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1 Light regulation of resistance to oxidative damage and magnetic crystal biogenesis in

2 *Magnetospirillum magneticum* mediated by a Cys-less LOV-like protein

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31

32 **Abstract**

33 Light–oxygen–voltage (LOV) proteins are ubiquitous photoreceptors that can interact with other
34 regulatory proteins and then mediate their activities, which results in cellular adaptation and subsequent
35 physiological changes. Upon blue-light irradiation, a conserved cysteine (Cys) residue in LOV
36 covalently binds to flavin to form a flavin–Cys adduct, which triggers a subsequent cascade of signal
37 transduction and reactions. We found a group of natural Cys-less LOV-like proteins in magnetotactic
38 bacteria (MTB) and investigated its physiological functions by conducting research on one of these
39 unusual LOV-like proteins, Amb2291, in *Magnetospirillum magneticum*. In-frame deletion of
40 *amb2291* or site-directive substitution of alanine-399 for Cys mutants impaired the protective
41 responses against hydrogen peroxide, thereby causing stress and growth impairment. Consequently,
42 gene expression and magnetosome formation were affected, which led to high sensitivity to oxidative
43 damage and defective phototactic behaviour. The purified wild-type and A399C-mutated LOV-like
44 proteins had similar LOV blue-light response spectra, but Amb2291^{A399C} exhibited a faster reaction to
45 blue light. We especially showed that LOV-like protein Amb2291 plays a role in magnetosome
46 synthesis and resistance to oxidative stress of AMB-1 when this bacterium was exposed to red light and
47 hydrogen peroxide. This finding expands our knowledge of the physiological function of this widely
48 distributed group of photoreceptors and deepens our understanding of the photoresponse of MTB.

49

50 **Key words:** LOV, magnetotactic bacteria, Amb2291, oxidative stress, reactive oxygen species.

51

52 **Key points**

53 We found a group of Cys-less light–oxygen–voltage (LOV) photoreceptors in magnetotactic bacteria,
54 which prompted us to study the light-response and biological roles of these proteins in these
55 non-photosynthetic bacteria.

56

57 The Cys-less LOV-like protein participates in the light-regulated signalling pathway and improves
58 resistance to oxidative damage and magnetic crystal biogenesis in *Magnetospirillum magneticum*.

59

60 This result will contribute to our understanding of the structural and functional diversity of the
61 LOV-like photoreceptor and help us understand the complexity of light-regulated model organisms.

62

63 **Introduction**

64 Most bacteria must cope with frequent changes in their environment; hence, they developed various
65 mechanisms to sense and respond to external stimuli for growth and survival. Bacteria can use light as
66 a source of energy when exposed to it, but they can also be damaged by ultraviolet (UV) radiation or
67 light-generated reactive oxygen species (ROS) (Metz et al. 2012). Different types of photoreceptors,
68 such as light-oxygen-voltage (LOV) sensing protein, photoactive yellow protein, phytochrome, sensor
69 of blue light using flavin adenine dinucleotide, cryptochromes, and sensory rhodopsin, are involved in
70 bacterial photosensing (Braatsch and Klug 2004; Losi 2007; Purcell and Crosson 2008; Spudich 2006;
71 Wilde and Mullineaux 2017).

72

73 Photoreceptor LOV belongs to a subset of the Per–Arnt–Sim (PAS) family that utilizes internally
74 bound flavin chromophores to sense changes in blue light or redox state (Pudasaini et al. 2017; Taylor
75 and Zhulin 1999; Wu et al. 2013). The flavin chromophore binds non-covalently to LOV in a dark
76 environment. Flavin photon absorption results in the formation of a flavin–C4(a)–cysteinyl adduct with
77 a conserved cysteine (Cys) residue on the PAS-motif, thereby initiating a cascade of structural
78 rearrangements within the LOV core, which are propagated to the domain boundaries (Pudasaini et al.
79 2015; Salomon et al. 2000). LOV proteins are widely distributed in bacteria. The first bacterial LOV
80 protein from *Bacillus subtilis*, YtvA (Akbar et al. 2001), was initially reported as a positive regulator of
81 general stress response (GSR). It binds to flavin mononucleotide (FMN) and undergoes a photocycle
82 similar to that of the LOV domains of the plant phototropins (Losi et al. 2002). In *Dinoroseobacter*
83 *shibae*, a blue-light photoreceptor of the LOV superfamily (*DsLOV*) regulates photopigment synthesis
84 (Endres et al. 2015). Purcell et al. suggested that bacterial LOV domain proteins may function as redox
85 sensors via flavin chromophore itself (Purcell et al. 2010). Furthermore, the data hint at a link between
86 *RsLOV* in *Rhodobacter sphaeroides*, carbohydrate metabolism and chemotaxis, and the response of
87 cells to photooxidative stress (Metz et al. 2012). The blue-light-modulating activity of *RsLOV* and
88 *RsAppA* is intended to maximize photosynthesis under favourable conditions and to minimize
89 photooxidative damage (Losi et al. 2015; Metz et al. 2012). In *Brucella abortus*, LOVhK is a cognate
90 sensor histidine kinase of PhyR that functions as a stress sensor (and GSR pathway activator). GSR can
91 be activated by red and blue lights and many other stimuli. Deletion of LOVhK severely impairs cell
92 survival under defined conditions of oxidative and acid stresses (Kim et al. 2014). In addition,

93 blue-light inhibition of *Listeria monocytogenes* growth is mediated by ROS and is influenced by σ^B and
94 the blue-light sensor Lmo0799 (O'Donoghue et al. 2016).

95

96 Previous research has found that replacement of the conserved adduct-forming Cys with alanine (Ala)
97 abolishes the photochemical reaction of LOV (Salomon et al. 2000). Recently, Yee et al. found that by
98 removing the conserved Cys, the LOV protein still undergoes light-induced photoreaction and signal
99 transduction through the signalling capability of neutral semiquinone (NSQ), which is photoreduced
100 from flavin (Yee et al. 2015). Interestingly, naturally occurring LOV proteins with proline (Pro) at the
101 position of the adduct-forming Cys residue can still respond to light. Additional studies are needed to
102 determine whether the light-induced naturally Cys-less LOV undergoing signal transduction is
103 widespread. Notably, we found a group of magnetotactic bacteria (MTB) that possess Cys-less
104 LOV-like proteins in which the Ala occupied the position of the adduct-forming Cys. Moreover, these
105 LOV-like proteins contain an N-terminal putative transmembrane domain. MTB can synthesize
106 intracellular membrane-enclosed, nanosized, and chain-arranged magnetite and/or greigite crystals
107 called magnetosomes (Komeili 2007; Spring and Bazylinski 2002). Magnetosome chains are the
108 magnetic sensors in MTB that act as an internal compass needle and help in the rapid orientation along
109 the geomagnetic field, a behaviour known as magnetotaxis (Faivre and Schuler 2008; Frankel and
110 Blakemore 1989; Komeili 2012). In addition to the magnetotaxis function, magnetosomes in
111 *Magnetospirillum gryphiswaldense* MSR-1 exhibit peroxidase-like activity that reduces intracellular
112 levels of ROS (Guo et al. 2012). Moreover, light irradiation can help *M. magneticum* AMB-1 cells
113 eliminate intracellular levels of ROS (Li et al. 2017). Other studies also revealed the photoresponses of
114 magnetotaxis in some MTB (de Melo and Acosta-Avalos 2017; Li et al. 2017; Qian et al. 2019;
115 Shapiro et al. 2011; Zhou et al. 2011). It is unknown whether and what kind of photoreceptor is
116 involved in this process. Analysis of AMB-1 genome reveals an unusual LOV-like photoreceptor,
117 Amb2291, of which the expected conserved adduct-forming Cys at the location of 399 was actually
118 replaced with Ala. To assess the function of the LOV-like protein, we mutated *amb2291* and analysed
119 the effects of irradiation and hydrogen peroxide (H₂O₂)-induced oxidative stress (photo-oxidative stress)
120 on cell growth and magnetosome biosynthesis in AMB-1 wild-type (WT) cells and *amb2291* mutants.
121 Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and dichlorofluorescein
122 diacetate (DCFH-DA) methods were used to analyse the requirement of *amb2291* in regulating the

123 expression of antioxidant genes as well as magnetosome formation genes, which scavenge intracellular
124 ROS under photooxidative stress conditions. In addition, deletion of *amb2291* or A399C substitution
125 affects the phototactic behaviour of AMB-1, which further confirms the role of Amb2291 in
126 antioxidant damage. Moreover, we purified the Amb2291 and A399C mutant proteins and analysed
127 their photochemical properties. This study contributes to the elucidation of the photoreaction
128 mechanism and deepens the understanding of the photoresponse of MTB.

