DNA Microarray Analysis of Human Gene Expression Induced by a Non-lethal Dose of Cadmium

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Abstract: Cadmium (Cd) is a hazardous heavy metal present in working and living environments. Cd affects many cellular functions, but little is known about the mechanisms of its toxicity and cellular defense against it. Recently, advanced gene expression analysis employing DNA microarrays provided us the means to profile the expression of thousands of genes simultaneously. We describe here a study of Cd-induced gene expression profile. Messenger RNA was prepared from HeLa cells exposed to a non-lethal dose of CdSO₄, and analyzed by the use of an array consisting of 7,075 human cDNAs. Many stress response genes including those coding for metallothioneins and heat shock proteins were observed to be induced by Cd. The cellular metabolism inclined toward the synthesis of cysteine and glutathione after Cd exposure. Anti-oxidant genes also appeared to be induced to protect cell components and to quench reactive oxygen species. Ubiquitin pathway was activated as well probably to degrade proteins which might not be renatured. These data suggest that human cells mobilize every genomic resource (induction of some genes and repression of others) to overcome cytotoxicity caused by Cd.

Key words: DNA microarray, Cadmium, Stress proteins, mRNA, Anti-oxidant

Introduction

Development of modern chemical industry has made our life convenient and comfortable. But we should not forget the fact that the products of the chemical industry give rise to many symptoms in the people who work in their production. To cite an example, cadmium (Cd) is required for commodities to sustain our daily life including pigments, plastic stabilizers, electroplating, alloys, nickel-Cd batteries and a number of other applications. In spite of unavoidable exploitation of Cd, the metal is very toxic, affecting many cellular functions^{1–3}, and carcinogenic^{4, 5}, possibly through stimulation and misregulation of proto-oncogenes^{3, 6, 7}. Although Cd is a non-Fenton metal and does not generate free radicals by itself, some Cd cytotoxicity has been shown to be mediated by the production of reactive oxygen species

(ROS) in the presence of metallothioneins (MTs) containing Fenton metals^{8–10}. Cd is also suspected to act as an endocrine disrupter at very low concentrations since it has various effects on estrogen receptor^{11–13}. Accordingly, this metal is still one of the real problems in industrial hygiene^{1, 14–16}. However, Cd is not rare on the earth, rather familiar to terrestrial lives. Therefore, they including human beings have no choice but to develop cellular devices to minimize danger from this metal in order to protect life. MTs¹⁷ and heat shock proteins¹⁸ (HSPs) are well known to be transcriptionally induced by Cd and play important protective roles. Despite the accumulation of knowledge of human genes whose expression is increased or decreased by Cd, its biological effects and the mechanisms of its toxicity are not yet well understood. If we could obtain the entire list of human genes whose expression is changed after Cd exposure, it would provide us with important information not only on mechanisms to protect against Cd exposure but also on those

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of the toxicity Cd exerts in human cells. The recently developed DNA microarray technology^{19, 20)} allows us to monitor the expression of thousands of genes simultaneously and provides the means of identifying genes whose expression is altered by exposure to toxicants. This paper describes a study in which gene expression changed by Cd exposure was examined with a microarray containing 7,075 human cDNAs.

Materials and Methods

Preparation of polyadenylated RNA

Culture of HeLa S3 cells, Cd exposure and preparation of total RNA were described previously²¹⁾. Briefly, cells were plated (4×10^6 cells/10 ml medium/100-mm Falcon plastic dish) and incubated for 3 days. Cultures were then incubated with or without 5 μ M CdSO₄ for 6 h before harvesting. Total cellular RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform extraction method²²⁾. Polyadenylated RNA was isolated from the total RNA with an Oligotex-dT30 ^{<super>} mRNA purification kit (Roche). The purity of the polyadenylated RNA was confirmed by the A₂₆₀/A₂₈₀ ratio (>2.05). No degradation of specific mRNA was observed in northern blot analysis employing digoxigenin-labeled probes for human β -actin and human metallothionein-II A (MT-IIA)²¹⁾ messages.

