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Synergistic Effect of *himA* and *gyrB* Mutations: Evidence that Him Functions Control Expression of *ilv* and *xyl* Genes

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We have constructed *Escherichia coli* strains containing mutations at two different loci, both originally selected for failure to support λ site-specific recombination: *himA* and *gyrB-him*(Ts). Although the *gyrB-him*(Ts) mutations by themselves reduce supercoiling at high temperature, the double mutants show a far greater effect on supercoiling. Our studies show that growth of phage λ is severely inhibited and that maintenance of plasmid pBR322 is extremely unstable in the double mutants. Physiological studies also reveal that the double mutants are isoleucine auxotrophs at 42°C. The fact that *himA* mutants are isoleucine auxotrophs at 42°C in the presence of leucine suggests that a significant component of the isoleucine auxotrophy of the double mutants is a result of the *himA* mutation. The *himA* gene encodes the α subunit of a protein called the integration host factor. Since mutations in the *hip* or *himD* gene encoding β, the other subunit of the integration host factor, also result in isoleucine auxotrophy in the presence of leucine, we suggest that the integration host factor regulates the synthesis of at least one of the enzymes in the *ilv* pathway, acetohydroxyacid synthase I, which is encoded by the *ilvB* gene. Studies of the utilization of various sugars as the sole carbon source suggest that the integration host factor controls expression of some gene(s) involved in the utilization of xylose.

An essential step in lysogeny by coliphage λ is the integration of the phage genome into the bacterial chromosome. Integration occurs by a site-specific recombination reaction between a unique site on the phage genome (*attP*) and a unique site on the bacterial chromosome (*attB*) (reviewed in references 22 and 27a). This site-specific recombination reaction can proceed in the absence of both the phage Red and host Rec systems for generalized recombination. Instead, λ site-specific recombination is catalyzed by another system that requires both the phage-encoded protein Int and two host-encoded products that make up the integration host factor (IHF). Excision of the prophage from the bacterial chromosome, the physical reversal of integration, requires an additional phage-encoded function, Xis, in addition to Int (11).

The bacterial contribution to λ site-specific recombination, first identified by the in vitro studies of Kikuchi and Nash (14), was shown subsequently to be biologically significant by the isolation of *Escherichia coli* mutants, *him* (host integration mediation) and *hip* (host integration protein), that fail to support either integrative or excisive recombination (17–19, 28). The *him* and *hip* mutations map in three unlinked loci: *himA* (min 38), *himB* (min 82), and *hip* or *himD* (min 21). Because there is some controversy over the nomenclature of the min 21 locus, we have chosen to use both names, *hip*/*himD*, to avoid confusion. In addition to failing to support site-specific recombination, these host mutants share a second phenotype, failure to support growth of bacteriophage Mu (18, 19, 28, 30).

Two-dimensional protein gel analysis has been used to demonstrate that the *himA* and *hip*/*himD* genes encode the two subunits of IHF, a 20,000-dalton protein. The α subunit (10,500 daltons) is encoded by the *himA* gene (21), and the β subunit (9,500 daltons) is encoded by the *hip*/*himD* gene (20). DNA sequence analysis of the *himA* gene (H. Miller, personal communication) and *hip*/*himD* gene (E. Flamm and R. Weisberg, personal communication) confirm these designations.

IHF is known to bind to DNA, and DNA footprinting experiments have led to the identification of a consensus IHF binding sequence (N. Craig and H. Nash, personal communication). Studies with *himA* and *hip*/*himD* mutants suggest that IHF can regulate expression of certain genes at the level of translation (12). Although IHF may play a role in *E. coli* physiology, the complete protein is clearly not an essential function because deletions of the *himA* gene are not lethal to the bacteria (H. Miller, personal communication).

As discussed in the accompanying paper (5), one class of mutations (originally called *himB*) causing a Him$^-$ phenotype map in the *gyrB* gene. The *gyrB* gene encodes one of the subunits of DNA gyrase, an enzyme that converts circular double-stranded DNA to a negatively supercoiled form (8, 9). A number of studies have shown that superhelical DNA is required for efficient λ site-specific recombination (1, 15). We have isolated two *gyrB* mutations that exhibit a temperature-sensitive Him phenotype and are therefore named *gyrB-him*(Ts) and given the numbers 230 and 231 (5). Bacteria with these mutations show reduced site-specific recombination as well as a failure to support bacteriophage Mu growth at 42°C, but are normal for these processes at 32°C.

