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Multiple Effects of Fis on Integration and the Control of Lysogeny in Phage λ

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Fis is a small, basic, site-specific DNA-binding protein present in *Escherichia coli*. A Fis-binding site (F) has been previously identified in the *attP* recombination site of phage λ (J. F. Thompson, L. Moitoso de Vargas, C. Koch, R. Kahmann, and A. Landy, Cell 50:901–908, 1987). The present study demonstrates that in the absence of the phage-encoded Xis protein, the binding of Fis to F can stimulate integrative recombination and therefore increase the frequency of λ lysogeny in vivo. Additionally, Fis exerts a stimulatory effect on both integration and lysogeny that is independent of binding to the *attP* F site. Maintenance of the lysogenic state also appears to be enhanced in the presence of Fis, as shown by the increased sensitivity of λ prophages encoding temperature-sensitive repressors to partial thermoinduction in a *fis* mutant. In the presence of Xis, however, Fis binding to F interferes with integration by stimulating excision, the competing back-reaction. Since Fis stimulates both excision and integration, depending on the presence or absence of Xis, respectively, we conclude that Xis binding to X1 is the key determinant directing the formation of an excisive complex.

One of the most thoroughly studied genetic regulatory systems is the bacteriophage λ lysis-versus-lysogeny decision. This seemingly simple system remains a paradigm for transcriptional activation and repression and their relationship to developmental pathways. After injecting the phage chromosome into a target cell, the vast majority of infecting λ phage follow a lytic pathway, involving phage replication and cell lysis to release progeny. Sometimes, however, the lytic pathway is bypassed and the lysogenic pathway is followed. In the lysogenic state, the phage genome is integrated into the host chromosome, genes encoding lytic functions are repressed, and the phage remains quiescent in a prophage form until an environmental signal induces a change to the lytic pathway (for recent reviews, see references 6 and 30).

Integration into the host chromosome ensures faithful replication of the lysogenic phage in the absence of phage-encoded replication machinery. Integration is a conservative, site-specific DNA recombination reaction between the 240-bp phage attachment site (*attP*) and a 25-bp target on the bacterial chromosome (*attB*) and is catalyzed by the phage-encoded recombinase Int (for a recent review, see reference 19). Integration requires the formation of a reactive complex, called the intasome (4), that is formed by complex interactions between DNA, Int, and integration host factor (IHF) proteins bound to multiple sites on the supercoiled *attP* (24) (Fig. 1).

The lysogenic state is maintained by repression of lytic functions by the phage-encoded repressor *c*I (15). Other phage and bacterial proteins (such as *c*II, *c*III, Cro, and Hfl) are directly or indirectly involved in the regulation of *c*I and Int expression and are therefore important in the establishment of lysogeny (9). Induction of lytic growth results from the loss of *c*I repression and is accompanied by the precise excision of the phage genome from the host chromosome at the two host-phage junctions, *attL* and *attR*. In addition to Int and IHF, excision requires the binding of the phage-encoded Xis protein (1, 33) and the host protein Fis (2, 32) to a region of *attR*. Fis binds a site called F in *attR* that is immediately adjacent to the X1 Xis-binding site (32). Fis has been shown to stimulate excisive recombination both in vitro (32) and in vivo (2) and is thought to exert its influence by bending the DNA it binds and by enhancing Xis binding to X1.

In this study, we have examined the role of Fis in promoting λ lysogeny. The effect of Fis on lysogeny could be expected to be complex, minimally involving one (or more) of at least three factors. First, Fis may have a direct effect on integration that is mediated through Fis binding at the F site. Some Int- and IHF-binding sites that stimulate excision are also required for integration (P′2, H2, and H′ [19]), so it is not unreasonable to suspect that Fis binding to the F site on *attP* may contribute to the assembly of the intasome. Conversely, binding of Fis to F might distort the intasome structure and inhibit integration. Second, the ability of Fis bound to F to cooperatively stimulate Xis binding to X1 could promote Xis-mediated inhibition of integration. In vitro experiments have demonstrated that binding of Xis to X1 can inhibit the frequency of integrative recombination (1), and Fis bound to F has been shown to enhance Xis binding to X1 (32). Therefore, the binding of Fis might result in a decrease in integrative recombination frequency. Since Fis binding to F stimulates excisive recombination, Fis could also decrease the yield of integration products by promoting the reverse reaction. Third, Fis might affect the expression of regulatory proteins involved in the establishment of λ lysogeny. For example, IHF is known to have a positive effect on the expression of *c*II (17) and the p_L promoter (12), as well as a direct function in the recombination reactions.

The results of this and the accompanying paper (2) indicate that Fis plays a number of roles in lambda biology. These include stimulation of excisive and integrative recombination, as well as establishment and maintenance of lysogeny. The latter effects are mediated at Fis-binding site(s) located outside of *attP*. These complex and sometimes conflicting effects of Fis on λ development suggest that Fis may act as an architectural protein whose functions λ has

