

Combined omics approaches reveal distinct responses between light and heavy rare earth elements in Saccharomyces cerevisiae

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1 Combined omics approaches reveal distinct responses between light and heavy rare earth

- 2 elements in *Saccharomyces cerevisiae*
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27 Highlights

- First proteo-transcriptomics analysis revealing yeast molecular response to REEs
- Rare earth elements trigger specific responses
- Yeast cells react differentially to light vs heavy REEs
- Cell wall organization and pheromone signalling pathways are key yeast responses
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33 Keywords

34 Lanthanides; REEs; lanthanum; ytterbium; proteomics; transcriptomics; cell wall

35 Abstract

- 36 The rapid development of green energy sources and new medical technologies contributes to the
- increased exploitation of rare earth elements (REEs). They can be subdivided into light (LREEs)

and heavy (HREEs) REEs. Mining, industrial processing, and end-use practices of REEs has led 38 to elevated environmental concentrations and raises concerns about their toxicity to organisms and 39 40 their impact on ecosystems. REE toxicity has been reported, but its precise underlying molecular effects have not been well described. Here, transcriptomic and proteomic approaches were 41 combined to decipher the molecular responses of the model organism Saccharomyces cerevisiae 42 to La (LREE) and Yb (HREE). Differences were observed between the early and late responses to 43 La and Yb. Several crucial pathways were modulated in response to both REEs, such as oxidative-44 reduction processes, DNA replication, and carbohydrate metabolism. REE-specific responses 45 involving the cell wall and pheromone signalling pathways were identified, and these responses 46 have not been reported for other metals. REE exposure also modified the expression and abundance 47 of several ion transport systems, with strong discrepancies between La and Yb. These findings are 48 valuable for prioritizing key genes and proteins involved in La and Yb detoxification mechanisms 49 that deserve further characterization to better understand REE environmental and human health 50 51 toxicity.

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53 1. Introduction

The rare earth element (REE) metal group consists of yttrium (Y), scandium (Sc), and 15 54 lanthanides, spanning from lanthanum (La) to lutetium (Lu). REEs can be divided into two different 55 groups known as light REEs (LREEs), which gather La, Ce, Pr, Nd, Pm, Sm, and Eu, and heavy 56 REEs (HREEs), which include Gd, Tb, Dy, Y, Ho, Er, Tm, Yb, and Lu (Cotton, 2006). These 57 elements display unique physical (magnetic) and chemical (catalytic) characteristics that make 58 them essential for advanced technologies (e.g., green energy production, communication, screens, 59 medicines, catalysts) (Ciacci et al., 2015). REEs are also used as fertilizers and food supplements 60 for cattle, pigs, and chickens (He et al., 2001; He and Rambeck, 2000; Hu et al., 2004; Panichev, 61 2015). The expanding exploitation of REEs owing to their increased use and low recyclability 62 63 (Binnemans et al., 2013; Ciacci et al., 2015) has generated concern regarding their subsequent environmental and toxicological impacts. High REE concentrations are found in the vicinity of 64 REE mining sites and impact the surrounding environment, as well as human health (Lee and Wen, 65 2018; Noack et al., 2014; Wei et al., 2013). In this context, REEs have been categorized as 66 67 emerging contaminants (Gwenzi et al., 2018; Souza et al., 2021).

REEs were initially considered harmless and nonessential elements (Leeuw and Academic, 2000; 68 69 Setua et al., 2010). However, several recent studies demonstrated the ecotoxicological effect of such elements on both aquatic and terrestrial organisms, including mammals (Gonzalez et al., 2014; 70 Pagano et al., 2015; Rim et al., 2013). REEs have been shown to display antibacterial, antifungal 71 72 and nematicidal activities (Wakabayashi et al., 2016). They also affect urchin embryogenesis (Pagano et al., 2016; Trifuoggi et al., 2017) and human health (Pagano et al., 2015; Wei et al., 73 2013). More specifically, it was shown that REEs induced oxidative stress through the production 74 75 of reactive oxygen species, lipid peroxidation, and the modulation of superoxide dismutase, catalase, and glutathione peroxidase in plants, fish, rats and mice and in animal cell lines (including 76 77 human cells) (Babula et al., 2015; Gonzalez et al., 2014; Liang and Wang, 2013; Pagano et al., 2015; Xu et al., 2016). Other effects have been observed, such as membrane permeability 78 alterations (El-Ramady, 2008; Gao et al., 2017; Técher et al., 2020; Weiwei et al., 2007; Yufeng et 79 al., 2007), mitotic and chromosomal aberrations (Pagano et al., 2015), and the overproduction of 80 exopolysaccharides (Fitriyanto et al., 2011) in microorganisms. However, these aspects of REE 81 toxicity were investigated in diverse organisms, making it difficult to draw comprehensive 82

conclusions. Moreover, the precise underlying molecular mechanisms are not well described, and
 only a few studies have hypothesized that LREEs and HREEs might induce different cellular

85 responses as a result of lanthanide contraction (a decreasing ionic radius with an increasing atomic

86 mass) or due to their reactivity and chemical differences (Gonzalez et al., 2014; Wang et al., 2017).

87 For instance, plants that accumulate REEs display specific enrichment of either LREEs or HREEs

88 (Grosjean et al., 2020, 2019). Taken together, these few available studies support the need to 89 elucidate the cellular and molecular mechanisms involved in the response to REEs, similar to work

90 performed on the specific REE yttrium (Grosjean et al., 2018).

To decipher the cellular response to new stressors and pollutants such as REEs, combining different 91 and complementary "omics" analyses can offer many benefits (Taymaz-Nikerel et al., 2016). There 92 is a considerable amount of data regarding the exposure of yeast cells to various toxic metals 93 (Wysocki and Tamás, 2010), and large-scale omics methods are powerful approaches for 94 investigating metal stress in yeast (Hosiner et al., 2014; Jin et al., 2008; Taymaz-Nikerel et al., 95 2016). Such analyses with model organisms are especially relevant as a first step to better 96 97 comprehend the functions involved in areas that have received little research attention. In our study, we investigated the unicellular eukaryotic model Saccharomyces cerevisiae, which has been 98 remarkably informative and extensively used as a proxy for unravelling eukaryotic cellular 99 machinery and mechanisms (Botstein et al., 1997; Kachroo et al., 2017). We hypothesized that 100 REEs would impact the yeast physiology and that LREEs and HREEs would trigger different 101 responses. To test our hypotheses, we chose La and Yb as a representative LREE and HREE, 102 respectively. La was selected as it is the first element of the lanthanide series while Yb is almost 103 104 the last and La and Yb are among the most extracted and the least recycled LREEs and HREEs, respectively (Ciacci et al., 2015). Additionally, we investigated whether REE-triggered responses 105 106 would be the same as for other well-known toxic metals.

