In Vivo Transcription of nrdAB Operon and of grxA and fpg Genes Is Triggered in Escherichia coli Lacking both Thioredoxin and Glutaredoxin 1 or Thioredoxin and Glutathione, Respectively*

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We have previously described (1) that Escherichia coli maintains a balanced supply of deoxyribonucleotides by a regulatory mechanism that up-regulates the levels of ribonucleotide reductase with the lack of its main hydrogen donors thioredoxin, glutaredoxin 1, and glutathione (GSH). By using a semi-quantitative reverse transcription/multiplex polymerase chain reaction fluorescent procedure that enables simultaneous analysis of up to seven mRNA species, we now demonstrate that regulation operates at the transcriptional level. Double mutant cells lacking both thioredoxin and glutaredoxin 1 had increased transcription of the nrdAB operon, as compared with the corresponding wild type parent (maximal induction of 10- and 9-fold for mRNA of nrdA and nrdB genes, respectively). Likewise, a dramatic increase of 36-fold in grxA mRNA was observed in bacteria simultaneously deficient in thioredoxin and GSH (the physiological reductant of all glutaredoxins). The increased expression of the grxA gene in trxA gshA double mutant bacteria was mimicked in trxA single mutant cells by depletion of GSH with diethylmaleate (DEM). This induction of grxA transcription was rapid since maximal increase was detected upon 10 min of DEM exposure. Like grxA expression, the basal level of fpg mRNA, encoding formamidopyrimidine-DNA glycosylase, was increased (about 4-fold) in a trxA gshA double mutant strain; this expression was also induced upon exposure to DEM (11-fold maximal induction). These results suggest that transcription of grxA might share common redox regulatory mechanism(s) with that of the fpg gene, involved in the repair of 8-oxoguanine in DNA.

Deoxyribonucleotides required for DNA synthesis are formed de novo by the enzyme ribonucleotide reductase (RRase)1 (2).

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1 The abbreviations used are: RRase, ribonucleotide reductase; NrdAB, RRase coded for by the nrdAB operon; NrdEF, RRase coded for by the nrdEF operon; Trx, thioredoxin; Grx, glutaredoxin; GSH, reduced glutathione; NrdH, protein coded for by the nrdH gene; RT/ MPCR, reverse transcription/multiplex polymerase chain reaction; PCR, polymerase chain reaction; DEM, diethylmaleate; GAPDH, 

RRases, which catalyze the reduction of ribonucleotides to deoxyribonucleotides, are divided into three main classes according to the mechanism employed to generate the free radical required for catalysis (3). Class I RRases are aerobic enzymes present in all higher organisms and in Escherichia coli. This bacterium actually contains the genetic information for two different class I RRases. One of them (called NrdAB and coded for by the nrdAB operon) is essential for growth in the presence of oxygen, whereas the other (NrdEF, encoded by the separate nrdEF operon) is normally not fully functional (4). No class II RRase has been found in E. coli, and class III enzymes operate only in anaerobiosis (5).

The electrons for ribonucleotide reduction are supplied by thioredoxin (Trx) or glutaredoxin (Grx) in the case of class I RRase (6, 7). In the reduced form, both Trx and Grx contain two redox-active cysteine thiols, which by dithiol-disulfide interconversion reduce an acceptor disulfide in the active center of RRase. Reduced Trx is regenerated by thioredoxin reductase and NADPH, whereas oxidized Grx is reduced by two reduced glutathione (GSH) molecules with the formation of glutathione disulfide. The reduction of glutathione disulfide is catalyzed by glutathione reductase and NADPH. E. coli mutants defective in Trx, Grx, or glutathione reductase and those defective in GST biosynthesis have been named trxA, grx, gor, and gsh, respectively (8–11).

Apart from the first isolated glutaredoxin (Grx1 coded for by the grxA gene), E. coli contains two other glutaredoxins (called Grx2 and Grx3) (12). Like Grx1, both Grx2 and Grx3 show high activity as general GSH-disulfide oxidoreductases. Nevertheless, Grx3 is an inefficient hydrogen donor for RRase in comparison with Grx1 (about 5% of the catalytic activity of Grx1), whereas Grx2 lacks such activity (12). Recently, a glutaredoxin-like protein (called NrdH) with thioredoxin-like activity profile has been isolated from E. coli (13). NrdH is a functional hydrogen donor for RRase with higher specificity for the NrdEF than for the NrdAB enzyme. The physiological function of NrdH in E. coli is not well understood, since the nrdH gene is part of the poorly transcribed nrdEF operon (4).

