FEBS *Letters*

Optical manipulation of *Saccharomyces cerevisiae* cells reveals that green light protection against UV irradiation is favored by low Ca^{2+} and requires intact UPR pathway

Ileana C. Farcasanu [a],[*],[1], Radu Mitrica [b],[1], Ligia Cristache [b], Ioana Nicolau [a], Lavinia L. Ruta [a], Liliana Paslaru [b], Sorin Comorosan [c]

[a] *University of Bucharest, Faculty of Chemistry, Department of Organic Chemistry, Biochemistry and Catalysis, Sos. Panduri 90-92, Bucharest, Romania*
[b] *University of Medicine & Pharmacy "Carol Davila", Postgraduate Department of Biochemistry, Bucharest, Romania*
[c] *Interdisciplinary Research Group, Romanian Academy, Bucharest, Romania*

ARTICLE INFO

ABSTRACT

Optical manipulation of *Saccharomyces cerevisiae* cells with high density green photons conferred protection against the deleterious effects of UV radiation. Combining chemical screening with UV irradiation of yeast cells, it was noted that the high density green photons relied on the presence of intact unfolded protein response (UPR) pathway to exert their protective effect and that the low Ca^{2+} conditions boosted the effect. UPR chemical inducers tunicamycin, dithiotreitol and calcium chelators augmented the green light effect in a synergic action against UV-induced damage. Photo-manipulation of cells was a critical factor since the maximum protection was achieved only when cells were pre-exposed to green light.

1. Introduction

Visible light is that part of the spectrum which is usually associated with physiological processes such as vision and color perception. Being one of the fundamental constants of the environment, the visible light is perceived as a bio-friendly type of radiation to which the living organisms have thoroughly adapted. The interaction between visible light and the biological systems has always been a topic of interest and there is increasing evidence that it often results in stimulating or protective effects [1–4]. Green light (GL) is that part of the visible spectrum with wavelengths between 487–570 nm [5], thought to act as photoprotector against the production of free radicals [6–8], displaying a long-range interaction [9] with

the biological systems, with increased active range between 515–530 nm [10]. In particular, high density green photons were shown to protect UV-irradiated melanocytes in standard culture [8]. The use of high density green photons as a source of metastable states by polarization effects was first revealed within the Comorosan group, which termed this new type of matter as complex optical matter [11]. In this study, we used the eukaryotic microorganism *Saccharomyces cerevisiae* to identify molecular components which are relevant for the photoprotective effect of GL against the deleterious effects of UV irradiation. Combining chemical screening with photomanipulation of yeast cells, we found that GL acted synergistically with the unfolded protein response (UPR) chemical inducers tunicamycin (TM), dithiotreitol (DTT) and calcium chelators, thus indicating the UPR involvement in the GL photoprotective action against UV irradiation.

The UPR is a signal transduction pathway that transmits information about the folding status of the proteins within the endoplasmic reticulum (ER) lumen to cytosol and then nucleus with the final aim of increasing the protein folding capacity [for reviews, [12–14]. UPR is initiated by accumulated misfolded proteins in the ER lumen (generically known as ER stress) readily sensed by the Ire1p, the key component of the unfolded protein response (UPR)

pathway [15]. In yeast, the activated protein receptor Ire1p transmits the signal by removing an intron from the *HAC1* mRNA (unspliced [u]*HAC1* mRNA → spliced, intronless [i]*HAC1* mRNA) that translates into the Hac1p transcription activator of the UPR target genes [16,17], many of which possess an UPR-responsive element (UPRE) in their promoter sequence [18].

In this study, we provide evidence that the GL photoprotective effect against UV-induced cell damage in yeast requires an intact UPR pathway and that the protective effect is augmented by low Ca^{2+} conditions. Ca^{2+} is a notorious signaling factor, used universally by living organisms. In *S. cerevisiae* cells, Ca^{2+} is critical to signal various environmental stresses including salt, alkaline and oxidative stresses [19–21], when signaling is achieved through transient increase in cytosolic Ca^{2+} [22]. Once the signaling achieved, the cytosolic Ca^{2+} is restored to minimum level by an intricate system of channels, exchangers and pumps [23,24]. The cytosolic Ca^{2+} bursts are sensed by the Ca^{2+}-binding protein calmodulin, which binds to and activates calcineurin, a well-conserved protein phosphatase. Calcineurin, in turn, dephosphorylates Crz1p [24], which then translocates from the cytosol to the nucleus and activates genes involved in the adaptation to stress [25,26]. Lingering cytosolic Ca^{2+} is detrimental to cell survival and it often associates with apoptosis onset[27]. Inversely, Ca^{2+} depletion is equally bad, since it can ultimately lead to incorrect protein folding and ER stress [28]. Recently, it was shown that extracellular Ca^{2+} levels stimulate the yeast response to blue light via changes in the nuclear localization of transcription factors Crz1p, Msn2p and Msn4p [29]. In this study we revealed that the GL protection of the yeast cells against UV irradiation involved calcineurin, but not Crz1p, Msn2p or Msn4p by a mechanism which relates to calcium homeostasis.