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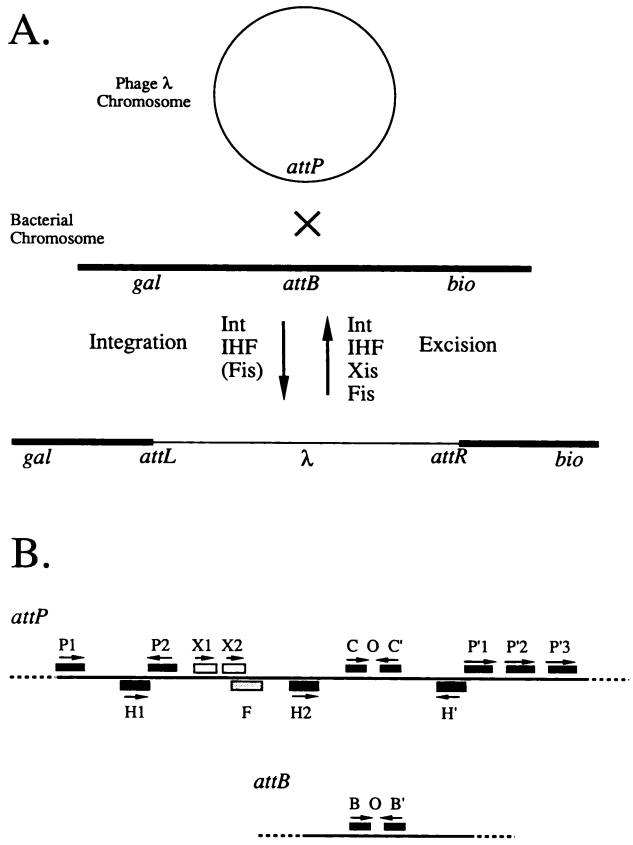

FIG. 1. (A) Schematic representation of λ recombination reactions. Integrative recombination occurs between the attachment site *attP* on the supercoiled phage chromosome and the attachment site *attB* at a specific site on the bacterial chromosome. The recombination event results in the formation of the two phage-host junctions, *attL* and *attR*. This reaction is catalyzed by the Int protein and is stimulated by the binding of IHF and, as shown in the text, Fis. Excision is the result of recombination between *attL* and *attR* to yield *attP* and *attB*. In addition to Int and IHF, excision requires Xis and is strongly stimulated in vivo by Fis. (B) Relative locations of protein-binding sites on *attP* and *attB* as determined by nuclease protection analysis (for a review, see reference 19). Besides binding the core sites that compose the points of strand exchange on both *attP* (C and C') and *attB* (B, and B'), Int binds five sites on flanking *attP* DNA (P1, P2, P'1, P'2, and P'3), as depicted (■). However, integrative recombination requires Int binding only to the P1, P'2, and P'3 flanking sites. In addition to Int, intasome formation requires the binding of the *E. coli* IHF to three accessory sites (▩) in *attP* (H1, H2, and H'). Excision of λ from the bacterial chromosome requires Int, IHF, Fis, and a phage-encoded protein, Xis, that has two adjacent binding sites, X1 and X2 (□). The Fis-binding site, F (▩), overlaps the X2-binding site, and binding of Fis to F mutually excludes Xis binding to X2. Arrows designate the relative orientations of the binding sites.

evolved to use to its advantage in a number of applications, rather that a specific regulator of recombination.

MATERIALS AND METHODS

Cell growth, media, and enzymes. Unless otherwise noted, cells were grown on either solid or liquid LB supplemented with 5 mM $MgCl_2$ as described previously (21). Phage were propagated and lysate titers were determined, using LE392 in T media supplemented with 5 mM $MgCl_2$ (27). Phage lysates were diluted into λCa buffer (10 mM Tris-HCl [pH 7.9], 20 mM $MgSO_4$, 5 mM $CaCl_2$). DNA restriction and modification enzymes used were purchased from Boehringer Mannheim, and antibiotics were obtained from Sigma.

Plasmid pickup assays. Plasmid pickup assays were performed essentially as described previously (10). Fresh overnight cultures of MC1000 or RJ1617 transformed with either pRJ994 or pRJ995 were subcultured into LB and grown at 37°C until to an optical density at 600 nm of 0.3. Then 0.5 ml of phage lysates (λY1063 or λY958) diluted into λCa buffer was added to 0.5 ml of cells at a multiplicity of infection (MOI) of 0.1. Cells were incubated at 37°C for 45 min, and lysates were prepared by adding chloroform to the cultures and pelleting the cell debris. Transductions were performed by using 0.1 ml of an overnight culture of RJ1654 and 0.1 ml of serial dilutions of the resulting phage lysates followed by incubation at 30°C for 60 min. Transductants were selected on LB plates containing 100 μg of ampicillin per ml.

Lysogen formation. Unless otherwise noted, cells were grown to an optical density at 600 nm of 0.3 and were incubated with an equal volume (0.2 ml) of phage diluted into λCa at an MOI of 0.1. After 60 min of incubation at 30°C (for λ*c*I857 phage) or 37°C (for λ*c*I$^+$ phage), 10^9 PFU of λRJ1000 was added to each tube, and the tubes were incubated for an additional 10 min before being placed on ice. Serial dilutions were plated onto LB plates seeded with 10^9 PFU of λRJ1000 and incubated at 30°C. Surviving colonies were scored 20 h after plating.

