Transcriptional Response of *Escherichia coli* to TPEN

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DNA microarrays were used to probe the transcriptional response of *Escherichia coli* to *N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). Fifty-five transcripts were significantly up-regulated, including all of the genes that are regulated by Zur and many that are regulated by Fur. In the same TPEN-treated cells, 46 transcripts were significantly down-regulated.

The transcriptional response of *Escherichia coli* to elevated levels of metal ions such as Zn(II), Cd(II), Co(II), Ni(II), and Fe(II) has been probed in an effort to understand the mechanisms by which the homeostatic levels of these metal ions are maintained (10, 15, 51, 95, 96). In contrast, very few studies have probed the global response of *E. coli* to low levels of metal ions, presumably due to the difficulty of sufficiently depleting the growth medium of metal ions. In this study, cDNA microarrays were used to probe the transcriptional response of *E. coli* to stress by *N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). TPEN (58, 81), a cell-permeative, divalent metal chelator, is often called “Zn(II) specific” (17, 33, 38, 48, 59, 64, 71, 79, 80, 83, 88). Our data show significant changes in the transcription of several genes in cells stressed with TPEN.

To identify genes that are differentially expressed in response to TPEN, *E. coli* BL21(DE3) cells were grown in minimal medium (76) until the cultures reached mid-log phase. TPEN was introduced, and the cells were cultured for 5 h; this time was chosen so that the results would correspond to previous transcriptional response studies with excess Zn(II) (51). The cultures containing 5 μ M TPEN were analyzed with DNA microarrays (*E. coli* K12 V2 array slides from MWG-BIOTECH), and the results were compared to those from *E. coli* cells grown in minimal medium containing no TPEN. A minimum of six slides was used for each experiment (three slides with one combination of Cy3 and Cy5 dyes and three slides with swapped dyes). Fifty-five transcripts were significantly up-regulated (twofold) (Table 1). Four genes (*ykgM*, *znuA*, *znuC*, and *yodA*) that are regulated by Zur, the Zn(II) uptake regulator (30), exhibited significant increases in expression. The remaining Zur-regulated transcript, *znuB*, was also up-regulated; however, this transcript did not meet our filtering criteria: (i) *P* values of ≤ 0.5 , (ii) ≥ 2 -fold changes in expression, and (iii) consistent data in all six slides. The expression of *zntA*, which is regulated by ZntR and encodes the high-affinity Zn(II) exporter in *E. coli* (7, 14, 78), was unchanged in *E. coli* cultures stressed with TPEN (see the complete set of DNA array data).

Twenty-nine of the up-regulated genes from TPEN-supple-

mented cultures are transcriptionally regulated by Fur, the iron uptake regulator (Table 1), and several are involved in Fe transport. The genes (*entB*, *entA*, *entD*, *entE*, and *fes*) which encode proteins involved with enterobactin synthesis and uptake (26, 29, 54) and the *fec* genes (*fecR* and *fecI*), which encode proteins involved in Fe import (23), were significantly up-regulated. Several other genes (*fhuA*, *exbB*, *exbD*, *fhuD*, *fhuC*, and *fhuF*) associated with ferrichrome transport in *E. coli* were also up-regulated (19, 56, 62).

Forty-six transcripts were significantly down-regulated in *E. coli* cells stressed with TPEN (Table 2). Two operons in *E. coli*, *flgBCDEFGHIJKL* and *flgAMN*, possess genes that encode proteins involved in flagellar biosynthesis (8). Some of these motility-related genes, namely, *flgB*, *fliM*, and *motB*, were previously reported as being up-regulated in *E. coli* cells stressed with excess Zn(II) (51). In addition, the expression of flagellar biosynthetic proteins in *E. coli* is affected by the concentration of copper, possibly exerting its effect via the OmpR or H-NS transcriptional regulators (42). All of the genes in the *cus* and *cue* systems, which confer copper tolerance to *E. coli* (68), were also down-regulated, although *cusA* and *copA* were filtered out of the data shown in Table 2. Previous studies have demonstrated that the expression levels of the *cus* and *cue* genes are dependent on aerobic/anaerobic conditions as well as on the levels of copper in the periplasm/cytoplasm (68). The expression levels of cytoplasmic ferritin (3) were also significantly down-regulated in TPEN-treated cells.

To validate the microarray data, two representative genes were selected for real-time PCR and assayed for the level of mRNA by a two-step, real-time PCR technique. The genes *yodA* and *pdxH*, which were up-regulated (21-fold) and exhibited no change (1.1-fold), respectively, were analyzed with real-time PCR. Real-time PCR results were as follows: *yodA* up-regulated, 17 ± 1 ; *pdxH* up-regulated, 1.1 ± 0.1 . In order to probe whether the changes observed in cells grown in the presence of TPEN were due to the chelator, RT-PCR was used to probe for the expression levels of *yodA* in *E. coli* cells grown in minimal medium containing 5 μ M TPEN and 30 μ M Zn(II). There was no change in transcript levels of *yodA* when *E. coli* was cultured in this medium.

