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Phenotypic Suppression of DNA Gyrase Deficiencies by a Deletion Lowering the Gene Dosage of a Major tRNA in *Salmonella typhimurium*

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One of the pleiotropic phenotypes of mutations affecting DNA gyrase activity in *Salmonella typhimurium* is the constitutive deattenuation of the histidine operon. In the present work, we isolated and characterized a suppressor mutation which restores *his* attenuation in the presence of a defective gyrase. Such a suppressor, initially named *sgdA1* (for suppressor gyrase deficiency), was found to correct additional phenotypes associated with defective gyrase function. These include the aberrant nucleoid partitioning of a *gyrB* mutant and the conditional lethality of a *gyrA* mutation. Furthermore, the *sgdA1* mutation was found to confer low-level resistance to nalidixic acid. The last phenotype permitted isolation of a number of additional *sgdA* mutants. Genetic analysis established the recessive character of these alleles as well as the position of the *sgdA* locus at 57 U on the *Salmonella* genetic map. All of the *sgdA* mutants result from the same molecular event: a deletion removing three of the four tandemly repeated copies of *argV*, the gene which specifies $tRNA_2^{Arg}$, the major arginine isoacceptor tRNA. These findings, combined with the observation of some Sgd-like phenotypes in a tRNA modification mutant (*hisT* mutant), lead us to propose that protein synthesis contributes, directly or indirectly, to the pathology of gyrase alterations in growing bacteria. We discuss plausible mechanisms which may be responsible for these effects.

DNA gyrase is a key enzyme in the biology of bacterial DNA. This type II DNA topoisomerase can increase the linking deficit of topologically constrained DNA by introducing negative supercoils (21) or by removing positive supercoils (59, 64). The classical role of the enzyme is to maintain cellular DNA in a state of negative superhelical tension (15, 49, 50). Through this function, DNA gyrase can indirectly influence the biological properties of DNA in transcription, replication, and site-specific recombination (reviewed in references 16, 20, and 62). The enzyme also plays an active role in some of these processes, as its activity is required for removal of positive DNA supercoils generated during the elongation phases of DNA replication (6) and transcription (37, 59, 64). Additional roles of DNA gyrase are connected with its ability to carry out unknotting and decatenation reactions. Inhibition of gyrase activity was shown to result in transcription-stimulated knotting of plasmid DNA (28) as well as in accumulation of catenated intermediates during site-specific recombination (1, 7). The decatenating activity of the enzyme is also thought to be required for proper segregation of newly replicated chromosomes prior to cell division. This was mostly inferred from the nucleoid partioning defects (the Par phenotype) exhibited by a number of gyrase mutants (27, 31, 45, 47, 55). The recent finding that a different topoisomerase activity, topo IV, can unlink newly replicated plasmid DNA has cast doubt on the presumed role of DNA gyrase in this reaction (2, 30, 40). The possibility remains that while topo IV intervenes specifically in the final stages of the chromosomal DNA decatenation process, gyrase activity is required throughout the growth cycle to remove random DNA entanglements (2). At present, knowledge of important details concerning the functional specialization of topo IV and gyrase activities is still lacking.

Not surprisingly, in light of the panoply of processes which rely directly or indirectly on gyrase function, mutations in the genes encoding the two subunits of the enzyme (*gyrA* and *gyrB*) are generally highly pleiotropic and often result in conditional growth. In most cases, these defects correlate with a measurable loss of negative superhelicity in both plasmid and chromosomal DNAs (17, 38, 56). However, the occurrence of gyrase mutants showing mild supercoiling defects and yet temperature sensitive for growth suggests that other mechanisms involving gyrase function can cause lethality in mutants (reference 18 and our unpublished results).

A phenotype associated with gyrase mutations which do affect negative DNA supercoiling levels in *Salmonella typhimurium* is the constitutive deattenuation of the histidine operon (references 17, 46, 52, and 58 and our unpublished results). This regulatory alteration appears to reflect a dependence on negative DNA supercoiling in the transcription of a separate locus (*hisR*) whose product, $tRNA^{His}$, is needed at high levels for proper functioning of the attenuation mechanism (29). A single base pair change in the *hisR* promoter sequence was shown to be sufficient to relieve the promoter dependence on gyrase activity and thereby restore *his* attenuation in a *gyrB* mutant background (17).

In the present study we tested whether the same genetic strategy which was applied for the isolation of the *hisR* promoter variant mentioned above could be used for identifying *trans*-acting factors contributing to this promoter's response to negative DNA supercoiling. Spontaneous mutations suppressing the His-constitutive phenotype of a *gyrB* mutant were isolated, one of which, found to be unlinked to *hisR* and *his* loci, was analyzed in detail. Contrary to what had been anticipated, the suppressor appeared to act not by specifically relieving the *hisR* promoter dependence on negative DNA

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TABLE 1. *S. typhimurium* strains[a] used

Strain	Genotype	Source
LT2	Wild type	Laboratory collection
TR5665	*cysC519 strA1*	J. Casadesús
TT521	*recA1 srl-202*::Tn*10*	J. R. Roth
TT10423	*proAB47*/F'128 *pro*$^+$ *lac*$^+$ *zzf-1831*::Tn*10*dTet	J. R. Roth
TT10427	LT2/pNK972	J. R. Roth
MA2842	*hisC9968*::Mu dJ(Km) *gyrB1820*	This work
MA2843	*hisC9968*::Mu dJ(Km) *gyrB1820*/F'[*hisO-E* (*E. coli*)]	This work
MA2844	*hisC9968*::Mu dJ(Km) *gyrB1820*/F'[*hisO-E* (*E. coli*)] *sgdA1*	This work
MA2846	*hisC9968*::Mu dJ(Km) *gyrB1820 zfh-6784*::Tn*10*dTet *sgdA1*	This work
MA2847	*zfh-6784*::Tn*10*dTet	This work
MA2849	*hisO1242*/F'400 *hisC9968*::Mu dJ(Km)	This work
MA2850	*hisO1242 zfh-6784*::Tn*10*dTet/F'400 *hisC9968*::Mu dJ(Km)	This work
MA2851	*pheA561*::Mu dA(Amp) *cysC519 strA1*	This work
MA2852	LT2/pNK2884	This work
MA2853	*hisC9968*::Mu dJ(Km) *srl-202*::Tn*10*	This work
MA2854	*hisC9968*::Mu dJ(Km) *gyrB1820 srl-202*::Tn*10*	This work
MA2855	*hisC9968*::Mu dJ(Km) *gyrB1820* DUP[(*srl-202*::Tn*10 sgdA*$^+$) (*srl*$^+$ *sgdA1*)]	This work
MA2856	*hisC9968*::Mu dJ(Km) *sgdA1*	This work
MA2857	*hisC9968*::Mu dJ(Km)	This work
MA2858	*hisC9968*::Mu dJ(Km) *zib-748*::Tn*10 gyrB1820 sgdA1*	This work
MA2859	*hisC9968*::Mu dJ(Km) *zib-748*::Tn*10 gyrB1820*	This work
MA2860	*hisC9968*::Mu dJ(Km) *zid-62*::Tn*10 hisR1223 sgdA1*	This work
MA2861	*hisC9968*::Mu dJ(Km) *zid-62*::Tn*10 hisR1223*	This work
MA2862	*proU2081*::Mu dJ(Km)	D. El-Hanafi
MA2863	*recA1*	This work
MA2864	*hisC9968*::Mu dJ(Km) *nalB561*	This work
MA2865	*sgdA1 zfh-6784*::Tn*10*dTet	This work
MA2866	*nalB561 zfh-6784*::Tn*10*dTet	This work
MA2867	*zfh-6787*::Tn*10*dCam	This work
MA2868	*hisC9968*::Mu dJ(Km) *zeh-105*::Tn*10 hisT1529 sgdA1*	This work
MA2869	*hisC9968*::Mu dJ(Km) *zeh-105*::Tn*10 hisT1529*	This work
MA2870	*hisC9968*::Mu dJ(Km) *zid-62*::Tn*10 hisR1223 zib-748*::Tn*10 gyrB1820 sgdA1*	This work
MA2871	*hisC9968*::Mu dJ(Km) *zid-62*::Tn*10 hisR1223 zib-748*::Tn*10 gyrB1820*	This work
MA2872	*hisC9968*::Mu dJ(Km) *gyrB1820 zfh-6784*::Tn*10*dTet *sgdA2*	This work
MA2873	*hisC9968*::Mu dJ(Km) *gyrB1820 zfh-6784*::Tn*10*dTet *sgdA3*	This work
MA2874	*hisC9968*::Mu dJ(Km) *gyrB1820 zfh-6787*::Tn*10*dCam *sgdA4*	This work
MA2875	*hisC9968*::Mu dJ(Km) *gyrB1820 zfh-6787*::Tn*10*dCam *sgdA5*	This work
MA2876	*sgdA1*	This work
MA2877	Wild type (isogenic to MA2876)	This work
MA2878	*zib-748*::Tn*10 gyrB1820 sgdA1*	This work
MA2879	*zib-748*::Tn*10 gyrB1820*	This work
MA2880	*hisC9968*::Mu dJ(Km) *zef-754*::Tn*10 gyrA208 zfh-6787*::Tn*10*dCam *sgdA1*	This work
MA2881	*hisC9968*::Mu dJ(Km) *zef-754*::Tn*10 gyrA208 zfh-6787*::Tn*10*dCam	This work
MA2903	*din-1001*::Mu dJ(Km) *zef-6792*::Tn*10*dCam *gyrA208 zeh-105*::Tn*10 hisT1529*	This work
MA2904	*din-1001*::Mu dJ(Km) *zef-6792*::Tn*10*dCam *gyrA208 zeh-105*::Tn*10*	This work

