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Specialized Lambda Transducing Bacteriophage Which Carries *hisS*, the Structural Gene for Histidyl-Transfer Ribonucleic Acid Synthetase

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A number of specialized lambda transducing bacteriophages which carry the *Escherichia coli* gene *guaB* were isolated from *E. coli*. One of these bacteriophages, λ cI857 *Sam7* d *guaB*-2, also carries *hisS*, the structural gene for histidyl-transfer ribonucleic acid synthetase (EC 6.1.1.21). Histidyl-transfer ribonucleic acid synthetase activities in induced and uninduced lysogens carrying λ*d guaB*-2 indicate that the phage carries the entire structural gene and that the gene is under the control of an *E. coli* promoter. These conclusions were confirmed by the in vivo production of a protein encoded by the phage which comigrates with authentic histidyl-transfer ribonucleic acid synthetase on two-dimensional polyacrylamide gels.

Specialized transducing phage carrying structural genes encoding proteins and RNA indispensable in transcription and translation have proven to be very valuable in genetic and regulatory studies. As a case in point, the transducing phage originally selected as carrying the structural gene for the β subunit of RNA polymerase (λ*drif*^{d}-18 [10]) has proved invaluable in studies of the polymerase, ribosomal proteins, tRNA's, rRNA's, and elongation factors (e.g., see 15, 30, 41). Such phage greatly simplify the genetic studies of regulatory regions preceding indispensable genes (e.g., see 19, 41).

The largest class of proteins involved in protein synthesis are the ribosomal proteins. Most of the structural genes for these proteins are clustered at a few loci in *Escherichia coli*. Specialized transducing phage carrying them have been isolated (5, 6, 8, 13, 42). The next largest class are the aminoacyl-tRNA synthetases. There must be at least 23 structural genes encoding these enzymes in *E. coli* (22). The only known cluster of these genes is one containing the genes for the two different subunits of phenylalanyl-tRNA synthetase and for threonyl-tRNA synthetase (7), although the structural gene for isoleucyl-tRNA synthetase is closely linked to a ribosomal protein structural gene (2, 6). The rest of the loci seem to be scattered about the chromosome.

The aminoacyl-tRNA synthetases seem to be regulated both individually in response to specific amino acid limitations and as a group in response to the overall demand of protein synthesis, although other modes of control have been described. The regulatory mechanisms involved are poorly understood and may differ for different enzymes (see 20, 22, 25 for review).

To begin genetic studies in *E. coli* on the regulation of one of these enzymes, histidyl-tRNA synthetase (EC 6.1.1.21), we have isolated a specialized transducing phage carrying *hisS*, the structural gene for this enzyme. Biochemical studies indicate that the complete gene is carried by the phage and that it is under the control of an *E. coli* promoter.

MATERIALS AND METHODS

Bacterial and viral strains. The bacterial strains used in this work are described in Table 1. Lysogens of strain JF298 containing λ cI857 *Sam7* were prepared by Poul Jørgensen (University of Copenhagen). Derivatives of JK67 lysogenic for different transducing phage are given new strain numbers to avoid confusion.

Media and growth conditions. The complex medium used was L-broth (12) supplemented with 0.2% (wt/vol) glucose, or 0.2% maltose when growing cells as recipients for λ transduction. The defined medium used was M9 (1) supplemented with 0.4% glucose, 10 μg of thiamine per ml, and, when necessary, 50 μg of L-amino acids and 20 μg of purine bases per ml. Cultures were grown aerobically in Erlenmeyer flasks with rotary shaking. Growth was monitored with a Klett-Summerson colorimeter.

Isolation of transducing phage. λ*d guaB* transducing phage were isolated by the general procedure described by Schrenk and Weisberg (34) from *att*λ deletion strains lysogenic for λ cI857 *Sam7*. A lysate prepared after heat induction of a mixed culture of these lysogens was used to infect strain JK67 (*guaB*) at a multiplicity of 5 to 10. The infected cells were

