JOURNAL OF BACTERIOLOGY, Mar. 1989, p. 1524–1530
0021-9193/89/031524-07$02.00/0

Vol. 171, No. 3

Mutations in the *Escherichia coli fnr* and *tgt* Genes: Control of Molybdate Reductase Activity and the Cytochrome *d* Complex by *fnr*

BRUNO FREY, GERLINDE JÄNEL, UWE MICHELSEN, AND HELGA KERSTEN*

Institut für Biochemie der Universität Erlangen-Nürnberg, Fahrstrasse 17, D-8520 Erlangen, Federal Republic of Germany

Received 24 June 1988/Accepted 7 December 1988

In eubacteria, the tRNA transglycosylase (Tgt) in specific tRNAs exchanges a guanine in the anticodon for 7-aminomethyl-7-deazaguanine, which is finally converted to queuosine. The *tgt* gene of *Escherichia coli* has been mapped at 9 min on the genome, and mutant pairs containing an intact or mutated *tgt* allele were obtained after transduction of the *tgt* locus by P1 bacteriophages into a genetically defined *E. coli* strain (S. Noguchi, Y. Nishimura, Y. Hirota, and S. Nishimura, J. Biol. Chem. 257:6544–6550, 1982). These *tgt* mutants grew anerobically with fumarate as an electron acceptor, while nitrate or trimethylamine *N*-oxide could not be reduced. Furthermore, molybdate reductase activity was almost lacking and the characteristic absorption maxima, corresponding to cytochrome a_1 and the cytochrome *d* complex, were not detectable in low-temperature reduced-minus-oxidized difference spectra in anaerobically grown cells. Transduction of the mutated *tgt* locus into another *E. coli* recipient resulted in *tgt* mutants without anaerobic defects. Transformation of the original *tgt* mutants with an *fnr* gene-containing plasmid reversed the anaerobic defects. Clearly, the original *tgt* mutants harbor a second mutation, affecting the anaerobic regulator protein Fnr. The results suggest that *fnr* is involved in anaerobic control of components of the cytochrome *d* complex and of the redox system that transfers electrons to molybdate. F′ plasmids containing a fused *lacI-lacZ* gene with the nonsense codon UAG at different positions in the *lacI* part were transferred to *E. coli* strains with a mutated or nonmutated *tgt* locus but intact in *fnr*. A twofold increase in the frequency of incorrect readthrough of the UAG codon, dependent on the codon context, was observed in the *tgt* mutant and is suggested to be caused by a $tRNA^{Tyr}$ with G in place of queuosine.

Escherichia coli mutants with a defined genetic background were isolated, containing or lacking the tRNA guanine transglycosylase (Tgt), and consequently containing or lacking queuosine (Q), 7-(((4,5-*cis*-dihydroxy-2-cyclopentene-1-yl)-amino)-methyl)-7-deazaguanosine, in tRNA (31). The transglycosylase enzyme catalyzes (in $tRNAs_{GUN}$ specific for Asn, Asp, His, and Tyr) the exchange of the guanine residue in the first position of the anticodon for 7-aminomethyl-7-deazaguanine, a precursor of Q (32, 33). The cyclopentenediol moiety of Q is then synthesized at the level of tRNA and involves epoxy-Q which is finally converted to Q by a cobamide-dependent enzyme system (7).

Noguchi et al. (31) isolated *tgt* mutants by random screening from a collection of *Escherichia coli* K-12 mutants obtained by treatment with *N*-methyl-*N*′-nitrosoguanidine. tRNA was isolated from about 400 strains, and three mutants were found with respective tRNAs containing the anticodon GUN, where N is one of the canonical nucleosides. The defective gene, named *tgt*, was mapped at about 9 min on the *E. coli* chromosome, and the gene order was shown to be *phoB tgt tsx*. The *tgt* locus was transferred into *E. coli* ANLO5 (see Table 1) by P1 bacteriophage transduction. The resulting mutants JE7336 *tgt* and JE7337 *tgt*, in contrast to the tgt^+ strains JE7334 and JE7335, do not contain tRNA transglycosylase; rather, they accumulate free 7-aminomethyl-7-deazaguanine, the precursor of Q. No clear biological defect is observed in the *tgt* mutant; the only phenotypic change is a marked reduction of viability when the cells are kept under unsuitable conditions of growth. It is therefore concluded that the presence of Q in tRNA of *E. coli* is important for survival in the natural environment (31).

We have compared *E. coli* JE7334 tgt^+ with JE7336 *tgt* and JE7335 tgt^+ with JE7337 *tgt* and have found that the *tgt* mutants, in contrast to the tgt^+ strains, are unable to synthesize the subunits of nitrate reductase under anaerobic growth conditions. Furthermore, the typical absorption maxima of cytochrome a_1 and the cytochrome *d* complex are lacking in low-temperature reduced-minus-oxidized difference spectra of cytochromes in whole cells (16, 18).

In *E. coli*, nitrate reductase and the anaerobic enzyme fumarate reductase, and possibly also trimethylamine *N*-oxide (TMAO) reductase, are under the control of the anaerobic regulator protein Fnr (4, 22, 34). Nitrate reductase and TMAO reductase require a molybdopterin cofactor (8, 39). We have therefore asked whether the *tgt* mutation might affect, in addition to nitrate reductase, other Fnr-regulated or molybdopterin cofactor-dependent redox systems. In the course of these studies, we observed that the *tgt* mutants are unable to reduce molybdate when grown anaerobically with an excess of molybdate, as described by Campbell et al. (2); also, they cannot reduce TMAO. Surprisingly, the anaerobic defects in the *tgt* mutants, including molybdate reduction and the deficiency of cytochromes a_1 and *d* in the absorption spectra, can be reversed by transformation of these mutants with a plasmid carrying the *fnr* gene. From the results presented here, we conclude that the *tgt* mutants harbor a

* Corresponding author.