107 This is the first study that combines an investigation of the transcriptome and proteome of *S.* 108 *cerevisiae* upon exposure to REEs. The transcriptional patterns were compared at two different 109 effective concentrations of two REEs, and time-dependent proteomic profiles were obtained to 110 provide the first insights into REE toxicity in this important eukaryotic model.

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112 2. Materials and methods

2.1. Yeast strain, chemicals and REE toxicity tests

The wild-type strain BY4741 of S. cerevisiae (MATa; his $3\Delta 1$; leu $2\Delta 0$; met $15\Delta 0$; ura $3\Delta 0$) was 114 used. REEs in their trichloride form were purchased from Sigma-Aldrich (MO, USA). To 115 determine the EC_{10} (the concentration at which 10% of the population is inhibited) and EC_{50} (the 116 concentration at which half of the population is inhibited), log-phase cells (OD_{600nm} 0.15) were 117 exposed to increasing REE concentrations in modified YNB-β-glycerophosphate liquid medium 118 (YNB medium lacking KH₂PO₄, substituted by 1 mM β-glycerophosphate to avoid REE 119 precipitation, pH 5.8), and growth (OD_{600nm}) was recorded for 10 h at 28°C under constant shaking 120 (180 rpm). The growth ratio (OD at 9 h over OD at 4 h) was measured in log-phase and plotted 121 against the REE concentrations. 122

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2.2. Sample preparation for RNA-seq analysis

The wild-type strain was grown in YNB-β-glycerophosphate liquid medium. Cultures were inoculated at an OD_{600nm} of 0.05, and once the cultures reached an OD_{600nm} of 0.6, the cells were exposed for 1 h to 50 or 160 μ M lanthanum or to 6 or 8 μ M ytterbium, corresponding to their EC₁₀ and EC₅₀, respectively. The cells were then harvested by centrifugation at 4000 rpm for 1 min at room temperature and flash frozen. After cell disruption by bead beating, total RNA was columnpurified using an RNeasy mini kit (Qiagen, Les Ulis, France) according to the manufacturer's
instructions, and the gDNA was removed using DNAse I (Sigma Aldrich). RNA integrity was
further assessed using a Bioanalyzer 2100 (Agilent, CA, USA). Strand-specific cDNA libraries
were prepared by Eurofins Genomics (Ebersberg, Germany) using the TruSeq-3 library kit.
Sequencing was carried out on an Illumina HiSeq 2500 with chemistry v4 applying the high-output
run mode and using 2x100 bp paired-end sequencing.

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2.3. Analysis of differentially expressed (DE) genes

The data obtained were analysed using the online platform Galaxy (<u>https://usegalaxy.org/</u>). The 138 reads were first trimmed using the Trimmomatic v0.36.1 tool to remove any remaining Illumina 139 adaptor sequences and low-quality reads, and FastQC v0.67 was used to check read quality before 140 and after the Trimmomatic step. Sorted trimmed reads were mapped to the reference genome 141 (S288C R64-2-1 of 2015-01-31 from https://www.yeastgenome.org/) of S. cerevisiae using TopHat 142 v2.1.1. Reads that were mapped to the reference genome were quantified using HTSeq-count 143 v0.6.1p1. Finally, differential gene expression analysis between treatments was carried out using 144 DESeg2 v1.14.1. Genes that were differentially expressed among the conditions were identified 145 and expressed as the log₂-fold change, with adjusted p-values calculated via the Benjamini-146 147 Hochberg test. A cut-off-adjusted p-value of < 0.01 was applied.

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2.4. Sample preparation for proteomics analysis

The wild-type strain was grown as mentioned in section 2.1. Cultures were inoculated at an OD_{600nm} 150 151 of 0.05. Once the cultures reached an OD_{600nm} of 0.6, the REEs were added at the EC₅₀ concentration and exposed for 1 or 4 h. The cells were harvested by centrifugation at 4000 rpm at 152 room temperature for 1 min. The cell pellets were washed twice with 50 mM PBS pH 7 and flash 153 frozen. Proteins were extracted from the frozen cells by bead beating with a Precellys 24 instrument 154 (Bertin) after the addition of 1X LDS (Invitrogen) consisting of 106 mM Tris-HCl, 141 mM Tris 155 base, 2% (m/v) lithium dodecyl sulfate, 10% (m/v) glycerol, 0.51 mM EDTA, 0.22 mM SERVA 156 Blue G250, 0.175 mM phenol red, buffered at pH 8.5 and supplemented with 2.5% (v/v) ß-157 158 mercaptoethanol. The samples were heated for 5 min at 95°C, loaded (25 µL per well) onto a 4-12% gradient 10-well NuPAGE denaturing gel (Invitrogen), and subjected to electrophoresis for 5 159 min as previously recommended (Hartmann et al., 2014). After rapid staining with Coomassie Blue 160 Safe (Invitrogen), polyacrylamide bands corresponding to the whole proteomes were cut out of the 161 gel, destained in ultrapure water, reduced with dithiothreitol, and treated with iodoacetamide before 162 performing proteolysis with Trypsin Gold Mass Spectrometry Grade protease (Promega) in the 163 presence of 0.01% (v/v) ProteaseMAX surfactant (Promega). 164

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2.5. Tandem mass spectrometry

167 Tryptic peptides were subjected to tandem mass spectrometry with a Q-Exactive HF tandem mass spectrometer (Thermo Fisher Scientific, Les Ulis, France) incorporating an ultrahigh field Orbitrap 168 analyser coupled in line to an Ultimate 3000 chromatography system (Thermo Fisher Scientific). 169 The tandem mass spectrometer was operated in the data-dependent acquisition mode as previously 170 described (Klein et al., 2016). Briefly, each sample (4 µL) was injected and desalted on a reverse-171 phase capillary C18 PepMapTM 100 precolumn (LC-Packing) and then peptides were resolved 172 based on their hydrophobicity on a nanoscale 500-mm C18 PepMap 100 column (5 mm x 300 µm 173 i.d., Thermo Fisher Scientific) using a 90-min gradient from 2.5% to 40% of 80% CH₃CN, 19.9% 174 H₂O, and 0.1% formic acid. Once separated, mass spectra of the peptides were acquired in the full-175 scan range of 350-1800 m/z at a resolution of 60,000 and an AGC target of 3.10⁶. Peptide ions with 176

177 charges of $^{2+}$ or $^{3+}$ were selected for fragmentation according to a Top20 method consisting of the 178 sequential analysis of each of the 20 most abundant peptide ions. MS/MS mass spectra were 179 acquired with an AGC target set at 10^4 , a loop count of 60 ms, an isolation window of 1.6 *m/z*, a 180 resolution of 15,000, a dynamic exclusion of 20 s, and a scan performed over a dynamic mass range 181 from the first mass detected up to 15 times the first mass.