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
NP2106	*hisS210 pheS11*	F. C. Neidhardt
PC0631	Hfr, Reeves 4 type, *carB671 metB1 purG48*	P. G. deHaan via CGSC[a]
AT2465	Hfr, Hayes type, *thi-1 relA1 guaA21* λ⁻	A. L. Taylor via CGSC
JF298	Δ(*gal-att*λ-*bio-uvrB*) *thi*	J. Schrenk via J. D. Friesen
JF564	S159 (*uvrA gal rpsL*) lysogenic for λpapa[b]	S. R. Jaskunas via J. D. Friesen
JK66	F⁻ *thi-1 his-68 tyrA2 trp-45 purC50 rpsL125 lacY1 gal-6 xyl-5 mlt-2 tonA2 tsx-70 supE44* λ⁻	26
JK67	As JK66 except *guaB22 purC*⁺	26
JK85	*glyA6 argH1 hisS210* λ⁻	Constructed for this study
JK86	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d*[c] *guaB-1*	Constructed for this study
JK87	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d guaB-2*	Constructed for this study
JK88	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d guaB-3*	Constructed for this study
JK89	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d guaB-4*	Constructed for this study
JK90	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d guaB-5*	Constructed for this study
JK91	As JK67 except lysogenic for λ *cI857 Sam7* and λ *cI857 Sam7 d guaB-6*	Constructed for this study
JK128	*argH1 purG48 upp-1 rpsL* λ⁻	Constructed for this study

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[b] λpapa is the Paris-Pasadena wild-type strain.
[c] *d* = defective lambda.

plated without guanine at 30°C. Colonies were picked and tested for their ability to produce lysates capable of transducing *guaB*⁺.

Phage preparations. Lysates were either used directly or purified by CsCl block gradients followed by equilibrium gradient centrifugation (18). Transductions with λ were done according to the method of Signer (36).

Radioactive labeling. Strain JF564 (lysogenic for λpapa) was inactivated with UV light, infected with transducing phage, and labeled with a reconstituted ^{14}C-protein hydrolysate (3 μCi/ml; 13 amino acids with 156 to 460 mCi/mmol) (38). Extracts were made as previously described (38).

Two-dimensional gels. Two-dimensional separation of proteins on polyacrylamide gels was carried out essentially as described by O'Farrell (24). The pH range in the isoelectric focusing dimension was 5 to 7, and the electrofocusing was carried out for 7,000 V h. The second dimension was a sodium dodecyl sulfate-10% polyacrylamide slab. Gels were stained with Coomassie brilliant blue (30). The gels were then dried under vacuum on filter paper, and autoradiograms were made using Kodak film NS54.

Preparation of subcellular extracts. Subcellular extracts were prepared by sonic disruption essentially as described by Parker and Neidhardt (28) except the buffer used throughout was 50 mM potassium phosphate (pH 7.4), 6 mM 2-mercaptoethanol, and 10% (vol/vol) glycerol.

Protein determinations. Protein content of subcellular extracts was determined by the method of Lowry et al. (14), using bovine serum albumin as a standard.

For determining total protein in a bacterial culture, the following procedure was used. A sample (5 ml) of culture was transferred to a tube containing 0.5 ml of 55% (wt/vol) trichloroacetic acid. The cells were pelleted by centrifugation at 12,000 × *g* for 5 min, resuspended in 5 ml of 5% trichloroacetic acid, and repelleted. The final pellet was resuspended in 1 ml of 1 N NaOH, incubated for 1 h at 37°C, and then diluted with 9 ml of water. This extract was then assayed for protein by the method of Lowry et al. (14).

Synthetase assays. Enzyme activity was measured by determining the rate of aminoacyl-tRNA formation at 37°C. The reaction mixture (0.2 ml) contained 100 mM sodium cacodylate (pH 7.5), 4 mM disodium ATP, 8 mM $MgCl_2$, 200 μg of tRNA, 4 mM 2-mercaptoethanol, 20 μg of bovine serum albumin, and 20 μM L-histidine (20 μCi of L-[^{14}C]histidine per μmol). The reaction was initiated by adding 1 to 4 μg of crude extract protein. The reaction mixture was incubated for 0 to 6 min, and the reaction was terminated with 3 ml of 5% trichloroacetic acid containing 1 mg of L-histidine per ml. The precipitate was washed and counted in a Beckman liquid scintillation counter (28).

Lysyl-tRNA synthetase was assayed as described, except the L-[^{14}C]lysine was 10 μCi/μmol and the reaction was terminated with 5% trichloroacetic acid containing 1 mg of lysine per ml.

