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Research Article

Global gene expression of fission yeast in response to cisplatin

L. Gatti[a], D. Chen[b], G. L. Beretta[a], G. Rustici[b], N. Carenini[a], E. Corna[a], D. Colangelo[c], F. Zunino[a], J. Bähler[b] and P. Perego[a,*]

[a] Istituto Nazionale Tumori, 20133 Milan (Italy), e-mail: paola.perego@istitutotumori.mi.it.
[b] The Wellcome Trust Sanger Institute, Cambridge CB10 1SA (United Kingdom)
[c] Università del Piemonte Orientale, Novara (Italy)

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Abstract. The cellular response to the antitumor drug cisplatin is complex, and resistance is widespread. To gain insights into the global transcriptional response and mechanisms of resistance, we used microarrays to examine the fission yeast cell response to cisplatin. In two isogenic strains with differing drug sensitivity, cisplatin activated a stress response involving glutathione-S-transferase, heat shock, and recombinational repair genes. Genes required for proteasome-mediated protein degradation were up-regulated in the sensitive strain, whereas genes for DNA damage recognition/repair and for mitotic progression were induced in the resistant strain. The response to cisplatin overlaps in part with the responses to cadmium and the DNA-damaging agent methylmethane sulfonate. The different gene groups involved in the cellular response to cisplatin help the cells to tolerate and repair DNA damage and to overcome cell cycle blocks. These findings are discussed with respect to known cisplatin response pathways in human cells.

Key words. Cisplatin; fission yeast; transcriptional profile; microarray; resistance.

Genetic alterations contributing to drug response/resistance have not been fully defined. Most of the information about mechanisms of cellular resistance and response comes from mammalian cell systems [1, 2]. Several mechanisms of resistance to cisplatin (DDP, cis-diamine-dichloroplatinum) have been described including reduced drug accumulation, enhanced repair, and increased expression of detoxication factors [3–5]. Recent evidence supports the concept that altered expression of subsets of genes may be important in determining the sensitivity/resistance to antitumor agents including DDP [6–8]. A few reports have addressed the mechanisms of DDP sensitivity/resistance in fission yeast [9, 10]. Yeast may be a good model system for the identification of determinants of drug response as the pathways involved in the cellular response to damage are conserved between yeast and mammalian cells [11].

DDP is a widely used antitumor drug, but the factors influencing its spectrum of activity and development of resistance have not been fully elucidated. Microarray technology provides an opportunity to explore global gene expression patterns, thereby giving valuable insights into cellular regulatory mechanisms. Expression profiling studies in *Schizosaccharomyces pombe* have defined a core environmental stress response (CESR) common to different stresses including those caused by the heavy metal Cd and by the alkylating agent methylmethane sulfonate (MMS) [12]. The response substantially overlaps with that of the distantly related budding yeast under similar conditions [13]. The available evidence supports a conservation of stress response mechanisms in eukaryotes.

* Corresponding author.

As altered expression of genes influencing different biochemical pathways may have relevance in cellular sensitivity and/or resistance to DDP, we examined global gene expression in response to the drug in *S. pombe* using two strains differing in sensitivity to DDP [14]. We used fission yeast because it provides an attractive model system for the study of basic cellular functions, including processes such as DNA repair and cell cycle control that are relevant for the cellular response to DNA damage induced by antitumor drugs [11]. Moreover, the well-characterized low-complexity genome and the availability of strains differing in their sensitivity to cisplatin, whose growth conditions can be tightly controlled, can give valuable insights into the determinants of chemosensitivity.

Understanding the relevant genetic/biochemical alterations of the DDP resistance/response pathway may provide a rational basis for improving therapy in resistant tumors and for the development of effective therapeutic strategies.

Materials and methods

Strains, growth conditions, and drug exposure

The *S. pombe* strain 972 (h^-, no auxotrophic markers) was the wild-type (wt) strain from which the DDP-resistant strain (previously designated wtr2, now Pt/R) was derived by exposure to increasing drug concentrations, the degree of resistance being around 5 [14]. Pt/R cells were routinely cultured in the absence of DDP, since after drug removal, the resistant phenotype was stable for at least 5 months [14]. Cultures were grown at 30 °C in EMM medium [15]. The wt and Pt/R strains were exposed to a DDP (Bristol-Myers Squibb, Princeton, N. J.) concentration inhibiting colony growth by 80% (1.28 mM) after 4 h drug exposure.

RNA extraction, cDNA labeling and microarray hybridization

Exponentially growing yeast cells were exposed to DDP for 4 h, harvested, and suspended in TES buffer (10 mM Tris pH 7.5, 10 mM EDTA pH 8, 0.5% SDS). Total RNA was isolated by phenol-chloroform extraction followed by sodium acetate precipitation. RNA quality was checked by gel electrophoresis.