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2.6. Peptide-to-spectrum assignment and protein identification

Peak lists were automatically generated from raw files with an in-house script using the Proteome 184 Discoverer 1.4.1 (Thermo Fisher Scientific) daemon conversion function and the following 185 options: minimum mass (400), maximum mass (5,000), grouping tolerance (0), intermediate scans 186 (0). and threshold (1,000). MS/MS spectra from the resulting *.mgf files were assigned to S. 187 *cerevisiae* peptide sequences with Mascot v2.5.1 (Matrix Science) with the following parameters: 188 full trypsin specificity with up to two missed cleavages allowed, static modification of 189 carbamidomethylated cysteine, variable deamidation of asparagine and glutamine, variable 190 oxidation of methionine, mass tolerance of 5 ppm on parent ions and mass tolerance on MS/MS of 191 0.02 Da. Peptide matches with a Mascot peptide score below a p-value of 0.05 were parsed with 192 IRMA (Dupierris et al., 2009). Proteins were validated when at least two different peptides were 193 detected. The false discovery rate for protein identification was less than 1%, as estimated with the 194 reverse database decoy Mascot search option. Spectral counts corresponding to the number of 195 MS/MS spectra assigned per protein were extracted for each sample without applying the 196 parsimony rule to avoid isoform quantitation bias. The normalized spectral abundance factor 197 198 (NSAF) for each protein was calculated as the total spectral count divided by its molecular mass in kDa and presented as a percentage. Spectral counts were compared between conditions using the 199 T-Fold method in PatternLab (Carvalho et al., 2012) after normalizing the spectral counts. The 200 statistical classes were defined as "Blue" for proteins that satisfied both $|T-fold| (\geq 1.5)$ and its 201 202 associated p-value (≤ 0.05), "Orange" for proteins with identifications that did not meet the T-fold criterion (but should be further investigated because they had low p-values), "Green" for proteins 203 204 that satisfied the fold criteria but were likely false positives, and "Red" for proteins that did not meet both fold and p-value criteria. 205

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2.7. GO term enrichment analysis

208 Overrepresented gene ontology (GO) terms among differentially expressed genes (DEGs) and proteins (DEPs) in each condition tested were obtained using the online Database for Annotation, 209 Visualization and Integrated Discovery (DAVID) tool (Huang et al., 2009a, 2009b). GO terms 210 significantly overrepresented for biological pathways, cellular compartment, and molecular 211 functions were selected with a set p-value cut-off of < 0.05. Kyoto Encyclopaedia of Genes and 212 Genomes (KEGG) analysis was also performed on DEGs and DEPs using the online DAVID tool 213 with the same cut-off. DEG and DEP heatmaps were obtained using the R package pheatmap 214 v1.0.10 with a complete clustering method. Euclidian clustering distance measurement was 215 selected for rows and columns. Venn diagrams were drawn using the R package Venneuler v1.1-216 217 0.

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219 **2.8. Elemental analyses**

Yeast cultures were grown in YNB- β -glycerophosphate as previously mentioned. The cultures were inoculated at an OD_{600nm} of 0.05, and once the cultures reached an OD_{600nm} of 0.6, lanthanum and ytterbium were added at 50 μ M and 6 μ M, respectively. After 1 h of exposure to the REEs, the yeast cells were harvested by centrifuging at 4000 rpm for 1 min at 4°C. The cell pellets were

washed three times with ice-cold MES (20 mM) – EDTA (10 mM) buffer (pH 6), three times with 224 ice-cold ultrapure water, and then dried for 48 h at 70°C. Triplicate samples were prepared. 225 Aliquots (125 mg) were mineralized with 1.75 mL of ultratrace HNO₃ and 0.5 mL of ultratrace 226 hydrogen peroxide in closed tubes placed in a block digestion system (DigiPREP, SCP Sciences, 227 Courtaboeuf, France). Gradual heating was used to achieve a final temperature of 100°C (total 228 heating time of 265 min). Ultrapure water was then added to a final volume of 12.5 mL. Blank and 229 reference materials were digested under the same conditions as the samples. Concentrations of Mn, 230 Mo and Zn were determined using inductively coupled plasma mass spectrometry (ICP-MS, X 231 Series II Model, Thermo Fischer Scientific, Courtaboeuf, France). Other elements were analysed 232 by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Radial ICAP6500 Model, 233 Thermo Fischer Scientific). The validity of the analytical methods was checked using certified 234 reference material (oriental basma tobacco leaves, INCT-OBTL-5, LGC Promochem, Molsheim, 235 France) (Table S1). 236

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2.9. Data availability

The mass spectrometry and proteomics datasets are available through the ProteomeXchange Consortium via the PRIDE partner repository (https://www.ebi.ac.uk/pride/) under the dataset identifier PXD010700 and project DOI 10.6019/PXD010700. The RNA-seq datasets are available in the NCBI GEO repository (GSE175826).

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244 **3. Results and discussion**

245 **3.1. REE toxicity assessment**

246 To compare the cellular toxicity of La and Yb, the yeast EC_{10} and EC_{50} were first established for the two elements. For this, S. cerevisiae was exposed to increasing concentrations of these two 247 REEs, and the cellular density was monitored every hour for nine hours (Fig. 1a,c). Different 248 249 concentration ranges were used for La and Yb because preliminary tests showed a higher toxicity 250 of Yb than La (data not shown). The growth ratio between 9 and 4 hours was then plotted against the REE concentration to determine the EC_{10} and EC_{50} values (Fig. 1b,d). Yb was 20 times more 251 252 toxic than La, which is in line with observations using Escherichia coli (Técher et al., 2020). The respective EC₁₀ and EC₅₀ values for La were calculated to be 50 μ M and 160 μ M and were 6 μ M 253 254 and 8 µM for Yb (Fig. 1b,d).

We also investigated the effect of La and Yb exposure on the elemental composition of the cells 255 (Fig. 1e). We observed an increase in the Mn (18 to 50%), Fe (29 to 42%), Zn (13 to 18%) and Cu 256 257 (80 to 84%) concentrations under both La and Yb exposures. Under Yb exposure, the Ca (10-fold) and Na (4-fold) concentrations increased, whereas their concentrations were less affected by 258 exposure to La (2-fold increase for Ca at La EC_{10}). The only decreased elemental concentration 259 observed was for K (-20%) under Yb exposure (Fig. 1e). The decrease in K was probably triggered 260 to counterbalance the increase in Ca and Na to maintain the cationic balance in the cells. Another 261 hypothesis is that REEs can induce plasma membrane permeabilization as observed in other 262 organisms (Ramos et al., 2016; Técher et al., 2020). Permeabilization of the plasma membrane 263 would allow the nonspecific influx of ions in the cells, explaining the observed increase of cellular 264 cation content following La and Yb exposure. In addition to these preliminary investigations of 265 266 REE-dependent ionome alterations and La- and Yb-triggered toxicity, the EC_{10} and EC_{50} were chosen to further study the common and potentially specific yeast cell responses to these different 267 lanthanides through transcriptomic and proteomic analyses (Fig. 1f). 268

269 Prior to describing the cellular mechanisms involved in the response to the different REEs, we first

compared the transcriptome of yeasts exposed to the EC_{10} and EC_{50} of the two REEs to test whether

271 different responses would be observed at these two concentrations. The time-evolving proteome

272 was also monitored and analysed.