Despite the fact that TPEN is often referred to as a Zn(II)-specific chelator (17, 33, 38, 48, 59, 64, 71, 79, 80, 83, 88), the analyses of our microarray data suggest that the levels of other metal ions may have been affected by the presence of TPEN.

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TABLE 1. Genes up-regulated in *E. coli* cells stressed with TPEN

Grouping	ID ^a	Gene	Increase (fold)	P value	Description	Reference
Zur regulated	B0296	<i>ykgM</i>	23	5.5E-04	Ribosomal protein; L31 paralog	70
	B1973	<i>yodA</i>	21	2.8E-03	Cd(II) induced; putative Zn(II) transporter	21, 75
	B1857	<i>znuA</i>	7.6	1.5E-03	Periplasmic component of Zn(II)-specific ZnuABC transporter	73
	B1858	<i>znuC</i>	2.2	1.1E-02	ATPase of ZnuABC	73
Fur regulated	B2673	<i>nrdH</i>	12	1.9E-04	Glutaredoxin-like protein	39
	B2674	<i>nrdI</i>	12	8.8E-03	Stimulates NrdH	39
	B0595	<i>entB</i>	9.7	2.4E-05	2,3-Dihydro-2,3-dihydroxybenzoate synthetase	26
	B0596	<i>entA</i>	8.7	6.1E-03	2,3-Dihydro-2,3-dihydroxybenzoate dehydrogenase	91
	B0583	<i>entD</i>	7.2	1.0E-04	Enterochelin synthetase, component D	26
	B3006	<i>exbB</i>	5.8	4.9E-04	Uptake of enterochelin; TonB-dependent uptake of b colicins	34
	B3005	<i>exbD</i>	5.3	1.3E-03	Enterochelin synthetase, component d	34
	B0594	<i>entE</i>	5.1	1.6E-02	2,3-Dihydroxybenzoate-AMP ligase	26
	B1683	<i>sufB</i>	5.1	3.6E-03	Component of SufBCD cysteine desulfurase activator complex	69
	B1682	<i>sufC</i>	5.1	4.2E-03	ATP-binding component of SufBCD cysteine desulfurase activator complex	69
	B0151	<i>fhuC</i>	5.1	1.1E-03	ATP-binding component of hydroxymate-dependent iron transport	16
	B4292	<i>fecR</i>	4.9	2.4E-03	Regulator for <i>fec</i> operon, periplasmic	22
	B4293	<i>fecI</i>	4.8	4.5E-03	Probable RNA polymerase sigma factor	4
	B0585	<i>fes</i>	4.5	1.9E-02	Enterochelin esterase	13
	B1102	<i>fhuE</i>	4.4	3.4E-03	Outer membrane receptor for ferric iron uptake	11
	B0150	<i>fhuA</i>	4.3	1.9E-03	Outer membrane protein receptor for ferrichrome, colicin m	24
	B4367	<i>fhuF</i>	2.10	4.7E-03	Ferrioxamine B reductase	62
	B1679	<i>sufE</i>	2.01	1.4E-03	Cysteine desulfurase	55
	B1252	<i>tonB</i>	3.9	5.2E-04	TonB	45
	B1681	<i>ynhC</i>	3.9	3.5E-03	Component of SufBCD cysteine desulfurase activator complex	69
	B1680	<i>sufS</i>	3.7	6.7E-03	L-Selenocysteine lyase	72
	B2676	<i>nrdF</i>	3.1	8.7E-04	Subunit of ribonucleoside-diphosphate reductase II	46
	B3908	<i>sodA</i>	3.0	2.0E-02	Mn superoxide dismutase	36
	B4290	<i>fecB</i>	3.0	4.4E-02	Periplasmic component of iron dicitrate ABC transporter	31
	B2155	<i>cirA</i>	2.9	3.3E-03	Outer membrane receptor for colicin I receptor	12
	B0153	<i>fhuB</i>	2.9	5.0E-02	Component of Fe(III) hydroxamate ABC transporter	16
	B0592	<i>fepB</i>	2.7	2.2E-02	Fe(III) enterobactin ABC transporter	84
	B1132	<i>ycfC</i>	2.6	2.1E-02	Membrane protein in operon with purB	28
	B0586	<i>entF</i>	2.4	2.5E-02	Apo-serine activating enzyme	26
SoxRS regulated	B1611	<i>fumC</i>	3.8	2.6E-02	Class II fumarase	89
	B2962	<i>yggX</i>	3.2	6.4E-03	Protects Fe-S clusters from oxidation	67
	B2963	<i>mltC</i>	2.6	4.6E-05	Component of lytic murein transglycosylase C	6
	B2237	<i>inaA</i>	2.2	3.1E-03	pH-inducible protein involved in stress response	93
	B3924	<i>fpr</i>	2.1	4.8E-02	Flavodoxin NADP ⁺ reductase	9
Other stress related	B0126	<i>yadF</i>	3.7	6.9E-03	Carbonic anhydrase	60
	B1053	<i>yceE</i>	2.5	1.3E-03	Drug resistance protein	65
	B0313	<i>betI</i>	2.4	5.9E-03	Transcription repressor of <i>bet</i> genes	49
	B1629	<i>rsxC</i>	2.1	2.9E-02	Component of SoxR reducing complex	47
	B4062	<i>soxS</i>	2.1	7.3E-03	Superoxide response regulon	52
	B1307	<i>pspD</i>	2.0	3.4E-02	Phage shock protein	61
Anabolic processes	B1264	<i>trpE</i>	5.3	3.7E-02	Anthranilate synthase component I	20
	B3161	<i>mtr</i>	2.9	7.6E-04	Trp transport protein	32
	B1265	<i>trpL</i>	2.7	3.1E-02	Trp operon leader peptide	43
	B2678	<i>proW</i>	2.6	2.4E-02	Pro and Gly betaine transporter	5
	B0311	<i>betA</i>	2.6	3.1E-03	Choline dehydrogenase	77
	B4245	<i>pyrB</i>	2.6	2.1E-02	Aspartate transcarbamoylase	41
	B2028	<i>ugd</i>	2.4	3.1E-03	UDP-glucose-6-dehydrogenase	85
	B1261	<i>trpB</i>	2.3	5.0E-02	Trp synthase β	44
	B3409	<i>feoB</i>	2.3	1.7E-02	Fe(II) transport protein	40
	B1981	<i>shiA</i>	2.3	4.7E-02	Shikimate transporter	92
	B0931	<i>pncB</i>	2.0	7.3E-03	Nicotinate phosphoribosyltransferase	94