One unit of aminoacyl-tRNA synthetase is defined as that amount of enzyme which attaches 1 nmol of amino acid to tRNA in 1 min at 37°C. Specific activity is defined as units per milligram of protein. All enzyme assays included multiple time points and protein concentrations. Enzyme assays were performed within 24

h of extract preparation.

Chemicals. *E. coli* tRNA was obtained from Boehringer-Mannheim. ^{14}C-labeled amino acids were obtained from New England Nuclear Corp. and Schwarz/Mann. Chemicals for electrophoresis are as given by O'Farrell (24). Other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Histidyl-tRNA synthetase (>90% pure) was generously supplied by Carlos Spears and Herbert Weissbach (Roche Institute).

RESULTS

Isolation of λ*d guaB* genes. We had previously mapped *hisS* at 53.8 min on the *E. coli* genetic map (26; see Fig. 1). The only *hisS* mutant of *E. coli* known has a histidine requirement only at high temperatures, making it unsuitable for direct selection with a temperature-inducible phage. Therefore, using the method of Schrenk and Weisberg (34), we isolated transducing phage carrying the structural gene *guaB*, a nearby gene that is readily selected and useful as an outside marker in *hisS* studies (26). Six phages were isolated which could transduce *guaB*, λ*d guaB*-1 through λ*d guaB*-6.

Genetic complementation. Lysates containing each of the six λ*d guaB* phage were then used to attempt to transduce loci other than *guaB*. All these transductions were performed where possible with the appropriate *E. coli* auxotrophs and plated at 30°C. The *upp* locus was screened by first transducing into a *upp purG* double mutant and selecting *purG*$^+$ recombinants at 42°C and then screening for *upp*$^+$ (6-azauracil sensitivity). The *hisS* locus was screened by transducing into a *hisS* mutant (temperature-sensitive auxotrophy for histidine) and selecting for *hisS*$^+$ recombinants at 42°C. The results of these transductions are indicated on Fig. 1. Unfortunately, the temperature-sensitive phenotype of the *hisS* mutation required the screening of recombinants rather than lysogens (which would be temperature sensitive because of the temperature-inducible lambda). For this reason these transductions only reveal that the phage carry at least enough of the gene to give wild-type recombinants when crossed with the mutant alleles used. Further characterization was necessary to determine whether the entire *hisS* gene and its promoter were present on λ*d guaB*-2.

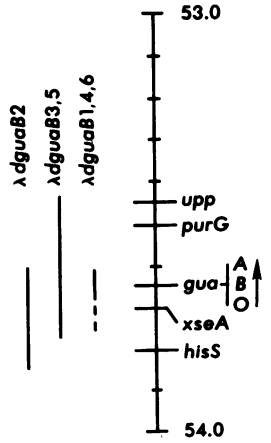

FIG. 1. *E. coli linkage map between 53.0 and 54.0 min. The distances and orientation of the genes on the chromosome are taken from Parker and Fishman (26). The orientation of the gua operon (11, 23) and the location of xseA are as given in reference 37. The lines at the left of the map indicate the extent of the E. coli genetic map as determined by transductional analysis. The xseA locus, the structural gene for exonuclease VII, was shown to be carried by at least λd guaB-2 and λd guaB-3 by J. W. Chase (personal communication).*

Histidyl-tRNA synthetase activity in lysogens. The specific activities of many aminoacyl-tRNA synthetases are found to increase in cells which are diploid for the structural gene (e.g., see 3, 4, 31, 40). We therefore measured the levels of histidyl-tRNA synthetase in extracts from lysogens containing each of the λ*d guaB* phages and from heat pulse cured derivatives of these lysogens. These data are presented in Table 2 along with the specific activity of lysyl-tRNA synthetase in the lysogens. All the data presented in this table are from measurements of extracts prepared in the same experiment. Although the activities of histidyl-tRNA synthetase are reasonably consistent within experiments (±10%), the absolute activities seem to vary between experiments so that internal con-

