Combined Proteome and Metabolite-profiling Analyses Reveal Surprising Insights into Yeast Sulfur Metabolism*

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Metabolomics is considered as an emerging new tool for functional proteomics in the identification of new protein function or in projects aiming at modeling whole cell metabolism. When combined with proteome studies, metabolite-profiling analyses revealed unanticipated insights into the yeast sulfur pathway. In response to cadmium, the observed overproduction of glutathione, essential for the detoxification of the metal, can be entirely accounted for by a marked drop in sulfur-containing protein synthesis and a redirection of sulfur metabolite fluxes to the glutathione pathway. A kinetic analysis showed sequential and dramatic changes in intermediate sulfur metabolite pools within the first hours of the treatment. Strikingly, whereas proteome and metabolic data were positively correlated under cadmium conditions, proteome and metabolic data were negatively correlated during other growth conditions, i.e. methionine supplementation or sulfate starvation. These differences can be explained by alternative mechanisms in the regulation of Met4, the activator of the sulfur pathway. Whereas Met4 activity is controlled by the cellular cysteine content in response to sulfur source and availability, the present study suggests that Met4 activation under cadmium conditions is cysteine-independent. The results clearly indicate that the metabolic state of a cell cannot be safely predicted based solely on proteomic and/or gene expression data. Combined metabolome and proteome studies are necessary to draw a comprehensive and integrated view of cell metabolism.

The yeast sulfur pathway has been extensively investigated at the genetic, enzymatic, and regulatory levels (3). The pools of most metabolites of the pathway have been analyzed (5, 6), and the $K_m$ values of many enzymes have been measured (3). However, some metabolic data such as the metabolite fluxes in the pathway and the concentration of the metabolites of the transsulfuration pathway (homocysteine and cystathionine) are lacking. Moreover, the levels of some sulfur metabolites are presumed to be modified in different mutants and under different physiological conditions (i.e. sulfur starvation, the presence of a sulfur metabolite, or a toxic metal in the medium), but the few quantitative data that are available are restricted to a small part of the pathway (5). Thus, it has been shown that cadmium (Cd$^{2+}$) strongly increases GSH synthesis (7), which is consistent with the primary importance of this metabolite in the Cd$^{2+}$ detoxification process (8), but the metabolic changes leading to GSH accumulation remain unknown. The γ-glutamyl-cysteine synthetase (Gsh1)$^1$ is considered as the rate-limiting enzyme of the GSH pathway since the homologous mammalian enzyme is inhibited in vitro by high levels of GSH (9), the end product of the pathway. Accordingly, previous work supports this notion in yeast (10), but no definitive proof has been provided, and this question remains opened.

Generally, studies based on proteomic and gene expression profiling are interpreted in terms of cell metabolism, assuming a trivial link between protein expression and metabolic states, but few combined transcriptome, proteome, and metabolome studies (2, 11–14) have been made to experimentally test these assumptions. The present study is intended to address the question of the correlation of metabolic data with the wealth of proteomic, genomic, and regulatory data currently available on one of the best investigated metabolic pathways. With the availability of complete and precise metabolic data, combined with genetic, enzymatic, and proteomic data, it is conceivable to model the sulfur pathway in further studies, thus providing an interesting first step in ambitious projects aiming at modeling the whole cell metabolism.

We recently developed a new method based on liquid chromatography-electrospray-mass spectrometry allowing the quantitative analysis of metabolomes by using $^{15}$N metabolic labeling of Saccharomyces cerevisiae (15). Remarkably, when applied to the yeast sulfur metabolic pathway and combined with proteomic analysis, it revealed novel and unanticipated insights into the regulation of this pathway. In particular, we found that the data from proteome and metabolite profiling can be either positively or negatively correlated, depending on growth conditions.