Ten micrograms of total RNA was labeled by directly incorporating Cy3- and Cy5-dCTP through reverse transcription and used to hybridize onto glass DNA microarrays containing probes for >99% of all known and predicted fission yeast genes (http://www.sanger.ac.uk/PostGenomics/S_pombe/) [16]. The microarrays were scanned using a GenePix 4000 Laser Scanner (Axon Instruments, Molecular Devices Corporation, Union City, Calif.). The data analyses (i.e., determination of the scanned fluorescence ratios) were performed with GenePixPro, initial data processing and normalization were performed using an in-house script [16], and GeneSpring (Silicon Genetics, Redwood City, Calif.) was used for data evaluation.

The experiments were performed three times independently. Samples were hybridized together with a labeled reference pool consisting of the RNA samples of the untreated wt strain from the three independent extractions. The reported ratios represent the expression levels at each experimental point relative to the expression levels of the untreated wt cells from the same experiment. Because of the relative importance of the measurements for untreated wt cells, we performed technical repeats of these arrays (with swapping of fluorochromes) and used the averaged data to 'zero-transform' the data of all points. Expression ratios of biological repeat experiments were averaged. In total, nine arrays were used in this study. The complete raw data set is available at http://www.sanger.ac.uk/PostGenomics/S_pombe/.

Data analysis, hierarchical clustering, and gene classification

GeneSpring was used to discard genes that did not behave reproducibly among triplicate experiments. Hierarchical clustering was performed with pre-selected log-transformed gene sets using GeneSpring, Cluster, and Tree View software [17]. Genes with >50% of data points missing were not used.

Glutathione S-transferase activity

Glutathione S-transferase (GST) activity was determined spectrophotometrically at 37 °C according to Habig et al. [18]. The reaction mixture (3 ml) contained 100 mM phosphate buffer pH 6.5 (1.4 ml), 30 mM reduced glutathione (GSH, 0.1 ml), and 30 mM 1-chloro-2,4 dinitrobenzene (CDNB, 0.1 ml). The mixture was pre-incubated at 37 °C for 5 min and the reaction was started by adding diluted cytosol (0.4 ml). The absorbance was followed for 5 min at 340 nm. Results were normalized with respect to protein content determined using Bradford's method. Three independent determinations were performed.

Western blot analysis

Denaturated protein extracts were prepared from 50 ml of untreated and DDP-treated yeast cells [15]. Electrophoresis and Western blot were performed as described elsewhere [19]. An anti-ubiquitin antibody (clone FK1; Affiniti Research Products, Exeter, UK) was used to probe the membrane. Equal loading was checked by Ponceau staining of the filter.

Assay of septation index

Cell cycle progress into mitosis after drug exposure was assessed microscopically in 4,6 diamidino-2 phenylin-

dole (DAPI)-stained cells as the fraction having a septum (septation index). Staining was performed by incubating cells with 1 µg/ml DAPI in ethanol-fixed cells [15]. The septation index was monitored microscopically and defined as the percentage of yeast cells containing a division septum. At least 100 cells per data point were scored.

Comparison to transcriptional responses to environmental stress data

Among the genes that were induced at least twofold by DDP exposure of wt yeast cells, we selected those that were in common with either CESR or non-CESR genes or with genes induced by Cd or MMS [12]. Complete processed datasets are available at http://www.sanger.ac.uk/PostGenomics/S_pombe/. The induced CESR genes were subtracted from the genes that were up-regulated twofold or greater in at least one stress condition (i.e., H_2O_2, Cd, heat shock, osmotic stress, MMS) to identify induced non-CESR genes.

Results

Experimental design: comparisons and data analysis

We monitored global gene expression in *S. pombe* DDP-sensitive wt and DDP-resistant (Pt/R) cells before and after DDP exposure. Three conditions were investigated: wt cells exposed to DDP, Pt/R cells exposed to DDP, untreated Pt/R cells. A 4-h exposure time was chosen to allow sufficient uptake of the drug. Untreated wt cells were used as reference for all conditions. A cluster analysis of the three experimental conditions indicated that: (i) there was a large overlap in the responses to DDP between wt and Pt/R cells, both for induced and repressed genes; (ii) Pt/R cells showed a stronger response of DDP-induced genes than wt cells, both with regard to gene number and fold changes; (iii) even in the absence of DDP, the Pt/R cells showed up- and down-regulation of several DDP response genes relative to wt cells (fig. 1A).

DDP-induced transcripts

Exposure to DDP up-regulated 133 and 237 transcripts in the wt and Pt/R strain, respectively (fig. 1B). Seventy-nine transcripts were induced in both strains, including GST, glutathione (GSH)-peroxidase, glutaredoxin, and mitochondrial superoxide dismutase. A biochemical approach indicated that GST activity was higher in Pt/R than in wt cells (0.23 ± 0.01 versus 0.11 ± 0.01 µmol/min per milligram), and exposure to DDP resulted in induction of GST activity in both wt (0.56 ± 0.02 µmol/min per milligram) and Pt/R strains (0.4 ± 0.06 µmol/min per milligram). The extent of transcript modulation paralleled the quantitative analysis of GST activity.

