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Genetics

his operons of *Escherichia coli* and *Salmonella typhimurium* are regulated by DNA supercoiling

(*hisW/hisU*/chromosomal superhelicity/tRNA metabolism/DNA gyrase)

KENNETH E. RUDD*† AND ROLF MENZEL‡§

*Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, and ‡Laboratory of Molecular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The *hisW* mutations of *Salmonella typhimurium* are highly pleiotropic mutations that elevate *his* operon expression, reduce *ilv* gene expression, alter stable RNA metabolism, and confer defective growth properties. The *hisW* mutations are highly linked to a naladixic acid-resistant *gyrA* mutation of *S. typhimurium*. Multicopy recombinant plasmids containing the *Escherichia coli gyrA* gene are able to complement both the growth defects and the elevated *his* operon expression associated with the *hisW* mutations. We conclude that *hisW* mutations are alleles of the *gyrA* gene. The *hisU1820* mutant of *S. typhimurium* exhibits many of the same phenotypes as *hisW* mutants. Several lines of evidence, including high transduction linkage to *recF*, suggest that *hisU1820* is an allele of *gyrB*. Finally, well-characterized *gyrA* and *gyrB* alleles of *E. coli* are also *his* regulatory mutations. We propose that a wild-type degree of chromosomal superhelicity is required for maximal production of histidyl-tRNA and normal *his* operon regulation.**

Regulatory mutations at several loci (*hisR,S,T,U,W*) that are unlinked to the *his* operon result in the increased expression of the *his* operon in *Salmonella typhimurium* and *Escherichia coli* (1–3). These mutations all affect histidyl-tRNA metabolism, resulting in either lowered levels of histidyl-tRNA or, in one case (*hisT*), undermodified histidyl-tRNA (3–5). When the intracellular level of histidyl-tRNA is low, translation of the *his* leader peptide is inefficient, impeding formation of the *his* attenuator and allowing increased transcription of the *his* structural genes (6).

Mutations *hisW3333*(Cs)(cold sensitive, lethal) and *hisU1820*(Ts)(thermosensitive, lethal) of *S. typhimurium* are highly pleiotropic. In addition to the *his* regulatory phenotype, these mutants have lowered levels of several tRNA species (including histidyl-tRNA) (4), reduced levels of enzymes involved in isoleucine–valine biosynthesis (7, 8), and are defective in the normal control of stable RNA accumulation (9–11). We show that the *S. typhimurium hisW* mutations are alleles of *gyrA*, the structural gene for the A subunit of DNA gyrase. In addition, we provide evidence that the *hisU1820*-(Ts) mutation is an allele of the *gyrB* gene, the structural gene for the B subunit of DNA gyrase. Further, we were able to show that well-characterized *gyrA* and *gyrB* alleles of *E. coli* are *his* constitutive regulatory mutations causing phenotypes similar to those of the *hisW3333*(Cs) and *hisU1820*(Ts) mutations of *S. typhimurium*.

DNA gyrase is the enzyme responsible for maintaining the bacterial chromosome in a state of negative superhelicity (12, 13). Chromosomal superhelicity has been implicated in the control of gene expression since the activities of many promoters are modulated by changes in the superhelical density of the template DNA both *in vivo* and *in vitro* (reviewed in refs. 13–15). In particular, the *hisR* multigene tRNA operon (encoding the sole *S. typhimurium* $tRNA^{His}$ gene) is transcribed efficiently *in vitro* only when the DNA template is supercoiled (Lionello Bossi, personal communication). We suggest that the *his* constitutive phenotypes of the *hisW(gyrA)* and *hisU(gyrB)* mutations are a consequence of reduced transcription initiation at the *hisR* promoter *in vivo*. This implies that expression of the $tRNA^{His}$ gene, and consequently the *his* structural gene operon, is controlled by DNA supercoiling.

