Transcriptional responses of *Escherichia coli* during recovery from inorganic or organic mercury exposure

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24 **ABSTRACT**

25 **Background:** The protean chemical properties of mercury have long made it attractive
26 for diverse applications, but its toxicity requires great care in its use, disposal, and
27 recycling. Mercury occurs in multiple chemical forms, and the molecular basis for the
28 distinct toxicity of its various forms is only partly understood. Global transcriptomics
29 applied over time can reveal how a cell recognizes a toxicant and what cellular
30 subsystems it marshals to repair and recover from the damage. The longitudinal effects
31 on the transcriptome of exponential phase *E. coli* were compared during sub-acute
32 exposure to mercuric chloride ($HgCl_2$) or to phenylmercuric acetate (PMA) using RNA-
33 Seq.

34 **Results:** Differential gene expression revealed common and distinct responses to the
35 mercurials throughout recovery. Cultures exhibited growth stasis immediately after each
36 mercurial exposure but returned to normal growth more quickly after PMA exposure
37 than after $HgCl_2$ exposure. Correspondingly, PMA rapidly elicited up-regulation of a
38 large number of genes which continued for 30 min, whereas fewer genes were up-
39 regulated early after $HgCl_2$ exposure only some of which overlapped with PMA up-
40 regulated genes. By 60 min gene expression in PMA-exposed cells was almost
41 indistinguishable from unexposed cells, but $HgCl_2$ exposed cells still had many
42 differentially expressed genes. Relative expression of energy production and most
43 metabolite uptake pathways declined with both compounds, but nearly all stress
44 response systems were up-regulated by one or the other mercurial during recovery.

45 **Conclusions:** Sub-acute exposure influenced expression of ~45% of all genes with
46 many distinct responses for each compound, reflecting differential biochemical damage

47 by each mercurial and the corresponding resources available for repair. This study is
48 the first global, high-resolution view of the transcriptional responses to any common
49 toxicant in a prokaryotic model system from exposure to recovery of active growth.
50 Many genes encoding the responses provoked by these two mercurials are highly
51 conserved evolutionarily, affording insights about how higher organisms may respond to
52 these ubiquitous metal toxicants.
53

54 **BACKGROUND**

55 The common metallic element mercury (Hg) has no beneficial biological function
56 but its chemical similarities to essential transition metals such as zinc, copper, and iron
57 make it highly toxic to all living systems. Global mercury emissions range from 6500 to
58 8500 Mg annually with estimates of 50% [1, 2] and even two-thirds [3] being
59 anthropogenic and the rest from volcanism. Mercury exists in multiple chemical forms
60 that are readily susceptible to abiotic and biotic inter-conversions [4]. Mercury occurs
61 naturally as the insoluble HgS ore (cinnabar), as inorganic complexes of Hg^{+2}, Hg^{+1}, or
62 $(Hg_2)^{2+}$ of varying solubility depending on available ligands, and as organomercurials
63 generated by microbial and anthropogenic processes.

64 Major sources of chronic mercury exposure in humans include dental amalgam
65 fillings [5, 6], consumption of fish containing methylmercury [7], and artisanal gold
66 mining operations [8]. Organomercurials, like phenylmercury, methylmercury, and
67 merthiolate (ethylmercury) have historically been used in medical, industrial and
68 agricultural applications as antimicrobial or fungicidal agents [9-11]. The toxic effects of

69 mercury exposure in humans are associated with neurological, kidney, liver,
70 gastrointestinal, and developmental disorders [9, 12-15].
71 Like other common electrophilic toxic metals such as arsenic, cadmium, and
72 lead, there is no single biochemical target for mercury damage. Mercury has a strong
73 affinity for sulfur and selenium [16, 17] and therefore targets the cellular thiol pool,
74 composed of glutathione and cysteine thiol groups of proteins [9] and selenocysteine, a
75 rare but critical amino acid in proteins involved in redox defense and thyroid function
76 [18]. Depletion of the cellular thiol pool and disruption of the cellular membrane potential
77 by mercury can induce oxidative stress and apoptosis pathways in mitochondria [19,
78 20]. However, there is no evidence that mercury itself undergoes Fenton-type chemistry
79 to generate reactive oxygen species like iron and copper [21].
80 In earlier work we used global proteomics to identify stable mercury-protein
81 binding sites in growing *E. coli* cells exposed to acute levels of organic or inorganic Hg
82 [22]. We found cysteine sites in several hundred proteins, many highly conserved
83 evolutionarily, that formed stable adducts with one or more of these mercurials,
84 consequently disrupting many cellular processes such as iron homeostasis and the
85 electrolyte balance [23]. Importantly, we found that organic and inorganic mercurials
86 had distinct effects on these cellular processes and distinct protein structural
87 preferences. Although the pathobiology of organic and inorganic mercurials has been
88 known for decades to differ, with methyl- and ethyl-mercury recognized as neurotoxic
89 and inorganic mercury as neurotoxic, nephrotoxic, hepatotoxic, and immunotoxic, no
90 previous studies at that time had assessed the biochemical underpinnings of these
91 distinctions on a global scale in any model system.

92 Motivated by our proteomics observations and by microarray data from *C.*
93 *elegans* showing distinct transcriptional single end point response and toxicity for
94 inorganic and organic mercurials [24], we applied RNA-Seq to examine the
95 transcriptional effects of $HgCl_2$ and phenylmercuric acetate (PMA) exposure on *E. coli*
96 K-12 MG1655 over time. This is the first study to examine the transcriptional response
97 to mercury exposure in a microorganism and the only study to compare directly the
98 effects of different compounds over time through recovery. The changes in gene
99 expression were idiosyncratic for each compound, confirming and extending the idea
100 that the cell suffers overlapping but distinct biochemical damage and marshals both
101 distinct and overlapping recovery processes in response to these chemically distinct
102 mercurials. Although our work was in a bacterium, the high evolutionary conservation of
103 many of the proteins we identified as mercury-vulnerable offers insights for the
104 toxicology of mercury compounds in higher organisms.
105
106 **METHODS**
107 ***Cell cultures.***
108 For each biological RNA-Seq replicate *E coli* K-12 MG1655 was subcultured from
109 cryostorage on Luria-Bertani (LB) agar overnight at 37°C. A half-dozen well-isolated
110 colonies were used to inoculate a 20 ml starter culture in Neidhardt MOPS Minimal
111 Medium (NM3) [25] (0.2% final glucose concentration) supplemented with 20 mg/L
112 uracil and 500 µg/L thiamine, which was incubated at 37°C with shaking at 250 rpm
113 overnight (~18 hr). The overnight starter culture was diluted 1:30 to initiate the
114 experimental culture and divided into three 500 ml flasks with 100 ml NM3 in each,

115 which were incubated at 37°C with shaking at 250 rpm. When cultures reached OD_{595} ≈
116 0.470 (~ 200 min), two cultures were made 3 μM mercuric chloride ($HgCl_2$) or 3 μM
117 phenylmercuric acetate (PMA) and the third was left as an unexposed control. Mercury
118 stocks were prepared fresh for each growth experiment: 10 mM $HgCl_2$ (Fisher) in water
119 and 5 mM PMA (Sigma) in 25% dimethyl sulfoxide DMSO (Fisher), which is 2.1 mM or
120 0.015% v/v final concentration DMSO in culture. These mercurial exposures were
121 chosen from prior pilot experiments to find exposure conditions (OD_{595}, mercurial
122 concentration and sampling times) that displayed a marked decrease in growth rate
123 relative to the unexposed control but allowed subsequent restoration of rapid growth
124 rate (i.e. recovery) within 1 hr (approximately one generation in NM3) after mercurial
125 exposure. Duplicate 1-ml aliquots of each culture were collected at 0 (unexposed
126 control only), 10, 30, 60 min after mercurial exposure and immediately centrifuged at
127 20,800 *x g* for 3 min at 4°C. Spent medium was aspirated and cell pellets were frozen at
128 -70°C within 5 min after collection. Seven biological replicates were prepared following
129 this protocol and the average variance for all replicates in culture optical density over
130 each 90-min experiment ranged from 0.0019 – 0.0073. The three biological replicates
131 with the lowest variance between growth curves (range from 0.0007 – 0.0017 for all
132 time points) were prepared for RNA-Seq.
133
134 ***Purification of mRNA***
135 One cell pellet from each condition and sampling time was thawed on ice; total
136 RNA was isolated by RNA*snap*™ [26] and stored at -70°C. DNA contamination was
137 removed by two treatments with Turbo-DNase (Ambion; Life Technologies). RNA

138 concentrations and A_{260}/A_{280} ratios were determined using a Nanodrop™ 1000
139 spectrophotometer (Thermo Scientific). Ribosomal RNA depletion was performed with
140 the Ribo-Zero™ rRNA removal kit for Gram-negative bacteria (Epicentre) and
141 concentrated using RNA Clean and Concentrator™ -5 columns (Zymo Research)
142 following the manufacturer's instructions. Purified mRNA was quantified using the
143 Nanodrop™ and stored at -70°C.
144
145 ***Library Preparation and Next-generation Sequencing***
146 The quality and quantity of rRNA-depleted RNA was assessed on a 2100
147 Bioanalyzer RNA pico chip (Agilent Technologies) using the manufacturer's
148 recommendations. Next-generation sequencing (NGS) libraries were prepared using the
149 Kapa biosystems NGS stranded library prep kit for RNA-Seq with dual indexed Illumina
150 adapters. Library insert size was ~150 bp, as determined by high-sensitivity NGS
151 fragment analysis kit for Fragment Analyzer™ (Advanced Analytical Technologies) using
152 the manufacturer's instructions. Quantification of each library was done by qPCR and all
153 30 libraries were pooled in equal concentrations. The library preparation, quality
154 analysis, and pooling were performed by the Georgia Genomics Facility
155 (http://dna.uga.edu). Paired-end (2 x 50 bp) sequencing of the pooled libraries using the
156 Illumina HiSeq 2000 platform was performed by the HudsonAlpha Institute for
157 Biotechnology Genomic Services Laboratory (http://gsl.hudsonalpha.org). See Table S1
158 for index and filename information for data uploaded to NCBI Gene Expression
159 Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) with accession ID: GSE95575.
160

161 ***Data Processing and Differential Expression Analysis***

162 Quality control processing of sequence data was performed using Galaxy
163 (https://galaxyproject.org) on the Georgia Advanced Computing Resource Center at the
164 University of Georgia. The FASTX tools in Galaxy
165 (http://hannonlab.cshl.edu/fastx_toolkit) were used for filtering by quality (80% of
166 sequence ≥ quality score of 20), then reads were trimmed at both 5' and 3' ends using a
167 window and step size of 1 with quality score ≥ 20. Forward- and reverse-read mate-
168 pairs were assembled and aligned to the *Escherichia coli* MG1655 K-12 genome using
169 Bowtie2 [27]. SAMtools [28] was used to convert Bowtie2 output (.bam file) to SAM
170 format. The number of sequence reads that aligned to features in the annotation file
171 (Escherichia_coli_str_k_12_substr_mg1655.GCA_000005845.2.24.gtf from
172 http://bacteria.ensembl.org) were tabulated from the resulting SAM alignment files using
173 the HTSeq-count program [29] with intersection non-empty mode. Mapped read counts
174 were analyzed for differential expression (false discovery rate of ≤ 0.01, fold-change ≥
175 2) using the baySeq package in R [30]. Within baySeq, two-way comparisons using
176 quantile normalization were made for all three biological replicate transcriptomes over
177 time for $HgCl_2$ exposure or PMA exposure versus the unexposed control. We also
178 examined changes over time in the unexposed control culture itself.

179

180 **RESULTS**

181 ***Effects of sub-acute mercury exposure on growth of MG1655.***

182 We defined sub-acute exposure as the concentration of mercury that clearly
183 inhibited growth relative to the unexposed control but allowed cells to resume growth

184 within 1 hour or approximately one generation in this medium (Figure 1a, Figure S1).
185 Based on pilot experiments the appropriate dose proved to be 3 μM for both mercurials.
186 Exposure to 4 - 5 μM $HgCl_2$ prevented growth resumption during 1 hr and the effects of
187 PMA exposure were similar at 3 and 5 μM; exposure to 2.5 μM of either mercurial did
188 not consistently retard growth (data not shown).
189 Cell-associated Hg (Table S2 and Supp Info Methods) declined slowly as has
190 been reported previously for low level $HgCl_2$ exposure of Hg sensitive cells and was
191 attributed to non-specific endogenous reductants [31-33]. Bound Hg in cells exposed
192 either to $HgCl_2$ or to PMA declined similarly from ~50% of total Hg added to culture at
193 10 min to ~20% at 30 min, after which Hg loss from PMA-exposed cells continued to
194 decline to 11% of input at 60 min. In contrast, cell-associated Hg in cells exposed to
195 $HgCl_2$ increased from 24% at 30 to 47% at 60 min. Presently, we have no simple
196 explanation for this unexpected difference in cell-bound Hg in late exponential phase
197 cultures, however it does echo our finding that cultures acutely exposed to 40μM or 80
198 μM PMA or $HgCl_2$ bound 24% or 208% more Hg(II) than PhHg, respectively [23]. Also
199 notable was a brief drop in the culture optical density immediately after PMA exposure
200 consistent with some cell lysis as has been reported [34]. The lack of apparent lysis
201 after divalent $HgCl_2$ exposure may be due to its ability to cross-link cell envelope
202 proteins via their cysteines, which is not possible for monovalent PMA.
203
204 ***Transcriptome benchmarks***
205 Paired-end libraries averaged over 9.5 million reads and mapped reads provided
206 an average of 143X coverage (Figure S2). The sequencing data were of high quality,

207 requiring removal of only 11% as low-quality reads. Of the high-quality reads, 97% of
208 reads mate-paired, 99.4% of paired reads mapped to the genome and 82% of reads
209 mapped to an annotated genome feature on average from all libraries.