Genes for repair proteins (putative translesion DNA repair polymerase, SPCC553.07c, and a repair enzyme for proteins inactivated by oxidation, SPAC29E6.05c) and

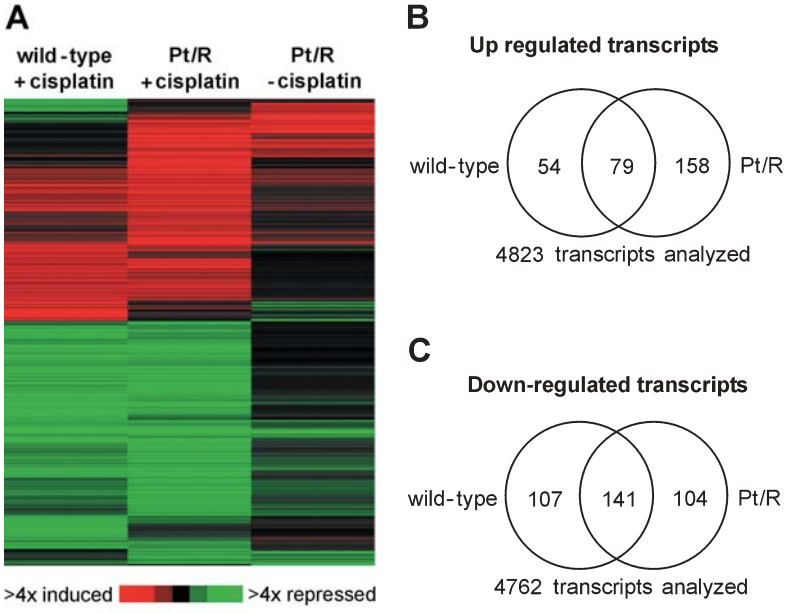

Figure 1. Clustering analysis of gene expression and Venn diagrams of modulated transcripts. A cisplatin-sensitive (wt) and -resistant (Pt/R) strain were exposed to cisplatin. The Pt/R strain was also analyzed in the absence of drug. In all three cases, a wt strain in the absence of cisplatin served as a reference. (*A*) 580 genes that were at least twofold induced or repressed in one or more of the three experimental conditions studied were hierarchically clustered. Horizontal strips represent genes, and columns represent the three experimental conditions. Induced genes are shown in red and repressed genes in green; the fold changes relative to the reference are color-coded as shown in the bar. (*B*) Venn diagram of the transcripts up-regulated in wt versus Pt/R cells after 4 h drug exposure. (*C*) Venn diagram of the down-regulated transcripts in wt versus Pt/R cells after 4 h drug exposure.

heat shock protein (HSP) genes (*swo1/hsp90, hsp9,* and *hsp16*) were also induced by DDP in the two strains. In both strains, DDP also induced the *ero1* gene (endoplasmic reticulum oxidoreductin) [20, 21]. DDP induced the expression of a flavoprotein involved in chromatin condensation and DNA fragmentation (SPAC26F1.14c) similar to human mitochondrial apoptosis-inducing factor [22–24].

An essential cell cycle regulator induced by DDP in both strains was Ppa2, the catalytic subunit of the major serine/threonine protein phosphatase, which is involved in controlling entry into mitosis, and could play a role in DNA repair pathways in mammalian cells [25–27].

Among the transcripts up-regulated only in the wt strain, genes involved in proteasome-mediated protein degradation were found (e.g., *pam1, rpn3, rpn12, uch2, ubc6, ubi4*). Thus, the wt strain reacts to DDP by activating the ubiquitin-proteasome pathway, thereby degrading damaged proteins. Proteasome activation can be detected by reduced accumulation of ubiquitinated proteins as a consequence of increased degradation [28]. Exposure of the wt strain to DDP resulted in a reduced smear of high molecular-weight proteins as shown using an anti-ubiquitin antibody (fig. 2). Genes encoding proteins implicated in stress response/tolerance (e.g., the peptidyl-prolyl cis-trans isomerase Wis2, Slt1 involved in caffeine resistance, the thioredoxin peroxidase required for oxidative stress response, and factors involved in resistance to H_2O_2) were also found. The heat shock-inducible 40-kDa cyclophilin-like (CyP40) protein Wis2 is a peptidyl-prolyl cis-trans isomerase (PPIase) implicated in intracellular protein folding, transport, assembly, and also in response to Cd stress, heat shock, and in mitotic control [29–31].

Some heat shock genes were up-regulated only in wild-type cells (*hsp10*, *hsp78*, and the two hsp90 cochaperones *sti1* and *wos2*). Wos2 plays a role in the activity of Wee1 and Cdc2, and cells lacking Wos2 are heat-shock sensitive [32]. The stability and activity of Wee1 from fission yeast is dependent on functional Hsp90 chaperones [33]. In the wt strain we found up-regulation of the recombinational repair gene *rhp51* (homolog to *RAD51* of *Saccharomyces cerevisiae*) encoding a RecA-like protein whose DNA-dependent ATPase activity is required for homologous recombination. Thus, in *S. pombe*, recombinational repair is triggered by DDP as shown for a variety of DNA-damaging agents that increase the levels of the *rhp51* transcript [34]. A putative damage-inducible deoxycytidyl transferase (SPBC1347.01c) possibly involved in mutagenic translesion DNA synthesis, was also induced by DDP.