We have recently proposed that, apart from an assorted set of hydrogen donors and RRase activities, E. coli maintains a balanced supply of deoxyribonucleotides by a regulatory network that compensates the RRase, Trx, Grx1, and GSH levels (1). Of particular relevance is the large increase in ribonucleotide reductase activity (from 19- to 23-fold) displayed by E. coli strains defective in both Trx and Grx1 (the two main hydrogen donors) (14) and the extremely high Grx1 content (55-fold) of △glyceraldehyde-3-phosphate dehydrogenase; Fpg, formamidopyrimidine-DNA glycosylase.
bacteria simultaneously lacking Trx and GSH (the physiological hydrogen donor of all glutaredoxins) (1). This study investigates whether that proposed balanced network is regulated at the transcriptional level. To this end, we have designed and optimized a semi-quantitative reverse transcription/multiplex polymerase chain reaction procedure (RT/MPCR) to simultaneously detect and quantify the expression level of up to seven different genes. The assay is based on competitive primer extension reactions using specific fluorophore-labeled primers and the subsequent DNA sequence analysis of PCR products.

**MATERIALS AND METHODS**

**Chemicals**—Phenol-saturated II, acrylamide/bis (19/1 mixture), and Tris-buffered EDTA were from Amresco (Solon, OH). GeneAmp RNA PCR kit, Prism Genescan-350 Tamra ladder, fluorescent-labeled primers, and dNTPs were from Perkin-Elmer (Norwalk, CT). DNase I (RNase-free) was from Boehringer Mannheim. TaqPlus-long was from TaqPlus-long was from Stratagene (La Jolla, CA). MPCR buffer 3 was from Maxim Biotech (San Francisco, CA). Diethylmaleate (DEM) and other chemicals were purchased from Sigma. DEM was dissolved in dimethyl sulfoxide from Merck.

**Bacterial Strains**—All bacterial strains were Escherichia coli K-12 and have been previously described (1, 14). UC5710 (arg, gapA, araD81, Δ(uvr-B-bio)) was considered the parental wild type. UC844 (ΔtrxA), UC858 (gsha::Tn10kan), UC827 (ΔtrxA, gsha::kan, zbi::Tn10), and UC859 (ΔtrxA, gsha::Tn10kan) were derivatives defective in Trx, GSH, Trx and Grx1, or Trx and GSH, respectively.

**Media**—The Luria-Bertani (LB) nutrient broth and the M9 minimal medium were prepared as described (15). The media were supplemented with kanamycin (50 μg/ml) and ampicillin (5 μg/ml). The minimal medium contained arginine (40 μg/ml), n-biotin (5 μg/ml), thiamine (5 μg/ml), glucose (2 g/liter), and casamino acids (2 g/liter). Samples were denatured at 94 °C, 15 s of annealing at 70 °C, and 30 s for enzymatic primer extension at 72 °C. The multiplex PCR amplification was performed in a mixture containing 1.5 units of TaqPlus-long, 2.5 μl of PCR buffer 3, 1.25 μl of each primer, and 2.5 μl of DNA. Products were analyzed by agarose gel electrophoresis. The fluorescence signals were quantified by using a Bio-Rad Gel Doc System. The calculated fluorescence intensities were compared with the fluorescence signal of the corresponding gene in the cell-free samples.

**Polymerase Chain Reaction**—After amplification, 2 μl of the multiplex PCR product was mixed with 0.5 μl of Primer Genescan-350 Tamra ladder, 2.5 μl of deionized formamide, and 0.5 μl of loading buffer. Samples were denatured at 95 °C for 2 min and run on a 6% polyacrylamide gel at 800 V in an ABI 373A Stretch Sequencer from Applied Biosystems (Foster City, CA). Samples produced bands of correct size for the primers used. Twenty-five cycles were run for each PCR reaction. Similar profiles were observed with the rest of the genes. Based on the results, the amount of cDNA was adjusted in the multiplexed PCR reactions reported herein to produce a fluorescence intensity in the range of linearity.