Strains and plasmids. Table 1 describes the bacterial and phage strains and plasmids used in this study. P1*vir* transduction was used to introduce the *fis*::*767* mutation (18) into different strains.

pJT173 and pJT175 contain the *attB* sequence inserted between the adjacent *Pfl*MI sites and the *attP* sequence inserted between the *Hin*dIII and *Bam*HI sites of pBR327 and were gifts of J. Thompson and A. Landy. In each case, two base changes in *attP* have generated an *Xho*I site that has no effect on in vitro recombination (31). pJT173 contains changes in 13 of 23 nucleotides, resulting in the replacement of the X2 Xis-binding site and the F Fis-binding site with a duplication of the X1 Xis-binding site. In vitro characterization of the *attPF* construct (31) demonstrated that Fis binding was abolished but excisive recombination occurred efficiently at high Xis concentrations.

pRJ994 and pRJ995 were constructed by removing the *attB*-carrying fragment of pJT173 or pJT175, respectively, by digestion with *Bst*EII and *Nru*I. The staggered end generated by *Bst*EII cleavage was filled in with deoxynucleoside triphosphates by using reverse transcriptase, and the plasmid was religated under dilute DNA conditions (26).

λRJ996 carries the *attPF* mutation from pJT173. Construction was initiated by infecting MC1000 transformed with pRJ999 with λY1028 (*int*). λY1028 can become *int*$^+$ if it acquires wild-type *int* sequences by recombining with pRJ999, a derivative of pJT173 whose construction is described below. At some frequency, such a recombination would also include the nearby *attPF* mutation. The resulting lysate was used in a red-plaque assay on galactose-tetrazolium plates to screen for *int*$^+$ recombinant phage (8). Lysates were grown from several red (*int*$^+$) plaques, and λ DNA was isolated by using the Promega λsorb procedure. The *int*$^+$ *attPF* recombinant phage named λRJ996 was identified by the presence of an additional *Nde*I site in *attP*, resulting from the base substitutions that created the *attPF* mutation. pRJ996 also contains the *Xho*I site that is present in pJT173.

TABLE 1. Strains and plasmids used

Strain	Genotype	Source or reference
Bacteria		
MC1000	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK strA*	M. Casabadan
RJ1617	MC1000 *fis*::*767*	18
RJ1654	HfrH, lysogenized with λY1	2
RJ1785	RJ1654 *fis*::*767*	2
LE392	*tonA lacY galK metB trpR hsdR hsdM*$^+$ *supE supF*	L. Enquist
Phages		
λY1	*c*I857	R. Weisberg
λY124	*c*I857 *xis1*	H. Nash
λY958	*c*I857 *int*$^+$ *xis*$^+$ *red3 h*$^+$ *gal49 bio936 attB*	R. Weisberg
λY1063	*c*I857 *int*$^+$ *xis*am6 *red3 h*$^+$ *gal49 bio936 attB*	R. Weisberg
λY1028	*c*I857 *int21*	R. Weisberg
λRJ996	*c*I857 *attPF*	This work
λRJ1000	*c*I *b*221	Laboratory collection
λW4	*c*I*ts*1	S. Gottesman
Plasmids		
pJT173	pBR327 containing *attPF* and *attB* inserts	J. Thompson and A. Landy
pJT175	pBR327 containing *attP* and *attB* inserts	J. Thompson and A. Landy
pRJ994	pJT173 without *attB* insert	This work
pRJ995	pJT175 without *attB* insert	This work
pRJ999	pJT173 with a 1.37-kb *Hin*dIII-*Eco*RI fragment of λ added adjacent to *attPF*	This work

pRJ999 was constructed by cleavage of pJT173 with *Hin*dIII and *Eco*RI and ligation with the 1.37-kb λ fragment generated by *Hin*dIII and *Eco*RI digestion as described elsewhere (26). Addition of this fragment adds a greater length of homology adjacent to *attP*, facilitating recombination of the *attPF* mutation onto λY1028. The 1.37-kb λ fragment was isolated by using Geneclean (Bio 101).

RESULTS

Because Fis binds to *attP* and efficiently stimulates the excisive recombination of phage λ, we wished to examine the role of Fis in the integration reaction in vivo. The initial experiments compared frequencies of lysogeny in isogenic *fis* and *fis*$^+$ cells. The formation of stable λ*c*I857 lysogens, as assayed by λ resistance or by phage-encoded antibiotic resistance, was found to be 4- to over 20-fold greater in the presence of Fis than in its absence. Some of the variability in the differences observed was shown to be dependent upon the bacterial strain, the MOI, and the precise culture conditions used. For example, in one experiment the reduction of lysogeny frequency in the absence of Fis was 11.5-fold with an MOI of 0.1 but only 3.7-fold with an MOI of 5.0. In another experiment, early exponentially growing cells that were starved for 30 min in λCa buffer prior to infection at an MOI of 0.1 gave 40-fold fewer lysogens in *fis* cells. Likewise, when an exponentially growing culture was diluted into fresh medium after phage absorption, the frequency of lysogen formation was increased as much as 50-fold in *fis*$^+$ cells but only 4-fold in *fis* cells. The effects measured in the last two experiments may be attributed to the growth-phase-dependent regulation of *fis* expression. Fis levels are much higher during early exponential growth than during late exponential or stationary phase (2b, 32). Dilution into fresh media or maintenance of early exponentially growing cells in buffer for an extended period after infection will maintain high cellular Fis levels. In the experiments described below, fresh overnight cultures were diluted 1/100 into LB and grown at 37°C for 90 min prior to the adsorption of phage at an MOI of 0.1.

Effects of Fis on integrative recombination in the absence of Xis. Among the ways to specifically measure integrative recombination in vivo is Int-mediated plasmid transduction, or the plasmid pickup assay (10). In this experiment, integrative recombination frequencies between an infecting phage growing lytically, which contains *attB* in the place of the phage attachment site, and an Amp^r plasmid carrying an *attP* were determined by monitoring the frequency of phage-mediated Amp^r transduction. Table 2 shows the averaged results of experiments performed in parallel, in the presence or absence of host-encoded Fis, of phage-encoded Xis, and of an F site within the *attP*. Comparison of the individual results allows the assessment of the relative contributions of the various factors that affect integrative recombination.

