SOS induction and autoregulation of the himA gene for site-specific recombination in Escherichia coli
(regulation by LexA and RecA/bacteriophage λ integration/gene fusion)

HARVEY I. MILLER, MARTIN KIRK, AND HARRISON ECHOLS

Department of Molecular Biology, University of California, Berkeley, California 94720

Communicated by Allan Campbell, July 27, 1981

ABSTRACT The himA gene of Escherichia coli controls the lysogenization of bacteriophage λ at the level of catalysis of site-specific recombination and expression of the λ int and cl genes required for lysogenic development. We have analyzed the regulation of himA by two methods: (i) β-galactosidase synthesis from a lacZ gene inserted into the himA gene and (ii) detection of radioactive HimA protein after fractionation by two-dimensional gel electrophoresis. We find that himA- mutations produce enhanced expression of the himA gene, indicating that HimA protein controls its own synthesis. The himA gene is also induced by treatment of cells with UV or mitomycin C, suggesting control by the inducible DNA repair (SOS) system regulated by the LexA and RecA proteins. Regulation of himA follows the pattern expected for a typical SOS gene: constitutive high expression in mutants that have inactive LexA or the altered RecA conferred by the recA441 (rif1) mutation and low noninducible expression in a mutant that has a deleted recA gene. We conclude that the himA gene is a component of the inducible SOS response, repressed by LexA and induced by the capacity of activated RecA to cleave LexA. We suggest that HimA may be subject to SOS induction because it functions as an "acquisitionase" for new genetic material and thus is of special utility under conditions of impaired capacity for growth of the bacterial population.

The chromosome of Escherichia coli specifies gene products active in at least two types of genetic recombination, general (homology dependent) and site specific. The product of the recA gene is a major component of general recombination pathways (1); the himA gene specifies a crucial component of site-specific recombination (2). The best studied recombination reaction in which the HimA protein participates is the integration of the DNA of bacteriophage λ into the E. coli chromosome (3).

In addition to its catalytic function in general recombination, the RecA protein has a regulatory role in the coupled induced response to DNA damage involving mutagenesis, inhibition of cell division, increased DNA repair capacity, and prophase induction: the "SOS response" (4, 5). One element of the SOS response is induction of the recA gene itself (6-11). This feature probably serves to provide enhanced capacity for recombinational repair (12). A number of other genes are also induced, although the roles of most have not yet been identified (13). Induction of recA (and other SOS genes) occurs by a chain of events in which the first, a signal of DNA damage that activates RecA as a protease, is followed by inactivation of theLexA repressor through RecA-mediated cleavage (5, 11, 13-15). Cleavage of LexA has been demonstrated directly (16). The cl repressor protein for λ is also cleaved by RecA, allowing the phage to escape the damaged cell (15, 17).

HimA protein also has both a catalytic and a regulatory role: It is a subunit of the integration host factor (IHF) required for integrative recombination by λ DNA (18), and it is also needed for efficient expression of the int and cl genes specific for the lysogenic response by λ (19, 20). Because of the multiple roles of HimA in prophage insertion, we expected that the himA gene might be subject to a variety of cellular control signals. In this paper, we show that himA is negatively regulated by its own gene product and is induced as an element of the SOS response. We also consider the possible significance of the SOS regulation.

MATERIALS AND METHODS

Bacteria and Bacteriophage. The basic E. coli strains used were K37Sm″Su″ and its himA42 derivative K536 (2), K5407, a muc″ lysogen of K37 also lacZ deleted, K5070, carrying a pro lac deletion (2); and K660, which has the F″148 plasmid that carries the himA gene and Tn10. Mutations were introduced into the appropriate strains by transduction with phase P1 to tetracycline resistance (Tet") from donors containing the Tet" transposon Tn10 linked to the mutation. The recA441 (rif1) (21), recAΔ306 (22), and lexA3 (23) mutations were introduced into strains JC10257, JC10289, and JC13519, respectively, of A. J. Clark. The spr-51 mutation was from DM1187 of D. Mount (24). Tet" (Tetracycline-sensitive) derivatives were selected by the method of Bochner et al. (25). The insertion of the mu-defective lacZ phage (mud) (26) is described below. All derivatives containing F″148 mud were tested for their ability to segregate Lac⁺ ampicillin-sensitive (Ap⁺) colonies (≈ 1%) to demonstrate that the mud fusion was maintained solely on the plasmid. Lysogens of muc" were selected by immunity to superinfecting phage.

