YjjG, a dUMP Phosphatase, Is Critical for Thymine Utilization by *Escherichia coli* K-12

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Exogenous thymine must be converted to thymidine to enable a *thyA* (thymidylate synthase) mutant to grow. The deoxyribose in the thymidine comes from dUMP, which must first be dephosphorylated. The nucleotidase YjjG is critical for this step. A *yjjG thyA* mutant cannot use thymine for growth on a glucose minimal medium.

Radio-labeled thymine is not efficiently incorporated into the DNA of wild-type *Escherichia coli* (reviewed in reference 7). Thymidine is better but only in the short term, before it is degraded by phosphorylase activity in the periplasm. However, thymine uptake may be greatly enhanced by supplying the cell with deoxyadenosine or by introducing a *thyA* (thymidylate synthase) mutation. The explanation is that in order for the thymine to be salvaged, it must have a source of deoxyribose-1-phosphate with which to condense. The resulting thymidine is then converted to TMP by thymidine kinase. Deoxyadenosine helps because it can produce deoxyribose-1-phosphate (plus adenine) through the action of purine nucleoside phosphorylase. In a *thyA* mutant, however, the source for deoxyribose-1-phosphate is dUMP, which accumulates in the cell because its conversion to TMP is blocked. dUMP must first be hydrolyzed to deoxyuridine, which is degraded by thymidine (deoxyuridine) phosphorylase to uracil and deoxyribose-1-phosphate (Fig. 1). The same enzyme then catalyzes the condensation of exogenous thymine and deoxyribose-1-phosphate to produce thymidine. In support of this mechanism, it was observed that the thymine requirement of *thyA* mutants was greatly reduced by the acquisition of mutations in *deoB* and *deoC*, which block the catabolism of deoxyribose-1-phosphate. However, the first step in this salvage pathway has been largely overlooked. The hydrolysis of dUMP to deoxyuridine is catalyzed by an unidentified enzyme.

*E. coli* has at least five enzymes with 5′ nucleotidase (nucleoside 5′ phosphomonoesterase) activity. The product of the *ushA* gene, which was originally designated a 5′ nucleotidase, was found to be primarily a UDP-sugar hydrolyase. However, it is located in the periplasmic space, where it would be inaccessible to phosphorylated cytoplasmic compounds, and it has a cytoplasmic inhibitor (15). Similarly, alkaline phosphatase (*phoA* gene) is located mainly in the periplasm, and it is both repressed and inhibited by levels of phosphate above growth-limiting conditions (17). A dUMP phosphatase was first described in 1973 (16). However, its gene was unknown and there were no available mutants, so its putative role in thymine salvage could not be tested. It had almost equal specificities for UMP, dUMP, and TMP. When tested with Mg2+ or Mn2+, it had an extraordinarily high *Kₘ* (−10 mM) for each of its three substrates. More recently, three other nucleotidases were discovered in a broad screen that used *p*-nitrophenylphosphate as a substrate (9). One, the YjjG protein, which was characterized in the form of a hexahistidine-tagged protein, had a substrate specificity that was similar to that of the earlier described dUMP phosphatase. Its activity on nucleotides was reported only for reactions in which Mn2+ but not Mg2+ was present, and under these conditions, it displayed much lower *Kₘ* (0.51 to 0.77 mM) than the earlier described enzyme, which was assayed with Mg2+. The enzymes are probably the same, and YjjG is a likely candidate for the dUMP nucleotidase of the thymine salvage pathway.

To see whether YjjG plays a significant role in thymine utilization, a *thyA* mutant and an isogenic *thyA yjjG* derivative were constructed (Table 1). LB medium (5) was used for routine growth. For *thyA* mutants, it was supplemented with thymidine at 125 µg/ml to prevent the emergence of low-thymine-requiring derivatives caused by spontaneous *deoB* or *deoC* mutations. Minimal media were enriched with thiamine and Norit-treated Casamino Acids as described previously (3). Tetracycline, chloramphenicol, and carbenicillin were used at 15 µg/ml, 20 µg/ml, and 100 µg/ml, respectively. Recombinant DNA and PCR methods were as described previously (13).

The *thyA::Tn10det* mutation was selected by trimethoprim resistance, a procedure that is highly specific for tight *thyA* mutants (1, 5). The mutant required more than 150 µM of thymine for optimum growth, thereby classifying it as a high-thymine-requiring mutant (7). The *yjjG* mutant lacked the first *92%* of the gene (Table 1). A sonicate of it had 50% of the wild-type dUMP phosphatase activity, which was measured as described previously (16). The residual activity is probably due to nonspecific phosphatases, as previously noted (16). The *thyA* and *yjjG* insertion mutations should not directly affect other genes. There are no large open reading frames that appear to overlap with those of *thyA* or *yjjG*, and the nearest downstream neighbors of *thyA* and *yjjG* are 184 nucleotides (nt) and 91 nt distant, respectively (11), so they are unlikely to be cotranscribed.

