Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12525–12529, December 1994
Genetics

A mutation in ribosomal protein L9 affects ribosomal hopping during translation of gene *60* from bacteriophage T4

KAREN L. HERBST*†, LINDY M. NICHOLS*†, RAYMOND F. GESTELAND*†, AND ROBERT B. WEISS†

*Howard Hughes Medical Institute and †Department of Human Genetics, University of Utah, Salt Lake City, UT 84112

Communicated by Raymond L. White, July 5, 1994

ABSTRACT **Ribosomes hop over a 50-nt coding gap during translation of gene *60* mRNA from bacteriophage T4. This event occurs with near-unitary efficiency when gene *60–lacZ* fusions are expressed in *Escherichia coli*. One of the components necessary for this hop is an RNA hairpin structure containing the 5′ junction of the 50-nt coding gap. A mutant *E. coli* was isolated and found to significantly increase hopping when carrying gene *60–lacZ* constructs with altered hairpins. The mutation, *hop-1*, changed Ser^{93} to Phe in *rplI*, the gene coding for ribosomal large-subunit protein L9. Ribosomal hopping on a synthetic sequence in the absence of a hairpin was also increased by this mutation. These data suggest that *hop-1* may substitute for the function of the hairpin during ribosomal hopping.**

During translation of bacteriophage T4 topoisomerase subunit gene *60* mRNA, ribosome-bound peptidyl-tRNA (ptRNA) disengages from glycine codon 46 (GGA) and reengages at an identical codon 50 nt downstream (1, 2). The production rate of single polypeptide chains from these two, disconnected, open reading frames encoding this single polypeptide approaches 100% *in vivo* in *Escherichia coli* (2). It has been surmised that during translational elongation, ptRNA bypasses, or hops, over the 50-nt coding gap. There are five discrete "recoding signals" in gene *60* mRNA required for the hop (2, 3); these signals recode gene *60* by altering the linear translation of mRNA codons. The five signals (Fig. 1) are (*i*) a cis-acting 16-aa stretch of the nascent peptide (KYKLQNNVRRSIKSSS) translated from codons 17–32 preceding the gap, (*ii*) a stop codon immediately following the takeoff site, (*iii*) a short stem–loop structure (hairpin) at the takeoff site, (*iv*) matching takeoff and landing sites [GGA (Gly) codons in the wild-type gene], and (*v*) an optimal 50-nt spacing separating codons 46 and 47.

Mismatching the takeoff and landing sites, changing the stop codon to a sense codon, increasing or decreasing the size of the coding gap, and altering the required peptide all reduce bypass of the coding gap. The putative wild-type hairpin of gene *60* contains an unusually stable CUUCGG tetraloop (2, 4). This tetraloop is common in RNA hairpin structures (5) and most likely is a biloop with a U·G base pair closing the stem, forming a structure more closely resembling a hairpin (6). Altering the sequence of the loop reduces hopping below 70%, and increasing the size of the loop or stem can reduce hopping below 5% (2), suggesting the importance of the hairpin sequence and structure.

The recoding signals important in ribosomal hopping during gene *60* expression are novel in their efficiency and the length of sequence they recode. There are many mRNAs, however, which contain natural or cryptic recoding signals (reviewed in ref. 7). For example, the ribosome can take off from a Leu codon, hop over a stop codon, and land on a Leu codon in the synthetic sequence CUU-UAG-CUA at an efficiency of 1% (ref. 8; see also *Results*). This sequence satisfies two of the requirements for hopping: there are (*i*) matching takeoff and landing sites (in this case, both Leu codons are read by the same tRNA) and (*ii*) a stop codon directly following the takeoff site. Hopping can also occur over sequences in which the takeoff and landing sites overlap as in the sequence GUGUA, where the tRNA hops from Val codon GUG to Val codon GUA (8, 9). If tRNAs are mutated to contain an extra base in the anticodon, hopping can occur over as many as three stop codons (9–11). Hopping has also been observed during overexpression of recombinant proteins in *E. coli* (12, 13). None of these cases of hopping, however, represents a natural event as efficient over such a distance as that for gene *60*.