TABLE 1. List of strains

Strain	Genotype	Reference
ANL05	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC phoB lacZ*	31
JE7334	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC⁺ phoB⁺ lacZ tgt⁺*	31
JE7336[a]	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC⁺ phoB⁺ lacZ tgt*	31
JE7335	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC⁺ phoB⁺ lacZ⁺ tgt⁺*	31
JE7337[a]	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC⁺ phoB⁺ lacZ⁺ tgt*	31
JE7337A[b]	F⁻ *leu purE trp* T5ʳ *sup*(Am) *lys metE thi ara xyl rpsL proC⁺ phoB⁺ lacZ⁺ tgt fnr⁺ zcj*::Tn*10*	31
LCB261	F⁻ *thi thr leu lacY ana rpsL175 supE44 tonA zcj*::Tn*10 nirR22(fnr)*	34
LCB268	F⁺ *thi thr leu lacY ana rpsL175 supE44 tonA zcj*::Tn*10*	34
PL2024	F⁻ *gal trpA9761 iclR trpR rpsL*	22
JRG861a[b]	F⁻ *gal trpA9761 iclR trpR rpsL fnr-1*	22
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 thi deoC relA1 rpsL150*	3
GG4104[b]	F⁻ *araD139* Δ(*argF-lac*)*U169 thi deoC relA1 rpsL150 proC*::Tn*5*	3
SJ1502[b]	F⁻ *araD139* Δ(*argF-lac*)*U169 thi deoC relA1 rpsL150 proC⁺ tgt⁺*	3
SJ1505[b]	F⁻ *araD139* Δ(*argF-lac*)*U169 thi deoC relA1 rpsL150 proC⁺ tgt*	3
JP3123	F⁻ *aroL478*::Tn*10 purE355 tyrR366 lac-352 rpsL tsx?*	12
X103B	F⁻ Δ(*pro-lac*)*103 trp-49 rpsL150*	12
XF4[b]	F⁻ Δ(*pro-lac*)*103 trp-49 rpsL150 aroL478*::Tn*10 tgt*	12
XF7[b]	F⁻ Δ(*pro-lac*)*103 trp-49 rpsL150 aroL478*::Tn*10 tgt⁺*	12

[a] In this paper it is shown that strains JE7336 and JE7337 harbor a second mutation in the *fnr* gene. They are listed here as originally described in reference 33.

[b] Constructed during this work.

second mutation in the anaerobic regulator gene *fnr*. Apparently, the *fnr* gene controls the expression of proteins involved in molybdate reduction and in formation of the cytochrome *d* complex.

Eucaryotic $tRNA^{Tyr}$ and $tRNA^{His}$ with G in place of Q have been shown to read through the nonsense codon UAG or to exhibit altered codon usage, respectively (for reviews of the literature, see references 19 and 20 and H. Kersten and W. Kersten, *in* C. H. W. Gehrke and K. C. Kuo, ed., Chromatographic and Other Analytical Methods in Nucleic Acids Modification Research, in press). *E. coli* strains containing mutations in *tgt* but not in *fnr* were therefore constructed, and the influence of the *tgt* mutation on readthrough of the UAG codons in a different context was investigated.

MATERIALS AND METHODS

Bacterial strains and plasmids. The genotypes and origins of the *E. coli* strains used in these experiments are listed in Table 1. The *fnr* gene-carrying plasmid pGS24 (40) was kindly supplied by G. Unden, Institut für Mikrobiologie, Frankfurt/Main, Federal Republic of Germany. The plasmid contains the *fnr* gene, including its promoter region in a 1.65-kilobase *Bam*HI-*Hin*dIII fragment. The plasmid contains, at the 5′ end, part of a gene X of unknown function.

Growth conditions. *E. coli* strains were grown at 37°C in L broth (24) or in minimal salt medium (6) with amino acids (40 mg/liter), purines (20 mg/liter), and thiamine (5 mg/liter) and various carbon sources (see Tables 2, 3, and 4, footnotes). Aerobic, liquid cultures were grown by vigorous shaking in Erlenmeyer flasks. For anaerobic growth, the flasks were tightly closed after the air was replaced by nitrogen gas. For anaerobic growth on agar plates, a cylindric compression vessel was used, filled with nitrogen gas to atmospheric pressure. For assay of fumarate reductase activity, all strains were grown anaerobically under comparable conditions in minimal salt medium supplemented with 0.8% glycerol–50 mM sodium fumarate. F′ plasmids carrying *pro*⁺ and a fused *lacI-lacZ* gene with nonsense mutations in the *lacI* part were kindly supplied by G. Björk, the University of Umea, Sweden (1), and were originally constructed by Miller and Albertini (27). Newly constructed *E. coli* strains, *tgt*⁺ or *tgt*, used as recipients for F′ plasmids are derived from strain X103B (12) (Table 1).