Measurements of integrative recombination frequencies

TABLE 2. Measurement of integrative recombination by plasmid pickup assays

Strain	Plasmid	Phage	% Recombination frequency (Amp^r transductants/input phage)[a]
MC1000 (*fis*$^+$)	pRJ995 (*attPF*$^+$)	λY1063 (*xis6*)	10.08
	pRJ994 (*attPF*)	λY1063 (*xis6*)	3.44
RJ1617 (*fis*)	pRJ995 (*attPF*$^+$)	λY1063 (*xis6*)	0.46
	pRJ994 (*attPF*)	λY1063 (*xis6*)	0.40
MC1000 (*fis*$^+$)	pRJ995 (*attPF*$^+$)	λY958 (*xis*$^+$)	0.60
	pRJ994 (*attPF*)	λY958 (*xis*$^+$)	1.17
RJ1617 (*fis*)	pRJ995 (*attPF*$^+$)	λY958 (*xis*$^+$)	0.19
	pRJ994 (*attPF*)	λY958 (*xis*$^+$)	0.15

[a] These results represent the averages of results obtained in three independent experiments performed in parallel.

are most directly assayed in the absence of Xis (with λY1063 encoding the *xis*am6 gene [16]), since potential Xis inhibition of integration, as well as promotion of excision, the reverse reaction, is eliminated. Comparison of the pickup frequencies between the *attP*$^+$ plasmid and λY1063 in *fis*$^+$ cells (10.08%) and *fis* cells (0.46%) shows an overall 22-fold stimulatory effect of the presence of Fis on the integration reaction. This 22-fold effect represents the sum of contributions from Fis binding to F and any effect of Fis exerted elsewhere in the host or phage. These contributions can be separately measured by further examination of the results. The contribution of Fis binding at F can be determined by contrasting the pickup frequency of *attP*$^+$ plasmids (10.08%) and *attPF* plasmids (3.44%) in *fis*$^+$ cells. In this experiment, the lack of the F site reduced integrative recombination about threefold, although effects up to sixfold have been measured in other experiments. An effect of Fis on integration other than that exerted at the F-binding site is suggested by the 8.6-fold difference between pickup frequencies with *attPF* plasmids in *fis*$^+$ cells (3.44%) and in *fis* cells (0.40%). The similarity of the pickup frequencies in *fis* cells, whether the *attP* is normal (0.46%) or lacks a Fis-binding site (0.40%), indicates that *attPF* is as reactive in integrative recombination as *attP*$^+$ is in the absence of Fis. Thus, any differences resulting from the substitution of 13 of 23 nucleotides in the mutant *attP* are negligible in this assay. One can conclude from these comparisons that in the absence of Xis, Fis significantly increases the efficiency of integration both by binding at F and by an additional mechanism.

Effect of Fis on integrative recombination in the presence of Xis. Use of the *xis*$^+$ λY958 in plasmid pickup assays allowed the contributions of the excision reaction and Xis inhibition of integration to be taken into account when examining the role of Fis in integration. The presence of Xis resulted in a large (16.8-fold) inhibitory effect on the integrative recombination reaction as determined by the comparison of pickup frequencies of *attP*$^+$ pRJ995 in *fis*$^+$ cells by either *xis6* (10.08% pickup frequency) or *xis*$^+$ (0.60% pickup frequency) phage. This inhibition by Xis has two potential causes: Xis binding at X1 directly inhibiting the integration reaction, as has been observed in vitro (1), or the presence of Xis allowing integration products to excise, yielding parental molecules. In the absence of Fis, Xis showed a small effect on integrative recombination since recombination frequencies involving *attP*$^+$ with *xis6* phage (0.46% pickup frequency) or *xis*$^+$ phage (0.19% pickup frequency) were not as dissimilar as the 17-fold difference observed in the presence of Fis. A similarly modest effect was seen when an *attPF* plasmid substrate was used in the presence of Fis (1.17% for *xis*$^+$ phage, 3.44% for *xis6* phage) and in its absence (0.15% for *xis*$^+$ phage, 0.40% for *xis6* phage). Thus, Xis has little inhibitory effect on integration in the absence of Fis or its binding site, most probably because it cannot bind efficiently to X1 in vivo without cooperative interactions with Fis.

In the absence of Xis, Fis was shown to have a 8.6-fold stimulatory effect on integration that is independent of binding to F (see above). In the presence of Xis, comparison of pickup of *attPF* pRJ994 in *fis*$^+$ cells (1.17%) and in *fis* cells (0.15%) showed a 7.9-fold difference, similar to the 8.6-fold difference observed in the absence of Xis. The similarity of these values suggests that the *attP* F-binding-independent effect of Fis on integration is of the same magnitude whether or not Xis is present. The overall effect of Fis on integration, as measured by the plasmid pickup assay, must take into consideration all the contributions of Fis binding to F on integration, excision, and Xis-mediated inhibition of integration, as well as the effects independent of F binding. This net effect is revealed in the 3.2-fold ratio between pickup of *attP*$^+$ plasmids by *xis*$^+$ phage in *fis*$^+$ (0.60%) and *fis* (0.19%) cells.