2. Materials and methods

2.1. Yeast strains, yeast manipulation, plasmids and growth media

The *S. cerevisiae* strains used in this study were isogenic to the "wild type" (WT) parental strain BY4741 (*MAT***a**; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*) [30] having the exact genotype of BY4741, except for the knock out mutations *yfg::kanMX4* of individual genes (Supplementary material, Table 1S). The individual knock-out mutants are referred to in text as *yfgΔ*. All strains were obtained from EUROSCARF (European *S. cerevisiae* Archive for Functional Analysis, Institute of Molecular Biosciences Johann Wolfgang Goethe-University Frankfurt, Germany). Cell storage, growth and manipulation were done as described [31]. Strains were stored and pre-cultured in standard YPD (1% yeast extract, 2% polypeptone, 2% dextrose). For irradiation experiments, cells were shifted to defined SD (synthetic dextrose) media supplemented with the necessary amino acids. For solid media, 2% agar was used. Glucose and the supplemental chemicals were added from filter-sterilized (Millipore, pore size 0.22 μm) stock solutions. Yeast transformation was performed by a modified lithium acetate method [32] and transformants were selected for growth on SD medium lacking uracil (SD-Ura). Plasmid pCZY1 (*URA3*, 2 μ) harboring the *lacZ* reporter gene driven by the *CYC* core promoter fused with the UPRE (unfolded protein response element) was generously provided by Professor Kenji Kohno (Nara Institute of Science and Technology, Japan) and used to monitor cellular UPR activity [18].

2.2. Optical manipulation of cells

Cells to be irradiated were inoculated from an overnight pre-culture at density 5×10^5 cells/ml in liquid SD (complete or lacking uracil) and grown with agitation (200 rpm) at 28 °C until reaching 5×10^6 cells/ml (approximately 6 h). Cell suspensions (500 μl) were shifted to multi-well transparent plates (Corning, well

diameter 2 cm) so that the cell suspension had approximately 1.5 mm thickness. Cells gently shaken (30 rpm) were irradiated in normal atmosphere with GL from underneath and with UV from above.

GL illumination of cells was performed from underneath using light emitting diodes (LEDs) of 100 lumens mounted on a copper ventilated radiator and passing through a window of the same size and shape as the well in which the cells were grown. A special geometry was arranged, to obtain a collimated monochromatic light $\lambda = 527$ nm with intensity on the target up to 10^5 Lx, determined with a digital Luxmeter LX-1102, Lutron (14.64 mW/cm^2). The same protocol was followed for control experiments with blue light (BL, $\lambda = 455$ nm, 14.64 W/cm^2) or white light (continuous visible spectrum, 3 mW/cm^2).

UV irradiation of cells was carried out with an UV-C lamp (Vilber Lourmat, Marne-la-Valée, France) emitting ultraviolet rays at 254 nm, which delivered uniform irradiation at a distance of 20 cm (10 mW/cm^2). For control experiments, UV-A and UV-B lamps emitting ultraviolet rays at 365 and 312 nm, respectively (10 mW/cm^2) were used.

2.3. Cell growth

Cell growth following irradiation was assessed in three different ways.

2.3.1. Growth in liquid media

Irradiated cells were shifted to a 28 °C dark incubator and grown overnight under mild agitation (100 rpm). The cell growth in liquid media was monitored by determining the optical density of cellular suspension at 660 nm (Shimadzu UV–Vis spectrophotometer, UV mini 1240, Kyoto, Japan) as described [33]. The growth was calculated relatively to the initial cell density of the non-irradiated, non-chemically stressed cells.

2.3.2. Cell growth spot assay

The irradiated cells were diluted in sterile water 10^0, 10^1, 10^2, 10^3 and 10^4-fold, then stamped on SD/agar plates using a replicator. Plates were photographed after 2 days incubation at 28 °C.

2.3.3. Cell viability

For viability test, thoroughly suspended cell samples were taken, suitably diluted with sterile deionised water and plated on YPD/agar. After 3–4 days of incubation at 28 °C, the colonies were counted and viability was expressed as percent of colony forming units (CFU) relatively to control (non-irradiated non-chemically stressed cells). Original cell suspensions had viability higher than 99%.

