Supporting Information

Fischer et al. 10.1073/pnas.1116843109

SI Text

Cloning of Plasmids. Sequences of all cloning primers are given in Table S2. The *singlet oxygen resistant 1* (*SOR1*) cDNA was created by reverse transcribing the corresponding RNA from *sor1* or 4A+ using the specific primer *SOR1_2534r*. Then, cDNAs were amplified with the same reverse primer and the forward primer *SOR1_1f* using Phusion High-Fidelity DNA polymerase (New England Biolabs) and subcloned into the pPCR-ScriptAmpSK(+) (Stratagene) cloning vector. Accuracy of the cDNA sequences was confirmed by sequencing. Then, the *SOR1* coding regions of *sor1* and 4A+ were amplified with primers *SOR1_186NdeI* and *SOR1_1248EcoRI* to add NdeI and *Eco*RI restriction sites, and again, they were subcloned into ScriptAmpSK(+). Finally, the coding regions were cloned into the pSL18 overexpression vector containing the *PSAD* promoter by NdeI and *Eco*RI restriction and ligation (1).

Cloning of *GPXH–arylsulfatase* (*ARS*) reporter constructs was described (2). For σ-class glutathione-*S*-transferase 1 (*GSTS1*) reporter constructs, the *GSTS1* promoter was amplified with either of the forward primers *GSTS1_f1* or *GSTS1_f2KpnI* and the reverse primer *GSTS1_r1EcoRV* to introduce a KpnI or *Eco*RV restriction site at the end of the fragments. PCR fragments were subcloned into pPCR-ScriptAmpSK(+) (Stratagene) and sequenced. Putative regulatory elements were deleted in a PCR-based approach where the whole plasmids containing the promoter fragments were amplified with the Phusion High-Fidelity DNA polymerase (New England Biolabs) using primers *GSTS1ΔPal1f*, *GSTS1ΔPal2f*, *GSTS1ΔPal3f*, or *GSTS1ΔCREf* containing a modified sequence of individual element together with the corresponding reverse primer *GSTS1ΔPal1r*, *GSTS1ΔPal2r*, *GSTS1ΔPal3r*, or *GSTS1ΔCREr*, respectively. PCR products were religated, transformed into *Escherichia coli*, and tested for accuracy of promoter fragments containing the desired mutation by sequencing. The triple palindrome mutation was achieved by subsequent repetition of the PCR with all three primer pairs. Then, 700-bp fragments of these *GSTS1* promoters were cut out with SacI/SpeI and used to exchange the SacI/NheI fragment of the *HSP70A*-luciferase construct (3) to get the various *GSTS1–Gaussia luciferase* (*GLUC*) constructs where the expression of the *GLUC* gene is driven by an individual *GSTS1* promoter. Similarly, replacement of a 1-kb KpnI-*Eco*RV promoter fragment of plasmid pASPro1 containing the *ARS* gene (4) with the KpnI/*Eco*RV fragments of the cloned *GSTS1* promoters led to the different *GSTS1-ARS* constructs.

1. Fischer N, Rochaix JD (2001) The flanking regions of PsaD drive efficient gene expression in the nucleus of the green alga *Chlamydomonas reinhardtii*. *Mol Genet Genomics* 265:888–894.
2. Fischer BB, et al. (2009) Function and regulation of the glutathione peroxidase homologous gene *GPXH/GPX5* in *Chlamydomonas reinhardtii*. *Plant Mol Biol* 71: 569–583.
3. Shao N, Bock R (2008) A codon-optimized luciferase from *Gaussia princeps* facilitates the in vivo monitoring of gene expression in the model alga *Chlamydomonas reinhardtii*. *Curr Genet* 53:381–388.
4. Leisinger U, et al. (2001) The glutathione peroxidase homologous gene from *Chlamydomonas reinhardtii* is transcriptionally up-regulated by singlet oxygen. *Plant Mol Biol* 46:395–408.

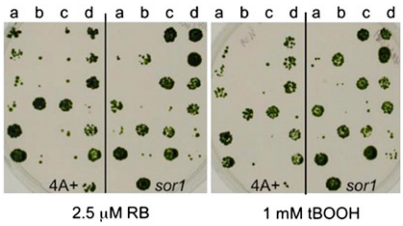

Fig. S1. Segregation of the reactive oxygen species resistance phenotype. *sor1* was backcrossed to the WT strain four times. Progeny from the fourth backcross was tested for resistance to rose bengal (RB) and *tert*-butylhydroperoxide (tBOOH). Shown are progeny from 12 complete tetrads arrayed in two columns. Individual members of a tetrad are labeled a–d. WT (4A+) and *sor1* controls were spotted at the bottom of each plate.