Media. Bacteria were propagated in LB medium containing ampicillin at 25 μg/ml where appropriate. P1 transductants to Tet" were selected on LB agar plates containing 0.01 M sodium pyrophosphate and tetracycline at 15 μg/ml. For assays of β-galactosidase from lacZ insertion strains, bacteria were grown in M9 liquid medium (27) with 0.1% casamino acids/0.2% glucose containing ampicillin at 25 μg/ml. For control experiments on induction of a normal lacZ gene, 0.5% glycerol was substituted for glucose and ampicillin was omitted.

Assay For β-Galactosidase. Cultures were assayed for β-galactosidase by hydrolysis of o-nitrophenylgalactopyranoside as described (27). One unit produces a change in A400 of 0.001/min in the standard reaction. Specific activity is given in units of enzyme per A420 unit of the culture. For lac operon induction, isopropylthiogalactoside was added to a concentration of 1 mM 20 min prior to assay. Only midlogarithmic phase cultures were assayed.

Radioactive Labeling of Bacterial Cultures and Gel Electrophoresis. Bacteria were grown at 37°C to a density of 2 × 10⁸/ml in M9 media/0.2% glucose containing 18 amino acids (no methionine or cysteine) at 10 μg/ml each, at which time 50 μCi of [35S]methionine (Amersham; 1240 Ci/mmol; 1 Ci = 37 g) were added. The cultures were shaken at 37°C for 30 min, and at this time the cells were harvested by centrifugation, resuspended in freshly made M9 medium, and incubated with 10 μg/ml chloramphenicol for 40 min. The cells were then harvested, resuspended in M9 medium, and incubated at 37°C for 45 min before isolation of the phosphorylase. Loading conditions were determined to optimize the gelation and separation of the proteins. After electrophoresis, the gel was cut into pieces, and the radioactivity of each was determined by liquid scintillation counting. The radioactivity recovered was always > 90% of that applied to the gel.
3.7 x 10^10 becquerels) was added to a 2-ml culture. After 2
min, the cultures were chilled, lysed, and subjected to two-
dimensional gel electrophoresis and autoradiography by the
technique of O'Farrell et al. (28) as described (18).

Selection for himA-lacZ Fusions. We have used the tech-
nique of gene fusion to study the transcriptional regulation of the
himA gene. Bacterial strains in which the promoter for the
himA gene (himAOP) is fused to the lacZ structural gene were
constructed by using the defective mu phage described by Cas-
daban and Cohen (26) that carries ampicillin-resistance and
lacZ genes [mud, (Ap, lac)] (Fig. 1). The mu phage is par-
icularly suited for this construction for two reasons: (i) it can insert
at random into the E. coli chromosome to provide for transcrip-
tion of the lacZ gene from the promoter of the target operon
and (ii) it carries a mutation that confers thermoinducibility and
thus high-temperature lethality for the lysogenic bacterium.
Because bacterial strains carrying himA mutations are able to
survive thermal induction of mu (2), selection for a mud lysogen
at inducing temperatures selects for mu insertions that inac-
tivate the himA gene. Approximately half of the Him^- mu lys-
ogens obtained by this procedure displayed a Lac^+ phenotype
on indicator media. One representative strain (K5333) of six
phenotypically identical insertions was chosen for further tests
indicating that transcription of the lacZ gene initiates at the
himA promoter.

