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NOTES

Characterization of *Escherichia coli* Strains with *gapA* and *gapB* Genes Deleted

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We obtained a series of *Escherichia coli* strains in which *gapA*, *gapB*, or both had been deleted. Δ*gapA* strains do not revert on glucose, while Δ*gapB* strains grow on glycerol or glucose. We showed that *gapB*-encoded protein is expressed but at a very low level. Together, these results confirm the essential role for *gapA* in glycolysis and show that *gapB* is dispensable for both glycolysis and the pyridoxal biosynthesis pathway.

Glycolysis is a common pathway for glucose breakdown in most living organisms. The phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) occupies a pivotal role in glycolysis, converting glyceraldehyde-3-phosphate into 1,3-biphosphoglycerate. The *gapA* gene of *Escherichia coli* was initially identified by mutations preventing cell growth on glucose (12, 13). No GAPDHase activity was observed in the resulting DF221 strain. However, this strain was shown to revert at high levels on glucose-containing media. Many of the revertants were reported to display a GAPDHase activity (12). *gapB*, a putative GAPDH gene, was identified by sequence identity with the bacterial GAPDHs. *gapB* is positioned within a gene cluster, where it is found immediately upstream of the *pgk* and *fda* glycolysis genes (1). This location is reminiscent of the transcriptional organization of glycolysis genes in other eubacteria, raising the question of whether this gene encodes a functional protein and plays a role in glycolysis. Recently, it has been proposed that *gapB*-encoded protein is involved in the biosynthesis pathway of pyridoxal phosphate (23).

We constructed plasmids pBS::EcogapA-Cm and pUGB-Erm to delete the chromosomal copies of the *gapA* and *gapB* genes, respectively, using *Escherichia coli* BJ5183 (Table 1), as previously described (14). Almost the entire coding regions of the genes, including the catalytic amino acids, have been deleted and replaced by an antibiotic resistance gene (Fig. 1C). In particular, the erythromycin resistance gene (Erm), which contains no transcriptional terminator, was used as a marker for *gapB* deletion. In order to obtain isogenic strains with deletions of either one or both of the *gapA* and *gapB* genes, the *gap* loci were transduced into two strains, MG1655 and W3110, respectively, which we now rename DS110 and DS120, respectively. Transduction was achieved with phage P1 as described by Miller (17) except that medium containing succinate and glycerol was used for deletion of *gapA*. Infection by phage P1 of the BJ5183 cells with *gapB* deleted (DS109) was quite efficient, as seen by the yield of phage (10^9 PFU/ml), compared with that of the Δ*gapA* strain (DS108), which could not be infected. We transformed the DS108 (Δ*gapA*) strain with plasmids overexpressing the GAPDH encoded either by the *E. coli gapA* gene (plasmid pBS::EcogapA) or the *Bacillus stearothermophilus gapA* gene (plasmid B1b) (6). Infection by phage P1 was restored in both transformed strains, and the latter was chosen for the phage P1 infection. One explanation for this result was a potential role of GAPDH, encoded by the *gapA* gene, for phage infection, which may suggest another biological function for the GAPDH. In this respect, it is worth noting that GAPDH has been found to associate with membranes of both gram-positive bacteria (19, 20) and eukaryotes (2, 9, 15, 21).

Strains DS113 and DS123, with deletions of both *gapA* and *gapB*, were obtained by two successive steps of P1-mediated transduction in strains DS110 and DS120. The *gap* locus from the deleted strains was checked by PCR (Fig. 1), confirming recombination at the *gapA* locus and deletion of the *gapA* gene in DS112, DS113, DS122, and DS123 strains. Likewise, DS123 and DS113 strains contained a disrupted *gapB* allele. This result implied that the *gapB* allele had been deleted in DS111 and DS121 strains, the parental strains for DS113 and DS123, respectively. The correlation between the antibiotic resistance and the modified *gap* alleles was confirmed by Southern blotting (Fig. 1B). Although the *gapB* gene lies upstream of the phosphoglycerate kinase (*pgk*) gene in the chromosome and its transcription was shown to affect the accumulation of the *pgk* mRNA (18), the specific phosphoglycerate kinase activity in the *gapB*-deleted strains was as high as that observed in the parental strain (1 μmol/min/OD_{280} unit in total extracts [OD_{280}, optical density at 280 nm]). Therefore, this result indicated that *gapB* inactivation by the erythromycin marker did not alter the expression of the *pgk* gene.