**Transcriptional Regulation, a Function of Trx, Grx1, and GSH**

**Reverse Transcription/Multiplex PCR**—Synthesis of cDNA was carried out with the GeneAmp RNA PCR kit. In short, RNA (1 μg) was retrotranscribed for 15 min at 42 °C with 2.5 units of murine leukemia virus reverse transcriptase, using random hexamers. The enzyme was inactivated by heating for 5 min at 99 °C. Each RNA sample was retrotranscribed on an average of three separate occasions. PCR amplification of cDNA was carried out using the primer pair sets listed in Table I. Primers were chosen to have high Tm and optimal ΔG for the three pentamers (18) in order to obtain the highest specificity and performance in multiplexed PCR reactions. Primers were designed with the Primer Select 3.0/96 (DNA Star, Madison, WI) and Oligo 5.0/96 (National Biosciences, Plymouth, MN) programs. PCR conditions were optimized so that only the desired products were produced. Twenty-seven cycles of PCR were performed. Each cycle consisted of 1 min of denaturation at 94 °C, 15 s of annealing at 70 °C, and 30 s for enzymatic primer extension at 72 °C. The multiplex PCR amplification was performed in a mixture containing 1.5 units of TaqPlus-long, 2.5 μl of PCR buffer 3, 1.25 μl of each primer, and the following amounts of primers: 3 pmol (gor), 1.25 pmol (grxA), 1.25 pmol (trxA), 2.75 pmol (nrdA), 3 pmol (nrdB), 2.5 pmol (gapA), and 2 pmol (guaA) in a final volume of 25 μl. The relationship between the fluorescence signal of PCR products and the input DNA target concentrations was investigated for each individual gene. Serial dilutions of genomic DNA from wild type bacteria were used as a target for the PCR step. Individual PCR amplifications were carried out under the standard conditions fixed for multiplexed PCR reactions. As exemplified in Fig. 1 for the nrdA, grxA, and trxA genes, there was a direct linear relationship between the fluorescence intensity and the number of target DNA molecules in the range from 102 to 106 molecules (coefficient of correlation r ≥ 0.98). Over 106 DNA molecules, the fluorescence signal was less intense than expected from the linear relationship; below 102 molecules, an insufficient signal was obtained with the number of PCR molecules, an insufficient signal was obtained with the number of PCR cycles used (27 cycles). Similar profiles were observed with the rest of the genes. Based on these results, the amount of cDNA was adjusted in the multiplexed PCR reactions reported herein to produce a fluorescence intensity in the range of linearity.

**Multiplex PCR Products Quantification**—After amplification, 2 μl of the multiplex PCR product was mixed with 0.5 μl of Primer Genescan-350 Tamra ladder, 2.5 μl of deionized formamide, and 0.5 μl of loading buffer. Samples were denatured at 95 °C for 2 min and run on a 6% polyacrylamide gel at 800 V in an ABI 373A Stretch Sequencer from Applied Biosystems (Foster City, CA). Samples produced bands of correct size for the primers used. Data were collected and analyzed with the ABI Collection 1.1/96 and ABI Analysis 2.1/97 software programs, respectively (Perkin-Elmer/Applied Biosystems). Fig. 2 shows a representative electropherogram pattern of RT/MPCR products from wild type cDNA samples and the corresponding gel image. Differences in amplification efficiencies among samples were normalized by referring the fluorescence intensity of each band to that resulting from gpaA amplification (used as reference gene, unless otherwise indicated). Significant variations in the ratios indicate relative differences in the initial miRNA levels among the bacterial strains or experimental conditions being compared. Samples from different bacterial strains were handled in parallel. Data are presented as mean ± S.E. from n
independent multiplexed PCR amplifications. Comparison between groups was done by a student’s t-test. Significances at the level of $p < 0.001$ are indicated in the text.

RESULTS

Gene Expression in trxA grxA and trxA gshA Double Mutant Strains of E. coli—To compare the levels of mRNA coding for glutathione reductase (gor), Grx1 (grxA), Trx (trxA), and NrdAB ribonucleotide reductase (nrdA and nrdB) from different E. coli strains, we designed and optimized a RT/MPCR procedure in which fluorescent PCR products were separated on acrylamide gels using an ABI 373A Stretch Sequencer and analyzed with the Genescan software.

d-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme of the glycolytic and gluconeogenesis pathways. In
E. coli, GAPDH is encoded by the household gapA gene, which is controlled by a very efficient multiple promoter system to allow the production of large amounts of gapA transcripts in a wide variety of environmental conditions (19). GAPDH activity is determined as previously described (21). The relative values compared with those of wild type are indicated in parentheses. The protein levels (ng/mg of protein) of Grx1 and Trx and the enzymatic activities of RRase (milliunits/mg of protein) and Grx1 (milliunits/mg of protein) are included for comparison with mRNA data. The RRase activity value is indicated for each gene.

