Two tRNA Gene Clusters Associated with rRNA Operons *rrnD* and *rrnE* in *Bacillus subtilis*†

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Sequence analysis of cloned rescued DNA fragments from a *Bacillus subtilis* strain with an inserted recombinant plasmid in ribosomal operon *rrnE* revealed the presence of two tRNA genes for Met and Asp at the 3' end of the operon. Probing chromosomal DNA from a strain carrying a plasmid inserted in *rrnD* with a fragment containing the genetically unassigned cluster of 16 tRNA genes revealed that the cluster is located immediately following the *rrnD* operon. Our findings show that all 10 *rrn* operons in *B. subtilis* are associated with tRNA gene clusters.

In *Bacillus subtilis*, most tRNA genes are highly clustered and closely linked to rRNA operons (20). Southern analysis has shown 10 *EcoRI* chromosomal fragments which hybridize to labeled tRNA (23). To date, 7 of the 10 fragments containing tRNA gene clusters, 6 of which are associated with *rrn* operons, have been sequenced. Two clusters are identical and are composed of spacer tRNA genes for Ile and Ala, found between the 16S and 23S genes of *rrnO* and *rrnA* (13, 16, 17). Four other clusters are located at the 3' end of rRNA operons, namely, a large cluster of 21 tRNA genes (*trnB*) downstream of *rrnB*, an unmapped cluster of 16 tRNA genes downstream of an *rrn* operon containing the minor 5S gene species (24), a cluster of 6 tRNA genes between *rrnI* and *rrnH* (23), and a cluster of 9 tRNA genes (*trnJ*) between *rrnJ* and *rrnW* (6). Only one unmapped cluster containing four tRNA genes (*trnY*) seems to be unassociated with any rRNA operon (20, 27).

In our search for additional tRNA gene clusters, we cloned and sequenced DNA downstream of operon *rrnE* located at 44° on the chromosomal map (9, 11). A small cluster containing tRNA^{Met} and tRNA^{Asp} was found 14 bases downstream of the 5S gene of *rrnE*. No promoter sequences were revealed, but a candidate terminator element has been identified. We designate this small cluster *trnE* because of its close proximity and possible regulatory relationship to ribosomal operon *rrnE*. Southern analyses of various integrant strains and strains with natural deletions of *rrn* operons (26) have revealed the location of the cluster of 16 tRNA genes downstream of the *rrnD* operon, which we designate *trnD*. In addition, we identified new *EcoRI* fragments, which may contain seven or more tRNA genes.

MATERIALS AND METHODS

Bacterial strains and plasmids. Some of the *B. subtilis* strains and plasmids were described previously (9, 11, 26). Plasmids pYR104, pGR111, and pWR103 are derivatives of plasmid pJH101 (3), containing a selectable Cm^r gene and a 2.3-kb *EcoRI*-*HindIII* 23S-5S fragment from *rrnA* (pYR104),

a 1.5-kb *EcoRI*-*SmaI* 23S fragment (pGR111), or a 1.0-kb fragment of *EcoRI*-*PstI* 16S-155 bp of the 23S sequence (pWR103) (9, 11). The *B. subtilis* strains are listed in Table 1 as parental and integrant strains. The table indicates the strains with a normal number of *rrn* operons and those with an interrupted *rrn* gene set due to plasmid integration (9, 11, 26). Plasmids pSR256-3'*rrnE* (containing 2.0 kb of DNA 3' to *rrnE*), pIR363-5'*rrnE* (containing 1.8 kb of DNA 5' to *rrnE*), pWR182-5'*rrnD* (containing 1.0 kb of DNA 5' to *rrnD*), and pWR179-5'*rrnO* (containing 3.5 kb of DNA 5' to *rrnO*) were rescued from integrant strains BD29-4, 168T-73, BD79-182, and BD170-179, respectively (Table 1). Transduction crosses in *B. subtilis* were done by using the mapping recipient kits 1 to 9 (2) as described previously (9, 11).