129

130 **Materials and Methods**

131 **Strains and culture conditions**

132 *Escherichia coli* DH5 α was used for general cloning. The WM3064 of *E. coli* was used for intergeneric
133 conjugation between *E. coli* and *M. magneticum*. *E. coli* strains were grown in LB medium
134 supplemented with apramycin (50 μ g/mL) or kanamycin (50 μ g/mL). *M. magneticum* AMB-1 strains
135 (ATCC 700264) were grown on modified enriched *Magnetospirillum* growth medium comprising 5
136 mL of 0.01 M ferric quinate, 0.68 g of potassium phosphate, 0.12 g of sodium nitrate, 0.74 g of
137 succinic acid, 0.05 g of L-Cys-HCl, 0.2 g of polypeptone, and 0.1 g of yeast extract. In addition, 15
138 μ g/mL apramycin or kanamycin was used for plasmid selection (Matsunaga et al. 2005; Matsunaga et
139 al. 1991; Yang et al. 2001). *M. magneticum* AMB-1 strains were cultured using a 100 mL Schott flask
140 bottle or a 15 mL cell culture flask and incubated at 30°C.

141

142 **Identification of the Amb2291 sequence**

143 Amb2291 was identified in the *M. magneticum* AMB-1 genomic database BLAST (Matsunaga et al.
144 2005). Amino acid sequences were aligned using DNAMAN. Domain was analysed by SMART
145 (<http://smart.embl-heidelberg.de>). The protein accession numbers were as follows: *M. magneticum*
146 AMB-1 (WP_070108660.1, Amb2291); *M. magnetotacticum* MS-1 (KIL98983.1); *M. gryphiswaldense*
147 MSR-1 (WP_106001729.1); *Magnetospirillum* sp. ME-1 (WP_085373061.1); *Magnetospirillum* sp.
148 XM-1 (WP_068434569.1); *H. hochstenium* (WP_008581736.1); *Microcoleus vaginatus*
149 (WP_006631677.1); *Populus trichocarpa* (XP_002315641.1); *Nectria* (XP_003042477.1); *Allium cepa*
150 (ACT22763.1); *Asticcacaulis* sp. YBE204 (WP_023462395.1); *Azospirillum halopraeferens*
151 (WP_029010434.1); *B. subtilis* (O34627.1); *Crocospaera watsonii* (WP_007308072.1);
152 *Gluconobacter frateurii* (WP_023942432.1); *Granulicella mallensis* (WP_014265984.1); *B. abortus*

153 (WP_002971240.1); *Methylocystis rosea* (WP_018406994.1); *Roseobacter denitrificans*
154 (WP_011568342.1); *Avena sativa* (PDB 2V0U); *Rhodobacter sphaeroides* (WP_009562854.1); and
155 *Dinoroseobacter shibae* (WP_044027762.1).

156

157 **Purification of protein**

158 The conserved soluble fragment (residues 351–714) of *amb2291* or *amb2291*^{A399C} was cloned into the
159 pET28a plasmid with N-terminal His6-SUMO-tagged fusion proteins. These proteins were expressed
160 in *E. coli* BL21 (DE3) cells and purified as previously described (Li et al. 2019). The expression levels
161 of Amb2291 variants were induced with 10 μM isopropyl-β-D-1-thiogalactopyranoside for 16 h at
162 16°C in the dark. For cell lysis, the soluble extract was filtered and incubated with 5 mM FMN on ice
163 for 20 min. After nickel chelate affinity chromatography, the proteins were further purified by size
164 exclusion chromatography on Superdex 200 column in Tris buffer (10 mM Tris/Cl, 200 mM NaCl, pH
165 8.0). All experiments were carried out in the dark.

166

167 **Genetic manipulation of AMB-1**

168 All plasmids and primers used in the study are listed in Tables S1 and S2. Deletion of 2045 nucleotides
169 of *Amb2291* (81-2125) was conducted by a CRISPR-Cas9 system using plasmid pCRISPomyces-2
170 (Cobb et al. 2015). The first step in constructing plasmid pCRISPR-sgRNA*amb2291* was to select the
171 proper sgRNA sequence. sgRNA carrying the 20 nt specific targeting sequence was synthesized
172 (Sangon Biotech, China) and inserted into the pCRISPomyces-2 through a *BbsI* site (Chen et al. 2018;
173 Xie et al. 2014). A 2.0 kb HDR DNA fragment of the editing template was amplified from the purified
174 genomic DNA of AMB-1 and inserted into the pCRISPR-sgRNA*amb2291* with the *XbaI* restriction
175 enzyme.

176

177 The vector used for the allelic exchange of WT *amb2291* for *amb2291*^{A399C} was constructed as follows.
178 PCR amplification of *amb2291* with 1.0 kb upstream and downstream sequences was conducted using
179 Q5 high-fidelity polymerase (NEB, United States). The resulting 4.2 kb fragment was incorporated into
180 pBBRI-mcs2. To prevent the sgRNA of the CRISPR plasmid from cutting the replaced *amb2291*^{A399C}
181 in AMB-1, we inserted a nonsense mutation into the sequence of PAM and the sgRNA sequence in the
182 *amb2291*^{A399C} fragment, so that sgRNA will not recognize and Cas9 will not cut *amb2291*^{A399C} in

183 AMB-1, guaranteeing the construction of the *amb2291*^{A399C} strain. Site-directed mutagenesis of the
184 resulting plasmid by using the NEBuilder HiFi DNA assembly reaction protocol was conducted to
185 introduce the A399C, PAM, and sgRNA sequences of the nonsense mutation (PAM: CCT to aCT and
186 sgRNA sequence: GTTCACCCTGCCCATGACCC to aTTCACCCTGCCCATGACCC). The
187 site-directed 4.2 kb fragment was inserted into the plasmid pCRISPR-sgRNA*amb2291* by using the
188 same method as above.

189

190 All pCRISPR plasmids were transformed into the *E. coli* strain WM3064 by chemical transformation
191 and transferred into AMB-1 through conjugation (Chen et al. 2018; Philippe and Wu 2010). All
192 deletions were verified by PCR and sequencing.

193

194 **Irradiation experiment and H₂O₂-induced oxidative stress in AMB-1**

195 We investigated the effect of photooxidative stress on the growth and magnetosome biomineralization
196 of AMB-1 cells. All cells were grown with the exposure to 20 μmol photons/m²/s from 625 nm or 450
197 nm LED light (Fig. S1a, Ouyingzhaoming, China) and were cultured in medium containing H₂O₂ at 0,
198 5, 7.5, 10, and 15 μM. In general, light exerts a certain decomposition effect on H₂O₂. Therefore, by
199 using the H₂O₂ Quantitative Assay Kit (Sangon Biotech, China), medium with 15 μM H₂O₂ was used
200 to analyse the H₂O₂ concentration changes versus the illumination time. The medium contains no iron
201 ions to prevent the detection of H₂O₂ (Fig. S1b). We also detected the change in the H₂O₂ concentration
202 in the dark, and the value remained unchanged within 20 h. The optical density (OD) and cellular
203 magnetisms (Cmag) of exponentially growing cultures were measured at 600 nm under a 4.5 mT
204 homogeneous magnetic field with a UV-visible spectrophotometer (Unico UV-2800, United States) and
205 an electromagnetic system (Schüler et al. 1995; Zhao et al. 2007).

206

207 **Quantitative RT-PCR assay**

208 The procedure for qRT-PCR was based on a previously described general protocol (Chen et al. 2018).
209 Analysis was performed with cells in the plateau phase. Total RNA was extracted using an RNAeasy
210 Plus Minikit (Qiagen, Germany). Single-strand cDNA was synthesized with a reverse transcription kit
211 (Takara, Japan). qRT-PCR assay was performed with the SYBR Green Real-time PCR Mix (Thermo
212 Fisher Scientific, United States) on an ABI QuantStudio 6 Flex Real-time PCR System (Applied

213 Biosystems, United States). The relative expression level was calculated by the $2^{-\Delta\Delta C_T}$ method using
214 the threshold cycle of 16S rRNA for normalization (Livak and Schmittgen 2001). The experiment was
215 repeated at least thrice, with each sample run in triplicate. All primers used for probing each gene in
216 this study are listed in Table S2.

217

218 **Detection of ROS generation**

219 Intracellular ROS levels were analysed using the DCFH-DA method (He and Hader 2002; Wang et al.
220 2013). After irradiation and H₂O₂ induction, the cells grew to the late stage of exponential growth.
221 Then, 2 mL of bacterial culture was immediately added to 10 μ L of 10 mM DCFH-DA and incubated
222 at 37°C for 30 min in the dark. The cells were centrifuged at 12,000 rpm for 6 min and washed thrice
223 with a PBS buffer. The fluorescence of all samples was measured using a fluorescence
224 spectrophotometer (Hitachi F-4500, Japan) at an excitation wavelength of 485 nm and emission
225 wavelengths of 500–600 nm.

