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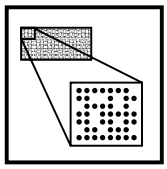

Research Article

Expression profiling of all protein-coding genes in wild-type and three DNA repair-deficient substrains of *Escherichia coli* K-12

Camilla Salmelin and Juhani Vilpo*

Leukemia Research Laboratory, Department of Clinical Chemistry, Tampere University Hospital and Tampere University Medical School, Tampere, Finland

*Correspondence to:
Department of Clinical Chemistry, Tampere University Hospital, P.O. Box 2000, FIN-33521 Tampere, Finland.
E-mail: juhani.vilpo@tays.fi

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Abstract

Gene chips or cDNA arrays of the entire set of *Escherichia coli* (*E. coli*) K12 genes were used to measure the expression, at the mRNA level, of all 4290 protein-coding genes in wild-type (WT) and three DNA repair-deficient derivative strains: (i) AB1157 (WT), (ii) LR39 (*ada*, *ogt*), (iii) MV1932 (*alkA1*, *tag-1*) and (iv) GM5555 (*mutS*). The aim was to investigate whether disruption of a single gene would result in significant deviation in the expression of other genes in these organisms. We describe here a simple approach for a stringent statistical evaluation of cDNA array data. This includes: (i) determination of intra- and interassay variation coefficients for different expression levels, (ii) rejection of biased duplicates, (iii) mathematical background determination, and (iv) comparison of expression levels of identical copies of a gene. The results demonstrated a highly significant correlation of gene expression when the mutants were individually compared with the wild-type. Altogether, 81 deviations of the expression of 59 genes were noted, out of 12,870, when 3-fold or greater up- or down-regulation was used as a criterion of differential expression. In the light of current knowledge of *E. coli* biology, the differential expression did not follow any logical pattern. In fact, the deviations may simply represent inter-assay variation. The results obtained here with a simple model organism are different from those obtained with most mammalian knockouts: disruption of the function of a single gene does not, under good growth conditions, necessarily result in great changes in the expression of other genes. **Copyright © 2002 John Wiley & Sons, Ltd.**

Keywords: ***Escherichia coli*; gene expression; cDNA array; DNA repair; mutant**

Introduction

Gene disruption techniques, also known as gene knockout, have proved to be important tools in assessing the functions of genes in various forms of life, ranging from bacteria to man. Knockout mice, for instance, are invaluable models for the study of mutations similar to those found in human diseases. Germ line gene disruption in mammals has resulted in unexpected phenotypes. This, on the other hand, is associated with a surprising functional redundancy of gene products as well as with an astronomic amount of possible interactions of proteins inside the cell. Mammalian cells remain very complicated systems in which to study the effect of single gene disruption on the expression and function of all other genes. The completion of the *E. coli* genome projects (Blattner *et al*., 1997) has permitted the development of new tools for genome-wide analysis in this model organism.

We have been interested in the biological effects of a therapeutic alkylating agent, chlorambucil. Wild-type (WT) and DNA repair-deficient *E. coli* strains have served as model organisms (Salmelin *et al*. 2000). The importance of DNA repair, shown by reversal of damage and attenuation of the toxicity of chlorambucil, was indicated by the susceptibility of cells lacking direct DNA repair or O^6-methylguanine-DNA methyltransferase I and II (*ada*, *ogt*). Similarly, the protective role of base

excision repair was substantiated by demonstration of even more increased susceptibility to chlorambucil among cells lacking 3-methyladenine-DNA glycosylase I and II (*alkA1*, *tag-1*). Cells deficient in mismatch repair (*mutS*) appeared to be only slightly more sensitive than normal cells to chlorambucil. These results clearly demonstrated that, dependent on the individual gene, gene knockout results in specific functional disturbance of *E. coli* cells. The traditional interpretation of this kind of outcome is straightforward: gene disruption results in paralysis of the corresponding function. In the present paper we report the assessment of another possibility, namely, that single gene disruption might cause unexpected changes in the homeostasis of the expression of genes whose involvement is not anticipated. To this end we analyzed, at the mRNA level, the expression of all protein-coding genes of these four *E. coli* strains. Specific emphasis was paid to the statistical interpretation of the results.

Materials and methods

E. coli strains and culture

The four *E. coli* strains and their respective proteins and functions have been reviewed briefly recently (Salmelin *et al.*, 2000). The strains (a generous gift from Dr. Lene Rasmussen, Department of Molecular and Cellular Toxicology, Harvard School of Public Health) were (i) AB1157 (WT): *argE3 hisG4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-1 thi-1 rpsL31 supE44 tsx-33*, (ii) LR39, as AB1157, but *ada*::Kan^r *ogt*::Cm^r, (iii) MV1932, as AB1157, but *alkA1 tag-1*, and (iv) GM5555, as AB1157, but *mutS*::Tn*10*.