MATERIALS AND METHODS

Bacteria, Media, and Growth Conditions. The bacterial strains used in this study are listed in Table 1 and below. The original strains used as sources of *hisW* mutations are TA800 (*hisW1821*), SB634 (*hisW1824*), TA805 (*hisW1825*), SB234 (*hisW1295*), SB230 (*hisW1291*), KRS2316 (*hisW1509*), and JL681 [*hisW3333*(Cs)]. JL681 was obtained from John Ingraham; the remainder were obtained from Phil Hartman. Minimal glucose and rich media used were described (19). Ampicillin, tetracycline, kanamycin, and naladixic acid (Nal) were used at concentrations of 100, 25, 30, and 10 μg/ml, respectively. L-Amino acids and other nutritional supplements were added at the concentrations recommended by Davis *et al.* (20). All incubations were carried out at 37°C unless otherwise noted. Cold-resistant growth is defined as the ability to grow on rich-medium plates at room temperature; thermoresistant growth is defined as the ability to grow on minimal-glucose plates at 42°C.

Chemicals. Antibiotics, *o*-nitrophenyl-β-D-galactoside, amino acids, 1,2,4-triazole-3-alanine (triazole alanine), and 3-amino-1,2,4-triazole were obtained from Sigma. Restriction enzymes, T4 DNA ligase, and mung bean exonuclease were obtained from either New England Biolabs or Bethesda Research Laboratories.

Genetic and Biochemical Techniques. Histidinol dehydrogenase (EC 1.1.1.23) was assayed using the toluenized cell method of Ciesla *et al.* (21). β-Galactosidase assays were performed as described by Miller (22). Triazole alanine plate tests were performed as described (19) except that 3-amino-1,2,4-triazole was used at 1.0 mM and only required amino acids were added. *S. typhimurium* transduction crosses were performed using bacteriophage P22 (HT105/1, *int-201*) as described by Maloy and Roth (23). *E. coli* transduction crosses using P1vir, genetic transformations, and plasmid DNA isolations were performed as described by Silhavy *et al.* (24). All *S. typhimurium* transformations were performed using strains derived from the transformable strain TR1859.

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Abbreviations: Nal, naladixic acid; Cs, cold sensitive lethal; Ts, thermosensitive lethal.

†To whom reprint requests should be addressed.
§Present address: E. I. du Pont de Nemours and Co., Experimental Station E328/155, Wilmington, DE 19898.

Table 1. Bacterial strains

Strains	Relevant genotype	Source or reference
E. coli		
KNK453	*gyrA43*(Ts)	Ref. 16
KRE447	*gyrA*$^{+}$*	This study
KRE449	*gyrA43*(Ts)*	This study
K124	*himA*$^{+}$ *gyrB*$^{+}$	Ref. 17
K1891	*himA*$^{+}$ *gyrB230*†	Ref. 17
K2030	*himA82 gyrB*$^{+}$	Ref. 17
K1972	*himA82 gyrB230*	Ref. 17
RM146	*gyrA204*(Nal)	This study
S. typhimurium		
KRS112	*rnpA1*(Ts)	This study
KRS1717	*gyrA1*(Nal)	This study
KRS2425	*hisW3333*(Cs)‡	This study
KRS2426	*hisW*$^{+}$‡	This study
KRS2446	*hisW3333*(Cs) *hisD9953*::MudJ‡§	This study
KRS2447	*hisW*$^{+}$ *hisD9953*::MudJ‡	This study
KRS2304	*recF521*::Tn*5*	This study
TR6673	*hisU1820*(Ts)	John Roth
TR5525	*dnaA1*(Ts)	John Roth

Additional strains used in this study are listed in *Materials and Methods* and in Table 3.
*Isogenic pair constructed using *zei-564*::Tn*10*.
†Isolated as a *himB* mutation (17, 18).
‡Isogenic pairs constructed using *zeh-754*::Tn*10*.
§See *Materials and Methods* for definition of MudJ.

