The effects of leader peptide sequence and length on attenuation control of the *trp* operon of *E.coli*

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Received November 19, 1990; Revised and Accepted January 18, 1991

ABSTRACT

We have examined the effects of changing the length and codon content of the *trp* leader peptide coding region on expression of the *trp* operon of *Escherichia coli*. It had previously been shown that coupling of transcription and translation in the *trp* leader region is essential for both basal level control and tryptophan starvation control of transcription attenuation in this operon. We have found that increasing the length of the leader peptide coding region by 55 codons allowed normal basal level control and normal tryptophan starvation control. As expected, the presence of a nonsense codon early in the leader peptide coding region decreased basal expression and eliminated starvation control. Introducing tandem rare codons had no effect on basal level expression, but eliminated the tryptophan starvation response. Frameshifting at tandem rare codons was tested as the most likely explanation for loss of the tryptophan starvation response, but the results were inconclusive.

INTRODUCTION

Although many features of transcription attenuation have been elucidated in studies with the *trp* and other biosynthetic operons (1–8), one of the remaining uncertainties is the significance of the length and codon content of the leader peptide coding region. In *E. coli* strains in which the initiation of synthesis of the *trp* leader peptide is prevented, basal level expression of the *trp* operon (expression in the presence of excess tryptophan) is reduced approximately 80% (9). In addition, in such mutants transcription termination in the leader region is not relieved upon tryptophan starvation (4,5). Therefore, translation of the *trp* leader peptide coding region affects both basal level expression of the operon, and the operon's response to tryptophan limitation. To examine the effect of leader peptide length and codon content on *trp* operon expression, we constructed altered *trp* leader regions in which these features were varied. Analyses of the regulatory effects of these alterations allowed us to determine whether the leader peptide coding region must be short, and whether rare codons (10,11) must be avoided, to obtain the coupling of transcription and translation that is essential for normal basal level expression and tryptophan starvation control.

MATERIALS AND METHODS

Plasmids and strains

E. coli strains W3110 *tnaA2 trpR* Δ *trpLE11-180* and W3110 *tnaA2 trpR miaA* Δ *trpLE11-180*, designated RK2 and RK3, respectively (9), were used generally, and were from our laboratory stocks. These strains have a deletion extending from *trpL* bp 11 to *trpE* bp 180. Strain W3110 *tnaA2 trpR trpA46PR9* Δ *trpLE11-180* (CY15100) was constructed for this investigation. Plasmid pRL415, a derivative of pUC119, was provided by Robert Landick of Washington University (12). This plasmid contains the promoter-leader region of the *trp* operon which has been engineered to contain unique *Pst*I and *Eco*RI restriction sites bracketing RNA segment 1 and designated *trpLEP* (see Fig. 1A). Altered *trpL* regions on plasmids were recombined into the *trp* operon on the *E. coli* chromosome by replacing the deletion Δ *trpLE11-180* and selecting for Trp⁺ recombinants (9). *trp* leader regions were then transferred to plasmid-free strains by generalized P1 transduction and selection for growth in the absence of tryptophan.

DNA Manipulations

A 165 bp *Pst*I fragment from the coding region of *con-10*, a conidiation specific gene from *Neurospora crassa* (13,14), was ligated into the unique *Pst*I site in the *trp* leader region of pRL415. Recombinant plasmids containing the *con-10* fragment in both orientations and a double insert in one orientation were isolated and sequenced. The sequences of the inserts (*trpL55A* and *trpL55B*) and the predicted amino acid sequence of the altered *trp* leader peptides are shown in Figure 1C.

trp leader regions containing rare codons were constructed by annealing complementary synthetic oligonucleotides encoding the rare codons and ligating them into the *Pst*I site of pRL415. Desired recombinants were detected by colony hybridization screening using ³²P labeled oligonucleotides. DNA sequence analysis was performed to confirm the presence of the expected sequence (15).