Bacterial growth and isolation of total RNA

Samples for gene expression analyses were taken from exponentially growing cultures. It was important to assess global gene expression under conditions similar to those where the susceptibility to chlorambucil had been determined, i.e. when the cells had been permeabilized by using polymyxin B nonapeptide (PMBN), as described in detail elsewhere (Salmelin *et al.,* 2000).

The cells were cultured in Luria-Bertani (LB) nutrient medium (Sambrook *et al.*, 1989). A single colony of each *E. coli* strain was used to inoculate LB medium and it was cultured at 37°C with shaking overnight. Next, 10 ml of this culture was added to a 250 ml Erlenmayer flask containing 40 ml fresh LB medium and it was incubated for 90 minutes at 37°C. After incubation, 10 μg PMBN/ml was added and the flasks were incubated for 2 hours at 37°C in a shaking incubator. This incubation time was chosen on the basis of the results of earlier experiments (Sambrook *et al.*, 1989). After the second incubation the Erlenmayer flasks were placed on ice and RNA isolation was started immediately.

Total RNA from logarithmic growth-phase cells was isolated according to the protocol recommended by Sigma-Genosys. In order to minimize errors originating from RNA preparation (Arfin *et al.*, 2000), two samples from each culture were preparated simultaneously and finally pooled for the cDNA array hybridization. The detailed protocol is available on the Internet at the Sigma-Genosys homepage: **http://www.genosys.com**. To remove contaminating genomic DNA from purified RNA, the samples were treated with RNase-free DNase I. The RNA was extracted again with three phenol (acidic) extractions followed by one phenol : chloroform : isoamyl alcohol (25 : 24 : 1) extraction and after that by one chloroform : isoamyl alcohol (24 : 1) extraction. RNA was precipitated (as in the protocol used for RNA isolation) and centrifuged at 12 000 x *g* for 30 minutes. The pellet was washed with 70% ethanol, re-centrifuged and dissolved in diethyl pyrocarbonate (DEPC)-treated water. The RNA pellet was stored in DEPC-treated water at -20°C after being quantified by absorbance at 260 nm. High quality of the RNA was confirmed by using agarose gel electrophoresis (1.2% agarose gel).

Hybridization, and analysis of DNA arrays

Expression profiling was performed as described in detail elsewhere (Panorama™ *E. coli* Gene Arrays, Protocol Booklet, available at **http://www.genosys.com**). In short, after RNA isolation the procedure consists of (i) generation of ^{33}P-labeled cDNA from the RNA samples, (ii) hybridization of labeled cDNA to duplicate arrays (Panorama™ *E. coli* Gene Arrays, Sigma-Genosys used in this work) representing 4290 PCR-amplified open reading frames, (iii) autoradiography of the arrays, and (iv) analysis of the expression patterns.

The primary data consisted of duplicate pixel intensities for all 4290 ORFs. For intra- and

inter-strain comparisons the data of each membrane were normalized by dividing all sampled intensities by the mean sampled intensity of all gene points (except the control points).

Results and discussion

Whole genome perspective

The expression profiles of the four *E. coli* stains were very similar, as indicated in the between-strain correlation analysis illustrated in Figure 1. Considerably smaller variation was observed in a within-strain comparison when duplicates of individual gene points were compared. Although the intra-strain variability in gene expression as a whole was smaller than the variability between the strains (Figure 2), this may simply represent an inter-assay effect rather than an overall difference in gene expression between the strains (see also Statistical Considerations, below). The inter-assay variation may also indicate variations between individual cDNA array membranes. This was shown to concern variable background levels of different membranes (cf Table 1 below). 'Between-slide' variation has been demonstrated by using comparative hybridization with fluorescent probes (Tseng *et al*., 2001).

We revealed a total of 130 genes in the three mutant strains whose expression at the mRNA level differed 2-fold or more from that of the WT. Sixty-six genes differed 3-fold or more and these genes were selected for further scrutiny as described below. This decision was based on two factors: (i) we wanted to examine whether there are gross inter-strain differences between the mutants versus WT, and (ii) previous work relying on very similar cDNA array hybridization methodology resulted in the conclusion that a 2.5-fold expression difference indicates significantly different expression, with 99% confidence in the two tails of the data (Tao *et al*., 1999).

Statistical considerations

The raw data consisted of pixel intensities, in duplicate, corresponding to relative hybridization signals of mRNAs representing all open reading frames (ORFs) of *E. coli*. There were a total of 34 320 data points representing gene expression. Corresponding pixel intensities of background signals and known hybridization standards were also obtained. We made use of these data to calculate the reliability of low values approaching background, and analytical precision at different levels of gene expression. This information was used to re-evaluate the reliability of the basic procedure. The primary selection concerned all genes that showed 3-fold or greater differences of expression when compared with the wild-type. Originally, 66 such genes were found.