TABLE 3. Lysogen formation in the presence or absence of Fis

Bacterial strain	Phage	% Lysogen frequency (λ^R colonies/input phage)[a]
MC1000 (*fis*$^+$)	λY1 (*attP*$^+$ *xis*$^+$)	0.59
	λRJ996 (*attPF xis*$^+$)	0.35
	λY124 (*attP*$^+$ *xis1*)	3.65
RJ1617 (*fis*)	λY1 (*attP*$^+$ *xis*$^+$)	0.14
	λRJ996 (*attPF xis*$^+$)	0.16
	λY124 (*attP*$^+$ *xis1*)	0.15

[a] These results represent the averages of three independent experiments performed in parallel. λ^R colonies are those able to grow on plates seeded with 10^9 PFU λRJ1000.

The results of the plasmid pickup assay show that Fis plays a complicated role in integrative recombination in vivo. Fis bound at *attP* F appears to enhance integrative recombination, yet also can inhibit it by allowing Xis-promoted inhibition of integration and excision. Additionally, Fis has a positive effect on integration by some means other than binding to *attP* F. We show below that Fis affects the establishment and maintenance of lysogeny in a manner that is independent of the integration reaction. Perhaps the mechanism by which Fis affects lysogeny (see below) also contributes to effects on integration rates. These three effects add up to a stimulatory effect of Fis on integrative recombination as measured by the plasmid pickup assay.

Effect of Fis on lysogen formation. Table 3 gives the results of parallel experiments designed to determine the frequency of lysogen formation in *fis*$^+$ and *fis* cells by using λY1 (*c*I857), λY124 (*xis1*), or λRJ996 (*attPF*) as the infecting phage. As with the plasmid pickup experiment, the use of the different strains and phages allows an assessment of the individual and cumulative contributions of different effects of Fis on the establishment of lysogeny. These include direct effects of Fis on the recombination reaction, as well as effects of Fis elsewhere on the regulatory pathways involved in establishment of lysogeny. In this experiment, the formation of lysogens of *attP*$^+$ *xis*$^+$ phage was reduced fourfold in the absence of Fis.

Effect of Fis on lysogen formation in the absence of Xis. Use of λY124 allows the examination of lysogen formation in the absence of any Xis-mediated integration inhibition or back reaction due to a missense mutation (*xis1*) that inactivates the *xis* gene (14). The contribution of these two effects is revealed in the 6.2-fold ratio between the lysogeny frequencies for λY124 (3.65%) and λY1 (0.59%) in *fis*$^+$ cells. However, no Xis effect is observed in the absence of Fis: lysogeny frequencies in *fis* cells are 0.15% for λY124 and 0.14% for λY1. Comparison of frequencies of lysogen formation by using λY124 in *fis*$^+$ cells (3.65%) and *fis* cells (0.15%) shows a very large (24.3-fold) disparity. This difference may be expected to be a function of either Fis binding to *attP* F or Fis regulation of lysogeny, or some combination of both. Thus, Fis is required not only for the Xis inhibition of integration, as demonstrated by the plasmid pickup assays, but also for the increased frequency of lysogeny observed in the absence of Xis.

To assay the effect of Fis binding to *attP* F in the absence of the back reaction, without changing any regulatory effects of Fis, lysogen formation in *fis*$^+$ cells with either λY124 (this phage has an F site but does not have any Xis binding to X1) or λRJ996 (this phage has no F site in *attP* and therefore is predicted to have little or no bound Xis) can be compared. In *fis*$^+$ cells, λY124 gives a 3.65% lysogeny frequency, 10.4-fold greater than that obtained for λRJ996. As a control, these phages give nearly the same lysogeny frequency in *fis* cells (0.15% for λY124; 0.16% for λRJ996). Therefore, one can conclude that in the absence of any back reaction, Fis binding to *attP* F stimulates lysogen formation about 10-fold.

Lysogen formation in the absence of Fis binding to *attP*. Any contribution of Fis in regulating the establishment of lysogeny that is unrelated to excision or binding at the *attP* F site can be measured by comparing the frequency of lysogeny of λRJ996 in *fis*$^+$ cells with that in *fis* cells. In this experiment, λRJ996 lysogenized twofold better in *fis*$^+$ cells (0.35% lysogeny frequency) than in *fis* cells (0.15% lysogeny frequency). In other experiments, this effect has been as high as 6-fold, and under starvation conditions, differences of over 10-fold are obtained. Since this effect cannot be attributed to the binding of Fis to F, it must reflect a role of Fis elsewhere in λ or the host in lysogen formation. These results are consistent with the plasmid pickup assays described above and the lysogeny challenge experiments detailed below.

From this experiment, one can conclude that Fis plays an important and complex role in the establishment of lysogeny. Under the conditions used in these experiments, Fis bound at *attP* F has an approximately 10-fold positive effect on lysogen formation, presumably by increasing integration efficiency. However, Fis binding to F also allows Xis to reduce lysogen formation sixfold by inhibiting integration and/or promoting excision. Additionally, Fis exerts a two- to over sixfold positive effect on lysogeny that is independent of binding to *attP* F.

Effect of Fis on maintenance of lysogeny. Since the plasmid pickup assays and lysogen formation experiments suggest a role for Fis in the establishment of lysogeny, we measured the effect of Fis on the maintenance of lysogeny. An experiment was performed to determine the resistance of *fis*$^+$ and *fis* strains to partial induction of a λ prophage. Exponentially growing cultures of λY1 (*c*I857) lysogens were switched from 30°C to the semi-inducing temperature of 36.5°C. Failure to maintain lysogeny would result in induction of the prophage and cell lysis. A plot of cell growth is shown in Fig. 2. Although *fis*$^+$ lysogens managed to survive the challenge to lysogeny relatively well, the lysogens in *fis* mutant cells suffered a large loss of viability soon after shifting to the semi-inducing temperature.