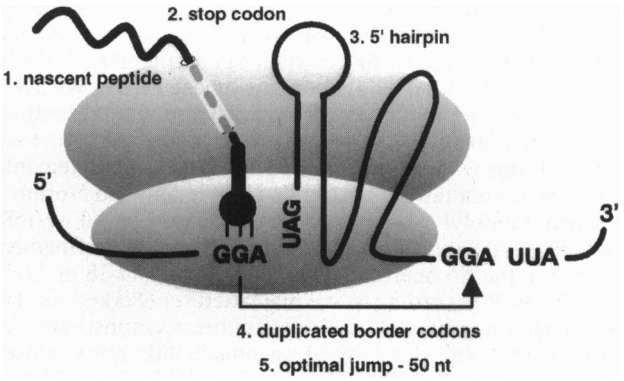

FIG. 1. Recoding signals for gene *60*. Shown are the five recoding signals necessary for maximal hopping during gene *60* expression.

The cellular components that promote hopping during gene *60* expression or, conversely, control hopping during normal translation are unknown. Here we describe a genetic screen for extragenic mutations that increase expression from gene *60* constructs with altered hairpins in the coding gap. The results suggest that L9 plays an important role in the control of hopping during normal translation.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *E. coli* strain 71-18 has been described (14). A library of strain W3110 CJ9797 containing $\approx 10^5$ genomic λ1098 mini-Tn*10* insertions (15, 16) was kindly provided by Constantine Georgopoulos (University of Utah). Isogenic strains containing Tn*10* insertions at specific sites in the genome (parentheses indicate insertion sites in minutes)—CAG18488 (93.75), CAG18427 (94.50), CAG12073 (95.75) and CAG12019 (96.75)—(17) were kindly provided by Carol Gross (University of Wisconsin, Madison).

Gene *60–lacZ* constructs BH2, BX1d, BX1j, BX1x, BX1z, BX1q, BX3, BX5a, BX5b, and BX5d have been described (2). Constructs BX1k and BX1c were made as described (8).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DES, diethyl sulfate; MMTV, mouse mammary tumor virus; ptRNA, peptidyl-tRNA.

The plasmid carrying *dnaX–lacZ* and the mouse mammary tumor virus (MMTV) plasmids were kindly provided by Norma Wills (University of Utah). The plasmid carrying the RF2–*lacZ* fusion and p163UAG have been described (2, 8). Plasmid BX1kACYC was constructed by ligating a *Dra* I fragment from BX1k containing all of the gene *60–lacZ* fusion into the *Eco*RV site of pACYC184, thereby inactivating the tetracycline-resistance gene.

Mutagenesis of Bacteria with Diethyl Sulfate (DES). Two drops of DES were added to a 15-ml dilution tube containing 5 ml of M9 medium plus 10 mM $MgSO_4$. After vigorous vortexing to form a saturated solution, the M9/DES solution was allowed to sit at 37°C for 10 min to allow excess DES to settle out. Aliquots (0.1 ml) of overnight cultures of *E. coli* 71-18 containing ampicillin-resistance construct BX5d, BX1j, or BX1k were added to the aqueous phase of the M9/DES solution, and at 5-min intervals up to 60 min, 0.2-ml samples were diluted into 10 ml of L broth. After overnight growth at 37°C, cultures were serially diluted in L broth and plated onto L agar plates containing ampicillin (100 μg/ml) and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal, 40 μg/ml).

Cloning and Sequencing of Genomic DNA. A DNA fragment containing the S6 operon was amplified from 71-18 or 71-18 *hop-1* by PCR according to the manufacturer (Perkin–Elmer/Cetus), although 10 μl of overnight culture was substituted for purified template. The pairs of oligonucleotide primers used were 5′-GGCCGGATCCGGGGGGTTGCAGAGGGAAAGATTTCTCG-3′/5′-GGCCGGATCCTTTATTGAGAGAAGAATTTGGAGCGGG-3′ and 5′-GGCCGAATTCGCCGACCTTTAACACGTTCCTTGCCTCCCCGGG-3′/5′-GGCCCCCGGGGATCTACTTGCGAAACTAATACCTGACG-3′ for the *glyV* and *S6* operons, respectively. All of the oligonucleotides contained a 5′-GGCC-3′ tail followed by a site for *Bam*HI restriction enzyme. After digestion with *Bam*HI, the fragments were ligated into pBR322 cut with the same enzyme, whose site is located in the tetracycline-resistance gene. The dideoxy chain-termination method of sequencing double-stranded DNA was employed throughout (18) except for the S6 operon amplified from 71-18 *hop-1* by PCR, which was sequenced according to Hultman *et al.* (19). Wild-type and mutant *rplI* genes were cloned into pTrc99A (20) and named pTrc*hop*$^+$ and pTrc*hop-1*, respectively.

