

# Nutrient Dependence of RNase E Essentiality in *Escherichia coli*

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*Escherichia coli* cells normally require RNase E activity to form colonies (colony-forming ability [CFA]). The CFA-defective phenotype of cells lacking RNase E is partly reversed by overexpression of the related endoribonuclease RNase G or by mutation of the gene encoding the RNA helicase DeaD. We found that the carbon source utilization by *rne* *deaD* doubly mutant bacteria differs from that of *rne*<sup>+</sup> cells and from that of cells mutated in *deaD* alone and that the loss of *rne* function in these bacteria limits conversion of the glycolytic pathway product phosphoenolpyruvate to the tricarboxylic acid (TCA) cycle intermediate oxaloacetic acid. We show that the mechanism underlying this effect is reduced production of the enzyme phosphoenolpyruvate carboxylase (PPC) and that adventitious overexpression of PPC, which facilitates phosphoenolpyruvate utilization and connects the glycolytic pathway with the TCA cycle, restored CFA to *rne* *deaD* mutant bacteria cultured on carbon sources that otherwise were unable to sustain growth. We further show that bacteria producing full-length RNase E, which allows formation of degradosomes, have nutritional requirements different from those of cells supplied with only the N-terminal catalytic region of RNase E and that mitigation of RNase E deficiency by overexpression of a related RNase, RNase G, is also affected by carbon source. Our results reveal previously unsuspected effects of RNase E deficiency and degradosome formation on nutrient utilization by *E. coli* cells.

RNase E was initially discovered in *Escherichia coli* as an endoribonuclease that processes 9S rRNA (1) and later was shown to have a multifunctional role in the processing of various other structural RNAs (1, 2, 3, 4), the global degradation of mRNA (5, 6, 7, 8), the control of plasmid DNA replication (9), and the maturation/processing of a variety of small catalytic RNAs (9, 10). Commonly, the cleavage of the targeted RNA by RNase E is followed by digestion of RNase E-generated fragments by other ribonucleases as tightly coupled events (11). DNA sequence analysis has predicted that orthologs of *E. coli* RNase E exist among dozens of evolutionarily disparate bacterial species (12, 13). The ribonucleolytic activity of RNase E resides in its N-terminal half, N-Rne (14), whereas the C-terminal half (CTH) includes binding sites for several proteins that form a multicomponent complex termed the “degradosome” (15, 16). Although degradosome formation is not essential for *E. coli* growth (14, 17, 18), deletion of the CTH has been found to reduce the rate of decay of some RNase E substrates (19, 20).

In *E. coli*, the actions of RNase E are essential for colony-forming ability (CFA) (21), and transfer of liquid cultures of *rne*(Ts) mutant bacteria to a nonpermissive temperature prevents normal cell division and results in the formation of elongated filamentous structures containing connected cell bodies (22). Multiple studies aimed at elucidating the basis of RNase E essentiality have been carried out (6, 23, 24, 25, 26). While CFA can be restored to *rne* deletion mutants by overproduction or mutation of RNase G (6, 24, 25, 27), an RNase E paralog (2, 4) that structurally resembles the amino-terminal catalytic region of RNase E (14), such cells grow more slowly than *rne*<sup>+</sup> bacteria and retain the filamentation phenotype characteristic of RNase E-deficient mutants in liquid culture (25). The filamentation of RNase G-overproducing  $\Delta$ *rne* mutants is reversed by overexpression of FtsZ; however, FtsZ-overproducing cells remain defective in their rate of division (25).

During recently reported investigations of insertion mutations that restore CFA to *E. coli* cells lacking RNase E (26), we observed that the survival of such  $\Delta$ *rne* cells was dependent on culture con-

ditions: colony formation was observed when the cells were grown on Luria-Bertani (LB) medium but not when they were grown on M9 medium containing glucose as the sole carbon source. This finding prompted us to investigate nutritional factors affecting the growth of  $\Delta$ *rne* bacteria, and the ability of cells containing mutations in the *deaD* gene (26), which encodes an RNA helicase (28), to propagate enabled us to do this in the absence of any complementing RNase. The results we report here show that a combination of a glycolytic pathway sugar plus a tricarboxylic acid (TCA) cycle sugar is needed for propagation of  $\Delta$ *rne* *deaD* doubly mutant bacteria, whereas no such requirement was observed for cells mutated in *deaD* alone. They further indicate, as shown by Western blotting and confirmed genetically, that RNase E-deficient cells are defective in production of the enzyme phosphoenolpyruvate carboxylase (PPC), which converts phosphoenolpyruvate to oxaloacetic acid and connects the glycolytic pathway with the TCA cycle, establishing a previously unsuspected role for PPC in RNase E essentiality.

## MATERIALS AND METHODS

**Strains and plasmids.** The strains used in this study are listed in Table 1. MT4000 was constructed by the method described by Lee et al. (6). MT4001, MT4002, MT4003, MT2105, and MT2005 were constructed by transformation of MT4000 by pNRNE4, MT4000 by pRNG2S, MT4000 by pRNG2S $\Delta$ H, CM2100 by pRNG2S $\Delta$ H, and MT2000 by pRNG2S $\Delta$ H, respectively. MT2113 was obtained by P1 transduction of *deaD*::Tn10 into CM2100, and colonies were selected on tetracycline (Tc) plates containing

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**TABLE 1** Bacterial strains and plasmids

