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Interaction of lead nitrate and cadmium chloride with *Escherichia coli* K-12 and *Salmonella typhimurium* global regulatory mutants

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SUMMARY

To investigate the interactions of heavy metals with cells, a minimal medium for the growth of enteric bacteria using glycerol-2-phosphate as the sole phosphorus source was developed that avoided precipitation of Pb^{2+} with inorganic phosphate. Using this medium, spontaneous mutants of *Escherichia coli* resistant to addition of $Pb(NO_3)_2$ were isolated. Thirty-five independent mutants all conferred a low level of resistance. Disk diffusion assays on solid medium were used to survey the response of *E. coli* and *Salmonella typhimurium* mutants altered in global regulatory networks to $Pb(NO_3)_2$ and $CdCl_2$. Strains bearing mutations in *oxyR* and *rpoH* were the most hypersensitive to these compounds. Based upon the response of strains completely devoid of isozymes needed to inactivate reactive oxygen species, this hypersensitivity to lead and cadmium is attributable to alteration in superoxide dismutase rather than catalase levels. Similar analysis of chaperone-defective mutants suggests that these metals damage proteins in vivo.

INTRODUCTION

The biological effects of lead are of great concern. Ingestion of lead-based paint by children is a major health problem. The synthesis and use of tetraethyl lead has contributed to pollution of the land, water and atmosphere. There is potentially much to be learned about the physiological effects of lead on cellular processes by using genetically well-defined bacteria as research tools. Nonetheless, the interaction of inorganic lead with the well-studied bacteria *Escherichia coli* and *Salmonella typhimurium* has received little attention. A reason for this may be that lead ions combine with phosphate found in most standard media to form an insoluble precipitate.

In this report we describe a growth medium for *E. coli* and *S. typhimurium* that allowed study of the inhibition of these bacteria by lead nitrate. The utility of this medium was demonstrated in its use for direct selection of lead-resistant mutants of *E. coli*. Furthermore, the medium was used to survey the interaction of lead and cadmium with a collection of *E. coli* and *S. typhimurium* mutants altered in their responses to a wide variety of environmental stresses.

MATERIALS AND METHODS

Chemicals and culture media

The rich LB medium and the minimal F-top agar have been described [19]. Heavy Metal MOPS Medium (HMM) is a modification of the medium described by Bochner and Ames [1] and consisted of 40 mM MOPS, pH 7.2, 50 mM KCl, 10 mM NH_3Cl, 0.5 mM $MgSO_4$, 0.4% glucose, 1 mM glycerol-2-phosphate, and 1 μM $FeCl_3$. The MOPS buffer, KCl, NH_3Cl and $MgSO_4$ were made as a 10× stock, filter-sterilized and stored in the dark at 4 °C. Glucose was added from an autoclaved 40% stock solution. Glycerol-2-phosphate (disodium salt hydrate, 98%, Aldrich Chemical Co., Milwaukee, WI, USA) was added from a 100-mM stock that had been filter-sterilized and stored at −20 °C. $FeCl_3$ was added from a 10-mM stock, stored at 4 °C in the dark. For solidified medium, Bacto-agar (Difco, Detroit, MI, USA, 1.5%) was added. Thiamine (0.2 $\mu g\ ml^{-1}$) was routinely added as a supplement to this medium. In some cases uracil (0.25 $\mu g\ ml^{-1}$) was also added since a number of *E. coli* strains are partial uracil auxotrophs [12]. Other growth factors were added, as required for growth due to auxotrophic requirements of particular strains, at standard media supplementation levels [6]. Stock solutions of $Pb(NO_3)_2$ (99.999%, Johnson Matthey Electronics, Royston, Herts, UK) and $CdCl_2$ (99.995%, Johnson Matthey Electronics) were made in ultra pure water, filter-sterilized and stored at −20 °C.