DNA microarray analysis

Microarray analysis was performed by Incyte Genomics Inc. (San Francisco, CA). Briefly, polyadenylated RNA from untreated cells or Cd-treated cells was reverse transcribed in the presence of Cy3 or Cy5-labeled nucleotides, respectively. These two sets of cDNA probes were hybridized to a UniGEM Human V cDNA microarray containing 7,075 immobilized cDNA fragments. The intensity of Cy3 and Cy5 fluorescence at a given spot on the microarray was used to quantify the differential expression of the corresponding gene.

Survey of upstream regulatory sequences

We performed a search for an enhancer sequence termed metal responsive element (MRE)^{23, 24)}, which contains a conserved core sequence TGCRCNC (R=purine; N=any base), in the upstream region of every gene listed in Table 1. The sequence of a given cDNA was used as a bait to find a full-length mRNA in the database of the National Center for Biotechnology Information (NCBI) by means of the Basic Local Alignment Search Tool (BLAST) program. The full-length mRNA was then employed as a secondary bait to

approach a genome sequence in the high throughput genome sequence (htgs) database of NCBI. The sequence more than 1 kb from the transcription start site was examined as to whether putative MREs were present or not. There were some cases in which we failed to determine the start site or obtain an upstream sequence longer than 1 kb. These are indicated as n.d. (for “not determined”) in Table 1.

Results and Discussions

To obtain an insight into the biological effects of Cd and to understand the cellular protection mechanism against it, we used microarray analysis to identify genes whose expression is changed upon treatment of HeLa cells with 5 μ M CdSO₄. Out of 7,075 human cDNAs examined, the expression was increased for 46 genes (Table 1) and was decreased for 10 genes (Table 2) twofold or greater.

Induction of stress response gene expression

The genes mentioned hereafter are correlated to Table 1 by indicating the gene numbers (shown in the lefthand column in Table 1) in brackets. Genes classified into the stress response genes and those relevant to gene regulation are indicated by circles in the “Stress response” and “Gene regulation” columns in Table 1, respectively.

MTs are low molecular weight cysteine-rich proteins which are known to be metal-inducible and to play a major role in heavy metal detoxification. MTs sequester heavy metals by chelating them¹⁷⁾. As expected from our previous data on Cd-induced MT gene expression in human cells^{16, 21, 25)}, MT genes were highly inducible [1, 5, 6 and 9]. However, in man, MT genes comprise a multigene family classified into four isoform gene groups (*MT-I*, *-II*, *-III* and *-IV*). Induction of the *MT-IL* [1] and *MT-III* genes [9] in HeLa cells is unlikely since the *MT-IL* gene is not transcribed²⁶⁾ and MT-III is found only in brain^{27, 28)}. The signals for *MT-IL* and *MT-III* might probably reflect hybridization with other functional MT isoform mRNAs (for example, *MT IIA*²⁹⁾ which is approximately 90% homologous to *MT-IL* and *MT-III* in nucleotide sequence). The apparent induction ratio of *MT-IL* is comparable to that of *MT-IIA* obtained by northern blot analysis²¹⁾. This case warns us to be prudent in interpreting the data from DNA arrays.

HSPs represent a major group of stress response proteins¹⁸⁾. Many of them including HSP110, HSP90, HSP70, HSP60 and HSP32 have been shown to be Cd-inducible^{3, 21, 30)}. Consistent with this, transcription of the *hsp70* isoform genes³¹⁾ was highly inducible by Cd: *hsp70-1* [2], *hsp70-6* [3], *hsp70-5* [4]. Their roles as chaperons might be important