Anthranilate synthase assays

Anthranilate synthase assays were performed essentially as described (16). Inocula were grown overnight in M9 minimal medium containing 0.05% acid hydrolyzed casein (ACH) and either no tryptophan or 50 μ g/ml *L*-tryptophan. The cells were diluted 1:50 into 10 ml of the same medium and grown to a

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RNA secondary structures were predicted using the RNAFLD program of Zuker and Steigler (17).

The effects of leader peptide length on transcription-translation coupling

We wished to examine the influence of the length of the *trp* leader peptide coding region on transcription attenuation in the *trp* operon of *E. coli*. A 165 bp *Pst*I fragment from the *con-10* gene of *Neurospora crassa* (sequence in Fig. 1C) was selected and was ligated into the unique *Pst*I site of pRL415 (Fig. 1A). The *con-10* segment was chosen because it contained no Trp or termination codons and was a convenient size. The insert is not

optimized for translation in *E. coli* and contains codons that are not preferred by *E. coli*, but has no runs of rarely used codons. Both orientations of the 165 bp fragment were isolated, as was an isolate with the proper phase insert in duplicate. In *trpL55A* 55 codons of *con-10* were fused in-frame within the leader peptide coding region (Fig. 1). The reverse orientation of the fragment, designated *trpL55B*, contains an in-frame stop codon at the 11th codon of the insert. These altered *trp* leader regions were introduced into the chromosome using P1-mediated transduction, replacing the short *trpL-trpE* deletion in the various recipients.

Two different types of strains were utilized to measure *trp* operon expression under tryptophan limiting conditions. One recipient strain carried the *trpA46PR9* mutation. This mutation results in the production of a tryptophan synthetase α chain that is only slightly active. Strains with this mutation are tryptophan-starved during growth in minimal medium and exhibit high levels of transcription readthrough at the *trp* attenuator. Thus they mimic the wild type strain subjected to tryptophan starvation. A second strain contained the *miaA* mutation. The *miaA* mutation eliminates isopentenylation of tRNA^{Trp} causing inefficient translation of Trp codons (18,19). *miaA* containing strains also have elevated levels of *trp* operon expression, even in the presence of excess tryptophan. Expression of the *trp* operon of each of the recombinant strains was determined by assaying the level of anthranilate synthase (the complex of the *trpE* and *trpD* polypeptides, products of the first two structural genes of the *trp* operon) produced in cells grown in the presence or absence of tryptophan.

The data in Table 1 show that the anthranilate synthase activity of strain *CY15102*, which contains *trpL55A*, is the same as that of the parental strain *CY15101*, when it is grown either in the presence or absence of tryptophan. It was shown previously that *trp* operon regulation in strains containing *trpLEP* (leader region containing unique *PstI* and *EcoRI* restriction sites; see Fig. 1) is indistinguishable from *trp* operon expression in wild-type strains (7,20,21). The results for *trpL55A* in a *miaA* mutant strain (strain *CY15107*) were similar to those in the *trpA46PR9* strain (Table 1). These findings demonstrate that the length of the *trp* leader

peptide coding region may be increased appreciably without affecting basal level or tryptophan starvation expression of the operon, even if the inserted codons are not optimal for *E. coli* translation. Strain *CY15103* (*trpL55B*), has an early stop codon in-frame with the leader peptide start codon (Fig. 1C). This strain has approximately one-fifth the anthranilate synthase level of the wild type strain when grown in the presence of tryptophan. The anthranilate synthase level of *CY15103* is not increased when it is grown under tryptophan limiting conditions. The decreased *trp* operon expression observed in *CY15103* is in agreement with results obtained with two previously isolated strains (*RK19*, *RK29*) containing the *trp* leader mutation *trpL29*, in which the *trp* leader peptide initiation codon was altered and there is little or no translation initiation (4,5).

A *trp* leader region with a tandem repeat (110 codons) of the in-frame *con-10* insert, *trpL110*, also was prepared. This insert was introduced into the chromosome in *miaA*⁺ and *miaA* strains. *CY15108* (*miaA*⁺) had anthranilate synthase levels similar to the wild type strain (Table 1), indicating that basal level expression of the *trp* operon was unaffected by the increased length of the leader peptide coding region. In the *miaA* mutant strain, *CY15109*, however, the anthranilate synthase level increased, but not to the same extent as in wild type or in the *trpL55A* strain (*CY15107*).