Transductions and Preparation of P1 Lysates. Bacteriophage P1 lysates were prepared according to Miller (21). Chromosomal DNA was transduced from one strain to another by adding 100 μl of a P1 lysate and 100 μl of 1 M $CaCl_2$ to 1.0 ml of an overnight culture grown in L broth. After incubation for 20 min, sodium acetate was added (20 mM) to chelate Ca^{2+} and the entire cell mixture was plated in 4 ml of R top agar (21) containing 20 mM sodium acetate and 10 μg of tetracycline per ml onto L plates containing the same additions.

β-Galactosidase Assays. The assays were performed according to Miller (21) as modified by Weiss *et al.* (8). Strains were grown overnight in L broth supplemented with the appropriate antibiotic and diluted 1:400 the following day in prewarmed L broth with the appropriate antibiotic. Isopropyl β-D-thiogalactopyranoside (IPTG) was added (2 mM) for strains carrying a repressor. β-Galactosidase units were expressed as [A_{420} (due to *o*-nitrophenol obtained by cleavage of *o*-nitrophenyl β-D-galactopyranoside) per μl per min per OD_{600} unit of culture] × 1000. IPTG was added (3 mM) for 71-18 carrying pTrc99A or derivatives of this plasmid. All assays were confirmed by triplicate samples on three separate days.

Nick-Translation and Probing the Kohara Library. Probes used to locate genomic sequences in the Kohara library were either total plasmid DNA or DNA fragments isolated from a 1% agarose gel (SeaKem; FMC) by spin dialysis in Spin-X cellulose acetate filter units (Costar). The Kohara library on a gene mapping membrane was from Takara Biochemical (Berkeley, CA). Preparation of, hybridization to, and washing of the membrane were done according to the procedures recommended by Takara Biochemical.

RESULTS

Strains of *E. coli* 71-18 (14), carrying one of four gene *60–lacZ* constructs with changes in the coding-gap hairpin, were mutagenized with DES and screened for genomic mutants which affect hopping. These constructs substantially reduce bypass of the coding gap when compared with the parent wild-type gene *60* construct BX3 (Table 1, part A). The reduced hopping levels make these constructs candidates for isolating extragenic mutations that suppress these particular defects in hopping. Constructs BX1k and BX5d have putative hairpins with greatly enlarged stems and possibly loop structures (Fig. 2). BX1z is unlikely to have a hairpin, and BX5c has a putative hairpin that is enlarged. In all of the constructs, gene *60* is fused to *lacZ* so that an increase in bypass of the gap is measured as an increase in β-galactosidase expression. Suppressor mutants are isolated as colonies with increased blue color intensity on X-Gal plates. One colony of ≈500,000 was chosen as a possible mutant affecting BX1k. The mutation in this strain is named *hop-1*. It causes no obvious growth defects.

Mutation *hop-1* shows a 10-fold increase in expression from BX1k (named 71-18 *hop-1*; Table 1, part A; Fig. 2). *hop-1* was shown to be genomic by curing 71-18 *hop-1* of BX1k, retransforming with fresh BX1k, and regaining a 10-fold increase in expression from this construct. A Tn*10* element linked to *hop*$^+$ at a distance of ≈10 kb was found by transducing in a phage P1 lysate from wild-type strain W3110 CJ9797, containing pooled Tn*10* insertions (15), and isolating transductants which had lost the 10-fold increase in β-galactosidase expression. The Tn*10* and surrounding genomic DNA was cloned into pUC19 and used to probe the Kohara library, revealing two overlapping clones at 95 min on the *E. coli* chromosome. Tn*10* sites at known locations around 95 min (17) were used to locate *hop*$^+$ more precisely at 95.75 min, as described below.

On a map of the *E. coli* genome, the closest operon to 95.75 min with genes involved in translation is the S6 ribosomal

Table 1. β-Galactosidase units for frameshifting and hopping constructs in 71-18 *hop*$^+$ or the L9 mutant 71-18 *hop-1*

	β-Galactosidase units		
	hop$^+$	*hop-1*	*hop-1*/*hop*$^+$
(A) Gene *60* constructs			
BX3	9,840	10,567	1.1
BX1Q	224	261	1.1
BX1d	894	4,478	5.0*
BX1z	545	3,050	5.6*
BX5d	228	696	3.1*
BX1k	34	362	10.6*
BX1x	5,214	6,187	1.2
BX5a	3,899	5,225	1.3
BX5b	2,725	6,137	2.3*
BX5c	556	5,581	10.0*
BH2	18	49	3.0*
BH3	111	327	3.0*
(B) Frameshifting or hopping constructs			
UGGA-1	22	16	0.7
UGGA-2	106	71	0.7
MMTV	124	119	1.0
p163UAG	294	875	3.0*

*Significant at the 95% confidence level; in all cases, standard deviations were <10% of the table value.