DNA manipulation, Southern blotting, and sequencing. Southern hybridizations were done as described previously (11). ³²P-labeled plasmids, purified rDNA fragments, or polymerase chain reaction (PCR) fragments (see below) were used as hybridization probes. The probes were prepared with the random primer extension kit as directed by the supplier (USB). For plasmid rescue of DNA 3' to the integration event, DNAs from integrant strains were digested with *SphI*, self-ligated at a low DNA concentration (2 µg/ml), and introduced into *Escherichia coli* HB101, and clones resistant to ampicillin (100 µg/ml) were selected. Rescue of DNA 5' to the integration event by using the enzyme *BclI* was described previously (8, 25, 26). To sequence the rescued downstream region of *rrnE*, two unique fragments (1.4 and 0.6 kb) from pSR256-3'*rrnE* were subcloned first into M13mp18 and later into vector pBS+/- (Stratagene) or pGEM-3Z(+)-CAT-1 (28) for dideoxy sequencing reactions. DNA sequencing was done by using the Sequenase kit (USB), double-stranded plasmid DNA as a template, and α³⁵-S-dATP (Dupont, NEN Research Products) as the label. Initially, the M13 universal reverse primer and the T7 forward primer were used. From the resulting sequence and the published sequence of the 23S-5S genes (5), the following four primers were used: (i) forward 5'-CGCAGGATGAGAGTGTC-3' and (ii) reverse 5'-CCTGCGTGACAGGCAGG-3' (both for the 1.4-kb fragment), designed to hybridize to positions 226 to 242 and 430 to 447, respectively (Fig. 1), and (iii) forward 5'-GATACATAGCAT TACTC-3' and (iv) reverse 5'-CACCGATTACAGTGA

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TABLE 1. Strains and plasmids used in the mapping of tRNA gene clusters

Strain or plasmid	Description	Origin of rescue ^a	No. of <i>rm</i> operons ^b
Parental strains			
NCTC3610	Prototroph		10
168T	<i>trpC2</i>		10
BD170	<i>trpC2 thrA5</i>		9 (<i>rmG</i>)
BD29	<i>leuB6 argA2</i>		9 (<i>rmG</i>)
CU420	<i>trpC2 leuB6 ilvC4</i>		9 (<i>rmW</i>)
Integrand strains			
BD170-10	pGR111, <i>trpC2 thrA5</i> Cm ^r		9 (<i>rmD</i>)
BD29-4	pYR104, <i>leuB6 argA2</i> Cm ^r		9 (<i>rmE</i>)
168T-41	pGR111, <i>trpC2</i> Cm ^r		9 (<i>rmB</i>)
Rescued plasmids			
pSR256-3' <i>rmE</i>	Am ^r Cm ^r , 3' to <i>rmE</i>	BD29-4/pYR104	
pIR363-5' <i>rmE</i>	Tc ^r Cm ^r , 5' to <i>rmE</i>	168T-73/pWR103	
pWR182-5' <i>rmD</i>	Tc ^r Cm ^r , 5' to <i>rmD</i>	BD79-182/pWR103	
pWR179-5' <i>rmO</i>	Tc ^r Cm ^r , 5' to <i>rmO</i>	BD170-179/pWR103	

^a See references 8, 9, and 11 for descriptions of strains and Materials and Methods for descriptions of integration plasmids.

^b Spontaneously deleted or interrupted genes due to plasmid insertions as shown in parentheses.

CT-3' (both for the 0.6-kb fragment), designed to hybridize to positions 674 to 691 and 1070 to 1048, respectively (Fig. 1).

PCR and DNA synthesis. All DNA oligomers were made on an Applied Biosystems DNA synthesizer, model 380A. The sequences for the 16-tRNA-gene cluster PCR primers

were obtained from the published report (21) and from C. Green. The 5' Asn PCR primer was 5'-CTCGAATTCCG AGTAGCTCAGTGGTAG-3' (the underlined bases represent the portion of the sequence designed to hybridize to positions 268 to 286 of the first tRNA gene in the *trnD*

FIG. 1. The sequence from the 3' region of *rmE-trnE* containing two tRNA genes. The sequence includes the end of the 23S gene (positions 1 to 78), the 5S gene (positions 134 to 250), tRNA^{Met} (positions 265 to 342), tRNA^{Asp} (positions 402 to 478), and an ORF (positions 499 to 1038). The sequences coding for the anticodons of each tRNA are indicated by broken lines. The region of dyad symmetry is indicated by dots and contains a weak ribosome binding site (RBS) 5' to the ORF.

