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Regulation of Lysyl-tRNA Synthetase Expression by Histone-Like Protein H-NS of *Escherichia coli*

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The *lysU* gene encoding lysyl-tRNA synthetase of *Escherichia coli* is normally silent at low temperatures and is expressed by certain metabolites and stimuli. A novel class of *lysU*-constitutive mutations were isolated by random insertion mutagenesis. These mutations nullified the *hns* gene encoding a histone-like protein, H-NS, and affected thermoregulation of *lysU*.

Escherichia coli has two distinct genes for lysyl-tRNA synthetase (LysRS), *lysS* and *lysU*, which is a rare exception to the rule of one synthetase per amino acid (8, 33; for a review, see reference 25). The two proteins have identical aminoacylation activities in vitro, so a rationale for this exception is not immediately obvious. The *lysS* gene is expressed constitutively under all measured growth conditions (13, 15). On the other hand, the *lysU* gene is normally silent at low temperatures. It is only expressed during growth at high temperatures, at a low external pH, or in the presence of certain metabolites such as L-alanine and L-leucine (1, 13, 14, 16). Recent studies have shed light on the control mechanism of *lysU* expression. For example, the leucine-responsive regulatory protein (Lrp), which controls the leucine regulon, also functions as a primary effector of *lysU* expression (9, 19, 23). However, it is not known how other stimuli induce *lysU* expression. Heat shock regulation of *lysU* expression eludes conventional reasoning, since thermoregulation of *lysU* appears to be independent of the heat shock factor σ^{32} and is distinct from Lrp-mediated regulation (19, 25).

Isolation of a novel class of *lysU*-constitutive mutants. Disruption of the *lysS* gene by insertion of a chloramphenicol acetyltransferase gene, *lysS1*::Cm^r, confers cold-sensitive growth to the cells because of the lack of expression of *lysU* at temperatures of <30°C (20). To study the mechanism of *lysU* expression, we have used the *lysS1* null mutant to select and characterize bypass mutations called *als* (abandonment of *lysS*) that derepressed *lysU* expression at 23°C and enabled the mutant cells to grow at low temperatures (20). Since most of the cold-resistant survivors have acquired mutations in the *lrp* gene (19), we used the strain RM545 (*lysS1*) lysogenic for the λRZ5 phage (2) carrying the wild-type *lrp* gene as a parental strain to isolate mutations other than in *lrp*. RM545 cells were infected with λ hop, λNK1323 (21), carrying the ΔTn*10* transposon, and two cold-resistant colonies, RM619 (*als-79*::ΔTn*10*) and RM620 (*als-81*::ΔTn*10*), were selected at 25°C and studied further.

P1 phage grown on these survivors cotransduced into strain RM545 the capacity for cold-resistant growth along with resistance to tetracycline (Tc^r). These markers did not segregate during crosses (100 of 100), suggesting that the ΔTn*10* transposon insertions are solely responsible for suppression of cold-sensitive growth in *lysS1*. Both the *als*::ΔTn*10* mutations were not linked to *lysU1*::Km^r (3) or *lrp*::ΔTn*10*Kan (19) by P1 transduction (data not shown). Instead, they cotransduced with *zch-3117*::Tn*10kan* (CGSC18551 [29]) at a frequency of 88% (49 to 56), showing that they are located at 27 min on the *E. coli* chromosome (Fig. 1).

The level of *lysU* expression was examined in MC4100 *als* mutants (19) by using a *lysU-lacZ* gene fusion carried on a λ prophage, λfRM460 (19). The gene fusion in λfRM460 contains a 2.7-kb piece of the 5′-flanking sequence and the first 1,296 coding nucleotides of *lysU* and was capable of synthesizing β-galactosidase in response to several inducers and environmental stimuli including thermoshift (19). The data showed an increase in the *lysU-lacZ* level of ca. 2.5-fold compared with that of the wild type in log-phase cultures at 28°C (Fig. 2, samples 1 and 2). Northern (RNA) blot analysis of *lysU* mRNA in the *als* mutants confirmed these results (data not shown). The degree of derepression of *lysU* in these *als* mutants became more evident on entry of the culture into stationary phase (data not shown).

Complementation of *als* mutations by the *hns* gene. The gene *hns*, encoding a protein called H-NS, maps to 27 min on the *E. coli* chromosome (10, 18; for a review, see reference 11). H-NS is the small (15.6-kDa), abundant, histone-like protein, previously purified as a major component of bacterial chromatin (11). To test whether the *als-79* and *als-81* mutations affect *hns*, we first examined the cotransduction frequency between these alleles and an *hns* deletion mutation tagged with a kanamycin resistance marker, Δ*hns*::Km^r (35). P1 phage was grown on strains RM619 (*als-79*::ΔTn*10*) and RM620 (*als-81*::ΔTn*10*) and used to infect to the Δ*hns*::Km^r strain, selecting for Tc^r colonies. None of the Tc^r transductants (0 of 200) was resistant to kanamycin, indicating that the *als* alleles are located at or near the *hns* gene.