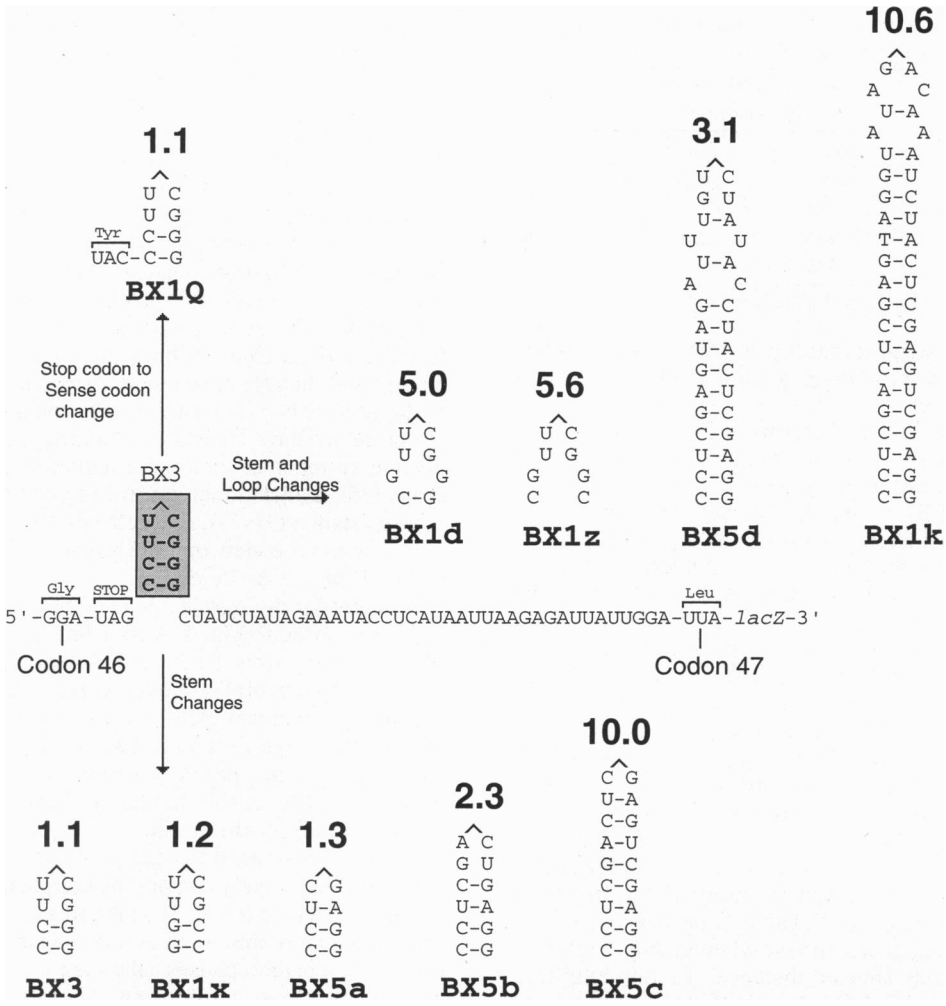

FIG. 2. Alterations in two recoding signals of gene *60*. The sequence of the wild-type gene *60* construct fused to *lacZ*, BX3, is shown from the glycine takeoff site to the first codon after the landing site. The structure of the putative wild-type hairpin is shown without the stop codon (see Fig. 4 for wild-type hairpin). Alterations in the boxed sequence of the hairpin are shown as putative hairpin structures. The entire sense codon is shown for a change in the stop to a sense codon (BX1Q). Numbers above alterations are the fold increase in β-galactosidase activity from these constructs in the presence of the mutation *hop-1*; see Table 1. Gene *60* constructs BX1d, BX1j, BX1x, BX1z, BX1Q, BX3, BX5a, BX5b, and BX5d have been described (2); BX1k was constructed by methods given in ref. 2.

operon at 95.5 min, containing four genes coding for ribosomal proteins S6 (*rpsF*), S18 (*rpsR*), L9 (*rplI*), and DNA replication protein *n* (*priB*) (22–25) (Fig. 3). The S6 operon was isolated by PCR from 71-18, cloned into pBR322, and transformed into 71-18 *hop*$^+$ and 71-18 *hop-1* carrying BX1kACYC. Expression of the wild-type S6 operon from the pBR322 clone reduced hopping to near wild-type levels during expression of BX1kACYC when *hop-1* was present (unpublished data). There was no effect on expression from BX1kACYC in 71-18 *hop*$^+$. These results strongly suggest that *hop-1* is in one of the genes of the S6 operon and that it is not a dominant mutation.