Strain or plasmid	Description	Reference(s) or source
<b>Strains</b>		
MG1655	<i>ilvG rfb-50 rph-1 fnr-267 eut</i>	<i>E. coli</i> Genetic Stock Center (CGSC 6300)
MG165D	Same as MG1655 but carrying <i>deaD::Tn10</i> (clone 2)	This study
NCM3416	Same as MG1655 but carrying <i>rph<sup>+</sup></i> and <i>zib-207::Tn10</i>	40
MT4000	Same as NCM3416 but carrying <i>rne::cat</i> and pBAD-RNE	This study
MT4001	Same as MT4000 but carrying pNRNE4	This study
MT4002	Same as MT4000 but carrying pRNG2S	This study
MT4003	Same as MT4000 but carrying pRNG2SΔH	This study
MT2000	<i>lacZ43(Fs) LAM<sup>-</sup> relA1 spoT1 thi-1 rne::cat</i> ; carries pBAD-RNE	25
MT2005	Same as MT2000 but carrying pRNG2SΔH	This study
MT2013	Same as MT2000 but carrying <i>deaD::Tn10</i> (clone 2)	26
CM2100	Same as MG1655 but carrying <i>rne::cat</i> and pBAD-RNE	This study
MT2103	Same as MG1655 but carrying <i>rne::cat</i> and pLAC-RNE2	This study
MT2105	Same as CM2100 but carrying pRNG2SΔH	This study
MT2113	Same as CM2100 but carrying <i>deaD::Tn10</i> (clone 2)	This study
MT2114	Same as MT2113 but carrying ASKA-ppc	This study
MT2115	Same as MT2113 but carrying pACYC177	This study
MT2116	Same as MT2113 but carrying pMT177Z	This study
MT2117	Same as MT2113 but carrying pCA24N(Ap)	This study
<b>Plasmids</b>		
pBAD-RNE	pSC101 <i>ori</i> Km <sup>r</sup> , <i>rne</i> under control of P <sub>BAD</sub>	6, 25
pLAC-RNE2	pSC101 <i>ori</i> Ap <sup>r</sup> , <i>rne</i> under control of <i>lacUV5</i> promoter	41
pNRNE4	P15A <i>ori</i> Ap <sup>r</sup> , His-tagged N- <i>rne</i> under control of <i>lacUV5</i> promoter	25
pRNG2	P15A <i>ori</i> Ap <sup>r</sup> , His-tagged <i>rng</i> (also N-terminally extended) under control of <i>lacUV5</i> promoter	Gift from Kangseok Lee
pRNG2S	P15A <i>ori</i> Ap <sup>r</sup> , His-tagged <i>rng</i> under control of <i>lacUV5</i> promoter	Gift from Kangseok Lee
pRNG2SΔH	P15A <i>ori</i> Ap <sup>r</sup> , natural short-form <i>rng</i> under control of <i>lacUV5</i> promoter	Gift from Kangseok Lee
pCA24N(Ap)	ColE1 <i>ori</i> Ap <sup>r</sup>	26
ASKA-ppc	ColE1 <i>ori</i> Ap <sup>r</sup> , <i>ppc</i> under control of P <sub>T5-lac</sub>	This study
pACYC177	P15A <i>ori</i> Km <sup>r</sup> Ap <sup>r</sup>	42
pMT177Z	P15A <i>ori</i> Ap <sup>r</sup> , <i>ftsZ</i> under control of natural promoter	25

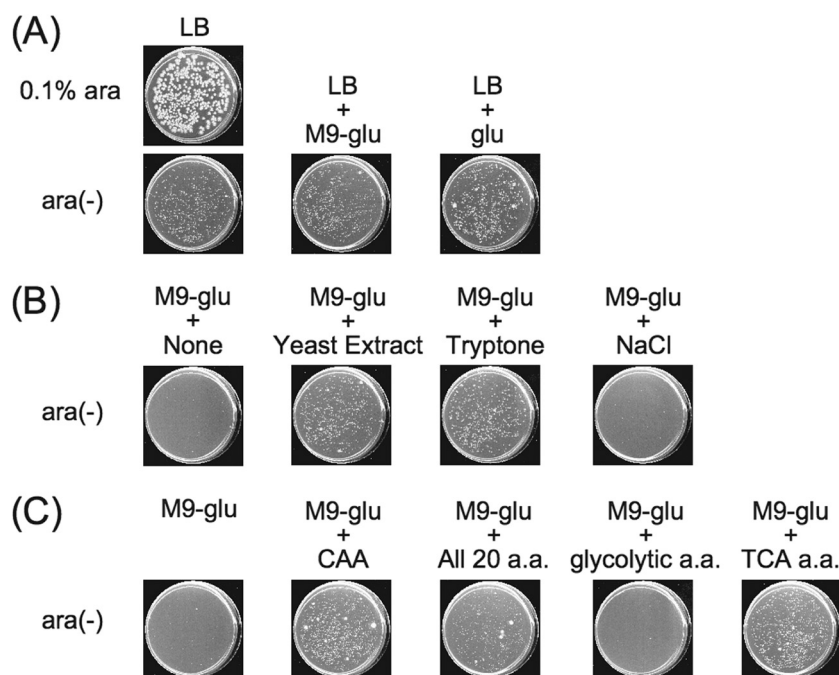
0.1% arabinose (0.1% Ara) to induce adventitious production of RNase E in the Δ*rne* strain (6, 26). P1(*deaD::Tn10*) was prepared from MT2013 as described previously (26). MT2103 was constructed by replacing pBAD-RNE of CM2100 with pLAC-RNE2 by the method described by Lee et al. (6). To test the effects of PPC overexpression, we introduced either a modified ASKA library plasmid (29) expressing PPC (i.e., ASKA-ppc) or an empty vector [pCA24N(Ap)] into the MT2113 strain, generating the MT2114 and MT2117 strains, respectively.