226

227 **Motility and phototactic behaviour analyses of AMB-1**

228 AMB-1 motility behaviour was analysed at a frame rate of 33 fps by using a long working distance 40 \times
229 Olympus objective. Cells (10 μ L) were observed on a glass slide with an Olympus microscope. Single
230 cells were tracked, and the average swimming velocities were calculated using the MTrackJ plugin for
231 ImageJ (Meijering et al. 2012; Murat et al. 2015; Zhang et al. 2017). Phototactic behaviour was
232 quantitatively analysed using a modified mini MTB collection vessel as previously described (Li et al.
233 2017). The cultures (80 mL) with and without H₂O₂ induction were pooled into the reservoir and into
234 the collecting tube. A microaerobic band formed after 30 min of growth in the dark prior to conducting
235 the phototactic experiment. The upper interface of the microaerobic zone is parallel to the collection
236 tube. Then, the device was placed in a cassette, where the collection tube was parallelly exposed to the
237 light beam. Control experiments were conducted in the dark. After 30 min of collection, we measured
238 the OD in the collecting tube and reservoir (OD_{collecting tube} and OD_{reservoir}, respectively). The ratio of
239 $(OD_{collecting tube} - OD_{reservoir})/OD_{reservoir}$ was used to determine the relative numbers of phototactic cells.

240

241 **Spectrophotometry analyses**

242 The absorbance spectra of purified Amb2291 WT and mutant proteins were determined from 700 nm to

243 250 nm at room temperature using a UV-visible spectrophotometer. Assays were recorded either in the
244 dark or after exposure to a blue LED lamp (450 nm) or red LED lamp (625 nm) (Ouyingzhaoming,
245 China). Fluorescence excitation and emission spectra were recorded at room temperature with a
246 fluorescence spectrophotometer (Hitachi F-4500, Japan). The emission wavelength was 530 nm, and
247 the excitation was at 450 nm.

248

249 **Statistical analysis**

250 All statistical analyses were performed using SPSS 22.0 (SPSS, IBM, United States). Hierarchical
251 clustering analysis (HCA) was performed to analyse the values of OD₆₀₀ and Cmag. ROS values and
252 the transcription-level results were analysed using two-tailed Student's t-test. Mann-Whitney U-test
253 was used to analyse phototactic behaviour. One-way analysis of variance was used to investigate the
254 difference in velocities under H₂O₂-induced oxidative stress. Each experiment was repeated three times
255 or more. All data were expressed as the mean±standard deviation. In all cases, statistical tests were
256 considered significant at $p<0.05$.

257

258 **Results**

259 **Genomes of *Magnetospirillum* sp. encode a group of natural Cys-less LOV-like proteins**

260 Genomic analysis allowed us to identify the *amb2291* as a CDS coding a LOV-like protein in AMB-1.
261 This 713-residue protein contains the LOV-like domain encompassing PAS and PAC domains,
262 histidine kinase, and histidine kinase-like ATPase (Fig. 1a). Notably, this LOV domain includes the key
263 residues of the canonical LOVs, such as the signalling relevant glutamine (Gln), the asparagines that
264 interact with the polar side of the isoalloxazine ring, and the arginines that interact with the phosphate
265 moiety of the flavin (Fedorov et al. 2003), but it does not contain the Cys in the highly conserved motif
266 GXNCRFLQ (Fig. 1b). The conserved Cys residue is involved in the photochemical generation of a
267 flavin–Cys adduct upon blue-light absorption by the flavin in LOV photoreceptors (Briggs 2007;
268 Christie et al. 1999). At the Cys position, an Ala substitutes for the strictly conserved Cys in the LOV
269 domain of *Amb2291* and the homologues from other *Magnetospirillum* sp. strains MS-1, MSR-1, ME-1,
270 and XM-1 (Fig. 1b). In other naturally occurring Cys-less LOV, this position can be Pro, glycine (Gly),
271 or lysine (Lys). This observation would challenge the photoresponse and physiological function of this
272 group of natural Cys-less, LOV-like proteins in magnetospirilla. Moreover, all *Magnetospirillum* sp.

273 LOV-like full-length proteins contain transmembrane domains with 1 to 3 predicted transmembrane
274 segments. To assess the function of this group of LOV-like proteins, we constructed an *amb2291*
275 deletion mutant and a site-directed A399C mutant in AMB-1 (substitution of Cys for Ala 399) (as
276 described in “Materials and Methods”).

277

278 **Characteristics of recombinant Amb2291 and Amb2291^{A399C} proteins**

279 To verify whether the Cys-less Amb2291 has the characteristics of LOV and to investigate the effect of
280 light illumination of Amb2291 or variant, the soluble LOV-like sensory domain of Amb2291 and
281 Amb2291^{A399C} from residues 351 to 714 (Fig. S2) were expressed in *E. coli* BL21 (DE3) as N-terminal
282 His6-SUMO-tagged protein and purified via nickel chelate affinity, SUMO protease treatment and size
283 exclusion chromatography. Recombinant proteins on denaturing gels of electrophoresis have a yellow
284 colour and an apparent size of ~40 kDa (Fig. S3). Based on the peak position of the elution profiles of a
285 Superdex 200-size exclusion column, Amb2291 and Amb2291^{A399C} displayed a molecular weight of
286 approximately 80 kDa (Fig. S3), thereby suggesting the dimer property of these proteins in solution.
287 Amb2291 and Amb2291^{A399C} exhibited a broad absorption band in the blue region that might be
288 composed of sharp peaks near 449 and 475 nm and a shoulder near 420 nm, revealing a mixture of
289 oxidized (450 nm) states of FMN. In addition, the absorption spectrum of Amb2291 had a double-peak
290 structure at 361 and 375 nm, whereas the component at 361 nm was reduced to a minor shoulder in the
291 absorption spectrum of Amb2291^{A399C}. The major UV-A absorption maximum occurred at 378 nm (Fig.
292 2a). Fig. 2b and c show the fluorescence emission and excitation spectra of the Amb2291 and
293 Amb2291^{A399C} proteins, respectively. Compared with Amb2291, the fluorescence emission spectrum
294 maximum of A399C mutant shows a redshift of 4 nm. The highest peaks of Amb2291 and
295 Amb2291^{A399C} proteins are at 529 and 533 nm, respectively, and those in the excitation spectrum are at
296 467 nm. Thus, both Amb2291 and Amb2291^{A399C} demonstrated the characteristic properties for the
297 LOV core, but a slight difference was obvious between the two chromophore binding domains.

298

299 Fig. 3 shows that dark-state LOV proteins contain an oxidized flavin cofactor that maximally absorbs
300 blue light at 449 nm in the ground state. Blue-light irradiation results in the bleaching of the ground
301 state and in the formation of a photoproduct, and absorption increased at 390 nm (Fig. 3a, b). The
302 results show a faster reaction of the A399C mutant to blue-light illumination compared with the WT

303 strain (Fig. 3c), demonstrating the efficiency of adduct formation in the presence of Cys. Moreover,
304 similar to the BAT P188C mutant of *H. hochstenium* (Yee et al 2015), Amb2291 and Amb2291^{A399C}
305 appear to form the hydroquinone (HQ) directly with little NSQ intermediate observed on our observed
306 time scale.

307

308 **Effects of *amb2291* deletion on cell growth under conditions with photooxidative stress**

309 To investigate the role of Amb2291, we performed a CRISPR-Cas9-based gene knockout experiment
310 to construct in-frame deletion of *amb2291* as described in the “Materials and Methods” section. The
311 effects of photooxidative stress on cell growth in AMB-1 WT cells and $\Delta amb2291$ mutant were
312 analysed. AMB-1 cells were treated with different concentrations of H₂O₂ and immediately incubated
313 for 20 h under multiple light ranges (red light 625 nm or blue light 450 nm, 20 $\mu\text{mol photons/m}^2/\text{s}$) or
314 dark conditions. OD at 600 nm was measured to evaluate the oxidative stress effect on growth.

315

316 As shown in Fig. 4, the OD values can be grouped into clusters a, b, and c by using the HCA method
317 when the squared Euclidean distance is equal to 4. Without irradiation in the dark, the OD of both
318 AMB-1 WT cells and $\Delta amb2291$ mutant decreased with the increase in the H₂O₂ concentration in the
319 growth media starting from 5 μM (Fig. 4, black curves). The OD of AMB-1 WT cells drastically
320 decreased from 10 μM H₂O₂ and continued to decrease with increasing H₂O₂ concentration under
321 blue-light irradiation, while the OD of $\Delta amb2291$ mutant decreased from 7.5 μM (group c, blue curves
322 in Fig. 4). The reason may be that blue light induces the formation of ROS that adds to the stress
323 induced by H₂O₂ addition. However, red-light irradiation abolished the deleterious effect of H₂O₂ on
324 the WT cells at all analysed concentrations (Fig. 4, red curve, group a). Interestingly, the sensitivity of
325 the $\Delta amb2291$ mutant to H₂O₂ under the red-light irradiation showed no significant change compared
326 with darkness treatment as well as WT cells under darkness (Fig. 4, red dashed-line, group b).
327 Collectively, these results revealed that red light protects the AMB-1 WT cells from the deleterious
328 effect of H₂O₂-generated oxidative stress, which was dependent on the function of Amb2291. For better
329 understanding the response of AMB-1 to photooxidative stress, red light was the main light source in
330 the following studies.