Two examples each of cloned S6 operons from 71-18 *hop*$^+$ and 71-18 *hop-1* were sequenced. In addition, uncloned PCR product from the S6 operon of 71-18 *hop-1* was sequenced directly to eliminate errors that might occur during amplification and cloning of PCR products or from replication of plasmids containing the S6 operon, since it is known that cells do not tolerate this operon on high-copy vectors (26).

When the sequence of the S6 operon in 71-18 *hop-1* was compared with the wild type, a single base difference was consistantly found in *rplI*. This mutation changes Ser^{93} to Phe (TCC to TTC; Fig. 3). To confirm that this is the suppressor mutation, the *rplI* genes from *hop-1* (*rplIhop-1*) and *rplIhop*$^+$ were cloned and examined for their dosage effect on expression from various constructs with hairpin changes (Table 2). As the gene dosage of *rplIhop-1* increased from only wild type to only *hop-1*, the activity of β-galactosidase from BX1kACYC increased up to 10-fold. These data confirm that the 10-fold increase in activity for BX1k in the presence of the chromosomal *hop-1* mutation (Table 1, part A; Fig. 2) is due to a mutation in *rplI*, the gene coding for ribosomal protein

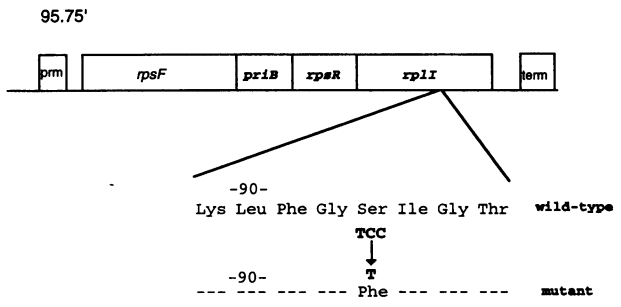

FIG. 3. *hop-1* in the S6 ribosomal operon in the *E. coli* chromosome. Shown is a stylized version of the S6 operon at 95.75 min on the *E. coli* chromosome. The operon is under the control of one promoter (prm) and is followed by transcription termination sequences (term). The *hop-1* mutation is a C-to-T transition changing Ser^{93} to Phe.

Table 2. β-Galactosidase activity from gene *60–lacZ* fusions in 71-18 *hop*$^+$ or 71-18 *hop-1* carrying pTrc*hop-1*

Gene *60* construct	β-Galactosidase units for pTrc/chromosome combinations			
	hop$^+$/ *hop*$^+$	*hop*$^+$/ *hop-1*	*hop-1*/ *hop*$^+$	*hop-1*/ *hop-1*
BX1k	16	39	102	155
BX5c	183	420	2501	2936
BX1z	163	346	2085	2913

pTrc*hop*$^+$ carries *rplI hop*$^+$, and pTrc*hop-1* carries *rplI hop-1*.

L9. These data also suggest that *hop-1* is not a null mutation but that the altered protein is a component of actively translating ribosomes.

hop-1 significantly affects expression from all hairpin alterations except those with unaltered stem–loop size but carrying compensating stem changes or altered loop sequences (BX1x and BX5; Fig. 2). The sequence changes in BX1x and BX5a most likely disrupt the enhanced stability of the CUUCGG hairpin but leave a potential structure of the same stem–loop size as BX3, which is also unaffected by *hop-1* (Table 1, part A; Fig. 2).

hop-1 does not affect expression from gene *60* constructs with gap sizes decreased to 45 or 35 nt, increased to 60 and 68 nt (unpublished data), or with a change from the stop codon to a sense codon (BX1Q; Table 1, part A; Fig. 2). The simultaneous slippage of tRNAs during expression of MMTV (27) and P-site slippage of tRNAs into the −1 or −2 frames (UGGA-1 and -2; unpublished data) are also unaffected by hop-1 (Table 1; part B). However, expression from constructs BH2 and BH3, with amino acid changes in the region of the nascent peptide important for hopping (2), increases 3-fold in the presence of *hop-1* (Table 1, part A).