In the Pt/R strain, DDP up-regulated genes involved in DNA damage recognition/repair including *cmb1*, coding for a protein with a high mobility-group domain, and *thp1*, encoding a mismatch-specific thymine DNA glycosylase [35]. We also found induction of Rhp54, a putative helicase involved in recombinational repair and processing replication-specific lesions [36].

Cdc18, essential for the DNA replication checkpoint and initiation of S phase [37], and *cdt1*, which cooperates with *cdc18* in checkpoint control [38], were up-regulated in Pt/R cells. Both genes play a role in the G2 phase by preventing the reinitiation of DNA synthesis until the next cell cycle [39]. As the deregulated expression of Cdc18 is sufficient to override the DNA replication checkpoint [39], we speculated that the Pt/R strain may not respond to damage with cell cycle arrest and may progress through mitosis despite damage. Indeed, septation index analysis indicated that Pt/R cells maintain the capability to progress through mitosis during DDP damage (fig. 3).

Among the genes induced only in the Pt/R strain, we found the transcription factor *ste11* [40], peroxisomal catalase A [41], and *ctr4*, encoding a high-affinity copper transporter [42].

Cisplatin exposure led to induced expression of genes involved in regulation of mating and meiosis (*ste4, mei2,*

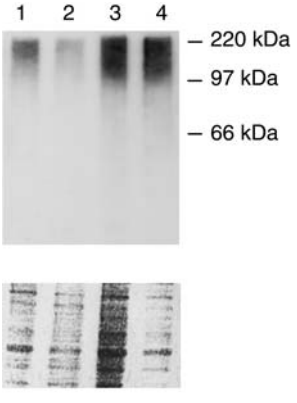

Figure 2. Western blot analysis of ubiquitinated proteins. Cisplatin-sensitive and -resistant yeast cells were exposed to cisplatin for 4 h, harvested, and processed for immunoblot analysis. Immunoblotting and Ponceau staining of the filter are shown. Control wt and Pt/R cells (lanes 1 and 3, respectively); cisplatin-treated wt and Pt/R cells (lanes 2 and 4, respectively).

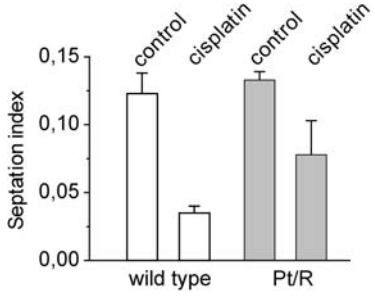

Figure 3. Analysis of septation index. Septation index of untreated and cisplatin-treated wt and Pt/R cells. Cells were exposed to cisplatin for 4 h and septation was monitored microscopically in DAPI-stained cells. Mean values (± SD, n = 3) are shown.

isp6, ste11 in wt cells; *meu8* in Pt/R cells), which appear unique to yeast.

DDP-repressed transcripts

Drug treatment induced down-regulation of 248 and 245 transcripts in the wt and Pt/R strains, respectively (fig. 1C). Among the genes negatively modulated in both strains, 141 were in common and included transporters (see below) and genes acting in RNA transcription (*rpb2*) and maturation (*dpb2*). The *ded1* (*sum3*) gene encoding a DEAD box RNA helicase was also down-regulated. Ded1 interacts with the checkpoint protein kinase Chk1, and is responsive to stress conditions that affect translation and could impact on cell cycle progression [43]. A helicase of the SNF2 family (SPAC25A8.01c) was down-regulated only in the resistant strain, whereas a putative transcriptional repressor involved in chromatin remodeling (*ecm5*, SPBC83.07) [44] and another helicase of the SNF2/RAD54 family (SPCC1235.05c), similar to *rhp54*, were down-regulated by treatment in wt and Pt/R cells. In both strains, DDP exposure also repressed the expression of a gene similar to *S. cerevisiae RAD50* (SPBC27.04), required for recombinational repair, and of the mitochondrial DNA polymerase-γ gene (*mip1*) [45].

Among cell cycle-regulated genes, down-regulation of *pub1*, whose protein product directly ubiquitinates Cdc25, and of *cdr2*, which negatively regulates the cyclin inhibitor Wee1 was observed. This modulation, possibly resulting in deregulation of Cdc25 and Wee1, could be involved in the failure of Pt/R cells to arrest before the onset of mitosis (fig. 3). In accordance with this is the observed repression of *pyp2*, encoding a tyrosine phosphatase (PTPase) that acts as a negative regulator of mitosis upstream of the Wee1/Mik1 pathway [46]. Interestingly, the transcription of *pyp2* is induced by the pathway involving Wis1 (MAPKK), Sty1/Spc1 (MAPK) and the transcription factor Atf1, and Sty1/Spc1 itself is inactivated by Pyp2 [47].