Assimilable sulfur is essential for all living organisms. The cell requirement for sulfur can be fulfilled by the uptake of sulfur-containing amino acids or by assimilation of inorganic sulfur into organic compounds such as cysteine or homocysteine (1, 2). In yeast, homocysteine is the precursor of methionine through the methyl cycle and of cysteine through the transsulfuration pathway (Fig. 1) (3). Cysteine is the sensor of the metabolic state in the sulfur amino acid pathway (4) and is required for the synthesis of GSH, an essential antioxidant molecule also important for detoxification.

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$^1$ The abbreviation used is: Gsh1, γ-glutamyl-cysteine synthetase.
FIG. 1. Schematic representation of the sulfur amino-acid pathway in yeast. The scheme is according to Ref. 3. Sam1 and Sam2, S-adenosylmethionine synthases; Sah1, S-adenosylhomocysteinease; Cys4, cystathionine β-synthase; Cys3, cystathionine γ-lyase; Str3, cystathionine γ-synthase; Str2, cystathionine β-lyase; Gsh1, γ-glutamylcysteine synthetase; Gsh2, glutathione synthetase; Glr1, glutathione reductase.

EXPERIMENTAL PROCEDURES

Reagents—Homocysteine, methionine, cadmium chloride, and sulfide were from Sigma. Acetonitrile was from SDS (Peypin, France). [15N]ammonium sulfate was from Eurisotop (Gif-sur-Yvette, France). Methanol, perchloric acid, trichloroacetic acid, and formic acid were from Merck. Water was deionized and filtered through a Millipore Milli-Q water purification system.

Strains and Growth Conditions—S. cerevisiae strain S288c (MATa SUC2 mal mel gal2 CUP1) was grown at 30 °C in a minimum medium corresponding to yeast nitrogen base medium modified for sulfate concentration (1 mM) and for ammonium concentration (20 mM). In these growth conditions, the generation time is 2 h. For supplementation experiments, mid-log cultures (Aο∞ = 0.4) were supplemented with methionine (0.5 mM), sulfate (0.5 mM), sulfide (0.5 mM), or homocysteine (0.5 mM) and collected after 3 h, 30 min for metabolite extraction. Concerning cadmium treatments, cells were treated with 50 μM Cd²⁺ and collected at the indicated times (0 h; 0 h, 30 min; 1 h, 10 min; 2 h; 3 h, 10 min; 4 h, 20 min; 6 h; 16 h; and 24 h) for extraction of metabolites. For sulfur starvation conditions, cells were grown for more than 10 generations with 50 μM sulfate instead of 1 mM and collected when growth was considerably slowed down (generation time greater than 10 h). For oxidative stress conditions, cells were treated with 0.6 mM H₂O₂ and collected after 40 min.

Proteome Analysis—Yeast cells were grown and treated with 50 μM Cd²⁺ or 0.5 mM methionine as described above. After 3 h of treatment, cells were labeled with 100 μCi of [15S]methionine. For sulfur starvation conditions, cells were grown for more than 10 generations with 50 μM sulfate instead of 1 mM and collected when growth was considerably slowed down (generation time greater than 10 h). For oxidative stress conditions, cells were treated with 0.6 mM H₂O₂ and collected after 40 min.

Metabolite Extraction—[35S]labeled metabolites were extracted as described below.

Metabolite Extraction—[35S]labeled cells were collected by centrifugation (4000 rpm), washed with cold water, boiled for 5 min in 0.1% perchloric acid (pH ranging from 2 to 2.5 in the extract), and centrifuged for 2 min at 4000 rpm. Unlabeled extracts were prepared in the same way. An aliquot of the unlabeled extract to be analyzed was mixed with an aliquot of the [35S]labeled reference extract. Methanol was added to the extract mixture (3/1, v/v) and centrifuged for 30 min at 18,000 × g to precipitate proteins. The methanol was then evaporated under nitrogen, and the resulting aqueous fraction of the sample was injected into the chromatographic system. The stability of sulfur metabolites was assessed in storage (−80 °C) and experimental conditions.

