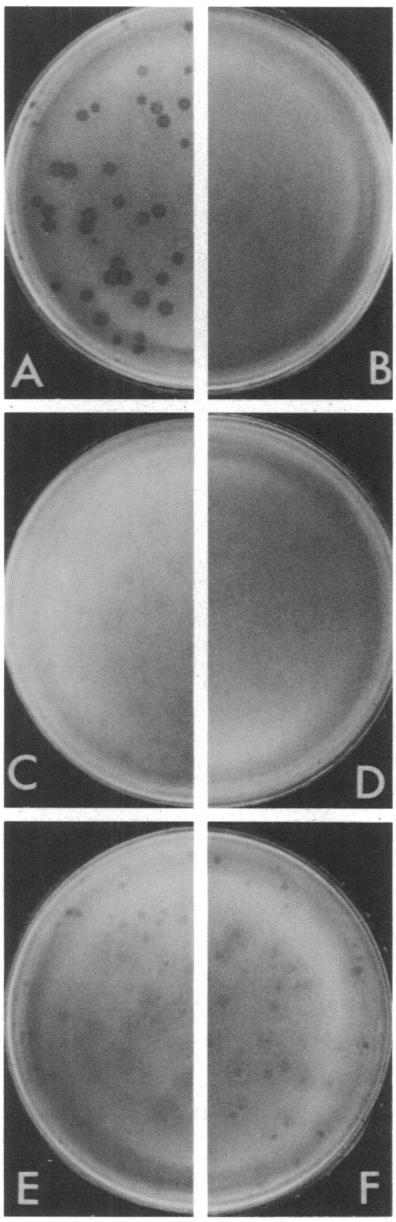

FIG. 1. Plaque formation by phage MS2 on *E. coli* 71-18 (A), ECL599 (B), ECL617 (C), ECL618 (D), ECL619 (E), and ECL620 (F). About 100 PFU of MS2 was incubated with about 10^8 cells for 10 min in 0.1 ml of mineral medium (5) containing 10 mM glucose. The sample was mixed with 1 ml of soft agar prepared with the same glucose medium and poured over hard agar of the same composition in petri dishes (6-cm diameter). The plates were incubated aerobically overnight.

with pili and the average number of pili per cell are given in Table 2. Among the wild-type cells, 80% possessed pili when growth was aerobic, versus 34% when growth was anaerobic. The number of pili per cell was also higher in aerobically grown cells than in anaerobically grown cells. Cells of strain ECL617 (*arcA1*) were pilus deficient when growth was aerobic. Surprisingly, when grown anaerobically they produced more pili per cell than did even wild-type cells grown aerobically. In contrast, ECL618 (*arcA2*) cells did not form pili under either growth condition, like cells of the F$^-$ strain ECL599. ECL619 (*arcB1*) and ECL620 (*arcB2*) cells showed normal pilus formation aerobically. Unexpectedly again, anaerobic growth increased the pilus formation per cell to levels exceeding that of aerobically grown wild-type cells. It appears that in addition to the *arc* and *cpx* products, another respiratory factor can potentially influence pilus formation.

According to Silverman, the extent of the Tra$^-$ phenotype (e.g., pilus formation) of *cpxA* mutations was greatly increased by a *cpxB* mutation, which by itself was not observed to produce a phenotype (7, 9). It might therefore be asked whether the failure to see an *arcB* effect on pilus synthesis was because of the *cpxB*$^+$ background. This possibility seems to be ruled out by a previous report that the *cpxB1* strain AE1010 produced pili (7). During the course of our work it was discovered that this strain inherited an *arcB6* mutation from strain JC411 (see footnote *a* of Table 1).

We then compared the activity levels of aconitase (1) and L-lactate dehydrogenase (6), two representative enzymes specified by genes of the *arc* modulon, in different genetic backgrounds. The *cpxA2* mutation did not affect the enzyme activity levels (Table 3; compare strain JC411-1 [*cpxB1*] with strain AE1019-1 [*cpxB1* *cpxA2*]). In contrast, both the *arcB1* and *arcB6* mutations showed anaerobic derepression of the two enzymes in a wild-type, *cpxB1*, or *cpxB1* *cpxA2* background. A control experiment showed as expected that the *cpxA2* mutation in the *cpxB1* background prevented pilus formation irrespective of the state of *arcB* (data not presented).