[a] Except for TR5665 and MA2851, the strains used in Hfr mapping are not listed. Most of these strains are described by Maloy (42).

supercoiling but rather by generally alleviating various defects associated with altered gyrase function. Intriguingly, identification of the suppressor gene revealed it to be an allele of a tRNA-encoding locus. The mutant allele results from a deletion removing three of the four tandemly repeated copies of *argV*, the gene which specifies the major arginine isoacceptor tRNA ($tRNA_2^{Arg}$) (60).

MATERIALS AND METHODS

Bacteria, media, and growth conditions. The genotypes and the sources of the strains used in this study are listed in Table 1. Except for strain TT10423, which is derived from *S. typhimurium* LT7, all strains are derived from *S. typhimurium* LT2. Bacteria were cultured at either 28 or 37°C in liquid media or in media solidified by addition of 1.5% agar (Difco). Nutrient broth (NB) (0.8%; Difco) containing 0.5% NaCl was used as a complex medium. Occasionally it was replaced by L broth (42). E and NCE media (42), supplemented, respectively, with 0.2% glucose or 0.2% sorbitol as the sole carbon source, were used as minimal media. The concentration of glucose in E medium was raised to 2% when colony morphology was scored. When needed, nutritional supplements were added to minimal media at the concentrations specified by Maloy (42). Lactose operon expression was monitored on MacConkey-lactose indicator plates (Difco) or on NB plates containing 25 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (Sigma) per ml. Antibiotics (Sigma) were used at the following final concentrations: tetracycline HCl, 25 μg/ml (10 μg/ml in minimal medium); kanamycin sulfate, 50 μg/ml; chloramphenicol, 10 μg/ml; and streptomycin sulfate, 1 mg/ml. The concentrations of nalidixic acid and novobiocin varied depending on the experiment and are indicated below. Nalidixic acid was added from a freshly made stock solution (50 mM in 0.5 M NaOH). Liquid cultures were grown in a New Brunswick gyratory shaker, and cell growth was monitored with a Milton-Roy Spectronic 301 spectrophotomer.

Genetic techniques. Transductional crosses using the high-frequency generalized transducing mutant of phage P22 (HT 105/1 *int-201*) and conjugational matings were carried out as previously described (26, 43).

Isolation and mapping of the *sgdA1* mutation. Strain MA2843 carries two copies of the *his* operon: one fused to the *lac* operon in the chromosome [a Mu dJ(Km) insertion] and the other intact on an F′ factor. Because of the presence of the *gyrB1820* mutation, this strain exhibits two phenotypes: high-level *lac* expression from the chromosomal *his-lac* fusion and a wrinkled colony morphology (on 2% glucose–E plates) resulting from high-level expression of *hisH* and *hisF* genes on the F′ factor (44). High-level β-galactosidase activity renders MA2843 particularly sensitive to ONPG (*o*-nitrophenyl-β-D-galactopyranoside), a lactose analog which yields a toxic product when cleaved by the *lacZ* gene product (43). This property was exploited to select for mutants expressing the *lac* operon at lower levels. ONPG (10 μl of a 0.3 M solution) was applied in the center of a 2% glucose–E plate previously spread with 0.1 ml of an overnight culture of MA2843. The concentration gradient created by ONPG diffusion ensured the recovery of mutants with different resistance levels. In practice, the effectiveness of the selection was somewhat variable because of considerable background growth. In one particular experiment, approximately 200 resistant colonies were clearly visible following a 2-day incubation period. Because of the difficulty in scoring colony morphology directly on the selection plate, 30 isolates were picked randomly and single-colony purified. The vast majority of these mutants formed wrinkled colonies on 2% glucose–E plates and were discarded. However, four isolates which formed smooth colonies were identified. When tested on MacConkey-lactose indicator plates, three of these strains formed white colonies, while one was pink. Further work concentrated on the white isolates. Only one of these mutants will be described hereafter, since the remaining, nonindependent isolates appeared to be siblings in subsequent tests. Preliminary transductional crosses indicated that this strain contained a mutation unlinked to either the *hisR* or *gyrB* locus which specifically suppressed the effects of the *gyrB* mutation on *his* expression. The mutant locus was named *sgdA* (for suppressor gyrase deficiency) and was further characterized.