The background values for each strain were determined from 22 dedicated replicate array points. The pixel intensities of background values were transformed to percentages of whole genome

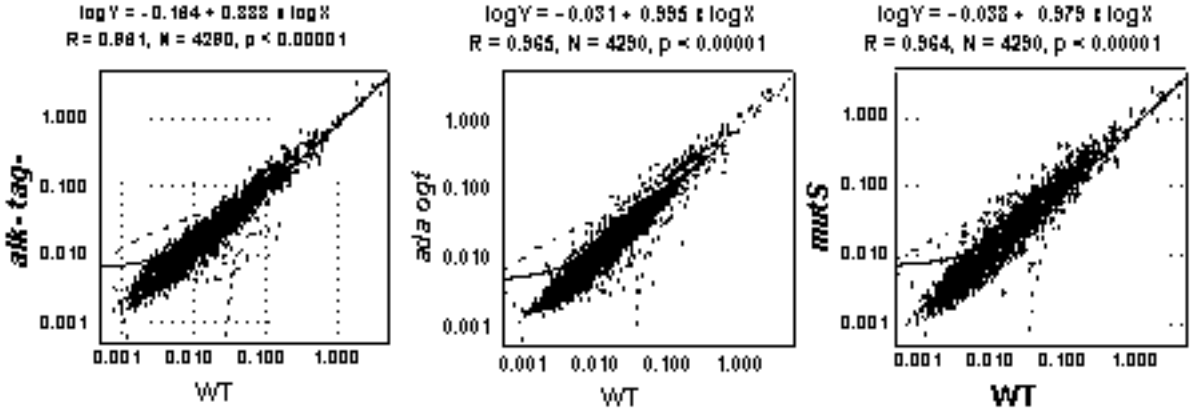

Figure 1. Gene expression in three DNA repair-deficient *E. coli* strains compared with WT. Least squares regression was computed according to the Pearson product-moment correlation method after logarithmic transformation of the data representing percentage expression of each individual gene. Confidence intervals were computed to correspond with 95% limits. The percentage transformation was carried out with normalized data

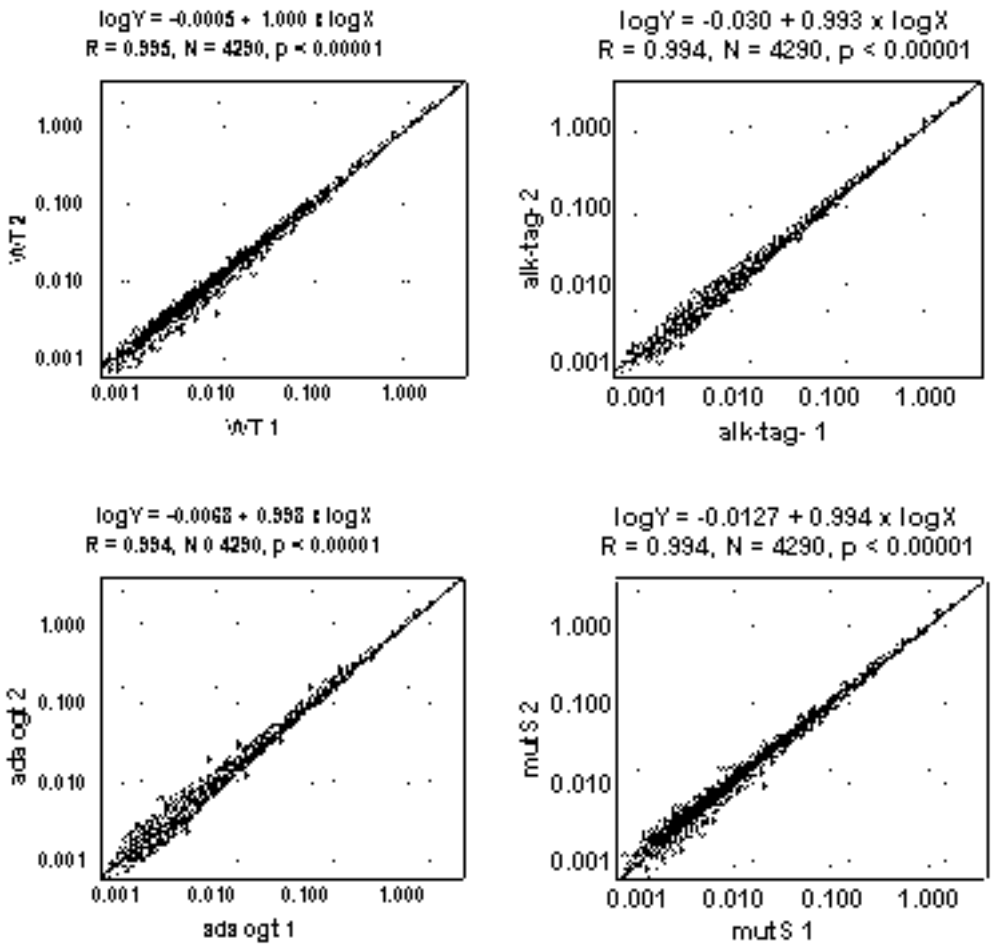

Figure 2. Correlations of duplicate analyses of the expression of all ORFs in WT and three DNA repair-deficient *E. coli* strains. Least squares regression was computed according to the Pearson product-moment correlation method after logarithmic transformation of the data representing percentage expression of each individual gene. Confidence intervals were computed to correspond with 95% limits (obscured by data points). The percentage transformation was carried out with normalized data