2.4. Reverse transcriptase-PCR (RT-PCR)

Analysis of *HAC1* mRNA splicing was done following the procedure described by Promlek et al. [34]. Total RNA was prepared from yeast cells using a SV Total RNA Isolation System (Promega, Madison, Wi, USA) following manufacturer's instructions. To obtain first strands of cDNA, total RNA samples (1 μg) were used for 20 μl-scale reverse transcription reaction with the GoScript Reverse Transcriptase kit (Promega, Madison, Wi, USA) and a dT15 oligonucleotide primer. The cDNAs of interest were amplified from 2 μl reverse transcription (RT) reactions with appropriate primer sets and Taq polymerase (Promega). To amplify the transcripts, 22 cycles for *HAC1* and 18 cycles for *ACT1* were used. The PCR products were then run on 2% agarose gels, and the ethidium bromide-stained fluorescent images were captured using a gel documentation system (Doc-Print II, Vilber Lourmat, France). The primers used were: for *HAC1*, forward: 5′-TACAGGGATTTCCAGAGCACG-3′, reverse: 5′-TGAAGTGATGAAGAAATCATTCAATTC-3′, and for *ACT1*,
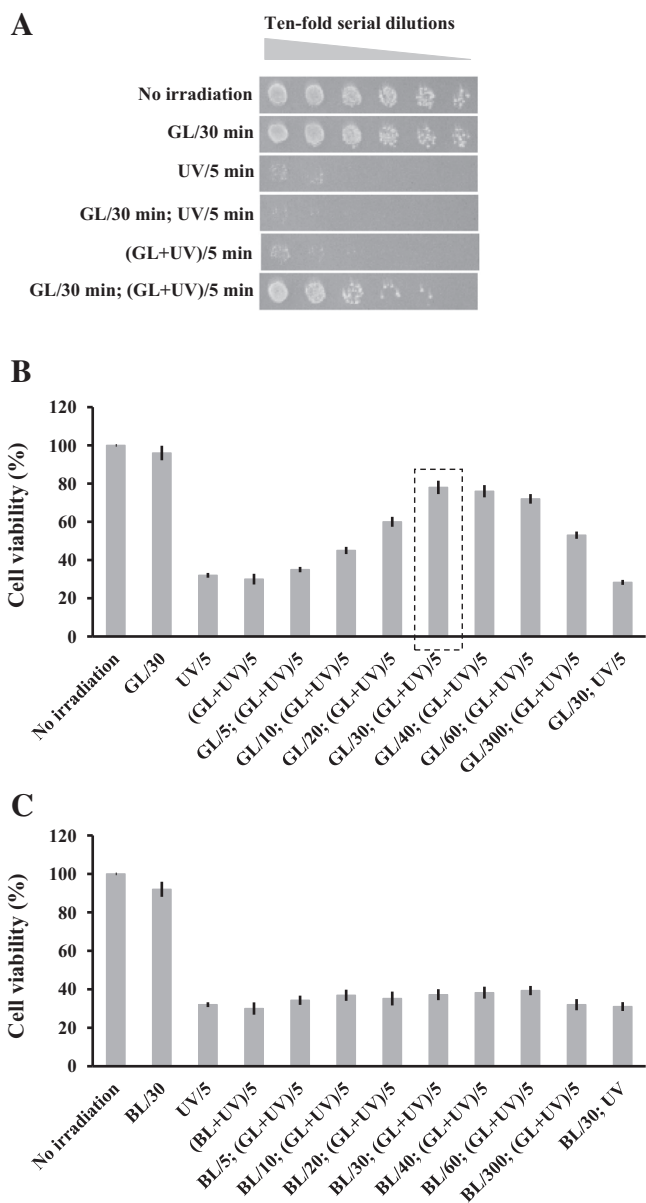
Fig. 1. Protective effect of GL upon UV-irradiated yeast cells. (A) GL rescued the UV-irradiated cells when applied on cells for 30 min before being used concomitantly with UV irradiation. Mid log growing cells (5×10^6 cells/mL) were placed in transparent wells to be illuminated with GL (527 nm, 14.64 mW/cm^2) from underneath and with UV (254 nm, 10 mW/cm^2) from above, as described in Section 2. Various irradiation schemes were performed in parallel, then individual cell suspensions were serially diluted in a 48-well plate before being stamped on YPD/agar by means of a pin replicator (approximately 4 μl/spot). The YPD plates were photographed after two days at 28 °C in a dark incubator. Experiments were repeated at least three times on different days and the results were similar. One representative plate is shown. (B) Influence of GL pre-exposure on the viability of cells irradiated with UV. Optically manipulated cells were diluted in sterile water and spread on YPD plates. Colony forming units (CFU) were counted after three days incubation at 28 °C in a dark incubator and viability was calculated relatively to non-irradiated cells. Each determination was repeated three times on different days, with no significant variations ($P < 0.05$). Values are expressed as the mean ± standard error (S.E.) of duplicate determinations of three independent experiments ($n = 6$). The data on the horizontal axis represent the type of optical manipulation and the numbers represent the exposure time (minutes) corresponding to each type of irradiation. Best protection achieved is highlighted by a dotted rectangle. (C) Influence of BL on the viability of cells irradiated with UV. The experimental setup was similar to that described in B, except for the GL which was replaced by BL (455 nm, 14.64 mW/cm^2).
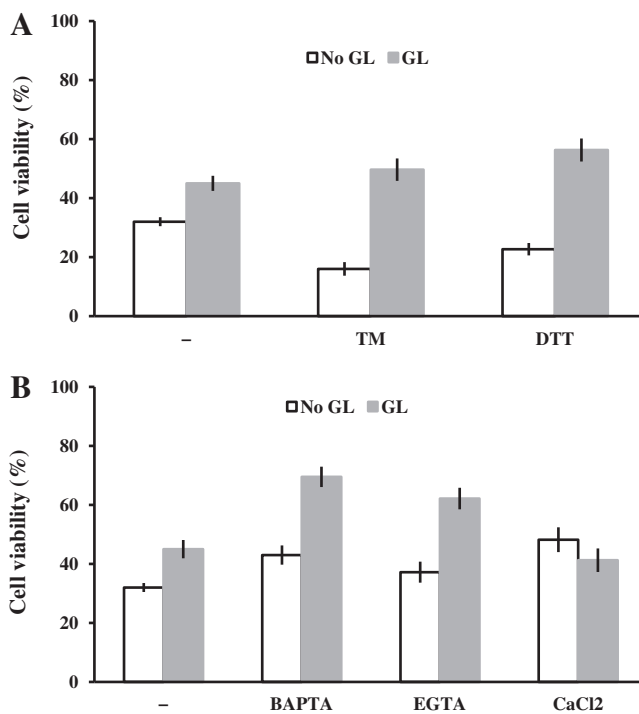
Fig. 2. Effect of chemicals on the protective action of GL against UV. (A) UPR chemical inducers tunicamycin (TM) and dithiothreitol (DTT) acted in synergy with GL to rescue the UV-irradiated cells. Mid log growing cells (5×10^6 cells/ml) pre-incubated for six hours with non-toxic concentrations of TM (2 μM) or DTT (0.5 mM) were placed in transparent wells and were illuminated with GL (10 min afore, then 2 min concomitantly with UV) in 2 cm wells as described in Section 2. Following irradiation, cells were diluted in sterile water and spread on YPD plates. Colony forming units (CFU) were counted after three days at 28 °C in a dark incubator. Cell viability was calculated relatively to non-irradiated cells grown in liquid SD medium with no added chemical. (B) Synergy between calcium chelators and GL in rescuing the UV-irradiated cells. The cells pre-incubated for 6 h with non-toxic concentrations of BAPTA (2 mM) or EGTA (1 mM) were further manipulated as in experiments described in A. Each determination was repeated three times on different days, with no significant variations ($P < 0.05$). Values were expressed as the mean ± standard error (S.E.) of duplicate determinations of three independent experiments ($n = 6$). The tests made in the presence of illumination (grey bars, GL) were paralleled by control experiments, in which the GL was switched off (white bars, no GL). GL (527 nm, 14.64 mW/cm^2); UV (254 nm, 10 mW/cm^2).