**Gene Expression under Different Growth Conditions**—To test whether the differences in gene expression between the mutant strains UC827 and UC859 and the wild type UC5710 showed variations along the exponential phase or with the growth medium, bacteria were grown in LB nutrient broth until the cell density reached an OD at 600 nm of 0.7. Under these growth conditions, the levels of both nrdA and nrdB mRNA in UC827 were 5.3- and 3.5-fold higher, respectively, than those from the wild type. Likewise, a 36.2-fold increase in the amount of grxA transcript was observed in the mutant strain UC859. As expected, bacteria carrying the null ΔtrxA and grxA::kan mutations had undetectable levels of the corresponding mRNA. Other increments reported in Table II were not significant at the \( p < 0.001 \) level, except the small increase (1.9-fold) in the amount of gor transcript detected in UC827. The enhanced expression of nrdAB operon in UC827 and of grxA gene in UC859 agrees with the increments in RRase activity and Grx1 protein level previously reported for these mutant strains under identical growth conditions (1), thus indicating a regulation at the transcriptional level.

**Table II**

| Gene | Protein UC5710 (wt) | mRNA UC5710 (wt) | Protein UC827 (trxA grxA) | mRNA UC827 (trxA grxA) | Protein UC859 (trxA gshA) | mRNA UC859 (trxA gshA) |
|------|-------------------|-----------------|--------------------------|------------------------|--------------------------|------------------------|
| gor  | 133 ± 14          | 0.50 ± 0.03     | 142 ± 9                  | 0.97 ± 0.16*           | 176 ± 20                 | 0.72 ± 0.09            |
| grxA | 129 ± 15          | 0.54 ± 0.03     | <0.96                    | (1.1)                  | 7093 ± 558              | 19.57 ± 1.94*          |
| trxA | 1911 ± 142        | 0.31 ± 0.01     | <0.54                    | (1.1)                  | 54.9                    | (36.2)                 |
| nrdA | 0.25 ± 0.10       | 0.48 ± 0.07     | 5.77 ± 0.47              | 2.54 ± 0.32*           | 0.97 ± 0.48              | 1.03 ± 0.20            |
| nrdB | 0.25 ± 0.10       | 2.01 ± 0.28     | 5.77 ± 0.47              | 6.95 ± 0.89*           | 0.97 ± 0.48              | 2.55 ± 0.39            |

**Fig. 3.** Gene expression under different growth conditions of trxA grxA and trxA gshA mutant strains of E. coli. Cells were grown in LB broth or M9 minimal medium to reach an OD\( _{600} \) of 0.4 and/or 0.7. The fluorescence signal of each PCR product was referred to that of gapA. Data were from an average of nine independent multiplexed PCR amplifications. S.E. values did not exceed 15% of the mean. The relative values compared with those of wild type were plotted for the different genes. Statistical significance \( p < 0.001 \) for comparisons with wild type are marked with an asterisk.

**Gene Expression Induction by Diethylmaleate**—DEM is an electrophilic compound that conjugates with GSH in a reaction catalyzed by glutathione S-transferase (22), an activity that is found in E. coli although at 2 orders of magnitude lower than in...
cytosolic fractions of rat liver (23). DEM is considered a very effective agent for in vivo glutathione depletion in eukaryotic cells (24).

The effects of GSH depletion by DEM on gene expression are summarized in Fig. 4. Bacteria defective in both Trx and GSH (UC859) and the corresponding single defective strains (UC858 and UC844) were used in comparison with the parental wild type (UC5710). Treatments with 30 mM DEM were for 10, 30, or 60 min. GSH depletion efficiency was tested by measuring the intracellular GSH contents, as previously reported (1). DEM decreased the bacterial GSH content to about 13% after a 60-min exposure (Fig. 4). Nevertheless, in all cases, the increase in the amounts of grxA transcripts was higher than that of fpg transcripts (e.g. 37 vs 11-fold for maximal DEM induction levels). In contrast to grxA and fpg, the amounts of the trxA, nrdA, and nrdB transcripts remained basically unchanged with or without DEM treatment.