expression. These figures and their statistical treatment were used to determine the sensitivity of the assay (Table 1). We chose an arbitrary detection limit such that 99.7% of all possible background values remained below this level. According to this sensitivity rule, the expression of eight out of 66 selected genes fell below the sensitivity level. Fold expressions of these genes were changed accordingly and two of these eight genes did not thereafter satisfy the '3-fold' rule. These were *b4273* (*yi22_6*) and *gatA* (see Table 2B).

The other statistical concern was the precision of the method. This information is necessary for validation of individual data points. The practical question was, how large is the maximal acceptable bias between duplicates? Duplicate assays provide a very convenient tool to determine intra-assay precision. To this end, we used the formula

$$\mathrm{SD} = \sqrt{\sum d^2/N}$$

where SD = standard deviation, d = difference between duplicates and N = total number of determinations (Reed and Henry, 1974). We determined the SD values for the three different expression ranges of all four strains examined. This allowed us to determine the coefficients of variation (CVs)

Table 1. Background values for four *E. coli* strains in the global analysis of 4290 ORFs at the mRNA level[a]

	Strains			
Parameters	**AB1157**	**MV1932**	**LR39**	**GM5555**
Mean	0.95	1.57	1.63	1.31
Median	0.92	1.58	1.46	1.31
SD	0.22	0.26	0.62	0.16
Minimum	0.61	1.16	1.17	0.11
Maximum	1.45	2.28	3.52	1.72
25%	0.078	1.41	1.34	1.20
75%	0.098	1.69	1.61	1.41
Sensitivity(mean + 3 SDs)	1.61	2.35	3.49	1.79

[a]The figures represent the statistics for 22 averages of 44 pre-selected control (blank) points in duplicate. The pixel numbers were first normalized to represent a percentage value of all genes plus control points (100%). To facilitate comparison, the percentage values were multiplied by 100 for this Table. For instance, the first figure 0.95 means that the average background for the AB1157 strain analysis was 0.0095% of the total expression (100%). The sensitivity limit represents the upper 99.7% of all possible background values.

for the different expression levels. Very similar intra-assay variations were observed (Table 3). As shown below, this information was applied to the acceptance or rejection of values represented by biased duplicates. Two recent analysis of global gene expression in *E. coli* did not take this opportunity into account (Tao *et al*., 1999; Arfin *et al*., 2000). Unfortunately, most published investigations do not give pertinent analytical variations. However, as shown here, random errors occur and must be corrected or data eliminated.

We arbitrarily chose to accept the duplicates if they were within $\pm$ 3 SDs of the average of the two values. This decision meant that all values within a 99.7% confidence interval would be accepted for further analysis. The procedure resulted in rejection of four of the selected 66 genes, as indicated in Table 2: *yhiE* in AB1157 and in LR39, *cysB* in LR39, *pnhA* in GM5555, and *b2640* in GM5555. This kind of validation process is possible only if duplicate cDNA arrays are used, or corresponding information of assay precision is otherwise available.

We estimated analytical precision also on the basis of comparison of gene expression in the wild-type and each of the three mutant strains. In this case, a mean value of each duplicate determination was used as illustrated in Figure 1. The SD values were calculated according to Reed and Henry (1974; equation 1). This allowed us to determine CVs for the different expression levels (Table 4). It shall be emphasized that this variation corresponds to the total variation (intra-assay, inter-assay and inter-strain). The use of duplicate means, on the other hand, results in a small underestimation of this total variation.

Another way to look at the global genomic data, not previously used, is to compare the results concerning mRNAs transcribed from identical copies of one gene. The cDNA of a gene should hybridize to all copies of the gene in a similar way. Examples are as follows: 6 identical copies of the *insA*-gene (*insA1 – insA6*) yielded the following fold expressions (MV1932/ WT): 1.4, 1.3, 1.4, 1.3, 1.3, 1.2. (LR39/ WT): 1.4, 1.5, 1.6, 1.5, 1.5, 1.2. (GM5555/ WT): 1.4, 1.5, 1.6, 1.4, 1.4, 1.1. The current assay system should not make any distinction between these similar mRNA species. This indicates a good assay precision, but deviations were also found. For instance, a similar comparison with 6 identical copies of the *yi22*-gene yielded less precise results in the strain LR39: (LR39/WT): -1.8, -2.9, -1.9, -1,8, -3.5, -3.5 compared to (MV1932/WT): 2.1, 2.2, 1.6, 1.9, 2.1, 1.7 and (GM5555/WT): -1.3, -1.3, -1.6, -1.2, -1.6, -1.7. Hence, the expression analysis of *yi22* remains insufficient in this case. In Table 5 some examples of these genes and their expression are shown. This approach led to rejection of two genes of the 66 selected genes, namely *yi22_5* and *b4273* (*yi22_6*). The gene *b4273* (*yi22_6*) is now rejected by two different criteria (see above). We recommend the application of this quality control approach in all cases where more than one copy of the pertinent gene is active.