Figure 1. Impact of REEs on *Saccharomyces cerevisiae* growth and ionome and the omics experimental setup approach. (a) Growth of yeast exposed to different La concentrations. (b) Growth ratio (OD at 9 h over OD at 4 h) of yeast exposed to increasing amounts of La, enabling the identification of the EC_{10} and EC_{50} , the concentrations that inhibited 10% and 50% of the growth, respectively. (c) Growth of yeast exposed to different Yb concentrations. (d) Growth ratio (OD at 9 h over OD at 4 h) of yeast exposed to increasing amounts of Yb, enabling the identification of the EC_{10} and EC_{50} . (e) Ionome analysis of cells exposed to La at EC_{10} , Yb at EC_{10} and La at the same concentration as that corresponding to Yb EC_{10} (6 μ M). Significant differences from the wild-type condition are indicated by different letters (ANOVA, Tukey HSD). Data are the means (±SD) of three independent cultures. (f) Experimental setup of yeast cultures used for RNA-seq and proteomic analyses. Cells were exposed to two concentrations of La and Yb (EC_{10} and EC_{50}) for two exposure durations (1 h and 4 h). Three independent experiments were carried out for each condition.

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3.2. Transcriptomic response to different effective concentrations (ECs) of La and Yb

We first aimed to identify the transcriptomic response of yeast cells to two different conditions of a high (EC₅₀) and a low (EC₁₀) toxicity concentration. The transcriptome of *S. cerevisiae* exposed for one hour to La and Yb at their respective EC₁₀ and EC₅₀ values was analysed (Fig. 1f, Fig. 2). Overall, we identified 1924 differentially expressed genes (DEGs) (applying a fold change (FC) cut-off of \geq 1.5 and p <0.01) in at least one condition (REE-stressed cells vs control condition).

280 The direct comparison of gene expression between EC_{50} and EC_{10} for the two REEs identified only a few DEGs (Fig. 2b). When exposed to La, 10 DEGs showed higher expression at EC_{10} than at 281 EC₅₀, while 14 DEGs were upregulated under EC₅₀ conditions (Fig. 2b, Table S2). A similar 282 observation was made for Yb. Twenty-two DEGs were more abundant under the EC₁₀ condition 283 than under the EC₅₀ condition, while this was the case for 13 DEGs under the EC₅₀ condition (Fig. 284 2b, Table S2). We extended the analysis to the entire set of genes that were significantly 285 differentially expressed relative to the control condition (p < 0.01). Again, a relatively high 286 similarity of expression patterns existed between the two exposure concentrations, with only a few 287

differences in the transcriptomic profiles between the EC_{10} and EC_{50} conditions (Fig. 2a, Table S2). In line with our observations, few differences in DEGs between EC_{10} and EC_{50} exposure were obtained for other metals such as Zn, Cd, Cu, Cr and Ag (Jin et al., 2008). For instance, when exposed to these different metals, only 6 DEGs (Cd), 52 DEGs (Cr) and 69 DEGs (Hg) were found to be different between the EC_{10} and EC_{50} conditions.

Given the small number of differences at the transcriptome level between the two tested

concentrations and to optimize the number of changes in protein abundance, only the EC_{50} for both

La and Yb was used to analyse the time-evolving proteome.



Figure 2. Comparative transcriptomic analyses of yeast exposed to La and Yb showed a similar pattern between the EC₁₀ and EC₅₀ treatments. (a) Heatmap of differentially expressed genes (DEGs) (blue, downregulated; orange, upregulated) under the two different REE concentrations (EC₁₀ and EC₅₀ for both La and Yb) in comparison to the control condition. The total number of DEGs ($P \le 0.01$) is specified in brackets. (b) Scatter plots of DE transcripts under La and Yb exposure. Significantly upregulated and downregulated transcripts (FC ≥ 1.5 , $P \le 0.01$) are shown in green and purple, respectively.

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3.3. Comparative proteomic analysis for two exposure times

298 Temporal response analysis was performed using a high-throughput shotgun proteomic approach to investigate differences in cellular responses to REEs after short (one hour, representing one 299 generation) and long (four hours, representing 4 generations) exposure times (Fig. 1f). We 300 leveraged label-free proteomic as a semi-quantitative method that quantify peptide peak areas, 301 which allows the direct comparison between samples, unlike label-based methods (iTRAQ, TMT, 302 SILAC). Combining every condition tested and all replicates, a total of 26,785 peptides were 303 304 detected, as supported by the 685,981 MS/MS spectra. This analysis allowed the successful identification of 2,630 proteins, among which 2,015 were certified and quantified. Identified 305 306 proteins with significant changes in abundance (FC \ge 1.5 and P \le 0.05) upon REE exposure were

used for the analysis. Many more differentially abundant proteins (DAPs) were found at one hour 307 than at four hours in both the La and Yb exposure conditions. As shown in Fig. 3a, 2.6 times more 308 proteins were identified as differentially abundant in the one hour exposure compared to the 4 hour 309 exposure, with total of 161 DAPs at one hour and 62 at four hours under La stress (Fig. 3a, Table 310 S3). Similarly, under Yb stress, 226 and 76 DAPs were found after one and four hours (3 times 311 more), respectively (Fig. 3b, Table S3). Only 10 (La) and 14 (Yb) DAPs were shared between the 312 two exposure times (Fig. 3a-c). These results suggest the establishment of an intense and faster 313 response during a short exposure time relative to durable acclimation after a long exposure time. 314 Similar conclusions were drawn from the direct comparison of DAPs between the one- and four-315 316 hour exposure for both REEs. This was also found in a previous study in which S. cerevisiae was exposed to other metals (Ag, Cd, Hg, Zn) and metalloids (As) and the transcriptomic response 317 under acute stress (30 min) and sustained stress (2 hours) was compared (Hosiner et al., 2014). 318 Under exposure to these metals, only 2.4% to 7.9% of the total DEGs were shared between the 319 acute and sustained stress (Hosiner et al., 2014). These results further support a time-course 320 adaptation process in yeast cells to cope with toxic metals. 321



Figure 3. Comparative proteomic analyses of *S. cerevisiae* exposed to La and Yb demonstrate a differential response over time. (a) Venn diagrams of differentially expressed proteins under La exposure vs control conditions after 1 h and 4 h. (b) Same as (a) but for Yb. (c) Heatmap of differentially expressed proteins (blue, downregulated; orange, upregulated) under the two different exposure times (1 h and 4 h). (d) Scatter plot of differentially abundant proteins between Yb and La after 1 hour of exposure. Significantly more abundant proteins under La exposure are shown in green, and those more abundant under Yb are in purple (FC ≥ 1.5 , P ≤ 0.05). (e) Same as (d) but after 4 hours. NSAF = normalized spectral abundance factor.