Liquid Chromatography-Tandem Mass Spectrometry—The system consisted of an Alliance HT model 2795 separation module (Waters, Saint Quentin en Yvelines, France) coupled to a Quattro-Micro mass spectrometer fitted with an electrospray source (Waters) and operating with Masslynx software 4.0 (Micromass, Waters). Chromatographic separations were performed on a TSK-Gel Amide 80 column (150 × 2 mm, 5 μm) (Interchim, Montluçon, France), as described previously (15).

Intracellular Concentration of Metabolites—The average diameter of S288c cells (4.4 μm) was determined by microscopic measurement of 100 different cells in the considered growth condition. The average diameter did not change significantly with culture conditions tested. Assuming that yeast cells are spherical, the intracellular volume of 10⁷ cells was calculated to be 4.5 × 10⁻⁵ liters. Intracellular concentrations were expressed in mol/liter.

Measurement of Protein Synthesis Rate and of Sulfur Metabolite Synthesis Rate—Exponentially growing cells (A∞₀ = 0.4) in minimum medium were treated with 50 μM Cd²⁺. At different times (0 min, 20 min, 50 min, 110 min, 170 min and 230 min), 4-ml aliquots were removed and labeled with [35S]sulfate (200 μCi) for 20 min. Cells were collected, washed twice with cold water, and resuspended in 50 μl of cold water. [35S] incorporation in cells was measured by scintillation counting. Metabolites were extracted by boiling the cells for 5 min. After centrifugation, the supernatant containing the metabolites was counted. The proteins in the pellet were precipitated with 30% trichloroacetic acid, washed with acetone, resuspended in 50 μl of 1 M sodium carbonate, and counted. Values were standardized to a cell concentration of 2 × 10⁶ cells/ml.

Determination of the Flux in the Glutathione Pathway—Mid-log phase cultures (2 ml, A∞₀ = 0.4) were supplemented with 0 or 50 μM Cd²⁺. After 1 h, cells were labeled with [35S]O₂⁻ (1 mCi). After 4 h, [35S]labeled cells (1 ml) were harvested, washed in 200 ml of cold water, and resuspended in 50 ml of cold water. The metabolites were extracted and separated by thin layer chromatography as described previously (16). The [35S]-labeled metabolites, GSH, cystathionine, and γ-Glu-Cys were quantified by PhosphorImager (Amersham Biosciences) as described previously (7).

RESULTS

Pools of Sulfur Metabolites under Standard Growth Conditions—Due to their high polarity, sulfur metabolites were analyzed on a liquid chromatographic system based on “hydrophobic interaction liquid chromatography,” which is suitable for electrospray mass spectrometric detection (17, 18). To limit matrix interferences that may lead to imprecise quantifications in the liquid chromatography-electrospray-mass spectrometry...
analysis (19, 20), we used as internal standards the uniformly
15N-labeled metabolites naturally present in a cell extract from
a yeast culture grown on a medium 99.8% enriched in [15N]am-
monium sulfate (21). The principle of the analysis consists of
mixing the 14N extract of interest with an aliquot of the 15N
reference extract before injection and separation of the metab-
olites on the liquid chromatography column. The 14N/15N ratio
can then be measured by mass spectrometry analysis for each
metabolite, and absolute levels of 14N metabolites are deduced
by comparison with calibration curves (Fig. 2). This method
allows an absolute and precise quantitation of nitrogen-con-
taining metabolites (15).

Table I displays the levels of sulfur metabolites in extracts
from cells grown under standard conditions. The intracellular
concentrations of most sulfur metabolites were in the range
30–80 μM except for homocysteine (<4 μM), methionine (close
to 0.5 mM), and GSH (~2 mM). The variation of metabolite
content for four different cultures of strain S288c did not ex-
ceed 25% of the mean value. Note that the intracellular con-
centrations of most sulfur metabolites is far below the
Km



of the relevant enzymes of the pathway (Table I), indicating that, in
standard conditions, the enzymes of the Cys/GSH pathway are
not saturated.