Enzyme activities. For quantitative analysis of molybdate reductase activity, the *E. coli* cells were grown aerobically at 37°C overnight in L-broth medium. Cell density (A_{578}) whenever necessary was measured after appropriate dilution. Thereafter, the cells were centrifuged and washed in 0.9% NaCl solution. The washed cells were suspended in an assay mixture containing 1% glucose–2 mM KH_2PO_4–40 mM sodium molybdate–0.01% chloramphenicol–100 mM NH_4Ac (pH 5.7)–200 mM NaCl, exactly as described by Campbell et al. (2). After incubation for 24 h at 30°C in tightly closed Microfuge tubes, the cells were pelleted and the A_{820} of the supernatant was determined. Relative activities were defined as described previously (2) (see also Table 4, footnote *b*).

The TMAO reductase reduces TMAO under anaerobic conditions to the basic trimethylamine. The alkalinity of the reduction product was detected on dye indicator agar plates. The plates contained MacConkey agar, 0.4% glucose, and TMAO (5 g/liter) (5).

Fumarate reductase activity was determined in cell extracts at 24°C in open 3-ml cuvettes as described elsewhere (23) by measuring the reoxidation of reduced benzyl viologen at 570 nm. Cell extracts were prepared by sonication. Cellular debris was removed by centrifugation at 10,000 × *g* for 30 min (see also Table 3, footnote *b*). Nitrate reductase activity was measured as described previously (16) (see also Table 2, footnotes *a* and *b*).

Transduction and transformation. All transductions were performed by the method of Miller (26), and the transfor-

mations were performed by the method of Maniatis et al. (25).

Low-temperature difference spectra of cytochromes. Dithionite-reduced minus ferricyanide-oxidized cytochrome spectra were taken in whole cells at the temperature of liquid nitrogen (77 K) in an Aminco DW-2UV/VJS spectrometer as described previously (16).

Characterization of the *tgt* mutation. All *tgt* mutants (Table 1) contain tRNA species of the Q family with an unmodified guanine residue in place of queuine. The amounts of these unmodified tRNAs can be determined in bulk unfractionated tRNA. In this assay, the purified tRNA-guanine transglycosylase from *E. coli* exchanges a guanine residue at position 34 of respective tRNAs by [^{3}H]guanine. The tRNA transglycosylase was prepared from *E. coli* MRE600 (32); only the last two steps of purification were omitted. The crude enzyme preparation exchanges 55 to 60 pmol of [^{3}H]guanine per A_{260} unit of tRNA within 2 h in unfractionated tRNA from all *tgt* mutants listed in Table 1 (31; Kersten and Kersten, in press).

Codon context-dependent readthrough. The position of the anticodon and the surrounding nonsense codons in F′ plasmids are listed (see Table 5; see also reference 1). In the F′ plasmids (del-14), the *lacI* gene is fused to the *lacZ* gene and the nonsense codon UAG is present in different positions of the *lacI* gene (29). Readthrough in F′ plasmid-transformed Δ*lac tgt*$^+$ and *tgt* strains was tested by measuring β-galactosidase activity (27). The *E. coli* strains containing an F′ plasmid were grown in L broth or in minimal salt medium at 37°C to about 2×10^8 cells per ml. Duplicate samples were analyzed for β-galactosidase activity (26). The relative readthrough of UAG codons was then calculated by referring to the β-galactosidase activity of the corresponding strain, set at 100%, with the same chromosomal genetic background that harbors the parental *lacI-lacZ* fusion (del-14) but without a nonsense mutation.

RESULTS

Characterization of *tgt* mutants. *E. coli tgt*$^+$ JE7334 and JE7335 and the corresponding mutants JE7336 *tgt* and JE7337 *tgt* were characterized with respect to anaerobic pathways. Although the *tgt* mutants cannot grow anaerobically with glycerol as a carbon source and nitrate as an electron acceptor (16), they do grow when nitrate is replaced by fumarate. The *tgt* mutants are unable to reduce TMAO and exogenously added molybdate under the conditions described in Materials and Methods.

We have transduced the mutated *tgt* locus from strain JE7337 *tgt* by infecting strain GG4104, originally derived from *E. coli* MC4100, with P1 phages. A resultant new mutant pair, SJ1502 *tgt*$^+$ and SJ1505 *tgt* (Table 1), was identified by analysis of the respective tRNAs. In Q-deficient tRNAs, the G residue in position 34 can be replaced by [^{3}H]guanine by using purified tRNA transglycosylase from *E. coli*. Into bulk tRNA from strain SJ1502 *tgt*$^+$, less than 2 pmol of [^{3}H]guanine per A_{260} unit of tRNA was incorporated, and into tRNA from the mutant SJ1505 *tgt* 60 pmol of [^{3}H]guanine was incorporated. A value of 55 to 60 pmol/A_{260} unit of tRNA indicates that the tRNA is completely unmodified with respect to Q (Kersten and Kersten, in press). Surprisingly, the mutant SJ1505 *tgt* grew anaerobically on nitrate as an electron source and reduced TMAO and exogenously added molybdate under appropriate anaerobic conditions, as did the corresponding *tgt*$^+$ strain SJ1502. In a cross experiment, the nonmutated *tgt* allele from strain JE7334 was transduced into the mutant JE7337 *tgt*. According to analysis of the tRNAs, a number of transductants were found to be *tgt*$^+$ and still showed the anaerobic defects. These results suggested to us that the original mutants JE7336 *tgt* and JE7337 *tgt* harbor an additional mutation that is involved in the control of anaerobic pathways. (Results that confirm this conclusion are presented below.)