Random transposon insertions in the *Salmonella* chromosome were obtained by inducing transposition of an F′-borne Tn*10*dTet element (Tn*10*Δ16Δ17 [63]) or of the Tn*10*dCam element on plasmid pNK2884 (32) in cells expressing a wild-type Tn*10* transposase gene (plasmid pNK972) or its ATS (altered target specificity) derivative (plasmid pNK2884 [32]), respectively. Various Tn*10*dTet and Tn*10*dCam insertions transductionally linked to the *sgdA* locus were identified. The Tn*10*dTet insertion showing the highest linkage to *sgdA1* was moved by transduction to a derivative of strain MA2849 in which the resident F′ *his* is integrated at the homologous chromosomal site. The resulting Hfr strain (MA2850) was mated with a set of known auxotrophic mutants, using a streptomycin resistance mutation (*strA1*) as marker for counterselection. The highest frequency of Tet^r colonies among the exconjugants (75%) was observed with strains carrying a *pheA* mutation (MA2851) or a *cysC* mutation (TR5665), indicating the Tn*10*dTet insertion site to be in the 55- to 60-U interval of the genetic map (53). Subsequent transductional crosses involving markers in this region showed transductional linkage of *sgdA* to the *proU* locus. On the basis of this information, the insertion was named *zfh-6784*::Tn*10*dTet. To further refine the *zfh-6784*::Tn*10*dTet and *sgdA1* map positions, three additional markers were used: *nalB*, *recA*, and *srl*. Mutations in *nalB* were selected on NB plates containing 3 μg of nalidixic acid per ml

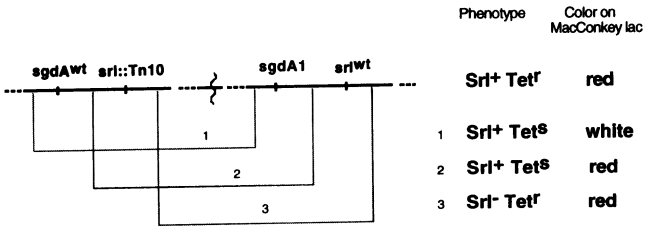

FIG. 1. Schematic representation of recombination events producing duplication-free segregants in strain MA2855. Accumulation of cells with the indicated phenotypes in nonselectively grown cultures of MA2855 is consistant with the presence of a duplication of the type illustrated in the figure. The red colony morphology of MA2855 indicates that *sgdA1* is recessive to its wild-type allele.

(23) and initially identified on the basis of their genetic linkage to *proU* (13). They were distinguished from *sgdA* mutations (which also confer Nal^r; see below) on the basis of their failure to suppress *gyrB1820*-induced *his* deattenuation and their slightly higher Nal^r level (5 μg/ml). NB plates containing 20 ng of mitomycin (Sigma) in 2.5 ml of soft top agar were used to distinguish *recA*$^+$ and *recA* mutant alleles. Transductional linkages between all of the above-described markers were determined in a series of two- and three-point crosses (see Results).

Dominance studies. A P22 lysate prepared on a strain carrying the *sgdA1* mutation (MA2844) was used to transduce strain MA2854 (*hisC9968*::Mu dJ *gyrB1820 srl-202*::Tn*10*) to Srl$^+$ on plates containing tetracycline. Concomitant selection for both wild-type and mutant alleles of the *srl* locus confined the possibility of yielding transductants to cells in the recipient population which carried a tandem duplication of the *srl* region (8). Rare Srl$^+$ Tet^r transductants were identified. When transferred onto MacConkey-lactose plates, most of these isolates exhibited the same red colony morphology as the parental strain MA2854. However, unlike the latter strain, these clones were unstable and segregated white Srl$^+$ Tet^s cells and red Srl$^-$ Tet^r or Srl$^+$ Tet^s cells at a high frequency. Such a segregation pattern would be expected in a strain carrying a duplication of the *sgdA* region with the *sgdA1* mutation in one copy of the duplicated segment (MA2855) (Fig. 1). The failure of *sgdA1* to suppress the *his* deattenuation phenotype in the presence of the wild-type *sgdA* allele (shown by the red colony morphology of the duplication-harboring strain) indicated the recessive nature of this mutation.

β-Galactosidase assays. β-Galactosidase activity was measured in toluene-permeabilized, NB-grown cells as described by Miller (43).

Measurements of plasmid DNA supercoiling levels. Bacterial strains were transformed with the plasmid pKK232-8 (10) by electroporation. Plasmid DNA extraction and electrophoretic separation of DNA topoisomers in chloroquine-agarose gels were carried out as described by Spirito et al. (54).

Cytological methods. For visualization of cell shape, unfixed bacterial preparations on glass slides were examined by differential interference contrast (Nomarski optics). For visualization of nucleoid morphology, bacteria were stained with 4′,6-diamino-2-phenyl-indole (DAPI) as described by Hiraga et al. (25) and examined with the appropriate fluorescence system (excitation wavelength, 365 nm). In both cases, cells were observed through a Reichert-Jung microscope and photographed with Kodak T-Max400 film.

PCRs. Chromosomal DNA was prepared from 1-ml full-density-grown bacterial cultures (in NB) as described previ-

ously (14). PCR mixtures contained 1 μl of genomic DNA (40 ng), 1 μl of each oligonucleotide primer (0.5 μg), 2 μl of 5 mM deoxynucleoside triphosphates, 2 U of *Taq* polymerase, 5 μl of 10× buffer (Boehringer Mannheim) (1× buffer is 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1 mg of gelatin per ml, pH 8.3), and H_2O to 50 μl, and reactions were performed in a Hybaid thermal reactor. Oligonucleotide primer sequences (GGCCGAGAGGCTGAAGGCGCTCCC CTGC and CCTCCGACCGCTCGGTTCGTAGCCGAGT AC) were complementary to portions of the *Escherichia coli* $tRNA_3^{Ser}$ and $tRNA_2^{Arg}$ genes (opposite strands), respectively. Following PCR amplification and a 1-volume chloroform extraction, samples were loaded directly on a 6% polyacrylamide gel.

Determination of tRNA levels by Northern (RNA) hybridization. RNA extracted from exponentially growing cells at 37°C and purified as described previously (17) was resuspended in 15% formaldehyde–10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to a final concentration of 0.7 μg/μl. Aliquots (3 μl) of the RNA solution were spotted in triplicate on sheets of a Hybond N-Nylon membrane (Amersham). Fixation was performed as described previously (24) except that UV exposure was for 3 min and the baking step was omitted. The three sheets were used in separate hybridizations with oligonucleotides previously phosphorylated with [γ-^{32}P] ATP and T4 polynucleotide kinase (Boehringer Mannheim). Probes were complementary to $tRNA_2^{Arg}$ (A25-A54 segment), $tRNA^{His}$ (A33-A54 segment) and $tRNA_3^{Ser}$ (A45-A65 segment). Hybridization signals were quantitated by scanning storage phosphor screens with a 400S phosphorimager (Molecular Dynamics, Sunnyvale, Calif.).