210 Overall 89% of annotated mapped reads were to coding regions (CDS) based on
211 raw un-normalized read counts per gene output from HTseq-count program [29](Figure
212 S3, Table S3, Table S4). Pearson correlations of raw read counts confirmed that no
213 strong biases were introduced in biological replicates for each condition (Figures S4-
214 S6). That dispersion is slightly greater in both mercury exposure conditions than in
215 unexposed cultures, especially at later time points, is consistent with perturbations of
216 multiple cellular processes.

217 Mapped reads to rRNA constituted only 0.3% of total reads (std. dev. = 0.425) on
218 average for all libraries (Table S3) consistent with effective Ribo-Zero™ rRNA removal.
219 In the unexposed culture non-coding RNA's (ncRNA) were 4% of total reads over all
220 time points, but their percentage increased in mercury exposure conditions indicating
221 greater differential expression of some ncRNA genes (details below). The very
222 abundant tmRNA (*ssrA*) needed for rescuing stalled ribosomes [35] was 6% of total
223 reads in the unexposed condition and although this percentage increased for mercury
224 exposure conditions, the tmRNA gene (*ssrA*) was not differentially expressed under any
225 condition. Pseudogenes accounted for less than 1% of total reads, but up to 35%
226 ($HgCl_2$) and 13% (PMA) of them were significantly up-regulated. The tRNA's were less
227 than 1% of total reads because the library preparation method we used was not
228 optimized for such small RNAs. However, approximately 35% of these observed tRNA

229 genes were significantly down-regulated during the first 30 min after exposure to either
230 mercurial.
231
232 ***$HgCl_2$ and PMA transcriptional responses are not the same.***
233 We expected that differentially expressed genes (DEGs) in the mercury exposure
234 conditions (compared to the unexposed cells) would change over time as the cells
235 transitioned from initial growth stasis back into a normal growth rate. We also expected
236 that some DEG responses would be similar because both mercurials are thiophilic and
237 will bind to the cellular thiol redox buffer, glutathione (GSH), and to protein cysteine
238 residues. However, since there are physiological differences and protein site
239 preferences for each compound in acute Hg(II)- or PMA-exposure [23 and Zink et. al. in
240 preparation] we aimed here at a low exposure using a longitudinal protocol to discern
241 more subtle distinctions between these mercurials as the cells experienced stasis and
242 then recovered their growth rate. In the following sections, we first describe the bulk
243 measures of gene expression over time and then describe differences in specific
244 functional pathways.
245
246 ***Differentially expressed genes: the view from 30,000 feet.***
247 *a. Differentially expressed genes (DEG) for each condition and time point*
248 DEGs were determined by pairwise comparisons of mercury exposure conditions
249 relative to the unexposed culture at the corresponding time point (Figure 1b and Table
250 S5). Ten minutes after exposure, expression of 41% or 49% of the 4,472 non-rRNA
251 genes changed significantly for $HgCl_2$ or PMA-exposed cells, respectively (Figure 1b).

252 At 30 min with growth still arrested, 32% of genes in the $HgCl_2$-exposed cells were
253 differentially expressed (Figure 1b), (Figure 1a, red). In contrast, PMA-exposed cells at
254 30 min began to recover their prior growth rate (Figure 1a, green), but 45% of their
255 genes remained differentially expressed (Figure 1b). By 60 min, the PMA-exposed cells
256 were growing at nearly their pre-exposure rate and only 1.5% of genes were
257 differentially expressed, whereas the $HgCl_2$-exposed cells were still growing more
258 slowly than pre-exposure with 13% of their genes still differentially expressed compared
259 to the unexposed cells (Figure 1b).
260
261 *b. Shared and unique genes at each time point for each exposure.*
262 The total distinct DEGs across all time points was slightly lower for $HgCl_2$ (2,327)
263 than for PMA (2,541) exposure (Figure 1b and Figure S7). More striking were the
264 differences in DEGs at each time point; PMA-exposed cells modulated 20% more genes
265 at 10 min (2,181 vs 1,821) and 40% more at 30 min (2,007 vs 1,422) than $HgCl_2$
266 exposed cells. This trend completely reversed by 60 min when DEGs declined in both
267 exposure conditions but $HgCl_2$ exposed cells were still modulating ~9-fold more genes
268 (563) than PMA-exposed cells (65), consistent with the latter recovering normal growth
269 sooner (Figure 1a). Also notable is the carryover of DEGs from one time point to the
270 next where $HgCl_2$ exposed cells have 1,001 DEGs in common at 10 and 30 min after
271 exposure but that number is 65% greater in PMA-exposed cells (1,650) (Figure S7).
272 However, $HgCl_2$ exposed cells have 52% more DEGs that are unique at 10 min
273 compared to PMA-exposed cells (804 vs 529), i.e. more $HgCl_2$ provoked DEGs occur
274 sooner after exposure than later. $HgCl_2$ exposure also yields more DEGs that occur at

275 all time points compared to PMA (Figure S7) since there are very few DEGs at 60 min
276 for PMA exposure.
277
278 c. *Up-regulated vs. down-regulated genes for each exposure*
279 Sorting expression simply into genes up-regulated or down-regulated by $HgCl_2$ or
280 PMA at each time point (Figure 2) revealed additional quantitative distinctions between
281 them. For up-regulated genes, PMA-provoked more unique DEGs than DEGs in
282 common with $HgCl_2$ exposure at all time points, in contrast to $HgCl_2$ which had more
283 DEGs in common than unique at all but the last time point. This trend was not continued
284 for down-regulated genes where more genes were in common for both compounds than
285 unique at all time points.
286 Thus, early in exposure the cell reduced expression of a similar number of genes
287 for both mercurials, but up-regulated expression of many more genes in response to
288 PMA than to $HgCl_2$, and these distinct trends persisted to the middle time point. By 60
289 min, gene expression of PMA-exposed cells closely resembled that of unexposed cells,
290 but $HgCl_2$ exposed cells still have many up- and down-DEGs, consistent with slower
291 growth rate recovery by $HgCl_2$ exposed compared to PMA-exposed cells (Figure 1a).
292
293 *d. Differentially expressed genes grouped by functional category.*
294 To sort our observations from a different perspective, the DEGs for each
295 condition were grouped by Clusters of Orthologous Groups (COGs) to identify
296 expression differences based on gene functions (Figure 3 and Table S6). For most
297 COGs, both mercurials elicited the strongest response, up or down, within the first 10

298 min of exposure (Figure 3); in most cases, the responses were of similar magnitude. At
299 30 min, the PMA (green) responses remained nearly the same, up or down, but $HgCl_2$
300 provoked responses (red) generally diminished, often sharply. By 60 min, expression in
301 PMA-exposed cells was barely distinguishable from the unexposed cells in all COG
302 categories, whereas $HgCl_2$ exposed cells had notable differential expression in most
303 COG categories.

304 The four well-defined COG categories with the most DEGs were energy
305 production (C), amino acid metabolism (E), carbohydrate metabolism (G), and
306 transcription (K), which are also categories with a large number of genes (284, 355,
307 381, and 294 respectively) in *E. coli* (Figure 3 legend). COG categories R and S
308 encoding poorly defined (261) or non-defined (203) genes were also represented
309 proportionally. These data suggest both mercurials broadly affect most metabolic
310 categories, albeit to different degrees and at different rates. However, four well-defined
311 COG categories have strikingly different responses to $HgCl_2$ and PMA. COG categories
312 for nucleotide metabolism (F), translation (J), motility (N), and intracellular trafficking (U)
313 have much less up-regulation in $HgCl_2$ exposed cells than in PMA-exposed cells. There
314 are relatively few DEGs involved in cell division (D), extracellular structures (W), and
315 mobile genetic elements (X) and genes within these categories responded similarly to
316 both mercurials. Thus, grouping DEGs by COGs emphasizes the broad functional
317 differences and similarities between $HgCl_2$ and PMA exposure. Furthermore, this
318 approach highlights that DEGs occur in all functional categories for both compounds,
319 but still display distinct differences in transcriptional response to each compound. We
320 provide gene-level detail on several of these functional groups below. Note that the

321 COG database only includes functional annotations for 3,398 of *E. coli's* 4,497 genes,
322 but functional categories discussed below in more detail are not limited to COG-
323 annotated genes.

324 A heat map of the DEGs log-fold changes (Figure 4) provides a more granular
325 look at all DEGs for both compounds across all time points. The heat map, using Ward's
326 minimum variance clustering method [36], shows considerable uniformity of up- and
327 down-regulated expression during the 30 min after PMA exposure. In contrast, although
328 $HgCl_2$ exposed cells grossly shared many DEGs with PMA-exposed cells (Figure 2), the
329 heat map reveals a more variegated response to Hg(II) in which different genes are up-
330 or down-regulated at all time points, in contrast to the relatively consistent response of
331 PMA during recovery. Overall, 34% of DEGs are unique to only one mercurial and ~3%
332 of all DEGs had an opposite response to each compound (Table S5). The most
333 dramatic differences were at 60 min when $HgCl_2$ exposed cells were still modulating
334 many genes but PMA-exposed cells had only minor differences with unexposed cells,
335 consistent with their faster recovery of normal growth (Figure 1a). We dissect some of
336 these differences in function-specific heat maps below.

337 We also used STRING (version 10.0) [37, 38] for unsupervised network analysis
338 to identify gene clusters that were up-regulated in response to each compound (Figure
339 S8 and Table S7). We focused on up-regulated genes on the working assumption that
340 they are more likely to contribute to recovery than genes whose expression is turned
341 down. Gene clusters were generated by STRING based on organism specific data
342 mining to identify genes with a functional association, such as a common biological
343 purpose, location within the same operon, or shared regulatory mechanism. Note that

344 this network algorithm does not consider fold-change intensity of response; it
345 enumerates only whether an up-regulated gene is present at a given time point. The up-
346 regulated DEGs (nodes) of $HgCl_2$ exposed cells formed several tight clusters
347 encompassing 16 gene-ontology functions (GOFs) at 10 min, nine GOFs at 30 min, and
348 seven GOFs at 60 min (Figure S8 and Table S7). In contrast, although there were more
349 nodes for PMA-exposure at 10 and 30 min, there were fewer edges yielding no well-
350 defined clusters at 10 min and only two GOFs at 30 min. This network analysis
351 suggests that, although PMA provokes more DEGs than Hg(II) does, there is less
352 functional congruence between the genes involved in the response to PMA. Specific
353 gene and function changes are discussed further in the next section.
354 Lastly, as a control for using RNA-Seq in a longitudinal experiment, we observed
355 DEGs at sequential time points in the unexposed control culture (Figure S9 and Table
356 S8). As expected, changes were gradual over time with no more than 5% of the
357 genome being differentially expressed from one time point to the next. At the 60 min
358 time point, as the cells approached stationary phase 815 genes were differentially
359 expressed compared to mid-log (time 0). Sorting these DEGs by COGs (Figure S9) and
360 by STRING network analysis (Figure S10 and Table S7) showed, as expected, many
361 DEGs were consistent with normal transitioning from mid-log to early stationary phase
362 [39, 40].
363
364 ***Higher resolution view of expression differences in specific functional groups***
365 ***during recovery from exposure to $HgCl_2$ or PMA***

366 Taking the perspective that a toxicant is a kind of signaling molecule, we
367 considered differences in gene expression for the two Hg compounds to reflect how the
368 cell senses the biochemically distinct damage produced by these two metallo-
369 electrophiles as manifest by what tools the cell calls upon to restore its viability. A quick
370 snapshot of the great extent of these compound-specific differences can be seen in the
371 genes with a >20-fold increase in differential expression after $HgCl_2$ exposure (Table 1)
372 or PMA exposure (Table 2). Here we emphasize up-regulated genes on the working
373 assumption they could contribute directly or indirectly to repairing damage caused by
374 mercurial exposure. The 25 genes highly up-regulated by $HgCl_2$ (Table 1) are involved
375 in altering the cell surface, oxidative stress response and repair, protein chaperones,
376 metals homeostasis, and ribonucleotide reductase. The vestigial prophage genes likely
377 play no rescue role for the cell and were simply activated by generalized stress
378 responses. The corresponding PMA response echoed only 8 of these 25 $HgCl_2$ high-
379 responders, and notably did not include the vestigial phage genes.
380 For PMA, the highly up-regulated genes are a distinct contrast to those for $HgCl_2$.
381 First, the maximum amplitude of the PMA-provoked differential expression is generally
382 much less than for Hg(II)-provoked high differential expression (Table 1), which could
383 reflect the lower uptake of PMA. Secondly, while 11 of the 17 PMA-provoked genes
384 were also on the $HgCl_2$ highly differential expression list, ion transport and antibiotic
385 resistance loci were more prominent with PMA and prophage genes were absent.
386 These two snapshot tables make the points that both mercurials generate broad,
387 but idiosyncratic, cellular responses. To place these “tips of many icebergs” in their
388 larger cellular context, we used heat maps and tables of subsets of functionally related

389 genes to discuss the differential effects of $HgCl_2$ and PMA on twelve canonical cellular
390 systems in the following sections.
391
392 i. INFORMATIONAL MACROMOLECULES
393 *(a) DNA replication, recombination and repair*
394 Of the 24 genes for initiation and maintenance, and termination of chromosome
395 replication, there were more genes down-regulated (8) than up-regulated (3) in
396 response to $HgCl_2$ and an equal number up- or down-regulated genes (7) in response to
397 PMA (Table S9). Of the 14 genes encoding the replicative polymerase holoenzyme, four
398 genes capable of translesion synthesis (*polB*, *dinB*, *umuCD*) were up-regulated more by
399 $HgCl_2$ exposure (Table S9), suggesting a greater degree of direct or indirect DNA
400 damage by $HgCl_2$ exposure. Of the 45 genes for repair and recombination proteins the
401 transcriptional response to each mercurial was very similar (11 up-regulated and 16
402 down-regulated for $HgCl_2$; 9 up-regulated and 18 down-regulated for PMA). But there
403 were repair genes unique to each compound: *xthA*, *uvrAB*, *mutM,* and *recN* were only
404 up-regulated by $HgCl_2$; and *mutH* and *mutY* were only up-regulated by PMA.
405 The *recA, recN,* and *xthA* DNA repair genes were the most highly up-regulated
406 (≥10 fold) in response only to $HgCl_2$. The *recA* gene, induced by double-strand DNA
407 breaks, serves multiple roles in DNA repair [41, 42]. Curiously, expression of *recBCD,*
408 which is needed for break repair, either did not change or declined compared to
409 unexposed cells for both mercurials. Expression of several genes involved in repair
410 (*recG*, *nth*, *hsdS*, and *mcrC*) were down-regulated by both compounds, but with much
411 larger negative fold-changes for PMA than $HgCl_2$. Thus, the cells responded quickly to