Large Variations in Sulfur Metabolite Pools under Different
Culture Conditions—We also analyzed the endogenous levels of sulfur metabolites under different physiological conditions. The addition of precursor sulfur metabolites methionine, homocysteine, or sulfide to the sulfate-based medium significantly increased all sulfur metabolite pools (Fig. 3 and not shown for sulfide supplementation). The strong increase in the end prod-
cuct (GSH) pool indicates that the metabolic flux in the pathway
was also augmented in each case. Thus, increasing the pool of
precursors is sufficient to increase the flux and the level of all
intermediate metabolites in the Cys/GSH pathway. These re-
results are consistent with the fact that, under standard condi-
tions, there is no saturating enzyme in the Cys/GSH pathway.

We also tested sulfur starvation conditions and found a general
drop in all sulfur metabolite pools (Fig. 4), particularly GSH.
The dramatic decrease of the content of this abundant sulfur
metabolite supports the notion that it can act as a sulfur
reserve (5) in addition to its other functions in the cell (22).

When cells were treated with cadmium (50 μM, 3 h), a strong
increase in all the metabolites of the sulfur pathway (except for
methionine) was observed (data not shown), confirming and
extending previous results (7). Finally, during oxidative stress
conditions (0.6 mM H2O2), no modification of sulfur metabolite
content was detected (not shown). Together, these analyses
showed a high range of variation in metabolite concentration
depending upon physiological growth conditions (up to 200-
fold). They also indicated a correlation between the flux in the Cys/GSH pathway and the content of intermediate metabolites in the pathway.

Kinetic Response of the Sulfur Metabolome during Cadmium Treatment—To detect putative transient variations in sulfur metabolite concentrations in response to cadmium treatment. As shown in Fig. 5, there was a rapid increase of homocysteine, S-adenosyl-homocysteine, and cystathionine peaking 2 h after cadmium addition followed by a wave of cysteine and γ-glutamyl-cysteine peaking at 3/4 h. The GSH pool increased regularly from the beginning of the treatment to a maximum at 5 h (15 mM) and then stabilized. After about 10 h, each pool reached a stable and constant value, indicating the establishment of a steady state at later times and representing an equilibrium between the synthesis and metabolism of the sulfur metabolites.

**Fig. 3.** Changes of sulfur metabolite pools depending on culture conditions. Cultures were supplemented with 0.5 mM homocysteine (B) and 0.5 mM methionine (C) or not treated (A), respectively. Values are expressed as the mean of at least two determinations. Error bars correspond to extreme values from at least two independent determinations (except for methionine for which only one determination was done). *, not detected. **, higher than 100 mM.

**Fig. 4.** Sulfur metabolite pools decrease under sulfur starvation conditions. Values are expressed as the mean of at least two determinations. Error bars correspond to extreme values from at least two independent determinations. A, standard condition; B, sulfur starvation condition.
different metabolites. The transient increase of some metabolite pools could correspond to the transition from the standard metabolic state to the new steady state of slow growth in the presence of cadmium. The only metabolite that did not increase in response to the metal was methionine, the levels of which decreased continuously to less than 1/10 of its initial level after 16 h.

Estimation of Sulfur Fluxes in Response to Cadmium—Data concerning metabolite pools are generally not sufficient to predict the flux in the corresponding pathway. To address this issue, we estimated the flux of sulfur metabolites in the first part of the sulfate assimilation pathway by measuring $[^{35}S]$sulfate uptake in response to cadmium. As shown in Fig. 6A, sulfate transport was not significantly modified by the treatment. In this experiment, we also estimated the sulfur incorporated into proteins and the various metabolites and found that the protein synthesis rate dropped rapidly after cadmium addition concomitantly with the increase in sulfur metabolism synthesis. This experiment and a previous one (7) also based on $[^{35}S]$sulfate labeling allowed us to estimate the balance of sulfur utilization. In standard conditions, the flux $F$ in the sulfate assimilation pathway is divided between the methionine cycle (0.6 $F$) and the Cys/GSH pathway (0.4 $F$) (Fig. 6B). In response to cadmium, the total sulfate assimilation flux is not significantly changed ($F$), but the proportions of sulfur entering the methionine cycle (0.2 $F$) and the Cys/GSH pathway (0.8 $F$) are strongly modified (Fig. 6C).