Table 1. Cd-induced gene expression detected by DNA microarray analysis

No	Induction (fold)	Gene Product	Stress response	Gene regulation	MRE core within -1 kb
1	58.8	MTI-IL*			5
2	11.6	HSP70-1			0
3	7.7	HSP70-6 (HSP70B')			0
4	7.1	HSP70-5 (GRP78)			0
5	6.5	MT-IE			4
6	5.3	MT-IB**			4
7	4.4	spermine/spermidine acetyltransferase			0
8	4.4	HSP110 α			n.d.
9	3.8	MT-III			7
10	3.4	ferritin, heavy polypeptide 1			3
11	3.3	arginine-rich protein			0
12	3.3	human hbc647 mRNA sequence			n.d.
13	2.9	interleukin 7 (IL-7) receptor			n.d.
14	2.9	spinocerebellar ataxia 2			n.d.
15	2.8	HSP40 (HDJ-1)			n.d.
16	2.7	EST			n.d.
17	2.6	soluble methyl glutaryl CoA synthase			1
18	2.5	keratin 17			2
19	2.5	Mn superoxide dismutase (Mn SOD)			n.d.
20	2.5	mafG			n.d.
21	2.4	retinoid X receptor γ (RXR γ)			n.d.
22	2.4	HSP70/HSP90-organizing protein (Hop)			0
23	2.3	amino acid transporter system A1 (ATA1)***			4
24	2.3	bactericidal/permeability-increasing protein			0
25	2.3	glucan (1,4- α -), branching enzyme 1			n.d.
26	2.2	ubiquitin B			1
27	2.2	EST			1
28	2.2	malic enzyme 1, soluble			n.d.
29	2.2	Mif1			1
30	2.1	EST			n.d.
31	2.1	interleukin 1, α (IL-1 α)			1
32	2.1	ferritin, light polypeptide			n.d.
33	2.1	H.sapiens polyA site DNA			n.d.
34	2.1	cytokine inducible nuclear protein, C193			n.d.
35	2.1	neuronal precursor cell-expressed developmentally down-regulated (Nedd4)			2
36	2.1	lysine hydroxylase 2			2
37	2.1	calcyclin binding protein			n.d.
38	2.1	annexin I (lipocortin I)			0
39	2.0	v-abelson murine leukemia oncogene (V-abl) homolog 2 product			1
40	2.0	EST			n.d.
41	2.0	γ -glutamylcysteine synthetase, catalytic (γ GCS)			2
42	2.0	importin (karyopherin) β 2			n.d.
43	2.0	HSP90 β			1
44	2.0	retinoic acid induced 3			n.d.
45	2.0	reduced in tumor 42kd (rit42, RTP, Ndr1, Cap43, TDD5, Drg1)			n.d.
46	2.0	HSP60-1 (chaperonin)			6

Clones showing >98% identity over 300 nucleotides were assumed to be identical to known sequences, if not otherwise indicated. *shows 87% identity of 296 nucleotides to MT1L. ** shows 87 % identity of 239 nucleotides to MT1B. ***shows 95% identity of 600 nucleotides to ATA1. n.d., "not determined"; MT, metallothionein; HSP, heat shock protein; EST, expressed sequence tag.

Table 2. Cd-repressed gene expression detected by DNA microarray analysis

No	Repression (fold)	Gene Product
1	2.9	natural killer group 2D (NKG2D)
2	2.7	methionine adenosyltransferase II, alpha (MAT II α)*
3	2.4	hypothetical protein MGC2217
4	2.4	never in mitosis gene a (NIMA)-related kinase 3
5	2.4	dermatan sulphate proteoglycan 3**
6	2.2	cerebellar degeneration-related protein (34kD)
7	2.1	regulatory factor X, 5
8	2.1	KIAA0535 gene product
9	2.1	one twenty-two (OTT)
10	2.1	high mobility group protein isoform I-C (HMGIC)

Clones showing >98% identity over 400 nucleotides were assumed to be identical to known sequences, if not otherwise indicated. *shows 100% identity of 273 nucleotides to MAT. **shows 96% identity of 475 nucleotides to dermatan sulphate proteoglycan 3. n.d., "not determined".

to renature cellular proteins impaired by Cd^{18, 32, 33}. The level of *hsp70-1* induction [2] was consistent with the results of northern blot analysis²¹. In addition to this, many other *hsp* genes (*hsp110* [8], *hsp40* [15], *hsp90* [43], *hsp60* [46]) and an *hsp*-related gene (*Hop* [22]) were induced, which are expected to be relevant to protein renaturation and gene regulation, etc.