pRNG2SΔH, which expresses the natural form of RNase G (i.e., no N-terminal or C-terminal extension), was constructed by ligating ClaI- and EcoRI-digested PCR products encoding the C-terminal region of RNase G lacking a hexahistidine tag into pRNG2S using the same restriction enzyme sites. The PCR primers used were *rng*-948F (5'-TGTCGGTC ATCGCAATCT-3') and 3'-*rng*-no-His (5'-AGACTAGTGAATTCACAT CATTACGACGTCAAACCTG-3'). pRNG2S, which expresses C-terminal His-tagged RNase G lacking any N-terminal extension, was constructed by ligating an NdeI- and NsiI-digested PCR product encoding the N-terminal region of RNase G containing its natural N-terminal sequence (ATGACGGCTGAA... ) into pRNG2 using the same restriction enzyme sites. The PCR primers used were 5'-*rng*-nat (5'-ATACATATG ACGGCTGAATTGTTAGTA-3') and *rng*-595R (5'-AGACGCGTTTCA GATAAG-3'). pRNG2, which expresses C-terminal His-tagged RNase G lacking any N-terminal extension, was constructed by ligating NotI- and SpeI-digested PCR DNA encoding the region of *rng* containing an extended N-terminal sequence (ATGAGAAAAGGGATAAAC) into pNRNE4 (25) using the same restriction enzyme sites. The PCR primers used were 5'-*rng*-2 (5'-GGATCCGCGCCGCTTTAAGAAGGAGATAT ACATATGAGAAAAGGGATAAAC-3') and 3'-*rng*-His (5'-GTCTAGA

CTAGTGAATTCAGTGGTGGTGGTGGTGGTGCATCATTACGACGT GCAAACCT-3'). The pRNG2SΔH, pRNG2S, and pRNG2 plasmids were kindly provided by Kangseok Lee (C. J. Moore, H. Go, K. Lee, and S. N. Cohen, unpublished data). Construction of an ASKA plasmid with an Ap resistance gene was described previously (26).

**Media and culture conditions.** LB medium or M9 minimal medium (30) containing 0.1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, and appropriate carbon sources at the following concentrations were used: D-(+)-glucose, 0.2%; D-mannitol, 0.2%; D-(-)-fructose, 0.2%; glycerol, 0.5%; sodium pyruvate, 0.2%; sodium acetate, 0.2%; succinic acid, 0.5%; L-(-)-malic acid, 0.5%; fumaric acid, 0.5%; and Casamino Acids (CAA) (see BD Bionutrients Technical Manual), 0.05%. Where indicated, LB components at the following concentrations were used: yeast extract (Bacto Yeast Extract; BD), 5 g/liter; tryptone (Bacto Tryptone; BD), 10 g/liter; and sodium chloride (Invitrogen), 10 g/liter. Where indicated, antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; chloramphenicol, 10 μg/ml; kanamycin, 20 μg/ml; tetracycline, 5 μg/ml; and streptomycin 50 μg/ml. For all experiments, agar plates were freshly prepared on the day used (never stored at 4°C or overnight on the bench), bacteria were streaked onto plates containing 0.1% L-arabinose from glycerol stock (containing 40% glycerol) and incubated at 37°C overnight, and colonies were picked for inoculations.

**Plating experiments.** For plating experiments, cells were freshly grown from a single colony (never grown overnight) to mid-log phase (optical densities at 600 nm [OD<sub>600</sub>] ranged from 0.3 to 0.6) at 37°C in LB medium containing the indicated supplements and antibiotics. Cells were diluted 10<sup>-4</sup> to 10<sup>-5</sup>, and 100 μl of diluted culture was spread on LB or M9 plates containing carbon sources, supplements, and antibiotics. The



**FIG 1** Effects of individual LB components on CFA of a  $\Delta rne deaD::Tn10$  strain. (A) A culture of MT2113 in LB containing 0.1% arabinose, which induces adventitious expression of plasmid-borne *rne*, was spread on LB containing or lacking 0.1% arabinose, LB containing the additional components of M9-glucose but lacking the inducer, or LB containing 0.2% glucose and no arabinose. (B) A culture of MT2113 was spread on M9-glucose lacking any additive or supplemented with yeast extract, a tryptic digest of casein (labeled as tryptone), or NaCl, all in the absence of arabinose. (C) Identification of amino acid requirements for CFA by a  $\Delta rne deaD::Tn10$  strain cultured on M9-glucose. A culture of MT2113 was spread on M9-glucose containing a variety of amino acid mixtures, in the absence of arabinose. A mixture of glycolytic pathway-derived amino acids, i.e., Ser, Cys, Gly, Trp, Tyr, Phr, Ala, Val, Leu, Ile, and His, is defined as “glycolytic a.a.,” and a mixture of TCA cycle-derived amino acids, i.e., Lys, Met, Thr, Asn, Asp, Glu, Gln, Pro, Arg, and His, is defined as “TCA a.a.” The concentration of each amino acid was 160  $\mu$ M. Plates were scanned after incubation for 2 days (A and B) or 4 days (C) at 37°C.

plates were incubated at 37°C for 2 to 7 days depending on the experiment and scanned, and colony number was determined. Different nutrient combinations resulted in different rates of colony growth, and where indicated, the incubation period was extended by up to 2 days to enable colonies to be better visualized. Incubation conditions for each experiment are described in the figure legends. The absence of detectable colony formation after 7 days of incubation was defined as a CFA-negative (CFA<sup>-</sup>) phenotype. Colony-forming efficiency (CFE) was determined by dividing the colony number observed under the indicated growth condition by the colony number observed under the same condition when an arabinose-inducible RNase E gene was expressed (26).