Protein and Metabolite Levels Can Be Positively or Negatively Correlated Depending on Growth Conditions—When interpreting proteomic and gene expression data, it is usually considered that induction of enzymes in a metabolic pathway indicates an increased flux in the pathway. Consistently, the analysis of the yeast cadmium response confirmed a clear correlation between the induction of enzymes involved in the GSH pathway and the increased GSH production (7, 16). Thus, this result may suggest that the assumed correlation between proteome and metabolic data can be effectively considered as a general rule. However, the sulfur metabolite analysis of sulfur starvation and methionine supplementation conditions revealed that proteome and metabolic data can also be negatively correlated. As shown in Fig. 7, enzymes involved in sulfur metabolism visible on two-dimensional gels were all found to be repressed upon methionine supplementation (compare Fig. 7A and B, for Met25, Met6, Cys3, Cys4, and not shown for Met16, Sam1, and Sam2), whereas the sulfur metabolite levels and flux were strongly increased in the pathway (Fig. 3). Conversely, sulfur starvation conditions induced the overexpression of Met25, Met16, and Cys3 (Fig. 7D), and no significant change in the expression of the other enzymes was seen. In this case, metabolite levels and pathway flux were reduced (Fig. 4) despite the strong induction of some important enzymes of the pathway. In these two growth conditions, proteome and metabolite data were thus negatively correlated. Although these observations may be rather surprising at first view, these negative correlations are not in contradiction with the properties of classical enzymes. Considering a Michaelis-Menten type enzyme, if the substrate concentration ($S$) is markedly lower than its $K_m$ (as is the case for most enzymes of the sulfur pathway), the rate of the enzyme reaction ($V$) can be approximated by the following formula: $V = K \times E \times S$, where $E$ is the enzyme concentration. Thus, a strong increase of the substrate concentration (as in the case of methionine supplementation) would lead to an increase of the reaction rate even if the corresponding enzyme is repressed. If this reasoning is valid for all enzymes of the pathway, the global flux in the pathway would increase.

DISCUSSION

Metabolomics, i.e. the global analysis of cellular metabolites, is becoming a powerful new tool for functional proteomics and for gaining insights into biological functions. Analyzing a large number of metabolites in biological medium and tracking their concentration changes under various physiological and genetic conditions should provide direct information on metabolic phenotypes complementary to gene expression and proteomic data (23–25). This work shows that although the well defined sulfur metabolic pathway has already been extensively analyzed by genetics, biochemical, and mRNA profiling studies, the metabolite profiling approach provides unanticipated insights into the regulation of the pathway, confirming its crucial importance for a complete and integrated description of cell metabolism. This approach revealed essentially four main insights:
Cadmium induces a strong decrease in protein synthesis and redirects the sulfur flux to glutathione synthesis. A. $^{35}$S incorporation in cells (■), proteins (□), and sulfur metabolites (▲) in response to Cd$^{2+}$ treatment (A). The experimental procedure is described under “Experimental Procedures.” Values were standardized to a cell concentration of 2 x 10³ cells/ml. Values are means of at least two determinations. The typical coefficient of variation was 15%. B and C, sulfur flux under standard conditions (B) and under cadmium conditions (C). The relative flux values are deduced from $^{35}$S-sulfate labeling experiments reported in Fig. 6A and from a previous study (7). F is the sulfate flux entering the cell under standard conditions (unchanged under cadmium conditions), and f and $f'$ are the unknown fluxes cycling at the end of the methyl cycle under standard and cadmium conditions respectively.