One sequence was chosen to test whether *hop-1* affected hopping over a much shorter distance. In this synthetic sequence, 5′-CUU-UAG-CUA-3′ (p163UAG; ref. 8), constructed in *lacZ*, the ribosome hops over a stop codon (underlined), taking off from a Leu codon before the stop and landing on a Leu codon immediately following the hop. *hop-1* increases expression from this construct 3-fold (Table 1, part B), presumably by stimulating hopping.

DISCUSSION

These results suggest an important role for ribosomal protein L9 both in the translational hop found in gene *60* and in controlling the level of hopping during normal translation. A mutation in *rplI* changing Ser^{93} to Phe in L9 is sufficient to induce significant increases in expression from gene *60* constructs with altered hairpins. Surprisingly, *hop-1* stimulates hopping on constructs whose alterations are either increases or decreases in the predicted size and stability of the stem–loop structure at the takeoff site. Expressing *hop-1* from a plasmid in a wild-type cell increases expression from constructs with hairpin alterations, but to a lesser degree than when *hop-1* is on both a plasmid and the chromosome, suggesting that L9 with the *hop-1* mutation can compete with wild-type L9 for incorporation into ribosomes that actively participate in translation.

L9 may be an essential ribosomal protein, since ribosomal mutants lacking L9 have not been found (28). L9 has been mapped in the ribosome by immune electron microscopy to a site near L1 (29); crosslinked to L2, L19, and L28 (30, 31); and crosslinked to 23S rRNA at nt 1484–1491 in helix 58 (32). These data suggest that L9 is in the lateral protuberance of the 50S subunit, close to the P site (33). L9 can also be crosslinked to ptRNA occupying the P site while aminoacyl-tRNA occupies the A site (34).

The crystal structure of L9 from *Bacillus stearothermophilus* has been reported (35). The structure comprises two domains joined by a long α-helix. It is postulated that both the N- and C-terminal domains are RNA-binding domains. Ser^{93}, conserved in all known L9 sequences (35), is located strategically in an exposed loop of the C-terminal domain and may be involved in the RNA binding site (35). The Ser^{93} mutation may disrupt one of two RNA binding sites on L9, leading to the enhanced hopping phenotype.

The *hop-1* mutation suggests that L9 may be involved in controlling the level of ribosomal hopping during normal translation. The suppression pattern of the gene *60* alleles tested provides clues to both the basic defect in the *hop-1* mutant and the role of secondary structure at the takeoff site of the gene *60* hop. Ribosomes are thought to melt secondary structure as they translate. Detailed examination of such diverse systems as bacterial attenuators in biosynthetic operons (36), translational control regions of inducible antibiotic resistance (37–39), and retroviral ribosomal frameshifts (40) supports the view that mRNA secondary structure melts 6–7 nt 3′ of the A-site codon.

A model for the role the gene *60* hairpin in stimulating the hop is proposed in Fig. 4. A translating ribosome (oval) with tRNA binding sites E, P, and A (exit, peptidyl, and aminoacyl, respectively) is shown with a region (triangle) where secondary structures melt 3′ to the A site. Gene *60* mRNA is shown with codons connected by dashed lines, and the coding gap with spaces between triplets. The region of melting activity in this model is chosen to be within 6 nt downstream from the A site.

As the ribosome translocates closer to the hairpin, the melter will eventually contact the hairpin and melt the hairpin to single-stranded RNA. The takeoff site is now in the A site. The stop codon enters the A site during the next translocation, the ribosome pauses, allowing time for the hairpin to snap back into structure, and this structure in the A site stimulates the hop.