We measured the erythrose-4-phosphate dehydrogenase (E4PDHase) activity as previously described (4) on total extracts from DS110, DS111, DS112, and DS113 strains (Table 2). The DS111 strain (Δ*gapB*) displayed an E4PDHase activity which is half that determined for the parental DS110 strain, whereas the DS113 strain (Δ*gapA* Δ*gapB*) showed no detectable activity. Therefore, based only on these results, it was

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TABLE 1. *E. coli* strains and plasmids used

E. coli strain or plasmid	Genotypes and comments	Origin, source, or reference
Strains		
XL1-blue	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F′[*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15 Tn*10*(Tet^r)]	Stratagene (7)
BJ5183	F′ *endA sbcB recBC galK met strR thi-1 bioT hsdR*(r_K^- m_K^+) λ^-	11
DF221	K10, *gap-2*(Am)	12
DS108	BJ5183 Δ*gapA*::Cm	This work
DS109	BJ5183 Δ*gapB*::Erm	This work
DS110	MG1655 (K-12, F^- λ^-)	3
DS111	MG1655 Δ*gapB*::Erm	This work
DS112	MG1655 Δ*gapA*::Cm	This work
DS113	MG1655 Δ*gapA*::Cm Δ*gapB*::Erm	This work
DS120	W3110 [K-12, F^- λ^- IN(*rrnD-rrnE*)]	3
DS121	W3110 Δ*gapB*::Erm	This work
DS122	W3110 Δ*gapA*::Cm	This work
DS123	W3110 Δ*gapA*::Cm Δ*gapB*::Erm	This work
Plasmids		
pTZ18-Cm$^+$	Carries the Cm fragment which confers resistance to chloramphenicol	P. Marlière
pTZ-Erm3	Carries the Erm fragment which confers resistance to erythromycin	P. Marlière
pBS::EcogapA	Carries *gapA* gene with a 480-bp upstream region in BSIISK$^+$, harbors a *Nde*I site at the ATG initiation codon.	10
pBS::EcogapA-Cm	Derivative of pBS::EcogapA by replacement of 589 bp of *gapA* coding sequence by the Cm fragment	This work
pBS::EcogapB	Carries the *gapB* coding sequence under the control of the *gapA* promoter	This work
pUGB100	Carries *gapB* gene with a 236-bp upstream flanking region in pUC18	This work
pUGB-Erm	Derivative of pUGB100 by replacement of 416 bp of *gapB* coding sequence by the Erm fragment	This work
B1b	Contains the *B. stearothermophilus* GAPDH gene in pBR322 plasmid	6

difficult to conclude whether *gapB*-encoded protein is expressed in *E. coli*. So we set up an alternative procedure to copurify the *gapB*-encoded protein with an overexpressed inactive C149G *gapB*-encoded protein mutant (4), which have the same physicochemical properties. The purified protein showed an E4PDHase activity of 15 nmol/min/mg when isolated from the DS110 strain, while no activity (≤1 nmol/min/mg) was detected in the DS111 (Δ*gapB*) strain. A value of 360 molecules of tetramers per cell was calculated from the purification yield (4) for *gapB*-encoded protein in DS110 strain. Two explanations can be advanced to account for the E4PDHase activity detected in total extract of DS111 strain. First, commercially available erythrose 4-phosphate (E4P) contains about 6% glyceraldehyde-3-phosphate (4, 23). Second, GAPDH A from *E. coli* displays a weak E4PDHase activity (kcat of 0.2 s^{-1}) (5) compared to the *gapB*-encoded protein enzymatic activity (kcat of 20 s^{-1} with E4P as a substrate) (4). Conversely, *gapB*-encoded protein itself displays a weak GAPDHase activity, so the trace of GAPDHase activity observed in DS112 could be that activity.