^a ID, identifier.

TPEN has been reported to bind Cd(II) ($K_d = 4.7 \times 10^{-17}$), Co(II) ($K_d = 2.6 \times 10^{-17}$), Ni(II) ($K_d = 2.8 \times 10^{-22}$), and Cu(II) ($K_d = 2.9 \times 10^{-21}$) more tightly than it binds Zn(II) (2). In addition, TPEN forms stable complexes with Fe(II) ($K_d =$

2.5×10^{-15}) (2). Since TPEN forms much tighter complexes with Cu(II), it is likely that the down-regulation of the copper homeostasis/export transcripts, *cueO*, *copA*, *cusA*, *cusB*, *cusC*, and *cusF*, is due to low levels of intracellular copper. Twenty-

TABLE 2. Genes down-regulated in *E. coli* cells stressed with TPEN

Grouping	ID ^b	Gene	Decrease (fold)	P value	Description ^a	Reference
Flagellar biosynthesis	B1074	<i>flgC</i>	7.9	3.1E-05	Cell-proximal portion of basal-body rod	8
	B1075	<i>flgD</i>	7.5	3.6E-04	Initiator of hook assembly	8
	B1078	<i>flgG</i>	4.9	1.2E-05	Cell-distal portion of basal-body rod	8
	B1080	<i>flgI</i>	4.3	3.3E-04	p ring of flagellar basal body	8
	B1079	<i>flgH</i>	4.0	8.7E-03	Outer membrane ring protein	8
	B1077	<i>flgF</i>	3.9	5.4E-05	Cell-proximal portion of basal-body rod	8
	B1081	<i>flgJ</i>	3.9	2.8E-03	Flagellar protein	8
	B1073	<i>flgB</i>	3.8	3.8E-04	Cell proximal portion of basal-body rod	8
	B1072	<i>flgA</i>	3.4	4.7E-02	Assembly of basal-body p ring	8
	B1082	<i>flgK</i>	3.1	2.6E-02	Hook filament junction protein	8
	B1071	<i>flgM</i>	2.3	3.6E-03	Anti-flia factor	8
Sugar metabolism	B2094	<i>gatA</i>	6.4	5.9E-03	Enzyme IIa of PTS	66
	B2095	<i>gatZ</i>	5.3	5.6E-03	Putative tagatose 6-phosphate kinase	66
	B2092	<i>gatC</i>	4.9	2.7E-02	Enzyme IIc of PTS	66
	B2091	<i>gatD</i>	4.7	2.4E-03	Galactitol-1-phosphate dehydrogenase	66
	B2167	<i>fruA</i>	4.0	2.2E-03	Fructose specific transporter	66
	B2093	<i>gatB</i>	3.7	1.3E-02	Enzyme IIb of PTS	66
	B2169	<i>fruB</i>	3.5	3.1E-03	Fructose specific IIa component	66
	B2168	<i>fruK</i>	3.2	2.8E-02	Fructose 1-phosphate kinase	66
	B2096	<i>gatY</i>	2.6	1.7E-02	Tagatose-bisphosphate aldolase	66
	B4034	<i>malE</i>	2.4	8.1E-03	Maltose binding protein	66
	B3417	<i>malP</i>	2.3	5.0E-03	Maltodextrin phosphorylase	66
	B1198	<i>ycgC</i>	2.3	6.5E-03	Dihydroxyacetone kinase M	66
	B1817	<i>manX</i>	2.1	1.5E-03	Mannose transporter	66
Fe and Cu metabolism	B1905	<i>ftnA</i>	5.3	3.8E-04	Cytoplasmic ferritin	82
	B0123	<i>yacK</i>	2.5	2.7E-03	CueO, Cu(I) oxidase	27
	B0573	<i>ylcC</i>	2.5	2.7E-03	CusF, copper efflux	27
	B0572	<i>ylcB</i>	2.4	2.0E-02	CusC, copper efflux	27