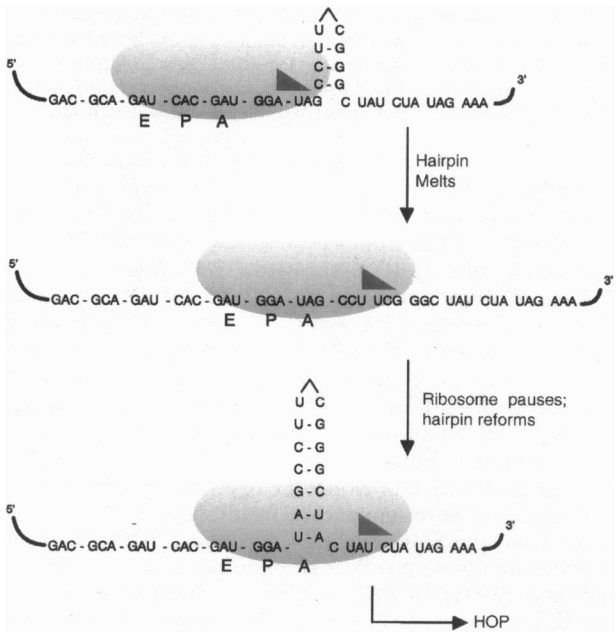

FIG. 4. Model for the location of the gene *60* wild-type hairpin during hopping. A highly stylized ribosome is shown with E, P, and A sites. A triangle depicts a region of the ribosome important in melting double-stranded RNA. The ribosome moves to its position at the start of the hop with the takeoff site in the P site and the stop codon in the A site. The wild-type hairpin is melted but snaps back to function during the hop.

Gene *60* constructs BX1d and BX1z have sequence in the region of the hairpin of equal length to that of wild type but are unlikely to form a hairpin next to the A site, since there is little or no possibility for base pairing in the hairpin region (Fig. 2). Hopping on these constructs is significantly increased by the *hop-1* mutation in L9, suggesting that *hop-1* functionally substitutes for the hairpin. When the hairpin is changed in sequence and stability but not structure, as in constructs BX1x and BX5a, hopping is unaffected by *hop-1*. In this case, the hairpin, even though not wild type, most likely stimulates the hop to a greater degree than *hop-1*. However, *hop-1* increases expression from BX5d, BX1k, and BX5c, which are predicted to form hairpins longer than wild type (Fig. 2). These hairpins have a greater number of base pairs than the wild-type hairpin and thus should have high stability. Why does *hop-1* affect these hairpins and not BX1x and BX5a? A simple explanation is that BX5d, BX1k, and BX5c do not form hairpin structures in the A site at the relevant moment. The tops of these hairpins are most likely engaged in the ribosomal melter due to the greater length of the hairpin, making a snap-back structure in the A site unlikely. In support of this hypothesis, hopping on these constructs is reduced from wild type, to levels comparable to those found for BX1d and BX1z, which lack hairpins. Thus, the suppression pattern of *hop-1* is consistent with a model in which the L9 mutation functionally substitutes for the role of the hairpin in stimulating hopping.

Expression from gene *60* constructs with changes in the region of the nascent peptide important for hopping is also increased 3-fold in the presence of *hop-1* (Table 1, part A). The hairpin structures are wild type in these constructs, suggesting that the five recoding signals important for hopping during gene *60* expression function as an integrated mechanism; ribosomal components affecting one recoding signal are likely to affect another. That *hop-1* did not affect changes in the size of the gap (unpublished data) or a change from the stop codon to a sense codon but did affect constructs with changes in the peptide may suggest a functional association between the nascent peptide and the hairpin.

The ability of *hop-1* in L9 to substitute for the gene *60* hairpin provides a link between the recoding signals in the mRNA of gene *60* and the ribosome. Use of the hopping phenotype and the L9 crystal structure will aid further studies of the role L9 plays within the 50S subunit and of the causes and prevention of ribosomal hopping. Such mutations may increase our understanding of the mechanism of hopping and also of the complex interactions between mRNA and the ribosome.