The site of the mud insertion was localized to the himA gene
by two methods: (i) the mud phage was 100% linked by P1
cotransduction to a Tn10 transposon inserted directly adjuc-
tant to the himA gene and (ii) the Him^- phenotype was converted to Him^- by lysogenization or recombination with AhimA, a
specialized transducing phage carrying the wild-type himA
and only four other E. coli genes (pheS, pheT, thrS, and infC)
(2, 18). Because the other four genes present on AhimA are es-
sential for growth of E. coli, the lesion resulting from mud
insertion must be confined to the himA gene and thus appears
to be an simple insertion of the type shown in Fig. 1.

The chromosomal region containing the mud insertion was
transferred, by homogenization, to an F'148 plasmid, and the
resulting episome (F'148himA::mud) was transferred by con-
jugation into a set of isogenic strains carrying a lacZ deletion, a
muc^- prophage, and additional mutations of possible regu-
latory significance.

RESULTS

Autoregulation of the himA Gene. The gene fusion system
used to study regulation of himA gene expression is shown in
Fig. 2; we measured ß-galactosidase from the lacZ gene in-
serted into the himA gene of an F' plasmid. In the standard
strain, a wild-type chromosomal himA gene is present, pro-
ducing a Him^- phenotype. Generation of a Him^- phenotype
by introduction of a missense, nonsense, or deletion mutation of
the himA gene into the chromosomal results in markedly el-
levated levels of ß-galactosidase (Table 1). From these results,
we suggest that HimA is an autoregulatory protein, negatively
regulating the himA gene. The basal level of expression of a
himA in the Him^- strain is substantial (Table 1), indicating a sensitive
balance to the regulatory system (and also that the himA gene
may be somewhat more repressed in a normal cell in which only
one copy of the operator region is present).

The HimA protein is one (IHF) of two subunits of the IHF
(18). The other subunit (IHFβ) is probably specified by an un-
linked gene, himD (see below); mutations in himD produce a
similar phenotype to himA^- lesions (unpublished work). The
himD mutations may alter the gene previously defined by the
hip157 mutation (3). Because of the close functional relationship
between the himA and himD gene products, we studied the effect
of a mutation in himD on expression of the himA gene; ß-
galactosidase is produced at the same high level found for
himA^- (Table 1). This result indicates that HimD protein is also
a negative regulator of the himA gene. HimA and HimD might
function separately, as the IHF dimer, or in a more complex
fashion.


Fig. 1. Construction of a himA-lacZ fusion. The expected struc-
ture is shown for a simple integration of mud (Ap^+, lac) into the himA
gene in the proper orientation such that the lacZ and lacY genes are
transcribed from the himA promoter (himAOP). K5070 was grown at
32°C to 2 x 10^8/ml in LB broth/0.05 M CaCl_2/0.01 M MgSO_4.
The cells were infected at room temperature with a mixed lysate of mud/82/
mud at 1 plaque former per cell. After 60 min of incubation, the in-
fected culture was centrifuged, the cells were suspended in 10 ml of
LB broth/0.01 M sodium pyrophosphate containing ampicillin at 25
µg/ml, and the fresh culture was incubated at 32°C overnight. The
overnight culture was centrifuged, the pellet was washed with 0.01 M
MgSO_4, and dilutions were plated at 32°C and 42°C on eosin/meth-
ylene blue plates containing ampicillin at 25 µg/ml. Survival at 42°C
was 10^-9. Colonies surviving at 42°C were screened for a integration
as described (29); clones exhibiting the integration-negative (Him^-)
phenotype (~5%) were screened for the Lac phenotype on MacConkey
lactose indicator plates. One Lac^- HIm^- strain, K5335, was chosen for
further study.