Genetic manipulations of *gyrA* and *hisW* mutations were facilitated by the use of transductionally linked Tn*10* elements *zei-564*::Tn*10* (in RM101, 15% linked to *E. coli gyrA*) and *zeh-754*::Tn*10* (in TT5371, 90% linked to *S. typhimurium hisW*). The *S. typhimurium hisU1820*(Ts), *dnaA1*(Ts), and *rnpA1*(Ts) mutations were genetically manipulated using *zia-748*::Tn*10* (in TT3920, 15% linked to *rnpA*). MudI1734 is a stable, kanamycin-resistant, ampicillin-sensitive derivative of MudI1(*lac*, Ap) constructed by Malcolm Casadaban and coworkers (25) and is referred to as MudJ throughout this paper. *S. typhimurium his* operon expression was monitored using the single copy, chromosomal *hisD9953*::MudJ operon fusion, which places the β-galactosidase gene under the control of the *his* promoter and attenuator.

Isolation of *S. typhimurium gyrA1, rnpA1,* and *recF521*::Tn*5* Mutants. KRS1717 [*gyrA1*(Nal)] was isolated as a spontaneous mutant resistant to Nal at 10 μg/ml. KRS2304 (*recF521*::Tn*5*) was isolated by transducing TR6673 [*hisU1820*(Ts)] to temperature resistance using bacteriophage P22 grown on a pool of independent Tn*5* insertion mutations. Kanamycin-resistant, temperature-resistant transductants were screened for UV sensitivity. The *recF521*::Tn*5* insertion is unusually stable since 300 of 300 kanamycin-resistant transductants of LT2 also became UV sensitive. KRS112 [*zia-748*::Tn*10*, *rnpA1*(Ts)] was isolated by hydroxylamine-induced localized mutagenesis (20). The RnpA phenotype was determined by observing the accumulation of tRNA precursors (ref. 26; Lionello Bossi and K.E.R., unpublished results).

Plasmid Constructions. An 11-kilobase (kb) *Bam*HI DNA restriction fragment containing the *E. coli gyrA* gene was subcloned from pMK90 (27) into pKO100 (28) to obtain pRM378 (see Fig. 1). The pRM386 and pRM389 subclones were obtained following the partial *Hin*dIII digestion of pRM378. The pRM387 plasmid was derived by inserting a blunt-ended chloramphenicol acetyltransferase cassette (from pBR325) into pRM386 at the unique *Sst* I site present at the 19th codon of *gyrA* (R.M. and M. Gellert, unpublished results). Prior to ligation of the chloramphenicol acetyltransferase cassette with T4 DNA ligase, the *Sst* I restriction cut was blunted with mung bean nuclease, which removed ≈200 base pairs.

RESULTS

The *hisW* Mutations Are Highly Linked to Nal Resistance by P22 Transduction. We have determined the genetic linkage between the *gyrA1*(Nal) and *hisW3333*(Cs) mutations of *S. typhimurium* by P22 transduction. Using P22 phage grown on KRS1717[*gyrA1*(Nal)], Nal-resistant transductants of JL681-[*hisW3333*(Cs)] were selected. Nal-resistant transductants also inherited the *hisW*$^{+}$ (cold resistance) phenotype 598 of 600 (99.7%). To confirm that selective pressure for Nal resistance was not causing preferential survival of *hisW*$^{+}$ cotransductants, we also determined the *gyrA1*(Nal) *hisW3333*(Cs) genetic linkage using both alleles as unselected markers in a three factor cross. In this experiment a nearby tetracycline-resistance transposable element (*zeh-754*::Tn*10*, 92% linked to *hisW*) was used as the selected marker. Tetracycline-resistant, Nal-resistant transductants of JL681-[*hisW3333*(Cs)] were cold resistant (374 of 375), again indicating a *gyrA1*(Nal) *hisW3333*(Cs) linkage of 99.7%. In addition, linkage of *gyrA1*(Nal) to all of the *hisW* alleles was determined. The triazole alanine-(a toxic histidine analogue) resistant phenotypes associated with the *hisW* mutations (*hisW1509, hisW1824, hisW1825, hisW1821, hisW1291,* and *hisW1295*) (1–3) are between 92% and 98% cotransducible with *gyrA1*(Nal).