In the wt strain, DDP repressed genes involved in cell cycle regulation (*pas1, slp1*, and *cut4/apc1*) and in spindle pole body formation, septation and cytokinesis (*slp1, rng2, moe1, bgs3*, and *bgs4*) [48, 49]. The observed pattern of regulation is consistent with the cell cycle arrest of the wt strain after DDP exposure (fig. 3).

In the Pt/R strain, DDP exposure down-regulated *crb3*, encoding a DNA damage/replication checkpoint control protein interacting with Cut5/Rad4 [50]. The serine/threonine protein phosphatase PP1-2 (Sds21), involved in cell cycle control and exit from mitosis, as well as *cdm1*, encoding a subunit of DNA polymerase δ, were also repressed by DDP in Pt/R cells. Interestingly, in Pt/R cells, DDP exposure repressed the cell cycle regulators *chk1/rad27*, *wee1*, and *flp1/clp1*. *chk1/rad27* encodes a serine/threonine protein kinase required for G2 arrest after DNA damage and is implicated in a Chk1-dependent G1/M checkpoint [51]. As in mammalian cells, Chk1 negatively regulates Cdc2 by inhibition of Cdc25 [52]. The Wee1 kinase also inhibits entry into mitosis by Tyr15 phosphorylation of Cdc2 [53]. The Flp1/Clp1 phosphatase regulates the G2/M transition and coordination of cytokinesis with cell cycle progression and is required for a cytokinesis checkpoint [54, 55]. The observed pattern of down-regulation may help Pt/R cells to retain the capability of progression through mitosis regardless of the damage (fig. 3).

Although both strains induced SPAC26F1.14c (homolog to human apoptosis-inducing factor), down-regulation of *cse1* (homolog to human CAS, cellular apoptosis susceptibility-gene) was found only in the DDP-treated Pt/R strain. The significance of these regulatory effects is unclear, as apoptosis is not a general response to stress in unicellular organisms.

Though we observed up-regulation of some HSPs in both DDP-treated strains, in the Pt/R strain, DDP induced down-regulation of two members of the Hsp70 protein family, *pss1/ssp1* and *bip1* [56–58].

Another gene down-regulated in Pt/R cells was *hmt2/cad1*, which codes for a mitochondrial enzyme oxidizing sulfide and participates in regulating sulfide levels so that the cell can synthetize phytochelatins in response to heavy-metal stress [59]. In the DDP-treated Pt/R strain we also found repression of *csn2*, encoding a COP9/signalosome subunit [60–63]. The signalosome complex has been located at the interface between signal transduction and ubiquitin-dependent proteolysis [63, 64]. The transcriptional repression is consistent with the lack of proteasome activation in treated Pt/R cells (fig. 2).

Comparison between untreated DDP-resistant and -sensitive cells

Differential gene expression between the untreated Pt/R strain versus the untreated wt strain involved a relatively low number of transcripts: 90 up-regulated and 43 down-regulated. In Pt/R cells, we found relatively increased expression of the stress response gene *rds1* [65], the heat shock genes *hsp16* and *hsp9*, the *ste11* transcription factor gene and the MAP kinase gene *spk1* [66]. We also found up-regulation of SPAC2F7.06c, a DNA polymerase of the X family involved in DNA repair and containing a BRCT domain in Pt/R cells.

Among the decreased transcripts in Pt/R cells, we identified genes for drug transporters of unknown specificity and two heat shock factors (Wis2 and Sti1). As Sti1 is an activator of Hsp70 and Hsp90 chaperones, its repression in the Pt/R strain is consistent with down-regulation of Hsp70 itself. This observation, together with a similar effect yielded by DDP on the expression of two other Hsp70-like proteins in the Pt/R strain (Pss1/Ssp1 and Bip1; see above), is consistent with a global down-regulation of Hsp70 family members in the Pt/R strain. The tran-

script SPBC2G2.17c for a possible member of the 'SUN' family proteins (involved in aging, oxidative stress, mitochondrial biogenesis, DNA replication, cell wall morphogenesis, and septation) [67] was also among the repressed genes in Pt/R. The *obr1* gene, encoding a target of the Pap1 transcription pathway conferring brefeldin A resistance [40, 68] was down-regulated in the Pt/R strain. This change was associated with down-regulation of *bfr1*, which also confers resistance to brefeldin A [69].

Modulation of transporters

Modulation of efflux [multidrug and major facilitator superfamily (MFS) transporters of unknown specificity] and 'non-efflux' transporters (permeases, ATPases, cation transporters, inorganic ion transporters) was observed. Overall, 17 efflux transporters were modulated by DDP. In particular, 2 were up- (1 in the wt and the other in the Pt/R strain) and 15 were down-regulated (8 in the wt and 7 in the Pt/R strain). Moreover, the efflux transporter Mam1 and the SPBC36.03c gene, encoding an MFS efflux transporter, were up-regulated in untreated Pt/R versus the wt strain, whereas genes encoding four efflux transporters were down-regulated in the untreated Pt/R strain, including SPAC1F8.03c, SPCC569.05c, SPCC794.14, and *bfr1/hba2*.