The overproduction of glutathione in response to cadmium can be entirely accounted for by a marked drop in sulfur-containing protein synthesis and a redirection of sulfur metabolite fluxes; 2) under cadmium conditions, Met4 is activated through a cysteine-independent mechanism; 3) under standard conditions, the γ-glutamyl-cysteine synthetase is not the rate-limiting enzyme of the glutathione pathway; and 4) proteome and metabolic data can be negatively correlated in certain growth conditions.

Our proteomic analyses of the cadmium response (7, 16) showed a strong induction of enzymes of the sulfur pathway concomitant with a saving of sulfur in the global proteome (i.e. sulfur-rich enzymes of glycolysis replaced by sulfur-depleted isoenzymes). These proteomic data were thus consistent with the idea that a strong increase of GSH synthesis is further enhanced by the increased availability of the sulfur amino acids not incorporated into protein. Our previous proteome data, when combined with the present metabolite profiling study, allow a precise description, in three steps, of the metabolic change leading to GSH accumulation.

A Decrease in Sulfur Utilization for Protein Synthesis—The proteins synthesized under cadmium conditions have a 30% reduced sulfur amino acid content when compared with proteins expressed under standard conditions (7). This sulfur sparing in proteins combined with a drop in global protein synthesis upon cadmium treatment results in a strong and rapid decrease in sulfur amino acid incorporation into proteins (a 3-fold decrease for cells treated with 50 μM Cd$^{2+}$ as seen in Fig. 6A), providing more sulfur (or homocysteine) for GSH synthesis. The drop in protein synthesis does not seem to be related to methionine and cysteine availability since the cysteine pool increases and the methionine content only slightly decreases during the first hours of cadmium treatment. The signaling pathway controlling this rapid drop in protein synthesis remains to be identified.

The Increased Homocysteine Pool Redirects the Sulfur Flux to the GSH Pathway—Cadmium treatment does not modify the rate of sulfur uptake. At the homocysteine step, the flux is divided between the methyl cycle (through Met6 or Mht1 and Sam4 (26)) for methionine synthesis and the Cys/GSH pathway (through Cys4). The dramatic increase in homocysteine concentration (from less than 4 μM to 100 μM) is probably due to the decreased utilization of methionine in proteins and to the recycling of homocysteine at the end of the methyl cycle (Fig. 1). Finally, the only outlet for homocysteine is its redirection to the Cys/GSH pathway.

Neosynthesis of Enzymes of the Sulfur Pathway—A previous analysis showed a strong induction of the synthesis of enzymes of the sulfur pathway upon cadmium treatment (16). Two-dimensional gel analyses and Coomassie Blue staining quantitation of the protein spots showed an increase in Met25, Cys4, and Cys3 by a factor of 1.5, 1.9, and 3.9, respectively, after a 4-h treatment (data not shown). The increased amount of these enzymes (particularly significant in the case of Cys3) may contribute to increasing the flux if one or some of these enzymes are limiting in the pathway under these growth conditions. In particular, 2 h after cadmium addition, the cystathionine pool reached values (1.5 mM) much higher than the $K_{m}$ of Cys3 (0.25 mM), indicating that Cys3 activity has reached its $V_{max}$ value and that the enzyme may not be abundant enough during the first 2 h to metabolize the increasing pool of cystathionine. The strong increase in the amount of Cys3 would then allow efficient metabolism of the precursor after 2 h and the subsequent decrease in homocysteine and cystathionine contents.
Previous genetic analysis demonstrated that the strong repression observed after methionine addition (3) is mediated by cysteine (4), suggesting that methionine is converted into cysteine and that elevated intracellular cysteine concentrations repress the pathway. The metabolic data presented here, i.e. the increase in cysteine pool upon methionine addition, support this notion. Also consistent with pathway activation being controlled by the level of the cysteine pool is the fact that under conditions of sulfur starvation (low level of cysteine), the genes of the sulfur pathway are induced (27). However, in the presence of cadmium, the genes and enzymes of the sulfur pathway are highly induced (7), although we found here that the cysteine content is also strongly increased. This observation suggests that in the presence of cadmium, the sulfur pathway is not repressed by cysteine or that this repression is overcome by another activation pathway. This putative pathway would activate the transcriptional activator Met4 since all the genes of the sulfur amino acid pathway are induced by cadmium in a Met4-dependent way (7). Consistent with our data, recent work showed that Met4, which is normally inactivated following methionine supplementation (through the increased pool of cysteine), remains active when methionine-supplemented cells are treated with cadmium (28, 29). Under cadmium conditions, Met4 is stabilized as a result of the dissociation of the Skp1-Met-30 interaction in the SCF<sup>Met30</sup> ubiquitin ligase complex (28) that targets the ubiquitylation and the degradation of Met-4 upon methionine addition (30).