Functional groups and protein functions of differentially expressed genes

The final number of selected genes having 3-fold or greater expression differences in mutant strains compared with WT was $59/(3 \times 4290)$. In other words, only 0.46% of the genes in the three mutants showed a 3-fold or greater difference compared with the WT. The functional groups, protein products, and fold differences of these genes are given in Table 4. Among these 59 genes, the expression of 40 was changed in one strain, that of 16 in two strains and only three were different from the wild type in all three mutant strains. The differentially expressed genes were evenly distributed among the 19 functional groups (Table 6). Furthermore, the three mutants had similar amounts of differentially

Table 2. Pixel intensities of duplicates and fold differences in expression of genes, initially selected on the basis of their expression being at least 3-fold compared with wild-type. The pixel levels had not been normalized between different hybridization arrays in this phase. (A) AB1157 (WT) versus MV1932. (B) AB1157 (WT) versus LR39. (C) AB1157 (WT) versus GM5555

(A)

Gene name	Pixels AB1157		Pixels MV1932		Fold[a]
b0465	1405	1458	2139	2165	3.0
b1374	7951	7470	1373	1120	−3.0
b1375	20852	20895	2937	2318	−5.2
b1544	20434	20138	2155	1517	−7.2
b1795	20975	24300	1815	2367	−7.1
b3913	14888	14832	2653	2417	−3.8
b3914	38773	36549	6843	6633	−3.6
cspA	106675	107571	8018	9502	−6.0
cspB	97906	107442	5508	5341	−9.3
cspG	15593	15652	1408	1303	−5.7
dps	23684	23567	1622	1550	−7.3
dsbB	1564	1728	2264	2787	3.1
ebgA	2431	2065	3691	3559	3.3
ftn	5622	5601	9909	10310	3.7
gadA	61603	61799	6674	6360	−4.5
gadB	83618	74947	6985	7371	−5.3
galT	39468	38854	2794	2306	−7.6
osmY	19337	17680	2499	3340	−3.1
proA	3199	3302	4737	5239	3.2
rhsB	8538	8856	1335	1500	−4.0
yhiX	13657	13801	1376	1472	−4.8

(B)

Gene name	Pixels AB1157		Pixels LR39		Fold[a]	Comments[b]
b0329	10956	11869	2104	2440	−3.1	
b1374	7951	7470	1369	1243	−3.8	F = −3.3
b1375	20852	20895	1799	1968	−8.1	
b1419	7657	8936	2397	2464	−3.0	
b1544	20434	20138	984	1435	−12.3	>3 SD F = −10.6
b1551	7010	7665	1288	1183	−4.3	F = −3.8
b1552	9125	8528	1951	1871	−3.4	
b1675	2697	2389	6458	5990	3.3	
b1826	1255	1207	2669	2766	3.0	
b4273	5187	5402	1032	938	−3.5	GC F = −2.3
cspA	106675	107571	21864	22955	−3.1	
cspB	97906	107442	6864	6408	−10.0	
cspD	12863	11900	23768	25247	3.1	
cspG	15593	15652	1627	1285	−6.9	F = −6.7
cyoC	8050	8571	1712	1921	−3.0	
cysB	17924	16974	3446	2571	−3.5	>3 SD
dppC	42615	43324	93627	90560	3.3	
fimI	19708	18784	2996	3478	−3.8	
galT	39468	38854	1868	1772	−13.9	
gatA	4685	5362	1087	997	−3.1	F = −2.1
gltA	37465	37595	4776	4469	−5.2	
himA	7542	6990	13755	15452	3.3	
hycF	26047	26657	58450	61829	3.5	
icdA	47893	47503	7944	7934	−3.9	
katE	14869	14732	2873	2626	−3.5	
melB	20636	18037	3209	3542	−3.7	
oppA	376884	360422	76431	67116	−3.3	

Table 2. Continued

(B)

Gene name	Pixels AB1157		Pixels LR39		Fold[a]	Comments[b]
melB	20636	18037	3209	3542	−3.7	
oppA	376884	360422	76431	67116	−3.3	
rhsA	5282	6699	1266	1314	−3.4	F = −3.1
rhsB	8538	8856	963	1222	−5.8	F = −4.5
rpmE	37276	37107	79147	77009	3.4	
thiH	63352	63262	13093	12665	−3.0	
ybgE	4731	4850	9152	8946	3.1	
yciD	4207	3598	10845	11140	4.4	
ydaC	1152	1138	3054	3132	3.7	
yfeC	10261	11107	27507	29246	3.6	
ygfE	22060	21575	4625	5052	−3.3	
yheE	8375	8525	29657	29676	4.8	
yhiE	23348	12109	3147	4997	−3.2	> 3 SD
yhiX	13657	13801	1727	1925	−4.9	
yi22_5	17850	17723	3600	3048	−3.5	GC
yqjF	4803	4865	11000	11762	3.2	