RESULTS

Isolation and mapping of the *sgdA1* mutation. Mutation *hisU1820* was isolated several years ago during a search for histidine regulatory mutants of *S. typhimurium* (3, 51). It was later found that *hisU1820* is an allele of *gyrB*, the gene encoding the B subunit of DNA gyrase (52). Hence, *hisU1820* has been renamed *gyrB1820* in the present study. Strains harboring this mutation fail to grow at 42°C and exhibit the His-constitutive phenotype throughout a wide range of permissive temperatures (28 to 37°C). In the initial phase of this work, we sought to find mutations capable of suppressing the *his* regulatory defect. Such suppressors were searched for by starting with a *gyrB1820* derivative carrying a *his-lac* transcriptional fusion (MA2843) and selecting isolates more resistant to ONPG, a lactose analog which is toxic to cells expressing high β-galactosidase levels (43). The presence of a second intact copy of the *his* operon on an F′ factor provided an additional phenotypic trait (colony morphology) which facilitated the identification of *trans*-acting mutations (see Materials and Methods). One such candidate was identified. Unlike the parental strain, which forms red colonies on MacConkey-lactose plates and wrinkled colonies on high-glucose minimal plates, this isolate (MA2844) formed colonies which were white and smooth, respectively, on these two media. Preliminary transductional tests confirmed that this clone still contained the *gyrB* mutation and that suppression of *his* deattenuation was due to a second-site event in a gene other than the $tRNA^{His}$-encoding locus (*hisR*) or the *his* operon. Failure of the suppressor mutation to correct deattenuation in other classes of *his* regulatory mutants (see below) suggested that this mutation acted by relieving the gyrase defect rather than by altering *his* promoter/attenuator functions. In light of this specificity, the suppressor was tentatively named *sgdA1* (suppressor gyrase deficiency) and further characterized. P22 transducing lysates prepared on pools of cells carrying random Tn*10*dTet chromosomal insertions were used to isolate a Tet resistance marker transductionally linked to the *sgdA* locus. The insertion showing the highest linkage (approximately 75%) was genetically mapped. Conjugational crosses using a set of auxotrophic mutants initially located the Tn*10*dTet element in the 55- to 60-U interval of the map (see Materials and Methods). The insertion site was more precisely defined, by P22 transduction, at 57 map units between the *proU* and *recA* loci. On the basis of these data, the transposon insertion was named *zfh-6784*::Tn*10*dTet (53). Further transductional analysis permitted a more precise localization of the *sgdA1* mutation and its genetic linkage to all relevant markers in the region. The results of this study are summarized in Fig. 2. These experiments provided further verification that the *sgdA1* phenotype resulted from a unique mutational event mapping at the indicated site. The possibility that the suppressor action required some uncharacterized feature of the *gyrB1820* genetic background was ruled out after observing the suppressed phenotype in wild-type-derived strains in which *sgdA1* was introduced prior to *gyrB1820* (see below).

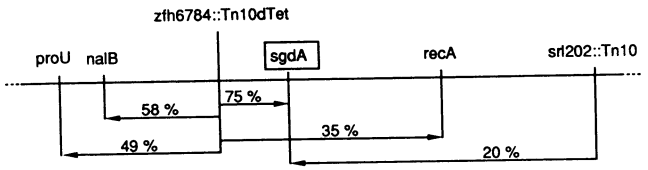

FIG. 2. Transductional linkages of *sgdA* to known genetic markers. Linkage between *sgdA1* and *zfh-6784*::Tn*10*dTet was established in a cross between MA2846 (donor) and MA2842 (recipient) in which Tet^r transductants (200) were screened for inheritance of the *sgdA1* mutation (conferring white colony color on MacConkey-lactose plates). A cross between strain MA2853 (donor) and MA2844 (recipient), selecting Tet^r and screening for the loss of *sgdA1* (100 transductants were analyzed), gave the linkage between *srl-202*::Tn*10* and the suppressor locus. Linkages between the *zfh-6784*::Tn*10*dTet insertion (in MA2847 as the donor strain) and each of the remaining markers in the recipient strains (*recA* in MA2863, *nalB* in MA2864, and *proU* in MA2862) were determined in further transductions (not fewer than 100 transductants were analyzed in each case). Gene order was established in two three-point crosses: (i) MA2862 (donor) × MA2865 (recipient); selected marker, *proU2081*::Mu dJ(Km); markers scored, *sgdA1* and *zfh-6784*::Tn*10*dTet (100 transductants analyzed); (ii) MA2862 (donor) × MA2866 (recipient); selected marker, *proU2081*::Mu dJ(Km); markers scored; *nalB561* and *zfh-6784*::Tn*10*dTet (100 transductants analyzed). Not included in the figure are the sites of two Tn*10*dCam insertions: *zfh-6787*::Tn*10*dCam to the left of *proU* (approximately 90% linked) and *zfh-6786*::Tn*10*dCam between *proU* and *sgdA*.

Specificity of suppression by *sgdA1*. The availability of selectable markers near *sgdA1* facilitated the genetic handling of the suppressor and its transfer to different strains. These experiments showed that *sgdA1* suppresses the His-constitutive phenotype of other *gyrB* mutants and can prevent *his* deattenuation in wild-type cells exposed to the gyrase inhibitor novobiocin (not shown). The latter property was useful in moving *sgdA1* into *gyrB*$^+$ backgrounds, since in the presence of a *his-lac* fusion (*hisC9968*::Mu dJ), inheritance of the suppressor mutation could be scored directly on MacConkey-lactose indicator plates containing sublethal doses of novobiocin (60 μg/ml). A pair of isogenic strains was constructed by transducing strain MA2853 (*srl-202*::Tn*10* *hisC9968*::Mu dJ) to Srl$^+$ with phage grown on an *sgdA1*-harboring strain and purifying one *sgdA* mutant and one *sgdA*$^+$ recombinant on the novo-

TABLE 2. Effect of the *sgdA1* mutation on expression of the *his-lac* operon fusion in the presence of different histidine regulatory mutations

Strain[a]	Relevant allele	β-Galactosidase activity[b]	Deattenuation ratio[c]
MA2857	Wild type	126	1
MA2856	*sgdA1*	72	1
MA2859	*gyrB1820*	3,481	28
MA2858	*gyrB1820 sgdA1*	574	8
MA2861	*hisR1223*	2,664	21
MA2860	*hisR1223 sgdA1*	1,787	25
MA2869	*hisT1529*	1,824	14
MA2868	*hisT1529 sgdA1*	1,642	23
MA2871	*gyrB1820 hisR1223*	3,098	24
MA2870	*gyrB1820 hisR1223 sgdA1*	1,642	23

[a] Overnight cultures (grown in NB at 28°C) were diluted 1:100 in NB and incubated with shaking at 37°C to the appropriate optical density at 600 nm. Cells were then processed for β-galactosidase activity measurements as described by Miller (43).

[b] In Miller units. Activity values are averages of at least three experiments.

[c] Deattenuation ratios are calculated for all *sgdA*$^+$ strains relatively to the activity measured in MA2857 and for all *sgdA1* strains relatively to the activity measured in MA2856.

biocin-supplemented MacConkey-lactose plates. These two strains (MA2856 and MA2857) constituted the starting material for the construction of most of the strains used in the work below. In one experiment, different histidine regulatory mutations (including *gyrB1820* as a control) were transferred into MA2856 and MA2857 by means of transductional Tn*10* insertions. In particular, we used two alleles, *hisR1223* and *hisT1529*, which cause constitutive deattenuation of the *his* operon by affecting, respectively, the production of tRNA^{His} (9) and the proficiency of this tRNA in translation (48). Expression of the *his* operon in the resulting strains was quantitated by measuring β-galactosidase levels. Computation of the deattenuation ratios (Table 2) confirms the specificity of *gyrB1820* suppression by *sgdA1* and shows, however, that suppression is not complete. From the absolute values of β-galactosidase activity it is apparent that a generalized decrease of *lac* expression levels (approximately twofold) is associated with the presence of the *sgdA1* allele. Finally, the data from strain MA2870 (Table 2) show that in the presence of *sgdA1*, the *hisR1223* mutation is epistatic to *gyrB1820*; that is, the *his* operon can still be fully deattenuated in a *gyrB1820 sgdA1* double mutant following the introduction of a mutation which reduces tRNA^{His} levels.