The same experiment was performed with λW4 containing the *ts*1 mutant repressor. The *ts*1 mutant repressor requires a significantly higher temperature to denature than does the *c*I857 repressor (20). At 41°C the *fis*$^+$ lysogens grew normally, while the *fis* lysogens induced, resulting in cell lysis (data not shown). Thus, the increased sensitivity to partial induction in *fis* cells can be demonstrated with two different temperature-sensitive repressors. The reduced ability to maintain the lysogenic state is not mediated through the *attP* F site, since *fis*$^+$ lysogens of λRJ996 (*c*I857 *attPF*) are as resistant to lysogeny challenge as are *fis*$^+$ lysogens of λY1 (*c*I857 *attPF*$^+$) lysogens.

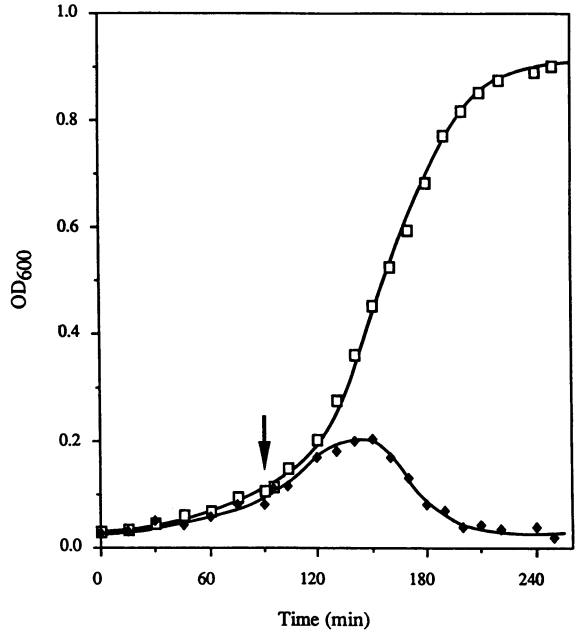

FIG. 2. Lysogeny challenge experiment. Lysogens of λ*c*I857 in *fis* or *fis*$^+$ cells were subcultured 1:100 into LB and grown at 30°C. After 90 min (arrow), the cultures were shifted to the semi-inducing temperature of 36.5°C. Cell growth, as measured by the optical density at 600 nm (OD_{600}), is plotted on the vertical axis, and the time of incubation is plotted on the horizontal axis. Symbols: □, cell density of *fis*$^+$ lysogens; ◆, growth of *fis* cells.

DISCUSSION

There are multiple effects of the Fis protein on λ lysogeny that involve both integrative recombination and the regulation of lysogen establishment and maintenance. The role of Fis in integration is further complicated by the large stimulatory effect of Fis on excision. However, since excision is dependent on the Xis protein (1), we are able to eliminate this effect of Fis by using *xis6* or *xis1* phage. We have directly measured the influence of Fis on integration in vivo by using *fis*$^+$ and *fis* mutant cells, as well as *attP* substrates lacking a Fis-binding site, in two in vivo assays: plasmid pickup and lysogen formation. In the absence of Xis, the presence of Fis increases integration frequencies over 20-fold; part of this increase is a direct effect of Fis binding to F. The mode by which Fis stimulates integration is not understood. Fis-induced DNA bending may facilitate association of distant sites required for the correct assembly of the intasome, as has been described for IHF (13). However, there is currently no in vitro evidence for cooperative binding interactions between Fis and Int or IHF (32).

Efficient in vitro integration reactions were developed in the absence of Fis, and no effect of Fis was observed in vitro when specifically tested (32). The lack of a measurable effect of Fis in vitro may be a function of different relative levels of proteins or subtle differences in substrate characteristics. In the latter, DNA in the cell is normally complexed with proteins and polyamines, causing the superhelical density of purified plasmid DNA to be higher than found in vivo (5, 28). Since λ integrative recombination requires a supercoiled *attP* (11, 22), the increased superhelical density of recombination substrates in vitro may relieve the requirement of Fis for efficient integration that is observed in vivo. An analogous situation has been found for transposition of phage Mu

(29). The stimulation of in vitro Mu transposition by IHF is observed only under conditions of physiological superhelical density.

The results of these experiments show that Fis binding to *attP* F acts to stimulate integration in the absence of Xis. However, in the presence of Xis, Fis inhibits integration, presumably either by directly inhibiting the integration reaction or by enhancing the reverse reaction. Because Fis bound at *attP* F stimulates both integrative and excisive recombination, one can conclude that Fis binding in itself does not exclusively bias a recombination complex toward either reaction. This suggests that the binding of Xis to X1 is solely responsible for creating an excisive complex from an integrative one. Xis, in the presence of Fis binding to *attP* F, dramatically reduces the efficiency of integrative recombination, either by directly inhibiting the reaction or by stimulating the reverse reaction. The negative effect of Xis on integration has been illustrated previously in vitro (1) and is demonstrated most directly in vivo by the results of the plasmid pickup assay. The presence of Xis inhibits integration 17-fold in the presence of Fis and an Fis-binding site, but only about 3-fold in the absence of Fis or its binding site, or both. Therefore, we conclude that Xis binding to X1 is the key determinant in the formation of an excisive recombination complex and also that Fis binding to F strongly stimulates the Xis-mediated assembly of such a complex.