Bacterial strains

The *E. coli* and *S. typhimurium* strains and their sources are shown in Table 1. Isogenic strains of *E. coli* and *S. typhimurium* with and without *recA* mutations were constructed as follows. *E. coli* strain TV4102 was made in two steps from strain MC4100. First, Plclr100 phage grown on strain JC10289 (F- *thr leu pro his arg thi ara lac gal xyl mtl rps tsx lambda- gln* Δ[*srlR-recA*]*306 srl*::Tn*10-84*; Yale University, *E. coli* Genetic Stock Center) was used for generalized transduction [19] of strain MC4100 selecting for tetracycline resistance and

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TABLE 1

Bacterial strains

Strain	Genotype	Source or reference
RFM443	*rpsL galK2 lacΔ74*	[18]
MP180	HfrH *thi-1*	[15]
UM122	MP180 *rpoS13::*Tn*10*	[15]
SA2600	F-*rpsL relA1 sup6 his cya*$^{+}$ *crp*$^{+}$	S. Adhya
SA2777	SA2600 *crp::Cm*^{R}	″
CF1648	+	M. Cashel [25]
CF1651	CF1648 Δ *relA251::Kan*	″
W3110	+	R. Matthews [7]
BE1	W3110 *lrp-201*::Tn*10*	″
BE2	W3110 *lrp-35*::Tn*10*	″
BE3	W3110 *lrp-1 zca::*Tn*10*	″
MC4100	F-*araD139 Δ(lacIPOZYA)U169 rpsL thi*	[3]
TV4102	MC4100 Δ*(srlR-recA)306 srlR*::Imprecise excision	This study
GC4468	F-*Δlac4169 rpsL*	[9]
JHC1092	GC4468 *Δ(soxR zjc-2205) zjc-2204*::Tn*10Km*	[9]
JTG936	GC4468 *soxR105*	[10]
JHC1096	GC4468 *zdd-239*::Tn*9Δ1738[soxQΔ]*	[9]
JHC1071	GC4468 *soxQ1 zdd2207*::Tn*10Km*	[9]
K-12	*oxyR*$^{+}$	G. Storz
GS08	*oxyR::Kan*	″
TA4110	*oxyR2*	″
CAG9333	MC4100 Δ*rpoH::Kan groE*(constitutive)	[13]
QC773	GC4468 *Insertion(sodB-kan)1-Δ2*	[2]
QC779	GC4468 *Insertion(sodB-kan)1-Δ2 sodA25::MudPR13*	[2]
UM255	*pro leu rpsL hsdM hsdR endl lacY katG2 katE12::*Tn*10 recA*	[20]
DA258	C600 *thr*::Tn*10* Cm^{R}(90% linked to *grpE*) Km^{R}(≈50% linked to *grpE*)	D. Ang and C. Georgopoulos
DA259	C600 *thr*::Tn*10* Km^{R} (≈50% linked to *grpE*) *grpE*::ΔCmR	″
CG799	C600 *thr*::Tn*10*	″
CG800	C600 *thr*::Tn*10 dnaK103*	″
CG2245	B178 *groE*$^{+}$ nearby Tn*10*	″
CG992	B178 *dnaJ::mini-Tet*	″
CG2244	B178 *groES619* nearby Tn*10*	″
JZ483	B178 *groES42* nearby Tn*10*	″
CG2239	B178 *groEL515* nearby Tn*10*	″
CG2241	B178 *groEL44* nearby Tn*10*	″
CG2246	B178 *groEL673* nearby Tn*10*	″
B178	F- W3110 *galE relA*	[8]
CG712	F- W3110 *galE relA groES30 zjd*::Tn*10*	[8]
CG714	F- W3110 *galE relA groEL140*	[8]
Salmonella typhimurium		
DS25	*Srl*::Tn*5*	This study
DS24	*recA1 srl*::Tn*5*	″
TT2385	*zii614*::Tn*10 oxyR*$^{+}$	G. Storz
TA4130	*zii614*::Tn*10 oxyRΔ2*	″
TA4129	*zii614*::Tn*10 oxyR1*	″

screening for sensitivity to ultraviolet irradiation. Subsequently, loss of the Tn*10* element from a tetracycline-resistant, UV-sensitive transductant was obtained by selection [17] yielding strain TV4102. Phage P22HT Δint-4 was grown on *S. typhimurium* strain LO451(F′*araE*$^+$ *zzf*::*535/serA790 lys-554 Δhis664 rpsL srl*::Tn*5 recA1*; University of Calgary, Salmonella Genetic Stock Centre). The resulting phage stock was used for generalized transduction [6] of strain LT2 (wild type). Kanamycin-resistant transductants were scored for sorbitol utilization on MacConkey medium and for UV-sensitivity. Strain DS24 was sorbitol-negative and UV-sensitive. Strain DS25 was sorbitol-negative and UV-resistant.