To assess the possibility of some type of interaction, direct or indirect, between DNA gyrase and IHF, we have constructed the double *himA*/*gyrB-him*(Ts) and *himD*/*gyrB-him*(Ts) mutants. We report studies characterizing these double mutants. Although we have focused our studies on the Δ*himA82*/*gyrB-him*(Ts) mutants, in some cases we have included data on other *him* combinations.

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MATERIALS AND METHODS

Construction of λ lysogens of Him⁻ strains. A lawn of the bacterial strain being lysogenized was poured on an LB plate. A mixture of equal amounts of λ^+ and a λ *himA*$^+$ transducing phage was spotted on the lawn. The next day bacteria were scraped from the zone of lysis on the plates and transferred with a sterile loop to an EMBO plate (10) spread with 10^9 λ *c*I60 phage to select for lysogens. Lysogens form pink colonies on these selective plates. Such colonies were obtained and tested for both λ lysogeny and maintenance of the appropriate Him phenotype. Bacteria fitting these criteria were isolated and purified.

Strains. K1299, a derivative of K37 that has a Tn*10* element from one end of which a deletion extends into the *himA* gene, and K1926, a derivative of K37 with the *himD74* mutation, were obtained from H. Miller. λ *himA*$^+$ was obtained from S. Adhya. All other bacterial and phage strains are described in the accompanying paper (5).

EOP. For measurement of efficiency of plating (EOP), see the accompanying paper (5).

Construction of Δ*himA82* and *himD74*/*gyrB-him*(Ts) double mutants. P1 lysates were prepared on donor strains that had Tn10 insertions adjacent to the marker being selected. With standard P1 transduction procedures (16) the Tn*10* and surrounding genetic material were transferred to recipients, and Tet^r transductants were tested for the appropriate phenotype, Cou^r and failure to support growth of phage Mu at 32°C.

Media. TB, LB, LBMM, and minimal M9 media as well as TMG buffer were described previously (18). For M9 minimal plates, sugars were added to give a final concentration of 0.2%, and amino acids were added to give a final concentration of 20 μg/ml.

Measurement of in vivo λ DNA supercoiling. See the accompanying paper (5).

RESULTS

Conditional auxotrophy for isoleucine. The effect of the Him mutations on bacterial growth is most dramatically illustrated when growth is tested on minimal plates at various temperatures. As shown in Table 1, the double *gyrB-him*(Ts)*himA* mutants are auxotrophic at 42°C, but not at 32°C. This auxotrophy is seen only at temperatures above 40°C (data not shown) and is relieved by the addition of isoleucine.

This requirement for isoleucine at high temperature is not limited to these particular combinations of Him mutations. The same requirement is observed when other mutations in either of the genes encoding the two subunits of IHF are combined with *gyrB-himB*(Ts) mutations. This is true when *himA42*, a point mutation, or *himD74* is combined with either *gyrB-him-230*(Ts) or *gyrB-him-231*(Ts) (data not shown). Other combinations of Him mutations have not been tested.

Although the *himA* (as well as the *hip*/*himD*) mutants by themselves are not isoleucine auxotrophs, the addition of leucine to the medium imposes a requirement for isoleucine (Table 1). This leucine-imposed auxotrophy is also temperature dependent and is only seen at higher temperatures (42°C). Data for the *himA* mutant only are shown in Table 1. At 40°C *himA* (and *hip*/*himD*) mutants grow well on minimal medium in the presence of leucine (data not shown). Leucine is known to repress the synthesis of one of a pair of enzymes required for isoleucine biosynthesis, acetohydroxyacid synthase (AHAS) III, encoded by the *ilvH* and *ilvI* genes (2). As argued below, this suggests that the block in the *himA*-*hip*/*himD* mutants can best be explained as a reduction in the *ilvB* gene expression encoding the other isoenzyme normally expressed in K12 strains, AHAS I.

