JOURNAL OF BACTERIOLOGY, Sept. 1987, p. 4124–4127
0021-9193/87/094124-04$02.00/0

Vol. 169, No. 9

Overproduction of *Escherichia coli* Integration Host Factor, a Protein with Nonidentical Subunits

HOWARD A. NASH,[1]* CAROL A. ROBERTSON,[1] ERIC FLAMM,[2] ROBERT A. WEISBERG,[2] AND HARVEY I. MILLER[3]

Laboratory of Molecular Biology, National Institute of Mental Health[1] and Laboratory of Molecular Genetics, National Institute of Child Health and Human Development,[2] Bethesda, Maryland 20892, and Cell Genetics Department, Genentech, Inc., South San Francisco, California 94080[3]

Received 17 March 1987/Accepted 15 June 1987

Integration host factor (IHF) is a small, basic protein that is needed for efficient recombination of bacteriophage λ, as well as for other host and viral functions. We have constructed strains in which the two subunits of IHF, encoded by the *himA* and *hip* genes of *Escherichia coli*, are expressed under the control of the λ p_L promoter. Separate overexpression of *himA* and *hip* led to the production of unstable and insoluble peptides, respectively. In contrast, the overexpression of both genes conjointly led to the accumulation of large amounts of active IHF. Extracts of such cells provided the starting material for a rapid purification procedure that results in milligram quantities of apparently homogenous IHF.

The DNA of bacteriophage λ integrates into the chromosome of its *Escherichia coli* host during the establishment of the lysogenic state (2). Early genetic studies (for a review, see reference 8) showed that a phage gene, *int*, is essential for this site-specific recombination, and subsequent biochemical studies uncovered an essential contribution from the host (15, 17). One of the required host proteins, integration host factor (IHF), is a direct participant in site-specific recombination reactions (10, 14). IHF appears to be a dimer containing two small, nonidentical polypeptides, IHF-α and IHF-β (18). The genes for these polypeptides, *himA* for IHF-α and *hip* (or *himD*) for IHF-β, are located far apart from each other on the *E. coli* chromosome (14). Recently, both genes were cloned and sequenced (5, 12). The binding of IHF to *attP*, the segment of the lambda chromosome that is essential for integration, is needed for efficient recombination (3, 7), but the way in which IHF binding activates *attP* is unknown. IHF also appears to be important for many processes other than lambda integration (for reviews see references 4 and 6). In some of these cases, an IHF-binding site is found at a position that suggests a direct involvement of IHF in the process; in other cases, the role of IHF is unclear.

IHF is not an abundant protein, and it has been difficult to obtain substantial quantities of highly purified material (18). In this paper, we describe the construction of strains in which both subunits of IHF are placed under the control of a regulatable promotor. We show that when these strains are induced, large amounts of active IHF accumulate. A simple purification scheme starting with such induced cells leads to large amounts of homogeneous material. Interestingly, the overexpression of each gene separately was not a usable method for overproducing the active protein; the presence of stoichiometric amounts of IHF-α appears to solubilize intracellular IHF-β, and the presence of stoichiometric amounts of IHF-β appears to stabilize intracellular IHF-α. This finding provides further evidence that the two subunits interact directly in vivo and offers a cautionary note to workers attempting to overproduce other proteins that are composed of more than one kind of subunit.

* Corresponding author.

MATERIALS AND METHODS

Construction of plasmids and description of strains. To overproduce IHF-α, the *himA* gene was inserted downstream from the p_L promoter, as diagrammed in Fig. 1. Transformants of strain K5607, a lambda lysogen of strain C600himA42 that had received the desired plasmid, were identified by screening ampicillin-resistant transformants for the ability to grow bacteriophage Mu (13). DNA was prepared from *himA*$^+$ transformants (1), and the orientation of the *himA* gene was determined by restriction enzyme cleavage. Plasmid pP_LhimA-1, which has the *himA* gene in the proper orientation for expression by the p_L promoter, was transformed into strain N5271, which contains a cryptic lambda prophage expressing the *c*I857 thermoinducible repressor (5), to yield strain K5770.