DISCUSSION

In this paper we demonstrate that the previously proposed regulatory network, in which the ribonucleotide reductase activity is balanced with the levels of its hydrogen donor systems (1), operates at the transcriptional level. Regulation of the E. coli nrdAB operon is a complicated regulatory sequence that includes Fis (factor for inversion stimulation) and DnaA (DNA replication initiation protein) binding sites (29) and an AT-rich sequence (30). The nrdAB operon could be activated by both Trx and GSH (UC859), carrying the null ghaA::Tn10kan allele that blocks the first step in the GSH biosynthetic pathway showed undetectable GSH levels as expected (1). DEM treatments decreased dramatically the amounts of gapA transcripts in all bacterial strains, making it difficult to use this as a reference mRNA. Therefore, variations in amplification efficiencies among samples were normalized in these experiments by referring the signals of each individual gene to that of gor. Fig. 4 shows the ratios of the values from DEM-treated cultures divided by those from the corresponding untreated controls.

In wild type cells (UC5710), DEM had no effect on gene expression after 10 min of treatment, but a moderate increase of approximately 4-fold and a large increment of 30-fold was observed in grxA expression after 30 and 60 min of treatment, respectively. Similar increases in grxA expression were observed in the GSH-defective bacterial strain (UC858). On the other hand, DEM rapidly triggered the grxA expression in bacteria lacking Trx (UC844); induction ratios from 11- to 15-fold were observed from the first 10 min of treatment. However, no significant increments were observed in bacteria lacking both Trx and GSH (UC859), probably due to the high steady-state level of grxA expression (16-fold increase as compared with wild type) in UC859 (Fig. 5).

The impairment of genetic integrity induced by 8-oxoguanine, the major oxidative product of DNA guanine, is prevented by the formamidopyrimidine-DNA glycosylase (Fpg), coded for the E. coli fpg gene (25). In close parallelism with grxA expression, transcription of the fpg gene was decreased in the presence of Trx or GSH (Fig. 5) and was oppositely increased upon DEM exposure (Fig. 4). Nevertheless, in all cases, the increase in the amounts of grxA transcripts was higher than that of fpg transcripts (e.g. 37 vs 11-fold for maximal DEM induction levels). In contrast to grxA and fpg, the amounts of the trxA, nrdA, and nrdB transcripts remained basically unchanged with or without DEM treatment.

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DISCUSSION

In this paper we demonstrate that the previously proposed regulatory network, in which the ribonucleotide reductase activity is balanced with the levels of its hydrogen donor systems (1), operates at the transcriptional level. Regulation of the E. coli nrdAB ribonucleotide reductase transcription is highly complex and not well understood. The expression of nrdAB genes increases when DNA synthesis is inhibited (26), and it is cell cycle-regulated (27) and sensitive to DNA supercoiling (28). The nrdAB operon has a complicated regulatory sequence that includes Fis (factor for inversion stimulation) and DnaA (DNA replication initiation protein) binding sites (29) and an AT-rich sequence (30). The cis-acting upstream AT-rich sequence is the essential element for the cell cycle regulation of nrdAB expression (30). DNA supercoiling is required for the positive regulation by Fis protein (28).

The results presented here add further complexity to the regulation of nrdAB operon, demonstrating for the first time that the lack of both Trx and Grx1 increases the transcription of nrdAB genes (maximal induction levels of 10- and 9-fold for nrdA and nrdB transcripts, respectively). Trx and Grx1 could affect nrdAB expression as redox-active proteins. Alternatively, the operation of ribonucleotide reductase in the absence of Trx and Grx1 could lead to an unbalanced deoxyribonucleotide production and disturbances similar to treatments that block DNA synthesis. Increments in nrdAB expression were
higher in bacteria at mid-exponential phase in LB broth or at late-exponential in M9 medium than at late-exponential in LB, a result that might be related with increased Fis level under those growth conditions (31). In all circumstances, the increments in nrdB expression were somewhat higher than in nrdA expression, in contrast to what might be expected from the existence of an extra promoter for nrdB gene (32). Nevertheless, it must be noticed that expression of nrdB gene is about 14-fold lower from its own promoter than from the nrdA promoter and, even more important, nrdB expression is stimulated by DNA damage only when it is transcribed from the nrdA promoter (32). Lower amounts of nrdB transcripts might be due to transcription termination after the nrdA gene, as previously indicated (33).