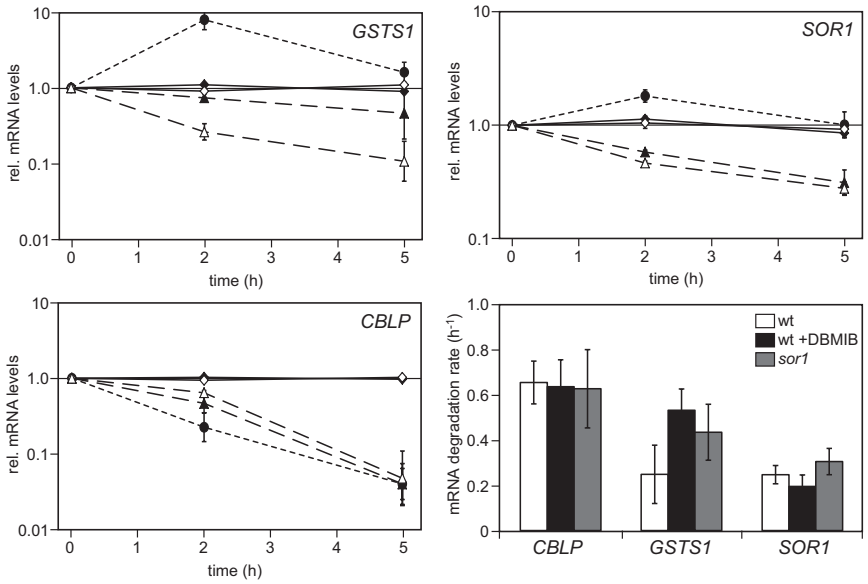

Fig. S2. Stability of the *GSTS1*, *SOR1*, and *CBLP* transcripts was determined in either the WT strain (closed symbols) or the *sor1* mutant (open symbols) by following mRNA levels after addition of actinomycin (50 μg/mL; dashed lines), actinomycin and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB; 2 μM; dotted lines), or no chemicals (full lines). DBMIB still caused a slight induction of *GSTS1* and *SOR1* even in the presence of actinomycin after 2 h, probably because of incomplete transcriptional inhibition at the beginning of the treatment. Comparison of degradation rates of *CBLP*, *GSTS1*, and *SOR1* mRNAs in *sor1*, the WT strain, and the WT exposed to DBMIB showed no significant difference in individual transcript stability between strains and treatments (degradation rates of DBMIB-treated samples were determined between 2 and 5 h of exposure).

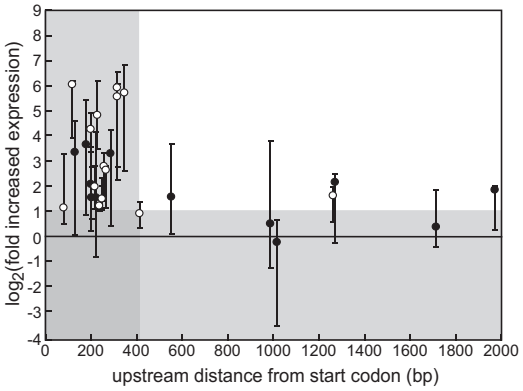

Fig. S3. Correlation of the positions of the 8-bp palindrome (electrophile response element) within the 2-kb promoter regions and the responses of the corresponding genes to the tested treatments. Circles show the median, and bars depict the minimal and maximal $\log_2$ fold increased response in the following conditions compared with the untreated WT strain: *sor1* grown in synchronized or nonsynchronized cultures or WT treated with DBMIB, 2*E*-hexenal, neutral red (NR), or tBOOH. The 14 genes overexpressed more than twofold in *sor1* are indicated by ○, and the 12 genes strongly induced by chemical treatments are indicated by ●. Gray areas help to visualize sections of less than twofold induction and the first 400 bp of the proximal promoter regions.