(C)

Gene name	Pixels AB1157		Pixels GM5555		Fold[a]	Comments[b]
b0753	10428	10141	30704	30735	4.3	
b1005	11839	14252	26884	28543	3.1	
b1375	20852	20895	3088	3398	−5.2	
b1544	20434	20138	2817	2630	−6.0	
b1675	2697	2389	8512	9183	4.3	
b1826	1255	1207	3588	3724	3.7	
b2640	2283	2522	7097	10539	4.5	> 3 SD
b3047	2706	2887	6900	6903	3.5	
b3975	2426	2899	11328	12228	5.5	
fliC	5781	6184	17999	17446	4.2	
gadA	61603	61799	178910	174069	4.1	
gadB	83618	74947	179769	174787	3.2	
gapC_1	8503	9434	29605	30425	4.8	
gapC_2	6997	7288	28058	27404	5.5	
gltA	37465	37595	8444	8452	−3.1	
hycF	26047	26657	66039	63623	3.5	
phnA	4048	3857	14088	6167	3.2	> 3 SD
rho	17832	18675	63873	63611	5.0	
rhsB	8538	8856	1357	1236	−5.4	
rpmE	37276	37107	85450	88405	3.4	
sdhD	16569	15864	42117	31032	3.2	
thiH	63352	63262	12642	11140	−3.7	
yccJ	10959	11151	25170	26524	3.4	
ydaC	1152	1138	5285	6654	6.4	
yehI	335524	323879	85312	85781	−3.1	
yfeC	10261	11107	33393	30886	3.7	
yheE	8375	8525	30641	29870	4.4	
yqjF	4803	4865	13689	12070	3.3	

[a]The primary fold values were obtained by comparing the normalized percentage expression of the mutant *E. coli* strains with those of the WT. Negative figures are used if the mutant showed lower expression than the WT. Signals that were higher in wild-type control cells (transcription repressed) were used in the numerator of the ratio, which was then converted to negative values.

[b]Fold values were changed in the indicated cases, since the values were below the accepted sensitivity of the assay. F = the new fold value based on acceptance of the sensitivity limit for the lowest acceptable value. On this basis, one gene (*gatA*) was rejected (Table 2B). The gene is marked '> 3 SD' and the pixel value is in italics if the bias between the duplicate values exceeded ± 3 SDs. The item was rejected unless the values fell below the sensitivity limit and unless the acceptance of the sensitivity limit as a lower value still resulted in a difference of 3-fold or more. Gene copies producing the same protein, but showing different expression from the other copies are marked 'GC'. On this basis two genes (b4273 and yi22_5) were rejected.

Table 3. The intra-strain coefficients of variation (CVs)[a]

	Coefficient of variation			
	Strain			
Expression level	wild-type	alkA tag-I	ada ogt	mutS
Low < 0.01 % of total	9.9%	12.5%	12.2%	11.6%
Middle 0.01-0.099% of total	7.0%	7.2%	9.1%	7.6%
High ⩾ 0.1%	8.2%	6.0%	6.3%	7.5%

[a]CVs were determined on the bases of duplicate observations according to Reed and Henry (1974) as described in Results and Discussion (Statistical considerations).

expressed genes. MV1932 had the lowest proportion (24%) of changes in gene expression, LR39 had the highest (43%), and GM5555 was in between (31%), when the wild-type was used as the expression reference. Of the 81 deviations of the 59 genes 38 were up-regulated (MV1932 13%, LR39 34% and GM5555 53%) and 43 down-regulated (MV1932 37%, LR39 51% and GM5555 12%).

Are the differences between the strains real?

The statistical approach revealed several significant gene expression differences between the four *E. coli* strains analyzed. However, none of these indicated a biological compensation of the primary gene defect. The other approach to the question of expression differences between the strains was to investigate the functions of the differentially expressed genes and to attempt to link this information to the possible consequences of the original disrupted gene function. In particular, differentially expressed operons would be valuable in this regard. It was not possible, however, to link any of the currently known functions, whether up- or down-regulated, to the conceivable consequences of the original disruption of the DNA repair genes in these three mutant *E. coli* strains. Some similarities in the mutants were noted, such as relatively low expression of the cold-shock proteins CspA and CspB in MV1932 and LR39. Furthermore *b1375 (ynaE)*, *b1544 (ydfK)* and *rhsB* showed low expression in all three mutants, but lack of information on the cellular functions of these proteins does not allow further interpretation of this observation. Furthermore, we cannot exclude the possibility that the genes undergoing 3 fold or greater differential expression in different mutant strains would have functions, in addition to the currently known ones, which could compensate for the functions of the deleted genes. It remains to be studied whether better compensation mechanisms exist in other knock-out strains or under more stressful conditions.