Among the 'non-efflux' transporters, 65 transcripts were down-regulated by DDP (39 in the wild-type and 26 in Pt/R), whereas only 1 (*ctr4*) was up-regulated in the Pt/R strain. Although a role for the human and *S. cerevisiae* homologs of *ctr4* in DDP uptake has been reported [70], the contribution of the gene to cisplatin resistance is controversial [71]. Indeed, in the Pt/R strain, resistance was not associated with down-regulation of *ctr4* and/or reduced drug uptake (data not shown).

We found modulation of PMA1 and Trk2, regulating pH homeostasis. In both treated strains, we observed down-regulation of *pma1*, encoding the major yeast plasma membrane H^+-ATPase (P-type ATPase cation pump) [72] required for cytosolic pH homeostasis, for the transport of nutrients/ions across the plasma membrane, and for maintaining the electrochemical membrane potential [73]. Among the transcripts for transporters that were down-regulated only in the wt strain, we found *trk2* that, together with *trk1*, defines the major K^+ transport system in fission yeast [74, 75]. The expression of *car1*, which encodes a transmembrane transporter belonging to the MFS family and confers resistance to the inhibitor of the Na^+/H^+ antiport, amiloride [76], was repressed in DDP-treated and the untreated Pt/R strain.

In the untreated Pt/R strain, on the whole, 11 'non-efflux' transporters were up-regulated and 10 were down-regulated compared to the wild-type strain. Both groups included transporters of different specificities (e.g., amino acids, glucose), not allowing us to define a relationship with the DDP-resistant phenotype.

Comparisons with environmental stress responses

The profiles of DDP-induced genes of the wt strain were compared with CESR genes defined based on their transcriptional response to different forms of stress [12]. Among a conservative list of 140 induced CESR genes, 48 overlapped with DDP-induced genes (an additional 20 of the DDP-induced genes fall into the CESR category when comparing with a less conservative CESR list containing 314 genes). The overlapping genes are implicated in protein folding/degradation (4 of 48) and in cellular oxidant defenses (3 of 48), metabolism (14 of 48), and other functions (table 1).

Among the genes up-regulated by DDP in the wt strain, 48 were in the CESR group, whereas the remaining 92 appeared as 'drug specific.' The latter genes included transcripts implicated in protein folding/degradation, signal transduction/repair, and cell cycle regulation. DDP also induced genes with antioxidant functions.

As DDP shares some determinants of sensitivity with stress induced by Cd [9], we compared transcriptional response of wt cells to DDP with responses to Cd. Commonly modulated CESR (48 of 81 common genes) included HSP and antioxidant genes. The induced transcripts did not include *hmt1*, encoding a transporter for vacuolar sequestration of phytochelatin/Cd complexes, as expected based on the fact that modulation of *hmt1* levels in *S. pombe* does not affect DDP tolerance [9]. A comparison between DDP-induced genes and Cd-specific genes indicated that the common non-CESR genes (33 genes) belonged to different pathways. Overall, both Cd and DDP appeared to share the capability of activating expression of genes controlling protein folding and degradation, heat shock response or antioxidant functions (data not shown).

Because of the pseudo-alkylating mode of DDP action, we looked for genes commonly up-regulated by DDP and the alkylating agent MMS. Of the 62 common genes, 42 belonged to the CESR group. Several genes related to GSH metabolism were found, thereby suggesting that activation of the GSH system represents a general defense mechanism.

Discussion

Our results support the view that a stress response involving GSH-dependent enzymes and HSPs, as well as recombinational repair, is activated by DDP in fission yeast, independently of the relative level of drug resistance (figs 1, 4). Induction of defense mechanisms involving GSH could be an aspect of the general drug response, as up-regulation of several GSH-related enzymes was observed in both strains (e.g., GST, GSH-peroxidase), although previous studies suggested a lack of involvement of GSH metabolism in response to DDP [9].

Table 1. Genes at least twofold up-regulated by cisplatin in wt cells overlapping with genes commonly up-regulated by enviromental stress.