***sgdA1* is a recessive mutation.** Dominance studies were performed by isolating a tandem duplication of the region of the chromosome which includes the *sgdA* locus and by examining the expression of the *his-lac* fusion in the presence of both mutant and wild-type alleles of the *sgdA* gene in a *gyrB1820* background (see Materials and Methods). The high β-galactosidase levels of the duplication-harboring strain indicated the failure of *sgdA1* to suppress the *his* attenuation defect. It was concluded that the *sgdA1* mutation is recessive to its wild-type allele.

Additional phenotypes of *sgdA1*. (i) Suppression of chromosomal DNA partitioning defects. An additional phenotype associated with the *gyrB1820* mutation became apparent upon microscopic examination of cell morphology. Cells from strains carrying the mutant allele were found to be very heterogeneous in size, with many cells being considerably longer than normal (Fig. 3B). These morphological alterations are unrelated to the filamentation phenotype of His-constitutive mutants (44), since they were observed in strains in which this operon either is disrupted by a Mu dJ insertion or has the filamentation-inducing *hisH* and *hisF* genes deleted (our unpublished results). Examination of cells by fluorescence microscopy showed that the irregularities in cell shape in the *gyrB1820* mutant are accompanied by an aberrant distribution of chromosomal DNA. A fraction of cells contains incompletely segregated nucleoids, while others are anucleate (Fig. 3E). Thus, as was previously shown for other gyrase mutants of *E. coli* (27, 31, 45, 47, 55), *gyrB1820* interferes with proper partitioning of bacterial nucleoids. The same analysis was done on the *gyrB1820 sgdA1* double mutant and showed that the presence of the suppressor mutation almost completely restores normal cell morphology and DNA distribution (compare panels B and C and panels E and F in Fig. 3). Therefore, in addition to partially suppressing the *his* regulatory defect associated with the *gyrB1820* mutation, the *sgdA1* allele corrects the Par phenotype also associated with this mutant allele.

(ii) Suppression of the conditional lethality of a *gyrA* mutation. Although correcting the morphology and nucleoid segregation defect of the *gyrB1820* mutant, the *sgdA1* mutation fails to restore growth of this strain at a restrictive temperature (42°C). This could be due to an inability of the suppressor to correct the DNA supercoiling defect caused by the *gyrB1820* mutation. Figure 4 shows that the relaxation of a reporter plasmid DNA in a strain harboring *gyrB1820* is not reversed to any significant extent upon introduction of the *sgdA1* allele. In spite of the limitation inherent to the use of a reporter plasmid, these results strongly suggest that *sgdA1* does not affect the superhelical density of DNA. We thought that *sgdA1* might be able to suppress the temperature-sensitive phenotype of gyrase mutants with less severe supercoiling alterations. A particular *gyrA* mutation (*gyrA208*; a generous gift of Molly Schmid, Princeton University) appeared suitable for such a test. An isogenic pair of strains harboring *gyrA208* in combination with *sgdA1* or its wild-type allele were constructed and tested for growth at the restrictive temperature. The results showed that the *sgdA1 gyrA208* double mutant displays a dramatically improved plating efficiency at 42°C compared with the strain carrying the *gyrA208* mutation alone (Fig. 5). Thus, the *sgdA1* mutation can suppress the lethal effects of an alteration in the *gyrA*-encoded subunit of DNA gyrase.

(iii) Low-level resistance to nalidixic acid. The proximity of the *sgdA1* mutation to the *nalB* locus (23), as indicated by the initial mapping results, led us to test the sensitivity of *sgdA1*-harboring strains to nalidixic acid. Although we subsequently established that *nalB* and *sgdA* are in fact separate loci (Fig. 2), this test revealed that the *sgdA1* mutation confers low-level resistance to the gyrase inhibitor. These findings suggested that additional *sgdA* mutations could be isolated by a selection for Nal^r. We tested this prediction on a strain carrying either the *zfh-6784*::Tn*10*dTet or the *zfh-6787*::Tn*10*dCam insertion in an otherwise wild-type background. Approximately 10^8 cells from two independent cultures of both MA2847 and MA2867 were spread on NB plates containing 3 μg of nalidixic acid per ml. Mutants resistant to this low drug concentration appeared at a high frequency (approximately 1,000 resistant colonies per plate after a 2-day incubation). Bacteria from each of the four plates were pooled, and P22 lysates were prepared on these pools. The four lysates were used to transduce strain MA2843 (*gyrB1820 hisC9968*::Mu dJ) to either Tet^r or Cam^r on MacConkey-lactose indicator plates, looking for the presence of white colonies. (Because of *gyrB1820*-induced *his* deattenua-

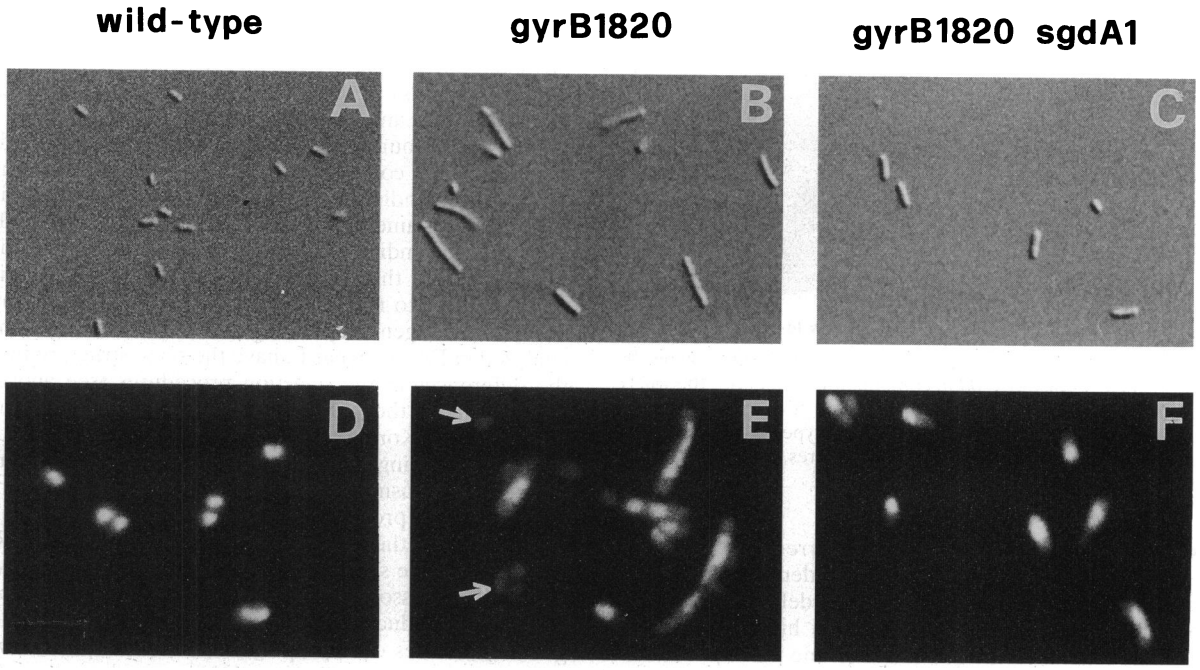

FIG. 3. Bacterial cell shape and nucleoid morphology of the *gyrB1820* mutant in the presence or in the absence of the *sgdA1* mutation. (A, B, and C) Overnight cultures grown in NB at 28°C were diluted 1:100 in the same medium and incubated at 37°C until they reached an optical density at 600 nm of 0.5. Bacterial cells were observed by differential interference contrast (40× objective). (D, E, and F) Overnight cultures grown in NB at 28°C were diluted 1:100 in the same medium and incubated at 28°C to an optical density at 600 nm of 0.2. At this point, bacterial cultures were incubated for 2 h at 42°C. Cells were stained with DAPI and examined under fluorescence microscopy as described in Materials and Methods. (A and D) MA2857; (B and E) MA2859; (C and F) MA2858. In panel E, arrows denote anucleate cells.