To test the possible role of *yjjG* in thymine salvage, the
FIG. 1. Thymine and thymidine salvage pathways, showing the putative role of yjjG. Arrows indicate the overall flow in a thyA mutant, which accumulates dUMP. Solid horizontal arrows portray the utilization of exogenous thymine and thymidine. The genes (and their corresponding products in parentheses) are thyA (thymidylate synthase), yjjG (a phosphomonoesterase for UMP, dUMP, and TMP), deoA (thymidine [deoxyuridine] phosphorylase), and tdk (thymidine [deoxyuridine] kinase). Chemical abbreviations: Pi, inorganic phosphate; dUrd, deoxyuridine; Ura, uracil; dRib-1-P, 2-deoxyribose-1-phosphate; Thy, thymine; and Thd, thymidine.

The growth requirements of a thyA and a thyA yjjG mutant were compared. If yjjG is needed to convert dUMP to deoxyuridine (Fig. 1), then the yjjG mutation should block the utilization of exogenous thymine by a thyA mutant. However, the double mutant should still be able to utilize thymidine for growth in a minimal medium. The growth requirements of the mutants were compared by growing them on a minimal agar medium supplemented with either thymine or thymidine (Fig. 2). The yjjG mutation blocked the utilization of thymine but not that of thymidine. Therefore, YjjG activity is essential in the salvage pathway.

The phenotype of the thyA yjjG double mutant BW1835 is identical to that of a thyA deoA mutant, which can use thymine but not thymine for growth because it lacks thymidine phosphorylase (7). However, BW1835 could grow on minimal media containing 5 mM thymidine as a sole carbon source, whereas strain BW934 (deoA) could not, indicating that thymidine phosphorylase is functional in BW1835.

To confirm that the phenotypic effect of the yjjG mutation was due to the yjjG gene itself, a complementation test was performed. A plasmid (pBAD28:yjjG) was constructed in which the yjjG gene was under the control of an arabinose-inducible, glucose-repressible promoter. When grown in LB medium containing 0.2% arabinose, the plasmid-bearing strain demonstrated an 11-fold increase in dUMPase specific activity over that of the wild-type strain or the glucose-repressed, plasmid-bearing strain. Strain BW1835 (thyA yjjG) containing the plasmid pBAD28:yjjG was spread on minimal agar containing thymine (100 μg/ml), carbenicillin (100 μg/ml), and either 0.2% glucose or 0.2% arabinose. Colonies were seen only on the plate containing arabinose, reaching a size of 2.73 ± 0.07 mm2 (mean ± standard error of the mean) after 24 h at 37°C. Thus, the inability of the thyA yjjG mutant to salvage thymine was complemented only when the yjjG gene of the plasmid was induced. Curiously, a control strain, BW1835 (pBAD28), which carried the vector without the yjjG gene while also not growing on the glucose plate, was able to produce tiny colonies (0.38 ±

### TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>Strains</td>
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<tr>
<td>BW934</td>
<td>Same as KL16 but with deoA22</td>
<td>3</td>
</tr>
<tr>
<td>BW1614</td>
<td>Same as KL16 but with thyA::Tn10det</td>
<td>This study</td>
</tr>
<tr>
<td>BW1670</td>
<td>λcI857 Δ(cro-bio) Δ(argD-lac)169 IN(mdR-mE) hsdR2 mdoB202::Tn10</td>
<td>P1(LCK8) × HME5</td>
</tr>
<tr>
<td>BW1670ΔyjjG strain</td>
<td>Same as BW1670 but with ΔyjjG::cat; replacement of nt 1 through nt 624 of yjjG (677 nt)</td>
<td>This study</td>
</tr>
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<td>BW1712</td>
<td>Same as KL16 but with ΔyjjG::cat</td>
<td>P1(BW1670 ΔyjjG) × KL16</td>
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<tr>
<td>BW1835</td>
<td>Same as KL16 but with thyA::Tn10det ΔyjjG::cat</td>
<td>P1(BW1712) × BW1614</td>
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<tr>
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<td>19</td>
</tr>
<tr>
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<td>CGSC</td>
</tr>
<tr>
<td>LCK8</td>
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<td>CGSC</td>
</tr>
<tr>
<td>Plasmids</td>
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<td>pBAD28</td>
<td>ori p15a, araC, arabinose P_BAD promoter, cat, bla</td>
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</tr>
<tr>
<td>pBAD28::yjjG</td>
<td>pBAD28 with SacI-XbaI segment replaced by PCR-amplified yjjG gene and synthetic ribosome binding site</td>
<td>This study</td>
</tr>
</tbody>
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All strains are derivatives of E. coli K-12 and are F− λ− unless stated otherwise. cat, tet, and bla are genes specifying resistance to chloramphenicol, tetracycline, and carbenicillin, respectively.