Fig. 2. Schematic representation of the system used for studying
himA gene regulation. The basic strain contains an F'148 episome car-
rrying the himA::mud fusion. This strain also carries a lacZ internal
deletion, so that the only source of ß-galactosidase is the mud fusion,
and a wild-type muc^- prophage, so that experiments can be conducted
at temperatures that would induce the thermoinducible repressor of
the mud phage. According to the mechanism proposed in this paper,
the product of the chromosomal himA gene represses both its own pro-
moter and the himA promoter controlling lacZ transcription. Thus, a
mutation inactivating the himA gene leads to maximal expression of
the lacZ gene.
Strain K5462 was constructed as follows. The F"148 plasmid carrying transposon Tn10 (from K660), was mated into the himA"- mud (Ap, lac) strain K5333 (selecting for TetR, ampicillin resistance (ApR)) to yield K5368. K5368 was mated with the SmR lacZ deletion K5407, and a SmR, ApR derivative was selected (K5451). Tn10 was eliminated from the plasmid by selection for tetracycline sensitivity to yield K5462. The structure of K5462 is shown in Fig. 2. K5466-K5469 are derivatives of K5462 prepared by transduction with phage P1. Bacteria were grown at 37°C to a density of 10^8/ml and assayed for β-galactosidase. Data presented are averages of duplicate determinations.

Table 1. Autoregulation of the himA gene

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>β-Galactosidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K5462</td>
<td>him+</td>
<td>572</td>
</tr>
<tr>
<td>K5468</td>
<td>himA42</td>
<td>1719</td>
</tr>
<tr>
<td>K5467</td>
<td>himA42m9</td>
<td>1413</td>
</tr>
<tr>
<td>K5468</td>
<td>himA42</td>
<td>1697</td>
</tr>
<tr>
<td>K5469</td>
<td>himD63</td>
<td>1752</td>
</tr>
<tr>
<td>K37</td>
<td>him+</td>
<td>0.9</td>
</tr>
<tr>
<td>K37*</td>
<td>him+</td>
<td>1192</td>
</tr>
<tr>
<td>K336</td>
<td>himA42</td>
<td>0.3</td>
</tr>
<tr>
<td>K336*</td>
<td>himA42</td>
<td>1016</td>
</tr>
</tbody>
</table>

To show that the Him" phenotype does not generally affect lacZ gene expression in some way, we measured β-galactosidase from a lacZ gene in the lac operon; there is no substantial difference between himA+ and himA- (Table 1).

If the himA gene is negatively regulated by HimA and HimD, bacterial strains carrying mutations in the himA or himD genes should exhibit elevated levels of HimA protein. We can establish this point qualitatively because we have identified the migration positions, after fractionation on two-dimensional acrylamide gels, of the himA+ and himA42 gene products (a and a42) and of the β-subunit of IHF (18). Based on this previous detailed analysis, we can use two-dimensional gel fractionation to estimate the levels of these proteins in cell extracts (Fig. 3). As previously shown, the IHFα and IHFB synthesized in wild-type E. coli is insufficient for detection by this method (18) (Fig. 3A); however, the normal migration positions of these proteins are indicated with respect to the position of ribosomal protein S10. The location of S10 can be seen in each gel from its characteristic migration with respect to marker proteins infC, ribosomal protein L5, and an additional ribosomal protein. The sites indicated for IHFα and IHFB have been confirmed by the use of purified IHFα and IHFB (18). In contrast to the wild type, fractionation of an extract from a himA- strain shows demonstrable production of IHFα42 and IHFB (and at least one unidentified third protein; Fig. 3B). Thus, we conclude that himA- mutation leads to derepressed synthesis of HimA (HIFα) and IHFB. The himD- extract exhibits wild-type IHFα and another protein, labeled β63, apparently identical in size to IHFB but shifted in charge (Fig. 3C). The relationship between IHFB and β63 is shown more clearly in the fractionated mixture of himA+ and himD- extracts (Fig. 3D); there is a similar molecular weight and charge heterogeneity characteristic of IHFB in this gel system (18). Thus, the gel analysis suggests that β63 is most likely the missense protein form of IHFB resulting from the himD63 mutation and that himD is probably the structural gene for IHFB. Fig. 3D also shows the relationship of a and α42 to ribosomal protein S10.