323 3.4. Combining transcriptomic and proteomic analyses

The proteome analysis of cells exposed for 1 hour to La or Yb allowed the identification of essential 324 genes and the related pathways that cells first activate upon exposure. There was a relatively low 325 number of downregulated proteins under both conditions, with 12 DAPs for La and 7 DAPs for Yb 326 (FC \geq 1.5 and p \leq 0.05), and these were mainly shared (Table S3, Fig. 3d). Furthermore, these 327 proteins were all involved in ribosome biogenesis (La) and rRNA processing (La) (Tables S4-S6). 328 For all DAPs with a p-value < 0.05, there was high representation of the NOP (nucleolar protein), 329 RPL (ribosomal protein of the large subunit) and RPS (ribosomal protein of the small subunit) 330 family proteins (Table S3). These data further support the downregulation of ribosome biogenesis 331 and consequently less protein synthesis under both La and Yb stress. A reduction in ribosome 332 abundance was reported under different metallic stresses as being a rapid, effective, and reversible 333 stress response to prevent misfolded proteins (Guerra-Moreno et al., 2015; Hosiner et al., 2014; 334 Pimentel et al., 2012; Taymaz-Nikerel et al., 2016; White et al., 1990). Guerra-Moreno et al. (2015) 335 suggested that when cellular ribosome levels are reduced, newly synthesized misfolded proteins 336 may be limited. Lower levels of misfolded proteins would enable proteolysis to more effectively 337 eliminate misfolded proteins (Guerra-Moreno et al., 2015). 338

In line with this observation, and when considering proteins with increased abundance when 339 340 exposed to REEs, most of the GO terms represented in the two data sets were proteolysis-related (Tables S4 and S5). In the Yb treatment, an increased abundance of transcripts and proteins 341 belonging to ubiquitin-dependent protein catabolism was also found (Table S7). The short-term 342 exposure of yeast cells to toxic ions appears to trigger a rapid detoxification process by degrading 343 344 proteins that have been damaged by metal stress. Proteolysis induction is a global cellular response to metallic stress and has been observed at toxic concentrations of Al, Ag, Cd, Mn, Ni, Zn, Hg, and 345 As (Guerra-Moreno et al., 2015; Hosiner et al., 2014; Jin et al., 2008). Additionally, under both 346 treatments, proteins involved in carbohydrate metabolism were more abundant (Tables S4 and S5). 347 348 Jin et al. (2008) grouped metal-induced biological processes under the term 'common metal responsive' genes (Jin et al., 2008). It appears that in response to acute stress (i.e., after one hour 349 350 of exposure), yeast cells preferentially alleviate toxic effects triggered by the accumulated REEs and then establish adaptation responses to sustained stress (Hosiner et al., 2014). 351

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When comparing the transcriptomic and proteomic profiles at one and four hours for both REEs, the highest correlations were observed between transcriptomic profiles obtained after one hour and proteomic profiles obtained after four hours (Fig. 4a,b, Table S8). Strikingly, there was hardly any overlap between differentially expressed mRNA and the corresponding differentially expressed proteins (Fig. 4c,d). For instance, under La exposure, the expression of 21 of the 30 (70%) more abundant proteins and 28 of the 22 lass abundant metating use not altered for the accents mBNAs.

abundant proteins and 28 of the 32 less abundant proteins was not altered for the cognate mRNAs (Fig. 4c). The same observation was found for Yb exposure, with as high as 50% of the proteins with increased abundance and 100% of those with decreased abundance for which the corresponding mRNA was not differentially expressed (Fig. 4d). These observations could be

explained by the relatively low number of DAPs (FC \ge 1.5 and P \le 0.05) in comparison with the number of DEGs (FC \ge 1.5 and P \le 0.01). A fourfold higher number of mRNAs than proteins were differentially expressed under La exposure, while it was 6.4-fold greater under Yb exposure (Fig. 4c,d).

Interestingly, several DAPs and DEGs were shared between the Yb and La treatments, but some were specific to one or the other (Fig. 3e). We thus analysed each data set for the enriched gene

368 ontology (GO terms) to identify modulated pathways and functions in response to either La or Yb

369 (Fig. 5a-d, Tables S9-S13).



Figure 4. Comparative transcriptomic and proteomic analyses of yeast exposed to REEs at the EC₅₀. (a) Correlation matrix (Pearson's correlation coefficient r) of transcriptomic and proteomic data sets. (b) Heatmap of differentially abundant (DA) proteins (Pro) and differentially expressed (DE) transcripts (RNA) at the different exposure times (1 h and 4 h, as indicated) under La or Yb stress (blue, downregulated; orange, upregulated). (c) Scatter plots and Venn diagrams of DA proteins (after 4 h) and mRNA (after 1 h) under La exposure vs control conditions. Significantly upregulated and downregulated proteins (FC ≥ 1.5 , P ≤ 0.05) or transcripts (FC ≥ 1.5 , P ≤ 0.01) are shown in green and purple, respectively. (d) Same legend as in (c) under Yb exposure. NSAF = normalized spectral abundance factor.

370 371

3.5. The cell wall as the first barrier to REEs

372 Regarding the response to La at the transcriptomic level, upregulated genes were mainly associated

with cell wall biogenesis and organization. These functions were conspicuously represented by four GO terms and associated with up to 31 different genes (Fig. 5a, Table S12). Accordingly,

fungal-type cell wall organization was also enriched in the differentially expressed proteins (Fig.

- 5b, Fig. 4c, Table S9). This finding was supported by the enriched GO cellular compartments
- 377 (Table S12). Compared to La, Yb exposure upregulated 28 genes involved in fungal-type cell wall

organization and cell wall organization (Fig. 5c, Fig. 4c, Table S7). Moreover, these two functions 378 were also found in the proteomic analysis under Yb stress. This was reinforced by the upregulation 379 of the chitin cell wall metabolic process and the 1,3-β-D-glucan biosynthesis (Fig. 5d, Table S10). 380 Mid2p acts as a sensor for cell wall integrity signalling and activates this pathway. Downstream of 381 the Mid2p signalling pathway, Slt2p, a serine/threonine MAP kinase involved in regulating the 382 maintenance of cell wall integrity, was also upregulated (Fig. 6a). GSC2 (FKS2) is known to be 383 triggered by cell wall stress and encodes a catalytic subunit of 1,3-B-glucan synthase (Inoue et al., 384 1995), and it was specifically upregulated under Yb stress. CWP1 and NCW2 were upregulated 385 under La and Yb exposure and were identified in the transcriptomic and proteomic analyses (Fig. 386 6a). Cwp1p is a cell wall mannoprotein linked to a β -1,3- and β -1,6-glucan heteropolymer, while 387 Ncw2p is thought to aid the organization of the ß-1,3-glucan structure of the cell wall. These two 388 proteins are needed for the proper organization of cell wall constituents (Lesage and Bussey, 2006; 389 390 Levin, 2005).