Secondly, the plasmid pGY3 carrying a 0.95-kb *Eco*RI-*Hin*dIII DNA fragment that encodes only the wild-type *hns* gene (a derivative of pKT02 [34]) (Fig. 1) was transformed into strains RM619 and RM620. Ampicillin-resistant (Ap^r) transformants selected at 42°C failed to grow at temperatures of <30°C (complementation [Table 1]), whereas those transformants carrying the control plasmid grew at high and low temperatures. These results strongly suggested that *als-79* and *als-81* affect *hns*.

Finally, the Δ*hns*::Km^r allele was introduced into strain KK696 (*lysS1*) by P1 transduction. The resulting *lysS1*-Δ*hns*

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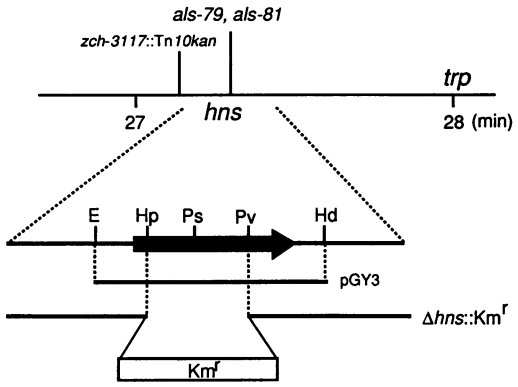

FIG. 1. Chromosomal structure of the *hns* region of *E. coli*. Genetic and physical maps are shown. The heavy line indicates the bacterial DNA segment cloned into the pGY3 plasmid. The arrow represents the location and orientation of the *hns* gene. The open box represents the insertion of the Km^r cassette. Only the relevant restriction sites are included: Ps, *Pst*I; Pv, *Pvu*II; E, *Eco*RI; Hd, *Hin*dIII; Hp, *Hpa*I.

double mutant grew at temperatures of <30°C, as did the original isolates, RM619 and RM620 (Table 1). These results led us to the conclusion that the *als-79* and *als-81* mutations affect the *hns* gene; hence, these mutations have been designated *hns-79* and *hns-81*, respectively.

Binding of H-NS to *lysU* DNA. H-NS binds to DNA as a dimer in a relatively nonspecific fashion in vitro (27, 30). However, recent studies have disclosed that H-NS binds preferentially to curved DNA (34). The 5′-flanking sequence of *lysU* contains short dA-dT stretches in a relatively periodic arrangement which mimics potential motifs of bent DNA (22). A theoretical nearest-neighbor method used to assess DNA

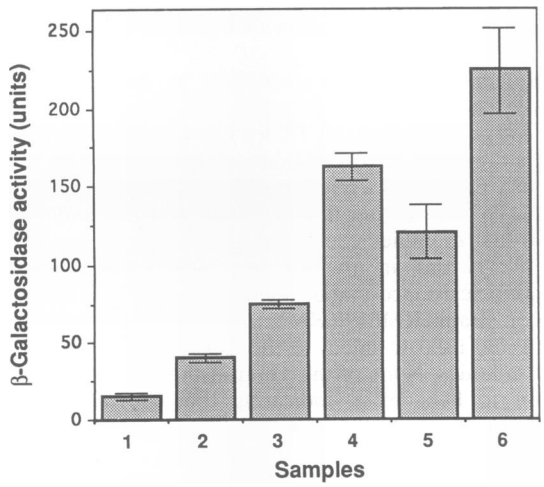

FIG. 2. Expression of the *lysU-lacZ* gene fusion upon temperature shift. Isogenic *hns*$^+$ and *hns* null strains lysogenic for λfRM460 were grown in LB medium at 28°C (samples 1 through 3) or exposed to 42°C for 60 min after exponential growth at 32°C (samples 4 through 6). Samples were assayed for β-galactosidase activities as previously described (19). Experiments were done independently at least four times, and the values were expressed as Miller units (24) with standard deviations. Host bacteria: samples 1 and 4, MC4100 (*hns*$^+$ *lrp*$^+$); samples 2 and 5, RM621-79 (*hns-79*::ΔTn*10*); samples 3 and 6, RM621-99 (*lrp-112*::ΔTn*10kan*).