Complementation of *gyrA* and *hisW* Phenotypes with Plasmids Containing the *E. coli gyrA* Gene. Fig. 1 shows the *E. coli* DNA fragments containing either an intact *gyrA* gene or various deletion derivatives that were used to transform several *E. coli* and *S. typhimurium gyrA* and *hisW* mutants. The *gyrA* gene has been sequenced, and its position and orientation relative to the restriction sites depicted are known (R.M. and M. Gellert, unpublished results; Stephen L. Swanberg and James C. Wang, personal communication). The recombinant plasmids pRM378 and pRM386 contain 11 kb and 6 kb of *E. coli* DNA, respectively. Both plasmids complement all of the *gyrA* and *hisW* phenotypes tested, including Nal resistance, thermosensitive- and cold-sensitive growth, small colony size, and constitutive *his* operon derepression (see Tables 2 and 3). The DNA insert present in pRM386 contains the intact *gyrA* coding sequence, 0.5 kb of downstream DNA, and 2 kb of DNA that precedes *gyrA*. The region distal to the carboxyl terminus of *gyrA* but upstream of the *Hin*dIII site (see Fig. 1) has the potential to code for a peptide with a maximum molecular weight of 18,000 and is thus unlikely to code for complementing activity (Stephen L. Swanberg and James C. Wang, personal communication). The pRM389 plasmid contains the first half of the *gyrA* gene

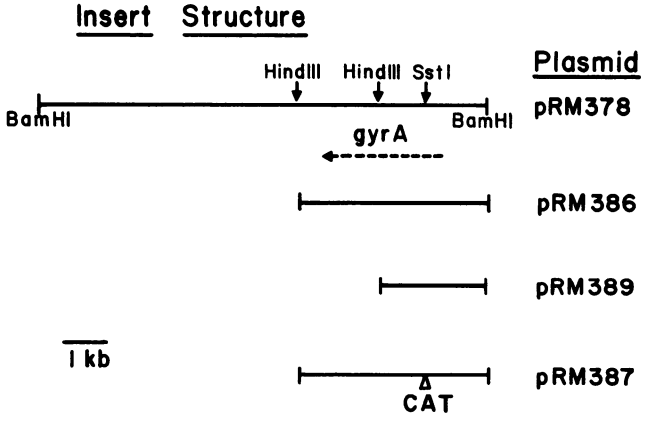

FIG. 1. Structure of cloned *E. coli gyrA* DNA.

in addition to the 2 kb of DNA that precedes the *gyrA* coding sequence. This plasmid has no *gyrA* and *hisW* complementing activity (Table 2), thus reducing the possibility that a gene other than *gyrA* is responsible for the complementation. Additionally, pRM387, in which a chloramphenicol acetyltransferase cassette has replaced ≈200 base pairs of *gyrA* DNA, also fails to show complementing activity (Tables 2 and 3). The pRM387 plasmid has the same downstream sequences present as does pRM386, the shortest complementing plasmid. To demonstrate that all of the previously studied *hisW* mutations of *S. typhimurium* constitute one complementation group, we transformed strains containing the *hisW* mutations (listed above) with pRM386 and pRM387 DNA. The small colony phenotypes of *hisW1509* and *hisW1824* and the *his* operon constitutive derepression phenotypes of all the mutants were completely reversed by pRM386 (*gyrA*$^+$) but not by pRM387 (*gyrA*::CAT). The genetic linkage data above, together with the observed complementation, indicate that all *hisW* mutations are alleles of *gyrA*.