TABLE 2. Distribution of integration events for rescued plasmids containing unique 3' and 5' sequences of *rmE*, *rmD*, and *rmO*

Donor plasmid	No. tested	No. (%) of integrants into locus ^b :				
		<i>rmO-rmG</i>	<i>rmE-trnE</i>	<i>sup-3-sup-44</i>	<i>rmD-trnD</i>	<i>rmB-trnB</i>
pSR256-3' <i>rmE</i>	50	18 (36)	24 (48)	7 (14)	1 (2)	0 (0)
pIR363-5' <i>rmE</i>	15	5 (33)	10 (67)	0 (0)	0 (0)	0 (0)
pWR182-5' <i>rmD</i>	77	54 (70)	5 (7)	4 (5)	11 (14)	3 (4)
pWR179-5' <i>rmO</i>	28	25 (89)	1 (4)	2 (7)	0 (0)	0 (0)

^a Plasmid used to transform *B. subtilis* SB25 (*trpC2 hisH2*). PBS1 lysates were then prepared from some of the resulting Cm^r transformants and used to transduce various auxotrophic strains to Cm^r.

^b Cm^r transductants were selected and the linkage to various auxotrophic markers was determined. Integrants at the following loci showed linkages as follows: *rmO-rmG*, 40 to 85% to *cysA14*; *rmE-trnE*, 39% to *purB6*; *sup-3-sup-44*, 18% to *aroI906*; *rmD-trnD*, 29% to *glyB13*, *rmB-trnB*, 41% to *aroG932*. For genomic locations see reference 9 and Fig. 3.

cluster) (24). The 3' Leu PCR primer was 5'-TTTTGTGCA TACCGGTGGTCGGGGTCTGAAC-3' (the underlined portion represents the part of the sequence designed to hybridize to positions 1957 to 1978, the last tRNA gene of the same cluster). The first nine nucleotides were made to accommodate the *EcoRI* site for the first primer and a *SalI* site for the second primer for cloning purposes. The PCR was as described by Green and Vold (6); 10 ng of *B. subtilis* DNA was amplified in 100 µl of 10 mM Tris-HCl (pH 8.3)–50 mM KCl–1.5 mM MgCl₂–200 µM each deoxynucleoside triphosphate–1 µM each primer–1.5 U of *Thermus aquaticus* DNA polymerase (Promega) or the Vent_R DNA polymerase (New England Biolabs). The reaction mixture was preincubated at 94°C for 5 min. It was then subjected to 25 cycles of 94°C for 30 s, 58°C for 60 s, and 72°C for 60 s on a Perkin-Elmer DNA Thermocycler. The PCR primers were used to amplify a 1.6-kb fragment generated either from genomic DNA or from a 1.8-kb PCR fragment covering the minor 5S gene and the 16 tRNA genes of *trnD* (24) kindly provided by C. Green.

Nucleotide sequence accession number. The sequence data presented here have been submitted to GenBank under accession no. L08236.

RESULTS

Sequence and organization of the *trnE* cluster. The DNA of integrant strain BD29-4 containing plasmid pYR104 inserted into *rmE* was chosen for this study (Table 1). Transductional crosses with mapping strain kits 2 and 3 (2) verified that the integration event occurred at the *rmE* locus (data not shown). To rescue fragments containing sequences downstream of *rmE*, chromosomal DNA from integrant strain BD29-4 was digested to completion with restriction enzyme *SphI* and self-ligated. The only site in the vector for *SphI* is located within the tetracycline gene, and no *SphI* sites are present within the entire rRNA operon (5). The resultant plasmid, pSR256-3'*rmE*, was recovered after transformation of *E. coli*, and it contained an additional 2.0 kb of *B. subtilis* DNA 3' to *rmE*. A 1.4-kb *PstI*-*HindIII* fragment which hybridized to the 23S-5S genes coding for rRNA (rDNA) and an adjacent 0.6-kb *HindIII*-*PstI* fragment with no rDNA sequences (data not shown) were subcloned and sequenced from both orientations (Fig. 1). The sequence revealed, in order, the 3' end of a 23S rDNA gene, a 5S rDNA major gene, genes for tRNA^{Met} and tRNA^{Asp}, and an open reading frame (ORF) which may encode a polypeptide of 179 amino acids. To date, a data base search has revealed no homology of this ORF with any known DNA or protein sequence. The two anticodons for the tRNAs, CAU (Met) and GUC (Asp), are identical to those found in *trnB* and *trnD* (20, 24) (*trnD* is assigned below). The same Asp anticodon was also found in