This work also demonstrates that the simultaneous deficiency in Trx and GSH (the physiological hydrogen donor of all glutaredoxins) results in a dramatic increase of 36-fold in grxA transcription (as compared with wild type bacteria). This up-regulation was not accompanied by a significant increase in nrdAB expression, in agreement with the inverse relation observed between RRase activity and the levels of its hydrogen donors (1). Actually, transcription of nrdAB genes was increased significantly in Trx- and GSH-defective bacteria only under growth conditions yielding increments in grxA expression lower than 30-fold. The increased expression of grxA gene in trxA gshA double mutant bacteria could be easily mimicked in trxA single mutant cells by depletion of GSH with diethylmaleate. This induction of grxA transcription was found to be rapid, since maximal increase could be detected after only 10 min of DEM exposure. With treatment over 60 min, DEM induced grxA gene expression also in a wild type genetic background. In this case, the inductive effect of DEM was not simply due to a transient depletion of GSH, since the GSH-negative strain was also responsive to the reagent. These results could be explained if Trx is inactivated, directly or indirectly, upon longer DEM exposure. In agreement with this, DEM treatment did not further enhance the high steady-state level of grxA mRNA in trxA gshA bacteria. Provocative enough was the finding that the basal level of fpg expression (like that of grxA gene) is substantially higher (about 4-fold) in the trxA gshA double mutant strain than in the wild type, this expression also being induced upon exposure to DEM (maximal induction level of 11-fold).

Little is known about the control of grxA transcription, except for a recent report indicating a mild 2-fold induction in the amount of grxA transcript by 100 μM hydrogen peroxide in an oxyR-dependent fashion (34). Additionally, it has been recently demonstrated that GSH and Grxl inactivate OxyR after oxidative stress (35). The large (36-fold) increased expression of grxA gene in trxA gshA double mutant bacteria grown under standard conditions might be explained by assuming that OxyR is fully oxidized in the absence of both Trx and GSH, hence triggering grxA expression. Nevertheless, an oxyR-independent mechanism cannot be excluded, since, in contrast to grxA expression, the transcription of gor, one of the genes whose expression is activated by OxyR (36), was unaffected in Trx- and GSH-defective bacteria. With regard to the E. coli fpg gene, our work represents a first example of variations in its expression and the first indication of a Trx- and GSH-mediated transcriptional regulation mechanism. However, it must be acknowledged that the activity of Fpg seems rapidly induced in E. coli by dioxygen and superoxide-producing agents and in parallel to the activity of superoxide dismutase, thus suggesting the possibility that both enzymes share common reduct regulatory mechanism(s) (37).

Glutathione has long been implicated in the regulation of gene expression in E. coli. Hence, Gardner and Fridovich (38) reported that GSH suppresses the in vitro transcription of the manganese-containing superoxide dismutase gene (denoted sodA), which is a member of the soxRS regulon (36). Interesting enough is the recent finding that GSH in aerobic solution disrupts the SoxR (2Fe-2S) clusters, releasing Fe²⁺ from the regulatory protein and eliminating the SoxR transcriptional activity (39). Such a reaction might occur in vivo, since induction of SoxR-dependent soxS transcription was found to be higher in a GSH-deficient E. coli strain than in its GSH-containing parent (39). One might speculate that a GSH-mediated disassembly of iron-sulfur centers of SoxR or other key regulatory proteins governs the transcriptional regulation of both grxA and fpg genes. In such a case, our data suggest that Trx might cause a similar disassembly of iron-sulfur clusters.

More experiments will be required to unravel the detailed mechanisms by which the in vivo transcription of nrdAB operon is controlled by Trx and Grx1 levels and by which those of grxA and fpg genes are suppressed by Trx and GSH. To this end, the multiplex fluorescence-based primer extension analysis described in this work will be of relevance. Key advantages of this procedure are its ease of design, potential for multiplexing and automation, and wide applicability and avoidance of radioisotopic labels. Its utility, sensitivity and reproducibility has been shown by comparing the expression of genes coding for glutathione reductase (gor), Trx (trxA), Grx1 (grxA), NrdAB ribonucleotide reductase (nrdA nrdB), and Fpg glycosylase (fpg) in a set of E. coli strains.

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