Determination of growth inhibition by zone diffusion assays

These assays, which result in a gradient of concentrations of the inhibitory substance, allow the effects of multiple concentrations to be determined in a single experiment. Bacterial cultures grown overnight in LB medium were collected by centrifugation and resuspended in an equal volume of sterile 0.8% NaCl. Aliquots of the resuspended cells (0.1 ml) were mixed with 2.5 ml F-top agar at 47 °C and poured onto HMM plates, containing supplements as required for the strains tested. Stock solutions (100 mM) of $Pb(NO_3)_2$ and $CdCl_2$ were spotted (20 μl) onto blank filter paper disks (6-mm diameter, BBL Microbiology Systems, Cockeysville, MD, USA) and placed on the surface of the plates seeded with bacteria. Following overnight incubation at appropriate temperature for the strains, the diameters of the resultant zones of growth inhibition were measured with a ruler. Experiments with mutant *E. coli* strains were conducted at least twice, independently, with comparable results. Representative data are reported.

RESULTS

Medium development

Two parameters were considered important in the development of an appropriate medium with which to study inhibition of *E. coli* and *S. typhimurium* growth by lead salts. Avoidance of lead precipitation with phosphate was critical for the lead to be available for entry into the bacterial cell. Another important factor was the effect of metal chelation. The MOPS medium of Neidhardt [21] as simplified by Bochner and Ames [1] was used as the basis for modifications. Disk diffusion assays indicated that precipitation interfered with the detection of *E. coli* growth inhibition by lead nitrate when inorganic phosphate was provided extracellularly in MOPS medium (Table 2). Glycerol-2-phosphate was substituted for inorganic phosphate as the sole phosphorus source, as suggested by Macaskie and Dean [16]. This substitution allowed growth of *E. coli*, indicating that glycerol-2-phosphate was broken down yielding intracellular inorganic phosphate. Furthermore, substantial growth inhibition by lead nitrate was observed (Table 2). Omission of tricine, a metal-chelating compound, from the modified MOPS medium containing glycerol-2-phosphate as the sole phosphorus source also resulted in enhanced sensitivity of *E. coli* to lead and cadmium (Table 2). The modified medium containing glycerol-2-phosphate as the sole phosphorus source, lacking tricine, and with a reduced amount of

TABLE 2

Factors effecting *E. coli* growth inhibition by lead nitrate[a]

Condition varied	Media supplementation	$Pb(NO_3)_2$ zone of inhibition, diameter (mm)
Phosphorus source	KH_2PO_4 (0.2 mM)	15 ppt, 18 clear
	Glycerol-2-phosphate (0.2 mM)	31 clear
Metal chelator	Tricine, 10 mM	16 clear
	Tricine, 7 mM	18 clear
	Tricine, 4 mM	21 clear
	Tricine, 2 mM	24 clear
	None	30 clear

[a] *E. coli* strain RFM443 was grown in LB medium (to log-phase for phosphorus-source test and overnight for Tricine test) and tested for $Pb(NO_3)_2$ sensitivity at 37 °C as described in Materials and Methods on HMM plates containing changes as indicated above.

$FeCl_3$ was designated as HMM (Heavy Metal MOPS testing medium).

Lead-resistant E. coli *mutants*

The minimal inhibitory concentration of $Pb(NO_3)_2$ in HMM plates for *E. coli* strain RFM443 was 100 μg ml^{-1} at 37 °C and 30 μg ml^{-1} at 42 °C. Spontaneous mutants of *E. coli* RFM443 were isolated under various selection conditions: 100 μg ml^{-1} $Pb(NO_3)_2$ at 42 °C, 30 μg ml^{-1} at 42 °C, and 100 μg ml^{-1} at 37 °C. The frequency of mutants varied from 5×10^{-7} to 1×10^{-6}. Thirty-five independent isolates were purified using the same conditions as the selection followed by a second, non-selective purification on LB agar plates. The mutants were characterized by checking the phenotypic markers of the parent strain, streaking to HMM plates containing $Pb(NO_3)_2$, and quantifying the zone of growth inhibition at 37 °C resulting from a disk containing 20 μl of 100 mM $Pb(NO_3)_2$ added to cells seeded on an HMM plate. All 35 putative mutants had Lac$^-$, Gal$^-$, and Streptomycin-resistant phenotypes, as did the parental strain. In contrast to the parental strain, all the putative mutants continued to demonstrate a Pb-resistant phenotype on the streak plate test. The degree of Pb-resistance as quantitated by the zone of diffusion assay, however, was weak for all mutants. The Pb-resistant mutants all had zones of growth inhibition of 25–27 mm in diameter compared to the zone of growth inhibition of the parent strain of 28–30 mm in diameter.