	B0574	<i>ylcD</i>	2.2	1.4E-02	CusB, copper efflux	27
Stress	B1656	<i>sodB</i>	3.7	2.0E-03	Fe superoxide dismutase	36
	B1379	<i>hslJ</i>	2.3	7.4E-03	Heat shock protein	53
	B3687	<i>ibpA</i>	2.1	4.7E-03	Heat shock protein	50
Metabolic proteins	B3936	<i>rpmE</i>	3.4	2.2E-02	L31 ribosomal protein	63
	B2142	<i>yohK</i>	2.5	3.0E-02	Serotonin transporter	43
	B2286	<i>nuoC</i>	2.5	1.3E-02	Component of NADH dehydrogenase	25
	B0972	<i>hyaA</i>	2.5	4.8E-02	Hydrogenase small subunit	37
	B4116	<i>adiY</i>	2.4	2.8E-02	Transcription activator of Arg decarboxylase	86
	B0411	<i>tsx</i>	2.3	2.8E-2	Nucleoside channel	57
	B1380	<i>ldhA</i>	2.3	1.1E-02	Lactate dehydrogenase	18
	B3209	<i>yhbL</i>	2.3	1.8E-02	Isoprenoid biosynthesis protein	35
	B0651	<i>ybeK</i>	2.3	1.9E-2	Ribonucleoside hydrolase	74
	B1245	<i>oppC</i>	2.2	7.2E-3	ABC transporter for peptides	87
	B0872	<i>hcr</i>	2.2	4.0E-02	NADH oxidoreductase	90
	B1241	<i>adhE</i>	2.0	2.1E-03	Acetaldehyde dehydrogenase and Fe-dependent alcohol dehydrogenase	18
	B1246	<i>oppD</i>	2.0	3.8E-02	ABC transporter for peptides	87
	B2284	<i>nuoF</i>	2.0	7.0E-03	NADH dehydrogenase	25

^a PTS, phosphotransferase system.^b ID, identifier.

nine Fur-regulated transcripts were up-regulated. Despite the fact that Fe(II) binds 1 order of magnitude less tightly to TPEN than Zn(II) (2), it is possible that TPEN lowered the intracellular concentrations of Fe(II), resulting in the transcription of Fur-regulated iron uptake proteins. The reduction in intracellular concentrations of Fe(II) could be due to direct chelation by TPEN or by oxidation of intracellular Fe(II) to Fe(III). The fact that several other SoxRS-regulated transcripts were up-regulated in cultures stressed with TPEN (Table 1) suggests oxidative stress in these cells (97). It is also

possible that the reduction of intracellular Zn(II) caused by the presence of TPEN resulted in an improperly folded Fur, which requires one Zn(II) for proper structure/function (1).

Taken together, these results strongly suggest that the intracellular levels of several metal ions in *E. coli* can be affected by TPEN, which indicates that caution should be exercised when TPEN is used in experiments to control intracellular concentrations of Zn(II) in cells.

Microarray data accession number. The microarray data have been loaded into the Gene Expression Omnibus (GEO)

with the accession number GSE5356 (www.ncbi.nlm.nih.gov/geo).

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