TABLE 2. *Aminoacyl-tRNA synthetase activities of lysogens and cured lysogens*

Strain	Sp act[a]		
	Histidyl-tRNA synthetase		Lysyl-tRNA synthetase (lysogen)
	Lysogen	Cured[b]	
JK86 (λ*d guaB*-1)	1.55 ± 0.31	1.06 ± 0.13	1.23 ± 0.07
JK87 (λ*d guaB*-2)	3.15 ± 0.40	1.03 ± 0.14	1.41 ± 0.04
JK88 (λ*d guaB*-3)	1.17 ± 0.14	1.04 ± 0.17	1.24 ± 0.10
JK89 (λ*d guaB*-4)	1.34 ± 0.15	0.93 ± 0.14	1.42 ± 0.12
JK90 (λ*d guaB*-5)	0.99 ± 0.08	0.90 ± 0.06	1.23 ± 0.09
JK91 (λ*d guaB*-6)	1.04 ± 0.18	1.00 ± 0.22	1.44 ± 0.10

[a] Specific activities for histidyl-tRNA synthetase and lysyl-tRNA synthetase were determined from crude extracts as described in the text. Histidyl- and lysyl-tRNA synthetase activities presented here are the average of eight and four assays, respectively, ± standard deviation, all of the same extract.

[b] Lysogens were heat pulse cured of helper and transducing phage by the method of Shimada et al. (35). The frequency of curing was 0.16 ± 0.02.

trols must always be run. It can be seen in this table that extracts from the λ*d guaB*-2 lysogen, JK87, contained significantly higher specific activities of histidyl-tRNA synthetase than the extracts from the other lysogens and that its cured derivative had the same activity as the others. The specific activity of lysyl-tRNA synthetase was the same in all extracts. This indicates that λ*d guaB*-2 contained the entire *hisS* gene and, since it was expressed in uninduced lysogens, that the gene is under *E. coli* promoter control.

Since the absolute differences between the histidyl-tRNA synthetase activities in the lysogens and the cured derivatives are not large, we measured the activity of histidyl-tRNA synthetase in extracts of JK87 and JK88 before and after heat induction. Data from the experiment are shown in Table 3 and Fig. 2. It can be seen that although the histidyl-tRNA synthetase specific activities of extracts of the two lysogens differed by less than a factor of 2 before induction, the activity in JK87 rose dramatically after induction. A differential plot of the data (Fig. 2) indicates that the rate of synthesis of histidyl-tRNA synthetase in the λ*d guaB*-2 lysogen increased abruptly at the time of the shift from 30 to 42°C, the final specific activity being over ninefold higher than in the other lysogen (Table 3). This experiment confirms the presence of *hisS* on λ*d guaB*-2.

Synthesis of proteins carried by λ*d guaB* transducing phage. Strain JF564 (lysogenic for λpapa) was inactivated with UV light and infected with λ transducing phage. Proteins were labeled by adding ^{14}C-amino acid mix. All proteins made under such conditions should be encoded by genes carried on λ but under *E. coli* control since the repressor already in the cell prevents expression of λ genes. (The only exception to this is the unmarked protein in Fig. 3C, the λE gene product. The reason for its synthesis in this experiment is not known.) Extracts were prepared and the proteins were separated on two-dimensional polyacrylamide gels. Figure 3 shows autoradiograms of such gels using λ*d guaB*-2, -3, and -6. These represent the three classes of transducing phage as determined by transductional analysis (see Fig. 1).

TABLE 3. *Histidyl-tRNA synthetase activities of lysogens after heat induction*

Time after induction[a] (min)	Histidyl-tRNA synthetase	
	JK87	JK88
Control (30°C)	3.03	1.86
30	7.99	1.72
60	12.3	1.56
90	13.6	1.49

[a] JK87 (λ*d guaB*-2) and JK88 (λ*d guaB*-3) were grown and heat induced as described in the legend to Fig. 2.