Response of global regulatory mutants to lead and cadmium

A second genetic approach to identification of the major targets of an inhibitory agent was also undertaken. A large number of *E. coli* and *S. typhimurium* strains containing mutations in various regulatory elements of many global control circuits were tested for their sensitivity to lead. This collection was also tested for sensitivity to cadmium, a better-studied heavy metal. Table 3 shows the results of this survey.

TABLE 3

Response of global regulatory mutants to lead and cadmium salts[a]

Regulatory circuit	Species	Strain	Controlling gene	Allele	Zone of growth inhibition	
					$Pb(NO_3)_2$	$CdCl_2$
Stationary phase	*E. coli*	MP180	*rpoS*	+	27 clear	33 clear, 34 turbid
	″	UM122	″	null	30 clear	35 clear
Limited carbon source	*E. coli*	SA2600	*crp*	+	31 clear	29 clear
	″	SA2777	″	null	35 clear	34 clear
Amino acid limitation	*E. coli*	CF1648	*relA*	+	26 clear	23 clear
	″	CF1651	″	null	32 clear	31 clear
Leucine	*E. coli*	W3110	*lrp*	+	30 clear	33 clear
	″	BE1	″	null	31 clear	33 clear
	″	BE2	″	null	34 clear	34 clear
	″	BE3	″	missense	31 clear	31 clear
DNA damage	*S. typhimurium*	DS25	*recA*	+	24 clear	31 clear
	″	DS24	″	null	25 clear	32 clear
DNA damage	*E. coli*	MC4100	*recA*	+	28 clear	32 clear, 49 turbid
	″	TV4102	″	null	26 clear	32 clear
Superoxide damage	*E. coli*	GC4468	*soxR soxQ*	+	29 clear	29 clear
	″	JHC1092	*soxR*	null	28 clear	29 clear
	″	JTG936	*soxR*	constitutive	29 clear	31 clear
	″	JHC1096	*soxQ*	null	29 clear	29 clear
	″	JHC1071	*soxQ*	constitutive	31 clear	30 clear
Peroxide damage	*S. typhimurium*	TT2385	*oxyR*	+	24 clear	27 clear, 30 turbid
	″	TA4130	″	null	32 clear	42 clear
	″	TA4129	″	constitutive	31 clear	35 clear
Peroxide damage	*E. coli*	K-12	*oxyR*	+	25 clear	28 clear
	″	GS08	″	null	25 clear	29 clear, 41 very turbid
	″	TA4110	″	constitutive	24 clear	29 clear, 41 turbid
Heat shock	*E. coli*	MC4100	*rpoH*	+	22 clear, 30 turbid	28 clear, 35 turbid
	″	CAG9333	″	null (with *groE* constitutive)	32 clear	44 clear

[a] The strains are grouped in isogenic series which were tested concurrently. The protocol for these experiments was as described in Materials and Methods. Thiamine was added to the F-top agar for all strains. Histidine was added to the HMM plates for experiments with strains SA2600 and SA2777. All tests were done at 37 °C except that comparing strains MC4100 and CAG9333 which was done at 30 °C. The diameter of the zone of growth inhibition was measured in millimeters.

Mutants altered in global responses to stationary phase, leucine concentration, DNA damage, or superoxide damage were not greatly different from isogenic control strains in their response to either lead or cadmium. Mutants altered in global responses to carbon- or amino acid-limitation were somewhat hypersensitive to lead and cadmium. The most dramatic differences in lead and cadmium sensitivity were between the isogenic controls and strains mutated in the *oxyR* regulatory gene or *rpoH* regulatory gene. *S. typhimurium* strains carrying either the null or constitutive allele of *oxyR* were hypersensitive to both lead and cadmium (Table 3). *E. coli* strains carrying either the null or constitutive alleles of *oxyR* were somewhat hypersensitive to cadmium. An *E. coli* strain carrying a deletion of the *rpoH* gene can only grow below 20 °C [13], thus a strain was tested which carried an additional mutation giving high level expression of the *groE* operon that can grow at temperatures up to 40 °C [13]. This mutant strain was dramatically more sensitive than an otherwise isogenic control strain to both lead and cadmium (Table 3).