TABLE 2. Nitrate reductase activity in *E. coli fnr* and the original *tgt* mutant transformed with plasmid pGS24 carrying the *fnr* gene

Strain	Generation time (min)[a]	Cell density[b]	Amt of nitrite (mmol)[c]
PL2024	60	3.0	5.4
JRG861a *fnr* × pGS24	90	1.6	3.2
JE7335 *tgt*$^+$	60	2.1	6.0
JE7337 *tgt* × pGS24	110	1.0	3.4

[a] Cells were grown anaerobically in salt medium with 0.05 M potassium lactate as a carbon source and 0.05 M potassium nitrate as an electron acceptor. Generation times were calculated from growth curves, monitored at A_{578} in special closed tubes.

[b] At the indicated cell densities ($A_{578} \times 10^{-1}$), cells were pelleted.

[c] The amount of nitrite was determined by using 20 μl of the supernatant added to 1 ml of an aqueous solution of 0.1% (vol/vol) sulfanilic acid–0.006% (vol/vol) α-naphthylamine and measuring the A_{526} (see also reference 16).

Nitrate reductase and fumarate reductase activities. In *E. coli*, the product of the *fnr* gene codes for the anaerobic regulatory protein Fnr (40). This was originally identified by the pleiotropic effects of *fnr* mutations on fumarate and nitrate reduction (22). The gene *fnr* is suggested to be identical to the *nirA* or *nirR* genes, all located at 29.5 min on the *E. coli* linkage map (30). From *E. coli* PL2024, the corresponding mutant JRG861a *fnr* was constructed. The mutant does not grow under anaerobic respiration conditions. This strain was therefore used to test the ability of plasmid pGS24, carrying the *fnr* gene, to complement for the anaerobic defects. The inability of the *fnr* mutant to use nitrate as a terminal electron acceptor was reversed upon transformation with the plasmid. Nitrite production during growth was almost identical in strain PL2024 and the mutant JRG861a *fnr* after transformation with the plasmid. When the original mutant JE7337 *tgt* was transformed with the plasmid, nitrate reductase activity was restored. The growth rate in the transformants was significantly lower than in the parent strains PL2024 and JE7335 (Table 2).

Under conditions of fumarate respiration, the mutant JRG861a *fnr* did not grow. Upon transformation with plasmid pGS24, the capacity to use fumarate as an electron acceptor was restored. Fumarate reductase activity was increased about eightfold by complementation. Although the mutant JE7337 *tgt* grew on fumarate at almost the same rate as the corresponding *tgt*$^+$ strain, JE7335, fumarate reductase activity was decreased by nearly one-third. Upon transduction of an unmutated *fnr* locus by P1 phages into strain JE7337 *tgt* (resulting in JE7337A *tgt fnr*$^+$), fumarate reductase activity achieved nearly the original levels. Transformation of the mutant JE7337 *tgt* with plasmid pGS24 caused an almost eightfold increase in fumarate reductase activity (Table 3). These results support the view that the mutant JE7337 *tgt* contains an additional mutation in the *fnr* gene. This mutant is designated hereafter the original *tgt* mutant.

Molybdate reduction. A deficiency in the molybdopterin-dependent enzymes nitrate reductase and TMAO reductase might also be caused by a failure in the uptake of molybdate.

TABLE 3. Fumarate reductase activity in *E. coli fnr* and the original *tgt* mutant transformed with plasmid pGS24 carrying the *fnr* gene

Strain	Generation time (min)[a]	Cell density[b]	Fumarate reductase activity (mmol/min per mg of protein)[b]
PL2024	100	4.2	690
JRG861a *fnr* × pGS24	130	3.3	4,160
JE7335 *tgt*$^+$	90	3.9	848
JE7337 *tgt*	95	3.6	280
JE7337A *tgt fnr*$^+$	90	3.8	620
JE7337 *tgt* × pGS24	110	3.0	2,331

[a] Cells were grown anaerobically in salt medium with 0.05 M glycerol and 0.05 M fumarate. At the indicated densities ($A_{578} \times 10^{-1}$), an appropriate amount of cells was harvested and cell extracts were prepared.

[b] Fumarate reductase activity was monitored in open cuvettes by following the fumarate-dependent oxidation of reduced benzyl viologen (23), using cell extracts as an enzyme source with a defined amount of protein. (Further details are described in Materials and Methods).

This defect can be overcome by growth with an excess of molybdate (9). *E. coli* strains containing or lacking mutations in *fnr* or *tgt* were therefore grown anaerobically in L broth with 10 mM molybdate in the presence of glucose. Surprisingly, strain PL2024 (intact in *fnr*) and the *tgt*$^+$ strain JE7335 turned deep blue, whereas the mutant JRG861a *fnr* and the original mutant JE7337 *tgt* remained white. The blue reduction product can be measured in the supernatant of anaerobically grown cells at 820 nm (Table 4). The reduction product interferes, to a certain extent, with measurements of cell density at 578 nm and with reagents used for protein determination. Cells were therefore counted after appropriate dilution; the final yield of each strain was in the range of 5×10^9 cells per ml. Molybdate reductase activity can be determined exactly when bacteria are grown to the stationary phase in L broth. An equal amount of cells from each strain is then transferred to phosphate-buffered medium containing molybdate and chloramphenicol and is incubated anaerobically for 24 h. Molybdate reduction occurs only in the presence and not in the absence of glucose as described by Campbell (2). The mutant JRG861a *fnr* exhibited a