The effect of rare codons in *trpL* on *trp* operon expression

Maximal basal level expression and tryptophan starvation expression of the *trp* operon is believed to depend on the translating ribosome reaching the stop codon or the Trp codons before synthesis of the terminator structure by RNA polymerase. Therefore it is thought that transcription and translation must be tightly coupled for proper basal level and tryptophan starvation expression. *E. coli* and other organisms do not utilize codons at random to specify their polypeptides. In highly expressed genes, a codon subset is used preferentially (10,11). Rare codons have been shown to slow the progress of translation in some cases (22–26). To examine the effect of rare codons on attenuation in the *trp* operon, several double-stranded synthetic oligonucleotides containing repeats of rare or common codons were ligated into the unique *PstI* site (Figure 1) of pRL415. The altered *trp* leader regions were then recombined into the chromosome, and these recombinant strains were grown and assayed for anthranilate synthase activity.

Tandem insertion of four copies of the rare AGG codon into the *trp* leader coding region had little effect on the basal anthranilate synthase level (Table 2, strain *CY15110*). However the four AGG codons prevented the increase in expression generally seen under tryptophan starvation conditions. The reverse orientation of the (AGG)₄ insert, (CCU)₄, gave a *trp* leader peptide coding region containing a relatively infrequently used proline codon, but one that is more common than AGG (10); approximately 10% of the *E. coli* proline codons that have been sequenced are CCU whereas <1% of the arginine codons are AGG (10). Regulation of the *trp* operon in *CY15111* containing *trpLCCU4* was essentially the same as in *CY15110*. That is, basal level expression of the operon was indistinguishable from wild type, and there was no tryptophan starvation response.

Strains containing two AGG or CCU codons inserted into the *trp* leader peptide coding region also were constructed. These leader regions were designated *trpLAGG2* and *trpLCCU2* and the strains containing them *CY15112* and *CY15113*, respectively. As shown in Table 2, the pattern of *trp* operon expression in *CY15112* was nearly identical to that of *CY15110* and *CY15111*.

Table 1. The effect of leader peptide length on *trp* operon expression

Strain	Relevant genotype ^b	ASase activity ^a	
		Basal level ^c	Starvation ^d level
<i>CY15101</i>	<i>trpA46PR9 trpLEP</i>	100	210+/-9.9
<i>CY15102</i>	<i>trpA46PR9 trpL55A</i>	95+/-10.7	203+/-12.8
<i>CY15103</i>	<i>trpA46PR9 trpL55B</i>	18+/-4.6	19+/-6.8
<i>CY15104</i>	<i>miaA</i> ⁺ <i>trpLEP</i>	102+/-4.5	—
<i>CY15105</i>	<i>miaA</i> <i>trpLEP</i>	—	190+/-13.8
<i>CY15106</i>	<i>miaA</i> ⁺ <i>trpL55A</i>	101+/-13.2	—
<i>CY15107</i>	<i>miaA</i> <i>trpL55A</i>	—	197+/-17.1
<i>CY15108</i>	<i>miaA</i> ⁺ <i>trpL110</i>	105+/-4.6	—
<i>CY15109</i>	<i>miaA</i> <i>trpL110</i>	—	142+/-9.2
<i>RK8</i>	<i>miaA</i> ⁺ <i>trpL29</i>	30+/-6.6	—
<i>RK9</i>	<i>miaA</i> <i>trpL29</i>	—	31+/-7.0

^a Anthranilate synthase (ASase) activity was measured as described in Materials and Methods. The activity observed with the various strains containing *trpLEP* was shown to be identical to that of the wild type *trpLUGA* leader construct. *CY15101* activity was normalized to 100. Standard deviations are given.

^b All strains are derivatives of W3110 *trpR miaA2 trp-11/180*.

^c Basal level expression was determined in cultures grown in the presence of excess tryptophan (50μg/ml).

^d To determine tryptophan starvation levels *A46PR9* strains were grown in the absence of tryptophan, *miaA* strains were grown with tryptophan.