331

332 **Expression of the *amb2291* gene in AMB-1 cells**

333 To investigate the functional differences between Amb2291 and Amb2291^{A399C}, we constructed an
334 allelic exchange of WT *amb2291* for the *amb2291*^{A399C} mutant. By qRT-PCR analysis, we observed
335 that red-light irradiation increased the expression of the *amb2291* gene in WT cells, regardless of the
336 H₂O₂ treatment (Fig. 5). The deletion of the *amb2291* gene from 81 to 2125 nucleotides substantially
337 enhanced *amb2291* expression, especially under the conditions of red-light irradiation and the presence
338 of H₂O₂ ($p < 0.01$), since the first 80 nucleotides of *amb2291* were still kept in $\Delta amb2291$, but the
339 transcription level is higher than that of WT and *amb2291*^{A399C}. By contrast, site-specific substitution
340 for the conserved Cys, *amb2291*^{A399C} mutation displayed the same phenotype of the WT strain. These
341 results unveil the autoregulation of *amb2291* to a certain extent of the transcription level compared
342 with the higher transcription level in $\Delta amb2291$ caused by the incomplete function of *amb2291*. In
343 addition, the conservation of Ala 399 is not required for the autoregulation, although it is indispensable
344 for the attenuation of photooxidative stress.

345

346 **Effect of H₂O₂ and light illumination on magnetosome formation in AMB-1 cells**

347 We analysed the effect of photooxidative stress on magnetosome formation by measuring Cmag in
348 AMB-1 WT, $\Delta amb2291$, and *amb2291*^{A399C} mutant cells (Schüler et al. 1995; Zhao et al. 2007). By the
349 HCA method, the results showed that Cmag can be divided into clusters (a) and (b) when the squared
350 Euclidean distance is equal to 4 (Fig. 6a). The Cmag value in WT cells under red-light irradiation and
351 with H₂O₂-induced oxidative stress formed cluster a, which was higher than all those under the other
352 conditions. This result suggested that Amb2291, red light, and oxidative stress contributed to the
353 maximum synthesis of magnetosomes.

354

355 We previously reported that magnetosome-formation-associated genes *mms13*, *mms6*, and *mmsF*
356 significantly responded to visible light (Li et al. 2017). To investigate the mechanism by which
357 photooxidative stress influences magnetosome formation in AMB-1 WT and mutant cells, we also
358 performed qRT-PCR to detect the expression levels of *mms13*, *mms6*, and *mmsF*. After 20 h of
359 illumination, the expression levels of *mms13* and *mmsF* were significantly upregulated in WT cells and
360 *amb2291*^{A399C} mutants compared with their dark controls (Fig. 6b, d, $p < 0.05$). Importantly, red- light
361 illumination led to significant upregulation of *mms13*, *mms6*, and *mmsF* in WT cells compared with
362 $\Delta amb2291$ and *amb2291*^{A399C} strains (Fig. 6b, c and d, $p < 0.05$). H₂O₂ treatment also led to significant

363 upregulation of *mms13* in WT cells compared with $\Delta amb2291$ or *amb2291*^{A399C} mutant strains (Fig. 6b,
364 $p < 0.05$). *mms6* expression was significantly downregulated in the H₂O₂ treatment of $\Delta amb2291$ strains
365 compared with dark controls (Fig. 6c, $p < 0.05$). Red light improved the magnetosome synthesis of WT
366 AMB-1 cells, which was related to the function of Amb2291.

367

368 ***amb2291* regulates the expression of antioxidant genes and helps AMB-1 scavenge intracellular** 369 **ROS**

370 At the end of exponential growth, after 20 h of incubation, we analysed the difference in the expression
371 of the stress-related genes (*oxyR*, *sod*) between the dark and light groups with or without H₂O₂ by
372 qRT-PCR. For WT and mutant cells, regardless of the H₂O₂ treatment, red-light illumination led to
373 significant upregulation of the *oxyR* gene compared with their dark controls (Fig. 7a, $p < 0.01$). By
374 contrast, red-light illumination significantly downregulated the expression of *sod* compared with the
375 dark controls in both the WT cells and the *amb2291* mutants (Fig. 7b, $p < 0.05$). The H₂O₂ treatment
376 without red-light illumination had no obvious effect on *oxyR* and *sod* expression. However,
377 simultaneous red-light illumination and H₂O₂ treatment increased the expression of *oxyR* and *sod* only
378 in the WT strain (Fig. 7, $p < 0.05$).

379

380 We further examined the involvement of the *amb2291* gene in controlling intracellular ROS levels by
381 the DCFH-DA method (Fig. 8). The ROS levels of WT cells under photooxidative stress conditions
382 were significantly lower than those of mutant cells ($p < 0.01$), whereas the ROS level significantly
383 increased ($p < 0.01$) in mutant cells under light irradiation compared with those in the dark. The
384 *amb2291* gene helps AMB-1 eliminate intracellular ROS under oxidative stress through the light
385 regulation system.

386

387 ***amb2291* affects the phototactic behaviour under photooxidative stress**

388 We analysed the difference in the phototactic behaviour in AMB-1 WT cells and mutants. The
389 phototactic behaviour was quantitatively analysed by a modified mini MTB collection vessel as
390 previously described (Li et al. 2017). To prevent the difference in phototactic behaviour caused by
391 H₂O₂ on the state of bacterial movement, we first examined the speed of bacteria movement under
392 different H₂O₂ concentrations. Low H₂O₂ concentrations (10, 20, 30, and 40 μ M) exhibited

393 insignificant effects on the velocities of AMB-1 WT cells and mutants. However, with increasing H₂O₂
394 concentration, the velocities of all cells showed a consistent downward trend (Fig. S4). Thus, we
395 studied the difference in phototactic behaviour between the WT and mutant strains under 0, 10, and 20
396 μM H₂O₂ treatments.

397

398 Phototrophic behaviour was observed only with red light for all three strains analysed. Phototactic
399 behaviour of the mutant strains all decreased with the increasing concentration of H₂O₂ (Fig. 9, $p < 0.05$).
400 Moreover, the photoresponse of WT cells was significantly stronger than that of $\Delta amb2291$ or
401 $amb2291^{A399C}$ mutants after adding H₂O₂ (Fig. 9, $p < 0.05$). When H₂O₂ was not added, all three strains
402 exhibited a photoresponse. Amb2291 is likely involved in the reduction of the toxicity of H₂O₂ to cells
403 and helped WT cells to actively swim towards light.

404

405 **Discussion**

406 Flavin-based photoreceptor proteins of the LOV superfamily are ubiquitous in plants, algae, fungi, and
407 prokaryotes (Losi et al. 2015). During photoreaction, the non-covalently bound flavin chromophore
408 forms a covalent adduct with a conserved Cys residue in LOV under blue light to initiate signal
409 transmission to various output domains (Losi 2007). Current studies have reported that some LOV
410 photoreceptors apparently retained biological activity even after the substitution of the Cys thiol for
411 non-reactive side chains (Aihara et al. 2012; Chen et al. 2010; Okajima et al. 2012; Vaidya et al. 2011;
412 Yee et al. 2015). In GenBank, 70 LOV-like proteins contain the strictly conserved LOV domain, except
413 that the adduct-forming Cys is replaced with other residues, including Ala, Pro, Gly and Lys (Yee et al.
414 2015). In the present study, a group of LOV-like proteins from *Magnetospirillum* sp. in which the
415 conserved Cys residue is substituted with Ala were reported. The Amb2291 and Amb2291^{A399C} mutant
416 proteins in *M. magneticum* AMB-1 were purified. Accordingly, the pale yellow Amb2291 and
417 Amb2291^{A399C} purified from overexpression in *E. coli* indicate the binding of FMN to the chromophore
418 domains and potential partial reduction of FMN, regardless of the presence or absence of the conserved
419 Cys. Combined with Fig. 2, these results demonstrate the characteristics of LOV-like proteins. Then,
420 we examined the spectral change of Amb2291 and Amb2291^{A399C} under blue-light illumination to
421 analyse the photoreaction. Consequently, a faster reaction of Amb2291^{A399C} to blue light was observed
422 compared with the WT strain, which shows that Cys is an effective electron donor to the photoexcited