forward: 5′-ATTCTGAGGTTGCTGCTTTGG-3′, reverse: 5′-GTGGTG-AACGATAGATGGAC-3′.

2.5. β-Galactosidase assay

The β-galactosidase activity of yeast extract was carried out on permeabilized cells as described [33]. Values are expressed in Miller units (MU) as the mean ± standard error (SE) of triplicate determinations of three independent yeast transformants.

2.6. Reproducibility of the results

All experiments were repeated independently on three different days. For each individual experiment values were expressed as the mean ± standard deviation (S.E.) of duplicate determinations on three independent days ($n = 6$). Multiple comparisons were performed with Student's t-test. The differences were considered to be significant when $P < 0.05$. Data analysis was performed with Statistical Package for Social Science 15.0 (SPSS 15.0) for Windows. The observed trends were fully consistent among the independent experiments. For visual results (photographs), a representative example is shown.

3. Results

3.1. Yeast cells pre-exposed to GL are protected from the deleterious effects of UV irradiation

GL was shown previously to have a protective effect on human melanocytes in cultures exposed to UV [8]. To further investigate the photoprotective action of GL ($\lambda = 527$) against UV exposure, we used the model eukaryotic microorganism *S. cerevisiae*. Thanks to a thick, rigid cell wall, the yeast cells are resistant to various environmental insults; nevertheless, UV exposure for around 5 min ($\lambda = 254$ nm, 10 mW/cm^2) severely impaired the yeast growth, killing more than 50% of the cells in the liquid suspension. GL irradiation (from 30 min to 24 h, 30 min increments) had no protective effect when performed either before or after the UV irradiation. Protection could be achieved though when UV irradiation was concomitant with GL exposure, but solely for cells subjected to GL pre-treatment (Fig. 1A). Maximum protection was recorded when concomitant UV- and GL irradiations were preceded by 30 min exposure to GL (Fig. 1B). The irradiation scheme: GL (30 min), immediately followed by concomitant exposure to both GL from underneath and UV from above (UV + GL, 5 min) systematically gave the best results in terms of cell survival and viability, therefore it was used in most subsequent experiments.

In the experiments described above, GL was shown to protect the yeast cells against UV-C (energy peak at 254 nm), therefore we wondered whether similar behavior could be observed for UV-B (energy peak at 312 nm) or UV-A (energy peak at 365 nm). Surprisingly, the protective action of GL, was weaker against UV-B and UV-A albeit following the same pattern as for UV-C (Supplemental material, Fig. 1S), suggesting a selective interaction between the green photons and the high-energy UV-C rays.