To characterize the phenotypes of the strains with deletions of the *gapA* and *gapB* genes, we used M63 medium (17) supplemented only with succinate and glycerol. When plated on agar plates, Δ*gapA* strains (DS112 and DS113) grew smaller colonies than the parental DS110 strain, with a tendency to aggregate and undergo lysis, which was not observed with the DS110 or DS111 (Δ*gapB*) strain (data not shown). The same phenotype was observed with strains DS120 to DS123, suggesting that this effect was independent of the strain background. Aggregation and lysis were both prevented, however, by diluting cells in 0.9% NaCl prior to spreading them on agar plates. The colony sizes were measured under these conditions. There were marked differences in growth between the Δ*gapA* strain and the Δ*gapB* strain, the latter behaving like the parental DS110 (Table 3). In liquid cultures, strains with a deletion of *gapA* or of both *gapA* and *gapB* showed an OD_{600} at saturation which had decreased markedly from those of the parental and Δ*gapB* strains (data not shown). Therefore, based on our growth data, deletion of the *gapB* gene alone confers no detectable phenotype.

Strains with deletions of the *gapA* gene (DS112) or both *gapA* and *gapB* genes (DS113) could grow on M63 medium supplemented with a combination of succinate and glycerol, but not on glucose or glycerol alone and, to that extent, behaved like the DF221 strain described previously (12). These data indicated that endogenous *gapB*-encoded protein, which is produced at a low level and displays very low GAPDHase

TABLE 2. Specific activities of E4PDHase and GAPDHase in crude extracts of *E. coli* strains[a]

Strain	GAPDHase activity[b]		E4PDHase activity[c]
	With K_2HPO_4	Without K_2HPO_4	
DS110	690 ± 140	4 ± 2	14 ± 2
DS111 (Δ*gapB*)	710 ± 60	1.5 ± 0.7	7 ± 2
DS112 (Δ*gapA*)	2.7 ± 0.8	1.3 ± 0.9	5.2 ± 0.2
DS113 (Δ*gapA* Δ*gapB*)	ND[d]	ND	ND
DS110(pBS::EcogapB)	680 ± 50	3 ± 2	500 ± 10
DS112(pBS::EcogapB)	13 ± 2	4 ± 1	480 ± 60

[a] Specific activity values are the average values for three different cultures (36 h in M63 medium supplemented with succinate and glycerol) and are expressed in nanomoles per minute per OD_{280} unit. The *E. coli* strains transformed with the plasmid construct permitting the overexpression of *gapB*-encoded protein are DS110(pBS::EcogapB) and DS112(pBS::EcogapB).

[b] GAPDHase activity was determined at 25°C in 40 mM triethanolamine buffer containing 0.2 mM EDTA, pH 8.9, in the presence or absence of 50 mM K_2HPO_4.

[c] E4PDHase activity was determined at 25°C in 40 mM triethanolamine buffer containing 0.2 mM EDTA, pH 8.9, with commercial E4P as previously described (4).

[d] ND, not detectable (≤1 nmol/min/OD_{280} unit).