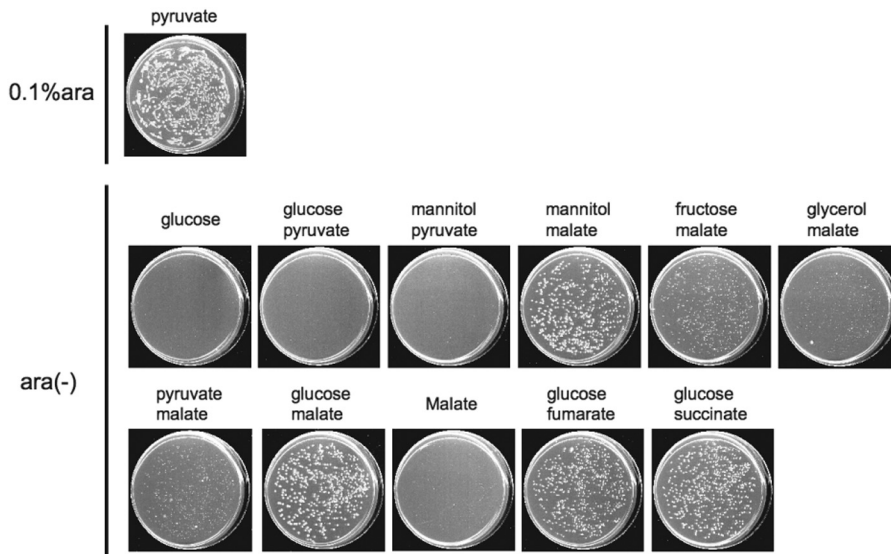
**Western blotting.** Cultures of CM2100, MT2113, MT2114, or MT2117 cells were grown overnight at 37°C on LB plates containing 0.1% arabinose and the appropriate antibiotics. Randomly selected colonies from each plate were suspended in LB and diluted to an OD<sub>600</sub> of 0.001 in fresh LB containing the appropriate selective antibiotics, with or without 0.1% arabinose (as indicated) and with 100  $\mu$ M isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) (for strains MT2114 and MT2117). The cultures were grown to an approximate OD<sub>600</sub> of 0.8, or for a minimum of 6 h, and then harvested and prepared for loading onto a polyacrylamide gel. The cells were diluted to an OD<sub>600</sub> of 0.01 per  $\mu$ l in water and SDS loading buffer and then boiled. Lysate from CM2100, MT2113, or MT2117 cells at an OD<sub>600</sub> of 0.24 cultured with inducing agents as indicated was loaded alongside lysate from MT2114 cells at an OD<sub>600</sub> of 0.1, and the proteins were separated on a Criterion XL 3 to 8% Tris-acetate polyacrylamide gel (Bio-Rad). The samples were transferred to a 0.1- $\mu$ m nitrocellulose membrane and probed with anti-PPC (Agriser rabbit polyclonal), anti-RNase E (mouse monoclonal), and anti-S1 (rabbit polyclonal) antibodies, in that order, with intervening membrane stripping. The numbers provided represent densitometric measurements of each band as determined by the

VersaDoc model 1000 imaging system (Bio-Rad), corrected for loading based on the S1 protein control and the difference in amount of total protein loaded between MT2114 and the other strains. Values were then normalized relative to the RNase E or PPC band from the CM2100 cells.

## RESULTS AND DISCUSSION

**RNase E is required for glycolysis in *E. coli*.** We recently reported that a Tn10 insertion mutation in the *deaD* gene, which encodes an RNA helicase, enables colony formation by *E. coli* N3433 cells lacking RNase E function (26). During those experiments, we observed that this ability was affected by the carbon source included in the medium. While N3433 is the *E. coli* strain in which RNase E was first discovered and has since been widely employed for study of RNase E essentiality, this strain contains multiple mutations that potentially affect nutritional requirements [i.e., *lacZ43(Fs)*, *relA1*, *spoT1*, and *thi-1*] and consequently affect the ability of even *rne*<sup>+</sup> cells to form colonies efficiently on some carbon sources (see Fig. S1 in the supplemental material); we thus chose to further investigate the nutritional effects of loss of RNase E function in *E. coli* strain MG1655, which is prototrophic at these loci, after confirming that a  $\Delta rne deaD$  derivative of MG1655 (i.e., strain MT2113) forms colonies on LB with 100% colony-forming efficiency relative to that of cells producing RNase E (Fig. 1A).

As seen in Fig. 1, in the absence of any arabinose to induce plasmid-borne RNase E, the  $\Delta rne deaD$  MT2113 strain formed colonies on LB medium or on M9 medium containing both glucose and a mixture of amino acids as carbon sources; however, no MT2113 colonies were observed when yeast extract or the mixture

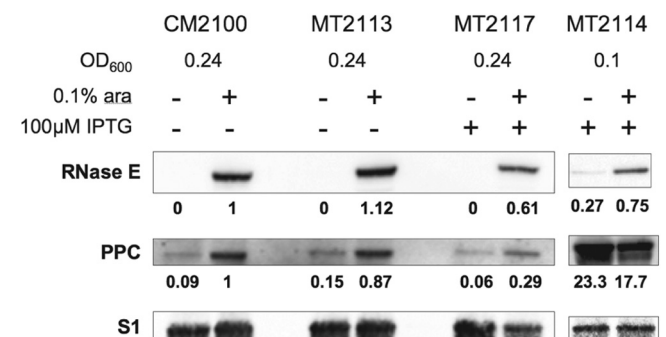


**FIG 2** Effect of carbon source combination on CFA of a  $\Delta rne\ deaD::Tn10$  strain. A culture of MT2113 in LB was spread on carbon sources as indicated. The presence or absence of 0.1% arabinose is indicated. Plates were scanned after incubation at 37°C for 4 days.

of amino acids was omitted (Fig. 1B). In contrast, in the absence of any amino acid supplement, strain MG165D, which is an MG1655 derivative containing an intact *rne* gene but mutated by *Tn10* insertion into *deaD*, formed colonies on M9 medium containing any of a series of tested sugars as a carbon source (see Fig. S2 in the supplemental material), arguing that the failure of MT2113 to form colonies in media lacking any supplemental amino acids and supplied with glucose as the sole carbon source results from defective RNase E function. Testing of additives of individual amino acids (Fig. 1C) indicated that amino acids specifically synthesized through the glycolytic pathway did not enable CFA by MT2113 supplied with glucose, whereas addition of amino acids generated through the TCA cycle did. Moreover, the addition of malate, fumarate, or succinate, which are all TCA cycle components and can generate TCA cycle-dependent amino acids, also enabled CFA on M9-glucose (Fig. 2). Together, our results indicate that the pathway to TCA cycle-dependent amino acid biosynthesis is intact in the  $\Delta rne\ deaD::Tn10$  mutant strain and suggest that a defect in the enzymatic connection between the glycolytic pathway and TCA cycle in the mutant bacteria prevents products of glycolysis from entering the TCA cycle.