Figure 5. Biological pathway (BP) GO terms overrepresented in the transcriptomic and proteomic analyses of yeast exposed to REEs at the EC₅₀. (a) BP GO terms of DE transcripts under La exposure. (b) BP GO terms of DE proteins under La exposure. (c) BP GO terms of DE transcripts under Yb exposure. (d) BP GO terms of DA proteins under Yb exposure. GO terms significantly enriched in upregulated (green) and downregulated (purple) proteins ($P \le 0.05$) and transcripts ($P \le 0.01$). GO terms found in both RNA-seq and proteomic analyses are in bold. Numbers indicate the number of proteins or mRNAs of the specified BP found in the data sets.

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The overrepresentation of these cell wall-related biological pathways (BPs) supports the role of this compartment in the first barrier yeast have against REEs. It also provides additional clues about

how the different REEs interact with the cell wall (Fig. 7). Ce (Jiang et al., 2010) and Yb (Jiang et al., 2010) a

al., 2012) were shown to adsorb to the cell wall of *S. cerevisiae*, *Bacillus subtilis*, and *E. coli*, with
affinity differences between LREEs and HREEs (Takahashi et al., 2010).



Figure 6. Expression heatmaps of GO terms enriched in proteomic and transcriptomic analyses for both REEs. (a) Fungal-type cell wall organization, (b) Glutathione metabolism, (c) Response to pheromones, (d) Ion transport-related GO terms (p < 0.05). The genes and proteins shown were all significantly differentially expressed.

397 Surface complexation modelling showed that LREEs preferentially associated with phosphate groups, while HREEs associated equally well with carboxyl and phosphate groups. Potentiometric 398 titration and FTIR spectroscopic analyses of S. cerevisiae showed a higher representation of 399 phosphate groups (68%) on the cell surface, while amino/hydroxyl (16%) and carboxyl groups 400 (16%) were less represented (Rogowska et al., 2018). The surface of the yeast cell wall, which 401 bears such groups, has been modelled to bind Ca ions, which could potentially be broadened to 402 REEs given their similarities (Rogowska et al., 2018). The higher representation of phosphate 403 groups on the surface and the high affinity of LREEs for these groups could explain the relatively 404 lower toxicity observed for La compared to Yb (Fig. 1). Thus, the upregulation of cell wall-related 405 genes appears to be essential in response to REE exposure and could allow the recycling or 406 selective remodelling of the yeast cell wall to cope with these elements. Modifications of the 407 exposed groups on the surface could ensue, with possible consequences for REE binding. Taken 408 together, these results support the involvement of the cell wall as a primary way in which REEs 409 interact with yeast cells and indicates that there are differences in La- and Yb-triggered effects. 410

411

412 **3.6.** Involvement of the pheromone signalling pathway

An investigation of the BPs related to sensing the extracellular environment indicated the 413 414 involvement of pheromone-related GO biological pathways. Indeed, two enriched GO biological pathways were downregulated under La stress, and both were involved in pheromone signalling 415 pheromone-dependent signal transduction (involved with cellular fusion) and the response to the 416 pheromone pathway (Fig. 5a, Fig. 6c, Table S12). Although these GO terms were specifically 417 enriched under La stress, genes belonging to these pathways were also significantly downregulated 418 under Yb stress, but to a lesser extent (Fig. 6c, Table S2). The GO terms included MFA1 and MFA2, 419 the two genes encoding mating pheromone a. The alpha-factor pheromone receptor gene STE2 and 420 its associated heterotrimeric G protein SST2 were also downregulated. 421

Two genes downstream of the regulation cascade triggered by STE2 were also downregulated: 422 FAR1 and FUS3. FAR1 is phosphorylated by FUS3 (a mitogen-activated serine/threonine protein 423 kinase involved in mating) in response to mating pheromone. Several other genes modulated by 424 the pheromone response pathways were also downregulated (Fig. 6c). Prm1p is a pheromone-425 426 regulated multispanning membrane protein that is involved in membrane fusion during mating, and Bar1p is an aspartyl protease secreted into the periplasmic space of mating type a cells (Mackay et 427 al., 1988). The latter cleaves and inactivates alpha factor, allowing cells to recover from alpha-428 429 factor-induced cell cycle arrest.

There is a possible link between the pheromone response pathway and the REE-triggered response. 430

It is known that some REEs are Ca analogues, particularly La (Fricker and Fricker, 2006) and can 431 be absorbed through the Ca influx system Cch1-Mid1 in yeast cells (Ene et al., 2015). Ca influx 432

- 433 occurs during the mating process and is initiated by mating pheromone detection (Carbó et al.,
- 2016; Cunningham, 2011). The response to mating pheromones notably includes increased 434 435 expression of the high-affinity Ca channels Cch1p-Mid1p and Ecm7p and increased expression of 436 the putative low-affinity Ca channels (Fig. 1p) (Cunningham, 2011; Muller et al., 2003). Thus,
- 437 considering that REEs can pass through Ca channels, the downregulation of pathways involved in the transcription of these channels would reduce the amount of REEs in the cytoplasm. However, 438 such a hypothesis requires further investigation of whether REE accumulation modifies pheromone 439 metabolism. In response to pheromones, yeast cells modify their cell wall to allow fusion with cells 440 of the other mating type, but they also induce cell cycle arrest (Chen and Thorner, 2007), thereby 441 442 leading to a more susceptible cell wall. Therefore, in line with previous observations, the 443 downregulation of genes and proteins involved in the pheromone-dependent signal transduction pathway could reduce cell cycle arrest and cell wall alteration processes, allowing cells to better
- 444

445 cope with La and Yb toxicity (Fig. 7).

446 447

3.7. Lanthanum and ytterbium impact amino acid, carbohydrate, and DNA metabolism

Under La stress, several metabolic and biosynthetic processes were altered at the transcriptomic 448 and proteomic levels (Fig. 5a, Table S12). The biosynthesis of purine nucleotides, through de novo 449 IMP (Fig. 5a) and AMP biosynthetic processes was significantly downregulated. Additionally 450 downregulated was the one-carbon metabolic process, which is related to glycine metabolism (Fig. 451 5a, Table S12). Conversely, the proteomic analysis demonstrated that lysine biosynthesis was 452 upregulated (10%) under La stress (Fig. 5b, Table S9). Interestingly, other than three upregulated 453 genes involved in proline transmembrane transport, no other amino acid-related GO terms were 454 identified under Yb stress (Fig. 5c, d, Table S7). 455

A relatively high number of upregulated genes were associated with carbohydrate metabolism 456 under both treatments, with eight upregulated genes under La stress and 26 under Yb stress (Fig. 457 5a,c). Many carbohydrate-related GO terms were enriched in the Yb condition, highlighting the 458

involvement of this pathway in the response to Yb. However, the induction of carbohydrate
metabolism was reported to be a common metal-responsive pathway and was also found when
yeast cells were exposed to several other toxic metals (Jin et al., 2008).