412 both mercurials, but some distinct responses suggest these two compounds directly or
413 indirectly yield different kinds of DNA damage.
414
415 *(b)Transcription*
416 Of the core RNA polymerase (RNAP) genes only PMA-exposure increased
417 expression of a single gene *rpoZ* (ω subunit), but expression decreased in the
418 remaining *rpoABC* core genes (Table S10). $HgCl_2$ exposure did not change expression
419 of any RNAP core genes except for a transient 3-fold drop in *rpoA* at 30 min. Only one
420 of the five termination factors, the Rho-directed anti-terminator, *rof,* increased and did
421 so for both mercurials with PMA again provoking a greater response.
422 Genes for three sigma factors displayed increased expression upon exposure to
423 either mercurial, with *rpoH* (heat shock sigma factor) and *rpoS* (stationary phase and
424 stress response sigma factor) increasing more following PMA-exposure and *rpoD*
425 (housekeeping sigma factor) only increasing after $HgCl_2$ exposure. The effects of $HgCl_2$
426 or PMA exposure on the regulation of genes within each regulon controlled by *E. coli's*
427 seven sigma factors are tabulated in Table S11. Many genes are modulated
428 differentially by $HgCl_2$ or PMA-exposure, but no single sigma factor is uniquely
429 responsible for increases or decreases in responses to these two compounds.
430 Many of the 203 transcriptional regulators annotated in the RegulonDB (Table 3)
431 [43, 44] and the 1,723 genes they control were expressed differently with the two
432 mercurials (Table S12). PMA provoked up-regulation of significantly more transcription
433 factor genes at 10 and 30 min than $HgCl_2$ exposure, but slightly fewer down-regulated
434 regulators (Table 3). Of all COG categories, transcription had the most up-regulated

435 genes for both mercurials (Figure 3 and Table S6). PMA up-regulated ~40% more
436 transcription related genes at 10 min and ~80% more genes at 30 min than $HgCl_2$. Six
437 activators (*mhpR, glcC, gadX, soxS, mlrA, phoB*) and three repressors (*mcbR, iscR,*
438 *betI*) were up at all times for $HgCl_2$, but *gadX* was the only activator gene up-regulated
439 at all times for both mercurials (Table S12). GadX is part of the RpoS regulon [45] and
440 activates the acid resistance system and multidrug efflux [46, 47]. Details of
441 transcription factors and their regulons are provided in Table S12.
442 Lastly*, E. coli* has 65 currently annotated (ASM584v2), small non-coding RNAs.
443 Although our RNA purification and library preparation methods were not optimized for
444 their enrichment, we observed differential expression for a number of them (Table S5,
445 feature type "ncRNA"). ncRNAs up-regulated for both mercurials are involved in
446 regulation of acid resistance (*gadY*), oxidative stress (*oxyS*)*,* and multiple transporters
447 (*gcvB* and *sgrS*). In contrast, adhesion and motility (*cyaR*), and anaerobic metabolism
448 shift (*fnrS*) were down-regulated by both compounds.
449
450 *(c)Translation*
451 Upon $HgCl_2$ exposure 83% and 74% of ribosomal proteins (r-proteins) were
452 down-regulated at 10 and 30 min, respectively, versus only 4% and 41% for PMA at the
453 corresponding times (Figures 5 and S9; data for all functional group heat maps are
454 shown in Table S13 and Table S14). Transcription of r-proteins is repressed directly by
455 binding of the nutritional stress-induced nucleotide ppGpp and DksA protein to RNAP
456 [48]. The ppGpp synthase genes, *spoT* and *relA*, were down-regulated or unchanged,
457 but expression of *dksA* was up-regulated for both $HgCl_2$ and PMA exposure. R-protein

458 expression can also be inhibited by excess r-proteins binding to and inhibiting
459 translation of their own mRNAs [49, 50].
460 Translation initiation and elongation factors were largely unchanged, but
461 expression of all three peptide chain release factor genes were down for PMA and the
462 ribosome recycling factor (*frr*) was up only for PMA, consistent with interruption of
463 translation. Eight tRNA-synthase genes declined with $HgCl_2$ but PMA caused only four
464 tRNA-synthase genes to decline and two to increase in expression (Table S14). Both
465 mercurials caused a relative decline in tRNA expression for most amino acids,
466 especially arginine, lysine, methionine, tyrosine, and valine tRNAs. With very few
467 exceptions ribosome assembly and translation were shut down for up to 30 min by both
468 compounds but returned to normal levels by 60 min.
469
470 *(d) Macromolecular turnover and chaperones*
471 Divalent inorganic mercury can stably crosslink proteins and their subdomains
472 via cysteines, disrupting 3-dimensional structures and allosteric movements [51-53].
473 Although monovalent PMA cannot cross-link, it forms a bulky adduct with cysteines
474 (LaVoie, 2015), which may compromise protein folding. The proteases and chaperones
475 of the heat shock response degrade or repair misfolded proteins [54] and we found their
476 expression was increased by both mercurials (Figures 6 and S12 and Table S13). At 10
477 min, expression of protease genes *lon, clpXP* and *ftsH* had risen 4- to 6-fold with $HgCl_2$
478 and *lon* and *clpXP* were up 3-fold with PMA. $HgCl_2$ provoked up-regulation of all 12 heat
479 shock protein (HSP) and chaperone genes by 10 min, but only chaperones *clpB* and
480 *ybbN* mRNAs remained elevated at 30 min. Two other HSP genes, Hsp15 (*hslR*)

481 involved in stalled ribosome recycling and Hsp31 (*hchA*) an amino acid deglycase, were
482 further up-regulated by $HgCl_2$ at 60 min. In contrast, at 10 min PMA had up-regulated
483 only five HSPs, increasing to six by 30 min and declining to three by 60 min. The *ibpA*
484 and *ibpB* chaperone genes were among the most highly up-regulated genes for both
485 $HgCl_2$ and PMA and persisted throughout recovery.
486 Of the 16 RNases and RNA processing enzymes only 3 increased: RNase R (3-
487 fold for $HgCl_2$ and 5-fold for PMA) for both compounds during first 30 min; RNase III
488 two-fold for PMA at 30 min; and RNase T two-fold for $HgCl_2$ at 60 min. The
489 degradasome complex subunit genes (*rne, eno, rhlB, pnp, ppk*) [55] were all down-
490 regulated for PMA during the first 30 min following exposure, except the helicase (*rhlB*),
491 but only enolase was down-regulated for $HgCl_2$ (Figures 6 and S12 and Table S13).
492 Expression of RNase II (*rnb)* was also down-regulated for both compounds, but with
493 greater fold-changes observed for PMA. It is unclear what effect these changes in gene
494 expression could have on RNA turnover and message decay rates while under mercury
495 stress. As with the DNA metabolism genes, expression of the transcriptional apparatus
496 shows that sufficient PMA was taken up to elicit both positive and negative responses
497 distinct from $HgCl_2$.
498
499 ii. ENERGY PRODUCTION
500 *(a) Electron transport chain*
501 Expression of approximately 50% of all electron transport chain (ETC) genes was
502 down-regulated during the first 30 min for $HgCl_2$ and PMA, with individual gene
503 responses being very similar for both compounds (Figures 7 and S13 and Table S13).

504 By 60 min, only 26% of these genes were down-regulated for $HgCl_2$, and none were
505 down-regulated for PMA. Expression of NADH:unbiquinone oxidoreductase genes was
506 down-regulated by both compounds, with 77% and 100% of these genes being down-
507 regulated at 10 and 30 min, respectively. The ATP-synthase subunit genes were also
508 strongly down-regulated by both mercurials at 10 and 30 min, but normal expression
509 was restored at 60 min.

510 The *torCAD* locus that encodes the trimethylamine N-oxide anaerobic respiratory
511 system was strongly up-regulated only by PMA exposure. This is likely an artifact of the
512 dimethyl sulfoxide used to dissolve PMA, although the final DMSO concentration,
513 0.015% vol/vol (2.1 mM), was not expected to have any biological effect [56] and the
514 anaerobic DMSO reductase genes (*dmsABC*) were down-regulated. It is unlikely that
515 either the *tor* or *dms* responses significantly affect growth rate [57] or afford protection
516 against either mercurial since over-expressed heme-dependent *torC* may be in the
517 apoprotein form [58].

518

519 *(b) Carbon metabolism*

520 Expression of genes for carbon metabolism decreased generally, but there were
521 more up-regulated genes in response to $HgCl_2$ and not all steps were affected equally
522 by both mercurials (Figure S14). Expression of five genes of the pentose-phosphate
523 pathway rose in at least one time point for $HgCl_2$, but only *pgl* increased for PMA at 10
524 min. The ribose-5-phosphate isomerase gene (*rpiB*), which is a backup enzyme for the
525 gene product of *rpiA* [59], was up-regulated 40-fold for $HgCl_2$ at 10 min; although
526 expression of *rpiA* did not differ from the unexposed cells for either mercurial at any

527 time. Glycolysis responded similarly to both mercurials, with the greatest number of
528 these genes being down-regulated at 30 min. The expression changes in TCA cycle
529 genes were distinct for $HgCl_2$ and PMA; six genes were up-regulated in at least one
530 time point for $HgCl_2$ and only one was up-regulated for PMA. Expression of several
531 carbohydrate transport genes was down-regulated by both mercurials (Table S6).
532
533 *(c) Nicotinamide adenine dinucleotide (NAD)*
534 Expression of genes for nicotinamide adenine dinucleotide (NAD) and NAD-
535 phosphate (NADP) synthesis and turnover pathways was repressed by mercury
536 exposure (Figure S15). The biosynthesis genes were moderately down-regulated, with
537 *nadB* being the only gene down for both mercurials at all times and *nadA* decreasing for
538 $HgCl_2$ at 30 and 60 min and for PMA at 30 min. Expression of the *pncABC* salvage
539 pathway did not change. The NAD reduction pathways were more affected than the
540 NADP reduction pathways, with only *pgi* down for both mercurials and *edd* down only
541 for $HgCl_2$. The transhydrogenase (*pntAB*) was down only for PMA at 10 and 30 min.
542 Expression of other genes for NAD to NADH reduction in glycolysis and the TCA cycle
543 were also down for both mercurials, which reflects the overall decrease in metabolism
544 and energy production pathways.
545 Globally, redox metabolism declined immediately after exposure and normal
546 gene expression levels were not restored until growth recovered to the pre-exposure
547 rate. KEGG maps created using iPath [60] depict system-wide metabolism changes
548 over time (Figures S16-S18 for $HgCl_2$ and Figures S19-S21 for PMA).
549

550 iii. CENTRAL METABOLISM

551 *(a) Amino acid metabolism and transport*

552 The two mercurials had distinct effects on expression of genes for biosynthesis of
553 amino acids (Figure 8 and S22). Since mercury targets cysteine thiol groups and will
554 deplete the cellular reduced thiol pool, we expected an increase in cysteine and
555 glutathione biosynthesis. Surprisingly, most genes for biosynthesis of these biothiols
556 and for general sulfur metabolism were down-regulated or no different from the
557 unexposed cells, with the exception of up-regulation of *cysE*, which is the first step in
558 the biosynthesis pathway from serine.

559 Methionine biosynthesis gene expression increased for 7 genes with $HgCl_2$, but
560 11 genes were down-regulated with PMA, especially *metE* dropping 187-fold with PMA
561 at 30 min (Figure 8 and S22). Expression of genes for histidine synthesis also
562 responded differently to each mercurial, rising dramatically with $HgCl_2$ at 30 to 60 min.
563 In contrast, all *his* genes expression dropped with PMA from 10 to 30 min. Genes for
564 the synthesis of leucine, isoleucine, and valine had the opposite response, with most
565 going down with $HgCl_2$ but increasing with PMA. Expression of other amino acid
566 biosynthetic pathways was largely unchanged or declined with both mercurials.
567 Branched-chain (*livKHMGF*), dipeptide (*dppABCDF*), and oligopeptide (*oppABCDF*)
568 transporters were also down for both mercurials, with greater negative fold-changes for
569 PMA (Table S5).