TABLE 4. Molybdate reductase activity in *E. coli fnr* and the original *tgt* mutant transformed with plasmid pGS24 carrying the *fnr* gene

Strain	A_{820} in supernatant[a]	Relative activity (A_{820}/A_{578})[b]
PL2024	0.66	0.88
JRG861a *fnr*	0.06	0.39
JRG861a *fnr* × pGS24	0.75	0.80
JE7335 *tgt*$^+$	0.63	0.46
JE7337 *tgt*	0.11	0.12
JE7337A *tgt fnr*$^+$	0.58	0.44
JE7337 *tgt* × pGS24	0.90	0.45

[a] The blue reduction product was measured in the supernatant of cells grown under strict anaerobic conditions (for details, see text).

[b] The enzyme system is present when *E. coli* is grown aerobically in L broth to the stationary phase. After growth, the cell density at 578 nm was determined, and the cells were pelleted and resuspended in the original volume of phosphate buffer containing glucose, sodium molybdate, and chloramphenicol. After 24 h of incubation at 30°C in closed tubes, molybdate reductase was determined. The relative activities were calculated by dividing the A_{820} by the corresponding cell yield (A_{578}). (Further details are described in Materials and Methods and elsewhere [2]).

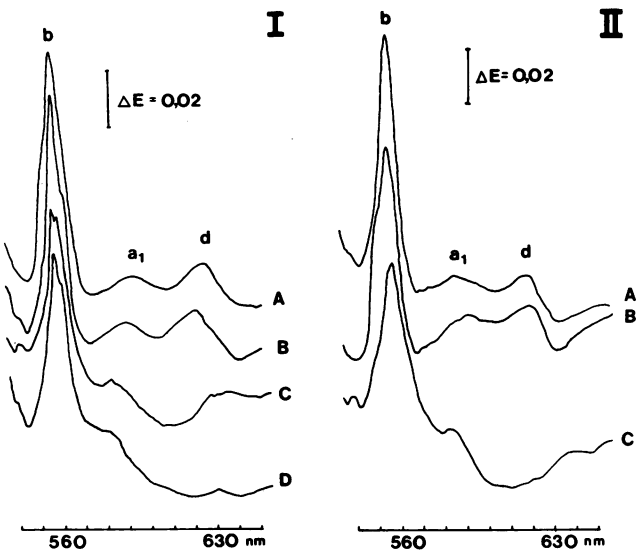

FIG. 1. Low-temperature, dithionite-reduced minus ferricyanide-oxidized difference spectra of cytochromes a_1, *b*, and *d* were measured in *E. coli* strains grown anaerobically in L broth to the stationary phase. (I) A, JE7335 *tgt*; B, LCB268; C, JE7337 *tgt* (this strain harbors the mutation in *fnr*); D, LCB261 *fnr*. (II) A, PL2024; B, JRG861a transformed with plasmid pGS24; C, JRG 861 *fnr*.

significantly lowered capacity to reduce phosphomolybdate compared with the parent strain PL2024. Transformation of the *fnr* mutant with plasmid pGS24 restored molybdate reductase activity. In the original mutant JE7337 *tgt*, molybdate reductase activity was considerably reduced. After transduction of the *fnr*$^+$ allele into this strain or after transformation of the original *tgt* mutant with plasmid pGS24, molybdate reductase activity went back to the level of the *tgt*$^+$ strain JE7335 (Table 4).

Low-temperature difference spectra of cytochromes. In *E. coli*, two enzyme complexes in the cytoplasmic membrane, cytochromes *o* and *d*, oxidize ubiquinone and directly reduce molecular oxygen to water. The cytochrome *d* complex has the highest affinity for O_2; it is formed preferentially when *E. coli* cells are grown under oxygen limitation or anaerobically on fumarate or in maximal media (for a review of the literature, see reference 13). In whole cells, absorption maxima are present in low-temperature, dithionite-reduced minus ferricyanide-oxidized difference spectra of cytochromes in the region of 560 nm and at 595 and 630 nm, corresponding to cytochromes *b*, a_1, and *d*, respectively (Fig. 1). Cytochrome *d* contains a chlorine hem and is synthesized coordinately with cytochromes a_1 and b_{558} (10, 11). Here we show that the trough of absorption in the region of the absorption maxima of a_1 and *d* cytochromes in the anaerobically grown original mutant JE7337 *tgt* is characteristic for a mutation in *fnr* (compare Fig. 1, IC and D and IIC). Changes virtually identical to those observed for the original *tgt* mutant were found in the low-temperature difference spectra of cytochromes of the mutants LCB261 *fnr* and JRG861a *fnr* (compare Fig. 1, IC and D). Upon transformation of these *fnr* mutants and the original mutant JE7337 *tgt* with the *fnr* gene-containing plasmid pGS24, the characteristic cytochrome a_1 and *d* absorption maxima occurred in the difference spectra of cells grown anaerobically in enriched medium (Fig. 1).