The *hisU1820* Mutation of *S. typhimurium* Is Probably an Allele of *gyrB*. Although complementation of the *hisU1820*-(Ts) mutation with cloned *gyrB* DNA has not been achieved, negative complementation results, phenotypic analysis, and genetic linkage studies indicate that *hisU1820*(Ts) is an allele of the *gyrB* gene of *S. typhimurium*. A clone of the *E. coli gyrB* gene that is capable of complementing known *gyrB* mutations has not been constructed. The pleiotropic *hisW3333*(Cs) and *hisU1820*(Ts) mutations cause a variety of strikingly similar phenotypes (see *Discussion*).

In *E. coli*, the genes near *gyrB* are (in order) *gyrB*, *recF*, *dnaN*, *dnaA*, *rpmH*, and *rnpA* (29, 30). Several *hisU* mutations of *S. typhimurium* have been shown to be *rnpA* (the structural gene for the protein subunit of RNase P) mutations, but *hisU1820*(Ts) is RnpA$^+$ (31). Using plasmids containing cloned DNA of *S. typhimurium*, which can complement *recF*, *dnaN*, *dnaA*, and *rnpA* mutations of both *S. typhimurium* and *E. coli* (Russell Maurer and K.E.R., unpublished results), we were unable to complement the *hisU1820*(Ts) mutation of *S. typhimurium*. Several restriction sites in the region (depicted in Fig. 2) are conserved between *E. coli* and *S. typhimurium*. The gene orders of the two organisms in this region are identical, and the cloned *S. typhimurium* DNA should contain only half of the *gyrB* gene. We also determined the P22 transductional linkages of *recF521*::Tn*5* to *rnpA1*(Ts), *dnaA1*(Ts) (32), and *hisU1820*(Ts). The results shown in Fig. 2 are consistent with the *E. coli* gene order in this region. The gene assignments of *recF521*::Tn*5* and *rnpA1*(Ts) were confirmed by complementation studies using cloned *E. coli* DNA (K.E.R., unpublished results). The assignment of *dnaA1*(Ts) was confirmed by complementation and marker rescue using cloned *S. typhimurium* DNA (Russell Maurer and K.E.R., unpublished results). We have also noted that growth of the *hisU1820* mutant is 10-fold more sensitive than wild type to the gyrase B subunit-specific antibiotic coumermycin A1. These results strongly suggest that the *hisU1820*(Ts) mutation of *S. typhimurium* is an allele of the *gyrB* gene.

Table 2. Complementation of *gyrA* and *hisW* phenotypes with *E. coli* cloned DNA

Plasmids	Phenotype			
	E. coli		*S. typhimurium*	
	gyrA204	*gyrA43*	*gyrA1*	*hisW3333*
pRM378(*gyrA*$^+$)	Nal^s	Tr	Nal^s	Cr
pRM386(*gyrA*$^+$)	Nal^s	Tr	Nal^s	Cr
pRM389(Δ*gyrA*)	Nal^r	Ts	Nal^r	Cs
pRM387(*gyrA*::CAT)	Nal^r	Ts	Nal^r	Cs
pKO100(vector)	Nal^r	Ts	Nal^r	Cs

Plasmid insert structures are depicted in Fig. 1. Strains used for transformation with various plasmids were RM146, KRE449, KRS2425, and KRS1717 (see Table 1 for genotypes). Plasmids had no effect on the phenotypes of wild-type strains. All transformants were selected and maintained on rich medium plus ampicillin at permissive growth temperatures [32°C for *gyrA43*(Ts); 37°C for *hisW3333*(Cs)]. Resistance (r) or sensitivity (s) to Nal was determined by the ability to form single colonies on Nal plates. Cold-resistant growth (Cr) and thermoresistant growth (Tr) were assessed. The small-colony phenotypes associated with both *gyrA43*(Ts) and *hisW3333*(Cs) at permissive temperatures were also complemented by the *gyrA*$^+$ plasmids.