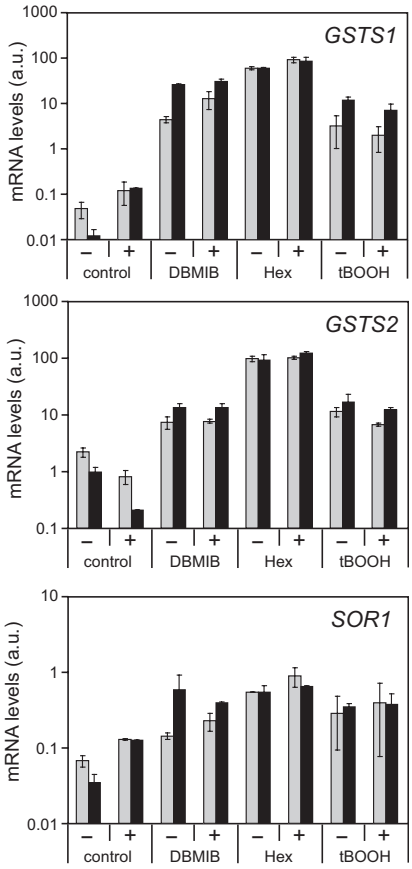

Fig. S4. Expression of the *GSTS1*, *GSTS2*, and *SOR1* genes in cultures pretreated with (+) or without (–) 35 μM cycloheximide for 20 min and exposed to DBMIB (2 μM), 2*E*-hexenal (0.3 mM), or tBOOH (100 μM) for 1 (gray bars) or 2 (black bars) h.

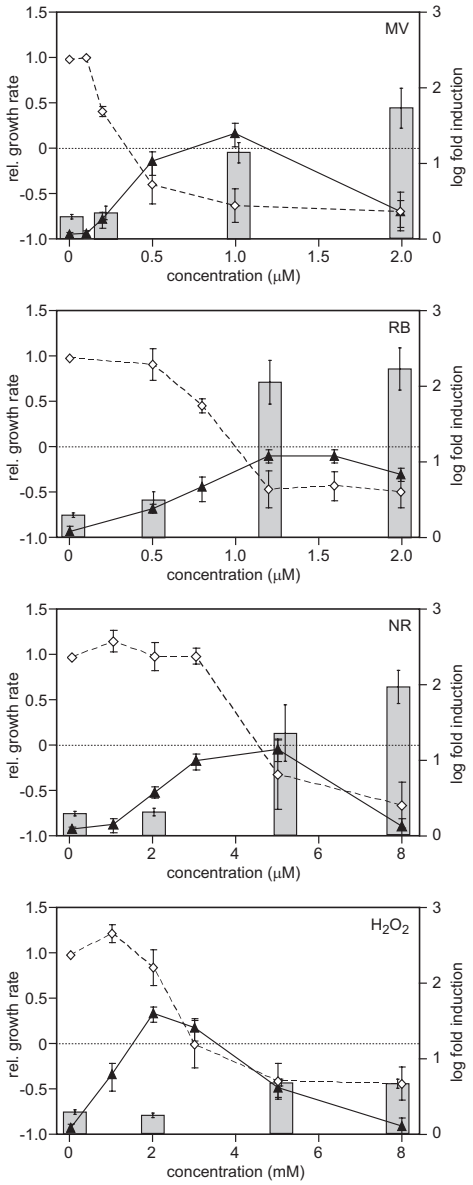

Fig. S5. Dose–response relationship of growth (◇), *GSTS1-GLUC* induction (▲), and the accumulation of the lipid peroxidation product malondialdehyde with thiobarbituric acid (gray bars) are shown for the four chemicals methyl viologen (MV), RB, NR, and hydrogen peroxide (H_2O_2).

Table S1. Distribution of the annotated genes differently overexpressed more than twofold in synchronized cultures of *sor1* compared with WT into different functional groups

		Up-regulation (>2.0-fold)		Down-regulation (<0.5-fold)	
Cellular function	No. of annotated genes	No. of genes	*P* value	No. of genes	*P* value
Cell: organization	127	6	0.56	5	0.17
Cell: division	50	5	0.03	0	
Cell: cycle	64	3	0.24	11	0.86
Cell: vesicle transport	48	2	0.22	4	0.04
Development	124	16	0.34	6	0.76
DNA: synthesis/chromosome structure	275	8	0.56	1	0.35
DNA: repair	59	2	0.35	1	0.26
Signaling	285	29	0.86	9	0.16
RNA: regulation of transcription	402	53	0.70	8	0.46
RNA: transcription	39	10	0.37	1	0.54
RNA: processing	185	18	0.40	6	0.18
Protein: amino acid activation	65	15	0.25	3	0.21
Protein: synthesis	226	33	0.39	11	0.22
Protein: posttranslation modification	662	71	0.81	9	0.32
Protein: targeting	131	11	0.85	10	0.62
Protein: degradation	468	64	0.47	5	0.55
Redox regulation	90	15	0.40	11	0.92
Stress response: abiotic	80	8	0.54	7	0.26
Stress response: biotic stress	22	0		4	0.10
Transport	437	36	0.04	33	0.20
Metal handling	12	2	0.46	0	
Hormone metabolism	67	9	0.62	1	0.09
Miscellaneous enzyme families	282	17	1.00	12	0.48
Not assigned/unknown	10,828	791	0.01	320	0.10