TABLE 1. Complementation of *als-79* and *als-81* mutations[a]

Strain (relevant genotype)	Plasmid (relevant genotype)	Growth at 25°C[b]
KK696 (*lysS1*::Cm^r)	None	−
RM619 (*lysS1*::Cm^r *als-79*::ΔTn*10*)	None	+
	pGY3 (*hns*$^+$)	−
RM620 (*lysS1*::Cm^r *als-81*::ΔTn*10*)	None	+
	pGY3 (*hns*$^+$)	−
RM574 (*lysS1*::Cm^r Δ*hns*::Km^r)	None	+

[a] Plasmid pGY3 was transformed into strains indicated by selecting for Ap^r at 42°C. These transformants were purified and scored for growth at 25°C on LB agar plates containing the selective antibiotic.
[b] Symbols: +, growth; −, no growth.

curvature, based on the conformational energy calculations of De Santis et al. (5; for the validity of this method, see reference 6), predicts moderate DNA curvature over 160 nucleotides between 130 and 290 bp upstream from the start codon (data not shown). This sequence appears to overlap with the Lrp-binding region (23).

We tested H-NS binding to the 5′-flanking sequence of *lysU* by using the gel retardation assay. H-NS protein was purified from *E. coli* JA221 *recA* cells by the published procedures (34). Briefly, cell lysates were fractionated by stepwise precipitation with ammonium sulfate, and the 40 to 60% saturated ammonium sulfate fraction was subjected to two-step chromatography with phosphocellulose P11 (Whatman) and fast protein liquid chromatography Mono-Q (LKB) columns. The resulting H-NS protein was >99% pure. A 540-bp DNA fragment containing the 160-bp bent sequence was generated by *Eco*RI and *Bam*HI digests of pRP92 DNA (19), and the 5′ termini were labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. A total of 10 ng of ^{32}P-labeled DNA and 5, 10, 20, or 40 pmol of purified H-NS protein were mixed, in the presence or absence of competitor DNAs, in a gel shift buffer (50 μl) containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM EDTA, 5 mM $MgCl_2$, 7 mM β-mercaptoethanol, 1 mM phenylmethanesulfonyl fluoride, and 10% glycerol. After incubation for 30 min at room temperature, 40 μl of the reaction mixtures was subjected to electrophoresis in 5% polyacrylamide gels and analyzed by using the BioImage Analyzing System 2000 (Fuji Film, Tokyo, Japan). H-NS protein clearly bound the *lysU* DNA probe (Fig. 3, lanes 2 through 5). Note that 20 or 40 pmol of H-NS protein yielded a band that migrates more slowly than that formed with 5 or 10 pmol of H-NS. Two competitor DNAs were used to challenge the *lysU* probe for binding: one, a synthetic noncurved DNA (233 bp), prepared from plasmid pS15-12 (34) by *Eco*RI-*Hin*dIII digestion, and the other, a curved DNA, prepared from plasmid pCU26 (32) which contains a ca. 830-bp bent DNA of the *proU* operon. A 25- or 100-fold excess amount of curved DNA competed for H-NS binding (Fig. 3, lanes 8 and 9), whereas the same amount of noncurved DNA did not (Fig. 3, lanes 6 and 7), showing that H-NS does not bind DNA nonspecifically under these experimental conditions. These results indicated that H-NS binds relatively specifically to a probably bent DNA region of *lysU*. The H-NS dose dependency for *lysU* DNA binding (Fig. 3, lanes 1 through 5) confirms the previous report that the DNA-binding affinity of H-NS is relatively lower than those of other DNA-binding proteins (34).

Partial involvement of H-NS in thermoregulation of *lysU*. The above observations suggested that H-NS functions as a transcriptional repressor of the *lysU* gene. We then asked whether the *hns* mutation affects thermoregulation of *lysU*

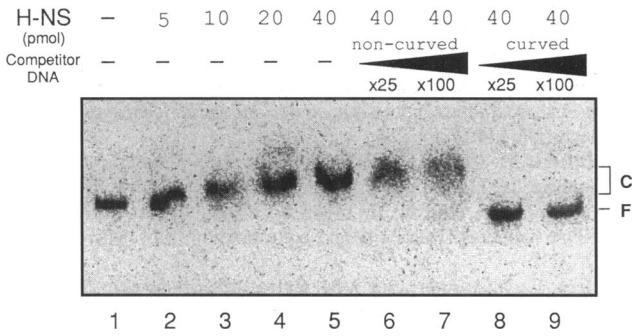

FIG. 3. DNA mobility shift analysis of the *lysU* promoter fragment. A 540-bp 5′-flanking DNA of *lysU* (10 ng) was end labeled with ^{32}P, incubated with (5, 10, 20, or 40 pmol) or without (−) purified H-NS and the indicated competitor DNAs, and fractionated by electrophoresis through a 5% polyacrylamide gel. Experimental conditions and procedures are essentially the same as described by Ricca et al. (26; see text). Competitor DNAs: lanes 6 and 7, 0.25 and 1.0 μg of noncurved DNA prepared from plasmid pS15-12, respectively; lanes 8 and 9, 0.25 and 1.0 μg of curved DNA prepared from plasmid pCU26, respectively. C and F, complex and free DNAs, respectively.