570

571 *(b) Inorganic ion transport and metallochaperones*

572 Inorganic Hg(II) can displace beneficial thiophilic metals from their native binding
573 sites in proteins, potentially affecting transport and disrupting transition metal
574 homeostasis [23], leading to expression changes for non-ferrous metal cation and
575 oxoanion transporters (Figures 9 and S23 and Table S13), iron homeostasis (Figures
576 10 and S24 and Table S13) and metal-binding proteins and enzymes (Table S15).
577 Inorganic mercury exposure releases labile iron, which could itself increase
578 oxidative stress via Fenton chemistry under aerobic growth [23, 61]. Most iron uptake
579 pathways declined early for both mercurials, consistent with the observed increase in
580 expression of the Fur repressor. The cytochrome *c* maturation genes that transport
581 heme to the periplasm (*ccmABCDE*) were also down for both mercurials. The putative
582 ferrous iron and zinc efflux pump, *fieF* [62] increased 2-fold for $HgCl_2$ at 10 min only,
583 suggesting it may have a brief role in restoring one or both of these homeostases.
584 There are two iron-sulfur (Fe-S) cluster assembly pathways in *E. coli* [63, 64].
585 Expression of the primary Isc system (*iscRSUA*, *hscBA*, and *fdx*) increased strongly for
586 both mercurials, but with greater changes for $HgCl_2$ (Figures 10 and S24 and Table
587 S13). The secondary Fe-S cluster assembly system *sufABCDSE*, which activates under
588 oxidative stress or iron limiting conditions also increased greatly, but only for $HgCl_2$.
589 These transcriptional responses confirm and extend biochemical findings [23] that Fe-S
590 clusters are more vulnerable to inorganic mercury than to organomercurials and the cell
591 quickly tries to repair this damage.
592 Zinc uptake via expression of *zupT* increased modestly from 3 to 5-fold for both
593 mercurials during the first 30 minutes (Figures 9 and S23 and Table S13). In contrast,
594 expression of the P-type ATPase zinc efflux pump, *zntA* [65], increased 20- to 40-fold

595 for both mercurials at 10 and 30 min and the periplasmic binding protein ZraP was up
596 throughout recovery. *E. coli* has two copper/silver efflux systems, Cue and Cus [66].
597 Surprisingly, the Cus system genes (*cusRS, cusCFBA)* primarily used under anaerobic
598 conditions were among the most down-regulated genes under PMA exposure. The Cue
599 system consists of the multi-copper oxidase, CueO, and a P-type ATPase, CopA, both
600 regulated by the MerR homolog, CueR. Both *cueO* and *copA* were up over 20-fold with
601 $HgCl_2$ at 10 and 30 min, but only 5-fold with PMA briefly at 10 min. The nickel uptake
602 system [67] (*nikABCDER*) was also very strongly down-regulated under PMA exposure
603 conditions through all times although expression of repressor NikR was unchanged,
604 except for a 3-fold increase with $HgCl_2$ at 10 min. Expression of the nickel and cobalt
605 efflux gene, *rcnA*, increased with $HgCl_2$ or PMA. Manganese (*mntH*), and magnesium
606 (*mgtA, corA*) uptake genes increased with both mercurials.
607 Inorganic anions used by *E. coli* include phosphate, sulfate, and molybdate and
608 genes for defense against arsenate, a phosphate mimic (Figures 9 and S23 and Table
609 S13). Expression of the ABC phosphate transport system (*pstSCAB*) genes increased
610 greatly for both mercurials, with PMA-provoked changes up to 165-fold for the
611 phosphate binding protein, *pstS*. The two-component phosphate regulatory system,
612 PhoBR increased 22-fold with $HgCl_2$ and 105-fold with PMA for *phoB*. Sulfate and
613 thiosulfate uptake by the ABC transporter (*cysPUWA*) decreased strongly with $HgCl_2$ at
614 30 min and PMA at 10 and 30 min. Expression of molybdate uptake (*modABC*)
615 increased with PMA during the first 30 min but only at 10 min with $HgCl_2$. The arsenate
616 resistance operon cannot effect Hg(II) resistance, but was highly induced by both

617 mercurials, perhaps through interacting with the three-cysteine metal-binding site of the
618 ArsR repressor [68].
619
620 iv. SURFACE FUNCTIONS
621 *(a) Cell wall biogenesis, porins, lps, efflux systems, and electrolyte balance*
622 The transcriptional response of peptidoglycan, membrane biosynthesis, and cell
623 division genes was similar for both mercurials (Figure S25 and Table S13). Expression
624 increased for roughly 20% of lipid biosynthesis genes, including those for cardiolipin,
625 and expression decreased for 20-30% of other lipid-related genes. Transcription of
626 genes for murein synthesis (*murCDEFGIJ*) in particular declined for both mercurials
627 during the first 30 min.
628 *E. coli* encodes several antibiotic resistance efflux systems that are up-regulated
629 by mercury exposure (Figures 11 and S26 and Table S13). The multiple antibiotic
630 resistance locus (*marRAB*), which increases drug efflux and also limits passive uptake
631 by decreasing porin expression [69], was strongly up-regulated by both mercurials with
632 greater changes observed for PMA. Though expression of some porin genes (*ompC,*
633 *ompF, ompT, ompW*) was repressed, three non-specific porins (*ompG, ompL, ompN*)
634 were up-regulated only by PMA. Genes from several TolC-dependent antibiotic efflux
635 systems were up-regulated by both mercurials as well, including *acrEF, emrD, emrKY,*
636 and several *mdt* genes [70]. $HgCl_2$ exposure alone also up-regulated two-component
637 sensor genes (*phoQP* at 10 min and *basSR* at 60 min) that regulate genes involved in
638 modification of the cell surface and increase polymyxin resistance [71], but most of
639 these genes were down-regulated or unchanged for PMA.

640 The response to osmotic stress and maintenance of electrolyte balance are
641 important membrane functions requiring adaptation in dynamic natural environments.
642 During $HgCl_2$ exposure the expression of the sodium antiporter, NhaA, increased 4-fold
643 at 10 min and the calcium/potassium antiporter, ChaA, was up 3-fold at 10 and 30 min
644 (Figures 9 and S23 and Table S13). In contrast, expression of genes for transport of the
645 major electrolyte, potassium, changed only modestly in some subunits of the *kdp, kef*,
646 and *trk* systems, without an obvious response pattern. However, transcription of genes
647 for defense against osmotic stress was uniformly up-regulated; betaine genes (*betABIT*
648 *and proP*), *osmBCEFY, and* mechanosensitive channel proteins (*mscL* and *mscS*)
649 increased for both mercurials, as did a putative osmoprotectant ABC permease
650 (yehYXW) [72] only with $HgCl_2$ at 30 and 60 min (Table S5).
651
652 *(b) Motility and biofilm*
653 Nearly all flagellar component genes were strongly down-regulated for both
654 mercurials, with negative changes being much greater for PMA than for $HgCl_2$ exposure
655 (Figures 12 and S27). Only PMA increased expression of fimbriae and curli fiber genes,
656 which alter motility and increase adhesion (Figure 13 and S28) [73]. Fifteen genes up-
657 regulated by PMA exposure were annotated as homologs of FimA, but with unknown
658 function. FimA is the major structural component of fimbriae, but these genes may serve
659 other functions. Motility genes whose expression dropped remained low until 60 min,
660 indicating that the structurally and energetically intensive motility systems are very slow
661 to recover.

662 $HgCl_2$ and PMA also provoked expression of several biofilm-related genes
663 (Figures 13 and S28 and Table S13). The *bhsA* and *bdcA* loci were among the most
664 highly up-regulated genes during $HgCl_2$ exposure, with much higher fold-changes than
665 observed for PMA (Tables 2 and 3). Neither gene is well characterized, but
666 independently each has been found to decrease biofilm formation and increase
667 resistance to external stressors [74, 75]. Only PMA increased expression of genes for
668 poly-β-1,6-N-acetyl-glucosamine (PGA) polysaccharide production [76] and biofilm
669 related genes, *ycgZ, ymgA, ariA, ymgC* [77]. Thus, PMA elicits a broader response that
670 potentially alters the cell surface and may increase adhesion and biofilm formation; in
671 contrast $HgCl_2$ only inhibits motility and does not activate adhesion pathways. It is
672 possible that some changes observed for motility and biofilm related genes following
673 PMA-exposure are an artifact of the DMSO, but other studies suggest that solvent
674 would have no effect or that much higher concentrations than used here would be
675 required to induce these changes [56, 78].
676
677 v. STRESS RESPONSES
678 *(a) Oxidative stress response and repair*
679 There are two oxidative stress response pathways in *E. coli*, the *oxyRS* and
680 *soxRS* regulons [61, 79]. OxyR, a LysR-family transcriptional regulator, uses a cysteine-
681 pair to sense oxidative damage and regulates 49 genes when oxidized [80]. $HgCl_2$
682 exposure increased expression of 22 OxyR regulon genes at 10 min; these then
683 declined to 13 genes by 60 min (Table S16). In contrast, PMA provoked expression of
684 16 OxyR regulon genes at 10 and 30 min, but none at 60 min. *OxyS*, a small non-

685 coding RNA regulated by OxyR, represses *rpoS*, *fhlACD* and other genes to prevent
686 redundant induction of stress response genes [81]. The *oxyS* gene was among the most
687 highly expressed genes with over a 1,000 fold-change with $HgCl_2$ at 10 and 30 min, and
688 a more modest change of 10-fold at 10 min and 6 fold at 30 min with PMA.

689 The SoxRS regulon is the other oxidative stress response system in *E. coli*.
690 SoxR, a MerR-family repressor-activator, uses the oxidation state of 2Fe-2S clusters to
691 respond to superoxide (O_2^-) stress and induce transcription of *SoxS* [82-85], which then
692 transcriptionally regulates 53 genes [79, 86] (Table S16). $HgCl_2$ or PMA exposure up-
693 regulated 22 or 25 genes, respectively, at 10 min and these had declined to 13 or 0
694 genes, respectively by 60 min.

695 Key genes in these oxidative stress regulons differentially expressed upon
696 mercury exposure include the ROS scavengers: catalase (*katG*), alkyl hydroperoxide
697 reductase (*ahpCF*), and superoxide dismutase (*sodA*) (Figures 14 and S29 and Table
698 S13). Thiol homeostasis genes included *gor*, *grxA,* and *trxC* (Figures 14 and S29 and
699 Table S13). Iron homeostasis and the Fe-S cluster assembly and repair genes (*fur, dps,*
700 *fldA, fpr, hemH, sufABCDES,* and *yggX*) were also up-regulated. PMA provoked
701 comparatively lower fold-changes than $HgCl_2$ for *grxA, trxC, ahpC, dps, fldA, hemH, and*
702 *yggx*. The manganese uptake protein, *mntH*, plays an important role in ROS resistance
703 [87] and was up for both mercurials. Oxidation-resistant dehydratase isozymes, *acnA*
704 and *fumC* [88, 89] also increased, but only for $HgCl_2$ exposure. Thus, both mercurials
705 triggered the Oxy and Sox oxidative stress responses, but $HgCl_2$ elicited greater fold-
706 changes overall than PMA compared to unexposed cells.

707 Because mercury poisons the cellular thiol pool [23], we expected that regulation
708 of redox homeostasis proteins such as glutaredoxins, thioredoxins and glutathione-
709 related genes would respond to mercury exposure (Figure 14 and S29). Glutaredoxin 1
710 (*grxA*) expression was up for both mercurials with a greater fold-change for $HgCl_2$, but
711 glutaredoxin 2 (*grxB*) was down for both mercurials, while glutaredoxin 3 (*grxC*) and
712 glutaredoxin 4 (*grxD*) were up only for PMA. Thioredoxin reductase (*trxB*) increased
713 two-fold with PMA (30 min only), but was up with $HgCl_2$ 8-fold (10 min) and 4-fold (30
714 min). Thioredoxin 1 (*trxA*) expression increased two-fold only with PMA (10 min), in
715 contrast to thioredoxin 2 (*trxC*), which was up for both mercurials, but much higher with
716 $HgCl_2$ than with PMA. However, the thiol peroxidase (*tpx*) was up modestly for PMA, but
717 did not change for $HgCl_2$. Thus, while each mercurial stresses the cell to maintain redox
718 homeostasis, a greater burden is created by $HgCl_2$ exposure.
719 Glutathione (GSH) serves as the cell's redox buffer and as a scavenger of
720 mercurials (Figures 14 and S29 and Table S13). Surprisingly, expression of GSH
721 biosynthesis and utilization genes increased only modestly. The γ-
722 glutamyltranspeptidase (*ggt*) increased late (60 min) by 4-fold only with $HgCl_2$. GSH
723 synthase (*gshA*) did not change and *gshB* was up 2-3 fold for both mercurials only at 10
724 min. The GSH importer (*gsiABCD*) may be a salvage pathway to recover GSH and
725 cysteine leaked into the periplasm by CydCD [90, 91], but it was down-regulated by
726 $HgCl_2$ or PMA through 30 min. The GSH reductase (*gor*) increased only with $HgCl_2$ at
727 10 min and several GSH S-transferase genes involved in detoxification [92] (*gstA, gstB,*
728 *yfcF, yqjG, yibF, yncG*) increased with both mercurials. Since all of these proteins have
729 Hg(II)-vulnerable cysteines in their active sites, it is surprising that neither Hg(II) nor

730 PMA-challenged cells provoked increased expression and suggests that their normal
731 mRNA levels are sufficient to replenish them.
732
733 *(b). Genes with delayed up-regulation*
734 Genes unchanged at 10 min but differentially expressed at both 30 and 60 min or
735 60 min alone may be those needed as cells transition out of stasis and towards normal
736 growth (Table S17). For $HgCl_2$ exposure, 95 genes were up-regulated and 140 genes
737 were down-regulated that display this delayed response pattern. Approximately half of
738 the up-regulated genes are involved in energy production, transport and metabolism
739 pathways based on COG annotations. Roughly 45% of these delayed $HgCl_2$ provoked,
740 up-regulated genes are the same as genes that were differentially expressed during the
741 first 30 min of PMA exposure. This overlap is consistent with slower recovery of growth
742 in $HgCl_2$ exposed cells and that some of the same pathways are used for recovery by
743 both compounds. In contrast, for the more quickly recovering PMA exposure, of the
744 genes that showed no change at 10 min only six were up-regulated at 60 min (Table
745 S17). Only two of these delayed-response genes for PMA exposure overlapped with up-
746 regulated genes for $HgCl_2$ exposure.
747
748 **DISCUSSION**
749 Mercury is a ubiquitous toxicant that serves no biologically beneficial role.
750 Exposure to any form of mercury negatively impacts the health of organisms from
751 microbes to humans. The biological effects of different forms of mercury are often
752 conflated and methylmercury is assumed to be the most toxic form. However, the

753 systemic biochemical and molecular differences between inorganic and organic mercury
754 compounds have yet to be well characterized from exposure through recovery in a
755 single model system.
756
757 ***BULK DIFFERENTIAL EFFECTS ON GROWTH AND GENE EXPRESSION***
758 The sub-acute mercury exposure conditions used in this study were chosen by
759 identifying a mercury concentration high enough to stop cells from doubling, but low
760 enough to allow restoration of the pre-exposure growth rate within one-hour (~1
761 generation period) after exposure (Figure 1a). Concentrations below 3 μM of either
762 compound did not consistently inhibit growth and higher concentrations of $HgCl_2$ did not
763 allow recovery within the desired time frame. Three micromolar Hg is well within the
764 range that bacteria can experience chronically from dental amalgam fillings [93] and in
765 highly contaminated environments, such as artisanal gold mining operations [8].
766 Mercury in tuna is 0.386 ppm compared to the proxy organomercurial, PMA, used here
767 at 3 μM or 0.6 ppm [94].
768 PMA-exposed cells recovered exponential growth faster (Figure 1) than those
769 with equimolar exposure to $HgCl_2$, perhaps owing to lower uptake of PMA (Table S2).
770 However, PMA-exposed cells differentially expressed more genes than $HgCl_2$ exposed
771 cells during the first 30 min of exposure (Figure 1b). These results agree with
772 observations in *C. elegans,* where MeHg exposure resulted in four times more DEGs
773 than did $HgCl_2$ for all concentrations tested [95]. However, in contrast to *E. coli,* whose
774 growth was inhibited more by inorganic $HgCl_2$, in *C. elegans* the effective toxic
775 concentration of methylmercuric chloride was lower than for $HgCl_2$ [95].