423 flavin. Despite of the change in a prolonged illumination studied here cannot be well compared with
424 those in a few minutes, FMN-bound Amb2291 indeed goes through photoreaction as a response to blue
425 light by some mechanism. As the spectrum does not show any change under the same dose of red light
426 (Fig. S5), it demonstrates that the effect of photodamage is negligible. In a conventional LOV
427 photocycle, blue-light absorption drives the formation of a covalent adduct between Cys and flavin,
428 promoting rotation of a conserved glutamine residue to initiate a cascade of structural rearrangements
429 within the LOV core that are propagated to the domain boundaries (Zoltowski and Gardner 2011) and
430 then generating downstream signalling responses. Signal transduction through flavin photoreduction to
431 the NSQ was recently found to be a general mechanism in natural Cys-less, LOV-like regulators. The
432 WT BAT of *H. hochstenium* shows properties similar to LOV proteins, reducing slowly to the NSQ
433 upon blue-light illumination. However, when the WT BAT is converted to a traditional LOV
434 mechanism by mutating the active-site Pro residue to Cys, the BAT P188C mutant shows a faster
435 reaction to blue-light illumination, appears to the fully reduced HQ with little NSQ intermediate
436 observed in their designed time scale (Yee et al. 2015). Interestingly, the absorption changes measured
437 for Amb2291 and Amb2291^{A399C} on exposure to blue light are similar to those of the BAT P188C
438 variant. Although photoreduction of the Cys-less Amb2291 is inefficient, the FMN-bound protein
439 readily undergoes complete chemical reduction to the fully reduced HQ, with little NSQ intermediate
440 observed on the carried-out time scale (Fig. 3a). Moreover, the aromatic residues of phenylalanine and
441 tyrosine close to the FMN-binding domain motif, as revealed in the sequence analysis in Fig. 1, may
442 generate reductive quenching, resulting in a short excited-state life time of Amb2291, which exhibits
443 inefficient photoreduction (Yee et al. 2015).

444

445 To analyse the biological functions of *amb2291* under photooxidative stress, we used the CRISPR
446 technique to construct $\Delta amb2291$ and *amb2291*^{A399C} mutants. Growth and magnetosome biosynthesis
447 of AMB-1 WT cells were better than that of $\Delta amb2291$ or *amb2291*^{A399C} mutants. Interestingly, we
448 found that red light protects the WT cells from the deleterious effect of H₂O₂-generated oxidative stress,
449 which depends on the function of Amb2291. Dual sensing of blue light and oxidative stress has been
450 observed in *R. sphaeroides*, wherein the blue-light-modulating activity of RsLOV and RsAppA is
451 intended to affect photosynthesis and minimize photooxidative damage (Losi et al. 2015; Metz et al.
452 2012). The structural biochemistry of a fungal LOV photoreceptor reveals an evolutionarily conserved

453 pathway that integrates light and oxidative stress (Lokhandwala et al. 2015). To some extent, the light
454 modulating activity of *amb2291* affected magnetosome synthesis and minimized photooxidative
455 damage. Red-light irradiation and H₂O₂ treatment upregulated the expression of the *amb2291* gene. In
456 addition, the expression results in *amb2291* deletion strains revealed the autoregulation of the *amb2291*
457 gene. Thus, Amb2291 played an important role in photooxidative stress.

458

459 Many environmental stimuli, including light, H₂O₂, and hyperthermia, generate high levels of ROS that
460 can damage cellular DNA and proteins, perturb the normal redox balance, and cause oxidative stress
461 (Lushchak 2001; Touati 2000). Therefore, organisms have developed methods to protect themselves
462 from ROS, enabling cells to survive oxidative stress. For example, *oxyR* and *sod* are stress-related
463 genes that regulate the transcription of defence genes via a rapid kinetic reaction in response to
464 oxidative stress (Jo et al. 2015; Lu et al. 2018). In the present study, the expression levels of *oxyR* and
465 *sod* genes were compared between dark and light conditions with or without H₂O₂ treatment.
466 Compared with the mutant controls, simultaneous red-light illumination and H₂O₂ treatment increased
467 the *oxyR* and *sod* expression levels only in the WT strains. This result is inconsistent with the
468 expression of *oxyR* and *sod* genes in the WT strains reported by Li et al. (2017), which may be due to
469 the differences among the concentrations of H₂O₂ and light treatment. Amb2291 is involved in the
470 regulation of oxidative stress genes and is beneficial for resisting the damage caused by photooxidative
471 stress. A previous study showed that singlet oxygen induces carotenoid biosynthesis in *Phaffia*
472 *rhodozyma* and *Myxococcus xanthus* and is presumed to be light dependent (Hodgson and Murillo
473 1993; Schroeder and Johnson 1995). Further studies showed that carotenoids can protect bacteria from
474 photooxidative stress damage (Glaeser and Klug 2005). Zeaxanthin accumulates only under
475 photooxidative stress and plays an essential role in protecting the photosynthetic apparatus via the
476 xanthophyll cycle (Demmig-Adams et al. 1999). The Amb2291 protein may also serve the function of
477 both pigments to protect cells from photooxidative stress damage. In addition, the ROS level of WT
478 cells was lower than that of mutant strains under the addition of H₂O₂, which is consistent with the
479 notion that light helps AMB-1 cells eliminate intracellular ROS (Li et al. 2017). These findings further
480 provide new evidence that Amb2291 reduces the increase in ROS through light regulation.

481

482 AMB-1 exhibited a photoresponse to red light (Fig. 9), which is consistent with our previous study

483 (Chen et al. 2011; Li et al. 2017). After H₂O₂ treatment, the number of *amb2291* deletion or
484 *amb2291*^{A399C} mutant cells swimming towards light was significantly less than that of the WT cells.
485 This finding might imply that red light under H₂O₂ conditions reduced the phototaxis of the mutant,
486 which may be related to the increased cytotoxicity of H₂O₂ or ROS in the absence of functional
487 Amb2291. Amb2291 is involved in light regulation to help eliminate ROS under oxidative stress,
488 thereby creating the optimum niche for bacterial growth. It has been shown that Fe₃O₄ magnetosomes
489 in MTB exhibit peroxidase-like activity, which can catalyse the photoFenton reaction according to the
490 following equation to scavenge the intracellular levels of ROS (Guo et al. 2012): Fe²⁺ + H₂O₂ = Fe³⁺ +
491 HO• + OH⁻ (Goldstein et al. 1993; Touati 2000). Moreover, light irradiation enhances this activity by
492 promoting the synthesis of magnetosome and helps MTB eliminate intracellular ROS (Li et al. 2017).
493 *amb2291* expression was significantly increased with red-light irradiation. Moreover, red-light
494 illumination and H₂O₂ treatment significantly increased the expression of magnetosome formation
495 genes (*mms13*, *mms6*, and *mmsF*) and antioxidative genes (*oxyR* and *sod*) in WT cells compared with
496 those in Δ *amb2291* and *amb2291*^{A399C} strains. Therefore, Amb2291 participates in the light-regulated
497 signalling pathway and further enhances the synthesis of magnetosomes and resistance to oxidative
498 damage, which helped clear intracellular ROS, thereby improving phototactic behaviour and bacterial
499 growth.

500

501 Collectively, Amb2291 showed a similar LOV spectra based on blue-light irradiation but was unable to
502 undergo the canonical photochemistry of LOV domains with the formation of a flavin–Cys covalent
503 adduct. Upon red-light irradiation, Amb2291 improved the resistance to oxidative stress by some
504 mechanism. Our understanding of the downstream physiological role of LOV protein is far less than
505 that of its biochemical properties, because only several of LOV proteins' roles had been identified (Wu
506 et al. 2013). Whether Amb2291 is directly involved in the red-light response needs further confirmation.
507 We propose the following assumptions. First, structural prediction analysis found that Amb2291 has a
508 specific extra membranous domain, while most LOV domains have no transmembrane structure in
509 general, except the one that is found in the cytoplasm and membrane components in *R. sphaeroides*
510 (Hendrischk et al. 2009). We are not sure whether the specificity of this protein structure plays a role.
511 Second, studies have shown that the red/far-red-light-sensing bacteriophytochrome (BphP) can form an
512 integrated signalling network with LOV in response to red, far-red, and blue light (Wu et al. 2013).

513 Strikingly, genomic analysis revealed the presence of BphP protein in AMB-1, named MagBphP,
514 which is a red/far-red photoreceptor that mediates photosensory responses (Brutesco et al. 2017; Wang
515 et al. 2019). We speculate that Amb2291 and the red/far-red-light-induced MagBphP may form an
516 integrated signalling network; one of the biological significances of light-mediated behaviours in
517 AMB-1 is to avoid environmental stress, such as oxidative stress. Fig. 10 shows the functional model
518 of LOV-like Amb2291. The Cys-less LOV-like Amb2291 protein participates in the light-regulated
519 signalling pathway and improves resistance to oxidative damage and magnetic crystal biogenesis in *M.*
520 *magneticum*. However, the present work does not provide enough data to allow for a conclusion about
521 the mechanism between Amb2291 and MagBphP signalling pathways. In addition, MTB usually live in
522 aquatic environments and navigate towards sediment. They may move to find better living
523 environments that contain trace oxygen through migration towards light (Wang et al. 2019). One of the
524 major challenges that ancient life may have adapted to is ROS. The ancestral role of intracellular
525 magnetosomes may have helped early life cope with oxidative stress through photoresponse (Lin et al.
526 2020). In short, Amb2291 acts as an LOV-like protein with a complex sensory role when cellular redox
527 state and/or light were functional input signals.