These studies have also uncovered an additional function(s) of Fis in the regulation of λ development that is manifested at a site(s) other than the *attP* F site. In the plasmid pickup assays, which primarily measure integration, a Fis-dependent increase in Int-mediated plasmid transduction of over eightfold was seen in the absence of the F site in *attP*. Similarly, the frequency of lysogen formation of λ*attPF* phage was increased by 2- to over 10-fold by the presence of Fis. This additional regulatory effect of Fis appears to vary significantly with respect to the physiological state of the cell, displaying a maximum under conditions in which cellular Fis concentrations are predicted to be at their highest level.

Fis also appears to influence the stability of the lysogenic state, as measured by the increased sensitivity of *fis* cells to partial induction of lysogens containing temperature-sensitive repressors. A similar effect by Fis has been observed for phage Mu, where in *fis* cells, Mu*cts*62 lysogens are more sensitive to induction at intermediate temperatures than other lysogens in *fis*$^+$ cells (3). The multiple effects on establishment and maintenance of λ lysogeny suggest that Fis may be directly or indirectly influencing the expression of λ regulatory genes, especially since no effect of Fis on lytic growth of λ has been found (2). There is increasing evidence that Fis acts in a number of systems in *E. coli* to modulate gene expression. Other studies have revealed that Fis functions as a transcriptional activator of a number of operons involved in rRNA, tRNA, and translation factor expression (23, 25) and as a repressor of its own synthesis (2b). One possible model for its effect on λ development is that Fis could be directly or indirectly affecting *c*I expression or function. For example, *c*II levels could be reduced in the absence of Fis. This would directly affect both establishment of lysogeny, by reducing initial *c*I levels, and Int expression, since the transcript responsible for the majority of Int synthesis in an infecting phage initiates at the *c*II-dependent P_I promoter (7). An alternative model is that the level of Cro protein may be increased in the absence of Fis, which would lead to reduced efficiency of lysogen formation initially and an increased propensity to commit to the lytic state upon partial induction. Further work is required to determine the relationship of these various additional effects of Fis on the control of λ development and the location(s) and mechanism(s) though which Fis is acting.

We have shown in the accompanying paper (2) that λ excision is strongly stimulated by Fis in vivo, even under maximal-induction conditions. It has also been observed in both Fis DNA-binding assays (32) and antibody assays (2b) that intracellular Fis levels are highest during early exponential growth and dramatically lower during the stationary phase. Likewise, λ yields are higher during exponential growth, when cells are robust and more able to support a good phage burst than during the stationary phase. The coincidence of high excision frequency and high intracellular Fis levels during exponential cell growth makes it attractive to postulate that Fis may play a role in regulating λ excision (30, 32). The presence of Fis bound at F during exponential growth would facilitate Xis binding to X1 during induction of lytic growth. On the other hand, no Fis would be bound during the stationary phase, and induction of lytic growth would not result in significant phage excision. However, we have failed to demonstrate any increase in phage yields from lysogens in the late exponential phase by artificially increasing Fis levels by expression from the *lac* promoter (2a). This suggests that during late exponential growth, when Fis levels are decreased by at least 10-fold compared with levels during early exponential growth (2b), intracellular Fis concentrations do not limit the success of excision. During the stationary phase, when Fis levels are low enough to be limiting for excision, the quiescent state of the cell prevents the expression of phage gene products that are required to achieve a successful induction. We are currently examining whether the levels of Fis are low enough to limit excision frequency when cells are growing slowly, but exponentially, in poor growth media.

Additionally, a model in which Fis specifically regulates excision does not accommodate the ability of Fis to also stimulate integration and enhance the establishment and maintenance of lysogeny. Indeed, *fis* lysogens appear to be more susceptible to induction, and yet induction in the absence of Fis leads to poor phage yields as a result of inefficient excision. The ability of Fis to affect different and sometimes conflicting functions suggests that Fis is used by λ because of its DNA-binding and DNA-bending properties, rather than its expression pattern.

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REFERENCES

1. **Abremski, K., and S. Gottesman.** 1982. Purification of the bacteriophage lambda *xis* gene product required for lambda excisive recombination. J. Biol. Chem. **257**:9658–9662.
2. **Ball, C. A., and R. C. Johnson.** 1991. Efficient excision of phage λ from the *Escherichia coli* chromosome requires the Fis protein. J. Bacteriol. **173**:4027–4031.

2a. **Ball, C. A., and R. C. Johnson.** Unpublished data.