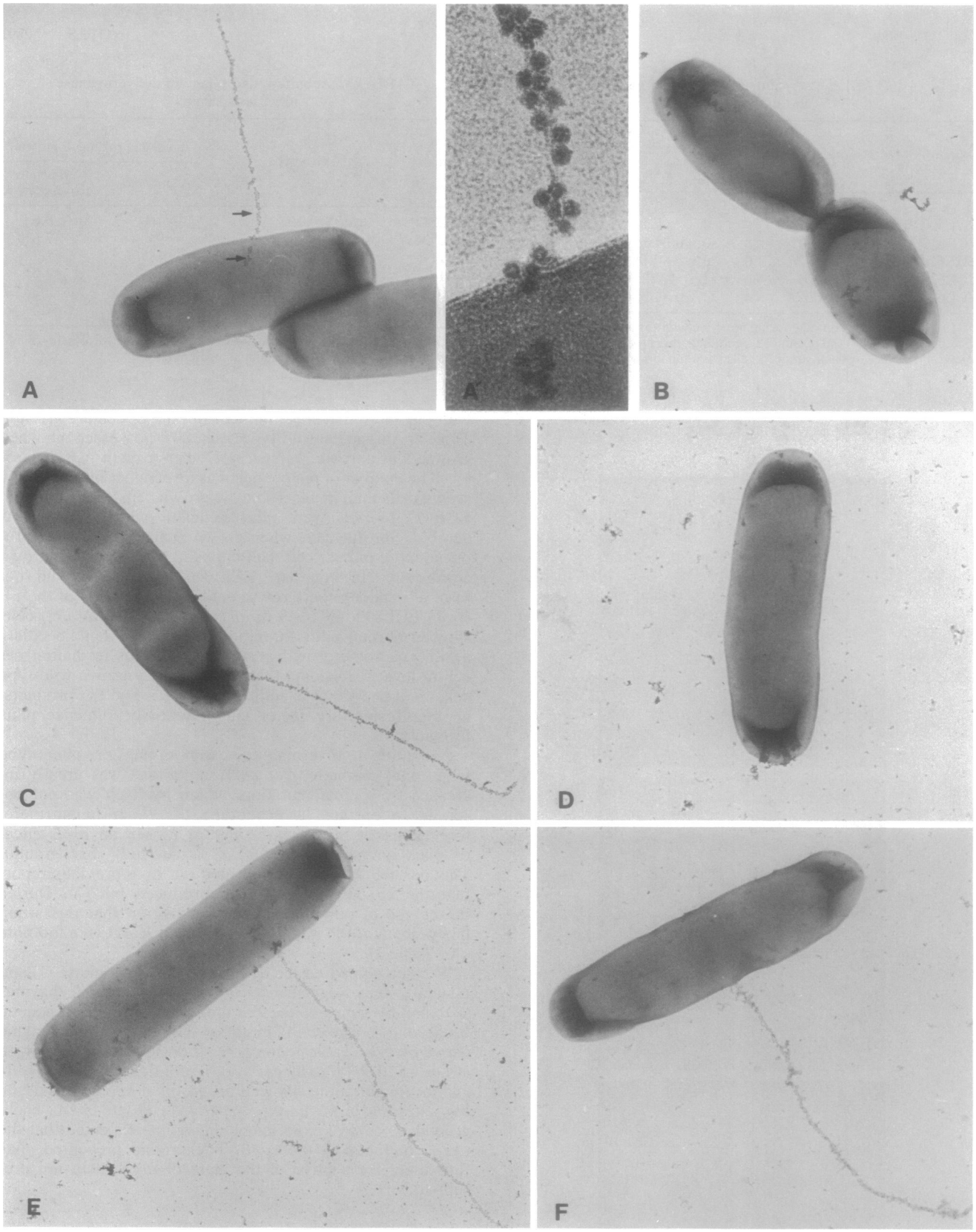

FIG. 2. Electron micrographs of cells of various *E. coli* strains. (A) A cell of the wild-type F′ strain 71-18 with a sex pilus. (A′) Enlargement of the section of panel A vertically demarcated by the arrows. Attached to the pilus are the readily identifiable MS2 phage particles. (B) A typical dividing cell of the wild-type F^- strain ECL599 which is devoid of the pilus. (C) An ECL617 cell (F′ *arcA1*) with a pilus. (D) A typical cell of strain ECL618 (F′ *arcA2*) which is devoid of the pilus. (E) An ECL619 cell (F′ *arcB1*) with a pilus. (F) An ECL620 cell F′ *arcB2*) with a pilus. Negative straining was done by a scaled-down version of the method of Valentine and Green (10), starting with 100-μl samples containing about 3×10^8 bacteria and 3×10^{10} PFU of phage MS2 per ml in 0.1 M 3-(*N*-morpholino)propanesulfonate) at pH 7.6. Samples were treated with 0.5% uranyl formate, and micrographs were taken at a magnification of ×10,000 or ×15,000 on a JEOL 100B electron microscope operated at 80 kV with a 50-μm condenser and objective apertures. Magnifications: panels A through F, ×18,000; panel A′, ×150,000.

Except for the hyperproduction of pili by the *arc* mutants under anaerobic conditions, the results from this and previous studies might be explained by invoking a signal transduction system with three components. The *arcA* product is a pleiotropic regulatory protein which can repress genes of aerobic function and activate genes related to sexual function. The protein can be altered in ways that affect its regulatory activity differently on the two sets of target genes, as in the *arcA1* mutant. The *arcB* product serves as a sensor for the redox or energy state of the cell. Null mutations in *arcB* shift the *arcA* product to the nonrepressive form. The *cpxA* product serves as a sensor for the sexual state of the cell (the gene also has additional host functions which are not yet understood [9]). Null mutations in *cpxA* shift the *arcA* product to the nonactivating form. Thus, the broad phenotypic changes resulting from a nonleaky *arcA* mutation represent the composite of the more limited phenotypic changes caused individually by *arcB* and *cpxA* null mutations.

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