528

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533

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538

539 **Compliance with ethical standards**

540 **Conflict of interests** The authors declare that they have no conflict of interest.

541 **Ethical approval** This article does not contain any studies with human participants or animals
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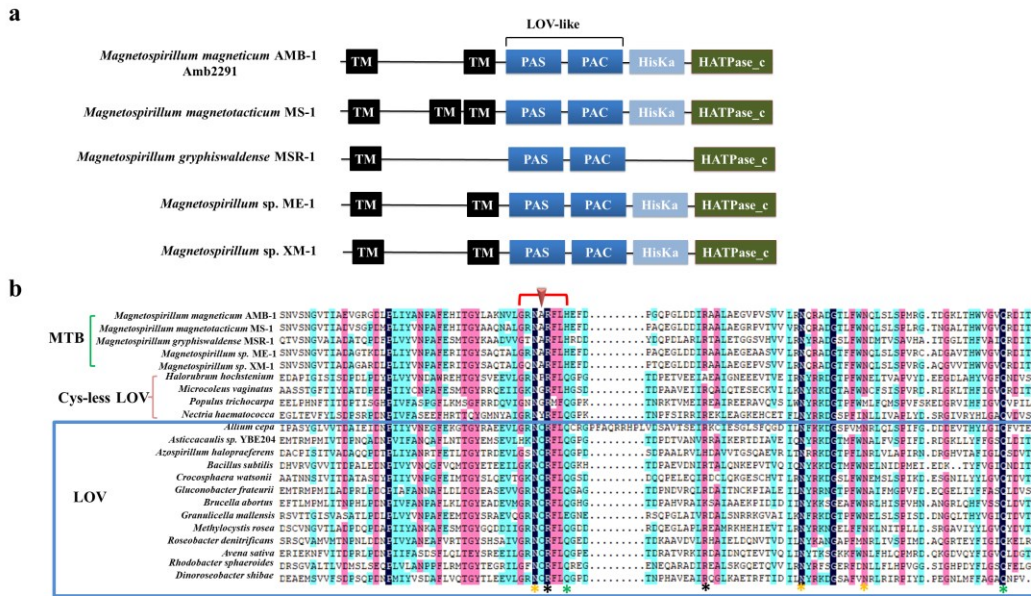
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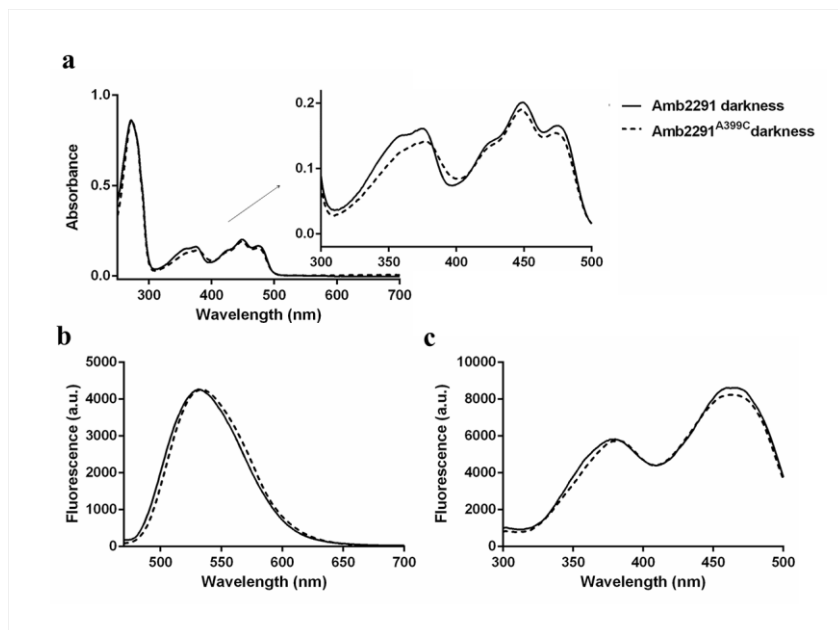
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753 **Figures**



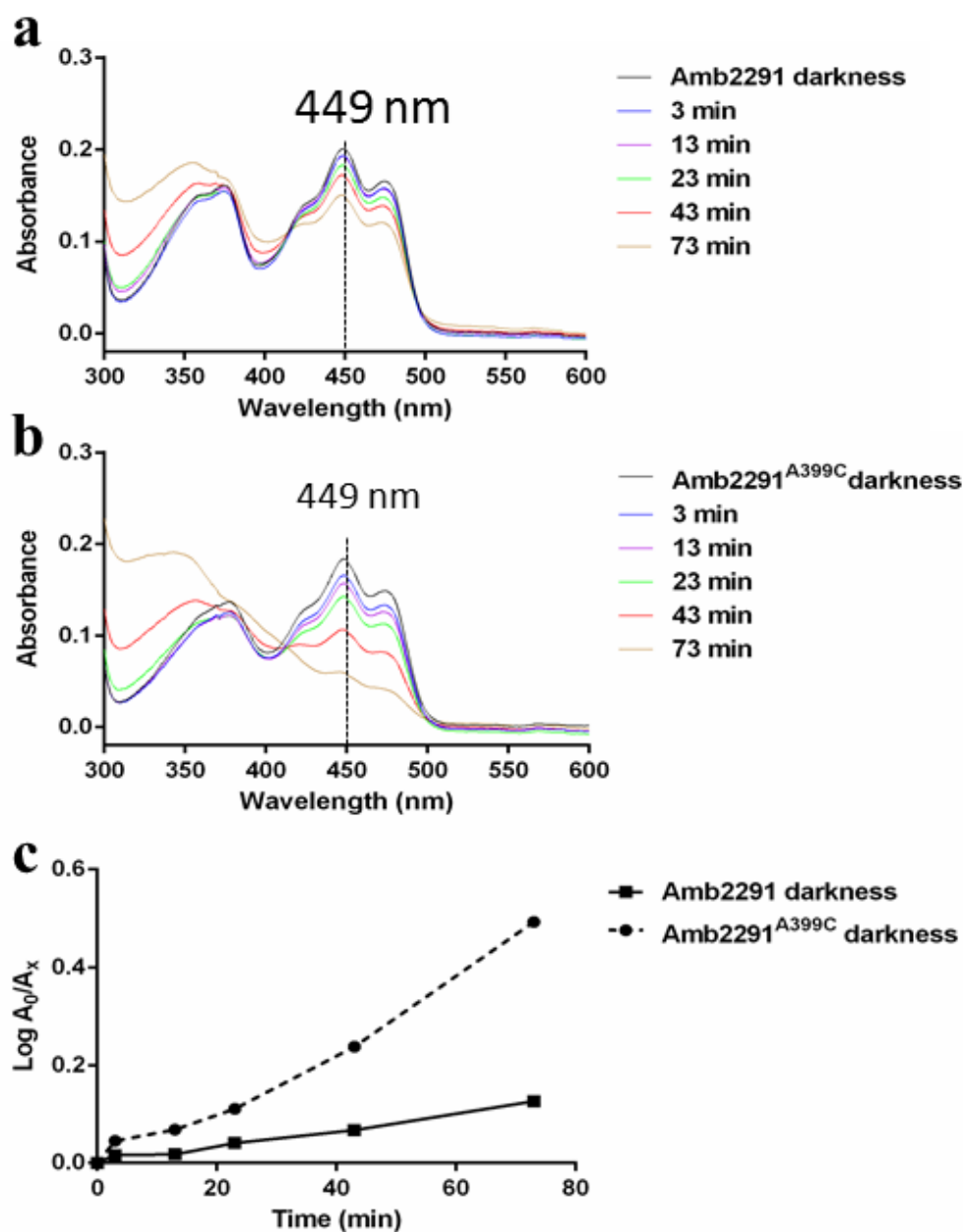
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755 **Fig. 1** Domain structures and amino acid sequence alignment of LOV proteins. **a** Domain structures of
 756 Amb2291 and other LOV-like proteins of MTB. TM, transmembrane segment; PAS, Per-Arnt-Sim
 757 domain; PAC, motif C-terminal to PAS motifs; HisKa, histidine kinase domain; HATPase, histidine
 758 kinase-like ATPase; The PAS-PAC domain is similar to LOV. **b** Alignment of selected LOV domains
 759 of MTB and similar regions of other Cys-less or LOV proteins. The red brace shows a conserved motif
 760 in LOV. The red triangle indicates the position of the conserved Cys involved in photoactivated FMN
 761 covalent binding. * Black shows the Arg residues that interact with the phosphate moiety of the flavin,
 762 * yellow shows the Asn residues that interact with the polar side of the isoalloxazine ring, and *green
 763 shows the Gln residues related to signalling.