FIG. 3. Two-dimensional gel analysis of proteins from strains carrying himA and himD mutations. Nonequilibrium two-dimensional gels of [35S]methionine-labeled extracts are shown for the following isogenic strains: (a) wild type (K37), (b) himA42 (K5366), (c) himD63 (K5283), and (d) a mixture of b and c extracts. Cultures were labeled, lysed, and subjected to electrophoresis and autoradiography. The locations of IHFα, IHFα42, IHFB; ribosomal proteins S10, L5, and RP; and infC (initiation factor S) have been described (18, 31). The proteins that we identify as IHFα in c and IHFB in b coelectrophorese in two dimensions with IHFα and IHFB from purified IHF (data not shown). The unlabeled arrow in b indicates a protein encoded by an unidentified gene that appears in extracts of himA- strains. The gel analysis was performed several times with qualitatively similar results; representative autoradiograms are shown.
From the qualitative agreement between the measurements of himA gene expression by lacZ fusion and identification of HimA protein in two-dimensional acrylamide gels, we consider the evidence to be strong that HimA is a negative regulator of the himA gene and that the lacZ fusion assay provides a valid way to measure himA regulation. The results of Fig. 3 also suggest a symmetrical relationship between regulation of himA and himD in which both HimA and HimD participate in the regulation of the himA and himD genes. However, this conclusion must be regarded as tentative until the himD gene product has been identified more definitively.

**SOS Regulation of the himA Gene.** To analyze the response of the himA gene to the SOS induction pathway, we used the lacZ fusion shown in Fig. 2 to measure β-galactosidase production in response to agents or mutations known to affect SOS regulation. The regulatory response and essential control experiments are shown in Fig. 4. After treatment with UV, the classical agent for SOS induction, β-galactosidase increases to a level comparable with that in a himA− strain. As for other SOS responses, this induction is blocked by introduction of a recA− mutation; a recA− mutation does not prevent β-galactosidase induction from the lac operon (in the presence of 1 mM cAMP) after UV irradiation (ref. 13; unpublished work). The induction is specific for the lacZ insertion in himA and not for the plasmid in general because no increase of β-galactosidase occurs in recA− bacteria carrying a lacZ fusion to a different promoter on the F plasmid.

Other methods of SOS induction show similar responses (Table 2). The lacZ fusion is also induced in response to treatment with mitomycin C. As for UV treatment, this increase is inhibited by mutations known to prevent SOS induction: e.g., a recA deletion or a lexA3 mutation (5, 23). The SOS pathway will undergo thermal induction in bacteria with the recA441 mutation (hfl) (5, 21). The introduction of recA441 into the standard lacZ fusion allows thermal induction of β-galactosidase. From the results of Fig. 4 and Table 2, we conclude that the himA gene is a component of the induced SOS response to DNA damage.

The induction of SOS-regulated genes is known to occur by at least two different mechanisms. Most appear to be repressed by LexA and derepressed when LexA is cleaved by RecA protease (16). Alternatively, a repressor other than LexA might be cleaved by RecA protease, as for the λ cI repressor (17). To distinguish between these two modes of induction, we measured β-galactosidase from the lacZ fusion in strains carrying a mutation that inactivates LexA, with or without an additional deletion mutation of the recA gene (Table 3). The lexA− mutation confers a high level of β-galactosidase synthesis in the absence or presence of the recA441 (hfl) mutation, indicating that LexA represses himA, either directly or by repression of RecA. The β-galactosidase level remains high for the lexA− recA− double mutant (recA− strains generally give slightly lower levels of β-galactosidase; cf. Table 2). From these results, we conclude that derepression of the himA gene can occur in the absence of RecA if LexA is inactivated by mutation. Thus, the LexA protein is probably a repressor of the himA gene, and the role of RecA is to cleave LexA.

The himA gene appears to be repressed, directly or indirectly, by both its own gene product and the LexA repressor. These two repressors might act independently or coordinately. If they act independently, we might observe SOS induction under conditions in which the himA gene product is absent. However, UV irradiation of a strain carrying a himA deletion does not further increase the level of β-galactosidase above that found prior to induction (Table 3). As himA− strains are not UV sensitive, induction of the SOS pathway presumably occurs normally (2). We suggest that the HimA and LexA proteins probably act coordinately to repress the himA gene.