Table 3. Complementation of the *his* operon derepression phenotype of *hisW3333*(Cs) using *E. coli gyrA* plasmids

S. typhimurium strains	*E. coli* plasmids	
	pRM386(*gyrA*$^+$)	pRM387(*gyrA*::CAT)
KRS2447(*his*$^+$)	59.8 ± 1.0	56.5 ± 2.3
KRS2446(*hisW3333*)	58.7 ± 1.0	208.7 ± 1.4*

Values given are units of β-galactosidase activity (nmol/min per ml) per OD_{650}. The *S. typhimurium* strains assayed were KRS2524-(KRS2447/pRM386), KRS2523(KRS2447/pRM387), KRS2522-(KRS2446/pRM386), and KRS2521(KRS2446/pRM387). Cells were grown in rich medium at 37°C. Assays were performed at 30°C. All of the β-galactosidase activity is due to the expression of the *hisD9953*::MudJ operon fusion.

*This value corresponds to a *his* operon derepression ratio of 3.7. This ratio is the same for KRS2446(no plasmid) at the growth-permissive temperature (37°C).

E. coli *gyrA* and *gyrB* Mutations with Known Defects in Supercoiling Are *his* Regulatory Mutations. If the *his* constitutive phenotype of *hisW*(*gyrA*) mutants is a result of reduced chromosomal superhelicity caused by a reduction in DNA gyrase activity, then the well-characterized DNA gyrase mutants of *E. coli* should also be constitutive for the expression of the *his* operon. We constructed an isogenic pair of strains containing either the *gyrA*$^+$ or the *gyrA43*(Ts) (16) alleles (see Table 1). These strains were tested for the triazole alanine resistance phenotype that is associated with *his* constitutive mutations (1, 2), and we assayed their histidinol dehydrogenase activity. In a similar fashion, we examined *his* operon expression in four isogenic strains containing either wild-type or mutant alleles of *himA* (encoding the A subunit of integration host factor) and *gyrB*. These strains have been examined for their ability to introduce supercoils into superinfecting λ DNA (17, 18). Table 4 shows that the presence of either the *gyrA43*(Ts) or the *gyrB230* alleles confers resistance to triazole alanine and causes a substantial elevation of histidinol dehydrogenase activity. This indicates that the *gyr* alleles of *E. coli* are *his* constitutive regulatory mutations. The presence of the *himA82* mutation causes a 2-fold elevation in *his* expression in either the presence or the absence of the *gyrB230* mutation; however the *himA* allele alone does not confer triazole alanine resistance (Table 4). This is consistent with the exacerbation of the *gyrB* supercoiling-defective phenotype by the *himA* mutation that was observed *in vivo* (17, 18), thus demonstrating a link between a defect in supercoiling and an elevation in the level of *his* expression.

DISCUSSION

We report that the *S. typhimurium hisW* mutations are highly linked to *gyrA*, are complemented by a *gyrA*$^+$ plasmid, and are, therefore, allelic to *gyrA*. Additionally, the *S. typhimurium hisU1820*(Ts) mutation is highly linked to *recF* and cannot be complemented by cloned *dnaA*$^+$, *dnaN*$^+$, *recF*$^+$, *rpmH*$^+$, or *rnpA*$^+$ DNA. Phenotypically, *hisW3333*(Cs) and *hisU1820*(Ts) are similar to each other (3, 7–11) and to *E. coli gyr* mutants (see below). Finally, *E. coli gyrA* and *gyrB* alleles