Gene name	Annotation
Carbohydrate metabolism	
SPACUNK4.16c	putative alpha-trehalose-phosphate synthase
SPAC22F8.05	putative alpha, alpha-trehalose-phosphate-synthase
SPAPB1A11.03	putative FMN-dependent dehydrogenase; similar to lactate dehydrogenase
SPBC215.11c	putative oxidoreductase; aldo-keto family
SPBC24C6.09c	similarity to transketolase; putative phospho-transacetylase
SPAC139.05	probable succinate semialdehyde dehydrogenase
SPAC19G12.09	putative aldose reductase
SPAC26F1.07	aldo/keto reductase
tps1	alpha, alpha-trehalose-phosphate synthase (UDP-forming)
SPAC4H3.03c	putative family 15 glycosyl hydrolase
SPBC1773.06c	alcohol dehydrogenase
Lipid or fatty acid metabolism	
SPAPB24D3.08c	putative NADP-dependent oxidoreductase
SPAC4H3.08	putative short-chain dehydrogenase
SPAC4D7.02c	putative glycerophosphoryl diester phospho-diesterase
Transporters	
SPCC965.06	putative potassium channel subunit
Protein folding and degradation	
hsp9	heat shock protein 9
hsp16	heat shock protein 16
SPAC2C4.15c	ubiquitin regulatory domain (UBX) protein
psi1	psi protein
Antioxidants	
grx1	thioltransferase
SPCC576.03c	thioredoxin peroxidase
gpx1	glutathione peroxidase
Others	
SPAC26F1.14c	putative flavoprotein; similar to human mitochondrial apoptosis-inducing factor
SPAC22E12.03c	THIJ/PFPI family protein; putative thiamine biosynthesis enzyme
SPAC26F1.04c	zinc-binding dehydrogenase; assembly of mitochondrial respiratory proteins
SPBC2A9.02	putative dyhydroflavanol-4-reductase
SPAC2E1P3.01	putative dehydrogenase by similarity
SPBC30D10.14	putative hydrolase
SPBC23G7.10c	putative NADH-dependent flavin oxidoreductase
SPBC725.10	similar to peripheral-type benzodiazepine receptor
SPAC513.07	putative cinnamoyl-CoA reductase
plr1	pyridoxal reductase
SPBC16A3.02c	putative quinine oxidoreductase
SPBC119.03	putative catechol o-methyltransferase

Genes commonly up-regulated by enviromental stress have been defined in Chen et al. [12]. Annotations are from *S. pombe* GeneDB http://www.genedb.org/genedb/pombe/index.jsp. Genes without a known function are not listed here (complete lists available at: http://www.sanger.ac.uk/PostGenomics/S_pombe/).

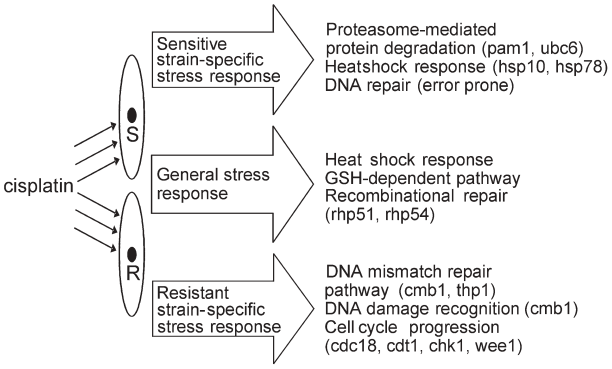

Figure 4. Cisplatin response in fission yeast. Schematic representation of the main cellular pathways activated by cisplatin treatment in fission yeast. S, wt cisplatin-sensitive cells; R, cisplatin-resistant Pt/R cells.

Among defense factors contributing to DDP resistance, an increased content of GSH and increased expression/activity of GSH-dependent enzymes have been described in mammalian models [5]. Moreover, induction of HSPs by DDP has been documented in mammalian cells using a variety of approaches. Hsp90 induction by DDP or other antitumor drugs has been associated with drug resistance [77, 78]. Finally, in this study, activation of recombinational repair after DDP damage was expected as *S. pombe* spends the majority of its time in the G2 phase, where repair of DNA lesions mainly occurs through recombination.

Transcriptional profiles of wt and Pt/R cells revealed strain-specific responses (figs 1, 4). The wt-specific response involved factors acting in proteasome-mediated protein degradation. The Pt/R-specific response suggested altered cell cycle progression and DNA damage recognition/repair. Up-regulation of genes involved in DNA damage recognition/repair (e.g., *cmb1* and *thp1*) was a feature of Pt/R cells. This is consistent with a role of the DNA mismatch repair pathway in response to DDP. Previous studies have shown that Cmb1 recognizes the cross-links produced by DDP [79, 80]. As DDP reacts with nucleophilic DNA residues including guanines, up-regulation of *thp1* could be an attempt of the cell to correct mispairs generated by Pt adducts. Up-regulation of *rhp54* is consistent with the observation that *rhp54* deletion mutants are hypersensitive to DDP (not shown). Altogether, the observed transcriptional profiles are consistent with additional biochemical evidence indicating activation of GST function after treatment of both strains, activation of the proteasome after DDP treatment of wt cells (fig. 2), and the ability of Pt/R cells to progress through the cell cycle despite of damage (fig. 3).

We observed modulation of transporters by DDP in both wt and Pt/R cells. The observed up- or down-regulations are possibly not directly related to drug sensitivity, but could be solely homeostatic changes after genotoxic

stress. Several transporters modulated in our study are members of the MFS and ATP-binding cassette superfamily. One of the most important and clinically relevant mechanisms of drug resistance in human malignancies is the active extrusion of toxic chemicals by broad-specificity multidrug resistance transporters [81, 82] and drug-specific efflux systems. However, in human cells, the multidrug resistant phenotype does not involve DDP, though a role for MRP2 in efflux of DDP-glutathione conjugates has been proposed [83].