In parallel, we tested other parts of the visible spectrum in terms of protective capacity against UV-C. Using a continuous spectrum of white light enhanced the UV-C damaging (Supplemental material, Fig. 2S), while blue light (455 nm) had no significant effect on cell survival (Fig. 1C). Although the protective potential of visible light with other specific wavelengths cannot be completely ruled out, we focused our further experiments solely on the optical manipulation of the yeast cells with high density green photons. In all the tests described below, only UV-C (254 nm) was employed, simply referred to as UV.

3.2. GL protects yeast cells against UV in synergy with chemicals which induce the UPR

The protection offered by GL against the UV irradiation was noteworthy; therefore correlations with various molecular mechanisms were attempted. For this purpose, we optically manipulated yeast cells grown under various chemical insults to eventually detect a synergism between GL and the chemicals tested in terms of UV protection. As most of the chemical used represented an extra stress upon yeast cells, a milder irradiation scheme was necessary. Wild type yeast cells pre-incubated with individual chemicals were subsequently exposed to GL (10 min) then GL + UV (2 min). The irradiated cells were shifted to a dark incubator and the synergy between GL and the chemical was assessed by cell relative growth. An array of chemicals was assayed, including sub-toxic concentrations of: (i) H_2O_2, *tert*-butylhydroperoxide, menadione, paraquat (for oxidative stress induction); (ii) antioxidants (ascorbate, α-tocopherol, quercetin, caffeic acid, epigallocatechin gallate, catechin, rutin); (iii) heavy metals (Mn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}); (iv) high Ca^{2+} (100–400 mM) and low Ca^{2+} achieved by chelators 1,2-bis(*o*-aminophenoxy)-ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA) or ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA); (v) tunicamycin (TM) and dithiothreitol (DTT) for UPR induction (see Supplemental material, Figs. 3S–7S). It was noted that GL protection of UV-irradiated cells was slightly
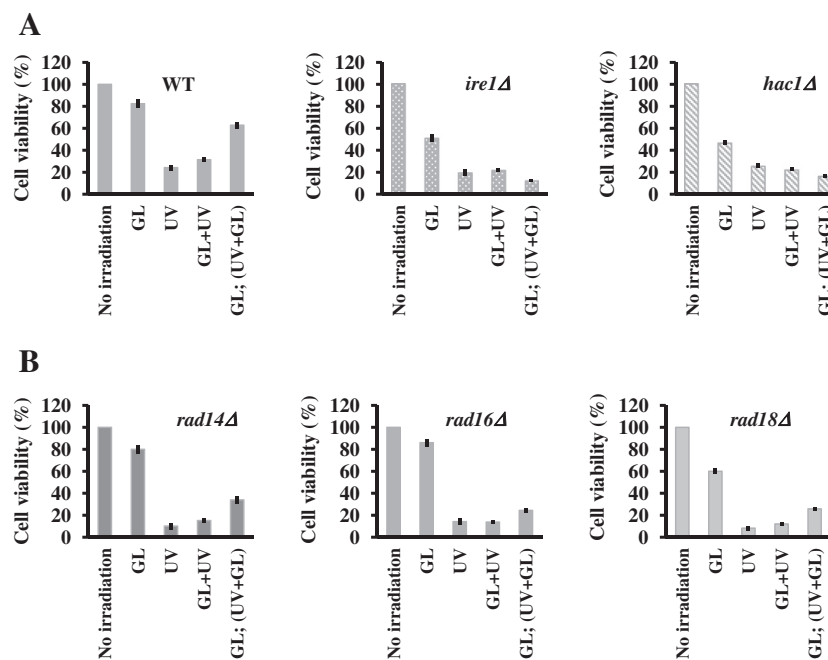
Fig. 3. GL protection against UV depended on UPR pathway status. (A) GL rescued the UV-irradiated yeast cells, providing they exhibit an intact UPR pathway. Wild type (WT) and knock-out mutants *ire1Δ* and *hac1Δ* grown to mid log phase (5 × 10^6 cells/mL) were placed in transparent wells under mild agitation (30 rpm) and were illuminated with GL from underneath (30 min afore, then 5 min concomitantly with UV from above (right-most bar). Control experiments with different irradiation schemes were also included. Following irradiation, cells were diluted in sterile water and spread on YPD plates. Colony forming units (CFU) were counted after three days incubation at 28 °C in the dark. Cell viability was calculated relatively to non-irradiated cells (left-most bar). (B) Effect of GL on the viability of UV-exposed mutants with defects in DNA repairs machinery. The knock-out mutants *rad14Δ*, *rad16Δ*, and *rad18Δ* were treated as in A. GL (527 nm, 14.64 mW/cm^2); UV (254 nm, 10 mW/cm^2).

augmented by TM and DTT (Fig. 3S), or by the calcium chelators EGTA or BAPTA (Fig. 4S). In contrast, no such effect could be noticed for any other chemical tested (see Supplemental material, Figs. 5S–7S). Further, the synergic action of GL and the responsive chemicals was checked by determining the viability of the wild type cells exposed to UV. In this case also, it was noted that both the UPR inducers (TM and DTT) and the calcium chelators (EGTA and BAPTA) augmented the capacity of GL to restore the viability of the UV-irradiated cells (Fig. 2A and B, respectively).