the *trnY* cluster (27). Both tRNA genes encode the 3' CCA sequence to which the aminoacyl residue is attached. In *B. subtilis*, 14 of the 62 tRNA genes that have been sequenced lack the CCA end and they occur without any obvious order in the tRNA gene clusters (6, 20). The order, tRNA^{Met} followed by tRNA^{Asp}, is the same in *trnE*, *trnD*, and *trnB*. However, the spacing between is quite different; in *trnD* and *trnB*, the genes are separated by similar sequences of 11 to 13 AT-rich nucleotides (24). In *trnE*, the genes are separated by 60 nucleotides with AT-rich sequences at both ends (Fig. 1). One can speculate that *rmE-trnE* represents a duplication of one of the large clusters followed by deletion of the tRNA genes on either side of tRNA^{Met} and tRNA^{Asp} and containing an insertion of extra sequences between them. In another large spacing of 56 nucleotides between tRNA^{Thr} and tRNA^{Gly} reported for *trnI*, a putative attenuation sequence is present (23). This feature is not apparent in *trnE* (Fig. 1).

A terminator-like element is located 7 nucleotides downstream of the tRNA^{Asp} gene (Fig. 1). The region of hyphenated dyad symmetry which creates the potential stem-loop is followed by a stretch of T's, as is commonly seen for termination signals. No discernible promoter sites are found in the entire region, and no potential termination site between the 5S and tRNA genes could be identified. Thus, it appears probable that the *rmE-trnE* genes can be expressed as a single transcript from the promoters of *rmE*. Transcription of the ORF may also be under the control of the *rmE* promoters. This ORF is preceded by a weak ribosome binding site which is part of the putative terminator sequence (Fig. 1) and could be expressed through an antitermination mechanism, i.e., binding of a ribosome to the ribosome binding site, which would prevent termination. In *E. coli*, transcription units encoding some single tRNA genes contain recognizable terminator-like sequences followed by a downstream ORF or protein gene not preceded by promoter-like elements (4). Similarly, this may be the first example in *B. subtilis* of an ORF functioning as part of an *rmE-trnE* transcription unit and being regulated through putative attenuator elements and associated proteins.

Integrative mapping of rescued plasmids containing *rmn* and *trn* sequences. If pSR256-3'*rmE* contains unique sequences, it should integrate at its original chromosomal locus more frequently than would a plasmid carrying only highly redundant sequences (i.e., rRNA genes). It should also serve as an integrative plasmid for mapping tRNA gene clusters, specifically those that contain tRNA^{Met} and tRNA^{Asp}. Table 2 summarizes transduction results for donor lysates of Cm^r clones that arose by transformation with the following four rescued plasmids: pSR256-3'*rmE*, pIR363-5'*rmE*, pWR182-5'*rmD*, and pWR179-5'*rmO*. As shown in Table 2, pSR256-3'*rmE* reintegrated into its original *rmE* locus with a rela-

FIG. 2. Autoradiograms of *Hind*III- and *Eco*RI-digested chromosomal DNAs from strains of *B. subtilis* with 10 or 9 *rrn* operons or integrant strains with insertions into *rrnE*, *rrnD*, or *rrnB*. (a) *Hind*III digests probed with a 2.0-kb *Pst*I fragment containing 1.2 kb of the 23S gene and 0.8 kb of vector sequences from plasmid pSR256-3'*rrnE*. See Table 1 for strain characteristics. (b) *Hind*III digests probed with a labeled 1.6-kb PCR fragment containing the amplified 16 tRNA genes of *trnD*. This part of the figure is a composite. To highlight some of the tRNA bands, we exposed the lower portion of the blot for an additional 48 h. (c) *Eco*RI digests probed with a labeled 23S fragment as in panel a. (d) *Eco*RI digests probed with a labeled *trnD* fragment as in panel b. (e) Generalized restriction map of a *B. subtilis* rDNA set described previously (9). The region between 16S and 23S rDNA with or without tRNA genes is hatched.

tively high frequency of 48% of the number of clones examined compared with 2% for plasmids containing only rDNA, as reported previously (9). We also observed that pSR256-3'*rrnE* exhibited a bias in integrating 14% of the time into the region containing the suppressor tRNA genes *sup-3* and *sup-44*, situated between *aroI* and *dal-1* (7, 12). Plasmid pWR179-5'*rrnO*, which contains tRNA^{Ile} and tRNA^{Ala} genes between the 16S and 23S genes, also integrated at the *sup* region, with a frequency of 7% (Table 2).