Sugar utilization. Previous studies with inhibitors of DNA gyrase (24) suggested that the degree of supercoiling influ-

TABLE 1. Growth of Him mutants on various sugars with and without isoleucine[a]

Temp (°C)	Carbon source	Ile	Leu	Growth of bacteria				
				K1299 Δ*himA82*	K1870 *gyrB-him-230*(Ts)	K1871 *gyrB-him-231*(Ts)	K1942 Δ*himA82* *gyrB-him-230*(Ts)	K1943 Δ*himA82* *gyrB-him-231*(Ts)
32	Glucose	−	−	+	+	+	+	+
42	Glucose	−	−	+	+	+	−	−
32	Glucose	+	−	+	+	+	+	+
42	Glucose	+	−	+	+	+	+	+
32	Glucose	−	+	+	+	+	+	+
42	Glocose	−	+	−	+	+	−	−
32	Glucose	+	+	+	+	+	+	+
42	Glucose	+	+	+	+	+	+	+
32	Galactose	+	−	+	+	+	+	+
42	Galactose	+	−	+	+	+	+	+
32	Maltose	+	−	+	+	+	+	+
42	Maltose	+	−	+	+	+	±	±
32	Ribose	+	−	+	+	+	+	+
42	Ribose	+	−	+	+	+	±	±
32	Arabinose	+	−	+	+	+	+	+
42	Arabinose	+	−	±	±	±	±	±
32	Xylose	+	−	+	+	+	+	+
42	Xylose	+	−	+	+	+	−	−

[a] Bacteria were grown on minimal agar plates. A small sample of bacteria was removed from a single colony with a sterile toothpick and suspended in a drop of TMG buffer. A sterile loop was used to transfer some of the suspension to each of the agar plates containing M9 salts and 0.2% of the indicated sugar listed in the Table. Plates were then incubated for 48 h at the indicated temperatures. Growth of the various mutants was compared with that of the K37 Him$^+$ parental strain. +, Good growth; ±, poor growth; −, no growth. Also indicated is whether isoleucine or leucine at 20 μg/ml or both are included.

ences the expression of some catabolite-sensitive genes. This led us to test the ability of the Him mutants to utilize a variety of sugars as the sole carbon source. A number of Him mutants and the isogenic parental strain, K37, were tested for the ability to utilize a variety of sugars as the sole carbon source (Table 1). At low temperature no significant differences were observed. All of the single and double Him mutants tested grew as well as the Him$^+$ control with each of the sugars. On the other hand, at 42°C, some differences were observed. Poorer growth for the double mutants, K1942 and K1943, was observed when the carbon source was either maltose or ribose. The single *himA* mutant, K1299, and the single *gyrB*-Him*ts* strains, K1870 and K1871, as well as the double mutants exhibited poorer growth on arabinose.

The most striking findings were those with xylose as the sole carbon source. All single mutants grew well at both temperatures. However, the double mutants, which utilize xylose effectively at lower temperatures, failed to grow on that sugar at 42°C.

Effect of *himA* mutation on growth rate of *gyrB-him*(Ts) mutants. To determine whether there was any other significant effect on bacterial growth imposed by the presence of both the Δ*himA82* and *gyrB-him*(Ts) mutations, we observed the growth rate of the mutants at high and low temperature in rich and minimal medium. When isoleucine is included in the medium and glucose is the carbon source, there is little difference between the growth of the double mutants, the parental single mutants and wild-type controls (data not shown).

λ growth. Previous studies have shown that hosts carrying either *himA* (19) or *gyrB-him*(Ts) mutations (5) show impairment in the ability to support growth of λ. In *himA* mutants, this impairment is best observed in the case of certain mutants of λ that interfere with the normal transcription of an essential operon; e.g., λ *cin*, which causes an enhancement of transcription termination (23) in the p_R operon. In the *gyrB-him*(Ts) strain the impairment is observed as a reduced burst size for what are essentially wild-type λ derivatives (5).

Growth of λ in the double *himA*/*gyrB-him*(Ts) mutants was studied by using EOP as a measure of the ability of a host to propagate the phage (Table 2). At 32°C the EOP was only slightly reduced in the various Him mutants; 80% of the control for the single mutants and 50 to 60% for the doubles. At 42°C the EOP in the two double mutants was $<10^{-6}$, whereas the EOP for the single mutants was not significantly changed from that seen at 32°C. Noticeably smaller plaques were observed on lawns formed from the Δ*himA82* mutant at both low and high temperatures. This small plaque size is reflected in a reduced burst size (data not shown). Smaller plaques were also observed at 32°C on lawns of the double mutants.