Table S2. List of primers used for quantitative PCR and cloning

Primer name	Sequence
GPXH	ACGTGTTTGACACGGTTATGAGA
	GCAATTGGCATGATGGATAGTG
GSTS1	CAGAGGTGAAAGGCGGATAC
	GTGTTGCAATGGACTTCAGC
GSTS2	ACCCCATCGGCAAAACAAG
	CCGGTATAGTATTGTAGCAGAATAACCA
SOR1	AAACCTTTTGCCGTTTGATGAA
	CATTGGGAGCCAGATCCATCT
CBLP	GCCACACCGAGTGGGTGTCGTGCG
	CCTTGCCGCCCGAGGCGCACAGCG
GSTS1_f2KpnI	acggtacCTCAGAACTGTGACGCTTGGACAC
GSTS1_r1EcoRV	acgatatcGAGACAGTGAGCGTTATATCGAGCGAAGGGCC
GSTS1ΔPal1f	CGTTTGGCGTGTGGCGACCCCGACCGGGCGATCCCGGCATCCCG
GSTS1ΔPal1r	CTGGCGTTCGAAGCGACACAG
GSTS1ΔPal2f	ATACGGGATGACGCAGGGGGCCCGACCGGACGAGTTGCTCGAGAAGGC
GSTS1ΔPal2r	CAACCGGGATTCCAACCTTGATG
GSTS1ΔPal3f	TGCTCGAGAAGGCGCGAAGCCCCGACCGCCTGTGCGTCTCCAGACTAT
GSTS1ΔPal3r	ACTCGTCCAACGTTGCCCCC
GSTS1ΔCREf	TGGAATCCCGGTTGATACGGGACCGAGCAGGGGGCAACG
GSTS1ΔCREr	ACCTTGATGTCGGTGCCAGGTTAC

Table S3. List of molecular markers used for mapping of the *sor1* mutation

Name	Primer sequences	Annealing temperature (°C)	Marker type	Size (bp) 137c	Size (bp) S1-C5
CHLH	TTGGCGGGTTGTGGTTGGACTAGG TCCTCGCGGAGCGCTCTCG	62	In/Del	127	375
SFA	CACAGCTCACACACACACGCACAA ACAGCATGCCCTGCAAGCTCGC TTGCATGGGCAGCACTGGTCGA	62.4	In/Del	330	211
CPN60B2	GCCGTATAAATTCAGGGCAGGCGC AGCTGCTTGGCAGCGGCTGTTG TGCAGCACAACTCCCGGCTGC	61	In/Del	260	621
C57_258	TGGAATTGGCGGTGCGAGCG GCTGTTAGGCCTGTCCTGTACTT TGACAAATTGAGTTGACCGACA	54	In/Del	161	171
C57_321	GCGTCTCTGCTCACTCCTATCC CGTGTGTATTGCGGTGTACAAG	54	+/–	800	—
C57_389	CCCTCCACCAAGTGCAATTGTTTAA GAGCTTTCGTGTGACGTCGATTC	63	dCAPS (DraI)	189	169 20
C57_407	GCTCAACTTCTGCTCTGACGA ATATGGTTTACACGCGCATTC	54	CAPS (AvaI)	390	370 20
C57_415	GGCGTTAGTTCCTGGTGATG CACTCAGCCCCTATCCTGAA	55	In/Del	598	480
C57_506	AACAGGGTCTGGTGCTGGTTG GGTGAATAGGGCTCCGCTTTG	64.5	CAPS (DdeI)	700 100	800
C57_554	GAGATTGTTGAGGCTCCCTTC GTACACTGCATCACGTCTTCGT	55	CAPS (SacII)	500 200	700
SOR1	GCGGGGTGGGAGGGGTATG GAGCACTGCAGTGTCAACCTGACAG	58	CAPS (*TAQ*1)	4A+ *sor1*	1,000 380 620

CAPS, cleaved amplified polymorphic sequence.

Other Supporting Information Files

Dataset S1 (XLSX)