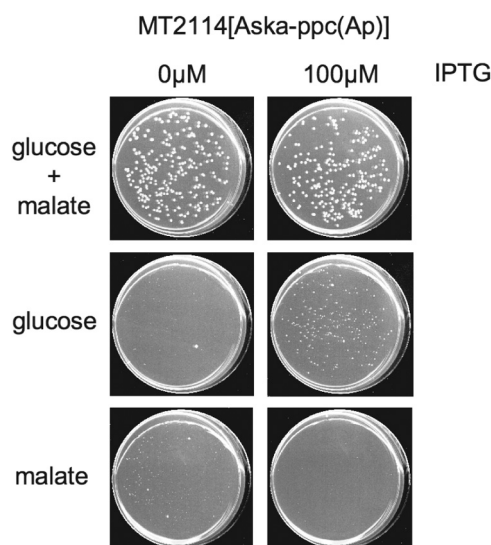
**Functional interactions between RNase E and PPC.** The glycolytic pathway and TCA cycle are connected by the enzyme phosphoenolpyruvate carboxylase (PPC), which converts phosphoenolpyruvate to oxaloacetic acid (31). To test the hypothesis that deficiency of PPC expression occurs in  $\Delta rne\ deaD$  bacteria, we used Western blotting to examine the effects of RNase E expression on the expression of PPC encoded by either the endogenous gene or a plasmid-borne PPC gene (Fig. 3). As seen, the expression of PPC protein is highly dependent on the expression of RNase E. When full-length RNase E was depleted to undetectable levels by growth in the absence of arabinose, the PPC protein was reduced by 79 to 91%. To determine whether overexpression of PPC rescues CFA in bacteria that fail to produce RNase E, we tested the ability of the MT2114 strain to form colonies on M9-glucose. As seen in Fig. 4, overexpression of PPC restored CFA to bacteria

cultured on M9-glucose medium. No restoration of CFA occurred in the absence of the *deaD* mutation (data not shown), which is consistent with the previously reported ability of mutation of *deaD* to suppress the need for RNase E in *E. coli* cells (26). Together, these findings suggest that while PPC production is necessary for CFA in cells lacking RNase E function, it is not sufficient, and thus that the CFA-promoting function mediated by mutation of *deaD* is distinct from the PPC-related function. Additionally, adventitious expression of PPC (Fig. 4) did not result in CFA on M9-malate medium, supporting the view that upregulation of PPC alone does not restore normality to  $\Delta rne\ deaD$  mutant cells. During these experiments, we noted that although faint “shad-



**FIG 3** Effect of RNase E expression on PPC expression in a  $\Delta rne$  strain, a  $\Delta rne\ deaD::Tn10$  strain, and a  $\Delta rne\ deaD::Tn10$  strain complemented with ASKA-ppc or an empty vector. Cultures of CM2100, MT2113, MT2117, and MT2114 were grown with and without 0.1% arabinose to induce or deplete full-length RNase E from the pBAD-RNE plasmid (MT2114 and MT2117 were additionally grown with 100 μM IPTG), separated on a 3 to 8% Tris-acetate gel, transferred to a nitrocellulose membrane, and probed with the appropriate antibodies for the presence of RNase E, PPC, and S1 proteins. Due to the high level of PPC overexpression from ASKA-ppc in MT2114 when induced by 100 μM IPTG, a smaller amount of culture was loaded per lane than for the other strains (as described in Materials and Methods). Protein expression was determined as described in Materials and Methods.





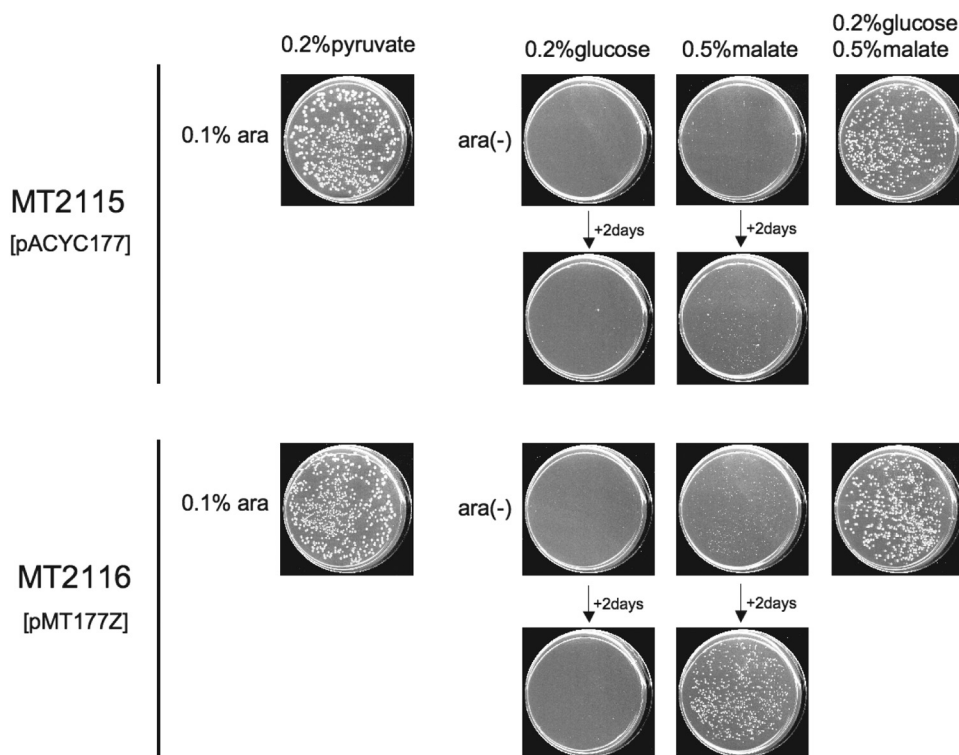
**FIG 4** Effect of PPC complementation on CFA of a  $\Delta rne\ deaD::Tn10$  strain cultured on M9-glucose or M9-malate. Cultures of MT2114 were spread on M9-glucose with 0.5% malate, M9-glucose, and M9-malate with or without 100  $\mu$ M IPTG in the absence of arabinose. Plates were scanned after incubation at 37°C for 7 days.