In *CY15113* however, the basal level of *trp* operon expression was slightly elevated, whereas tryptophan starvation gave anthranilate synthase levels comparable to those of the wild type strain.

The anthranilate synthase activities of strains with four AUA Ile codons and four UAU Tyr codons inserted into the *PstI* site of *trpLEP* also were determined (Table 2). AUA is a rare Ile codon in *E. coli* (<1%, ref. 10) and UAU is a commonly used Tyr codon (34%, ref. 10). In contrast to the results obtained with the *trpLAGG2* and *trpLAGG4* containing strains, *CY15114* (*trpLAUA4*) had nearly the same level of anthranilate synthase as the wild type strain in the presence of tryptophan, and exhibited a large tryptophan starvation response. The strain with 4 UAU codons, *CY15115*, had a slightly elevated basal level and the tryptophan starvation response was somewhat less than that of the wild type strain.

Analysis of the possibility of frameshifting

The simplest explanation for the lack of a tryptophan starvation response in strains with multiple rare codons in the leader peptide coding region is that ribosomal frameshifting occurs during

translation of these repeat rare codons. Frameshifting would prevent the translating ribosome from encountering the distal Trp codons or the normal stop codon. A deletion of 1 base in the *trp* leader coding region has been shown to have little effect on basal level expression, but it results in loss of the tryptophan starvation response (20). Tandem AGG codons have been shown to be capable of causing 50% ribosomal frameshifting in *E. coli* (23). In an attempt to detect frameshifting we inserted one and two extra base pairs between the four AGG codons and the Trp control codons (Table 3 legend). A frameshift in the +1 phase would cause the translating ribosome to read past the normal stop codon and terminate 17 codons beyond, at the UAA codon at position 100 (Fig. 1). A -1 frameshift would result in translation through the termination region to a stop codon located between this region and *trpE*, the first major structural gene of the operon. The +1 frameshift would be expected to have no effect on basal level expression of the operon because the translating ribosome would still disrupt the 1:2 pause structure, and it would reach a stop codon in the vicinity of its normal location. A -1 frameshift would be expected to significantly increase basal level expression of the *trp* operon, because translation through the terminator should prevent transcription termination. Since there are no Trp codons in either the +1 or -1 reading frame, frameshifting should eliminate the tryptophan starvation response. Addition of 1 or 2 bases between the AGG codons and the Trp control codons, should restore translation to the normal reading frame if a +1 or -1 frameshift occurred at the AGG codons. However if the frequency of frameshifting was less than 50%, it is unlikely that we would observe a significant change in the anthranilate synthase level. The altered leader regions were recombined into the chromosome of *miaA* and *miaA*⁺ strains and assayed for anthranilate synthase activity. As shown in Table 3, strains containing *trpLAGG+2n* (*CY15121*), but not *trpLAGG+1n* (*CY15117*) had slightly elevated anthranilate synthase activity in the presence of the *miaA* allele. However, the level was not as high as in the wild type strain containing *miaA*. This finding suggests that there may be some -1 frameshifting at the AGG codons. Thus frameshifting may be partly responsible for the lack of response of the *trpLAGG4* strains to tryptophan starvation. Complicating the interpretation of this result is the

Table 2. The effect of rare codons on *trp* operon expression

Strain	insert ^b	Met-----insert-----Trp Trp-----UGA ASase Activity ^a	
		Basal level ^c (+Trp)	Starvation level ^d (-Trp)
<i>CY15101</i>	<i>trpLEP</i>	100	210 +/- 9.9
<i>CY15110</i>	(AGG)4 Arg ₄	93 +/- 2.9	103 +/- 10.1
<i>CY15111</i>	(CCU)4 Pro ₄	92 +/- 19.6	95 +/- 8.0
<i>CY15112</i>	(AGG)2 Arg ₂	98 +/- 7.1	106 +/- 7.3
<i>CY15113</i>	(CCU)2 Pro ₂	139 +/- 14.2	231 +/- 24.7
<i>CY15114</i>	(AUA)4 Ile ₄	103 +/- 21.5	255 +/- 33.2
<i>CY15115</i>	(UAU)4 Tyr ₄	122 +/- 2.9	166 +/- 23.6

^a Anthranilate synthase (Asase) activity was measured as described in Materials and Methods. The values for the *trpLEP* containing strain grown in the presence of tryptophan was normalized to 100. The average results for four trials are presented along with standard deviations.