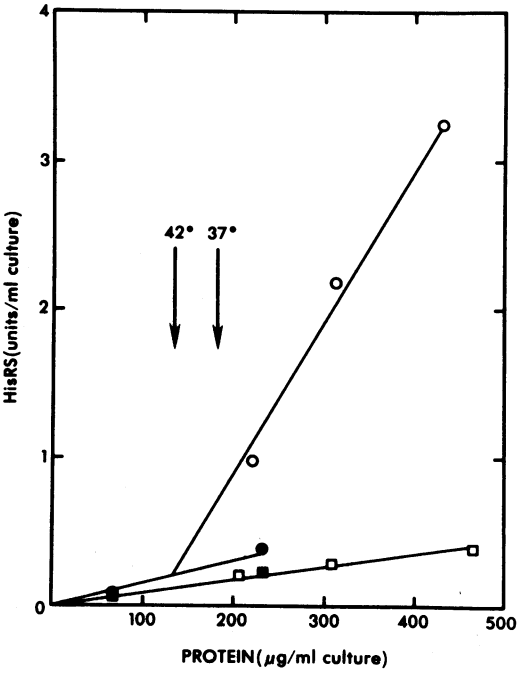

FIG. 2. *Differential rate of histidyl-tRNA synthetase synthesis in induced lysogens. Lysogens were grown at 30°C in L-broth to a Klett reading (filter no. 54) of 95, at which point a portion of each culture was heat induced by shifting to 42°C for 20 min and then to 37°C as indicated by the arrows. Histidyl-tRNA synthetase (HisRS) specific activities and total protein were measured before and after induction. Histidyl-tRNA synthetase activity per milliliter of culture was calculated assuming 100% recovery in crude extracts. Symbols: JK87 (λd guaB-2)—induced (○), uninduced (●); JK88 (λd guaB-3)—induced (□), uninduced (■).*

One of the radioactive proteins in the λ*d guaB*-2 extracts comigrates on the gel with purified histidyl-tRNA synthetase. This protein is labeled HisRS in Fig. 3A. This protein is found only with λ*d guaB*-2, as would be expected. The apparent molecular weight of the protein in this gel system is 49K compared to the reported subunit molecular weight of 42.5K (9).

There seem to be four proteins in common on the three gels, marked a, b, c, and d. It is not clear from our present analysis whether c is an independent gene product or a modified form of B. Although one would expect the products of the *guaA* and *guaB* genes to be among these common proteins, we have not yet identified them. The *guaA* product, GMP synthetase (EC

