SUPPLEMENTARY FIGURE AND TABLE LEGENDS

Figures S1. 3′ RACE assay of *galE* mRNA 3′ends from MG1655 cells harboring the plasmid-borne *gal DH1200* mutant with an additional E-hairpin at 1200 mutant operons (lane 2). The E-hairpin (42 nucleotides; 1137–1178) at position 1200 in the WT *gal* operon was inserted to generate the double-hairpin *gal* mutant DH1200 (1). Related to Figure 2.

Figure S2. Failure of *galT* translation initiation causes RDT. A) Northern blot with the E probe of *gal* mRNA from MG1655 cells harboring the plasmid-borne *galT* start^{o} mutant operon with or without the Rho inhibitor bicyclomycin (BCM). To determine whether RDT produces *galE* mRNA, we treated MG1655 cells (OD600 of 0.6) in LB medium with 25 μg/mL BCM for 10 min. Northern blotting was performed to analyze the total RNA from the cell culture (see *Materials and Methods*). Related to Figure 4A. B) Northern blot with the E probe of *gal* mRNA from GW20Δ*gal* cells harboring the plasmid-borne *galT* start^{o} mutant operon. Cells were cultured at both permissive (30°C) and nonpermissive (44°C) temperatures for analysis. Related to Figure 4B.

Figure S3. Northern blot with the E probe of *gal* mRNA from MG1655 cells harboring the plasmid-borne *galE* stop^{o}, *galT* start^{o}, and *galE-T* ONE frame mutant operons. A slight increase in the other *gal* mRNAs longer than *galE* in the *galE stop^{o}* mutant could have resulted from the fact that more transcription might have gone downstream of the 3′ end of *galE*.

Figure S4. Continuous translation without interruption by termination at the stop codon of *galT* could eliminate the break in transcription–translation coupling. The 3′ RACE assay compares the WT and *galE* stop^{o} mutant operon from GW20Δ*gal* (temperature-sensitive RNase E mutant) cells, where the entire *gal* operon is deleted from the chromosome. Cells were cultured at both permissive (30°C) and nonpermissive (44°C) temperatures for analysis. B) Relative band intensity for the 3′ RACE assay from (A).

Table S1. Primers used in this study and usage.

Fig. S1

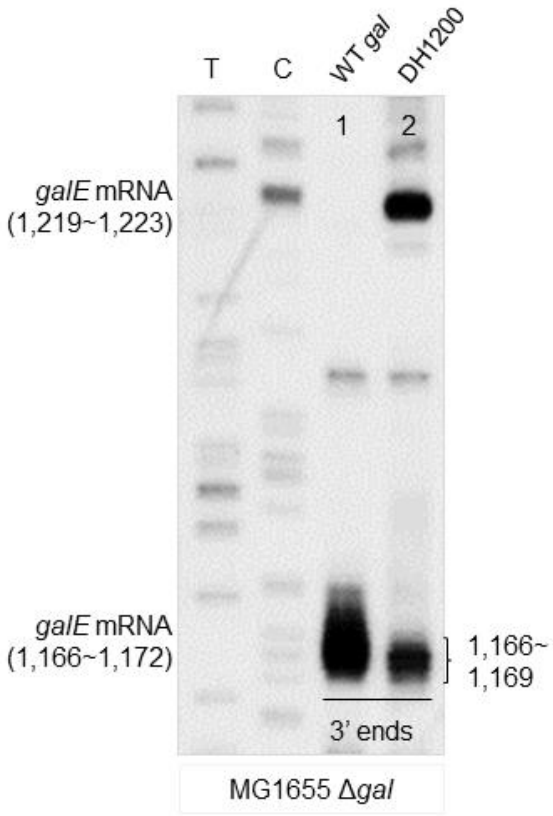
T
C
WT gal
DH1200
1
2
galE mRNA
(1,219~1,223)
galE mRNA
(1,166~1,172)
1,166~
1,169
3' ends
MG1655 Δgal