Influence of the *tgt* mutation on readthrough of the nonsense codon UAG. F′ plasmids were constructed by Miller and

TABLE 5. Readthrough of the UAG codon in various positions of the *lacI-lacZ* fusion strains containing (Tgt^+) or lacking (Tgt^-) tRNA transglycosylase

Medium	Position	Sequence	β-Galactosidase activity (%)[a]		
			Tgt^+	Tgt^-	Tgt^+/Tgt^- ratio
Enriched	84	AUU UAG UCU	2.0	2.0	1.0
	117	GCG UAG CGC	1.6	2.0	1.3
	181	CAG UAG AUC	1.6	1.6	1.0
	189	CCA UAG AGU	2.9	3.1	1.1
	220	GAC UAG AGU	3.1 (0.4)	6.6 (0.5)	2.1
	228	CAA UAG ACC	2.6	3.0	1.2
Glucose-salt	84	AUU UAG UCU	0.9	0.9	1.0
	117	GCG UAG CGC	0.7	1.0	1.4
	181	CAG UAG AUC	0.9	1.0	1.1
	189	CCA UAG AGU	2.5	3.6	1.4
	220	GAC UAG AGU	3.9 (0.5)	6.5 (0.5)	1.7
	228	CAA UAG ACC	1.0	1.2	1.2

[a] The β-galactosidase activity is relative to that of the corresponding F′ plasmid with the *lacI-lacZ* fusion but without a nonsense mutation, which was set at 100%. The activities were calculated as the mean value of two to four measurements (standard deviations for the relevant strains are given in parentheses). The β-galactosidase activities of the F′ plasmid del-14 for XF4 *tgt* and XF7 were 204 and 235 U, respectively, in enriched medium and were 180 and 226 U, respectively, in glucose-salt medium. (Further details are described in Materials and Methods and elsewhere [1, 27]).

Albertini (27) in which the *lacI* gene carrying a UAG codon at different positions has been fused in frame to the *lacZ* gene (27). The fusion strains serve as a system with which the influence of the tRNA modification in the anticodon on suppression of the nonsense codon UAG can be determined (1). The F′ plasmids were transferred to newly constructed *tgt* mutants. For this purpose, the *tgt* allele from strain JE7337 *tgt* was transduced by P1 phages into the recipient Δ*lac* strain X103B. According to the transglycosylase assays, the derived mutant Δ*lac* XF7 contains an unmutated *tgt* locus and the Δ*lac* XF4 contains the mutated *tgt* locus. β-Galactosidase activities were measured in F′ plasmid-transformed strains during growth. The observed activities were referred to those obtained from a *lacI-lacZ* fusion without a nonsense mutation (del-14). Thus, the β-galactosidase values represent a direct measure of the readthrough of the indicated UAG codons (1) (Table 5). A low but significant increase (about twofold) of incorrect readthrough of a UAG codon was observed in the *tgt* mutant in which the UAG codon is in position 220 and next to the codon GAC.

DISCUSSION

The *E. coli* mutants JE7336 *tgt* and JE7337 *tgt* and the corresponding tgt^+ strains JE7334 and JE7335 (31) have been used to study the function of tRNAs of the Q family with G or Q in the first position of the anticodon. Previous findings that the two *tgt* mutants, in contrast to the corresponding tgt^+ strains, are unable to synthesize the subunits of nitrate reductase when grown anaerobically were thought to be consequences of Q deficiency in specific tRNAs (16). Eucaryotic $tRNAs^{Tyr}$ with G in place of Q partially suppress the termination codon UAG in tobacco mosaic virus RNA during translation. In *Drosophila* sp., $tRNA^{His}$ with a G in place of Q prefers the His codon CAC over CAU (for a review of the literature, see E. Kubli, *in* C. W. Gehrke and K. C. Kuo, ed., Chromatographic and other Analytical Methods in Nucleic Acids Modification Research, in press). The absence of Q modification in specific tRNAs of eucaryotes is therefore suggested to be responsible for the observed distinct changes in the expression of lactate dehydrogenase enzymes and phosphoproteins (19, 20).

The observation that molybdopterin cofactor-dependent enzymes and molybdate reductase activity are lacking in the original mutants JE7337 *tgt* and JE7336 *tgt* led us first of all to suggest that the *tgt* gene is linked to another gene involved in the synthesis of the molybdopterin cofactor system. This hypothesis was based on the fact that pteridines and Q are derived from GTP and that the biosynthetic pathways share several common steps (15). The *tgt* mutants, however, harbor a second mutation in the *fnr* gene, localized in a different region, at 29.5 min on the chromosome. Interestingly, *chl* mutants with defects in the synthesis of the molybdopterin cofactor were also found to harbor a second mutation in the *fnr* gene (38).

In *E. coli*, fumarate reductase and nitrate reductase are under the positive control of the Fnr protein (22, 37). The original *tgt* mutants do grow on fumarate but not on nitrate; therefore, a mutation in *fnr* was not considered in our previous work (16). Further analysis of fumarate reductase reveals a significant decrease in activity of this anaerobic redox system. Possibly a partially active Fnr protein is produced which is still capable of stimulating the expression of the *frd* operon at a low rate, as in the case of another *frd* mutant (17).

During anaerobic growth in the presence of glucose, *E. coli* reduces exogenously added molybdate, but neither the redox chain involved nor its biological significance is known (2). Here we present data suggesting that the anaerobic redox system in question is under the control of the Fnr protein. Other anaerobic respiratory enzymes controlled by Fnr also reduce electron acceptors different from their actual substrates (4, 22, 30). Molybdenum cofactors can act as a coinducer of the *narC* operon and simultaneously as a corepressor of the *frd* operon (14). Molybdate-reducing enzyme systems might therefore modulate the functional properties of such molybdenum-containing cofactors, depending on the redox state of the cell.