776 We found that most DEGs peaked at 10 min after exposure for both compounds
777 with $HgCl_2$ provoking more down-regulation and PMA yielding more up-regulated genes
778 throughout the exposure period (Figure 1b). Even though the optical density (OD) of the
779 $HgCl_2$ exposed culture showed no growth recovery from 10 min to 30 min, DEGs
780 decreased by 22%, while PMA-exposed cells over the same period had a moderate
781 increase in OD, but only an 8% decrease in DEGs (Figure 1). In contrast in a eukaryotic
782 system, the livers of $HgCl_2$ exposed zebrafish continuously increased in DEGs
783 throughout the observed 96 hour exposure period as mercury accumulated in their cells
784 [96].
785 In *E. coli,* during the first 30 min post-exposure, 50-70% of both up- and down-
786 regulated genes were the same for both compounds (Figure 2), but at the level of
787 individual genes there were both qualitative and quantitative differences in expression
788 (Table S5), consistent with idiosyncratic transcriptional responses to each compound.
789 The nematode *C. elegans* also manifested distinct and even some opposite
790 transcriptional responses to inorganic and organic mercury exposure in a single end-
791 point microarray experiment [24].
792 As there are not yet other studies of the transcriptional response of a bacterium
793 to mercury exposure, on the basis of the findings in eukaryotes and our proteomic work
794 [23] we have organized our observations here into those we had expected and those we
795 did not expect from any yet published work.
796
797 ***EXPECTED AND UNEXPECTED GENE-SPECIFIC CHANGES***
798 *Expected transcriptional changes:*

799 *(a)* Thiol homeostasis. The millimolar cytosolic pool of glutathione (GSH) can sequester
800 mercurials and thereby protect protein thiols from binding strongly to these soft metal
801 toxicants. However, if the GSH pool becomes depleted by mercury complexation, the
802 cell loses this primary defense mechanism. Since proteins of the stress response and
803 repair pathways all contain active site thiols, often as part of Hg(II)-vulnerable Fe-S
804 centers, it is not obvious how a cell that has lost much of its available thiols to Hg(II)
805 chelation can restore its metabolism. Given this, we expected cysteine and glutathione
806 biosynthesis pathways to be up-regulated. However, cysteine biosynthesis was down
807 (Figures 8 and S22 and Table S13) and GSH biosynthesis was mostly unchanged
808 (Figures 14 and S29 and Table S13) for both compounds, in contrast to the eukaryotic
809 response to mercury [96-98] and H_2O_2 exposure [99], which increase GSH and
810 metallothionein production. However, although thiol biosynthesis did not increase,
811 genes involved in maintaining cellular thiol homeostasis did increase (Figures 14 and
812 S29 and Table S13); thioredoxin (*trxC*) and glutaredoxin (*grxA*) were among the most
813 highly up-regulated genes with $HgCl_2$ and PMA exposure. Others have also found in *E.*
814 *coli* that glutathione reductase increased with $HgCl_2$ and both compounds increased
815 expression of glutathione oxidoreductase and S-transferase genes, which protect
816 against oxidative stress and xenobiotics [100].
817
818 *(b)* Iron homeostasis. We also expected inorganic mercury to disrupt iron-sulfur clusters
819 with consequent effects on Fe homeostasis generally [23, 101]. Iron uptake was down-
820 regulated with both mercurials, consistent with excess intracellular free Fe(II) and
821 general oxidative stress, but expression of the uptake repressor (*fur*) was only up for

822 $HgCl_2$ (Figure 10 and S24). *Fur* expression is activated by either OxyR or SoxS [99,
823 102] and Fur represses Fe uptake pathways with ferrous iron as a co-repressor [103].
824 Fur can also bind other divalent metals [104], so Hg(II)-Fur might mimic Fe-Fur as an
825 iron uptake repressor under these conditions to limit Fenton-mediated damage from
826 excess iron. Although both mercurials increased expression of the primary Fe-S cluster
827 assembly and repair system (*isc*), only $HgCl_2$ induced the secondary system (*suf*),
828 which is normally induced under oxidative stress or iron limiting conditions [63, 105]
829 (Figures 10 and S24 and Table S13). Also, only $HgCl_2$ exposure increased expression
830 of iron storage proteins: ferritin, bacterioferritin, and Dps (Figure 10 and S24). The DNA
831 binding protein Dps which binds free iron to protect DNA from ROS damage [106] was
832 one of the most highly up-regulated genes with $HgCl_2$ exposure (Table 1).
833
834 *(c)* Oxidative stress response. The known close link between iron homeostasis and
835 oxidative stress [61] explains the high fold-changes upon $HgCl_2$ exposure in genes that
836 respond to oxidative stress (Table S16) and echoes mercury's long known stimulation of
837 oxidative damage in rat kidney mitochondria [107]. The small non-coding RNA *oxyS*
838 was the second most highly up-regulated gene upon $HgCl_2$ exposure with differential
839 expression more than 100-fold greater than observed for PMA (Table 1). The ROS
840 scavenger *ahpF* was also highly up-regulated, along with *katG* (early) and *sodA*
841 (delayed) but only for $HgCl_2$ (Figures 14 and S29 and Table S16). Other ROS resistant
842 enzymes, aconitase A [89] and fumarase C [108] (Table S16) also increased only for
843 $HgCl_2$, as did the manganese-dependent alternative ribonucleotide reductase genes
844 (*nrdHIEF*) [109]. The glutaredoxin-like protein that functions like thioredoxin, *nrdH* [110],

845 was highly up-regulated by $HgCl_2$ and might support other thioredoxin and glutaredoxin
846 proteins. These striking differences in gene expression illuminate how *E. coli* modulates
847 expression of specific genes not only to deal with compromised function of specific
848 $HgCl_2$ modified proteins, but also to manage the consequent cascade of reactive oxygen
849 species.
850
851 *(d)* Heat shock response. Mercurials bound to protein cysteines could disrupt protein
852 folding, subunit assembly, and allosteric movements [52, 111] and inorganic mercury
853 can crosslink neighboring cysteines leading to aggregation [51]. Increased expression
854 of heat shock response genes was expected as a consequence of such anticipated
855 protein misfolding problems [112]. Indeed, expression of heat shock chaperonins and
856 protease genes increased for both mercurials, with more genes up early in response to
857 $HgCl_2$ than to PMA (Figures 6 and S12 and Table S13). Genes for the small chaperone-
858 like proteins, *ibpA* and *ibpB*, were among the most highly increased for both
859 compounds, especially for $HgCl_2$ (Table 1), consistent with their role in aiding Lon
860 protease in the degradation of misfolded proteins [113-115].
861
862 *(e)* Translational apparatus. Thiophilic Cd^{2+} exposure in *E. coli* has been shown to
863 decrease expression of ribosomal proteins [116]. In our proteomics work (Zink et al. in
864 preparation) we observed fourteen r-proteins (7 for each ribosomal subunit) that formed
865 stable adducts with either PMA or Hg(II), so it was reasonable to expect this to be
866 reflected in transcription of r-proteins. Indeed, $HgCl_2$ exposure repressed expression of
867 up to 83% of r-protein genes at 10 min and 74% at 30 min (Figure 5, S11, and Table

868 S14), whereas PMA only briefly repressed expression of 41% of r-proteins at 30 min.
869 Divalent inorganic mercury's ability to cross-link proteins may interfere with ribosome
870 assembly resulting in translational feedback and repression of r-proteins transcription.
871 Disruption of ribosome assembly could also contribute to the slower recovery of growth
872 after inorganic Hg(II) exposure.
873
874 *(f)* Energy production. The dependence of most energy production pathways on redox-
875 active transition metals and redox-active sulfur compounds made them obvious targets
876 of mercurial disruption, e.g. three ATP-synthase subunits form stable adducts after in
877 vivo exposure to Hg(II) or PMA (Zink et al. in preparation). Intriguingly, expression of
878 genes within this functional category was largely down-regulated early in exposure, with
879 all nine ATPase subunits down for $HgCl_2$ (10 min) and eight down for PMA (30 min)
880 (Figure 7 and S13). Although others have found that Cd exposure in *E. coli* repressed
881 aerobic energy metabolism genes and induced anaerobic pathways [116], we found that
882 both aerobic and anaerobic energy metabolism were repressed by $HgCl_2$ and PMA.
883 Even though expression of the oxygen-sensing *fnr* [117] and *aer* [118, 119] activators of
884 the anaerobic shift were moderately up for PMA and unchanged for $HgCl_2$ (Table S5).
885 Similarly, glucose metabolism genes were also predominantly down during early
886 periods for both compounds, especially with PMA exposure (Figure S14). Thus, with
887 severely compromised energy production systems, it is not surprising that amino acid,
888 carbohydrate and nucleotide metabolism genes, and the energy-dependent transport of
889 these molecules (Figure 3 and Table S6), are also largely depressed initially.
890

891 *(g)* Homeostases of non-ferrous metals. We expected mercurials to disrupt electrolyte
892 balance [23], but expression of the potassium efflux pumps' subunit genes (*kcpABC,*
893 *kefBC, and trkAGH*) were not uniformly up-regulated, although the need to restore the
894 pH balance was indicated by briefly increased expression of the H^+/Na^+ antiporter
895 (*nhaA*) for both compounds (Figures 9, S23 and Table S5). It may be that normal levels
896 of the proteins involved in maintaining cellular electrolyte balance are sufficient to
897 respond to mercury exposure and a significant change in transcriptional expression is
898 not required for these genes.
899 Mercury is also expected to disrupt non-ferrous metal homeostasis because it
900 can displace other metals, such as zinc and copper, as enzyme cofactors [120, 121].
901 Expression of metal uptake genes decreased and of metal efflux genes increased for
902 zinc, copper, nickel and cobalt, with generally greater fold-changes for $HgCl_2$. Zinc efflux
903 by ZntA is regulated by the MerR homolog ZntR, which can respond to Hg(II) [122], but
904 has not been shown to confer resistance to Hg(II) exposure. Indeed, *in vitro* the ATPase
905 activity of the ZntA efflux pump is stimulated by Hg-thiolate complexes [65, 123]. This
906 could be a possible source for the decline in inorganic Hg content observed over time
907 (Table S2) once bound by glutathione or cysteine, but would not explain the increase at
908 60 min or decline in PMA, unless other efflux systems such as antibiotic resistance
909 systems are effective as well. Manganese may protect iron metalloenzymes under
910 oxidative stress conditions [87] and Mn uptake by *mntH,* as part of the OxyR regulon,
911 was correspondingly up-regulated for both compounds in response to mercury-induced
912 oxidative stress.
913

914 *Unexpected transcriptional changes:*

915 *(a)* Motility and chemotaxis. Energetically costly flagellar motility and chemotaxis were
916 strongly down-regulated by both mercurials and were among the slowest to recover
917 normal transcription levels (Figures 12 and S27 and Table S13). Motility gene
918 expression is regulated by σ^{28} (*fliA*) and FlhDC [124] and the expression of these two
919 regulatory genes declined with $HgCl_2$ but was unchanged with PMA. Repression of
920 motility may occur through sigma factor competition for binding to RNAP between σ^{28}
921 and increased expression of σ^{S} [125] and/or through repression of the flagellar
922 transcriptional activator FlhDC by increased expression of the small ncRNAs *oxyS* and
923 *gadY* [126]. Interestingly, $HgCl_2$ exposure impaired locomotion in *C. elegans* [24],
924 although through a very different mechanism of motility from *E. coli*.

925

926 *(b)* Surface appendages and biofilm synthesis. Surprisingly, there were large increases
927 in expression of genes involved in biofilm formation and adhesion or dispersal (Figures
928 13 and S28 and Tables S13). Expression of *bhsA* and *bdcA,* which function in biofilm
929 dispersal or reduced biofilm formation [74, 75], were the first and third, respectively,
930 most up-regulated genes by $HgCl_2$ (Table 1) and were also up for PMA, but with much
931 lower fold-changes (Table 2). Expression of *bhsA* is also up-regulated by other diverse
932 stressors and may decrease cell permeability [75, 127]. PMA exposure especially
933 increased expression of genes for the polysaccharide PGA, which aids in adhesion in
934 biofilm formation [76], and other biofilm formation (*ycgZ, ymgA, ariA, ymgC*) genes [77]
935 (Figures 13 and S28 and Table S13).