TABLE 3. Maintenance of pBR322[a]

Host	Relevant genotype	Fraction of cells with plasmid Amp^r/total cells	
		32°C	42°C
K37	*him*$^+$	1.0	1.0
K1299	Δ*himA82*	1.1	1.0
K1870	*gyrB-him-230*(Ts)	1.0	0.08
K1871	*gyrB-him-231*(Ts)	0.9	0.20
K1942	Δ*himA82*/*gyrB-him-230*(Ts)	0.2	0.01
K1943	Δ*himA82*/*gyrB-him-231*(Ts)	0.8	0.03

[a] Bacteria were first grown at 32°C in LB-ampicillin (30 μg/ml) to maintain the plasmid, and then samples were subcultured into LB broth and incubated with shaking overnight at 32 and 42°C. This permitted about eight generations of growth. The fraction of plasmid-containing Amp^r bacteria was determined by comparing bacterial titers on LB and LB-ampicillin places.

Plasmid maintenance. In the accompanying paper (5), we show that in hosts with *gyrB-him*(Ts) mutations, maintenance of both an F′ *gal* and pBR322 is significantly reduced at high, but not low, temperature. We therefore extended our studies of the double mutants by determining whether there was any synergistic effect of *himA* and *hip*/*himD* mutations on plasmid maintenance in the presence of the *gyrB-him*(Ts) mutations. Employing the methods for studying plasmid maintenance described in the accompanying paper (5), we measured the maintenance of both pBR322 and F′ *gal* in the various Him mutants.

First we examined the maintenance of pBR322 (Table 3). In these experiments plasmid-carrying bacteria were diluted into fresh medium and grown for about eight generations at the appropriate temperature. The major effect of the *him* mutations was observed at 42°C. Both the Him$^+$ parental and Δ*himA82* mutant exhibited no significant loss of the plasmid. As shown in the accompanying paper (5), 80 to 90% of the *gyrB-him*(Ts) mutants are cured of the pBR322 when bacteria are grown at 42°C. In the double mutants the curing goes up to 97 to 99% at 42°C. Even at 32°C there is some loss of the plasmid; in particular the combination with the *gyrB-him-230*(Ts) mutation appears to show less plasmid stability. This observation reflects a consistently greater loss of pBR322 in hosts with the *him230*(Ts) mutation compared with those with the *him-231*(Ts) mutation. This is true, to a greater or lesser extent, at both temperatures and in hosts with either single or double *him* mutations. Control experiments outlined in the preceding paper (5) show that we are indeed observing curing and not just failure to express Amp^r at high temperature.

TABLE 2. EOP of λ on Him mutants[a]

Temp (°C)	EOP of Bacteria					
	K37 *him*$^+$	K1299 Δ*himA82*	K1870 *gyrB-him*-230(Ts)	K1871 *gyrB-him-231*(Ts)	K1942 Δ*himA82* *gyrB-him-230*(Ts)	K1943 Δ*himA82* *gyrB-him-231*(Ts)
32	1	0.8[b]	0.8	0.8	0.5[b]	0.6[b]
42	1	1[b]	0.7	0.9	$<10^{-6}$	$<10^{-6}$

[a] Dilutions of a lysate of λcI60 (a derivative of λ that has a mutation in the gene encoding the repressor) were plated with 2 drops of a fresh culture of the bacterial strain being tested on TB plates. Bacteria were grown in LBMM. The EOP was determined by dividing the titer of the phage on a lawn of the bacterium being tested by the titer on the K37 (Him$^+$) control.

[b] Small plaques were formed.

Similar studies with derivatives containing F′ *gal* revealed no significant differences in the loss of the plasmid from the single *gyrB-him*(Ts) mutants (5) compared to the double Δ*himA82*/*gyrB-him*(Ts) mutants (data not shown). In this case curing was assayed by determining the number of Gal⁻ colonies.