To overproduce IHF-β, we used plasmid pKT23-hip323, which contains a fusion of the IHF-β-coding sequence to the bacteriophage lambda p_L promoter (Fig. 1). pKT23-hip323 was introduced into N5271 to give strain E443 (5). To facilitate the selection of pKT23-hip323 in the presence of another plasmid, we changed its selectable marker from ampicillin resistance (*bla*$^+$) to chloramphenicol resistance (*cat*$^+$). A *cat*-containing fragment was isolated from plasmid pBR325 as described by Flamm and Weisberg (5) and was inserted into the unique *Sca*I site in *bla*. The ligated DNA was introduced into strain E403, which carries a *hip* mutation and which synthesizes temperature-sensitive λ repressor (5), and chloramphenicol-resistant transformants were selected at low temperature. One such transformant (E735) was *hip*$^+$ and ampicillin sensitive; it therefore appears to carry a *bla cat*$^+$ derivative of pKT23-hip323 (pE735).

To generate a strain that overproduces both subunits of IHF, we transformed E735 with plasmid pP_LhimA-1, selecting a transformant (E738) that had become ampicillin resistant and had retained chloramphenicol resistance. The generation of a second strain that overproduces both subunits of IHF depended on the construction of plasmid pP_Lhip himA-5, which was made as diagrammed in Fig. 1. *himA*$^+$ transformants of strain K5607 were identified as described above, and the plasmid DNA was analyzed by restriction digestion. In all cases where the plasmid structure was

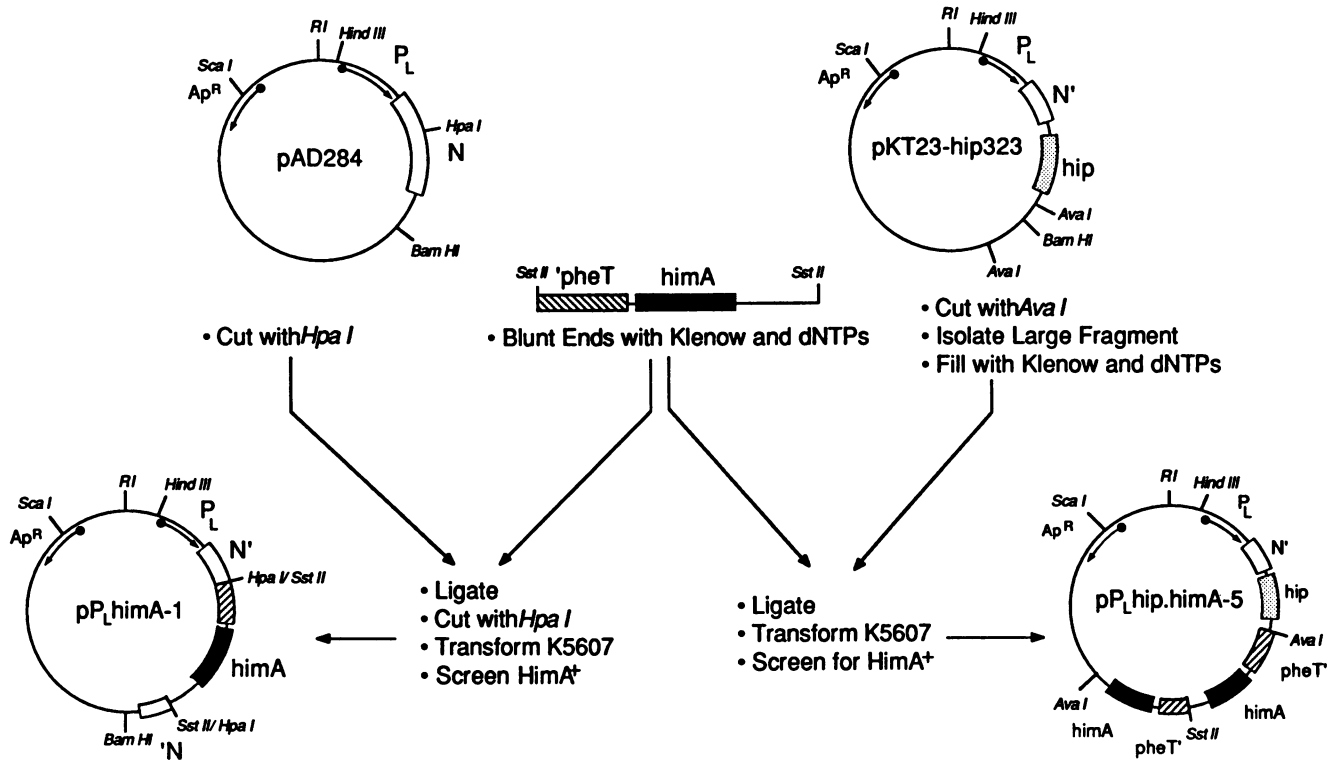

FIG. 1. Structure of plasmids that overproduce the *himA* and *hip* gene products. Plasmid pKT23-hip323 (5), plasmid pAD284 (9), and the transducing phage which was used as a source of the *himA* fragment (12) have been described. Only restriction sites relevant to the construction are shown. *RI*, *Eco*RI; dNTPs, deoxynucleotide triphosphates.