The two analytical methods identified INO1 and its encoded protein Ino1p as the most upregulated 462 representatives under both La and Yb exposure (Table S8). The inositol-3-phosphate synthase 463 Inolp is involved in the synthesis of inositol phosphates and inositol-containing phospholipids. 464 Under phosphate limitation conditions, genes implicated in inositol phosphate uptake and 465 metabolism, phosphate metabolism, and metabolite phosphorylation were induced (Taymaz-466 Nikerel et al., 2016). It has recently been shown that inositol-3-phosphates are involved in the 467 localization of metal uptake transporters in Arabidopsis thaliana (Agorio et al., 2017). Thus, an 468 increase in inositol-3-phosphate through the upregulation of INO1 could be important for 469 membrane transporter localization in response to REEs. Another explanation would be that 470 increased inositol phosphates help to chelate REEs, thus decreasing their toxicity and reactivity. 471 Indeed, all inositol phosphates are strong chelators of metal ions such as Ca, Mg, Zn, Cu, Fe, Mn 472 and Al (Chastukhina and Sharipova, 2014; Oh et al., 2006). 473

473 and AI (Chastukinia and Sharipova, 2014, On et al., 2000).
474 Finally, aside from the ion transport-related GO terms for La, almost all downregulated proteomic

pathways for both REEs were related to DNA replication and regulation (Fig. 5b,d, Tables S9,
S10). Proteins involved in DNA replication were downregulated under both La and Yb exposure,
and proteins involved in transcription regulation were downregulated under La exposure. Yb stress
also reduced translation, RNA processing and ribosome small subunit assembly at the
transcriptomic level (Fig. 5c). It has been strongly suggested that DNA replication is a target for
Cd toxicity (Serero et al., 2008). The downregulation of DNA replication by La and Yb could be
evidence of toxicity targets similar to Cd.

482 483

3.8. Ytterbium triggers oxidative stress response

484 In Yb-exposed yeast, the most represented process was the GO term oxidation-reduction (47 genes) (Fig. 5c Table S7). In addition, 18 genes involved with the cellular response to oxidative stress 485 were also upregulated, suggesting that Yb triggers oxidative stress and NADPH regeneration (Fig. 486 5c, Table S7). While only seen with the Yb stress in our study, it has been extensively shown that 487 other REEs can also generate oxidative stress (Gonzalez et al., 2014; Liang and Wang, 2013; 488 Pagano et al., 2015). Several genes involved in glutathione (GSH) homeostasis were upregulated 489 490 specifically under Yb stress. GSH can act at three different levels. First, GSH can chelate toxic metals to form a complex that can subsequently be sequestered into the vacuole. GSH can also act 491 as a protective agent against metal-induced oxidation (Jozefczak et al., 2012). Finally, protein 492 glutathionylation can occur, resulting in a protective role against irreversible metal binding and/or 493 oxidative damage to proteins (Deponte, 2017; Wysocki and Tamás, 2010). 494

495 Among the upregulated genes involved in GSH homeostasis under Yb stress, we identified GTT1 (ER) and ECM4 (GTO2, cytoplasm), and, to a lesser extent, GRX1 (cytosol) and GRX2 (cytosol 496 and mitochondria) (Fig. 6b, Table S2). Gtt1p and Ecm4p are glutathione-S-transferases (GSTs), 497 while Grx1p and Grx2p are glutaredoxins that can fill the same role as GSTs (Morano et al., 2012). 498 GSTs catalyse the conjugation of GSH with a substrate (e.g., Cd) or the isomerization of GSH to 499 form oxidized glutathione (Daniela et al., 2009). Furthermore, the upregulation of the gene 500 encoding the gamma-glutamyltranspeptidase ECM38 (Table S2), a major glutathione-degrading 501 enzyme localized into the vacuole, suggests the translocation of oxidized glutathione into the 502 vacuole, with a subsequent need for recycling (Cordente et al., 2015). Thus, along with ROS 503 production inducing oxidative stress, Yb could be internalized through GSH-Yb complexes via the 504 vacuole (Fig. 7). The ABC transporter Ybf1p, which was more abundant under Yb exposure, might 505

be involved in this process (Fig. 6d). This latter hypothesis requires further investigation to verifythe involvement of glutathione in Yb detoxification.



Figure 7. Schematic model of detoxification responses in yeast under acute REE stress. Upregulated functions are indicated by a green arrow, while downregulated functions are indicated by a red arrow. La-P and Yb-P correspond to phosphate-bound REEs, while Yb-C corresponds to carboxylate-bound Yb. Grey squares represent putative ligands that bind HREEs. Dashed arrows indicate REE movement, and plain arrows indicate response mechanisms.

508 509

3.9. Lanthanum and ytterbium affect plasma membrane transport

Reducing the import of toxic ions into the cell is another mechanism by which cells can adapt to 510 xenobiotic compounds. Of the downregulated genes under Yb and La exposure, several were linked 511 to plasma membrane ion transport (Fig. 7). Under Yb exposure, three transport-related GO terms 512 were enriched: arsenate ion transmembrane transport, high-affinity Fe ion transmembrane transport 513 and Fe assimilation by reduction and transport (Fig. 5c, Table S7). Under La exposure, four 514 downregulated proteins were involved in ion transport, and two of these were related to Zn ion 515 transport (Fig. 5b, Table S12). Regarding ion transport GO terms, the two genes encoding the two 516 517 components of the high-affinity Fe transport system Fet3p-Ftr1p were downregulated under both La and Yb exposure (Fig. 6d). A similar finding was identified at the protein level. Fet3p is a 518 multicopper oxidase that oxidizes ferrous Fe to ferric Fe for subsequent cellular uptake by the 519 transmembrane permease Ftr1p (Stearman et al., 1996). Other ion transport-related genes and 520 proteins also had modified expression, as evidenced by transcriptomic or proteomic analyses (Fig. 521 6d). Along with Fet3-Ftr1, the plasma membrane high-affinity Zn transporter Zrt1p was also 522 downregulated in the presence of both REEs (Fig. 6d). Also downregulated was the Zn transporter 523 Zrt3p, which localizes to the vacuolar membrane (Fig. 6d, Table S8). Zrt3p transports Zn ions from 524 vacuolar storage to the cytoplasm. Conversely, some genes encoding transmembrane ion 525 transporters were upregulated. Among them were *PCA1*, encoding a cadmium efflux transporter, 526 and SMF1, encoding a divalent metal transporter. A closer investigation of the roles played by 527 these different metal transport systems would help to elucidate novel REE uptake pathways in yeast 528 cells. Furthermore, the modulated expression and abundance of these nutrient transporters could 529