In conclusion, in spite of individual deviations from the common expression patterns of many genes in these four *E. coli* strains, no systematic

Table 4. Inter-strain coefficients of variation (CVs)[a]

	Coefficient of variation		
	Strains		
Expression level	alk- tag- and WT	ada ogt and WT	mutS and WT
Low < 0.01% of total	47%	32%	39%
Middle 0.01–0.099% of total	36%	40%	43%
High ⩾ 0.1%	66%	62%	72%

[a]CVs were determined on the bases of duplicate observations according to Reed and Henry (1974) as described in Results and Discussion (Statistical considerations)

Table 5. Examples of the fold induction of some groups of identical copies of a gene (*cpxP*, *insA*, *insB*, *tra8* and *yi22*). All copies have the same end product (protein)

	Fold induction		
Gene	MV1932/WT	LR39/WT	GM5555/WT
cpxP (b3913)	−3.8	1.1	1.0
cpxP (b3914)	−3.6	1.2	1.3
insA_1	1.4	1.4	1.4
insA_2	1.3	1.5	1.5
insA_3	1.4	1.6	1.6
insA_5	1.3	1.5	1.4
insA_6	1.3	1.5	1.4
insA_7	1.2	1.2	1.1
insB_1	1.4	1.4	−1.6
insB_2	1.4	1.4	−1.4
insB_3	1.4	1.4	−1.5
insB_4	1.5	1.3	−1.6
insB_5	1.6	1.6	−1.6
insB_6	1.4	1.2	−1.7
tra8_1	2.1	−1.1	1.1
tra8_2	2.6	1.1	1.1
tra8_3	2.6	−1.1	1.1
yi22_1	2.1	−1.8	−1.3
yi22_2	2.2	−2.9	−1.3
yi22_3	1.6	−1.9	−1.6
yi22_4	1.9	−1.8	−1.2
yi22_5	2.1	−3.5	−1.6
yi22_6 (b4273)	1.7	−3.5	−1.7

Table 6. Expression ratios, functional groups and protein functions of the genes whose expression differed ⩾ 3-fold from that of the wild-type

Functional group[a]	Gene name	Gene product	Expression in wt (% of total)[b]	Fold change as compared to the WT		
				MV1932	LR39	GM5555
Carrier (13; 2.6%)	ftn	Cytoplasmic ferritin (an iron storage protein)	0.84	3.7		
Enzyme (952; 0.63%)	cyoC	Cytochrome o ubiquinol oxidase subunit III	1.24		−3.0	
	dsbB	Reoxidizes DsbA protein following formation of disulfide bond in P-ring of flagella	0.25	3.1		
	ebgA	Evolved beta-D-galactosidase, alpha subunit; cryptic gene	0.34	3.3		
	gadA	Glutamate decarboxylase isozyme	9.03	−4.5		4.1
	gadB	Putative arylsulfatase regulator	11.6	−5.3		3.2
	gapC_1	Glyceraldehyde 3-phosphate dehydrogenase C (first fragment)	1.34			4.8
	gapC_2	Glyceraldehyde 3-phosphate dehydrogenase (second fragment)	1.07			5.5
	galT	Galactose-1-phosphate uridylyltransferase	5.86	−7.6	−13.9	
	gltA	Citrate synthase	5.62		−5.2	
	icdA	Isocitrate dehydrogenase, specific for NADP+	7.14		−3.9	
	katE	Catalase; hydroperoxidase HPII(III)	2.22		−3.5	
	proA	Gamma-glutamylphosphate reductase	0.48	3.2		
	sdhD	Succinate dehydrogenase, hydrophobic subunit	2.43			3.2
	thiH	Thiamin biosynthesis, thiazole moiety	9.27		−3.0	−3.7
Factor (71; 0.93%)	himA	Integration host factor (IHF), alpha subunit; site specific recombination	1.06		3.3	
	rho	Transcription termination factor Rho; polarity suppressor	2.67			5.0
IS, phage, Tn (80; 1.25%)	b1374	Putative transposon resolvase	2.14	−3.0	−3.3	
Leader (12)			None			
Membrane (42)			None			
Orf (1402; 0.76%)	b0329	Hypothetical protein	1.67		−3.1	
	b0465	Putative alpha helix protein	0.21	3.0		
	b1005	Hypothetical protein	1.91			3.1
	b1375	Hypothetical protein	3.80	−5.2	−8.1	−5.2
	b1419	Hypothetical protein	1.51		−3.0	
	b1544	Hypothetical protein	3.69	−7.2	−10.6	−6.0
	b1551	Hypothetical protein	1.33		−3.8	
	b1675	Hypothetical protein	0.46		3.3	4.3
	b1795	Hypothetical protein	4.12	−7.1		
	b1826	Hypothetical protein	0.22		3.0	3.7
	b3913	Hypothetical protein	2.70	−3.8		
	b3914	Hypothetical protein	6.85	−3.6		
	b3975	Hypothetical protein	0.48			5.5
	rhsA	rhsA protein in rhs element	1.09		−3.1	
	rhsB	rhsB protein in rhs element	1.58	−4.0	−4.5	−5.4
	ybgE	Hypothetical protein	0.70		3.1	
	yccJ	Hypothetical protein	1.62			3.4
	ydaC	Hypothetical protein	0.21		3.7	6.4
	yfeC	Hypothetical protein	1.94		3.6	3.7
	ygfE	Hypothetical protein	3.97		−3.3	
	yqjF	Hypothetical protein	0.88		3.2	3.3
Phenotype (115; 1.45%)	b1552	Cold shock-like protein	1.61		−3.4	
	cspB	Cold shock protein; may affect transcription	15.4	−9.3	−10.0	
	cpsD	Cold shock protein	1.85		3.1	
	cspG	Homolog of Salmonella cold shock protein	2.34	−3.1		
	osmY	Hyperosmotically inducible periplasmic protein	2.77	−3.1		