Responses possibly mediated by oxidative stress

Since Cd is not one of the Fenton metals, Cd itself is not redox active and does not generate free radicals^{8, 9}. But it has been shown that Cd produces hydroxyl radicals by displacing protein-bound Fenton metals (Fe or Cu, for example)⁹. Accordingly Cd can cause oxidative stress and lipid peroxidation as if it were redox active. Among the stress response genes indicated in Table 1, there are several antioxidant genes such as spermine/spermidine acetyl transferase³⁴ [7], heavy³⁵ [10] and light [32] polypeptides³⁶ of ferritin, Mn superoxide dismutase (SOD)³⁷ [19] and γ -glutamylcysteine synthetase (γ GCS)³⁸ [41] which synthesizes glutathione. The amino acid transporter system A1 (*ATA1*) gene [23] was also induced, which transports short-chain neutral amino acids including methionine, serine and glutamine³⁹ into cells. These amino acids are easily converted into one another, and their positions in the metabolic map are very close to glutathione (GSH). Cellular demands for these amino acids would be increased in Cd-exposed cells since cysteine and methionine residues are particularly vulnerable to ROS⁴⁰. Malic enzyme⁴¹ [28] catalyzes production of NADH which would help to reduce oxidized substances.

In addition, several Cd-induced changes also appear to

be relevant to the damage caused by ROS. Soluble methylglutaryl-CoA synthase⁴² [17] supplies starting substrates for the synthesis of sterols and fatty acids essential for cell membrane, which is impaired by lipid peroxidation caused by ROS. ROS not only modifies amino acid residues but also attacks the backbones of proteins⁴⁰. Breakage of peptide bonds can no longer be renatured by chaperons¹⁸, and the final remedy of the proteins might be degradation of themselves. It has been reported that *S. cerevisiae* strains lacking ubiquitin (Ub)-dependent proteolysis is sensitive to Cd⁴³, suggesting that the elimination of the damaged proteins is important to circumvent Cd toxicity. In HeLa cells major ubiquitins, UbB [26] and UbC⁴⁴ (1.9 fold induced; not shown in Table 1), and one of Ub-protein ligase, *Nedd4* (neuronal precursor cell-expressed developmentally down-regulated 4)⁴⁵ [35] are induced by Cd. It appears likely that a similar mechanism works in human cells.

Induction of other genes by Cadmium

Expression of *rit42* (reduced in tumor 42kd) gene [45] (also named *RTP/Drg 1/Cap43/TDD5/Ndr1*)⁴⁶ was increased twofold. It has been reported that *rit42* mRNA is expressed in a cell-cycle dependent manner: high levels in the G₁ and G₂-M phases and low levels in the S phase⁴⁷. In addition, many tumor cells do not express *rit42* and its overexpression decreases cell proliferation. These facts suggest that the induction of *rit42* gene by Cd would result in growth inhibition which is probably advantageous to cells in recovering from damage.

The "Gene regulation" column of Table 1 indicates genes whose products are known to affect the expression of other genes. These include interleukin 7 (IL7) receptor⁴⁸ [13],

interleukin 1 (IL1)⁴⁹⁾ [31] and C193⁵⁰⁾ [34] and *mafG*⁵¹⁾ [20] and retinoid X receptor (RXR) γ ⁵²⁾ [21]. Abelson murine leukemia oncogene (V-abl) encodes a protein tyrosine kinase. It is known to be negatively regulated by retinoblastoma protein and positively regulated by DNA damage signals, and is assumed to affect the expression of other genes⁵³⁾ although little is known about its target genes. The *v-abl* homolog 2 [39] gene product is expected to have a similar activity. Importin [42] supports the nuclear transport of many proteins containing histones, ribosomal proteins, p53 and heat shock transcription factors⁵⁴⁾. Cd-induced changes in the expression of the above-mentioned genes are expected to influence a number of cellular functions, many of which may probably be involved in the protective mechanisms.

Upstream MRE of the listed genes

Heavy metal-dependent induction of mammalian MT genes is mediated by repeats of the MRE enhancer sequence, which contains a conserved core sequence TGCRCNC and a less conserved GC-rich region. The core sequence has been reported to be essential for mediating metal response^{23, 24)}. Out of the genes listed in Table 1, 14 genes other than those coding for MTs were found to have the MRE consensus sequence within 1 kb of their upstream region. These genes include the gene encoding γ GCS which is known to be controlled by MRE and MRE-binding transcription factor 1 (MTF-1)³⁸⁾. Also some additional genes might actually have functional MREs. Since certain MRE sequences are known to be inactive⁵⁵⁾, an MRE-like sequence should be carefully inspected to see whether it is truly functional or not before conclusion.