The hypersensitivity of the *oxyR* regulatory mutants sug-

gested that the ability of the cells to respond to the oxidative damage caused by the heavy metals was critical. To more closely examine which enzymes may be important in the oxidative damage response, strains mutated in superoxide dismutase and catalase isozymes were tested for sensitivity to lead and cadmium. *E. coli* strain QC779 lacking both superoxide dismutases was hypersensitive to both lead and cadmium when compared to an isogenic control strain (Table 4). In contrast, *E. coli* strain UM255, lacking both catalases did not appear dramatically hypersensitive to either lead or cadmium, although an isogenic control strain was not available (Table 4).

The hypersensitivity of the *rpoH* regulatory mutant suggested that the ability of the cell to respond to protein damage was also critical. Mutants in some of the genes encoding molecular chaperones controlled by the *rpoH* gene were tested for sensitivity to lead and cadmium. These strains were tested at both 30 °C and 37 °C because many of the mutant alleles are temperature-sensitive, thus it was expected that any differences from the parental strains would be increased at the higher temperature. As the data in Table 5 indicate, strains mutated in *grpE*, *dnaK*, *dnaJ*, *groEL* and *groES* were hypersensitive to both lead and cadmium.

DISCUSSION

Avoidance of lead ion precipitation by the phosphate found in most standard media used for cultivation of *E. coli* and *S. typhimurium* was necessary prior to analysis of growth inhibition by lead. The use of glycerol-2-phosphate as a phosphorus source for growth of many microorganisms has been described [16]; the medium used, however, was buffered with Tris which is not optimal for growth of *E. coli* [21]; thus the modification of MOPS buffered media was made. Use of the HMM medium described in this report allows readily detectable growth inhibition by $Pb(NO_3)_2$.

The isolation of mutants resistant to specific toxic agents such as antibiotics and herbicides is often a useful genetic approach by which the mode of action of such agents and the biological responses of cells to presence of the agents is uncovered. Spontaneous *E. coli* mutants resistant to $Pb(No_3)_2$ were isolated on HMM medium containing added $Pb(NO_3)_2$. Although mutants were not difficult to obtain, each displayed a low level of resistance to $Pb(NO_3)_2$. Mutagenesis with diethyl sulfate also did not yield mutants dramatically more resistant to $Pb(NO_3)_2$ than the parental strain (data not shown). Thus, rather than further investigate these weak mutants, an alternative approach to understanding biological effects of $Pb(NO_3)_2$ was taken.

Global regulatory networks in bacteria direct transcription of specific genes in response to a variety of adverse environmental conditions, allowing the cell to survive the hostile environment. Cells that lack the ability to respond to a particular type of stress are expected to be hypersensitive to agents producing that stress. The principal type of damage caused by heavy metals may thus be discerned by analysis of the metal sensitivity of strains carrying mutations in controlling genes of various global regulatory networks. Such experiments reported here suggest that the ability of the cell to respond to oxidative damage and protein damage are critically important for their ability to mount a defense to the heavy metals lead and cadmium.

S. typhimurium strains mutated in *oxyR* were hypersensitive to both lead and cadmium (Table 3). Surprisingly, strains bearing both the null and constitutive alleles were hypersensitive to these heavy metals. This is in contrast to their sensitivity to hydrogen peroxide; the null allele confers hypersensitivity and the constitutive allele resistance [4]. Nevertheless, this result points to the importance of oxidative stress responses when microbial cells encounter heavy metals. The hypersensitivity of an *E. coli* strain devoid of superoxide dismutase

TABLE 4

Superoxide dismutase- and catalase-deficient mutants[a]

Strain	Relevant genotype	Zone of growth inhibition (mm)			
		Minimal medium		Enriched minimal medium	
		$Pb(NO_3)_2$	$CdCl_2$	$Pb(NO_3)_2$	$CdCl_2$
GC4468	+	25 clear	29 clear	23 clear	23 clear, 24 turbid
QC773	*sodB*	28 clear	34 clear	24 clear	28 clear, 29 turbid
QC779	*sodA sodB*	44 clear[b]	70 clear[b]	27 clear	32 clear
UM255	*katE katG*[c]	23 clear	26 clear, 28 turbid	Not tested	Not tested

[a] $Pb(NO_3)_2$ and $CdCl_2$ zones of growth inhibition were measured at 37 °C as described in Materials and Methods. The F-top agar contained thiamine for all experiments. For the data set labeled minimal medium, no other additions were made to the HMM plates except leucine and proline were added for strain UM255. For the data set labeled enriched minimal medium, the following amino acids were added: valine, isoleucine, leucine, methionine, threonine, tryptophan, tyrosine, phenylalanine, histidine, alanine, and glycine. The diameter of the zone of growth inhibition was measured in millimeters.