3.3. Protection against UV-related damages through exposure to GL requires intact UPR pathway

TM and DTT are notorious inducers of the UPR pathway. At the same time, the calcium chelators BAPTA or EGTA create an extracellular environment with low concentrations of free Ca^{2+}, causing Ca^{2+} shortage which is sometimes associated with the occurrence of misfolded proteins. Under such circumstances, it became apparent that the UPR pathway may relate to the protective effect of GL against UV irradiation. To test this possibility, yeast mutants with disrupted UPR pathway were subjected to the irradiation scheme that best worked for the wild type cells (Fig. 1B). For this purpose, cells with knock out mutations in the two main components of UPR pathway, *ire1Δ* and *hac1Δ*, were used. We noticed that while in the case of wild type cells GL restored the viability of UV-irradiated cells, no such effect could be observed for either *ire1Δ* or *hac1Δ* cells (Fig. 3A). These observations suggested that GL photoprotection against UV requires a functional UPR pathway.

UV attacks biomolecules randomly, and apart from proteins, it strongly damages the DNA, therefore we further investigated whether GL could reduce the UV sensitivity in yeast with defects in the DNA repair machinery. For this purpose we used three UV-sensitive knock-out mutants, *rad14Δ*, *rad16Δ* and *rad18Δ* which lack Rad14p, Rad16 (proteins that recognize and bind damaged DNA during nucleotide excision repair) [35] and Rad18p (E3 ubiquitin ligase required for postreplication repair) [36], respectively. In the case of all UV-sensitive mutants, the protective action of GL, albeit present, was rather weak (Fig. 3B). This observation suggested that under the experimental conditions used, GL did not significantly relieve the DNA damage caused by the much stronger UV.

3.4. GL optical manipulation induces the UPR

Ire1p and Hac1p are two key components of the UPR pathway in yeast: the former has the ability to sense the misfolded proteins within the ER and to transduce the signal further to the Hac1p transcription factor via the splicing of *HAC1* mRNA, thus activating the transcription of effector genes [12–14], many of which have UPRE in their promoters [18]. To detect the effect of GL upon UPRE-regulated gene transcription, we transformed yeast cells with a reporter plasmid having the *LacZ* gene under the control of UPRE [18] and subjected the transformed cells to optical manipulation. UPR induction was monitored by the presence of β-galactosidase activity in permeabilized yeast cells. We revealed that under normal conditions, the wild type cells exhibited a background β-galactosidase activity which was wiped out when cells were subjected to UV irradiation (Fig. 4A). In contrast, the β-galactosidase activity was restored and even increased in GL-protected UV-irradiated wild type cells. This could be correlated to the UPR induction, as the galactosidase activity was very low in both *ire1Δ* and *hac1Δ* cells subjected to similar optical manipulations (Fig. 4A). The GL ability to activate UPR through Ire1p-Hac1p system was also tested by determining the level of *HAC1* mRNA splicing in wild type cells. Total RNA was extracted from cells that underwent various irradiation schemes and the *HAC1* mRNA splic-

ing was assessed by RT-PCR (Fig. 4B). It was noted that GL alone could induce the *HAC1* mRNA splicing, which was not detected in UV-irradiated cells (Fig. 4B). *HAC1* mRNA splicing could be also detected in co-irradiated cells (UV + GL) and was highest in cells pre-exposed to GL (GL; GL + UV) (Fig. 4B), suggesting that GL protection against UV correlates with conditions fit for UPR activation.

3.5. Protection against UV through exposure to GL depends on cell calcium

As GL exhibited synergism with calcium chelators in the protective action against UV, the question was raised if the GL protective effect could be related to environmental or cellular Ca^{2+}. It was shown previously that increased extracellular Ca^{2+} levels stimulate the blue light-induced responses of three transcription factors, Crz1p, Msn2p and Msn4p and that Crz1p activation is mediated by calcineurin [29]. We subjected the knock-out mutants *crz1Δ*, *msn2Δ* and *msn4Δ* to the GL-UV irradiation scheme best fitted for the parental strain, but no significant difference from the wild type behavior could be noticed and that GL had similar protective effect on all the three mutants (Fig. 5A). In contrast, the cells lacking the regulatory subunit of calcineurin, *cnb1Δ*, became even more sensitive to UV when GL photomanipulated (Fig. 5B). As this
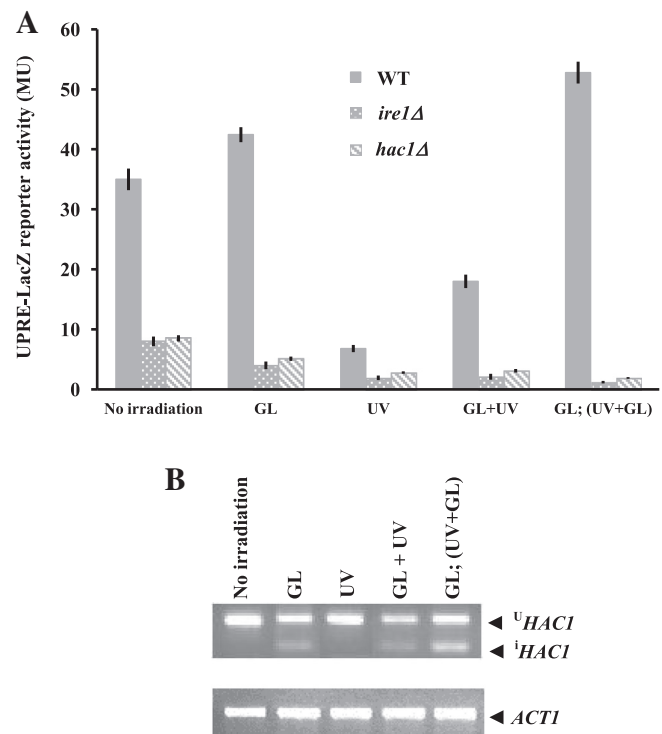