tion, MA2843 forms red colonies on these plates. If *sgdA* mutants were represented among the pooled bacteria, cotransduction of these mutations with the Tet^r or Cam^r markers would be expected to produce white transductants.) White clones were identified on all four plates at a frequency of about 3 in 1,000 transductants. The majority of these isolates were still Kan^r (ruling out a loss of the Mu dJ-*lac* insertion) and had acquired a low-level resistance to nalidixic acid. In subsequent tests, representatives from each of the four independent groups were conclusively identified as *sgdA* mutants (see below). From the above-described results, it appears that *sgdA* mutations account for a tiny fraction (0.1 to 0.2%) of low-level Nal^r mutants. Wondering about the frequency of mutations causing an *sgdA*-like phenotype, we repeated the Nal^r selection with strain MA2843 and examined the fraction of resistant isolates showing a concomitant decrease in *lac* expression. In four independent experiments, 15 to 25% of the colonies selected on 3 μg of nalidixic acid per ml were either white or light pink on MacConkey-lactose indicator plates. Thus, it would appear that many different genes can mutate to confer an Sgd phenotype (see Discussion).

Molecular characterization of the *sgdA* suppressors. All of our efforts to clone the *sgdA* locus on a multicopy plasmid vector were unsuccessful. Nonetheless, in one of these attempts we were able to obtain a recombinant plasmid carrying the *sgdA*-linked *zfh-6784*::Tn*10*dTet insertion. Using this plasmid DNA as a probe for Southern hybridization of chromosomal DNA restriction enzyme digests, we noticed that the hybridization patterns of DNA from all of the *sgdA* mutants included a fragment which was slightly shorter than the corresponding fragment in *sgdA*$^+$ strains. On closer examination, the difference could be attributed to a deletion of

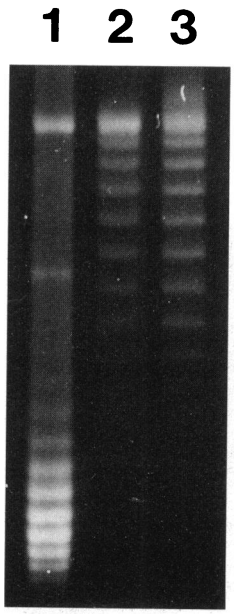

FIG. 4. Topoisomer profiles of plasmid DNA extracted from different strains. DNA of plasmid pKK232-8 (10) was purified from exponentially growing cells at 37°C (optical density at 600 nm = 0.35) as described previously (54) and loaded on a 1.2% agarose gel containing 10 μg of chloroquine per ml. At this chloroquine concentration, DNA topoisomers with a lower linking number migrate faster in the gel. Lane 1, pKK232-8 DNA from MA2877 (wild type); lane 2, pKK232-8 DNA from MA2879 (*gyrB1820*); lane 3, pKK232-8 DNA from MA2878 (*gyrB1820 sgdA1*).

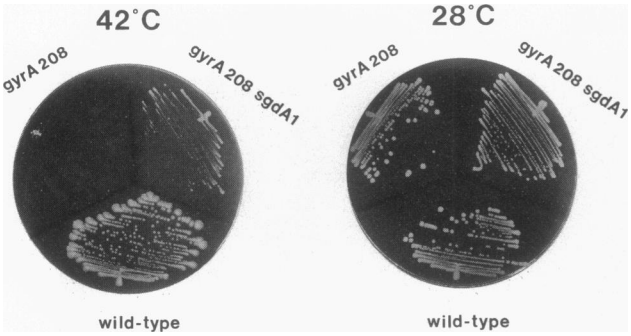

FIG. 5. Suppression of *gyrA208* thermosensitive phenotype by *sgdA1*. Strains harboring *gyrA208* in combination with either the *sgdA1* mutation (MA2880) or the wild-type allele of this locus (MA2881) were streaked on NB plates, along with a wild-type LT2 control, and incubated for 2 days at the indicated temperatures.

approximately 500 bp. The apparent occurrence of the same fragment length change in five independent *sgdA* isolates suggested the presence of a hot spot for deletion formation near or within the *sgdA* gene. With this hint in mind, we examined the map of the *E. coli* chromosome in the *proU-recA* interval, looking for possible clues. We found that at approximately the same position of the *sgdA* locus in *S. typhimurium*, a particular tRNA gene cluster is present on the *E. coli* map (5). This tRNA operon includes four identical copies of *argV*, the gene for the major arginine isoacceptor tRNA ($tRNA_2^{Arg}$), and the single-copy $tRNA_3^{Ser}$ gene, *serV* (33). The corresponding locus in *S. typhimurium* was recently sequenced and found to have the same overall organization and identical sequences in the tRNA coding region (59a, 60). Considering that the tandemly repeated *argV* sequences could provide a substrate for intragenic recombination and consequent deletion formation, we set out to analyze the structure of the *argV-serV* region in the different *sgdA* mutants. Synthetic oligonucleotides complementary to portions of the $tRNA_2^{Arg}$ and $tRNA_3^{Ser}$ genes (on opposite strands) were annealed to chromosomal DNA from various strains and used as primers for PCRs. The results showed that four different fragments are amplified when template DNA comes from $sgdA^+$ strains, whereas only one band, corresponding to the smallest fragment in the wild-type pattern, is obtained with DNA from *sgdA* mutants (Fig. 6). These results indicate that *sgdA* mutants contain a deletion which removes three of the four $tRNA_2^{Arg}$ gene copies and which is likely to result from a recombination event involving the outermost gene copies. Significantly, all five *sgdA* mutants analyzed in Fig. 6 appear to have the same structure in spite of the differences in the selection procedures (see above). Our failure to clone the *argV* locus on a multicopy plasmid matches the findings of Komine et al., who were also unable to subclone the corresponding *E. coli* locus from a lambda recombinant phage into a plasmid vector (33). Thus, overproduction of the *argV-serV* gene products appears to be lethal to the cell.