In the present work, we examined changes in the gene expression profile of HeLa cells after exposure to 5 μ M CdSO₄ for 6 h. This non-lethal condition^{10, 16)} was chosen to see mainly cellular protective responses, rather than the results of metal toxicity. In summary, the cells appear to use all their genomic resources to overcome the burden as follows. MTs are induced to sequester the metal by chelating. HSPs function as chaperons to renature cellular proteins. Metabolism inclines toward synthesis of cysteine and glutathione. Anti-oxidant genes and some other genes are also induced to quench ROS and to repair cell components, respectively. A great many cellular functions are controlled by induced regulatory genes. The Ub pathway is activated to degrade some proteins which cannot be renatured.

The array used in this study contained 7,075 genes randomly selected from the human cDNA library. Since the total number of human genes is estimated to be about

30,000⁵⁶⁾ to 50,000⁵⁷⁾, 4 to 7 times more genes than those we inspected remain to be examined. Moreover, detection of Cd-induced changes in gene expression was limited since dose- and time-dependent changes could not be addressed by the present analysis. Higher concentrations of Cd, for example, would give much different expression profiles, probably reflecting to a greater extent the expression of cytotoxicity. Further studies are required to see the entire image of Cd effects.

As documented above with an example of Cd exposure, microarray analysis is a powerful tool for studying toxicant-induced gene expression profiles. Analysis of altered gene expression after exposure to industrial hazardous factors would contribute much to understanding the mechanisms of toxicity and protection against it, and also to practical aspects in occupational health such as providing indices for biological effects of the hazardous factors and human sensitivity to them.

References

- 1) W.H.O. (1992) Environmental health criteria 134: Cadmium, 1st Ed., World Health Organization, Geneva, Switzerland.
- 2) Vido K, Spector D, Lagniel G, Lopez S, Toledano MB, Labarre J (2001) A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. *J Biol Chem* **276**, 8469–74.
- 3) Beyersmann D, Hechtenberg S (1997) Cadmium, gene regulation, and cellular signaling in mammalian cells. *Toxicol Appl Pharmacol* **144**, 247–61.
- 4) Hart BA, Potts RJ, Watkin RD (2001) Cadmium adaptation in lung—a double-edged sword? *Toxicol* **160**, 65–70.
- 5) Waalkes MP (2000) Cadmium carcinogenesis in review. *J Inorg Biochem* **79**, 241–4.
- 6) Jin P, Ringertz NR (1990) Cadmium induces transcription of proto-oncogenes c-jun and c-myc in rat L6 myoblasts. *J Biol Chem* **265**, 14061–4.
- 7) Wang Z, Templeton DM (1998) Induction of c-fos proto-oncogene in mesangial cells by cadmium. *J Biol Chem* **273**, 73–9.
- 8) Thévenod F, Friedmann JM, Katsen AD, Hauser LA (2000) Up-regulation of multidrug resistance p-glycoprotein via nuclear factor- κ B activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. *J Biol Chem* **275**, 1887–96.
- 9) O'Brien P, Salacinski HJ (1998) Evidence that the