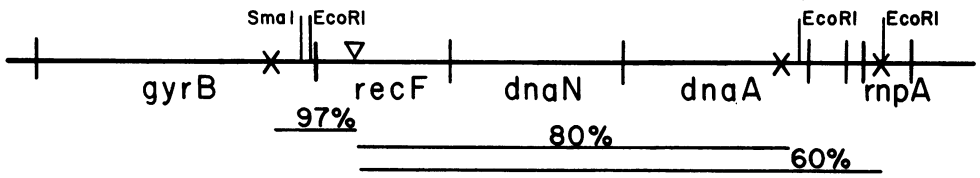

FIG. 2. *gyrB recF* region of the *S. typhimurium* chromosome. Cotransduction data, restriction analysis, and complementation analysis are consistent with this organization of genes in the *gyrB* region of the *S. typhimurium* chromosome (identical to *E. coli*, see *Results* and refs. 29, 30). The following mutations were used to determine cotransduction frequencies: *hisU1820*(Ts)(*gyrB*), *recF521*::Tn*5*, *dnaA1*(Ts), and *rnpA1*(Ts) (see *Results*). Either kanamycin resistance (Tn*5*) or temperature resistance was selected. Cotransduction data [selected marker in ()]: (*gyrB*)-*recF*, 146 of 150; *gyrB*-(*recF*), 98 of 100; *dnaA*-(*recF*), 79 of 100; (*rnpA*)-*recF*, 111 of 200; *rnpA*-(*recF*), 126 of 200.

are shown to be *his* regulatory mutations. Thus, it appears likely that the degree of chromosomal superhelicity regulates expression of the *his* operons of both *E. coli* and *S. typhimurium*. We believe that this is due to a dependence of the *hisR* ($tRNA^{His}$ gene) promoter on supercoiling for maximal activity. This model is supported by the demonstration of a very strong supercoiling requirement for the *in vitro* transcription of the *hisR* promoter (Lionello Bossi, personal communication). Reduced levels of histidyl-tRNA allow readthrough of the *his* attenuator and cause elevated *his* expression (4, 6). The *hisW* and *hisU1820*(Ts) mutations reduce total (aminoacylated and nonaminoacylated) $tRNA^{His}$ levels by ≈50%, resulting in a severalfold increase in *his* operon expression (4). Thus, expression of the *his* operon is a sensitive indicator of defects in $tRNA^{His}$ production due to defective gyrase function.

Identification of *gyr* mutations as *his* regulatory mutations will facilitate genetic analysis of *gyr* mutations, particularly in the isolation of mutations with supercoiling defects manifested during exponential growth. It should be possible to correlate *his* expression to the extent of chromosomal DNA supercoiling, at least in the domain containing the *hisR* gene. This may provide a method to easily quantitate the degree of supercoiling defect *in vivo*.

The *E. coli gyrB230* mutant is defective in its ability to supercoil superinfecting λ DNA, and this defect is exacerbated by *himA82* (17, 18). The *himA82* mutation alone did not detectably impair *in vivo* supercoiling function in these experiments. In contrast to this synergistic effect, we observed a 2-fold effect of *himA82* on *his* expression in both the presence and absence of *gyrB230* (Table 4). The $himA^+$ function responsible for the *his* phenotype could be positive control of *gyrA* expression (17), facilitation of DNA gyrase action (18), or gyrase-independent regulation of *his* expression. Nonetheless the *himA gyrB* double mutant is more defective in both supercoiling function and *his* regulation than the *gyrB* single mutant, consistent with the model that *his* expression is controlled by the degree of chromosomal superhelicity at the *hisR* locus.

Table 4. The *his* operon derepression phenotypes of *E. coli gyr* mutations

E. coli strains	*his* operon derepression ratio	Triazole alanine phenotype
KRE447($gyrA^+$)	1.0	Sensitive
KRE449(*gyrA43*)	3.6	Resistant
K124($himA^+gyrB^+$)	1.0	Sensitive
K2030(*himA82* $gyrB^+$)	1.9	Sensitive
K1891($himA^+$ *gyrB230*)	6.8	Resistant
K1972(*himA82 gyrB230*)	16.2	Resistant

E. coli cells were grown in minimal glucose medium at 37°C and assayed for histidinol dehydrogenase activity. The activities (in units, 1 unit = nmol/hr per ml) for *gyr* wild-type strains (defined as 1.0) were: KRE447, 3.9 units/OD_{650}; K124, 4.8 units/OD_{650}. The value for wild-type *S. typhimurium* in this assay was 3.1 units/OD_{650}. The triazole alanine (a toxic histidine analogue) phenotype was determined.