To evaluate the effects of the *argV* deletion on $tRNA_2^{Arg}$ levels and at the same time assess the possible suppression of the *gyrB1820*-associated defect in $tRNA^{His}$ gene expression (see the introduction), RNA extracted from exponentially growing cultures of appropriate strains was spotted on nylon membranes and hybridized to tRNA-specific probes. Hybridization signals were quantitated by phosphorimaging. The results in Table 3 allow a number of points to be made. First, the effects of the *argV* deletion on $tRNA_2^{Arg}$ levels are exactly as one might have anticipated from the decrease of gene dosage. Second, the *gyrB1820* mutation is also seen to cause a reduction in the concentrations of all three tRNA species which were measured. This is consistent with the idea that the reduction in $tRNA^{His}$ levels in the gyrase mutant is not specific but reflects a general impairment of tRNA gene expression (see Discussion). As a result, *gyrB1820* enhances the $tRNA_2^{Arg}$

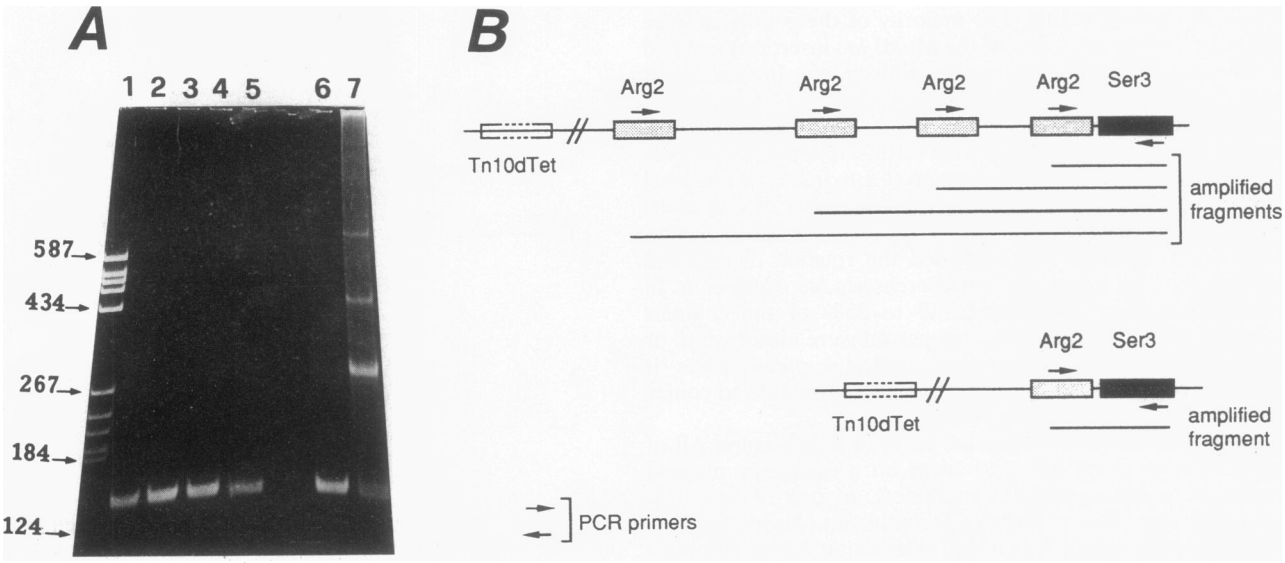

FIG. 6. PCR amplification of the *argV-serV* region of the *Salmonella* chromosome from wild-type and *sgdA* mutant strains. Chromosomal DNA was extracted from different strains and hybridized to oligonucleotide primers complementary to opposite strands of the *serV* ($tRNA_3^{Ser}$) and *argV* ($tRNA_2^{Arg}$) genes. Intervening DNA was amplified by PCR as described in Materials and Methods. (A) Polyacrylamide (6%) gel of PCR products. Lane 1, pBR322-*Hae*III; lane 2, MA2872 (*sgdA2*); lane 3, MA2873 (*sgdA3*); lane 4, MA2874 (*sgdA4*); lane 5, MA2875 (*sgdA5*), lane 6, MA2856 (*sgdA1*); lane 7, LT2 (wild type). (B) Diagram of amplified fragments from wild-type (top) and *sgdA* (bottom) strains. The wild-type amplification pattern is consistent with the results of Tuohy et al. (60).

TABLE 3. tRNA levels in strains harboring different combinations of mutant and wild-type *gyrB1820* and *sgdA1* alleles

Strain	Relevant allele(s)	Level[a] of:			Generation time in L broth (min)[b]
		$tRNA^{His}$	$tRNA_2^{Arg}$	$tRNA_3^{Ser}$	
MA2877	Wild type	1	1	1	30
MA2876	*sgdA1*	0.81	0.26	0.85	42
MA2879	*gyrB1820*	0.36	0.45	0.42	39
MA2878	*gyrB1820 sgdA1*	0.49	0.17	0.56	64

[a] RNA extraction and dot-blot hybridization to tRNA-specific probes were carried out as described in Materials and Methods. Hybridization signals were quantitated by phosphorimaging. For each of the three tRNAs, values are expressed as ratios between mutant and wild-type levels. These data represent the averages of two independent determinations.

[b] Generation times were determined during exponential growth at 37°C by monitoring the optical density at 600 nm.

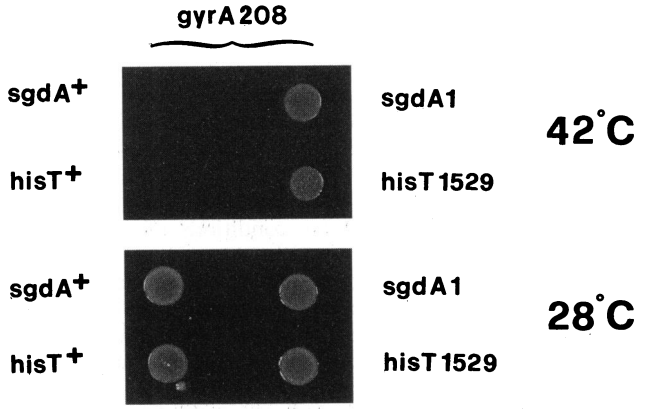

FIG. 7. Suppression of *gyrA208* thermosensitive phenotype by *hisT1529*. Dilutions (10^{-4}) of overnight cultures of strains harboring *gyrA208* in combination with the mutant or wild-type *hisT* allele (MA2903 and MA2904, respectively) were spotted on NB plates and incubated for 24 h at the indicated temperatures. The suppression by *hisT1529* is compared with that by *sgdA1*.

deficiency of the *sgdA* mutants. On the other hand, the slightly higher $tRNA^{His}$ and $tRNA_3^{Ser}$ levels in the *gyrB1820 sgdA1* double mutant, compared with those in the strain carrying the *gyrB* mutation alone, suggest that the impairment of tRNA gene expression by *gyrB1820* is somewhat alleviated in the presence of *sgdA1* (a similar trend may apply to $tRNA_2^{Arg}$ but be hidden by the nature of the *sgdA1* mutation itself).

The reduced availability of $tRNA_2^{Arg}$ in *sgdA* mutants is likely to seriously affect the rate at which arginine codons are translated in the cell. This can be inferred from the lower growth rates of *sgdA1*-harboring strains, particularly when *gyrB1820* is also present in the genetic background (Table 3).

sgdA-like phenotypes of a *hisT* mutant. A key question raised by the above-described results is whether the effects of the *argV* deletion on gyrase-dependent functions reflect a specific property of $tRNA_2^{Arg}$ or rather are a general consequence of slower translation. Thus, we sought to test other translational mutants for possible *sgdA*-like phenotypes. Mutations in the *hisT* gene affect the enzyme responsible for the synthesis of pseudouridine in the anticodon stem-loop regions of several tRNAs (61). Lack of modification interferes with the normal functioning of tRNA in translation, causing *hisT* mutants to show lower rates of protein synthesis (48). Since $tRNA_2^{Arg}$ is not a substrate of the *hisT*-encoded enzyme (61), these mutants appeared to be a suitable choice for examining the specificity problem. The results in Fig. 7 show that mutation *hisT1529* allows a strain carrying the temperature-sensitive *gyrA208* allele to grow at 42°C. Furthermore, when transferred into a wild-type genetic background, *hisT1529* was found to confer Nal^r levels comparable to those of *argV* mutants (data not shown). Albeit incomplete (suppression of *gyrB1820*-induced *his* deattenuation could not be tested because *hisT* mutants are themselves His constitutive [51; Table 2], and the *gyrB1820* Par phenotype was not examined), these results suggest that the Sgd phenotypes are not specifically coupled to *argV* deletion mutants. Relevant to the following discussion is the observation that like *argV* mutants, a strain harboring *hisT1529* shows an increased doubling time (data not shown).