obvious, two copies of *himA* had been ligated as a tandem direct repeat into the vector. It is not known if the presence of two copies of the *himA* gene on this plasmid is demanded by the selection, but it should be recalled that a single copy of the *himA* gene in plasmid pP_LhimA-1 is sufficient to complement a *himA* mutant. Plasmid pP_LhiphimA-5 was used to transform strain N5271 to yield strain K5746.

Growth, extraction, and labeling of cells. Cells were grown in shaking water bath at 31°C in TBY medium (10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride per liter). At mid-log phase (optical density at 650 nm, ca. 0.6), the cells were shifted to a 42°C water bath and shaking was continued. Typically, 300 ml of culture was centrifuged and suspended in 0.6 to 0.9 ml of TEG (20 mM Tris hydrochloride [pH 7.4], 1 mM sodium EDTA, 10% glycerol) containing 20 mM NaCl. The cells were disrupted with six 20-s bursts of sonication, with 40 s between each burst. A portion of the sonic extract was centrifuged in a Sorvall SS34 rotor for 20 min at 15,000 rpm. Samples of the sonic extract were analyzed by sodium dodecyl sulfate (SDS) gel electrophoresis as described previously (11). The IHF activity of the extracts was assessed as described before (18) by the intermolecular recombination assay, except that purified Int was prepared as described previously (16) from a strain carrying the *himAΔ82* allele (12). Pulse-labeling of proteins with [^{35}S]methionine and their analysis have been described previously (5).

Purification of IHF. A 3.6-liter batch of cells was induced for 3 h as described above. All subsequent steps were carried out at 0 to 4°C. The cell pellet from 3.3-liters was suspended in 10 ml of TEG containing 20 mM NaCl to give a total volume of 29 ml; this suspension was disrupted in two batches, each receiving six bursts of 3 min of sonication separated by 90-s intervals. The sonic extract was centrifuged for 20 min at 15,000 rpm, yielding 16.9 ml of clarified extract. A 10% (vol/vol) solution (1.1 ml) of polymin P (BDH Chemicals Ltd.) was added slowly to the clarified extract; after being stirred for 20 min, the mixture was centrifuged for 30 min at 10,000 rpm. The resulting pellet was suspended in 10 ml of TEG containing 500 mM NaCl; after being stirred for 15 min, the mixture was centrifuged for 20 min at 12,000 rpm. The supernatant (10.3 ml) was adjusted to 50% saturation by the addition of 3.2 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting supernatant was adjusted to 70% saturation by the addition of 1.64 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting pellet was suspended in 1 ml of TG (50 mM Tris hydrochloride [pH 7.4] containing 10% glycerol) and dialyzed against two changes of TG. The dialyzed material (2.0 ml) was loaded onto a 1-ml column (0.5 by 5.8 cm) of phosphocellulose (P11; Whatman, Inc.) that had been equilibrated with TG. The column was washed with 3 ml of TG and developed with 20 ml of a linear gradient (0 to 1.2 M) of KCl in TG. Fractions of 0.5 ml were collected, stored at −20°C, and assayed for IHF as described above.

RESULTS AND DISCUSSION

Overproduction of individual subunits. As a first step toward overproducing IHF, we studied the expression of separate α and β subunits in plasmid constructs where the *himA* gene or the *hip* gene was placed under the control of the lambda p_L promoter (Fig. 1). Our attempts at overproduction of each subunit with these constructs were disappointing. As reported before (5), the *hip* plasmid accumu-