be the result of REEs indirectly impacting the overall nutrient balance in cells. Interestingly, we 530 observed an overall increase of the cell content of several cations (Fig. 1e) that might be a result of 531 a partial membrane permeabilization by REEs (Ramos et al., 2016; Técher et al., 2020), triggering 532 a stress response illustrated by the higher abundance of Hog1p, and higher expression of ENA1 533 encoding a Na efflux system (Haro et al., 1991) in response to Yb (Tables S2-S3). The Hog1p 534 MAPK pathway is involved in the response to high osmolarity and has been hypothesized to take 535 part in the tolerance to As and other metals (Hosiner et al., 2014). However, this Hog1p mediated 536 stress response is likely due to the plasma membrane permeability induced by REEs rather than an 537 hyperosmolarity given the very low REE concentration used. In response to this uncontrolled ion 538 influx, metal-homeostasis mechanisms would be triggered to reduce metal uptake by inhibiting 539 metal transporters expression and translation. Additionally, high concentration of toxic metals such 540 as Cd downregulate the expression of the zinc transporter Zrt1p to prevent uptake of toxic Cd 541 (Gitan et al., 1998; Wysocki and Tamás, 2010). The high concentrations of REEs could also 542 contribute to this deregulation of membrane influx systems. The altered Fe and Zn homeostasis 543 under La or Yb stresses could rapidly be alleviated by downregulating the corresponding uptake 544 systems (Fet3p-Ftr1p, Zrt1p and Zrt3p) to reduce Fe and Zn loading into the cytosol. 545

546

547 **4.** Conclusion

548 Here, we report the first cellular and molecular insights into the REE toxicity response in the eukaryotic model organism S. cerevisiae. The use of complementary methods at different levels of 549 regulation (mRNA and protein) has furthered our understanding of the potential issues related to 550 551 these emerging metal contaminants. Some responses have previously been shown for other toxic elements, such as Cd. Among these shared responses, proteolysis, ribosome biogenesis regulation, 552 and oxidative stress were specific to Yb and were not seen for La. However, other pathways and 553 compartments appeared to be important for detoxification and/or the avoidance of the toxic impacts 554 555 of La and Yb. Unlike the toxicity triggered by other metals, here we found that the cell wall and pheromone signalling pathways were key response pathways for yeast cells exposed to La and Yb 556 557 (Fig. 7). Interestingly, several differences were observed between La (LREE) and Yb (HREE) exposure, indicating the heterogeneity of impacts and responses triggered by REEs. Other toxic 558 metals, such as Cd, can be taken up through Zrt1p, Smf1p, Fet4p and Mid1p (Wysocki and Tamás, 559 2010). We found in the present study previously unidentified mechanisms of nonspecific REE entry 560 into the cytosol through different transporters. Taken together, these findings provide valuable 561 information at the cellular and molecular levels to better assess the impacts and behaviour of REEs 562 towards eukaryotic organisms. 563

564

565 Credit authorship contribution statement

Nicolas Grosjean: Methodology, Data acquisition, Data curation and treatment, Data analysis,
Writing - original draft, review & editing. Marie Le Jean, Damien Blaudez: Conceptualization,
Methodology, Formal analysis, Funding acquisition, Supervision, Writing - review & editing. Jean
Armengaud: Proteomics data acquisition. Michel Chalot: Data acquisition, Writing - review &
editing. Adam Schikora, Elisabeth M. Gross: Writing - review & editing.

571

572 **Declaration of Competing Interest**

573 The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

575

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580 **Conflicts of interest**

581 Authors have no conflicts of interest to declare.

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803 Appendix A. Supplementary material

Table S1: ICP-AES and ICP-MS validity data. The data include the percentages of recovery of
the oriental basma tobacco leaves certified material (INCT-OBTL-5, LGC Promochem, Molsheim,
France), the limit of detection (LOD) and limit of quantification (LOQ).

Table S2: Summarized RNA-seq results of yeast exposed to La or Yb for 1 h. Each condition was compared to the non-REE-exposed control condition. Data are the log2FC expression. The left table presents differentially expressed genes with a padj < 0.01, while the right table includes the fold change values regardless of the padj value.

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Table S3: Summarized proteomics results of yeast exposed to La or Yb at their respective EC₅₀ for 1 h or 4 h. The left table presents differentially abundant proteins with a padj < 0.05, while the right table includes the fold change values regardless of the padj value.

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818 Table S4: GO terms significantly represented in the set of differentially abundant (DA) 819 proteins under La vs Ctrl exposure after 1 h. The tables are separated into biological process 820 (BP), cellular component (CC) and molecular function (MF). Significantly enriched GO terms are 821 identified in the DA downregulated proteins (blue), DA upregulated proteins (orange), or all DA 822 proteins (grey).

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Table S5: GO terms significantly enriched in the set of differentially abundant (DA) proteins
under Yb vs Ctrl exposure after 1 h. The tables are separated into biological process (BP),
cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
identified in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA
proteins (grey).

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Table S6: GO terms significantly enriched in the set of differentially abundant (DA) proteins
under Yb vs La exposure after 1 h. The tables are separated into biological process (BP), cellular
component (CC) and molecular function (MF). The significantly enriched GO terms are identified
in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
(grey).

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- Table S7: GO terms significantly enriched in the set of differentially expressed (DE) genes under Yb vs Ctrl exposure after 1 h. The tables are separated into biological process (BP),

cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
identified in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes
(grey).

- Table S8: Summarized RNA-seq and proteomics results. Each condition was compared to the non-REE-exposed control condition. Cells were exposed to La or Yb at their respective EC_{50} for 1 h or 4 h, as indicated. The left table presents differentially expressed genes with a padj < 0.05 (PRO) and < 0.01 (RNA), while the right table includes the fold change values regardless of the padj value.
- 847

Table S9: GO terms significantly enriched in the set of differentially abundant (DA) proteins
under La vs Ctrl exposure after 4 h. The tables are separated into biological process (BP), cellular
component (CC) and molecular function (MF). The significantly enriched GO terms are identified
in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
(grey).

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Table S10: GO terms significantly enriched in the set of differentially abundant (DA) proteins
under Yb vs Ctrl exposure after 4 h. The tables are separated into biological process (BP),
cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
identified in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA
proteins (grey).

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Table S11: GO terms significantly enriched in the set of differentially abundant (DA) proteins
under Yb vs La exposure after 4 h. The tables are separated into biological process (BP), cellular
component (CC) and molecular function (MF). The significantly enriched GO terms are identified
in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
(grey).

Table S12: GO terms significantly enriched in the set of differentially expressed (DE) genes
under La vs Ctrl exposure after 1 h. The tables are separated into biological process (BP), cellular
component (CC) and molecular function (MF). The significantly enriched GO terms are identified
in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes (grey).

870

871 Table S13: GO terms significantly enriched in the set of differentially expressed (DE) genes

- under La vs Yb exposure after 1 h. The tables are separated into biological process (BP), cellular
 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
- in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes (grey).