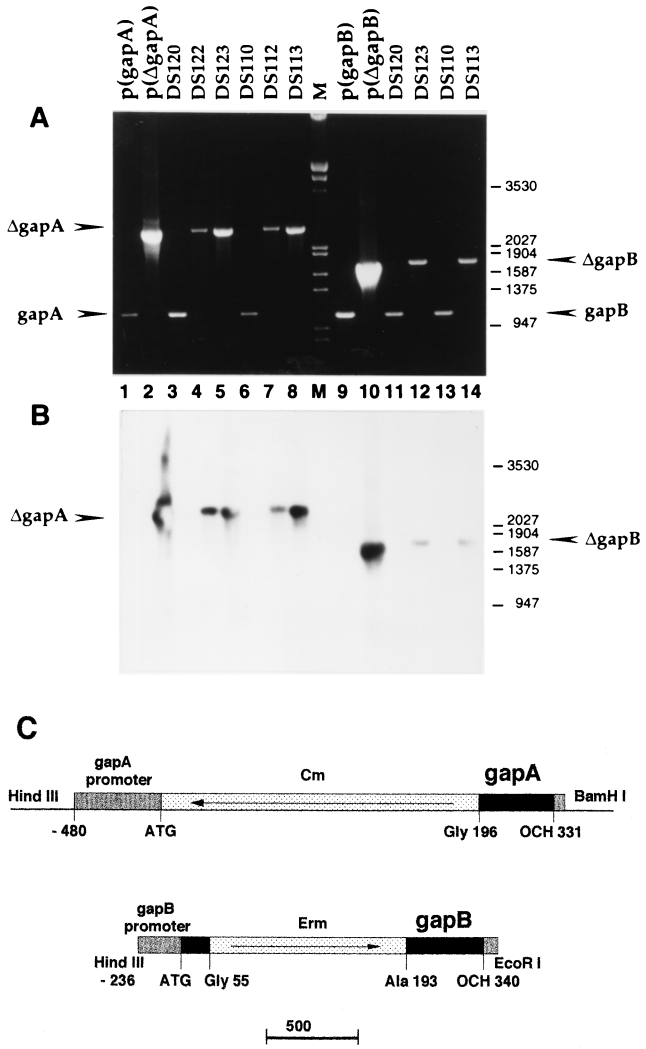

FIG. 1. Construction of the strains with deletions of *gapA*, *gapB*, or both. (A) PCR on the chromosomal DNA. Two sets of oligonucleotides (5′-CAGCTTTA GCAGCACC-3′/5′-ATATACGCCGTCACGC-3′ and 5′-TGGAATAAAGCTT CCCACAA-3′/5′-ACGTCGGATCCTGAATCCTCTCGTTGA-3′) that bind to the promoters and to the 3′ ends of the *gapA* and *gapB* genes, respectively, were used for PCR. The PCR products, obtained for the various strains or with the control plasmids, were separated on an agarose gel and stained with ethidium bromide. The bands corresponding to PCR products are indicated by arrowheads. (B) Hybridization of the PCR DNAs. The DNA fragments from the agarose gel shown in panel A were transferred to a filter and hybridized with a mixture of ^{32}P-labeled pTZ18-Cm$^+$ and pTZ-Erm3 plasmids. The autoradiograph is shown. The recognized bands are indicated by arrowheads. Amplification was done with *gapA* (lanes 1 to 8) and *gapB* (lanes 9 to 14) oligonucleotides. Lane 1, plasmid pBS::EcogapA; lane 2, plasmid pBS::EcogapA-Cm; lane 9, plasmid pUGB100; lane 10, plasmid pUGB-Erm; lane M, molecular weight markers. The relevant molecular weights of the markers are indicated to the right of the gels. (C) DNA constructs used for *gapA* and *gapB* deletions. The DNA fragments shown were used for the deletions of *gapA* and *gapB* genes of *E. coli*. The extents of the *gapA* deletion (from the ATG codon to glycine 196) and the *gapB* deletion (from glycine 55 to alanine 193) are indicated. DNA outside the *gapA* gene comes from the BSIISK$^+$ plasmid (thin line). The lengths of the promoters are indicated in nucleotides under the map. *gapA* and *gapB* coding sequences are indicated by the black bars, while *gapA* and *gapB* noncoding DNAs are indicated by heavily stippled bars. The DNA fragments encoding resistance to antibiotics (chloramphenicol [Cm] and erythromycin [Erm]) are indicated by lightly stippled bars. The orientations of the corresponding genes are indicated by arrows. Bar, 500 bp.