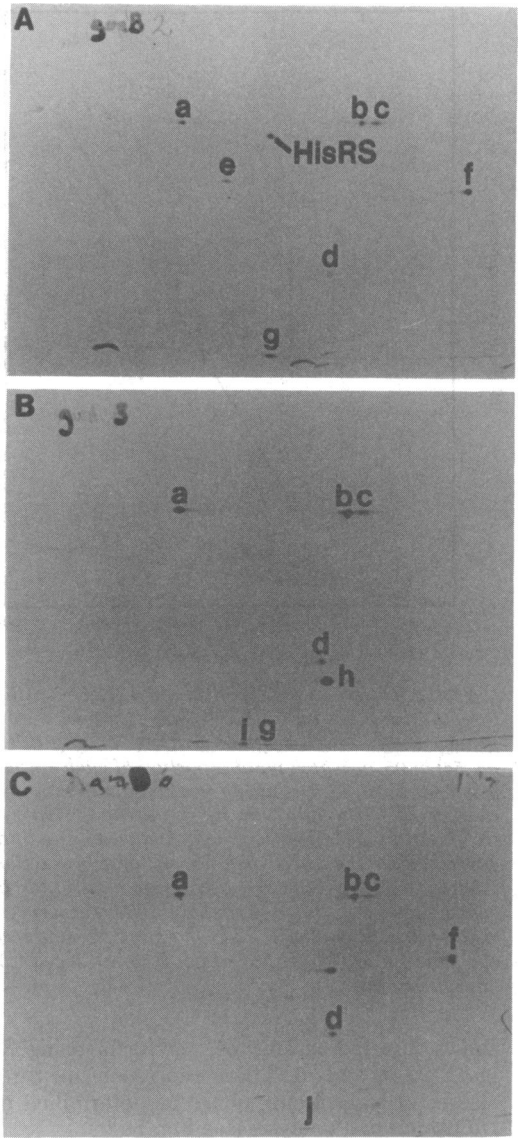

FIG. 3. *Autoradiograms of two-dimensional gels of E. coli proteins encoded by λd guaB phages. Strain JF564 (λpapa) was inactivated with UV light for 6 min (to 1% residual rate of protein synthesis), by exposing it to a 15-W Atlantic UV germicidal lamp at a distance of 35 cm, and then infected with purified transducing phage as described in the text. (A) λd guaB-2 infection; (B) λd guaB-3 infection; (C) λd guaB-6 infection. Protein synthesized under these conditions was labeled with a mixture of ^{14}C-amino acids. Extracts were made, two-dimensional gels were run, and autoradiograms were made as described in the text. Nonradioactive, stained E. coli proteins served as molecular weight markers. The molecular weights of the proteins indicated in this figure are: a, b, and c, 54,000; d, 22,500; e, 39,000; f, 38,500; h, 21,000; g, i, and j, ≤14,000; and histidyl-tRNA synthetase (HisRS), 49,000. The autoradiograms are oriented with the acidic proteins to the right.*

6.3.4.1, XMP aminase), has been purified from *E. coli* B and found to have a subunit molecular weight of 63K (33). Proteins a, b, and c all have apparent molecular weights of 54K. Interestingly, protein a is the major contaminant (<5%) of the purified histidyl-tRNA synthetase.

λd guaB-3 is the only one of these three phages to carry *upp* and *purG*. On our gel system there are only two proteins unique to this phage, h and i. Although it is tempting to speculate that these are the products of *upp* and *purG*, there are other possibilities. First, the actual products of these genes might have isoelectric points outside the range of our gel (pH 5 to 7). Second, there is no evidence that all genes are expressed in UV light-killed cells.

DISCUSSION

The genetic characterization of *λd guaB-2* and the biochemical analysis of cells lysogenic or infected with the virus demonstrated that it carries the entire structural gene for histidyl-tRNA synthetase and that this gene is under *E. coli* control. Since the pattern of regulation of the enzyme is not well known, it is not possible to say conclusively that it is under its normal control. Although the possibilities of genetic rearrangements during excision exist, we have no reason to suspect that any have occurred with *λd guaB-2*. In addition to *hisS*, *λd guaB-2* carries the *gua* operon and *xseA*, the structural gene for exonuclease 7 (J. W. Chase, personal communication).

The identification of the histidyl-tRNA synthetase protein on the two-dimensional gel system will greatly facilitate measurement of the amount of enzyme present and its rate of synthesis under a variety of conditions (21, 32). Although the correlation between activity measurements and chemical measurements has proved excellent for some synthetases (arginyl- and valyl-tRNA synthetases; 21, 27), it has not proved so for at least one other (leucyl-tRNA synthetase; 21, 28). In the latter case activity measurements showed, at best, a slight increase of leucyl-tRNA synthetase with increasing growth rate (28), whereas chemical measurements showed a much greater increase, similar to that of the other aminoacyl-tRNA synthetases (21). Activity measurements of histidyl-tRNA synthetase from cells at different growth rates show no clear tendency of change (S. E. Fishman, unpublished data). It must be remembered, though, that slight changes in histidyl-tRNA synthetase activity would be difficult to

quantitate (see Table 2). Furthermore, although our assay system has been optimized for measuring histidyl-tRNA synthetase, specific activities of arginyl-tRNA synthetase measured in the same system are 10-fold higher. It is unclear whether this represents a 10-fold difference in the amount of the enzyme or some problems in measuring histidyl-tRNA synthetase. The ability to chemically identify the histidyl-tRNA synthetase protein might also allow measurement of the molecule under conditions which lead to an apparent lability of the enzyme activity (39). This is particularly interesting since these conditions, histidine deprivation, should lead to both an increased rate of histidyl-tRNA synthetase synthesis (16, 17) and a very high level of mistranslation (29).

The finding that the major contaminant of highly purified histidyl-tRNA synthetase is encoded by a gene near *hisS* is not necessarily a coincidence. In *Salmonella typhimurium* a pleiotropic mutation, *strB* (40), seems to affect histidyl-tRNA synthetase regulation and maps very close to *hisS*. Furthermore, intergeneric merodiploid studies have shown that the wild-type allele of this gene is present in *E. coli* at approximately the same map location (40). It is possible, then, that protein a (Fig. 3A, B, and C) is the product of the *E. coli* equivalent of the *S. typhimurium strB* gene and that it interacts with histidyl-tRNA synthetase.

Physical mapping of λ*d guaB*-2 and in vitro synthesis should allow us to identify that segment of the DNA carrying *hisS* and its control region and the other genes carried by this phage.

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