936 Expression of fimbriae (*fim)* and curli fibers (*csg*), important for adhesion in
937 biofilm formation [73] were also up-regulated only by PMA (Figure 13 and S28), as were
938 15 of 22 FimA homologs of unknown function. Outer membrane vesicle formation
939 (OMVs) could also play a role in detoxification, since an increase in formation of these
940 vesicles has been associated with heat shock, oxidative stress response, and biofilm
941 formation [128], which are responses up-regulated to varying degrees by both
942 mercurials. These are distinct differences between PMA and $HgCl_2$ response. PMA
943 provocation of biofilm formation and adhesion genes might be an artifact of its DMSO
944 solvent, but it is not obvious why $HgCl_2$ induces such high increases in biofilm dispersal
945 genes.
946
947 *(c)* Phosphate metabolism. Phosphate uptake genes were among the most highly up-
948 regulated genes for PMA exposure (Figure 9, S23, and Table 2). The PhoBR two-
949 component system controls expression of phosphate transport genes, as well as some
950 genes that increase virulence including those for fimbriae and biofilm formation [129].
951 Since expression of PhoBR and its regulon increases under phosphate limiting
952 conditions [130], it may be that PMA inhibits phosphate uptake by an unknown
953 mechanism, possibly through direct interaction with highly up-regulated PstS or this
954 could be an artifact of DMSO.
955
956 *(d)* Amino acid biosynthesis. Expression of most amino acid pathways was turned down
957 by both compounds, but a few responded uniquely to each mercurial (Figures 8 and
958 S22 and Table S13). Since methionine auxotrophy occurs under oxidative stress due to

959 ROS susceptibility of methionine synthase (MetE) [131], expression of methionine
960 biosynthesis genes may have been increased by $HgCl_2$, which provoked a stronger
961 oxidative stress response than PMA. However, since PMA did provoke some oxidative
962 stress response, it is curious that Met operon expression was strongly down under PMA
963 exposure. Next to its affinity for cysteine sulfur, Hg(II) binds the imino nitrogen of
964 histidine very strongly [132], so it was intriguing that histidine biosynthesis genes were
965 also up-regulated by $HgCl_2$ but down-regulated by PMA. It remains unclear how these
966 differences or the opposite responses for leucine, isoleucine and valine biosynthesis
967 help the cell survive mercurial exposure.
968
969 *(e)* Miscellaneous genes. Multiple antibiotic efflux systems and polymyxin resistance
970 surface modifications were up-regulated by $HgCl_2$ exposure, and even more so by PMA
971 (Figures 11 and S26 and Table S13). Chronic mercury exposure contributes to the
972 spread of multiple antibiotic resistant bacteria through co-selection of plasmid-borne
973 antibiotic and mercury resistance genes [133, 134]. Increased expression of antibiotic
974 resistance and surface components hint that low-level mercury exposure could prime
975 cells for increased antibiotic resistance. However, the ubiquity of plasmid- and
976 transposon-borne Hg resistance loci suggests that expression of these chromosomal
977 genes offers insufficient protection against the antibiotic or mercurial levels encountered
978 in clinical practice.
979 A handful of vestigial e14 or CPS-53 prophage genes were up-regulated by
980 $HgCl_2$ (Table 1) or PMA (Table S5), respectively. Some are known to increase

981 resistance to osmotic, oxidative, and acid stressors [135, 136], but their roles and
982 mechanisms have not been well defined.
983
984 *(f)* Differential expression of genes required for the same functional protein complex.
985 In many instances we observed that transcripts for subunits of the same enzyme,
986 protein complex, or component of a tightly articulated pathway were differentially
987 expressed. In some cases these proteins lie in distinct transcripts, which may
988 experience different turnover rates and in other cases the differences could be due to
989 transcriptional polarity. We have chosen not to deal explicitly with such paradoxes in
990 this work, which is sufficiently complex as it is, but will address them in future work.
991
992 **CONCLUSIONS**
993 The effects of mercury exposure in multicellular organisms have long been
994 studied at the physiological level but a global, fine grained understanding of the
995 differences in the precise biochemical sequelae of inorganic and organic mercury
996 exposure has been lacking. This study is the first to examine not only the transcriptional
997 response differences between inorganic mercury ($HgCl_2$) and an organomercurial
998 (phenylmercuric acetate) in a model microorganism, but also first to examine
999 longitudinally how the cell recovers from these chemically distinct compounds. Taken
1000 together with global identification of vulnerable protein targets (Zink et al. in preparation)
1001 and of damage to thiol and metal ion homeostasis upon acute mercurial exposure [23],
1002 the current work provides a quantitative systems-level description of the effects of *in*
1003 *vivo* mercury exposure in *E. coli*. What was striking and most challenging with this study

1004 was the breadth and diversity of the systems whose expression was affected by these
1005 two chemically distinct mercurials. Sub-acute exposure influenced expression of ~45%
1006 of all genes with many distinct responses for each compound, reflecting differential
1007 biochemical damage by each mercurial and the corresponding resources available for
1008 repair. Energy production, intermediary metabolism and most uptake pathways were
1009 initially down-regulated by both mercurials, but nearly all stress response systems were
1010 up-regulated early by at least one compound. These results echo the wide functional
1011 variety of proteins stably modified by these mercurials owing to the widespread
1012 occurrence of cysteines found in nearly all *E. coli* proteins. Microbiome studies are
1013 rapidly unveiling the importance of commensal bacteria to the health of all higher
1014 organisms. Our findings in this model commensal organism provide insights into how
1015 chronic mercury exposure might affect such complex microbial communities and,
1016 consequently, the health of the host. This work also serves as a foundation for studies
1017 now underway of how the widely found mobile Hg resistance (*mer*) locus assists the cell
1018 in recovery from Hg exposure.
1019
1020 **LIST OF ABBREVIATIONS:**
1021 Hg = mercury
1022 $HgCl_2$ = mercuric chloride
1023 PMA = phenylmercuric acetate
1024 PhHg = phenylmercury
1025 DMSO = dimethyl sulfoxide
1026 LB = Luria-Bertani medium

1027 NM3 = Neidhardt MOPS minimal medium

1028 NGS = next generation sequencing

1029 DEGs = differentially expressed genes

1030 CVAA = cold vapor atomic absorption

1031 GSH = glutathione

1032 Cys = cysteine

1033 MDR = multidrug resistance

1034 ncRNA = non-coding RNA

1035 COGs = clusters of orthologous groups

1036 GOFs = gene-ontology functions

1037 RNAP = RNA polymerase

1038 HSP = heat shock protein

1039 ETC = electron transport chain

1040 PGA = poly-β-1,6-N-acetyl-glucosamine

1041

1042 **DECLARATIONS**

1043 **Ethics approval and consent to participate:** Not applicable

1044 **Consent for publication:** Not applicable

1045 **Availability of data and materials:** The tabulated datasets supporting the conclusions

1046 of this article are included as additional files. The read counts and raw sequence data

1047 (.fastq) are stored in the Gene Expression Omnibus database

1048 (http://www.ncbi.nlm.nih.gov/geo/) with accession ID: GSE95575; they are available to

1049 reviewers upon request and will be open to the public when the manuscript has
1050 been published.

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1053 **Authors' contributions:** SL conceived and designed experiments, prepared biological
1054 samples, extracted ribosomal depleted RNA for RNA-Seq, performed all data analysis,
1055 and drafted manuscript. AOS was a major contributor in experimental design, feedback
1056 on data analysis, and in editing the manuscript. All authors read and approved the
1057 manuscript.

1058

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1066

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1453 **Table 1: Genes with ≥ 20 fold-change for at least two time points after $HgCl_2$ exposure (n = 25).**

Gene ID	Gene Name	Product Description	Hg t10	Hg t30	Hg t60	PMA t10	PMA t30	PMA t60
b1112	**bhsA**	Biofilm, cell surface and signaling defects, YhcN family	**1949**	**563**	**94**	**46**	**21**	n.s.
b4458	oxyS	OxyS sRNA activates genes that detoxify oxidative damage	**1524**	**1292**	**150**	10	6	n.s.
b4249	bdcA	c-di-GMP-binding biofilm dispersal mediator protein	**596**	**79**	7	16	2	n.s.
b3686	**ibpB**	Chaperone, heat-inducible protein of HSP20 family	**402**	**124**	**281**	**37**	**211**	9
b0812	dps	Stress-induced Fe-binding and storage protein	**339**	**52**	n.s.	4	2	n.s.
b4248	yjgH	Putative reactive intermediate deaminase, UPF0076 family	**296**	**24**	3	6	2	n.s.
b0849	grxA	Glutaredoxin 1	**246**	**33**	n.s.	14	4	n.s.
b1144	ymfJ	Function unknown, e14 prophage	**185**	**39**	n.s.	3	3	n.s.
b2582	**trxC**	Thioredoxin 2, zinc-binding; Trx2	**134**	**50**	7	**24**	**24**	n.s.
b1147	ymfL	Function unknown, e14 prophage	**95**	**29**	n.s.	n.s.	3	n.s.
b1146	croE	Cro-like repressor, e14 prophage	**90**	**40**	n.s.	n.s.	n.s.	n.s.
b3687	**ibpA**	Chaperone, heat-inducible protein of HSP20 family	**86**	19	**41**	10	**28**	4
b2531	iscR	Transcriptional repressor for isc operon; contains Fe-S cluster	**62**	**48**	9	12	18	n.s.
b1148	ymfM	Function unknown, e14 prophage	**49**	**21**	n.s.	n.s.	n.s.	n.s.
b4599	yneM	function unknown, membrane-associated; regulated by PhoPQ	**38**	**36**	n.s.	3	3	n.s.
b2673	nrdH	NrdH-redoxin reducing oxidized NrdEF	**35**	**127**	**21**	7	14	n.s.
b4030	**psiE**	Pho regulon, regulated by phoB and cAMP	**35**	**64**	17	**38**	**59**	n.s.
b1684	sufA	Scaffold protein for assembly of iron-sulfur clusters	**32**	**39**	5	n.s.	3	n.s.
b1748	**astC**	Succinylornithine transaminase; carbon starvation protein	**30**	**31**	**25**	**34**	**36**	n.s.
b4663	**azuC**	Function unknown; membrane-associated	**27**	**25**	**36**	**41**	**49**	n.s.
b0484	copA	Copper-, silver-translocating P-type ATPase efflux pump	**25**	**20**	n.s.	5	n.s.	-10
b2674	nrdI	Flavodoxin required for NrdEF cluster assembly	**21**	**73**	**22**	4	7	n.s.
b1747	astA	Arginine succinyltransferase, arginine catabolism	10	**20**	**29**	9	14	n.s.
b1020	**phoH**	ATP-binding protein, function unknown	8	**71**	**221**	**61**	**365**	n.s.
b4002	zraP	Zn-dependent periplasmic chaperone	3	**27**	**43**	n.s.	n.s.	-3

1454 Table is sorted by Hg at 10 min column. n.s. = not significantly different from unexposed culture and boldface highlights actual values

1455 ≥ 20-fold. Gene names in boldface have a ≥ 20 differential expression response to both Hg and PMA in at least one time point (n =

1456 8).

1457 **Table 2: Genes with ≥ 20 fold-change in at least two time points for PMA exposure (n= 17).**

Gene ID	Gene Name	Product Description	Hg t10	Hg t30	Hg t60	PMA t10	PMA t30	PMA t60
b3728	pstS	ABC phosphate transport system; periplasmic binding protein	n.s.	16	8	**86**	**165**	n.s.
b1020	**phoH**	ATP-binding protein, function unknown	8	**71**	**221**	**61**	**365**	n.s.
b0996	torC	c-Type cytochrome	n.s.	n.s.	n.s.	**55**	**65**	**77**
b4060	**yjcB**	Function unknown	**33**	11	12	**52**	**35**	n.s.
b0399	**phoB**	Positive response regulator for pho regulon	4	**22**	3	**51**	**105**	n.s.
b1530	**marR**	Transcription repressor of multiple antibiotic resistance	**26**	7	n.s.	**48**	**29**	n.s.
b1531	marA	Transcriptional activator for multiple antibiotic resistance;	15	9	n.s.	**46**	**31**	n.s.
b1112	**bhsA**	Biofilm, cell surface and signaling defects, YhcN family	**1949**	**563**	**94**	**46**	**21**	n.s.
b1532	marB	marRAB multiple antibiotic resistance operon	16	8	n.s.	**45**	**27**	n.s.
b4663	**azuC**	Function unknown; membrane-associated	**27**	**25**	**36**	**41**	**49**	n.s.
b4030	**psiE**	Pho regulon, regulated by phoB and cAMP	**35**	**64**	17	**38**	**59**	n.s.
b3686	**ibpB**	Chaperone, heat-inducible protein of HSP20 family	**402**	**124**	**281**	**37**	**211**	9
b1748	**astC**	Succinylornithine transaminase; carbon starvation protein	**30**	**31**	**25**	**34**	**36**	n.s.
b3469	**zntA**	Zn(II), Cd(II), and Pb(II) translocating P-type ATPase	**40**	19	8	**29**	**22**	n.s.
b2582	**trxC**	Thioredoxin 2, zinc-binding; Trx2	**134**	**50**	7	**24**	**24**	n.s.
b4354	yjiY	Predicted transporter, function unknown	n.s.	n.s.	n.s.	**20**	**77**	n.s.
b1625	cnu	OriC-binding complex H-NS/Cnu	n.s.	10	n.s.	**20**	**24**	n.s.

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1459 Table is sorted by PMA at 10 min column. n.s. = not significantly different from unexposed culture and boldface highlights actual

1460 values ≥ 20-fold. Gene names in boldface have a ≥ 20 differential expression response to both Hg and PMA in at least one time point

1461 (n = 11).