ows” of colonies often appeared following plating of such bacteria on M9-malate plates (see also Fig. 5), these presumed minute colonies did not increase in size during further incubation and upon replating did not result in colony formation (data not

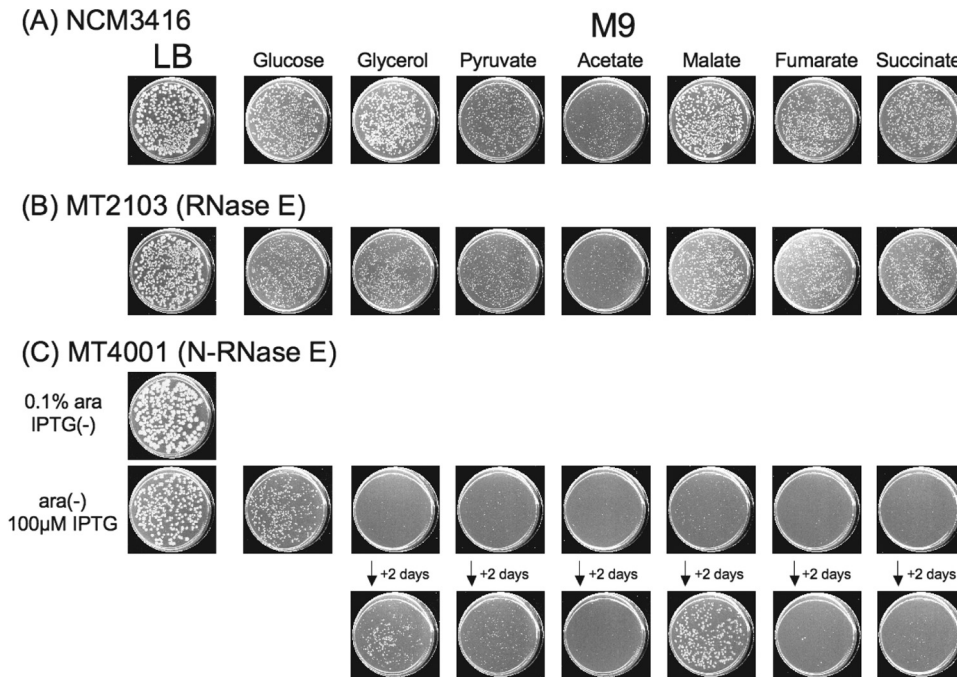
shown). We also observed that overproduction of PPC protein from the ASKA-ppc plasmid was increased by RNase E deficiency, in contrast to what was observed in cells expressing PPC from the endogenous gene. As the ASKA-ppc plasmid expresses the open reading frame of the PPC gene but lacks the 5' and 3' untranslated regions, this result suggests that RNase E production may have a dual role in PPC synthesis by affecting both mRNA synthesis and translation.

Interestingly, introduction of an FtsZ-producing plasmid (pMT177Z), which previously has been shown to reverse filamentation of RNase E-deficient cells (25) (see Fig. S3 in the supplemental material), into  $\Delta rne\ deaD::Tn10$  bacteria enabled CFA on M9-malate but not on M9-glucose in the absence of amino acids whose synthesis is dependent on the TCA cycle (Fig. 5). As the genes expressed from the *ftsQAZ* operon are not known to contribute to glycolysis or gluconeogenesis, the effect of FtsZ on the nutritional requirements of RNase E-deficient cells suggests that an additional step in the glycolytic or gluconeogenic pathway (32, 33) is impaired in these RNase E-deficient bacteria.

**Role of the degradosome in carbon source utilization by *E. coli*.** The C-terminal half of RNase E functions as a binding site for multiple other proteins (15); one of these is the glycolytic pathway enzyme enolase, which converts 2-phosphoglycerate to phosphoenolpyruvate (34), which is the cellular substrate for PPC. Interaction between RNase E and enolase normally is not required for propagation of *E. coli*, as the N-terminal catalytic region of RNase E (N-Rne), which lacks the enolase binding site (35), is sufficient for colony formation by *rne* mutant bacteria (14). As both mannitol and glucose are upstream of the glycolytic pathway



**FIG 5** Effect of FtsZ complementation on CFA of a  $\Delta rne\ deaD::Tn10$  strain on M9-glucose or M9-malate. Cultures of MT2115 and MT2116 were spread on M9 plates containing glucose and/or malate, as indicated, and containing or lacking 0.1% arabinose. Plates were scanned after incubation at 37°C for 4 days or, where indicated, after 2 additional days of incubation.



**FIG 6** (A and B) Growth of wild-type *E. coli* (A) or a full-length RNase E-complemented  $\Delta rne$  strain (B) on the indicated carbon sources. Cultures of NCM3416 or MT2103 were spread on LB or M9 plates as indicated (10  $\mu$ M IPTG for MT2103). (C) Effect of RNase E degradosome formation on growth of *E. coli* on different carbon sources. Cultures of MT4001 were spread on LB or M9 plates containing or lacking 0.1% arabinose as indicated, and 100  $\mu$ M IPTG was added as indicated. Plates were scanned after incubation at 37°C for 2 days and, where indicated, after 2 additional days of incubation.

step that requires enolase (i.e., conversion of mannitol to pyruvate) and combinations of glucose plus pyruvate or mannitol plus pyruvate did not restore CFA to RNase-E deficient cells (Fig. 2), the sugar-related defect in CFA during RNase E deficiency is necessarily independent of enolase.