- reactions of cadmium in the presence of metallothionein can produce hydroxyl radicals. *Arch Toxicol* **72**, 690–700.
- 10) Szuster-Ciesielska A, Stachura A, Slotwinska M, Kaminska T, Sniezko R, Paduch R, Abramczyk D, Filar J, Kandefer-Szerzen M (2000) The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. *Toxicology* **145**, 159–71.
 - 11) Stoica A, Katzenellenbogen BS, Martin MB (2000) Activation of estrogen receptor- α by the heavy metal cadmium. *Mol Endocrinol* **14**, 545–53.
 - 12) Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB (1994) Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *J Biol Chem* **269**, 16896–901.
 - 13) Guével RL, Petit FG, Goff PL, Métivier R, Valotaire Y, Pakdel F (2000) Inhibition of rainbow trout estrogen receptor activity by cadmium. *Biol Reproduct* **63**, 259–66.
 - 14) Kjellström T (1979) Exposure and accumulation of cadmium in populations from Japan, the United States, and Sweden. *Environ Health Perspect* **28**, 169–97.
 - 15) McDiarmid MA, Freeman CS, Grossman EA, Martnik J (1996) Biological monitoring results for cadmium exposed workers. *AIHA J* **57**, 1019–23.
 - 16) Yamada H, Kiozumi S (2001) Lymphocyte metallothionein-mRNA as a sensitive biomarker of cadmium exposure. *Ind Health* **39**, 29–32.
 - 17) Klaassen CD (Ed.) (1999) *Metallothionein IV*. Birkhäuser Verlag, Basel.
 - 18) Morimoto RI, Tissières A, Georgopoulos C (Eds.) (1990) *Stress proteins in biology and medicine*. Cold Spring Harbor Laboratory Press.
 - 19) Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467–70.
 - 20) Maxwell SA, Davis GE (2000) Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines. *Proc Natl Acad Sci USA* **97**, 13009–14.
 - 21) Murata M, Gong P, Suzuki K, Koizumi S (1999) Differential metal response and regulation of human heavy metal inducible genes. *J Cell Physiol* **180**, 105–13.
 - 22) Chomezynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction *Anal Biochem* **162**, 156–9.
 - 23) Stuart GW, Searle PF, Palmiter RD (1985) Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences. *Nature* **317**, 828–31.
 - 24) Culotta VC, Hamer DH (1989) Fine mapping of a mouse metallothionein gene metal response element. *Mol Cell Biol* **9**, 1376–80.
 - 25) Kiozumi S, Sone T, Otaki N, Kimura M (1985) Cd-induced synthesis of metallothionein in HeLa cells. *Biochem J* **227**, 879–86.
 - 26) Stennard FA, Holloway AF, Hamilton J, West AK (1994) Characterization of six additional human metallothionein genes. *Biochim Biophys Acta* **1218**, 357–65.
 - 27) Tsuji S, Kobayashi H, Uchida Y, Ihara Y, Miyatake T (1992) Molecular cloning of human growth inhibitory factor cDNA and its down regulation in Alzheimer's disease. *EMBO J* **11**, 4843–50.
 - 28) Palmiter RD, Findley SD, Whitmore TE, Durnam DM (1992) MT-III, a brain-specific member of the metallothionein gene family. *Proc Natl Acad Sci USA* **89**, 6333–7.
 - 29) Karin M, Richard RI (1982) Human metallothionein genes-primary structure of the metallothionein-II gene and a related processed gene. *Nature* **299**, 797–802.
 - 30) Goering PL, Fisher B, Kish CL (1993) stress protein synthesis induced in rat liver by cadmium precedes hepatotoxicity. *Toxicol Appl Pharmacol* **122**, 139–48.
 - 31) Tavaría M, Gabriele T, Kola I, Anderson RL (1996) A hitchhiker's guide to the human Hsp70 family. *Cell Stress Chaperons* **1**, 23–81.
 - 32) Liao V H-C, Freedmann JH (1998) Cadmium-regulated genes from the nematode *C. elegans*, Identification and cloning of new cadmium-responsive genes by differential display. *J Biol Chem* **273**, 31962–70.
 - 33) Wickner S, Maurizi MR, Gottesman S (1999) Posttranslational quality control: folding, refolding, and degrading proteins. *Science* **286**, 1888–93.
 - 34) Xiao L, Casero RA (1996) Differential transcription of the human spermidine/spermine *N*¹-acetyltransferase (SSAT) gene in human lung carcinoma cells. *Biochem J* **313**, 691–6.
 - 35) Tsuji Y, Ayaki H, Whitman SP, Morrow CS, Torti SV, Torti FM (2000) Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. *Mol Cell Biol* **20**, 5818–27.
 - 36) Jang MK, Choi M, Park YB (1999) Regulation of ferritin