Fig. S2

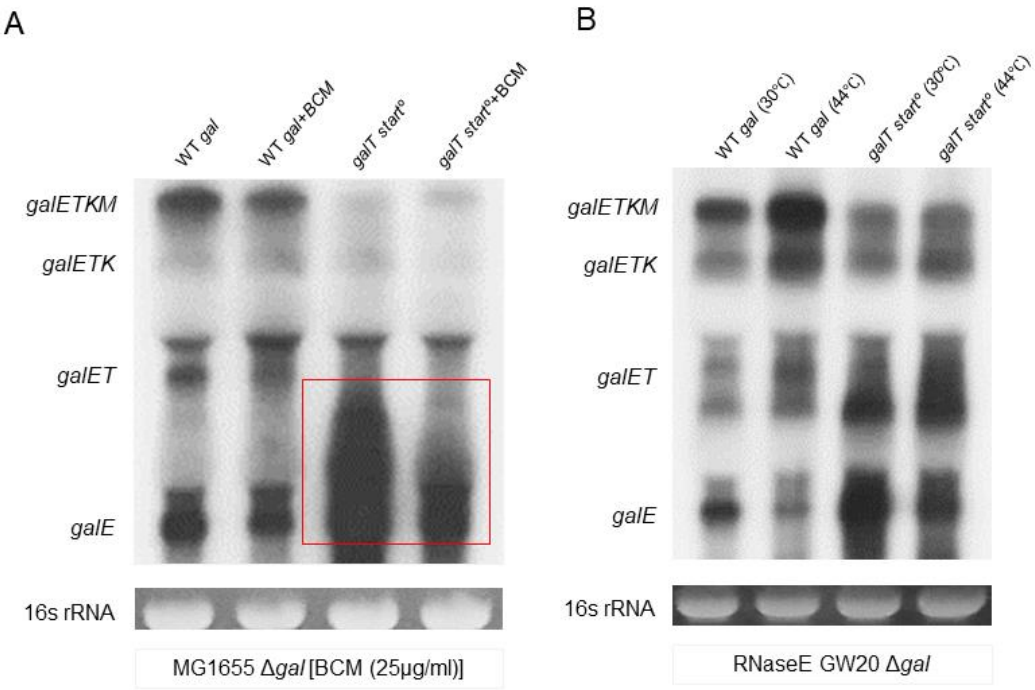
A
WT gal
WT gal+BCM
galT start°
galT start°+BCM
galETKM
galETK
galET
galE
16s rRNA
MG1655 Δgal [BCM (25μg/ml)]
B
WT gal (30°C)
WT gal (44°C)
galT start° (30°C)
galT start° (44°C)
galETKM
galETK
galET
galE
16s rRNA
RNaseE GW20 Δgal

Fig. S3

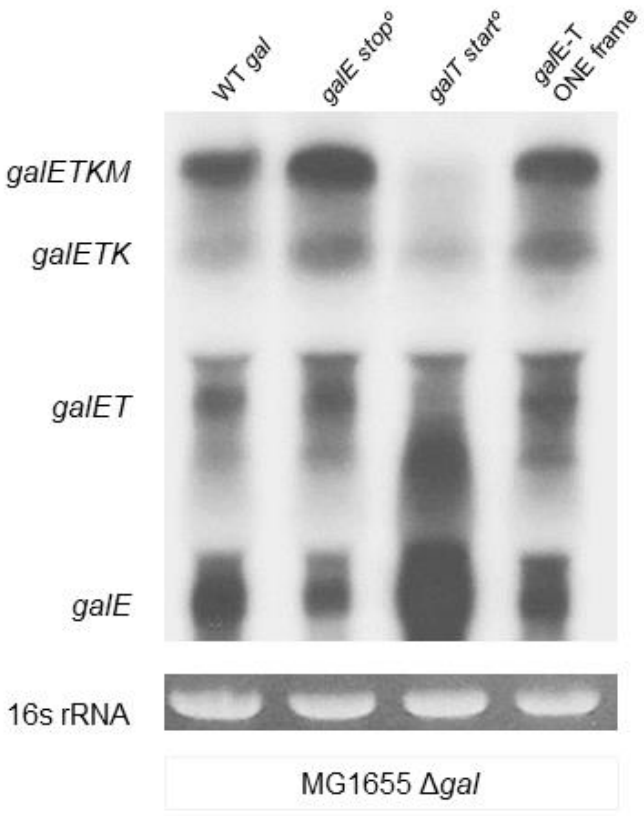
WT gal
galE stop°
galT start°
galE-T ONE frame
galETKM
galETK
galET
galE
16s rRNA
MG1655 Δgal