^b All *trp* leader regions were in W3110 *tnaA2 trpR* +/- *trpA46PR9*.

^c Strains were grown in the presence of 50 µg/ml tryptophan.

^d Strains were grown in media lacking tryptophan.

Table 3. ASase activities of strains with *trp* leader frameshift mutations

Strain	<i>trp</i> leader insert	ASase Activity ^a	
		Basal level <i>miaA</i> ⁺	Starvation level <i>miaA</i>
<i>CY15104</i> , <i>CY15105</i>	<i>trpLEP</i>	100	190 +/- 13.8
<i>CY15116</i> , <i>CY15117</i>	<i>trpLAGG4+1n</i>	121 +/- 19.8	119 +/- 7.1
<i>CY15120</i> , <i>CY15121</i>	<i>trpLAGG4+2n</i>	93 +/- 16.1	141 +/- 9.7

Insert sequences:

Leu His Arg Arg Arg Arg Pro Ala Glu Arg Leu Val Ala
AGG4+1n CTG CAC AGG AGG AGG AGG CCT GCA GAA AGG TTG GTG GCG
 ↑
 Leu His Arg Arg Arg Arg Pro Cys Arg Lys Val Gly Gly
AGG4+2n CTG CAC AGG AGG AGG AGG CCG TGC AGA AAG GTT GGT GGC
 ↑↑

^a Anthranilate synthase (Asase) activity was measured as described in Materials and Methods. *trpLEP miaA*⁺ values were normalized to 100. The sequences of the inserts are shown, with added sequence underlined and added bases in bold and marked by arrows. The predicted amino acid sequences of the in-phase translation products are shown. The nucleotides of the natural Trp codons are overlined.

unexpected finding that basal level expression is not elevated in the *trpLAGG4+2n* strain, although translation in this reading frame (a -1 frameshift) is predicted to disrupt the terminator structure.

DISCUSSION

The molecular features of transcription attenuation in the *trp* operon of *E. coli* have been studied extensively (4–9,21). Perhaps the most important relationship in this example of transcription attenuation, is the position of the ribosome translating the leader peptide coding region relative to the location of the RNA polymerase molecule transcribing the leader region. The translating ribosome is thought to affect RNA polymerase location and movement in the *trp* leader region in at least three ways. First, ribosome translation of the initial segment of the leader peptide coding region disrupts the RNA pause structure, 1:2 (Fig. 1), allowing RNA polymerase to resume transcription. This event presumably synchronizes translation and transcription. When a ribosome is prevented from translating the leader peptide coding region, expression of the *trp* operon decreases by 70–80% (4,5). Second, when the translating ribosome stalls at the tandem Trp control codons at the base of RNA segment 1, the antiterminator (structure 2:3, Fig. 1) forms, and prevents formation of the RNA terminator (structure 3:4, Fig. 1)—the transcription termination signal (6). Third, release of the translating ribosome at the leader peptide stop codon is essential in establishing the basal level expression typical of this operon (7,21). When the ribosome releases from this stop codon on a fraction of the *trp* operons being transcribed, the leader transcript presumably folds to form either structure 1:2 or 2:3. Occasional formation of the RNA antiterminator prevents formation of the terminator (9).

From the above considerations it is apparent that the codon content and length of the *trp* leader peptide coding region could have a profound influence on the effectiveness of attenuation in controlling *trp* operon expression. We examined these parameters and observed that increasing the length of the leader peptide coding region by 55 codons permitted normal *trp* operon expression. Enlarging the coding region by 110 codons had no effect on the basal level, but did reduce the elevated expression generally observed under tryptophan starvation conditions. When translation was stopped early in the leader coding region, basal level expression was reduced by 75–80%. These findings show that extending the length of the coding region does not prevent the coupling of transcription and translation that is essential for proper attenuation regulation of the operon. It should be noted that the 55 codon insert was from a *N. crassa* gene. *N. crassa* has a different codon bias than *E. coli*, and the inserted sequence has several codons that are rarely used in *E. coli* (Fig. 1 and ref. 10). Nevertheless, regulation was normal. It is conceivable that the lengthened coding region is inefficiently translated, but the half-life of the RNA polymerase pause complex is sufficiently long that the slower translating ribosome can catch up with the paused polymerase.