Fig. 4. Effect of optical manipulation on UPR response. (A) WT, *ire1Δ* and *hac1Δ* cells were transformed with plasmid pCZY1 (harboring a UPRE-*LacZ* reporter) [18] and grown in selective medium until mid log phase. Cell suspensions were optically manipulated as described in Fig. 3A, and then shifted to a shaking incubator (200 rpm, 28 °C). Samples were taken after 60 min and UPRE-LacZ reporter activity was assessed by β-galactosidase activity (Miller units). The assay was done on permeabilized cells with the colorimetric substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG). Each determination was repeated three times on different days, with no significant variations (*P* < 0.05). Values were expressed as the mean ± standard error (S.E.) of triplicate determinations on three independent transformants (*n* = 9). (B) Effect of optical manipulation on *HAC1* mRNA splicing. Total RNA prepared from wild type cells optically manipulated as in Fig. 3A was subjected to RT-PCR to detect the unspliced (less active ^uHAC1) or the sliced (active ^iHAC1) forms of *HAC1* on 2% agarose gels. *ACT1* RT-PCR was done as a loading control. The experiment was done three times and the results were similar. One representative gel is shown. GL (527 nm, 14.64 mW/cm²); UV (254 nm, 10 mW/cm²).
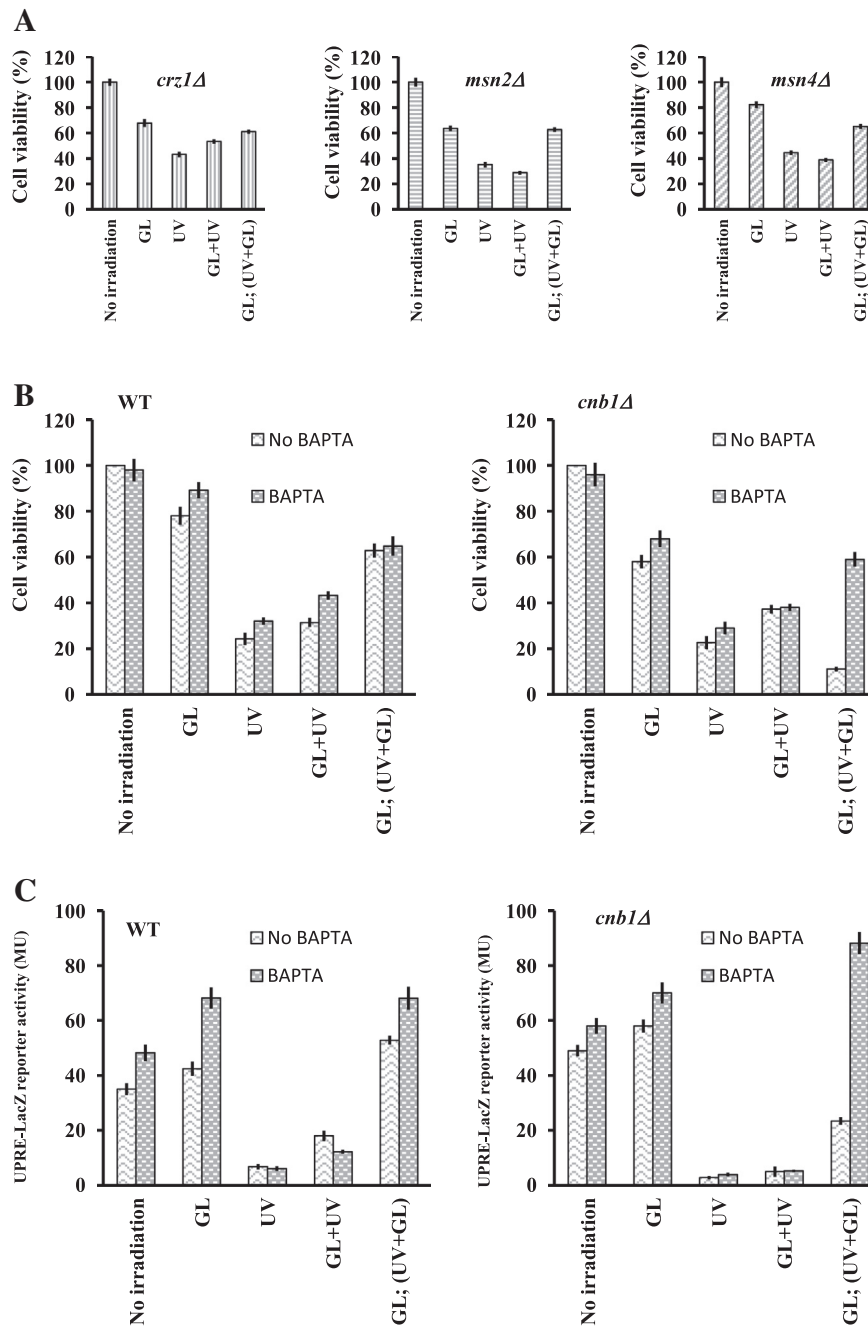
Fig. 5. GL protection against UV-cells was favored by low environmental calcium. (A) Deletions of genes encoding transcription factors which were activated by blue light [29] did not interfere with the photoprotective activity of GL against UV-related cell viability. Knock-out mutant *crz1Δ*, *msn2Δ* and *msn4Δ* cells in mid log growing phase (5×10^6 cells/ml) were manipulated as in Fig. 3A. Following irradiation, cells were diluted in sterile water and spread on YPD plates. Colony forming units (CFU) were counted after three days incubation at 28 °C in the dark. Viability was calculated relatively to non-irradiated cells. Each determination was repeated three times on different days, with no significant variations ($P < 0.05$). Values are expressed as the mean ± standard error (S.E.) of duplicate determinations of the three independent experiments ($n = 6$). (B) Effect of calcium chelator BAPTA on optically manipulated cells defective in calcineurin. Wild type cells (left) or cells lacking the regulatory subunit of calcineurin (*cnb1Δ*, right) were pre-grown in liquid SD or in SD containing non-toxic concentrations of BAPTA (2 mM) until mid log phase (5×10^6 cells/ml). Following irradiation, cells were manipulated as in A to determine the viability. (C) Effect of calcium chelation on UPR response of optically manipulated cells. Wild type cells (left) or *cnb1Δ* cells (right) were transformed with plasmid pCZY1 (harboring a UPRE-*LacZ* reporter) [18] and grown in SD-Ura supplemented or not with BAPTA, until reaching the mid log phase. Cell suspensions were optically manipulated as described in Fig. 3A, then shifted to a shaking incubator (200 rpm, 28 °C). Samples were taken after 60 min and UPRE-LacZ reporter activity was assessed by β-galactosidase activity (Miller units). β-Galactosidase assay was done on permeabilized cells using the colorimetric substrate *o*-nitrophenyl-β-ᴅ-galactopyranoside (ONPG). Each determination was repeated three times, with no significant variations ($P < 0.05$). Values are expressed as the mean ± standard error (S.E.) of triplicate determinations on three independent transformants ($n = 9$). GL (527 nm, 14.64 mW/cm^2); UV (254 nm, 10 mW/cm^2).