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1465 **Table 3: Changes in transcription factor gene expression.** The sum of transcription

1466 factor genes either up-regulated or down-regulated is shown with the percentage of the

1467 total transcription factor genes in parenthesis; percent's do not total 100 because genes

1468 with no change compared to unexposed cells are not tabulated here. See details in

1469 Table S12.

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Transcription Factors (n = 203)						
	Hg_t10	**Hg_t30**	**Hg_t60**	**PMA_t10**	**PMA_t30**	**PMA_t60**
up	63 (31)	46 (23)	14 (7)	86 (42)	78 (38)	1 (0.5)
down	28 (14)	28 (14)	13 (6)	22 (11)	23 (11)	2 (1)

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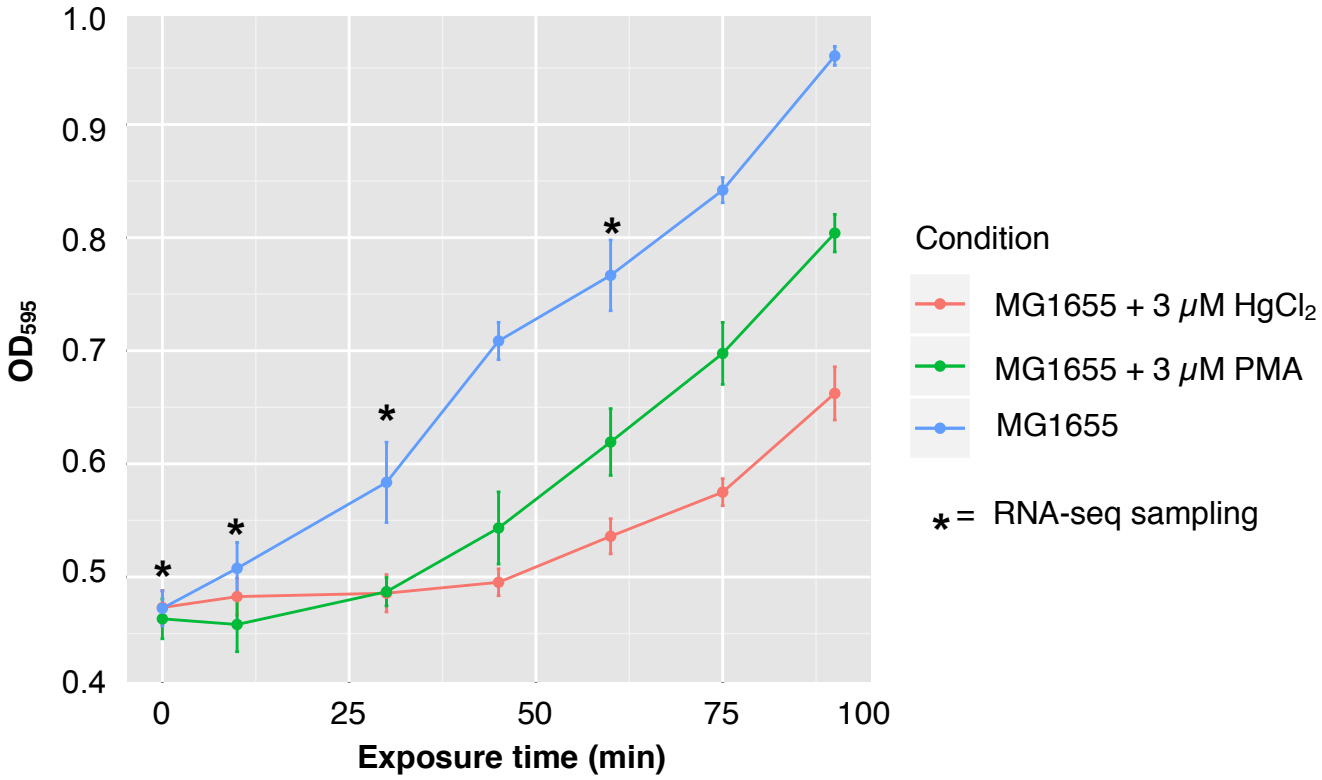
1486 **Figures:**

1487 **a**



1497 **b**

	t10	t30	t60
3μM $HgCl_2$	1,821 (894↑927↓)	1,422 (637↑785↓)	563 (259↑304↓)
3μM PMA	2,181 (1,261↑920↓)	2,009 (1,156↑853↓)	65 (36↑29↓)

1501 **Figure 1: Effects of sub-acute mercury exposure on growth of MG1655. (a)** *E. coli*
1502 K12 MG1655 grown in MOPS minimal medium, unexposed (blue) or exposed to 3 μM
1503 $HgCl_2$ (red) or 3 μM PMA (green) during mid-log phase. Asterisks indicate sampling
1504 times for RNA-seq. Error bars are standard error (SEM) of 3 biological replicates for
1505 each culture condition. See Figure S1 for full growth curve. **(b)** Differentially expressed
1506 genes (DEG) counts (up or down) for $HgCl_2$ and PMA relative to unexposed control
1507 culture at each time point.

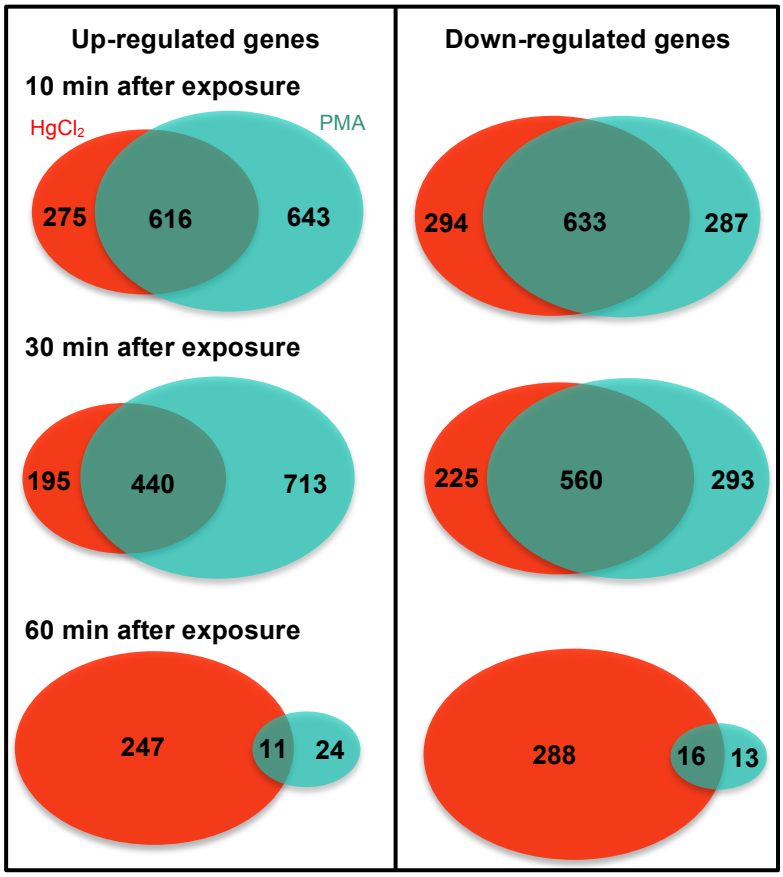


Figure 2: Overlap between differentially expressed genes at each sampling time

The 3 μM $HgCl_2$ exposure is in red and the 3 μM PMA exposure in green. Ovals are to scale only at each time point, but not between between time points in a panel nor between left and right panels.

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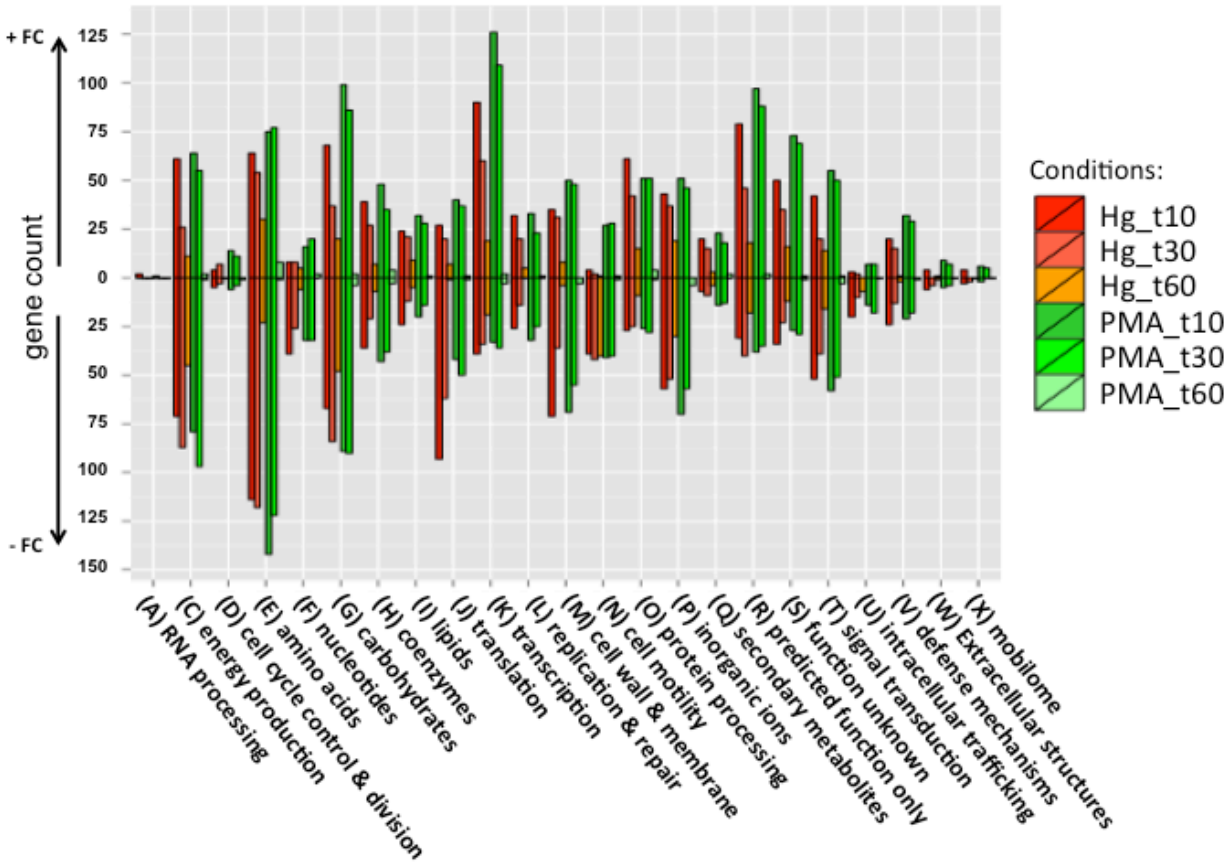

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1533 **Figure 3: Counts of differentially expressed genes for each condition grouped by**

1534 **COG functional category.** Genes with a $\log_2$ fold-change ≥ 1 for each condition were

1535 grouped by COG group. Positive counts represent observed up-regulated genes and

1536 negative counts represent observed down-regulated genes. COG code, number of

1537 proteins encoded by genome and category description: (A, 2) RNA processing and

1538 modification; (C, 284) Energy production and conversion; (D, 39) Cell cycle control, cell

1539 division, chromosome partitioning; (E, 355) Amino acid transport and metabolism; (F,

1540 107) Nucleotide transport and metabolism; (G, 381) Carbohydrate transport and

1541 metabolism; (H, 179) Coenzyme transport and metabolism; (I, 121) Lipid transport and

1542 metabolism; (J, 236) Translation, ribosomal structure and biogenesis; (K, 294)

1543 Transcription; (L, 139) Replication, recombination and repair; (M, 242) Cell wall,
1544 membrane and envelope biogenesis; (N, 102) Cell motility; (O, 156) Post-translational
1545 modification, protein turnover, chaperones; (P, 223) Inorganic ion transport and
1546 metabolism; (Q, 68) Secondary metabolites biosynthesis, transport and catabolism; (R,
1547 261) General function prediction only; (S, 203) Function unknown; (T, 191) Signal
1548 transduction mechanisms; (U, 50) Intracellular trafficking, secretion, and vesicular
1549 transport; (V, 91) Defense mechanisms; (W, 31) Extracellular structures; (X, 60)
1550 Mobilome, prophages, transposons.
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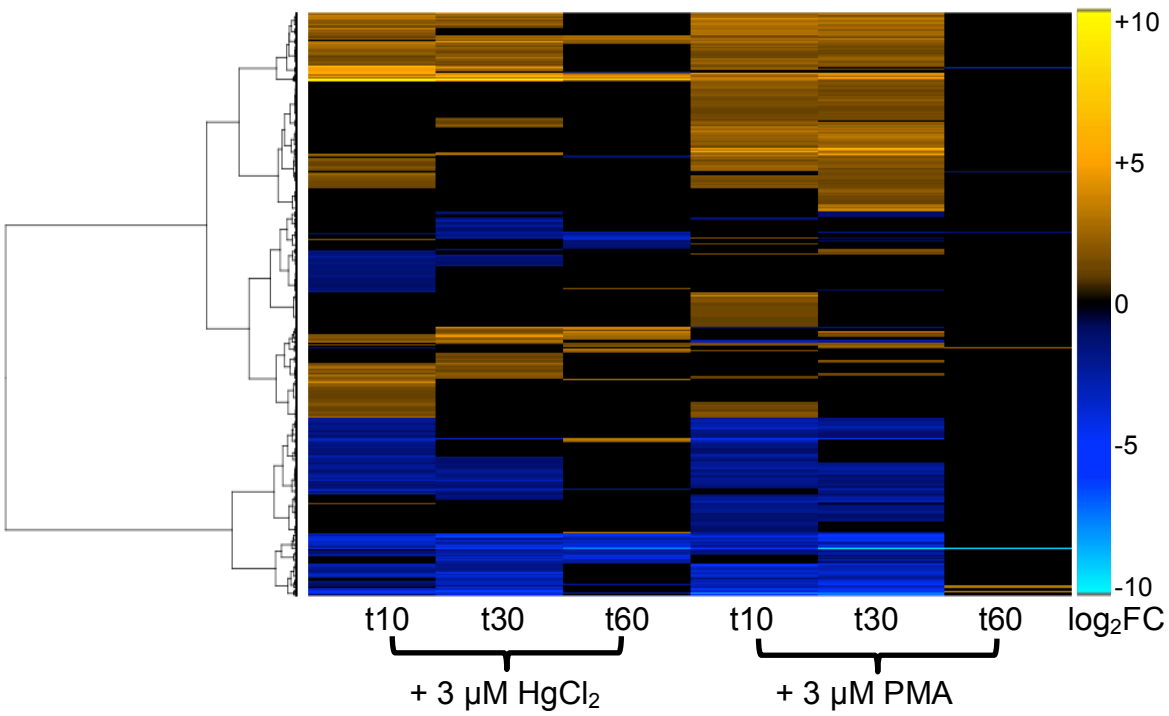

Figure 4: Differentially expressed genes at each RNA sampling time. Heat map of all genes that were differentially expressed in at least one mercury exposure condition (n = 3,149). Genes were clustered by row using Ward's minimum variance method [36] with non-squared $\log_2$ fold-change input values.