In strains containing tandem rare codons, *trpLAGG4*, *trpLAGG2*, and *trpLCCU4*, anthranilate synthase levels did not increase under tryptophan starvation conditions. However in these strains basal level expression of the operon was not appreciably different than in wild type. This was true although tandem repeated AGG codons have been shown by others to be inefficiently translated (23,26). The simplest interpretation of our data is that the

translating ribosome was able to translate past the inserted rare codons, disrupt the RNA pause structure, and reach the leader peptide stop codon. A possible explanation for the lack of a tryptophan starvation response in these strains is that translational frameshifting occurs at some frequency at the added rare codons and thus the translating ribosome rarely encounters the Trp codons in phase. Translation shifted into either the $+1$ or -1 reading frame in the *trp* leader peptide coding region would be expected to allow the translating ribosome to disrupt the pause structure and release RNA polymerase for continued transcription. The possibility of ribosomal frameshifting is supported by findings in two previous studies. First, Landick *et al.* have shown that a $+1$ frameshift in the *trp* leader peptide coding region eliminated the tryptophan starvation response but had little effect on basal level expression (20). Second, ribosomal frameshifting at AGG codons in *E. coli* has been demonstrated by Spanjaard and Van Duin (23); they observed 50% frameshifting into the $+1$ phase when the coding region examined contained tandem AGGs.

We attempted to demonstrate ribosomal frameshifting by making strains in which either one or two nucleotides were added between the repeated AGGs and the Trp codons. *CY15120* (*trpLAGG4+2n*) did display a significant increase in *trp* operon expression in the absence of tryptophan (Table 3), but the elevation was only about one-half that of the wild type strain. The most plausible explanation for this result is that frameshifting did occur at the AGG codons, but the extent was insufficient to restore most translation to the normal reading frame. However, interpretation of results with our system is complicated by the expectation that a -1 frameshift would allow the translating ribosome to translate beyond the terminator segment of the leader transcript. Ribosome movement over the terminator should disrupt it and decrease transcription termination. We have no suitable explanation for our failure to observe such a decrease.

The effect of inserting rare codons into *trpL* on the tryptophan starvation response varied depending on the codons tested. Either 2 or 4 tandem AGGs eliminated tryptophan starvation induction. Four tandem CCU codons led to loss of tryptophan starvation induction, while 2 tandem CCU codons did not. Perhaps this difference is due to the fact that AGG is more rarely used than CCU in *E. coli* (10). However the AUA isoleucine codon is used as infrequently as AGG in *E. coli* (10), yet *trp* operon expression in the *trpLAUA4* strain increased more than two fold when it was starved of tryptophan (Table 2). It should be noted that the *trpL55A* insert introduces two AGG codons (at positions 8 and 41) but the *trpL55A* strain showed a normal increase in operon expression when grown in the absence of tryptophan. These observations suggest that the loss of induction upon tryptophan starvation may not be solely attributable to the rarity of the codon used, but may also depend on other features, such as the identity of the codon or its neighbors.

The effect of translation of rare codons in a leader peptide coding region has also been examined by Bonekamp and Jensen (26). They inserted three contiguous AGG codons into the *pyrE* leader region and measured the effect on *pyrE* operon expression. Expression of this operon is normally dependent on transcription-translation coupling, and is sensitive to changes in the UTP concentration (low UTP concentrations slow transcription, increase coupling, and increase transcription readthrough) (27). Strains containing *pyrE* leader regions with AGG codons were not induced to the same extent as the wild-type strain, suggesting that translation of the rare codons was slow. Slow translation presumably uncoupled transcription from translation.

