

**Secondary Structure of Bacteriophage T4 Gene 60 mRNA:
Implications for Translational Bypassing**

by

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For Edochan.

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List of Abbreviations

16S	rRNA for the small ribosomal subunit
30S	small ribosomal subunit (bacteria)
³⁵ S-Cys	35-sulfur labeled cysteine
³⁵ S-Met	35-sulfur labeled methionine
50S	large ribosomal subunit (bacteria)
6 FAM	6-carboxyfluorescein
70S	complete ribosome (bacteria) with both subunits, third rRNA?
7-deaza-dGTP	7-deaza-2'-deoxy-guanosine-5'-triphosphate
A	adenine
Ala	alanine
aSD	anti-Shine Dalgarno sequence (3' end of 16S rRNA)
Asp	aspartic acid
C	cytosine
c7dGTP	7-deaza-2'-deoxy-guanosine-5'-triphosphate
CAT	chloromphenicol acetyl transferase
Cys	cysteine
D	aspartic acid
ddNTP	dideoxy nucleotide triphosphate
dGTP	deoxy guanosine triphosphate
dITP	2'-deoxyinosine 5'-triphosphate
dNTP	deoxy nucleotide triphosphate
DTT	dithiolthreitol
G	guanosine
GFP	green fluorescent protein
Gly	glycine
gp60	gene 60 protein
HE	homing endonuclease
HEG	homing endonuclease gene
HEX	6-carboxy-2',4,4',5',7,7'-hexachlorofluorescein
IC	initiation complex
kb	kilobase
Met	methionine
mRNA	messenger ribonucleic acid
mSD	mutant Shine-Dalgarno
mut aSD	mutant anti-Shine-Dalgarno
NTP	nucleotide triphosphate
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
phage	bacteriophage
Phe	phenylalanine
RF1	release factor 1

RNA	ribonucleic acid
ROX	6-carboxy-x-rhodamine
rRNA	ribosomal ribonucleic acid
SD	Shine Dalgarno sequence
SDS	sodium dodecyl sulfate
T2	bacteriophage T2
T4	bacteriophage T4
T7	bacteriophage T7
TAMRA	5'carboxytetramethylrhodamine
tRNA	transfer ribonucleic acid
Tyr	tyrosine
U	uracil
UTR	untranslated region
x	any amino acid

Abstract

Translational bypassing is a unique phenomenon of Bacteriophage T4 gene 60 in which the ribosome generates a single polypeptide chain from a discontinuous open reading frame. In about half of translational events, the ribosome skips over a 50 nucleotide segment in the open reading frame to generate a full-length subunit of a Type II DNA topoisomerase; the rest of the time the ribosome terminates at an in-frame stop codon at the 5' edge of the untranslated region or coding gap. Mutational and computational analyses suggest a stable structure forms in the coding gap to induce bypassing, yet there is no direct evidence of structure in this region. We have probed the structure of gene 60 mRNA with Tb³⁺ ions and the selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) reagent 1M7 and constructed a secondary structure model compatible with experimental data. Our model confirms the presence of a predicted UUCG-loop hairpin at the 5' edge of the coding gap that aids in efficient translational bypassing. Contrary to several previously proposed models, however, the rest of the untranslated region is highly reactive to both probing reagents suggesting it possesses little structure. Furthermore, mutational analyses reveal that the untranslated region does base pair to any other region of gene 60 mRNA. The autonomous nature of the coding gap is consistent its role as a foreign genetic element inserted into gene 60 mRNA to inhibit cleavage by the MobA homing endonuclease. These results indicate that the 5' hairpin may be the only structural element required to stimulate translational bypassing.