Transductions with phage P1 dam rev6 (14) are described as follows: P1( donor) × recipient. CGSC, E. coli Genetic Stock Center, Yale University, New Haven, CT (http://cgsc.biology.yale.edu).

Random mini-Tn10 insertions were produced from λ1048 as described previously (18), and a thymine-requiring mutant was selected by trimethoprim resistance (5).

The thyA::Tn10det mutation was then transduced into strain KL16 by selection for tetracycline resistance.

Tetracycline-resistant transductants were screened for the restriction-negative (HsdR−) phenotype by cross-streaking to test for resistance to phage kix on E. coli C and sensitivity to xin on E. coli K-12.

Transformation (19) of BW1670 was carried out with a PCR product of plasmid pKD3 (2). The primers used were 5′-TCATGGCCGTGCAATACGTATGTA ATACAAGTGGAAATGTTACGCGTGGAGCTGCTCTC-3′ and 5′-CCAGTTCGTCGCAAGAAGACGCTTCGAGTGGCCGCGCTATAGTATCCTATTG-3′.

Using strain KL16 DNA as a template, yjjG was amplified by PCR with Vent DNA polymerase (New England Biolabs) and the following two primer pairs: (i) 5′-GCTTCCAGAAGGAACTCACCATGTCATTGAGGAGCTGATTTC-3′ and 5′-CTAGATCGTACGTTACACGAGCGTGGCGTG-3′ and (ii) 5′-CAAGAACAAATCCATGAAAGTGGGACCTGATTTC-3′ and 5′-ATACGTTTGACACGAGCGTGGCGTG-3′. The PCR products were purified with a QIAquick PCR spin column (QIAGEN Corp.) and then combined, heat denatured, and reannealed so that one-fourth of the molecules had protruding tetranucleotide ends that could be ligated to pBAD28 that had been treated with SacI and XbaI.
0.01 mm²) in the presence of arabinose. It is possible, therefore, that there may be a minor second dUMP phosphatase that is subject to catabolite repression by glucose.

In *Escherichia coli*, the de novo synthesis of nucleic acid precursors occurs at the nucleotide level; the sugar residues are phosphorylated before the nucleobases are formed. However, salvage and catabolism occur at the nucleoside level and below (7). Therefore, a nucleotidase such as YjjG is at a critical branch point between synthesis and catabolism. It is opposed by thymidine (deoxyuridine) kinase. The balance between these two enzymatic reactions determines the overall metabolic flow. The two enzymes, which each catalyze an irreversible reaction, constitute a reversible pathway or substrate cycle.

A similar role has been postulated for nucleotidases in human cells. Human mitochondria contain dNT-2 (10, 12), a 5',3' nucleotidase which, like YjjG, is a phosphatase of the haloacid dehalogenase superfamily and is specific for uracil- and thymine-containing nucleotides. dNT-2 is an Mg²⁺-requiring enzyme with much lower *Kₘ*ₜₜ than those for YjjG. It was surmised that the mitochondrial enzyme participates with thymidine kinase in a cycle of concomitant phosphorylation and hydrolysis. This cycle should regulate the amount of nucleotides available for DNA synthesis and prevent a possibly toxic accumulation of TTP in resting cells (10).

Although thymidine kinase has been the subject of numerous studies, there have been only two on YjjG (9, 16). No evidence of its regulation was found (16), perhaps explaining why it failed to attract much interest. However, its high *Kₘ*ₜₜ may give us a clue as to how it operates in the pathway. From the results of growth yield experiments, we should expect Mg²⁺ to be 20 to 80 times more abundant than Mn²⁺ in the cell (8). In the presence of Mg²⁺, the enzyme has *Kₘ*ₜₜ for dUMP and TMP of about 10 mM (16), and even in the presence of Mn²⁺, its *Kₘ*ₜₜ are no lower than 0.5 mM (9). However, the concentration of dUMP is less than 0.01 mM in wild-type cells and about 0.5 mM in a *thyA* mutant (6). Therefore, in the cell, YjjG will remain mostly unsaturated, and its activity will be almost proportional to the concentration of its substrates. Thus, an enzyme with a high *Kₘ* has the same advantage as one that is genetically or allosterically regulated: the more the enzyme is needed, the more active it is. In a *thyA* mutant, which accumulates dUMP, the enzyme will be more active on dUMP than on TMP, thereby favoring a net flow from dUMP to TMP in the salvage pathway.

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I am grateful for the capable technical assistance of Carrie L. Flood.

REFERENCES


FIG. 2. The effect of a *yjjG* mutation on the utilization of thymine and thymidine by a *thyA* mutant. Cultures of BW1614 (*thyA* mutant) and BW1835 (*thyA yjjG* mutant) were plated on glucose minimal medium supplemented with various concentrations of (A) thymine or (B) thymidine and incubated for 24 h at 37°C. Each datum point represents the mean ± standard error of the mean for 25 to 50 uncrowded colonies.