Interpretation of these results is complicated by the fact that +1 frameshifting at the inserted AGG codons would cause translation to terminate at a UAG codon 30 nucleotides upstream of the normal leader peptide stop codon. Thus frameshifting and premature translation termination would uncouple transcription and translation and prevent the normal response to low UTP concentrations.

ACKNOWLEDGEMENTS

We are grateful to Paul Gollnick, Barry Hurlburt, Oded Yarden and Dan Ebbole for helpful discussions. We also thank Susan Lacoste for preparation of the manuscript. This study was supported in part by United States Public Health Service Grant GM-09738. J.R. was a Postdoctoral Fellow of the National Institutes of Health (GM 12090). C.Y. is a Career Investigator of the American Heart Association.

REFERENCES

1. Kolter, R. and Yanofsky, C. (1982) *Annu. Rev. Genet.*, **16**, 113–143.
2. Bauer, C.E., Carey, J., Kasper, L.M., Lynn, S.P., Waechter, D.A. and Gardner, J.F. (1983) In Davies, J. and Gallant, J. (eds.), *Gene Function in Prokaryotes*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 65–89.
3. Landick, R. and Yanofsky, C. (1987) In Neidhardt, F.C. (ed.) *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*. American Society for Microbiology, Washington, D.C. pp. 1276–1301.
4. Zurawski, G., Elseviers, D., Stauffer, G.V. and Yanofsky, C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5988–5992.
5. Zurawski, G. and Yanofsky, C. (1980) *J. Mol. Biol.* **142**, 123–129.
6. Landick, R., Carey, J. and Yanofsky, C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4663–4667.
7. Roesser, J.R., Nakamura, Y. and Yanofsky, C. (1989) *J. Biol. Chem.* **264**, 12284–12288.
8. Winkler, M.E. and Yanofsky, C. (1981) *Biochemistry* **20**, 3738–3744.
9. Kolter, R. and Yanofsky, C. (1984) *J. Mol. Biol.* **175**, 299–312.
10. Ikemura, T. (1985) *Mol. Biol. Evol.* **2**, 13–34.
11. Grantham, R., Gautier, C., Gouy, M., Mercier, R. and Pavé, A. (1980) *Nucl. Acids Res.* **8**, r49–62.
12. Landick, R., Carey, J. and Yanofsky, C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1507–1511.
13. Berlin, V. and Yanofsky, C. (1985) *Mol. Cell. Biol.* **5**, 849–855.
14. Hager, K.M. and Yanofsky, C. (1990) *Gene* (In press).
15. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
16. Creighton, T.E. and Yanofsky, C. (1970) *Methods Enzymol.* **17**, 365–380.
17. Zuker, M. and Steigler, P. (1981) *Nucl. Acids Res.* **9**, 133–148.
18. Eisenberg, S.P., Yarus, M. and Soll, L. (1979) *J. Mol. Biol.* **135**, 111–126.
19. Buck, M. and Griffiths, E. (1982) *Nucl. Acids Res.* **10**, 2609–2624.
20. Landick, R., Yanofsky, C., Choo, K. and Phung, L. (1990) *J. Mol. Biol.* (In press).
21. Roesser, J.R. and Yanofsky, C. (1988) *J. Biol. Chem.* **263**, 14251–14255.
22. Robinson, M., Lilley, R., Little, S., Emtage, J.S., Yarrenton, G., Stephens, P., Millican, A., Eaton, M. and Humphreys, G. (1984) *Nucl. Acids Res.* **12**, 6663–6671.
23. Spanjaard, R.A. and Van Duin, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7967–7971.
24. Petersen, S. (1984) *EMBO J.* **3**, 2895–2898.
25. Bonekamp, F., Andersen, H.D., Christensen, T. and Jensen, K.F. (1985) *Nucl. Acids Res.* **13**, 4113–4123.
26. Bonekamp, F. and Jensen, K.F. (1988) *Nucl. Acids Res.* **16**, 3013–3025.
27. Bonekamp, F., Clemmesen, K., Karlstrom, O. and Jensen, K.F. (1984) *EMBO J.* **3**, 2857–2861.