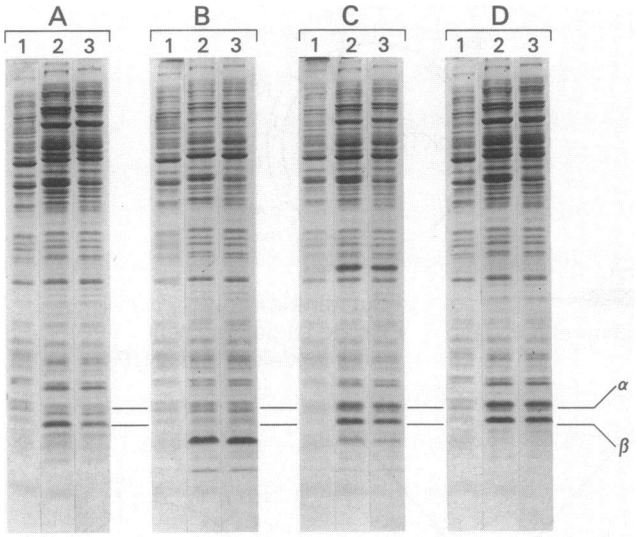

FIG. 2. Electrophoresis of cell extracts. (A) *hip* overproducer (strain E443); (B) *himA* overproducer (strain K5770); (C) IHF overproducer (strain E738); (D) IHF overproducer (strain K5746). Sonic extracts were made from cells that were grown to mid-log phase or grown to mid-log phase and induced for 3 h. A portion of each induced extract was clarified by centrifugation. A sample (0.3 μl) of each extract was boiled in the presence of SDS and beta-mercaptoethanol and applied to an SDS-urea 18% polyacrylamide gel. The positions of IHF-α and IHF-β are marked. Lanes: 1, uninduced extract; 2, induced extract; 3, clarified induced extract.

lated large amounts of IHF-β (Fig. 2A, lanes 1 and 2). However, much of this material was insoluble and was removed by a brief centrifugation (Fig. 2A, lanes 2 and 3). Moreover, little or no polypeptide of the size expected for the *himA* gene product accumulated after induction of the *himA* plasmid (Fig. 2B, lane 1 and 2). The failure of these plasmids to overproduce native α or β subunits was confirmed by the lack of increase in α or β-complementing activity (14) in the extracts of induced cells (data not shown). Thus, it appears that when overproduced separately, neither of the individual subunits of IHF is able to adopt a structure that is consistent with a stable, active product.

Our experience with these plasmids suggests that in the absence of a partner subunit, the individual subunits of IHF are rapidly degraded in vivo. First, induction of the *himA* plasmid generates large amounts of a polypeptide that is shorter than the *himA* gene product (Fig. 2B, lanes 1 and 2). However, when this plasmid is induced and then pulsed for 30 s with radioactive [^{35}S]methionine, the label is incorporated into a peptide that comigrates with IHF-α, as well as into the faster-migrating species (data not shown). This finding suggests that strain K5770 synthesizes full-length IHF-α that is rapidly converted to the smaller species seen in Fig. 2B. This view was supported by our observation that the smaller species cross-reacts with antibodies raised to purified IHF (data not shown). The second observation suggesting instability of the individual subunits is that, as measured by Coomassie blue staining, induction of the *hip* plasmid in a *himA*$^+$ host (strain E443) leads to a much higher accumulation of IHF-β than does induction of this plasmid in the *himAΔ82* host, strain E484 (data not shown). Pulse-chase experiments indicated that the difference is due primarily to greater stability of the β subunit in the *himA*$^+$ host, rather than to greater synthesis. Induced E443 and E484 cells incorporated similar amounts of [^{35}S]methionine into a polypeptide that comigrates with IHF-β after a 30-s chase of a 1-min pulse. However, after a 30-min chase of a 1-min pulse, E443 cells retained up to 50 times as much label in the β subunit as did E484 cells (data not shown). Apparently, the overproduced β subunit is unstable in the complete absence of IHF-α, although it is stable, but insoluble and inactive, in the presence of substoichiometric amounts of IHF-α. We do not know why a small amount of IHF-α stabilized the β subunit (Fig. 2A), but small amounts of IHF-β did not protect IHF-α from degradation (Fig. 2B). In any case, our data lead us to believe that the two subunits of IHF interact directly, a hypothesis that is in agreement with earlier biochemical data (18).