764

765 **Fig. 2** Spectral properties of purified Amb2291 and Amb2291^{A399C} proteins. **a** Absorption spectra of
 766 Amb2291 (solid line) and Amb2291^{A399C} (dashed line) in the dark. **b** Fluorescence emission spectra for
 767 Amb2291 and Amb2291^{A399C}. Fluorescence excitation was monitored at 450 nm. **c** Fluorescence
 768 excitation spectra for Amb2291 and Amb2291^{A399C}. Emission was at 530 nm.



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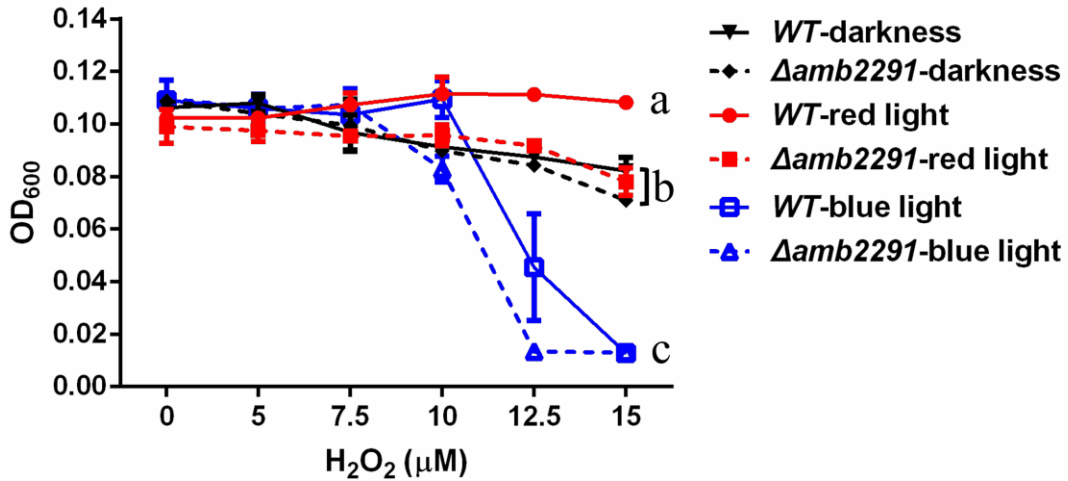
770 **Fig. 3** Photoreduction of purified Amb2291 and Amb2291^{A399C} proteins by blue-light irradiation.

771 Absorbance changes measured for Amb2291 (**a**) and Amb2291^{A399C} (**b**) on exposure to high-intensity

772 blue light (1700 $\mu\text{mol photons/m}^2/\text{s}$). **c** The kinetic data were obtained by following the absorbance

773 changes at 449 nm for Amb2291 and Amb2291^{A399C} proteins. A_0 represents the amount of unreacted

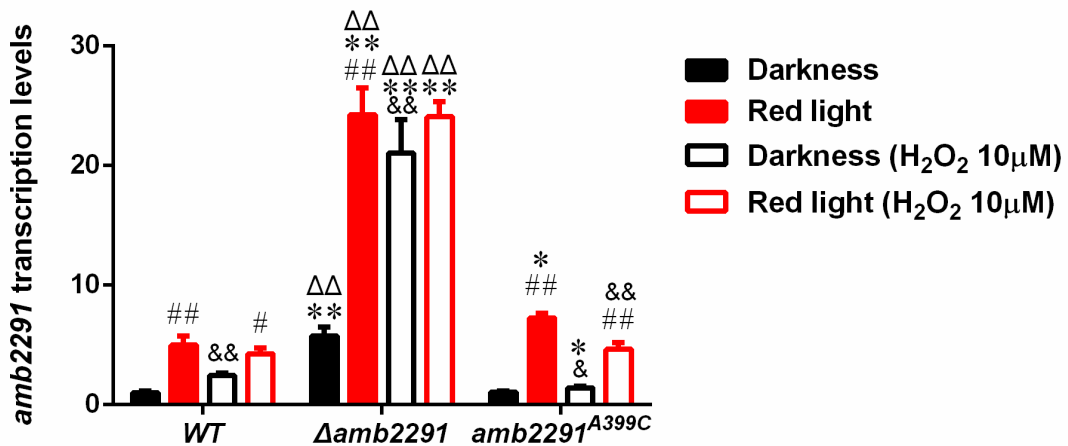
774 flavoprotein at time zero. A_x represents the corresponding amount of unreacted flavoprotein at time x.



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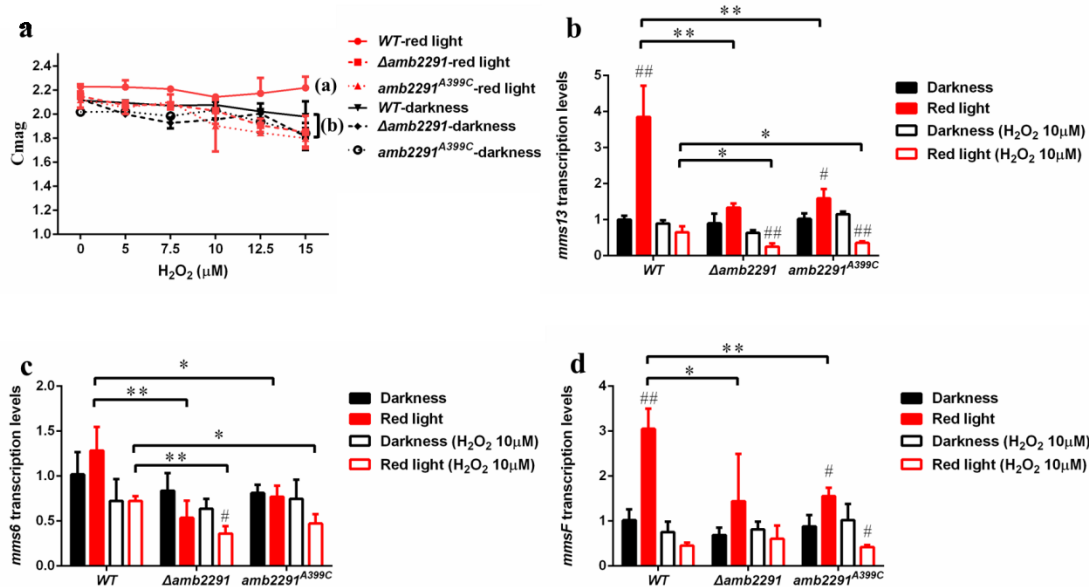
776 **Fig. 4** Evaluation of the H₂O₂-generated oxidative stress effect on AMB-1 cells. The OD values of
 777 AMB-1 WT cells (solid lines) and Δ*amb2291* mutant (dashed lines) under irradiation or in the dark at
 778 different concentrations of H₂O₂ were the average of four independent experiments with standard
 779 deviations (error bars). The OD values are grouped into a, b, and c using the HCA method.

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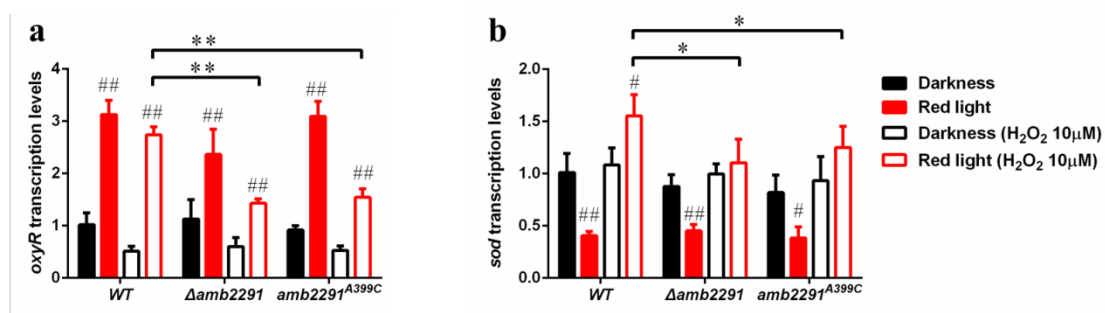
782 **Fig. 5** Relative expression levels of the residual *amb2291* gene determined via qRT-PCR under red
 783 light and/or by adding H₂O₂ to the medium (*n*=3). # represents the statistical analyses performed
 784 between red light and dark. & shows the statistical analyses between the H₂O₂ group and their
 785 respective normal controls. * describes the statistical analyses between the mutants and WT control. Δ
 786 shows the statistical analyses between Δ*amb2291* and *amb2291*^{A399C} mutant cells.



787

788 **Fig. 6** Effects of *amb2291* deletion or mutant on magnetosome formation under photooxidative stress
 789 conditions. **a** Value of Cmag under red-light irradiation or dark at different concentrations of H₂O₂ in
 790 AMB-1 cells (*n*=4). The Cmag of each group is shown and analysed using HCA. Relative expression
 791 levels of *mms13* (**b**), *mms6* (**c**), and *mmsF* (**d**) determined via qRT-PCR under red light and/or by
 792 adding H₂O₂ to the medium (*n*=3). Level of significance of the differences observed between light and
 793 dark control is expressed as hashtag sign (#*p*<0.05 and ##*p*<0.01). The significant differences between
 794 the two groups depicted by the lines in the graph are indicated by one or two asterisks (**p*<0.05,
 795 ***p*<0.01).