DISCUSSION

This paper describes the unprecedented finding that a deletion lowering the gene dosage of a major tRNA species ($tRNA_2^{Arg}$) can influence various phenotypes associated with alterations of DNA gyrase function. The same structural alteration was isolated by two independent selection procedures: as a mutation suppressing the histidine regulatory defect of a *gyrB* mutant strain (*gyrB1820* mutant) or as a mutation conferring increased resistance to the gyrase inhibitor nalidixic acid. Besides these two phenotypes, the *argV* deletion partially suppresses the nucleoid segregation defect of the *gyrB1820* mutant and the conditional lethality associated with a different gyrase allele, *gyrA208*. In contrast, neither the temperature sensitivity nor the DNA supercoiling alterations associated with the *gyrB1820* allele are suppressed by the deletion.

As expected, an approximately 75% reduction in $tRNA_2^{Arg}$ levels could be demonstrated in one of the *argV* mutants (*sgdA1* mutant) by Northern hybridization analysis. These experiments revealed that the *gyrB1820* mutation also independently affects $tRNA_2^{Arg}$ levels (reduced by about 50%). The latter findings are consistent with measurements performed prior to elucidating the nature of this allele (formerly *hisU1820* [36]) and support the idea that gyrase activity is required for optimal expression of many different tRNA genes. Because of the *gyrB* effects, the concentration of $tRNA_2^{Arg}$ is reduced to 17% of the wild-type levels in the *sgdA1 gyrB1820* double mutant.

Previous examples of tRNA mutations affecting important cellular function such as cell division and DNA replication have been reported for *E. coli* (11, 19, 35, 57). In general, these findings have been interpreted by suggesting that some tRNAs (particularly some minor species reading rare codons) can control the expression of certain polypeptide products of key importance for these processes. It seems unlikely that this type of interpretation could be applied to the *argV* deletion mutants. $tRNA_2^{Arg}$ is the main arginine isoacceptor tRNA and the only one reading triplets CGC, CGU, and CGA, which altogether account for 90% of the arginine codons in *E. coli* messages (4), a bias which increases to 98.5% in messages encoding abundant proteins (22). Therefore, one expects that lowering the concentration of this tRNA would have a generalized effect on translational rates as opposed to causing differential changes in protein levels. Indeed, our preliminary data suggest that the phenomenon described here is independent of the nature of the tRNA involved. First, some of the properties of *argV* mutants (namely, low-level nalidixic acid resistance and ability to suppress *gyrA208*'s conditional lethality) are mimicked by a *hisT* mutation which alters the modification patterns of several tRNAs which do not include

$tRNA_2^{Arg}$ (61). Second, the existence of a large number of potential *sgd* loci could be inferred from the high frequency of Nal^r mutations which concomitantly suppress the *gyrB1820*-induced *his* regulation defect (see Results). Taken together, these two sets of data suggest that Sgd and Nal^r phenotypes result from any condition which causes protein synthesis to slow down.

The possibility that some undefined mechanism could cause gyrase levels to increase under conditions of slowed-down translation was not directly tested in our study. However, this possibility is made unlikely by the Nal^r phenotype as well as by the supercoiling data. Because of the mechanism of action of nalidixic acid (whose toxicity depends on gyrase function), higher gyrase levels might be expected to increase sensitivity and not resistance to the drug. One might also expect that higher gyrase levels would tend to reduce the DNA supercoiling defect of the *gyrB1820* mutant. However, no such effects were detected upon comparing plasmid DNA linking number distributions in strains carrying *gyrB1820* in the presence or absence of the *sgdA1* allele (see Results). These considerations make us favor the idea that what is affected by the slowdown of protein synthesis is not gyrase expression but rather the cell's ability to cope with a defective gyrase. Two alternative scenarios can be envisioned. The first possibility comes from the observation that the translational defects in *argV* and *hisT* mutants slow down bacterial growth. Slower growth might limit the cellular demand for gyrase activity, making gyrase mutations more easily tolerated. For example, longer generation times could allow a defective gyrase to complete its DNA-unlinking tasks before septation so as to reduce the defect in nucleoid partitioning. Concomitantly, a slight increase in nalidixic acid resistance could result from more time being available for the repair of nalidixic acid-induced lesions. As an initial test for this model, we tested whether any of the properties of *argV* mutants could be mimicked by growing *gyrB1820*- and *gyrA208*-harboring strains on poor carbon sources. We saw no suppression of mutant phenotypes (data not shown). However, it remains unclear how medium-induced slow growth affects the normal regulation of gyrase. In particular, it is conceivable that growth on a poor carbon source might also reduce gyrase expression in a fashion that will not allow suppression.

An alternative model for interpreting our findings is based on the possibility that protein synthesis actually recruits gyrase activity. Gyrase is thought to intervene in the relaxation of positive DNA supercoils generated by RNA polymerase movement during transcription elongation (37, 59, 64). In bacteria, the coupling of translation to RNA chain elongation greatly enhances the supercoiling action of RNA polymerase and the consequent stimulation of topoisomerase activities (34, 39, 41, 54). It follows that translation might directly affect the level of gyrase activity solicited by the transcription process. If so, one can envision that a generalized slowdown of protein synthesis will lower the enzyme's participation in transcription, making the enzyme (particularly if defective) more available for other tasks. A decrease in transcription-driven gyrase activity might also limit the accumulation of trapped enzyme-DNA intermediates in the presence of nalidixic acid (12, 34) and thus possibly result in a slightly increased resistance to the drug.

In conclusion, both of the above-described models provide a conceptual framework in which the properties of *argV* can be rationalized. Only the suppression of the *gyrB1820*-induced His-constitutive phenotype remains somewhat elusive, considering the apparent lack of effects on negative DNA supercoiling and the modest increase of $tRNA^{His}$ levels resulting from the *argV* deletion in a *gyrB* mutant background. Perhaps combined with the slight increase in $tRNA^{His}$ absolute levels, a more extensive aminoacylation of this tRNA, as a result of $tRNA_2^{Arg}$-limited translational rates, specifically improves reading of *his* leader RNA (which contains seven histidine codons and only one arginine codon) and thus restores some attenuation. The alternative possibility that the effects of the *argV* deletion result from a direct involvement of $tRNA_2^{Arg}$ in the *his* regulatory mechanism is ruled out by our finding that the *his* operon can still be deattenuated in a *gyrB1820 argV* double mutant (see Results). Finally, in the absence of any change in plasmid DNA average supercoiling levels, the slight enhancement of $tRNA^{His}$ (and $tRNA_3^{Ser}$) gene expression in the *gyrB1820 argV* mutant might suggest that the sensitivity of tRNA gene promoters to gyrase activity reflects local, as opposed to global, topological requirements.

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