Table 6. Continued

Functional group[a]	Gene name	Gene product	Expression in wt (% of total)[b]	Fold change as compared to the WT		
				MV1932	LR39	GM5555
Putative carrier (8)			None			
Putative enzyme (472; 0.14%)	hycF	Probable iron-sulfur protein of hydrogenase 3 (part of FHL complex)	3.94		3.5	3.5
Putative factor (57)			None			
Putative membrane (59; 1.14%)	b3047	Putative membrane protein	0.42			3.5
	yciD	Putative outer membrane protein	0.58		4.4	
Putative regulator (163; 0.82%)	b0753	Putative homeobox protein	1.51			4.3
	yehI	Putative regulator	60.0			−3.1
	yhiX	Putative ARAC-type regulatory protein	2.05	−4.8	−4.9	
Putative RNA (4)			None			
Putative structure (51)			None			
Putative transport (288; 0.23%)	yheE	Putative general secretion pathway for protein export	1.54		4.8	4.4
Regulator (183; 0.55%)	cspA	Cold shock protein 7.4, transcriptional activator of hns	16.0	−6.0	−3.1	
	dps	Global regulator, starvation conditions	3.54	−7.3		
Structural component (91; 1.46%)	fimI	Fimbrial protein	2.88		−3.8	
	fliC	Flagellar biosynthesis; flagellin, filament structural protein	0.90			4.2
	rpmE	50S ribosomal subunit protein L31	5.46		3.4	3.4
Transport (227; 0.44%)	dppC	Dipeptide transport system permease protein 2	6.43		3.3	
	melB	Melibiose permease II	2.89		−3.7	
	oppA	Oligopeptide transport; periplasmic binding protein	55.2		−3.3	
Total (1.38%)	59/4290 genes		2.9 % of WT total mRNA	21	35	25

[a]The revised classification to 19 functional groups was used. The figures in the parenthesis represent the number of all *E. coli* genes belonging to the pertinent category and the percentage of differentially expressed genes from this whole group.
[b]The column illustrates the proportion of each gene-specific mRNA from the whole expressed mRNA. In order to facilitate the comparison, the percentage values were multiplied by one hundred (100). The bottom of the column gives the real percentage of the sum mRNA in the wild-type.

patterns were revealed at the 3-fold sensitivity level used in this investigation. We emphasize the importance of careful assessment of the precision of the method as well as individual scrutiny of every gene accepted in the final expression analysis. The cDNA arrays with at least two replicas for each gene provide a very versatile tool to determine the pertinent assay precision. The current results demonstrated a good interassay precision as evaluated by using different *E. coli* substrains.

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References

Arfin SM, Long AD, *et al*. 2000. Global gene expression profiling in *Escherichia coli* K12: The effects of integration host factor. *J Biol Chem* **275**: 29672–29684.

Blattner FR, Plunkett G 3rd, Bloch CA, *et al*. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453–1474.

Reed HR, Henry RJ. 1974. Accuracy, Precision, Quality Control, and Miscellaneous Statistics. In *Clinical Chemistry Principles and Technics*, Henry RJ, Cannon DC, Winkelman JW (eds). Harper & Row Inc.: Hagerstown; 287–341

Salmelin C, Hovinen J, Vilpo J. 2000. Polymyxin permeabilization as a tool to investigate cytotoxicity of therapeutic aromatic alkylators in DNA repair-deficient *Escherichia coli* strains. *Mutat Res* **467**: 129–138.

Sambrook J, Fritsch E, Maniatis T. 1989. *Molecular Cloning. A laboratory manual*. Cold Spring Harbor Laboratory Press.

Tao H, Bausch C, Richmond C, Blattner FR, Conway T. 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J Bacteriol* **181**: 6425–6440.

Tseng GC, Oh MK, Rohlin L, Liao JC, Wong WH. 2001. Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. *Nucleic Acids Res* **29**: 2549–2557.

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