In anaerobically grown *E. coli*, the mutation in *fnr* but not the mutation in *tgt* is correlated with the absence of absorption maxima of cytochrome a_1 and the cytochrome *d* complex in low-temperature reduced-minus-oxidized difference spectra. The function of cytochrome a_1 is still unknown; cytochrome a_1 is presently suggested to be identical to hemoprotein b-590 (36), implying that it is not part of a terminal oxidase. The cytochrome *d* complex serves as a terminal oxidase and exhibits a high affinity for molecular oxygen. Two subunits have been described for the cytochrome *d* complex (21, 28). Both subunits are encoded by the *cyd* operon at 16.5 min on the *E. coli* chromosome (10, 11). It has been suggested that the synthesis of the cytochrome *d* complex may depend not on the oxygen tension per se but on the ability of the cells to utilize oxygen (35). The cytochrome *d* complex possibly serves to oxidize traces of oxygen under almost anaerobic conditions. From the results presented here, it seems plausible that this redox system is responsible for the observed molybdate reductase activity.

A reexamination of consequences of the *tgt* mutation, using the *tgt* mutant with an intact *fnr* locus, confirms the previously published results (31) that the *tgt* mutants have significantly lower viability in resting cells (results not

shown). The disadvantage of the *tgt* mutants for survival under unfavorable conditions might be caused by an increase in the frequency of errors during translation that occur in a specific codon context, e.g., when two Q-deficient tRNAs are adjacent on the ribosome. We observe that incorrect readthrough of a UAG nonsense codon (1:10,000) in the *tgt lacI-lacZ* fusion mutants increased about 2-fold when a GAC codon was adjacent to the nonsense codon. GAC codes for $tRNA^{Asp}$; the nonsense codon UAG is occasionally recognized by $tRNA^{Tyr}$. Both tRNA species belong to the Q family and both contain G in place of Q in the *tgt* mutant.

ACKNOWLEDGMENTS

We thank A. Böck, Institut für Mikrobiologie, München, Federal Republic of Germany; M. Chippaux, Laboratoire de Chimie Bacterienne, Marseille, France; and G. Unden, Institut für Mikrobiologie, Frankfurt/Main, Federal Republic of Germany, for providing us with requested strains, phages, and plasmids and for helpful discussions. We thank Sonja Noeth for her excellent help with the manuscript.

This work was supported by Deutsche Forschungsgemeinschaft grant Ke 98/19-1 and by the Fond der Chemischen Industrie.