- light chain gene expression by oxidized low-density lipoproteins in human monocytic THP-1 cells. *Biochem Biophys Res Commun* **265**, 577–83.
- 37) Melov S, Coskun P, Patel M, Tuinstra R, Cottrell B, Jun AS, Zastawny TH, Dizdaroglu M, Goodman S, Huang T, Mizioro H, Epstein CJ, Wallace DC (1999) Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proc Natl Acad Sci USA* **96**, 846–51.
- 38) Günes Ç, Heuchel R, Georgiev O, Muller KH, Lichtlen P, Bluthmann H, Marino S, Aguzzi A, Schaffner W (1998) Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcription activator MTF-1. *EMBO J* **17**, 2846–54.
- 39) Wang H, Huang W, Sugawara M, Devoe LD, Leibach FH, Prasad PD, Ganapathy V (2000) Cloning and functional expression of ATA1, a subtype of amino acid transporter A, from human placenta. *Biochem Biophys Res Commun* **273**, 1175–9.
- 40) Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* **272**, 20313–6.
- 41) Yang Z, Batra R, Floyd DL, Hung H, Chang G, Tong L (2000) Potent and competitive inhibition of malic enzymes by lanthanide ions. *Biochem Biophys Res Commun* **274**, 440–4.
- 42) Hegardt FG (1999) Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthetase: a control enzyme in ketogenesis. *Biochem J* **338**, 569–82.
- 43) Jungmann J, Reins H, Schobert C, Jentsch S (1993) Resistance to cadmium mediated by ubiquitin-dependent proteolysis. *Nature* **361**, 396–71.
- 44) Fornace AJ, Alamo I, Hollander MC, Lamoereaux E (1989) Ubiquitin mRNA is a major stress-induced transcript in mammalian cells. *Nucleic Acids Res* **17**, 1215–30.
- 45) Hamilton MH, Tcherepanova I, Huibregtse M, McDonnell DP (2001) Nuclear import/export of hRPF1/Nedd4 regulates the ubiquitin-dependent degradation of its nuclear substrates. *J Biol Chem* **276**, 26324–31.
- 46) Agarwala KL, Kokame K, Kato H, Miyata T (2000) Phosphorylation of RTP, an ER stress-responsive cytoplasmic protein. *Biochem Biophys Res Commun* **272**, 641–7.
- 47) Kurdistani SK, Arizti P, Reimer CL, Sugrue MM, Aaronson SA, Lee SW (1998) Inhibition of tumor cell growth by RTR/rit42 and its responsiveness to p53 and DNA damage. *Cancer Res* **58**, 4439–44.
- 48) Hofmeister R, Khaled AR, Benbernou N, Rajnavolgyi E, Muegge K, Durum SK (1999) Interleukin-7: physiological roles and mechanisms of action. *Cytokine Growth Factor Rev* **10**, 41–60.
- 49) Gubler I, Chua AO, Stern AS, Hellmann CP, Vitek MP, Dechiara TM, Benjamin WR, Collier KJ, Dukovich M, Familletti PC, Fiedler-Nagy C, Jenson J, Kaffka K, Kilian PL, Stremlo D, Wittreich BH, Woehle D, Mizel SB, Lomedico PT (1986) Recombinant human interleukin 1 α : purification and biological characterization. *J Immunol* **136**, 2492–7.
- 50) Chu W, Burns DK, Swerlick RA, Presky DH (1995) Identification and characterization of a novel cytokine-inducible nuclear protein from human endothelial cells. *J Biol Chem* **270**, 10236–45.
- 51) Suzuki T, Blank V, Sesay JS, Crawford DR (2001) Maf genes are involved in multiple stress response in human. *Biochem Biophys Res Commun* **280**, 4–8.
- 52) Casaar-Malek I, Marchal S, Rochard P, Casas F, Wrutniak C, Samarut J, Cabello G (1996) Induction of c-Erb A-AP-1 interactions and c-Erb A transcriptional activity in myoblasts by RXR. *J Biol Chem* **271**, 11392–9.
- 53) Wang JY (2000) Regulation of cell death by the abl tyrosine kinase. *Oncogene* **19**, 5643–50.
- 54) Bonifaci N, Moroianu J, Radu A, Blobel G (1997) Karyopherin β 2 mediates nuclear import of a mRNA binding protein. *Proc Natl Acad Sci USA* **94**, 5055–60.
- 55) Koizumi S, Suzuki K, Ogra Y, Yamada H, Otsuka F (1999) Transcriptional activity and regulatory protein binding of metal-responsive elements of human metallothionein-IIA gene. *Eur J Biochem* **259**, 635–42.
- 56) Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J,

- Wei M, Wides R, X Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X (2001) The sequence of the human genome. *Science* **291**, 1304–51.
- 57) Hogenesch JB, Ching KA, Batalov S, Su AI, Walker JR, Zhou Y, Kay SA, Schultz PG, Cooke MP (2001) A comparison of the Celera and Ensembl predicted gene sets reveals little overlap in novel genes. *Cell* **106**, 413–5.