expression. MC4100 *hns* (or *lrp* for control) cells lysogenic for λfRM460 carrying a *lysU-lacZ* fusion were grown in Luria-Bertani (LB) medium at 28 or 32°C and shifted to 42°C for 60 min and then assayed for β-galactosidase activity as previously described (19). The *lysU-lacZ* level at 28°C was increased 2.5- and 4.7-fold by *hns* and *lrp* mutations, respectively, and further increased upon thermoshift of cell cultures to 42°C (Fig. 2). The increase of thermoinduction in *hns* and *lrp* strains was both 3-fold (compare samples 2 and 5 or 3 and 6), much lower than that in the wild type (approximately an 11-fold increase between samples 1 and 4). These results indicate that *hns* and *lrp* null strains are capable of thermal expression of *lysU*, but only partially with respect to the fold induction. These apparent defects reflect, at least in part, derepression of *lysU* at 28°C by *hns* and *lrp* mutations, revealing indirect (or direct) involvement of both proteins in thermoregulation. However, the level of *lysU* expression at 42°C was markedly different in both strains; the *lrp* null strain acquired the wild-type level at 42°C, while the *hns* null strain showed a significant decrease in the *lysU-lacZ* level at 42°C even though there is an increase of the basal β-galactosidase level at 28°C in this strain (Fig. 2). These results could be interpreted as indicating that *lysU* is normally repressed by H-NS at least in part, and this repression is abolished in response to thermoshift. In other words, the thermal induction of *lysU* is only partial in the *hns* mutant and there probably is another regulatory factor, other than H-NS (Lrp protein?), of *lysU* under heat shock conditions.

The H-NS protein has been characterized biochemically by several groups. It binds tightly to double-stranded DNA under physiological conditions in vitro and increases the thermal stability of DNA (11). Although purified H-NS protein binds relatively nonspecifically to DNA and can affect transcription from a number of promoters in vitro (27, 30), recent genetic studies have revealed that mutations in the *hns* gene exhibit relatively specific phenotypes (11). In fact, mutations altering expression of some unlinked genes, i.e., *bglY* (4), *cur* (7), *pilG* (31), *osmZ* (12), and *virR* (17), have been identified or are very likely to affect H-NS activity (10, 11, 18). This study revealed that *lysU* expression is specifically derepressed by *hns* mutations. Thus, H-NS does not seem to be restricted to a purely structural role but appears to influence gene expression, probably through the maintenance of DNA topology.

The work presented here demonstrated that thermoregulation of *lysU* is controlled at least in part by H-NS. This finding is consistent with the notion that *lysU* has some heat shock regulation but is independent of σ^{32} (for a review, see reference 25). There is increasing evidence that DNA topology in bacterial cells changes in response to environmental signals such as osmolarity, anaerobicity, temperature, and nutrient status, and these alterations involve H-NS activity (11). For instance, transcription of a regulatory cistron, *papI*, in the pilus-adhesin gene cluster of uropathogenic *E. coli* strains is thermoregulated by a silencer action of H-NS (10). It is also noteworthy that the *cadBA* operon located about 2 kb upstream of *lysU* is regulated in a fashion similar to *lysU* in response to harsh environmental conditions such as low pH and anaerobiosis (for a review, see reference 25). Recently, Bennett and colleagues have demonstrated that *cadA* gene expression can be modulated by H-NS (28). Therefore, one can speculate that the regulatory regions of the *lysU* and *cadBA* genes are assembled into common microdomains of nucleoid, in association with H-NS (and some other proteins), that are susceptible to environmental stimuli (sensory nucleoid structure). A preliminary experiment suggested that low pH inducibility of *lysU* is also affected by the *hns* null mutation (unpublished observation). A change in temperature, pH, or anaerobicity may thereby cause altered conformational properties of DNA, and local alterations in topological microdomains of this sensory nucleoid structure may influence protein-DNA interactions, thereby modulating expression of *lysU* and *cadBA* genes coordinately. In summary, it is now obvious that thermoregulation of *lysU* is distinct from heat shock regulation controlled by σ^{32} and is mediated, at least in part, by the *E. coli* nucleoid protein H-NS.

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