[b] Weak growth.

[c] Not isogenic to GC4468.

TABLE 5

Molecular chaperone deficient mutants[a]

Strain	Relevant genotype	Zone of growth inhibition			
		30 °C		37 °C	
		$Pb(NO_3)_2$	$CdCl_2$	$Pb(NO_3)_2$	$CdCl_2$
DA258	+	18 clear, 21 turbid	28 clear	14 clear, 17 turbid	28 clear
DA259	*grpE*	21 clear	30 clear	20 clear	32 clear
CG799	+	15 clear, 18 turbid	29 clear	16 clear, 20 turbid	27 clear
CG800	*dnaK*	19 clear	30 clear	28 clear	40 clear
CG2245	+	22 clear	28 clear	24 clear	27 turbid
CG992	*dnaJ*	22 clear	26 clear	29 clear[b]	38 clear[b]
CG2244	*groES*	22 clear	28 clear, 34 turbid	24 clear, 27 turbid	32 clear
JZ483	*groES*	28 clear, 29 turbid	27 clear, 34 turbid	26 clear[b]	32 clear, 43 turbid[b]
CG2239	*groEL*	24 clear	28 clear	24 clear, 26 turbid	32 clear
CG2241	*groEL*	22 clear	28 clear	24 clear, 27 turbid	30 clear
CG2246	*groEL*	24 clear	31 clear	26 clear, 28 turbid	32 turbid
B178	+	23 clear	27 clear	22 clear, 24 turbid	28 clear
CG712	*groES*	25 clear	33 clear	26 clear	35 clear
CG714	*groEL*	22 clear	28 clear, 35 turbid	24 clear	30 clear

[a] The *E. coli* strains are grouped in isogenic sets. These strains were grown overnight in LB medium at 30 °C and used for determination of growth inhibition as described in Materials and Methods. Thiamine was added to the F-Top agar. The HMM plates contained uracil and 18 amino acids (aspartate, alanine, asparagine, arginine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, proline, phenylalanine, serine, tryptophan, tyrosine, threonine and valine). Diameters of $Pb(NO_3)_2$ and $CdCl_2$ zones of inhibition formed following incubation at the temperatures indicated were measured in mm.
[b] Weak growth.

(Table 4) was also consistent with the idea that heavy metals cause oxidative damage to cells.

An *E. coli* strain carrying a null allele of *rpoH*, the heat shock regulatory gene, was dramatically more sensitive to lead and cadmium salts than was an isogenic control strain (Table 3). The *rpoH* gene product controls the synthesis of about 20 genes that are induced by heat shock and a number of other environmental stresses [22]. The common signal for induction by these various stresses is thought to be the presence of non-native proteins in the cell [5,14]. Many genes controlled by *rpoH* are molecular chaperones which could be involved in renaturing non-native proteins and proteases which degrade severely damaged proteins [23]. The molecular chaperones encoded by *grpE*, *dnaK*, *dnaJ*, *groEL* and *groES*, appear to be important in the stress response of *E. coli* to cadmium and lead because of the hypersensitivity of strains containing temperature-sensitive mutations in those genes (Table 5).

Another approach to the analysis of the effects of cadmium on *E. coli* that has been taken is two-dimensional gel analysis of induced proteins. Results from VanBogelen et al. [24] using such an approach are, in general, in agreement with the results reported here. $CdCl_2$ was found to induce many proteins of the heat shock and *oxyR* regulons. VanBogelen et al., however, also reported induction of the *recA* gene; our analysis of a *recA* mutant does not suggest the critical importance of the SOS response to heavy metal stress. Furthermore, several other unknown proteins were also found to be induced by $CdCl_2$ [24]. These may be members of an as-of-yet-unidentified global regulatory network for cadmium stress, as Hartman has suggested may exist [11].

The responses of the global regulatory mutants to lead and cadmium salts were, in general, similar, suggesting common biological effects of these heavy metals. It seems unlikely, however, that all the biological effects of both metals are identical. The key to understanding distinctions may lie in the analysis of differential gene induction by the two metals. Useful understanding of the biological effects of lead and cadmium may also arise from selection of suppressors of the metal-hypersensitive global regulatory mutants.

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