1. Huang, W. M., Ao, S.-Z., Casjens, S., Orlandi, R., Zeikus, R., Weiss, R., Winge, D. & Fang, M. (1988) *Science* **239**, 1005–1112.
2. Weiss, R. B., Huang, W. M. & Dunn, D. M. (1990) *Cell* **62**, 117–126.
3. Gesteland, R. F., Weiss, R. B. & Atkins, J. F. (1992) *Science* **257**, 1640–1641.
4. Tuerk, C., Gauss, P., Thermes, C., Groebe, D. R., Gayle, M., Guild, N., Stormo, G., d'Aubenton-Carafa, Y., Uhlenbeck, O. C., Tinoco, I., Brody, E. N. & Gold, L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1364–1368.
5. Woese, C. R., Winker, S. & Gutell, R. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8467–8471.
6. Varani, G., Cheong, C. & Tinoco, I. (1991) *Biochemistry* **30**, 3280–3289.
7. Atkins, J. F., Weiss, R. B. & Gesteland, R. F. (1990) *Cell* **62**, 413–423.
8. Weiss, R. B., Dunn, D. M., Atkins, J. F. & Gesteland, R. F. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52**, 687–693.
9. O'Connor, M., Gesteland, R. F. & Atkins, J. F. (1989) *EMBO J.* **8**, 4315–4323.
10. Falahee, M. B., Weiss, R. B., O'Connor, M., Doonan, S., Gesteland, R. F. & Atkins, J. F. (1988) *J. Biol. Chem.* **263**, 18099–18103.
11. Hughes, D., Thompson, S., O'Connor, M., Tuohy, T., Nichols, B. P. & Atkins, J. F. (1989) *J. Bacteriol.* **171**, 1028–1034.
12. Kane, J. F., Violand, B. N., Curran, D. F., Staten, N. R., Duffin, K. L. & Bogosian, G. (1992) *Nucleic Acids Res.* **20**, 6707–6712.
13. Benhar, I. & Engelberg-Kulka, H. (1993) *Cell* **72**, 121–130.
14. Yanisch-Perron, C., Viera, J. & Messing, J. (1985) *Gene* **33**, 103–119.
15. Way, J. C., Davis, M. A., Morisato, D., Roberts, D. E. & Kleckner, N. (1984) *Gene* **32**, 369–379.
16. Morisato, D., Way, J. C., Kim, H.-J. & Kleckner, N. (1983) *Cell* **32**, 799–807.
17. Singer, M., Baker, T. A., Schnitzler, G., Deischel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. & Gross, C. A. (1989) *Microbiol. Rev.* **53**, 1.
18. Chen, E. Y. & Seeburg, P. H. (1985) *DNA* **4**, 165.
19. Hultman, T., Stahl, S., Hornes, E. & Uhlen, M. (1989) *Nucleic Acids Res.* **17**, 4937–4964.
20. Amman, E., Ochs, B. & Abel, K. J. (1988) *Gene* **69**, 301–315.
21. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
22. Schnier, J., Kitakawa, M. & Isono, K. (1986) *Mol. Gen. Genet.* **204**, 126–131.
23. Bachmann, B. J. (1990) *Microbiol. Rev.* **54**, 130–197.
24. Allen, G. C. & Kornberg, A. (1991) *J. Biol. Chem.* **266**, 11610–11613.
25. Zavitz, K. H., DiGate, R. J. & Marians, J. (1991) *J. Biol. Chem.* **266**, 13988–13995.
26. Fallon, A. M., Jinks, C. S., Yamamoto, M. & Nomura, M. (1979) *J. Bacteriol.* **138**, 383–396.
27. Weiss, R. B., Dunn, D. M., Shuh, M., Atkins, J. F. & Gesteland, R. F. (1989) *New Biol.* **1**, 159–169.
28. Dabbs, E. R. (1979) *J. Bacteriol.* **140**, 734–737.
29. Stöffler-Meilicke, M. & Stöffler, G. (1990) in *The Ribosome*, eds. Hill, W. E., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D. & Warner, J. R. (Am. Soc. Microbiol., Washington, DC), pp. 123–133.
30. Walleczek, J., Martin, T., Redl, B., Stöffler-Meilicke, M. & Stöffler, G. (1989) *Biochemistry* **28**, 4099–4105.
31. Walleczek, J., Redl, B., Stöffler-Meilicke, M. & Stöffler, G. (1989) *J. Biol. Chem.* **264**, 4231–4237.
32. Osswald, M., Greuer, B. & Brimacombe, R. (1990) *Nucleic Acids Res.* **18**, 6755–6760.
33. Nag, B., Glitz, D. G., Tewari, D. S. & Traut, R. R. (1991) *J. Biol. Chem.* **266**, 11116–11121.
34. Graifer, D. M., Babkina, G. T., Matasova, N. B., Vladimirov, S. N., Karpova, G. G. & Vlassov, V. V. (1989) *Biochim. Biophys. Acta* **1008**, 146–156.
35. Hoffman, D. W., Davies, C., Gerchman, S. E., Kycia, J. H., Porter, S. J., White, S. W. & Ramakrishnan, V. (1994) *EMBO J.* **13**, 205–212.
36. Landick, R. & Yanofsky, C. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Magasanik, B., Low, K. B., Schaechter, M., & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC) pp. 1276–1301.
37. Gryczan, T. J., Grandi, G., Hahn, J. & Dubnau, D. (1980) *Nucleic Acids Res.* **8**, 6081–6097.
38. Horinouchi, S. & Weisblum, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7079–7083.
39. Alexieva, Z., Duvall, E. J., Ambulos, N. P., Kim, U. J. & Lovett, P. S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3057–3061.
40. Jacks, T., Madhani, H. D., Masiarz, F. R. & Varmus, H. E. (1988) *Cell* **55**, 447–458.