Figures and figure supplements

Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*

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Figure 1. The sak1 mutant is defective in singlet oxygen acclimation. (A) Acclimation phenotype of WT and sak1. The cells were pretreated in the dark (−) or under light (+) in the presence of rose bengal (RB), which requires light for generation of $1\text{O}_2$. Pretreatment was followed by a subsequent higher concentration of RB (Challenge) as indicated under light. (B) Cells grown in low light were either kept in low light (−) or transferred to high light (+) for an hour before challenge in the light with increasing RB concentrations. (C) $F_v/F_m$
values were measured after each time point indicated. Pretreatment (PreT) with 0.5 μM RB was applied for 30 min with (+PreT) or without (−PreT) light. After the pretreatment, RB was added to both dark and light samples to a final concentration of 3.75 μM RB (challenge), and \( F_v/ F_m \) was measured for 90 min at 30 min intervals (total 120 min). First arrow: addition of pretreatment; second arrow: addition of challenge.

(D) \( sak1 \) has wild-type sensitivity to other photo-oxidative stresses. Serial dilutions of WT and \( sak1 \) were spotted onto minimal (HS) plates at the indicated light intensity or on TAP plates containing the indicated inhibitor. DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; low light (LL), 80 μmol photons m\(^{-2}\) s\(^{-1}\); high light (HL), 450 μmol photons m\(^{-2}\) s\(^{-1}\). (E) Gene expression of a known \( \cdot O_2 \)-responsive gene, \( GPX5 \), is induced during acclimation, while two genes associated with \( H_2O_2 \) response, \( APX1 \) and \( CAT1 \), are not. WT cells were mock-pretreated without RB (white bars) or pretreated with RB in the light (black bars).

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Figure 1—figure supplement 1. Pretreatment with RB does not increase resistance to high light or norflurazon in cells grown on plates.

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Figure 2. qRT-PCR analysis of genes identified to be \( \cdot \)O$_2$-responsive by RNA-seq. (A) The error bars indicate standard deviation of biological triplicates. The locus of the transcript (v5) and gene name if annotated, are indicated. *SOUL1 was named gene in v4 but not in v5. (B) Comparison of fold change values from RNA-seq data and qPCR. Fold change values were calculated for RNA-seq as described in ‘Material and methods’, and the values for qPCR are averages obtained from biological triplicates.

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Figure 3. Differentially expressed genes from pair-wise comparisons. (A) Venn diagram representing differentially expressed genes in WT and sak1. Mapman functional classes distribution of differentially expressed genes (passing criteria of fold change greater than 2\(^{\text{up}}\) or smaller than 2\(^{-1}\) [down] with FDR <1%) during acclimation in (B) WT and (C) sak1. (D) Differentially expressed genes when comparing WT and sak1 in basal conditions (i.e., before exposure to \(\text{O}_2\)). The functional classes represented by the numbers are listed; asterisks indicate classes that were enriched compared to the genome.
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Figure 4. Genetic and molecular analysis of sak1. (A) The insertion of a zeocin resistance gene and the RB sensitivity phenotype are linked. Twelve complete tetrads from a backcross of sak1 to wild type are shown. Numbers indicate independent tetrads, and letters (a-d) indicate the individual progeny from tetrads. (B) Gene
Figure 4. Continued

structure of SAK1 and the insertion site. Gray boxes indicate positions of primers used for qPCR. (C) Transformation of sak1 with a genomic fragment containing SAK1 rescues the acclimation phenotype. sak1(gSAK1)-1 and sak1(gSAK1)-2 are two independent transformants. (D) sak1(gSAK1)-1 and sak1(gSAK1)-2 show recovery of \( \text{O}_2 \) target gene expression. Y-axis indicates fold change during acclimation to \( \text{O}_2 \). (E) qRT-PCR of SAK1 in WT and sak1 mutant using primers for 5′- and 3′-UTR shown in panel B. (F) SAK1 protein is induced in WT and detected as higher molecular weight bands during acclimation to \( \text{O}_2 \) generated by RB. (G) SAK1 transcript probed for 5′-UTR in cells transferred from low light to high light for 1 hr. Error bars indicate standard deviation of biological triplicates.

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Figure 5. SAK1 contains an uncharacterized domain present in some bZIP transcription factors. Schematic of relative positions of SAK1 and bZIP domains. One protein (Cv28) contains a mitochondrial termination factor (mTERF) domain. The letters and numbers in the abbreviated names represent initials of the species and numbers listed in Table 8. Proteins with italicized names contain bZIP domains that were recognized by Pfam but scored below significance.

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Figure 5—figure supplement 1  Multiple sequence alignment of SAK1 domains.
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Figure 5—figure supplement 2. Secondary structure prediction of SAK1 domain. DOI: 10.7554/eLife.02286.018

Figure 5—figure supplement 3. Prediction of phosphorylation sites in SAK1. DOI: 10.7554/eLife.02286.019
Figure 6. SAK1 is a phosphorylated protein that is in the cytosol. (A and B) SAK1 is detected in the cytosol and not in other subcellular fractions. (C) SAK1 is not enriched in nuclear extracts. Approximately 30 μg of protein was loaded into each well except for mitochondrial fractions that were loaded approximately 7.5 μg protein due to low protein yield in isolated fractions. Subcellular markers: Chloroplast (CP), PSAD; Endoplasmic reticulum (ER), KDEL; Cytosol, NAB1; Mitochondria (mito), cytochrome c (Cyt c); Nuclear, histone 3 (H3). The arrowhead indicates the band corresponding to Cyt c. (D) Protein extracts from cells treated with increasing concentrations of RB were then treated with phosphatase (+) or only with buffer (--) before detection of SAK1 by immunoblot analysis.
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