Effect of *himA* mutation on supercoiling of DNA. Supercoiling of λ DNA in vivo was measured in the Δ*himA82*/*gyrB-him-230*(Ts) double mutant at 42°C 15 min after infection. As shown in the accompanying paper (5), in the *gyrB-him*(Ts) mutant alone λ supercoiling is considerably slowed, being grossly defective at 5 min after infection. By 15 min after infection, the bulk of the DNA in the single mutant has nevertheless become supercoiled (Fig. 1c). Supercoiling in the double mutant is much slower; after 15 min the DNA is still almost fully relaxed (Fig. 1d). Taking these results as an approximate measure of in vivo gyrase activity, we conclude that the level of DNA gyrase is considerably lower in the double mutant than in the *gyrB-him*(Ts) mutant alone.

DISCUSSION

We have reported studies characterizing the effects of combining two classes of *him* mutations in *E. coli* K12. One class of mutations, primarily represented in our studies by *himA* mutations, is in either of two genes encoding the subunits of IHF. The *himA* gene encodes the α subunit (21), and the *hip*/*himD* gene encodes the β subunit (20). The second class of mutations is in the *gyrB* gene, which encodes the B subunit of DNA gyrase (8, 9).

In the accompanying paper (5), we demonstrate that only a special class of *gyrB* mutations, referred to as *gyrB-him*, influences site-specific recombination. Moreover, two of the mutations that we have isolated are temperature sensitive, exhibiting a Him⁻ phenotype at 42°C, but not at 32°C. Although the *gyrB-him*(Ts) mutants reduce supercoiling of an infecting λ genome, we show in this paper that there is a substantially greater effect on supercoiling in the double Δ*himA82*/*gyrB-230*(Ts) mutant. In fact, 15 min after infection at 42°C there is essentially no supercoiling of DNA in the double mutant, whereas there is substantial supercoiling in the Δ*himA82* single mutant and only a slight reduction in the *gyrB-him-230*(Ts) single mutant.

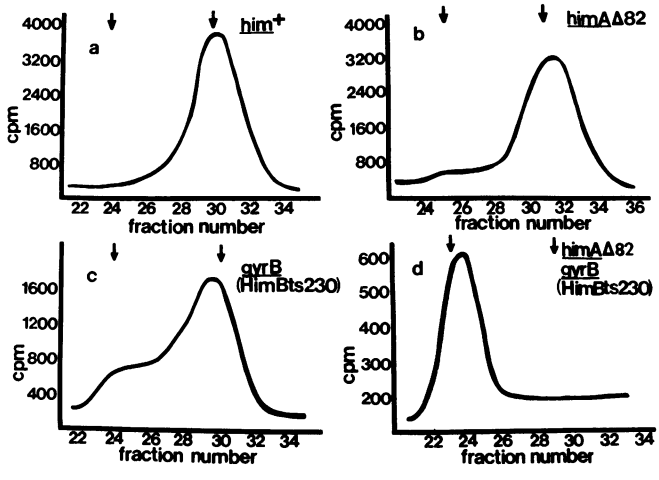

FIG. 1. CsCl-ethidium bromide gradient of λ DNA isolated after infection. All bacteria were λ lysogens. Lysogens were constructed as outlined in the text. Lysogens were infected with λ and incubated for 15 min at 40°C, and then the DNA was isolated and analyzed by the methods presented in the accompanying paper (5).

To what can we ascribe this profound effect on supercoiling of combining the *himA* and *gyrB-him*(Ts) mutations? One possibility is suggested by the studies (7) indicating that in the presence of the Δ*himA82* mutation the rate of synthesis of *gyrA* gene product is reduced fourfold. According to this explanation, the reduction in gyrase activity caused by the defect in GyrB is compounded by the reduction in amount of the GyrA subunit. A second, but not mutually exclusive, possibility is based on the fact that IHF (in part the product of *himA*) is a DNA-binding protein (N. Craig and H. Nash, personal communication). IHF, through its DNA-binding activity, might facilitate action of DNA gyrase; therefore in a *himA* mutant, gyrase might fail to act at certain sites on the DNA. According to this explanation IHF might be one of a number of proteins that could act as pilots directing gyrase to specific sites. Thus, in a mutant defective in IHF synthesis, one set of activities dependent on gyrase would be lost, whereas others would be relatively unaffected. Regardless of the mechanism underlying the severe reduction of supercoiling in the double mutants, our studies clearly delineate a number of activities severely restricted in the double mutants.