2b. **Ball, C. A., R. Osuna, K. C. Ferguson, and R. C. Johnson.** Unpublished data.
3. **Betermier, M., V. Lefrere, C. Koch, R. Alazard, and M. Chandler.** 1989. The *Escherichia coli* protein, Fis: specific binding to the ends of phage Mu DNA and modulation of phage growth. Mol. Microbiol. **3**:459–468.
4. **Better, M., C. Lu, R. C. Williams, and H. Echols.** 1982. Site-specific DNA condensation and pairing mediated by the Int protein of bacteriophage lambda. Proc. Natl. Acad. Sci. USA **79**:5837–5841.
5. **Bliska, J. B., and N. R. Cozzarelli.** 1987. Use of site-specific recombination as a probe of DNA structure and metabolism *in vivo*. J. Mol. Biol. **194**:205–218.
6. **Echols, H.** 1986. Bacteriophage λ development: temporal switches and the choice of lysis or lysogeny. Trends Genet. **2**:26–30.
7. **Echols, H., and G. Guarneros.** 1983. Control of integration and excision, p. 75–92. *In* R. W. Hendrix, J. W. Roberts, F. Wong-Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Enquist, L. W., and R. A. Weisberg.** 1976. The red plaque test: a rapid method for identification of excision defective variants of bacteriophage lambda. Virology **72**:147–153.
9. **Friedman, D. I., E. R. Olsen, C. Georgeopoulos, K. Tilly, I. Herskowitz, and F. Banuett.** 1984. Interactions of bacteriophage and host macromolecules in the growth of bacteriophage λ. Microbiol. Rev. **48**:299–325.
10. **Gardner, J. F., and H. A. Nash.** 1986. Role of *Escherichia coli* IHF protein in lambda site-specific recombination: a mutational analysis of binding sites. J. Mol. Biol. **191**:181–189.
11. **Gellert, M. K., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA **73**:3872–3876.
12. **Giladi, H., M. Gottesman, and A. B. Oppenheim.** 1990. Integration host factor stimulates the phage lambda pL promoter. J. Mol. Biol. **213**:109–121.
13. **Goodman, S. D., and H. A. Nash.** 1989. Functional replacement of a protein-induced bend in a DNA recombination site. Nature (London) **341**:251–254.
14. **Gottesman, S., and K. Abremski.** 1980. The role of HimA and Xis in lambda site-specific recombination. J. Mol. Biol. **138**:503–512.
15. **Gussin, G., A. Johnson, C. Pabo, and R. Sauer.** 1983. Repressor and *cro* protein: structure, function, and role in lysogenization, p. 93–121. *In* R. W. Hendrix, J. W. Roberts, F. Wong-Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Hoess, R. H., C. Foeller, K. Bidwell, and A. Landy.** 1980. Site-specific recombination functions of bacteriophage λ: DNA sequence of regulatory regions and overlapping structural genes for Int and Xis. Proc. Natl. Acad. Sci. USA **77**:2482–2486.
17. **Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols.** 1982. Control of phage λ development by stability and synthesis of cII protein: role of the viral *cIII* and host *hflA*, *himA* and *himD* genes. Cell **31**:565–573.
18. **Johnson, R. C., C. A. Ball, D. Pfeffer, and M. Simon.** 1988. Isolation of the gene encoding the Hin recombinational enhancer binding protein. Proc. Natl. Acad. Sci. USA **85**:3484–3488.
19. **Landy, A.** 1989. Dynamic, structural, and regulatory aspects of λ site-specific recombination. Annu. Rev. Biochem. **58**:913–949.
20. **Mandel, N. C., and M. Lieb.** 1976. Heat sensitive DNA binding activity of the cI product of bacteriophage lambda. Mol. Gen. Genet. **146**:299–302.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Mizuuchi, M., and K. Mizuuchi.** 1979. Integrative recombination of bacteriophage λ: *in vitro* study of the intermolecular reaction. Cold Spring Harbor Symp. Quant. Biol. **43**:1111–1114.
23. **Nilsson, L., A. Vanet, E. Vijgenboom, and L. Bosch.** 1990. The role of Fis in *trans* activation of stable RNA operons in *E. coli*. EMBO J. **9**:727–734.
24. **Richet, E., P. Abcarian, and H. A. Nash.** 1986. The interaction of recombination proteins with supercoiled DNA: defining the role of supercoiling in lambda integrative recombination. Cell **46**: 1011–1021.
25. **Ross, W., J. F. Thompson, J. T. Newlands, and R. L. Gourse.** 1990. *E. coli* Fis protein activates ribosomal RNA transcription *in vivo*. EMBO J. **9**:3733–3742.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Silhavy, T., M. L. Berman, and L. W. Enquist.** 1984. Experiments in gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Sinden, R. R., S. S. Broyles, and D. E. Pettijohn.** 1983. Perfect palindromic *lac* operator DNA sequence exists as a stable cruciform structure in supercoiled DNA *in vitro* but not *in vivo*. Proc. Natl. Acad. Sci. USA **80**:1797–1801.
29. **Surette, M. G., and G. Chaconas.** 1989. A protein factor which reduces the negative supercoiling requirement in the Mu DNA strand transfer reaction is *Escherichia coli* integration host factor. J. Biol. Chem. **264**:3028–3034.
30. **Thompson, J. F., and A. Landy.** 1989. Regulation of bacteriophage lambda site-specific recombination, p. 1–22. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
31. **Thompson, J. F., H. F. L. Mark, B. Franz, and A. Landy.** 1988. Functional and structural characterization of stable DNA curvature in lambda *attP*, p. 119–128. *In* W. K. Olsen, M. H. Sarma, and M. Sundaralingam (ed.), Structure and expression, vol. 3. DNA bending and curvature. Adenine Press, Schenectady, N.Y.
32. **Thompson, J. F., L. Moitoso de Vargas, C. Koch, R. Kahmann, and A. Landy.** 1987. Cellular factors couple recombination with growth phase: characterization of a new component in the lambda site-specific recombination pathway. Cell **50**:901–908.
33. **Yin, S., W. Bushman, and A. Landy.** 1985. Interaction of the λ site-specific recombination protein Xis with attachment site DNA. Proc. Natl. Acad. Sci. USA **82**:1040–1044.