It has been repeatedly suggested (3–5, 33) that the *hisW* and *hisU1820*(Ts) mutations confer defects in tRNA modification; however, no evidence of this has ever been presented. In fact, this interpretation has been discounted due to the fact that rRNA accumulation is also affected (10). Ames has alternatively suggested (33) a defect in tRNA biosynthesis as a possible explanation for the observation that several tRNAs are affected by the *hisW* mutations. The levels of several specific tRNAs (in addition to $tRNA^{His}$) are low and the overall accumulation of 4S RNA is reduced in the *hisW-3333*(Cs) and *hisU1820*(Ts) mutants (10, 11, 26).

A definitive analysis of the *in vivo* effects of supercoiling on stable RNA synthesis has been elusive. *In vitro*, both tRNA and rRNA transcription can be stimulated by template supercoiling (34, 35). However, *in vivo* analyses have been contradictory and inconclusive (34, 36). Analysis of a variety of *gyr* mutants whose exponential growth and temperature-shifted gyrase defects can be graded on the basis of quantitative *his* operon expression will help identify problems with allele specificity or phenotypic leakiness of specific *gyr* mutations. To definitively sort out primary and secondary effects of *gyr* mutations will require very thorough investigations.

We have not yet concluded our analysis of the other $RnpA^+$ alleles of *hisU*, and it is possible that some of them could be *dnaA* mutations since the *dnaA* protein has been shown to be a transcriptional regulatory protein (37, 38). The failure of $dnaA^+$ plasmids to complement *hisU1820*(Ts) makes it unlikely that it is a *dnaA* allele.

The phenotypes of the *hisW* mutants, particularly the well-characterized *hisW3333*(Cs), can now be attributed to defects in DNA gyrase function. The defects in isoleucine–valine biosynthesis caused by the *hisW3333*(Cs) and *hisU1820*(Ts) mutations (7, 8) are consistent with the observation that the *gyrB230* and *gyrB231* alleles of *E. coli* confer isoleucine auxotrophy in the presence of the *himA82* mutation (18). The failure of the *hisU1820*(Ts) mutant to control stable RNA accumulation during carbon and energy starvation was attributed to a problem with RNA degradation, not synthesis (11). This may reflect the highly pleiotropic nature of the *gyr* mutations; the expression of certain RNase genes may be controlled by supercoiling. The effects of the *E. coli gyr* mutations on DNA synthesis are consistent with the observation that both *hisW3333*(Cs) and *hisU1820*(Ts) form filamentous cells, a phenotype associated with a cell division defect (refs. 14, 15; K.E.R., unpublished observations). Another interesting observation taken from the *his* literature, which we have corroborated, is that the *his* constitutive phenotype of *hisW*(*gyrA*) mutants is substantially suppressed by growth in rich media (ref. 3; K.E.R., unpublished results). Reevaluation of the published characteristics of the *hisW* and *hisU1820*(Ts) mutations should enrich our understanding of the cellular effects of DNA supercoiling.

It is possible that tRNA gene expression, and consequently attenuator-regulated amino acid biosynthetic operon expres-

sion, is controlled by the degree of chromosomal superhelicity as part of a global regulatory system. This putative global control system must be quite complex in light of the opposite responses of the *ilv* and *his* operons to *gyr* mutations. The function of such a global control system would depend on the ability of the cell to modulate its level of supercoiling in response to environmental conditions or possibly timing within the cell cycle.

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