Coexpression of α and β subunits. We constructed two strains that simultaneously overproduced both subunits of IHF (see Materials and Methods). One strain (E738) contains separate p_L-*hip* and p_L-*himA* fusions, and the second strain (K5746) contains a p_L-*hip-himA* double fusion. SDS gel electrophoresis of crude extracts of both strains (Fig. 2C and D) showed that induction leads to the accumulation of large amounts of polypeptides that comigrate with IHF-α and IHF-β. (Inducing strain E738 also leads to the accumulation of a polypeptide that migrates about 0.6 times as fast as the IHF subunits. We believe that this species arises from overproduction of a vector gene; it does not cross-react with anti-IHF antibody.) Most of the overproduced IHF polypeptides are soluble (Fig. 2C and D, lanes 2 and 3). It thus appears that the insolubility of the β subunit is overcome when a roughly equimolar amount of the α subunit is present. It is also interesting to note that the rapidly migrating polypeptide that is made in large amounts by the *himA* overproducer (Fig. 2B, lane 2) is greatly diminished in one strain (Fig. 2C, lane 2) and is undetectable in the other strain (Fig. 2D, lane 2). It appears that the β subunit suppresses the formation of this material and simultaneously stimulates the formation of the full-length α subunit. This suggests to us that the β subunit affects the synthesis or the stability of α subunit, either by altering the length of the effective message or, more likely, by protecting the α subunit against degradation.

The overproduced IHF is highly active in promoting integrative recombination in vitro. When either strain is induced to express IHF for 90 to 180 min, there is a great increase in the amount of extractable IHF activity above the low or undetectable level present in uninduced cells (data not shown). A conservative estimate is that after induction, the extracts of either strain contain at least 100 times more activity than do the extracts of wild-type cells. The IHF activity in these extracts is stable with storage at −20° and with repeated freezing and thawing. We conclude that the overexpression of both subunits of IHF is an effective way to generate large quantities of active, stable protein.

Purification of IHF. The purification of IHF from conventional sources is a lengthy procedure that is accompanied by large losses of activity (18). We have used one of the overproducing sources of IHF to develop a much more

TABLE 1. Purification of IHF from strain K5746

Fraction	Total vol (ml)	Total amt of protein (mg)	Total U (10^3)	Sp act (U/mg)
Crude extract	16.2	1,976	12,150	6,150
Polymin P-ammonium sulfate	2.0	98	3,000	30,000
Phosphocellulose	5.5	12.1	2,062	166,000

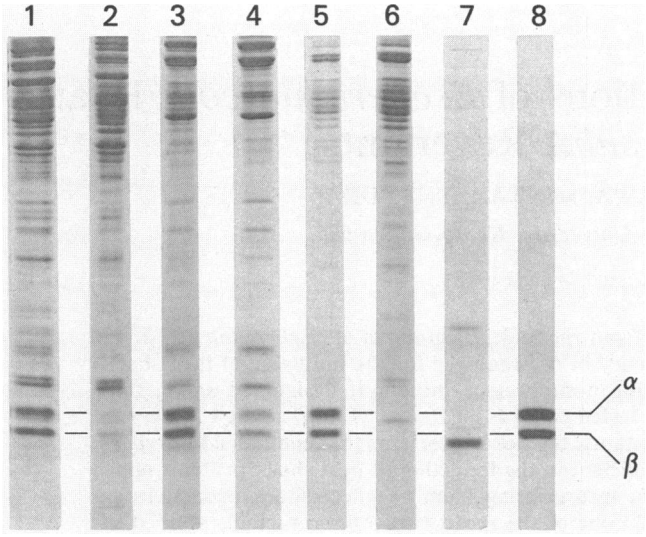

FIG. 3. Purification of IHF. Samples of IHF at various steps in the purification procedure were treated and electrophoresed as described in the legend to Fig. 2. Lanes: 1, 0.5 μl (61 μg) of clarified sonic extract; 2, 0.5 μl (32 μg) of polymin P supernatant; 3, 0.5 μl (20 μg) of polymin P extract; 4, 0.15 μl (13.5 μg) of 0 to 50% ammonium sulfate precipitate; 5, 0.15 μl (7.4 μg) of 50 to 70% ammonium sulfate precipitate; 6, 0.5 μl (13 μg) of a phosphocellulose flowthrough fraction; 7, 2 μl (2.1 μg) of a phosphocellulose fraction before the peak of IHF activitiy (the prominent band that migrates faster than IHF-β has the mobility of protein HU); 8, 2 μl (4.4 μg) of pooled phosphocellulose fractions with maximal IHF activity.

effective and simple purification scheme. First, the scale was drastically reduced; from conventional sources we normally process an extract from 300 liters of *E. coli*, but successful purification was obtained with the extract from 3.3 liters of the overproducing strain (Table 1). Second, the purification procedure was truncated from four column steps to one; the first column chromatography now results in very pure IHF. The recovery of activity was substantial, and the purified material had a specific activity greater than we have observed from any conventional source (Table 1).