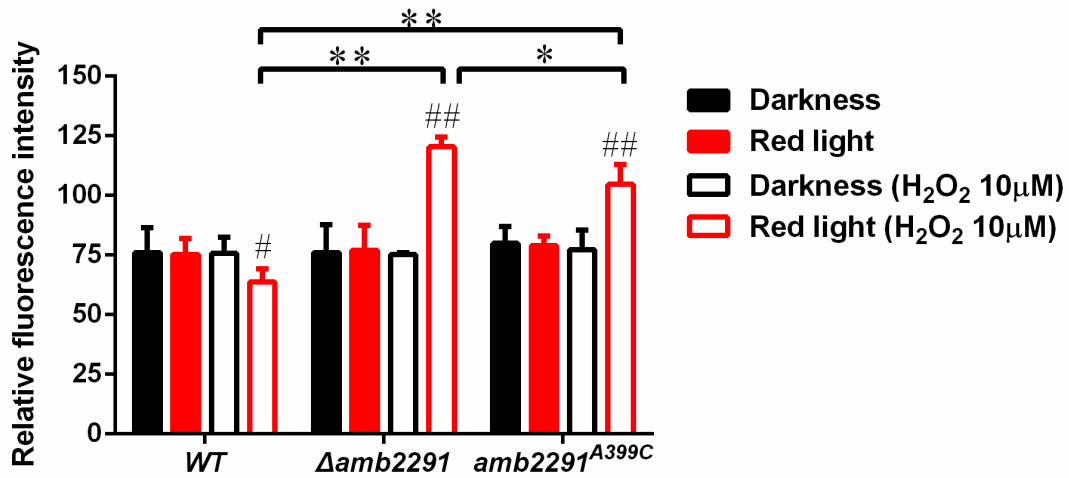
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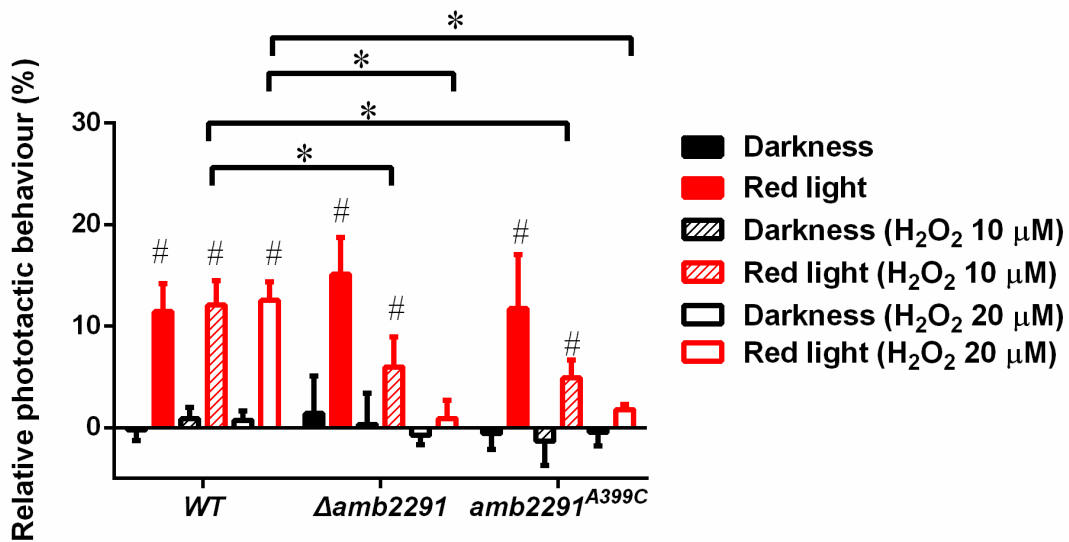
798 **Fig. 7** Relative expression levels of *oxyR* (**a**) and *sod* (**b**) genes determined under red light and/or by
 799 adding H₂O₂ in the medium (*n*=3). The level of significance of the differences observed between light
 800 and dark control is expressed as hashtag sign (#*p*<0.05 and ##*p*<0.01). The significant differences

801 between the two groups depicted by the lines in the graph are indicated by one or two asterisks
 802 (* p <0.05, ** p <0.01).



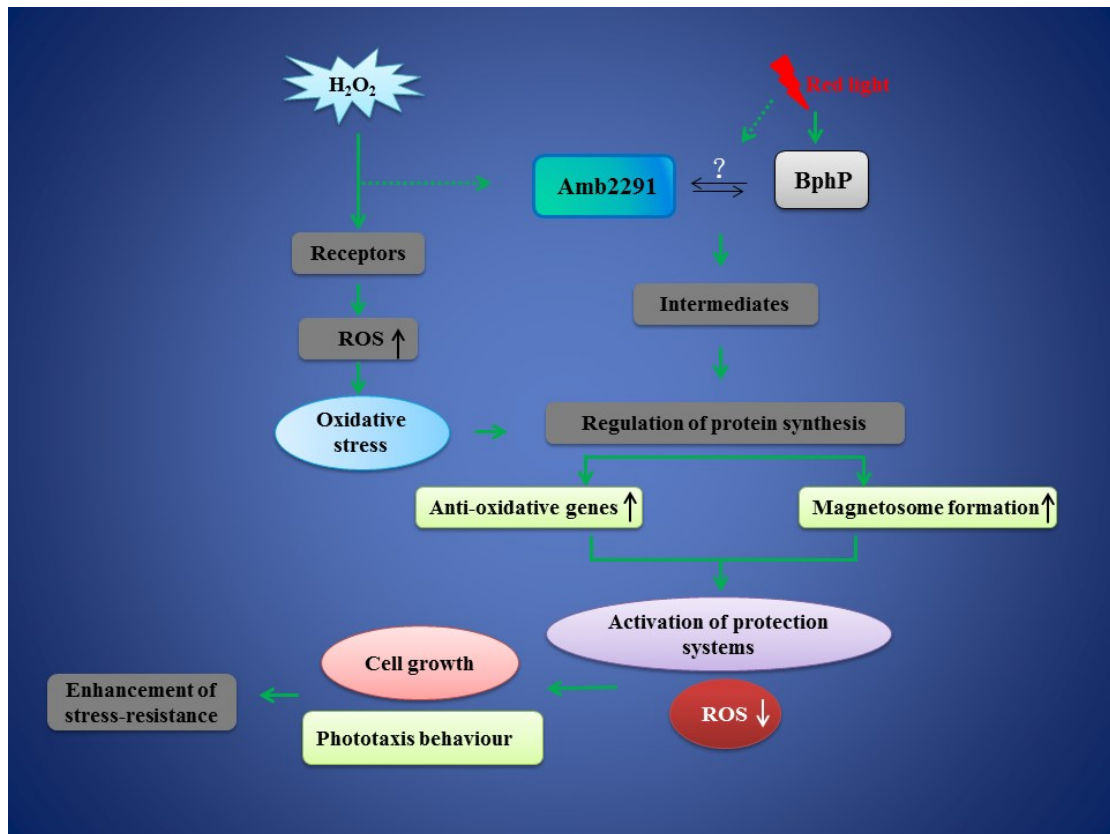
803
 804 **Fig. 8** Effects of Amb2291 on intracellular ROS level in AMB-1 cells ($n=6$). The level of significance
 805 of the differences observed between the light and dark control is expressed as hashtag sign (# p <0.05
 806 and ## p <0.01). The significant differences between the two groups depicted by the lines in the graph
 807 are indicated by one or two asterisks (* p <0.05, ** p <0.01).

808



809
 810 **Fig. 9** Effects of *amb2291* deletion or site-directed mutagenesis on the phototactic behaviour of
 811 AMB-1 cells. Relative phototactic behaviour (%) of the WT and mutant strains ($n=4$). Level of the
 812 significance of the differences observed between light and dark control is expressed as a hashtag sign
 813 (# p <0.05). The significant differences between the two groups depicted by the lines in the graph are

814 indicated by asterisk (* $p < 0.05$).



815

816 **Fig. 10** Functional model of LOV-like Amb2291 protein. Red-light illumination and oxidative stress
817 can regulate the LOV-like protein Amb2291 to enhance the expression levels of antioxidant genes and
818 stimulate the formation of magnetosomes and then reduce intracellular ROS levels. The general
819 function is to protect magnetotactic bacteria (MTB) cells from the deleterious effects of oxidative stress.
820 The arrows show the red-light signal transduction pathway; Amb2291 may interact with
821 red/far-red-light-induced bacteriophytochrome (BphP) by some mechanisms to form the signalling
822 network response to red light. The signal transduction intermediates can be anti-oxidative genes, such
823 as *oxyR* and *sod*, and magnetosome, the magnetite crystal in MTB.