LITERATURE CITED

1. **Bouadloun, F., T. Srichaiyo, L. A. Isaksson, and G. R. Björk.** 1986. Influence of modification next to the anticodon in tRNA on codon context sensitivity of translational suppression and accuracy. J. Bacteriol. **166**:1022–1027.
2. **Campbell, A. M., A. Campillo-Campbell, and D. B. Villaret.** 1985. Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. Proc. Natl. Acad. Sci. USA **82**:227–231.
3. **Casabadan, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage. In vitro probe for transcriptional control signals. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
4. **Chippaux, M., D. Giudici, A. Abou-Jaoude, F. Casse, and M. C. Pascal.** 1978. A mutant leading to the total lack of nitrite reductase activity in *Escherichia coli* K12. Mol. Gen. Genet. **160**:225–229.
5. **Davidson, A. E., H. E. Fukumoto, C. E. Jackson, E. L. Barrett, and G. W. Chang.** 1979. Mutants of *Salmonella typhimurium* defective in the reduction of trimethylamine oxide. FEBS Lett. **6**:417–420.
6. **Fraenkel, D. G., and F. C. Neidhardt.** 1961. Use of chloramphenicol to study control of RNA synthesis in bacteria. Biochim. Biophys. Acta **53**:96–100.
7. **Frey, B., J. McCloskey, W. Kersten, and H. Kersten.** 1988. New function of vitamin B_{12}: cobamide-dependent reduction of epoxyqueuosine to queuosine in tRNAs of *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **170**:2078–2082.
8. **George, G., R. C. Bray, F. F. Morpeth, and D. H. Boxer.** 1985. Complex with halide and other anions of the molybdenum centre of nitrate reductase from *Escherichia coli*. Biochem. J. **227**:925–931.
9. **Glaser, J. H., and J. A. DeMoss.** 1971. Phenotypic restoration by molybdate of nitrate reductase activity in *chlD* mutants of *Escherichia coli*. J. Bacteriol. **108**:854–860.
10. **Green, G. N., and R. B. Gennis.** 1983. Isolation and characterization of an *Escherichia coli* mutant lacking cytochrome *d* terminal oxidase. J. Bacteriol. **154**:1269–1275.
11. **Green, G. N., J. E. Kranz, and R. B. Gennis.** 1986. Cloning the cyd locus coding for the cytochrome d complex of *E. coli*. Gene **32**:99–106.
12. **Grodzicker, T., and D. Zipser.** 1968. A mutation which creates a new site for the re-initiation of polypeptide synthesis in the Z gene of the lac operon of *Escherichia coli*. J. Mol. Biol. **38**:305–314.
13. **Ingledew, J. I., and R. K. Poole.** 1984. The respiratory chains of *Escherichia coli*. Microbiol. Rev. **38**:222–271.
14. **Iuchi, S., and E. C. C. Lin.** 1987. Molybdenum effector of fumarate reductase repression and nitrate reductase induction in *Escherichia coli*. J. Bacteriol. **169**:3720–3725.
15. **Jacobson, K. B., W. R. Farkas, and J. R. Katze.** 1981. Presence of queuine in *Drosophila melanogaster*: correlation of free pool with queuosine content of tRNA and effect of mutations in pteridine metabolism. Nucleic Acids Res. **9**:2351–2366.
16. **Jänel, G., U. Michelsen, S. Nishimura, and H. Kersten.** 1984. Queuosine modification in tRNA and expression of the nitrate reductase in *Escherichia coli*. EMBO J. **3**:1603–1608.
17. **Jones, H. M., and R. P. Gunsalus.** 1987. Regulation of *Escherichia coli* fumarate reductase (*frdABCD*) operon expression by respiratory electron acceptors and the *fnr* gene product. J. Bacteriol. **169**:3340–3349.
18. **Kersten, H.** 1984. On the biological significance of modified nucleosides in tRNA, p. 59–114. *In* K. Moldave and W. E. Cohn (ed.), Progress in nucleic acid research and molecular biology. Academic Press, Inc., New York.
19. **Kersten, H.** 1986. Anpassung des Zellstoffwechsels an Umweltveränderungen. Naturwissenschaften **73**:593–604.
20. **Kersten, H.** 1988. The nutrient factor queuine: biosynthesis, occurrence in transfer RNA and function. Biofactors **1**:27–29.
21. **Kita, K., K. Konishi, and Y. Anraku.** 1984. Terminal oxidases of Escherichia coli, aerobic respiratory chain. I. Purification and properties of cytochrome b_{562}-o complex from cells in the early exponential phase of aerobic growth. J. Biol. Chem. **259**: 3375–3384.
22. **Lambden, P. R., and J. R. Guest.** 1976. Mutants of *Escherichia coli* K12 unable to use fumarate as an anaerobic electron acceptor. J. Gen. Microbiol. **97**:145–160.
23. **Lemire, B. D., and J. H. Weiner.** 1986. Fumarate reductase of *Escherichia coli*. Methods Enzymol. **126**:377–386.
24. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage PI. Virology **1**:190–206.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Miller, J. H., and A. M. Albertini.** 1983. Effects of surrounding on the suppression of nonsense codons. J. Mol. Biol. **164**:59–71.
28. **Miller, J. H., and R. B. Gennis.** 1983. Purification and characterization of the cytochrome d terminal oxidase complex from *Escherichia coli*. J. Biol. Chem. **248**:9159–9165.
29. **Müller-Hill, B., and J. Kania.** 1974. Lac repressor can be fused to β-galactosidase. Nature (London) **249**:561–562.
30. **Newman, B. M., and J. A. Cole.** 1978. The chromosomal location and pleiotropic effects of mutations of the $nirA^+$ gene of *Escherichia coli* K12: the essential role of $nirA^+$ in nitrite reduction and in other anaerobic redox reactions. J. Gen. Microbiol. **106**:1–12.
31. **Noguchi, S., Y. Nishimura, Y. Hirota, and S. Nishimura.** 1982. Isolation and characterization of an *Escherichia coli* mutant lacking tRNA-guanine transglycosylase. J. Biol. Chem. **257**: 6544–6550.
32. **Okada, N., and S. Nishimura.** 1979. Isolation and characterization of a guanine insertion enzyme, a specific tRNA transglycosylase, from *Escherichia coli*. J. Biol. Chem. **254**:3061–3066.
33. **Okada, N., S. Noguchi, H. Kasai, N. Shindo-Okada, T. Ohgi, T. Goto, and S. Nishimura.** 1978. Novel mechanism of post-transcriptional modification of tRNA. J. Biol. Chem. **254**:3067–3073.
34. **Pascal, M.-C., J.-F. Burini, and M. Chippaux.** 1984. Regulation of the trimethylamine N-oxide (TMAO) reductase in *Escherichia coli*: analysis of tor::Mudl operon fusion. Mol. Gen. Genet. **195**:351–355.
35. **Poole, R. K., and B. Chance.** 1981. The reaction of cytochrome o in *Escherichia coli* K12 with oxygen. Evidence for a spectrally and kinetically distinct cytochrome o in cells from oxygen-limited cultures. J. Gen. Microbiol. **126**:277–287.
36. **Poole, R. K., and W. J. Ingledew.** 1987. Pathways of electrons to oxygen, p. 170–200. *In* F. C. Neidhardt, J. L. Ingraham, K. B.

Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society for Microbiology, Washington, D.C.

37. **Shaw, D. J., and J. R. Guest.** 1982. Nucleotide sequence of the fnr gene and primary structure of the Fnr protein of *Escherichia coli*. Nucleic Acids Res. **10**:6119–6130.
38. **Stewart, V., and C. H. MacGregor.** 1982. Nitrate reductase in *Escherichia coli* K-12: involvement of *chlC*, *chlE*, and *chlG* loci. J. Bacteriol. **151**:788–799.
39. **Takagi, M., T. Tsuchiya, and M. Ishimoto.** 1981. Proton translocation coupled to trimethylamine *N*-oxide reduction in anaerobically grown *Escherichia coli*. J. Bacteriol. **148**:762–768.
40. **Unden, G., and J. R. Guest.** 1985. Isolation and characterization of the Fnr protein, the transcriptional regulator of anaerobic electron transport in *Escherichia coli*. Eur. J. Biochem. **146**: 193–199.