Although we routinely assay the purification of IHF by determining recombination activity, the purification scheme can also be easily followed by SDS gel electrophoresis. We found few or no α or β polypeptides in fractions that were discarded during the purification (Fig. 3). It should also be noted that the HU protein, a troubling contaminant in our previous IHF preparations (18), eluted early from the phosphocellulose column (Fig. 3, lane 7), and the most purified fractions of IHF were virtually free of this and other visible contaminants (Fig. 3, lane 8).

The recovery of more than 10 mg of apparently homogeneous material from 3.3 liters of cells indicates that it should be straightforward to prepare amounts of IHF sufficient to carry out crystallization, spectrophotometric, and chemical studies. Indeed, more than 500 mg of purified IHF has been recovered from a small portion of a 300-liter fermentor growth of the overproducing strain K5746 (K. Appelt, personal communication). Finally, we note that by placing the synthesis of IHF under the control of a regulatable promoter, the constructs described in this study should expedite the investigation of the in vivo role of IHF in various physiological processes.

ACKNOWLEDGMENTS

We thank Nagaraja Ramaiah for reading the manuscript and Krzysztof Appelt for communicating unpublished results.

LITERATURE CITED

1. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
2. **Campbell, A. M.** 1962. Episomes. Adv. Genetics **11**:101–145.
3. **Craig, N. L., and H. A. Nash.** 1984. E. coli integration host factor binds to specific sites in DNA. Cell **39**:707–716.
4. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histonelike proteins of bacteria. Microbiol. Rev. **51**:301–319.
5. **Flamm, E. L., and R. A. Weisberg.** 1985. Primary structure of the *hip* gene of *Escherichia coli* and of its product, the β subunit of integration host factor. J. Mol. Biol. **183**:117–128.
6. **Friedman, D. I., E. R. Olson, C. Georgopoulos, 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.
7. **Gardner, J. F., and H. A. Nash.** 1986. Role of *Escherichia coli* IHF protein in lambda site-specific recombination. J. Mol. Biol. **191**:181–189.
8. **Gottesman, M. E., and R. A. Weisberg.** 1971. Prophage insertion and excision, p. 113–138. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **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.
10. **Kikuchi, Y., and H. A. Nash.** 1978. The bacteriophage λ*int* gene product. J. Biol. Chem. **253**:7149–7157.
11. **Lange-Gustafson, B. J., and H. A. Nash.** 1984. Purification and properties of Int-h, a variant protein involved in site-specific recombination of bacteriophage λ. J. Biol. Chem. **259**:12724–12732.
12. **Miller, H. I.** 1984. Primary structure of the *himA* gene of *Escherichia coli*: homology with DNA-binding protein HU and association with the phenylalanyl-tRNA synthetase operon. Cold Spring Harbor Symp. Quant. Biol. **49**:691–698.
13. **Miller, H. I., and D. I. Friedman.** 1980. An E. coli gene product required for λ site-specific recombination. Cell **20**:711–719.
14. **Miller, H. I., A. Kikuchi, H. A. Nash, R. A. Weisberg, and D. I. Friedman.** 1979. Site-specific recombination of bacteriophage λ: the role of host gene products. Cold Spring Harbor Symp. Quant. Biol. **43**:1121–1126.
15. **Mizuuchi, K., M. Gellert, and H. A. Nash.** 1978. Involvement of supertwisted DNA in integrative recombination of bacteriophage lambda. J. Mol. Biol. **121**:375–392.
16. **Nash, H. A.** 1983. Purification and properties of the bacteriophage lambda Int protein. Methods Enzymol. **100**:210–216.
17. **Nash, H. A., K. Mizuuchi, R. A. Weisberg, Y. Kikuchi, and M. Gellert.** 1977. Integrative recombination of bacteriophage lambda—the biochemical approach to DNA insertion, p. 363–373. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Nash, H. A., and C. A. Robertson.** 1981. Purification and properties of the *Escherichia coli* protein factor required for λ integrative recombination. J. Biol. Chem. **256**:9246–9253.