Comparison between transcriptional responses to DDP and environmental stresses supports the idea that DDP injury activates a response at least in part common to most stresses (tables 1, 2). Up-regulation of genes related to GSH metabolism induced by MMS and DDP is consistent with the electrophylic nature of the two stresses.

Comparison of untreated Pt/R versus untreated wt cells suggests a role for stress response factors in the DDP-resistance phenotype. The factors found by comparing the two untreated strains were limited in number in relation to those modulated by treatment and included HSPs (Hsp16, Hsp9) and an X family DNA polymerase whose homologs are involved in protection against alkylating agent-induced cell death in higher eukaryotes [84]. X family DNA polymerases include mammalian/yeast error-prone β-polymerases acting in the single-base excision repair pathway after DNA alkylation and in repair of oxidative lesions of bases [85, 86]. Human β DNA polymerase is recognized to perform post-replicative error-prone translesion synthesis of DDP-DNA adducts as it bypasses Pt drug adducts [87]. Increased DDP resistance has been observed in cell lines with increased β polymerase expression [84].

Our findings should help to clarify mechanisms of cellular response and resistance to cisplatin in multicellular organisms, despite evident differences between fission yeast and mammalian cells that need to be taken into account. Indeed, diverse and more complex biological responses to DNA damage are expected and have been documented in higher organisms. For example, the regulatory control module involving the tumor suppressor gene p53 is missing in *S. pombe*, in which the major control point is the transition from G2 to M [88], and repair mainly occurs through recombination. Therefore, our study may have missed additional mechanisms that are operative in mammalian cells. On the other hand, fission yeast has additional pathways that are absent in mammalian cells. For example, a second nucleotide excision repair pathway involving *rad18* has been reported [89], but the pathway was not modulated under our conditions. Among the determinants of tolerance to cisplatin and heavy metals of fission yeast, a role for increased sulfide production has been reported [9]. However, this pathway is likely to represent a response specific for unicellular organisms. Among the pathways modulated by cisplatin, we found defense systems similar to those described in mammalian cells, including heat shock genes, GSH-related genes as well as genes controlling RNA splicing and stability and recombinational repair/DNA damage recognition [90–91]. To our knowledge, this study represents the first evidence revealing an induction of proteasome subunits by cisplatin. The role of proteasomal degradation of proteins, in particular proteins involved in cell cycle control, is an emerging area with potential pharmacological implications [92]. We identified *ero1*, which encodes a membrane glycoprotein required for protein oxidation and folding [20, 21], as a novel cisplatin-responsive gene in this study; *ppa2*, encoding the catalytic subunit of the protein phosphatase PP2A, whose mammalian homolog is involved in DNA repair [25–27], and the cell cycle-regulating genes *cd18* and *cdt1* [37–39]. *cdc18* and *cdt1* function in DNA replication, and for the human homolog of *cdc18* (CDC6), a role in cell death activation after damage has recently emerged [93]. Though the relevance of apoptosis in unicellular organisms is unclear, our study raises the possibility that genes of the apoptotic pathway (e.g., SPAC26F1.14c similar to human apoptosis-inducing factor [22–24] and *cdc18*) are present in fission yeast cells and are modulated by cisplatin exposure. In this perspective, yeast could become a useful model system to investigate the effects of drugs targeting specific cell death mechanisms.

In conclusion, various gene groups are involved in the cellular response to cisplatin in fission yeast. Some of the pathways which we found modulated (e.g., heat shock and GSH-related genes) have been described as being activated in response to DDP treatment in mammalian cells, thereby suggesting that the signaling pathways involved in the cell response to DNA damage are conserved from lower to higher eukaryotic cells. Our findings, obtained using the microarray technology in two isogenic yeast strains displaying differential sensitivity to DDP, are consistent with triggering of cell death signals by DDP in the wt strain, whereas the profile of gene expression of the

Table 2. Glutathione metabolism genes commonly modulated by cisplatin and MMS.

Gene name	Annotation	CESR
SPCC965.07c	GST	–
SPCC191.09c	putative GST	–
grx1	thioltransferase	+
SPCC576.03c	thioredoxin peroxidase	+
gpx1	glutathione peroxidase	+
SPAC824.07	putative hydroxyacyl-glutathione hydrolase	–
SPBC12C2.12	lactoylglutathione lyase	–

Annotations are from *S. pombe* GeneDB http://www.genedb.org/genedb/pombe/index.jsp. (complete lists available at: http://www.sanger.ac.uk/PostGenomics/S_pombe/).

Pt/R strain reflects an increased ability to repair or tolerate DNA damage (fig. 4). Thus, the induced genes could help Pt/R cells to survive in the presence of DDP. Multiple factors underlie resistance to DDP in yeast cells as is well documented in mammalian models. These findings are expected to provide insights into general mechanisms of the cellular response to DDP in eukaryotic organisms. Better understanding of the relevant alterations of DDP resistance and response pathways should provide useful clues to optimize therapy with platinum compounds.

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