Our data indicate that, under standard conditions, there is no limiting enzyme in the cysteine/glutathione pathway. This conclusion is based on the fact that the addition of a precursor metabolite to the medium (sulfide, homocysteine, or methionine) is sufficient to strongly increase the flux in the pathway, although the levels of the corresponding enzymes are not increased in these conditions (not shown and Fig. 7). According to these results, the limiting step (if any) would more likely be the first part of the sulfur pathway, i.e. the sulfate assimilation/reduction pathway. Intriguingly, it is commonly accepted that the limiting enzyme of the cysteine/glutathione pathway is the γ-glutamyl-cysteine synthetase Gsh1. This assumption is based on the fact that the homologous mammalian enzyme is inhibited <i>in vitro</i> by a high level of GSH, the end product of the pathway (9). Consistent with this hypothesis, strains overexpressing Gsh1 present a significant increase in GSH content (10). However, this increase may also be interpreted as the consequence of the regulation of Met4 activity. As the γ-glutamyl-cysteine synthetase activity is strongly augmented in this strain (10), the pool of cysteine, the precursor metabolite of Gsh1, is expected to be reduced, leading to a significant derepression of the sulfur assimilation pathway and an increased flux in the pathway. Further analyses will be necessary to identify unambiguously the rate-limiting step of the pathway.

A striking result of this work is the observation that the proteome and the metabolome of the sulfur pathway can be positively correlated under some conditions (<i>i.e.</i> cadmium) and negatively correlated under other conditions; when cells were starved for sulfate, the metabolite level and the flux were reduced in the sulfur pathway, whereas the corresponding enzymes were induced. Conversely, methionine supplementation enhanced the flux and metabolite pools in the pathway, whereas the enzymes were strongly repressed. These counterintuitive results can in fact be interpreted simply as the consequence of cysteine-mediated repression; increased (or decreased) flux in the GSH pathway increases (decreases) the level of cysteine, which in turn represses (induces) the enzymes of the pathway. According to this simple logic, the negative correlation between data from proteome and metabolite profil-
ing might appear to be the rule in this pathway. Interestingly, the positive correlation observed for cadmium response is thus an exception to this rule since the cysteine-mediated repression seems absent in the presence of cadmium as shown above. Altogether, these data emphasize the necessity to carefully confirm predictions regarding cellular metabolism inferred from gene expression data. They also show the essential contribution of metabolite-profiling analysis to the interpretation of proteomic data, thus underscoring the complementarity of the two approaches.

Cell metabolic regulations are multiple and intricate. Metabolite levels are regulated by changes in enzyme expression and activity. In turn, these processes are regulated by metabolite levels, and these reciprocal regulations feed intricate networks for the maintenance of the cellular metabolic balance. In this context, the present study shows the essential contribution of metabolome analyses to the interpretation of the data provided by proteomics and transcriptomics as well as genetic and biochemical approaches, thus drawing an integrated view of cell physiology.

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