On the other hand, rescued plasmid pWR182-5'*rrnD*, which does not contain any tRNA genes, also integrated at the same *sup* locus, with a frequency of only 5% (Table 2). This result may be explained by the presence of an additional 65-bp leader sequence between promoter P2 and the 16S rDNA common to operons *rrnO*, *rrnD*, and *rrnB* (5, 8, 16). This leader sequence can form a stable secondary structure (16) which mimics, in part, a tRNA structure and may have a homologous counterpart at the *sup-3-sup-44* locus. These

results suggest that the *sup-3-sup-44* locus may contain a cluster of tRNA genes for Ser (12), Met and/or Asp, and Ile and/or Ala.

Assignment of the 16-tRNA-gene cluster to *trnD*. To physically map tRNA gene clusters associated or unassociated with *rrn* operons, it was first necessary to assign the known clusters to chromosomal restriction fragments. We chose enzymes *Hind*III, which cuts once in each *rrn* operon 92 bp from the start of the 23S gene, and *Eco*RI, which cuts each three times (Fig. 2e) (5). Downstream of the 5S genes, the *Hind*III and *Eco*RI restriction sites are at variable distances, allowing for the resolution of specific restriction fragments for each operon. Southern analysis of *B. subtilis* 168T genomic DNA yields 7 *Hind*III fragments that hybridize to the 23S or 23S-5S labeled rDNA probes instead of the expected 10 (Fig. 2a). On the basis of sequence and restriction analyses, a cluster of four bands with sizes of 3.0 to 3.1 kb can be assigned to *rrnO*, *rrnA* (16), *rrnI* (23), and *rrnG* (19). The 5.0- and 5.8-kb bands were assigned to *rrnH-rrnG* and *rrnJ-trnJ-rrnW*, respectively, because they are missing in strains BD170 (or BD29) and CU420 (Table 1). These strains are known to contain a deletion of a gene set by a recombination event between either pair of the above operons (26). In strains 168T-41, BD29-4, and BD170-10, which contain plasmids integrated into *rrnB*, *rrnE*, and *rrnD*, respectively, fragments of 7.9, 3.3, and 5.6 kb were replaced by high-molecular-weight bands corresponding in size to the integrated vector plus the missing fragment (Fig. 2a). The high-molecular-weight band of BD29-4, which separately hybridized to vector sequences without rDNA, comigrates with the 7.9-kb fragment of *rrnB-trnB* (data not shown). When a *Hind*III blot of wild-type DNA from strains BD170 and BD170-10 or strain 168T (not shown) was probed with a 1.6-kb PCR fragment containing only the amplified 16-tRNA-gene cluster, all bands that hybridized to the 23S rDNA probe except the 3.0- to 3.1-kb cluster and the 5.0-kb fragment, which do not contain *trn* genes, were detected (Fig. 2b) (6). The 5.6-kb band assigned to *rrnD* gave the strongest signal (Fig. 2b), and therefore, it is likely to represent the *rrnD-trnD* operon containing the minor 5S gene and the 16-tRNA-gene cluster. To confirm this result, an *Eco*RI blot hybridized with the same PCR probe showed that the 4.2-kb *Eco*RI band known to contain the 16-tRNA-gene cluster (24) also gave the strongest signal (Fig. 2d).

The 16-tRNA-gene probe also revealed eight unidentified *Hind*III bands ranging from 1.7 to 4.2 kb which did not cohybridize with *rrn* sequences when the 23S probe was used (Fig. 2a and b). The 2.3-kb band represents the *trnI*-5'-*rrnH* cluster as predicted from the published sequence (23). One or more of these bands should represent cluster *trnY* (27) and/or the *sup-3-sup-44* region. The 3.3-kb tRNA fragment comigrates with the *rrnE-trnE* fragment and becomes evident when the DNA of strain BD29-4 is probed (data not shown). Southern hybridizations of *Eco*RI digests served to clarify the states of these additional tRNA bands. When hybridized with 23S rDNA, an *Eco*RI blot shows 10 fragments (Fig. 2c) (26). The assignment of the fragments from 2.3 through 3.9 kb was described previously (6, 26). The 6.2-kb fragment hybridized to the ORF downstream of *rrnE-trnE* (data not shown). On the basis of sequence data (24), the 4.5-kb fragment was assigned to *rrnB-trnB*. The 7.5- and the 9.3-kb fragments belong to the 3' ends of *rrnO* and *rrnA*, respectively, on the basis of restriction mapping of the *oriC* region (17). When an *Eco*RI blot was hybridized to the 16-tRNA-gene probe, three bands of 2.9, 7.0 (not present in BD170), and 19 kb, which did not cohybridize with 23S