TABLE 3. Growth of *E. coli* strains on M63 minimal medium with different supplements

Strain	Colony diam (mm)[a]		
	Glucose	Glycerol	Succinate + glycerol
DS110	2.1 ± 0.3[b]	0.9 ± 0.3[c]	1.8 ± 0.3[b]
DS111 (Δ*gapB*)	2.0 ± 0.2[b]	0.8 ± 0.4[c]	1.6 ± 0.2[b]
DS112 (Δ*gapA*)	–[d]	–	0.8 ± 0.3[c]
DS113 (Δ*gapA* Δ*gapB*)	–	–	0.6 ± 0.2[c]

[a] Final isolates of the strains were tested for growth on supplemented M63 medium containing the appropriate antibiotics.
[b] Colony diameter after 48 h.
[c] Colony diameter after 72 h.
[d] –, no growth.

activity (4), was not sufficient to complement a *gapA* deletion and thus played no relevant role in glycolysis. Expression of *gapA* gene in the DS113 strain totally restored the ability of this strain to grow on glucose (data not shown), confirming the essential role of *gapA* for glycolysis. The Δ*gapA* (DS112) and Δ*gapA* Δ*gapB* (DS113) strains did not give rise to revertants on glucose, in contrast to the DF221 strain (12), indicating that the Δ*gapA* phenotype was not suppressed by mutations in *gapB* or by other genetic events.

When DS112 and DS113 strains were transformed by the *gapB* expression vector and streaked on glucose, no growth was observed. This correlated well with the low GAPDHase activity of *gapB*-encoded protein, i.e., kcat = 0.12 s^{-1} (4). Moreover, similar results were obtained when instead of *gapB*-encoded protein, GAPDH A mutants displaying a kcat of ≤0.2 s^{-1} were tested (data not shown). However, the accumulation of a large amount of *gapB*-encoded protein or GAPDH A mutants with a kcat of ≤0.2 s^{-1} (by growing a 36-h liquid culture on medium containing succinate and glycerol) allowed strains to grow on plates containing glucose. As a control, the overexpression, under similar conditions, of an inactive mutant of *gapB*-encoded protein (C149G) failed to complement Δ*gapA* strains on glucose (data not shown). This indicated that the GAPDHase activity of *gapB*-encoded protein can complement a Δ*gapA* strain, provided that *gapB*-encoded protein is first highly overexpressed.

Surprisingly, in contrast to *E. coli* strains with deletions of transketolase (22) and PdxB genes (16), the Δ*gapB* (DS111) strain is still capable of growing on glycerol- or glucose-containing medium (without vitamin B_6 supplement). Similar results were reported for a *Vibrio cholerae* strain with deletions of the *gapB* gene (8). A possible explanation is that GAPDH A, which displays a low E4PDHase activity (4), supplies the *gapB*-encoded protein activity. The fact that Δ*gapA* (DS112) and Δ*gapA* Δ*gapB* (DS113) strains can grow on succinate plus glycerol makes this hypothesis unlikely. Moreover, the involvement of another enzyme displaying an E4PDHase activity is also unlikely because total extracts from the strain DS113 were devoid of E4PDHase activity. These findings suggest that, although *gapB*-encoded protein could be involved in the cellular oxidation of erythrose-4-phosphate into 4-phosphoerythronate, it is not essential in the pyridoxal phosphate biosynthesis pathway. While it is clear that our strains are useful tools for investigating cellular functions of GAPDH proteins, additional work will be required to establish the relevant role of *gapB*-encoded protein in the metabolism of *E. coli.*

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