Many of the activities selectively reduced at high temperature in the double mutants have previously been shown to be sensitive to interference with DNA gyrase activity, e.g., plasmid maintenance (5, 29) and expression of certain genes (13, 24, 27). The fact that both maintenance of pBR322 and expression of some genes are reduced in the double mutants at high temperature is consistent with the temperature-sensitive nature of the component *gyrB* mutations. However, the finding that the double mutants are isoleucine auxotrophs and unable to utilize xylose at high temperature was unexpected. The isoleucine auxotrophy clearly represents at least in part a primary defect imposed by the *himA* mutation. We find that at 42°C single *himA* mutants are isoleucine auxotrophs in the presence of leucine.

Because we find a similar defect in the presence of a *hip*/*himD* mutation, we propose that the defect is due to an absence of IHF, whose two subunits are encoded by the *himA* and *hip*/*himD* genes. To consider these findings, it is necessary to briefly summarize certain aspects of the regulation of isoleucine biosynthesis (reviewed in reference 2). The critical step for our discussion is the synthesis of α-acetohydroxy butyrate from α-ketobutyrate. This reaction is catalyzed by AHAS. In *E. coli* K12 there are two AHAS isoenzymes; AHAS I, encoded by *ilvB*, and AHAS III, encoded by *ilvH* and *ilvI*. Although AHAS I and III are isoenzymes, AHAS III is specialized for synthesis of isoleucine (2). In minimal medium leucine represses the expression of AHAS III. However, under normal conditions the bacterium can still synthesize isoleucine by using AHAS I. Thus, we conclude that the inability of the *himA* or *hip*/*himD* mutants to grow at 42°C in the presence of leucine suggests that expression of *ilvB* must be reduced. It follows, then, that failure of the double mutant to synthesize isoleucine must also result in a block in this step, and this leads us to conclude that the *gyrB-him*(Ts) mutation limits expression of *ilvH* and *ilvI*, leading to reduction in AHAS III. Considering these observations we suggest that the isoleucine auxotrophy of the double mutant can be explained in the following way; the *himA* or *hip*/*himD* mutation leads to reduced AHAS I synthesis, and the *gyrB-him*(Ts) mutation leads to reduced AHAS III synthesis.

Based on the activities of IHF and gyrase it is possible to

postulate roles for these products in isoleucine biosynthesis. The DNA binding of IHF could regulate gene expression at the level of transcription. On the other hand, IHF has been shown to regulate λ *c*II gene expression at the level of translation (12). Thus, we suggest that the *ilvB* operon is under IHF control. In the case of the *ilvH* and *ilvI* genes, we suggest that the degree of supercoiling is critical in the expression of these genes and that the reduced gyrase activity in the *gyrB-him*(Ts) mutants at 42°C reduces expression of these genes.

One result left unexplained is why isoleucine auxotrophy in the presence of leucine is only seen in the *himA* or *hip/himD* mutants at temperatures at or above 42°C. We offer one possible explanation. The *ilvB* gene is regulated by attenuation (3). It has been argued that transcription termination is more active at higher temperatures (4). If attenuation in the *ilvB* leader is more active at higher temperature, then coupling higher temperature with a *himA* defect could lead to levels of expression of *ilvB* (AHAS I) too low to permit isoleucine synthesis when *ilvH* and *ilvI* expression (AHAS III) is repressed by leucine.

Because little is known about the genes involved in xylose utilization, we cannot propose a specific model to explain the observations on the effect of Him mutations on growth on xylose. Studies with the closely related species *Salmonella typhimurium* show that genes involved xylose utilization are clustered and under positive control (26). The expression of at least one of these genes is regulated by catabolite repression (25). The finding that gyrase plays a role in the expression of xylose utilization genes is consistent with the findings of Sanzey (24) that operons regulated by catabolite repression are sensitive to changes in supercoiling. The strong effect on xylose utilization seen with the double mutant suggests that at least one of the genes encoding a product in the pathway for xylose utilization may be under IHF control. Interestingly, *ilvB* expression is also regulated by cyclic AMP-cyclic AMP receptor protein (3).

In sum, by combining two types of Him mutations, *himA* and *gyrB*, we have constructed conditions that enabled us to uncover heretofore undescribed roles for both gyrase and IHF. Further studies with the double mutants should prove useful in discovering more roles for these gene products.

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