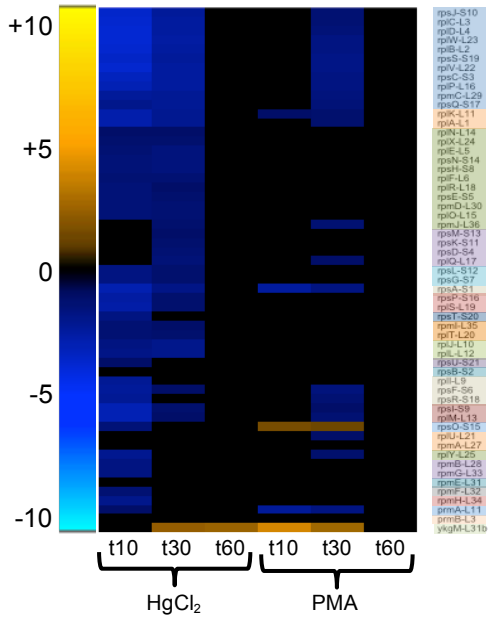

Figure 5: Ribosomal subunit protein genes. Genes are grouped and colored by operon (see larger Figure S11 and Table S13 for details).

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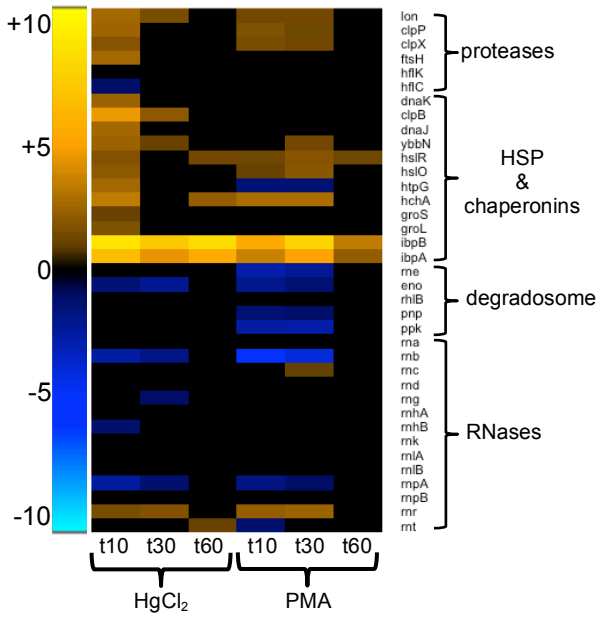

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1623 **Figure 6: Protein and RNA turnover and repair**. (see larger Figure S12 and Table

1624 S13 for details).

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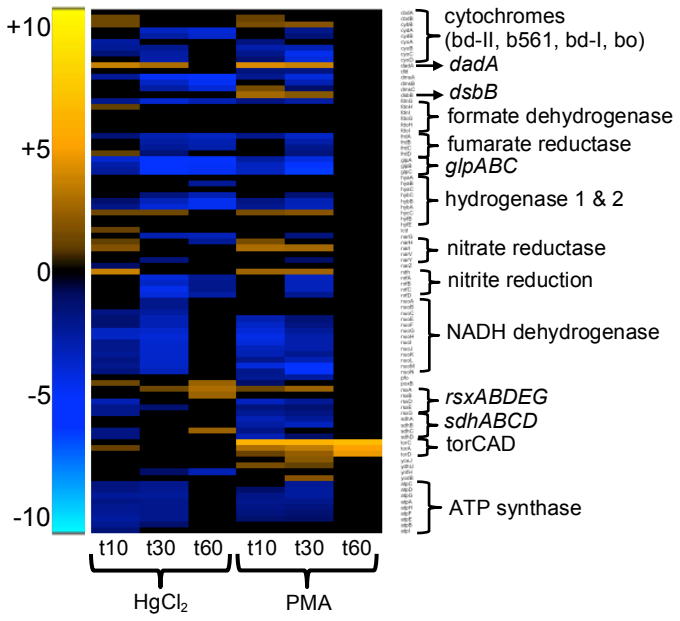

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1646 **Figure 7: Electron transport chain and ATP-synthase.** (see larger Figure S13 and

1647 Table S13 for details).

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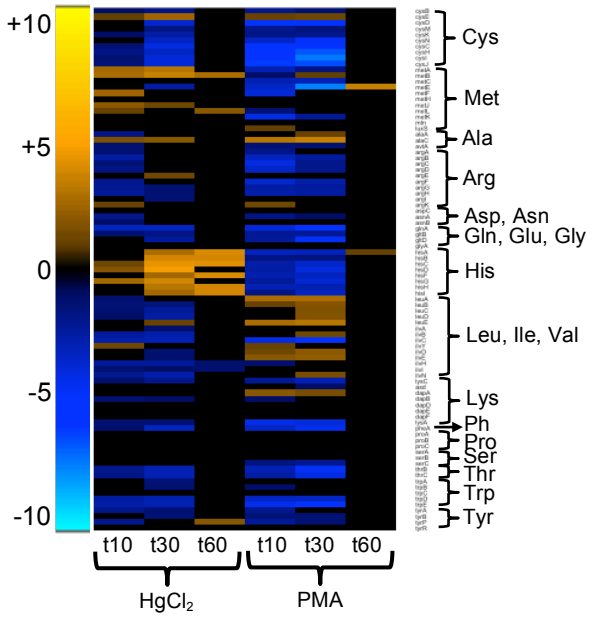

Figure 8: Amino acid biosynthesis. (see larger Figure S22 and Table S13 for details).

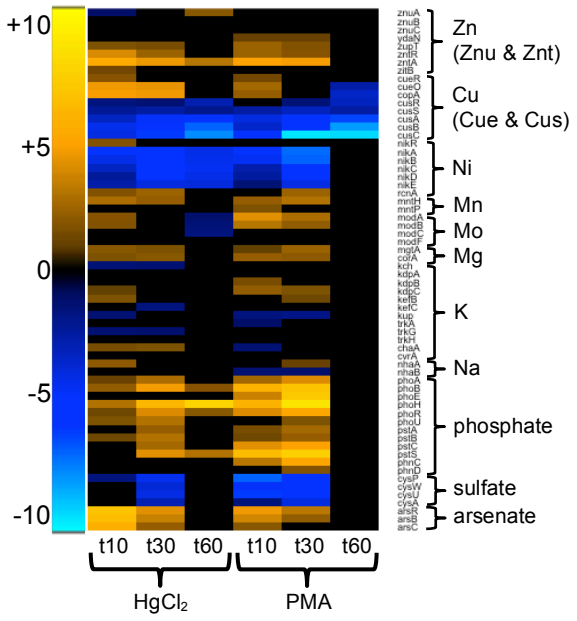

Figure 9: Non-ferrous metals homeostasis. (see larger Figure S23 and Table S13 for details).

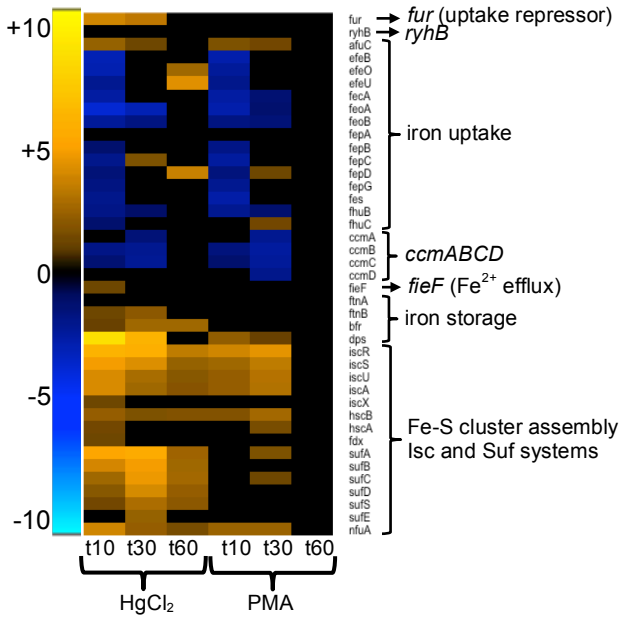

Figure 10: Iron homeostasis. (see larger Figure S24 and Table S13 for details).

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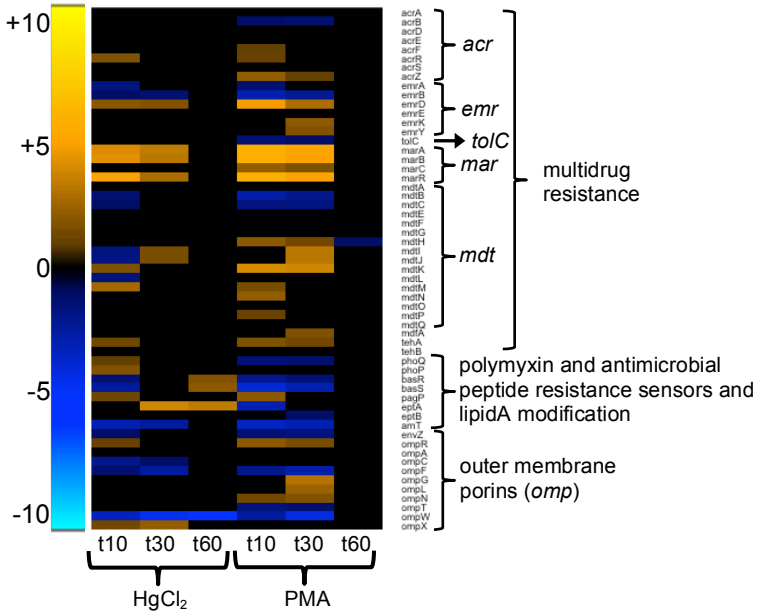

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1739 **Figure 11: Antibiotic resistance and outer membrane porins.** (see larger Figure S26

1740 and Table S13 for details).

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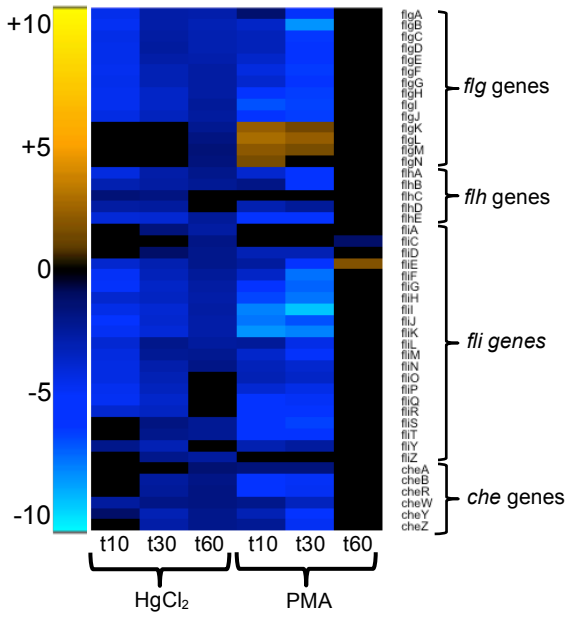

Figure 12: Flagella components and chemotaxis. (see larger Figure S27 and Table S13 for details).

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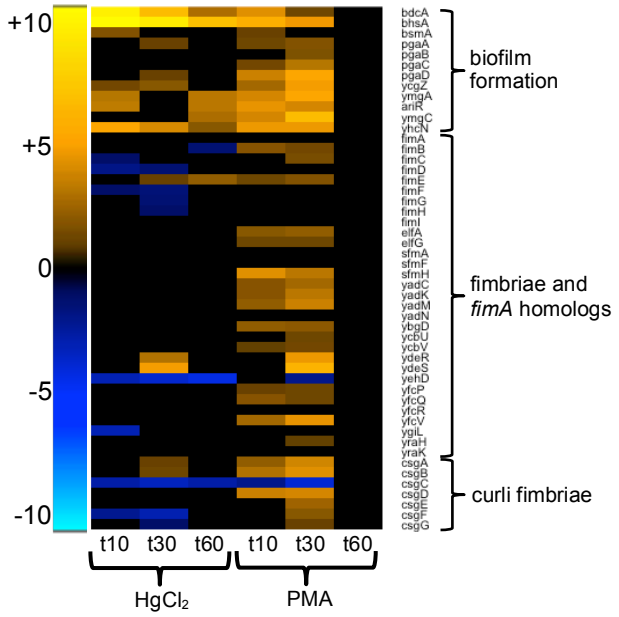

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1785 **Figure 13: Biofilm formation and fimbriae.** (see larger Figure S28 and Table S13 for

1786 details).

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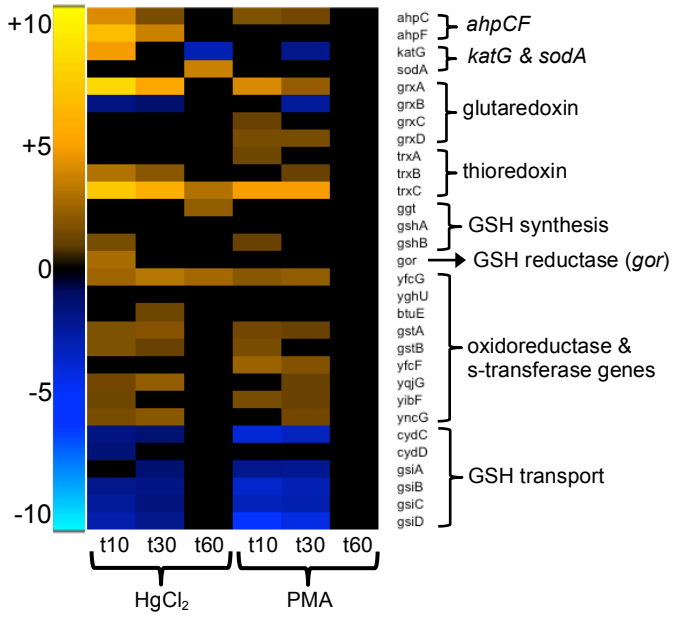

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1808 **Figure 14: Oxidative stress defense and thiol homeostasis.** (see larger Figure S29

1809 and Table S13).

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