As PPC production is impaired in RNase E-deficient bacteria and a component of the RNase E-based degradosome generates the cellular substrate for PPC (i.e., phosphoenolpyruvate), we hypothesized that the presence of a scaffold region on RNase E may affect the carbon source utilization by *E. coli* cells. To test this notion, wild-type bacteria and a *deaD*<sup>+</sup> *rne* deletion mutant strain complemented by either N-Rne or full-length Rne were tested for their ability to grow on M9 plates supplemented with specific sugars. As shown in Fig. 6, both wild-type bacteria (Fig. 6A) and a  $\Delta rne$  strain carrying a plasmid expressing full-length Rne (Fig. 6B) formed normal-size colonies on all tested carbon sources except acetate after 2 days of culture on M9 plates. However,  $\Delta rne$  bacteria carrying a plasmid expressing N-Rne [i.e., MT4001

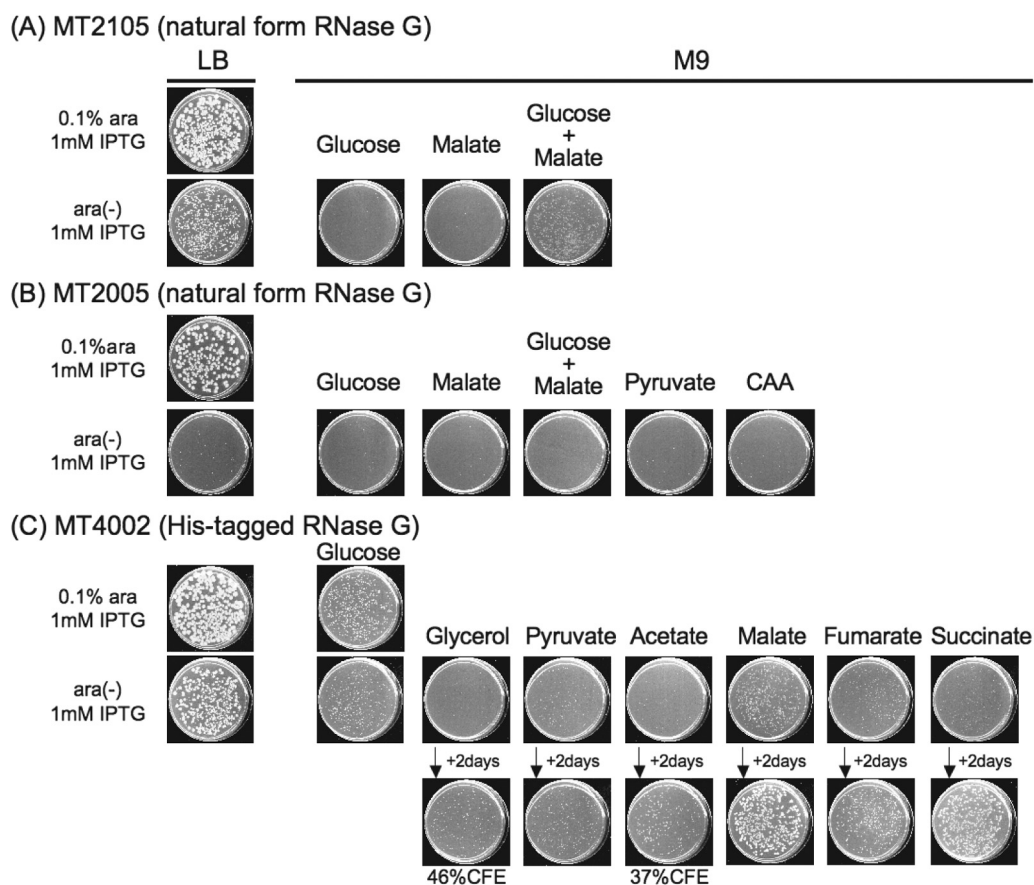
(pNRNE4)] did not form colonies on some of the carbon sources that are utilized effectively by full-length RNase E (i.e., fumarate and succinate), and colony growth on certain other carbon sources occurred much more slowly than was observed for  $\Delta rne$  cells complemented in *trans* by a plasmid expressing full-length RNase E (Fig. 6C and Table 2). Thus, while degradosome formation was not necessary for utilization of glucose by *E. coli* (Fig. 6C), the absence of the scaffold region of the enzyme affected utilization of other sugars. It should also be noted that overproduction of full-length RNase E in a  $\Delta rne$  strain (MT2103) did not enable formation of colonies on M9 medium containing 0.2% lactose, although wild-type NCM3416 bacteria were able to form colonies (data not shown). The basis for this effect currently is not known.

**Complementation of  $\Delta rne$  bacteria by different forms of RNase G results in differential sugar utilization.** Lee et al. reported that overproduction of His-tagged RNase G restores CFA to  $\Delta rne$  bacteria both on LB plates and in LB liquid medium (6). Subsequent investigations have shown that complementation of

**TABLE 2** Summary of plating experiments

Strain	Result <sup>a</sup> of plating on:							
	LB medium	M9 with:						
		Glucose	Glycerol	Pyruvate	Acetate	Malate	Fumarate	Succinate
NCM3416	ND	○	○	○	△	○	○	○
MT4001(pNRNE4)	○	○	△	△	×	△	×	×
MT4002(pRNG2S)	○	○	46% CFE, slow	△	37% CFE, slow	○	○	○
MT2105(pRNG2SΔH)	○	×	ND	ND	ND	×	ND	ND
MT2113 ( <i>deaD</i> ::Tn10)	△	×	ND	△	ND	×	ND	ND

<sup>a</sup> ○, 100% CFE; △, 100% CFE but slow growth; ×, no growth.