Fig. S4

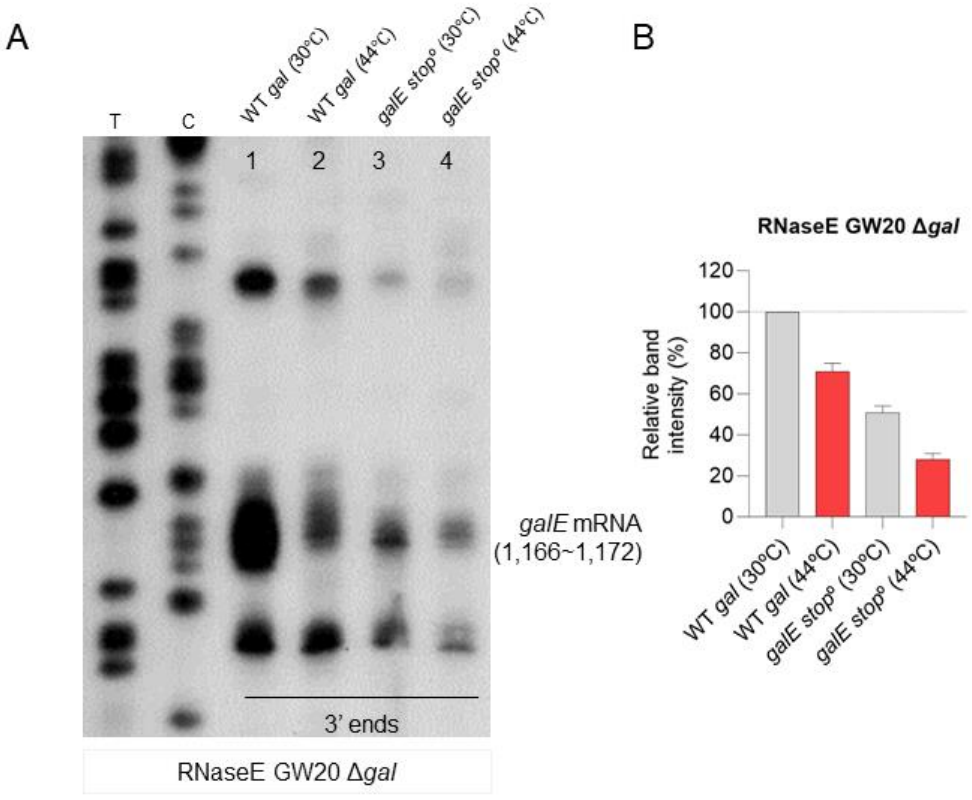
A
T
C
WT gal (30°C)
WT gal (44°C)
galE stop° (30°C)
galE stop° (44°C)
1
2
3
4
3' ends
RNaseE GW20 Δgal
galE mRNA
(1,166~1,172)
B
RNaseE GW20 Δgal
Relative band intensity (%)
120
100
80
60
40
20
0
WT gal (30°C)
WT gal (44°C)
galE stop° (30°C)
galE stop° (44°C)

Table. S1

Primer name	Primer sequences (5′→3′)	Usage
galBamHI-R	CCGGATCCGGTGATTTGAACAATATGAG	Cloning
galHindIII-F	CACCGTTTATGGCGATCAGCCC	
galMluI-R	GCGTTTTCAGTCAGTATATGACG	
EHMM2-F	CGGCCCCTGGCAGGGGGCGCAGGAAA	Site-directed mutagenesis
EHMM5-F	CGCGGCCTGGCAGGGGGCGCAGGAAA	
ENO-stop-R	GGTCGTTCCTTTATCCGGATATC	
TNO-start-F	TAAGGAACGACCAAAACGCAA	
ET-one frame	AAAGGAACGACCAAAACGCAA	
DH1200aatII-F	GTTACCTGCGCACGATCCAGACGTC	
DH1200aatII-R	ACATTACCTGCGCAGAGGAAGACGTC	
850 MluI-R	CCATTACGCGTATGGTATGAAATAACC	
850 PstI-R	CAAATTCACGCCTGCAGGCGCCTGATT	
E1-F	ATGAGAGTTCTGGTTACCGGTGGTAG	E-probe generation for Northern blot
E1-R	TGGGCTTTTTTGCAGATCGGTGAGGA	
3RP	AGCATGCGGCCGCTAAGAAC	3' RACE – RT and PCR primers
3′RNA Oligo	UUCACUGUUCUUAGCGGCCGCAUGCU	
E3-F	CATCGCCCAGGTTGCTGTAG	
NewT6_1123-F	TTTCACCGCACCGCGCTAA	3' RACE extension primer
T1, T-ext1,T6-F	ATGACGCAATTTAATCCCGTTGATCATC	
5s-F	GAGAGTAGGGAACTGCCA	5′ RACE PCR primer
K2-R	AGCCTACAAACTGGTTTTCTGCTTCC	
M2-1-R	CATCTGAACTCAGGGCAAACA	
EText-R	AGAATCCATTGCCCGGTGAG	5`RACE extension primer
TKext-R	ATGGTGTGAGTGGCAGGGTA	
KMext-R	TGCCAGTGCGGGAGTTTCGT	