**DISCUSSION**

Autoregulation of the himA Gene. We have examined the regulation of the himA gene by two methods: (i) genetic fusion of the himA gene promoter to the lacZ gene and (ii) identification of radiolabeled himA gene product in autoradiograms of two-dimensional gels of cell extracts. Although these two techniques probe different stages in the expression of the himA gene, their results are consistent. The himA promoter is inducible by UV or mitomycin C, and its activity is repressed by LexA (Table 2). Moreover, induction of himA expression occurs after UV irradiation, mitomycin C treatment, or RecA441 introduction (hfl) (5, 21). These results suggest that the himA gene is a component of the SOS response to DNA damage.

**Table 2. SOS Induction of the himA gene**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>β-Galactosidase activity after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Strain</td>
</tr>
<tr>
<td>K5462</td>
<td>None</td>
<td>572</td>
</tr>
<tr>
<td>K5465</td>
<td>recA306</td>
<td>390</td>
</tr>
<tr>
<td>K5556</td>
<td>lexA3</td>
<td>517</td>
</tr>
<tr>
<td>K5504</td>
<td>recA441(tifl)</td>
<td>650</td>
</tr>
</tbody>
</table>

Procedures for cell growth and assay are described in the legend to Fig. 4, except for the inducing treatment. For mitomycin or thermal induction of recA441, mitomycin was added to 1 μg/ml or the culture was shifted to and maintained at 43°C, respectively. Assays were performed 8 hr after induction. The recA306, recA441, and lexA3 strains were prepared by P1 transduction.
gene—transcription for the lacZ fusion and translation for the gel—their results are in qualitative agreement. Because we infer that both transcription and protein synthesis from the himA gene are elevated in himA mutants, we conclude that the himA gene is negatively regulated by its gene product. At present, the most likely mechanism seems to be for HimA protein, IHFa, or the IHFa/β complex to repress transcription of himA; however, more complicated models are possible at this level of analysis.

We have also found that a mutation in the himD gene results in derepression of the himA gene. Our data implicate himD as the structural gene for the β subunit of IHF, although this point has not been rigorously established. Thus, there may be a symmetrical regulatory interaction in which HimA and HimD each participate in control of both the himA and himD genes, serving to maintain an appropriate stoichiometry between the two subunits of IHF.

**SOS Regulation.** Our results indicate that the himA gene is a component of the induced SOS response to DNA damage, normally repressed by LexA and induced by cleavage of LexA by activated RecA. The most direct interpretation of the data on auto- and SOS regulation is for HimA and LexA to be joint repressors. However, the apparent regulatory interaction between the himA and himD genes allows LexA, HimA, and HimD to regulate himA in a number of different ways, and further work will be required to clarify the control hierarchy.

Why is the himA gene regulated in coordination with genes known or suspected to be active in recovery from DNA damage? Although himA mutants are not UV sensitive (ref. 2; unpublished work), the HimA protein might be involved in some subtle way in repair of damaged DNA. However, we think that a more likely possibility is the capacity of HimA to facilitate the acquisition of completely new genetic material through site-specific recombination. Thus, induction of the himA gene increases the likelihood that potentially helpful genes can be acquired by a cell population in distress. Other SOS genes without an identified function might have a similar role (e.g., in transposon mobility). The mutagenic DNA repair associated with the SOS response might have also evolved in response to a need for more varied genetic potential. If present in organisms other than bacteria, an induced capacity for genetic variation might serve in a general way to enhance the rate of evolutionary change under conditions of environmental stress.

In addition to its possible role for the cell, the SOS induction of HimA also presumably helps the phage because it ensures a high level of the host component required for excision of a DNA during prophage induction.

We thank R. Crowl, A. J. Clark, and D. Friedman for helpful discussion. We also thank N. Story for excellent illustrations and photographic displays and C. Walker for preparation of this manuscript. This work was supported by National Institute of General Medical Sciences Grant GM 17078 and by National Science Foundation Grant PCM-7921724. H. I. M. was supported by California Division—Cancer Society Senior Fellowship S-34-81.