**FIG 7** Growth of a natural-form RNase G-complemented  $\Delta rne$  strain on LB and a variety of carbon sources. (A and B) Cultures of MT2105 (A) or MT2005 (B) were spread on LB or M9 plates containing or lacking 0.1% arabinose (as indicated), and 1 mM IPTG was added as indicated. Plates were scanned after incubation at 37°C for 2 days (A) and 4 days (B). (C) Growth of a  $\Delta rne$  strain expressing His-tagged RNase G. Cultures of MT4002 were spread on LB or M9 plates containing or lacking 0.1% arabinose and the indicated carbon sources, and 1 mM IPTG was added as indicated. Plates were scanned after incubation at 37°C for 2 days and, where indicated, after 2 additional days of incubation.

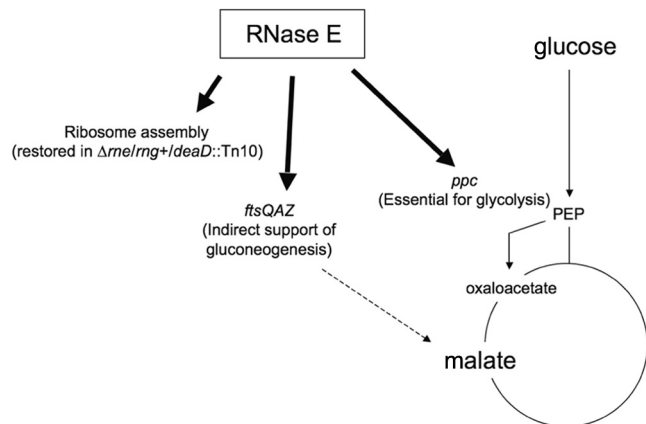
*rne*-defective mutants by RNase G is facilitated by unnatural extensions at the termini of the RNase G protein, and it was consequently concluded that RNase G function is constrained by the termini of the enzyme (24). In other instances, mutations in the catalytic region of RNase G have been reported to facilitate complementation of *rne* mutants (27). Given such information and the results we have described above, we were interested in learning whether  $\Delta rne$  cells complemented to viability by overproduction of the natural versus elongated forms of RNase G have different nutritional requirements.

As shown in Fig. 7, overproduction of the natural form of RNase G restored CFA when introduced into an MG1655-derived  $\Delta rne$  strain plated on LB medium but failed to enable CFA when cells were plated on M9 containing any tested sugar except for the combination of glucose and malate (Fig. 7A). In contrast, His-tagged RNase G enabled CFA with 100% efficiency, not only on LB but also on M9 medium containing glucose, pyruvate, malate, fumarate, or succinate, indicating that the nutritional requirements for such complementation resemble those of bacteria complemented by full-length RNase E (Fig. 7C). However, relative to cells complemented by plasmid-encoded RNase E, CFA occurred in  $\Delta rne$  cells at a reduced efficiency for His-tagged RNase G complementation during growth on glycerol (46% CFE) or acetate

(37% CFE) (Fig. 7C). Complementation by the natural form of RNase G was not observed, even on LB, for the N3433-derived  $\Delta rne$  strain (Fig. 7B), which as noted above differs from MG1655 derivatives in carbon sources needed for CFA, suggesting that strain differences may account for the disparate results reported in investigations of complementation of RNase E deficiency by RNase G overexpression (6, 24, 27, 36, 37).

**Proposed mechanism for the role of the glycolytic pathway and TCA cycle in RNase E essentiality.** The results reported here indicate that the absence of RNase E in *E. coli* made viable by a second-site *Tn10* insertion mutation in *deaD* (26) results in defective production of PPC, which links glycolysis with the TCA cycle (Fig. 8) (31). We conclude that a deficiency of this enzyme results in the inability of cells to use glycolytic pathway carbon sources for the synthesis of amino acids generated by the TCA cycle. Consequently, whereas *rne deaD* mutant cells failed to grow solely on glucose or other glycolytic pathway intermediates, they formed colonies on minimal medium containing these sugars when the cellular connection between the glycolytic pathway and TCA cycle was enhanced by adventitious overexpression of a cloned *ppc* gene or when TCA cycle amino acids were added adventitiously. However, defective PPC synthesis is not the only nutritional consequence of RNase E deficiency, as cells overexpressing PPC re-





**FIG 8** Proposed model for RNase E effects on the glycolytic pathway and TCA cycle. PEP, phosphoenolpyruvate.

mained unable to form colonies when supplied with certain sugars. Moreover, even the combination of glucose and malate did not support CFA in *Δrne* cells that lack an additional mutation of *deaD*. Thus, RNase E essentiality is not attributable to a single gene or pathway.

Whereas the model we have proposed explains the bulk of the findings we have observed, some findings remain unexplained. (i) A Keio collection *E. coli* K-12 BW25113 strain deleted in *ppc* (JW3928-1) (38), as expected from the proposed model (see also reference 39), did not form colonies on M9 medium containing either glucose or pyruvate as a sole carbon source. However, *rne deaD* doubly mutant bacteria can form colonies on M9-pyruvate, as we reported previously (26). This finding is consistent with the conclusion that inactivation of PPC alone does not fully explain the modified metabolism observed in *E. coli* in the absence of RNase E. (ii) M9 minimal medium containing a CAA mixture as the sole carbon source did not confer CFA to *rne deaD* mutant cells, contrary to what was expected. As the combination of pyruvate (a glycolytic pathway intermediate) and malate (a TCA cycle intermediate) supported CFA (as shown in Fig. 2), all glycolytic pathway (and gluconeogenic pathway) steps upstream from pyruvate should in principle be active in the cultured *rne deaD* mutant cells. Thus, we anticipated that providing a mixture of glycolytic pathway-derived and TCA cycle-derived amino acids (i.e., CAA) would fulfill the nutritional requirements for cell growth. The basis for the absence of CFA by *rne deaD* mutant bacteria cultured on medium containing such a mixture has not been determined.

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