rDNA, appeared (Fig. 2d). The sizes of these fragments correspond to the DNA estimated from seven of the eight tRNA *Hind*III fragments, which add up to 24.5 kb of DNA (Fig. 2b). The 2.9-kb tRNA fragment probably represents *trnY* (Fig. 2d) (27) and is seen in the deletion strain BD170 (or BD29) lacking the 2.9-kb *rrnG* fragment (26). This *Eco*RI pattern is somewhat at odds with the previously published results of Southern blots which revealed a 5.0-kb band but no 19-kb band (23). This difference may be due to differences in the probes or differences in strains. Given that we found two *Eco*RI fragments that hybridized only to the tRNA gene probe and similarly to seven *Hind*III fragments, we conclude that there are at least two more unmapped tRNA gene clusters residing on the 7.0- and 19-kb fragments, containing at least seven *trn* genes.

DISCUSSION

A chromosomal map with the locations of all known tRNA gene clusters and all the rRNA genes is shown in Fig. 3. It includes the two tRNA gene clusters, *trnE* and *trnD*, assigned in this paper and a cluster of nine tRNA genes (*trnJ*) between *rrnJ* and *rrnW* recently reported by Green and Vold (6). At least three additional tRNA clusters have not been mapped either genetically or physically; one of these is *trnY* (27), another corresponds to the *sup-3-sup-44* locus (7, 12), and neither of these is associated with any *rrn* operon. A total of 62 tRNA genes have been characterized for *B. subtilis*, compared with the 78 tRNA genes reported for *E. coli* (10). The number of tRNA genes in *B. subtilis* is at least 69, if one includes the uncharacterized *Hind*III and *Eco*RI tRNA homologs (Fig. 2b and d) and the *sup-3* and *sup-44* genes (7, 12).

Compared with the tRNA genes, the chromosomal organization of the rRNA genes in *B. subtilis* and *E. coli* is relatively conserved. In *B. subtilis* and *E. coli*, the majority of the rRNA operons (7 of 10 and 5 of 7, respectively) are located near the replication origin on one side of the chromosome (Fig. 3) (4, 10). Furthermore, in both organisms all *rrn* genes contain or are adjacent to *trn* genes. In contrast, the degree of clustering of the *trn* genes differs greatly between the two organisms. In *B. subtilis*, the 62 tRNA genes that have been sequenced to date are distributed in eight clusters, each containing from 2 to 21 tRNA genes. Of the eight clusters, seven are associated with the *rrn* operons. In *E. coli*, all 78 tRNA genes are distributed among 40 different locations, each containing 1 to 4 tRNA genes; only 7 of these loci are proximal to *rrn* operons (10). On the other hand, in *B. subtilis*, 19 of the 62 tRNA genes are located near *oriC* in association with seven *rrn* operons (Fig. 3). However, the two largest clusters, containing the majority of tRNA genes, *trnB* (21-tRNA-gene cluster) and *trnD* (16-tRNA-gene cluster), are symmetrically situated the farthest distances away from the *oriC* region (Fig. 3). These two *rrn* operons have counterparts in *E. coli* but, again, without large tRNA gene clusters (4, 10).

Three hypotheses may explain the differences and similarities between these two organisms. The high densities of *rrn-trn* gene sets near the origin of replication in *B. subtilis*, *E. coli*, and *Salmonella typhimurium* (9, 10, 16, 20) may provide the high gene dosage required to support the high rate of rRNA and tRNA synthesis in rapidly dividing cells. We have found that operons nearer to the origin (i.e., *rrnO*, *rrnJ*, and *rrnW*) are expressed at much higher levels than the ones further away (i.e., *rrnD* and *rrnB*) (24a). The tRNA genes may be transcriptionally linked with their respective

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