sensitivity could be reverted by the cell-impermeant calcium chelator BAPTA (Fig. 5B), it became apparent that GL photoprotection against UV was favored by low environmental Ca^{2+}. Additionally, BAPTA stimulated the UPR in the *cnb1Δ* cells subjected to the GL/30, (GL + UV)/5 irradiation scheme (Fig. 5C), indicating that

low environmental Ca^{2+} augments the GL protective effect against UV via UPR activation. To double check the role that cell calcium has in mediating the protective effect of GL against UV, we employed various yeast mutants defective in Ca^{2+} transport and homeostasis. In this experiment, *cch1Δ*, *mid1Δ*, *pmc11Δ*, *yvc1Δ*,
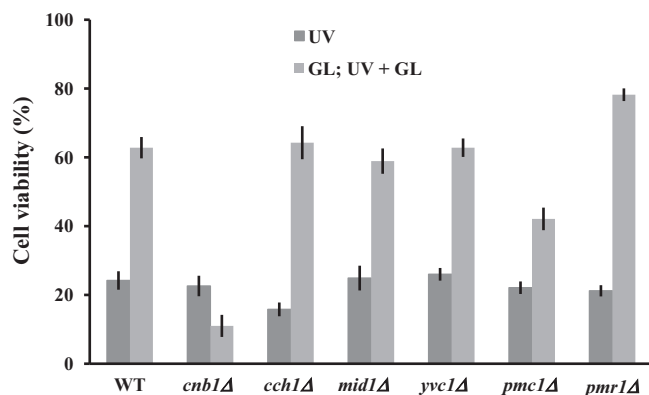
Fig. 6. The photoprotective action of GL against UV depends on the calcium status of the yeast cells. Yeast cells with various defects in calcium transport and homeostasis were grown to mid log phase (5×10^6 cells/ml) and were placed in transparent wells under mild agitation (30 rpm). The cells were UV-irradiated for 5 min (control, dark grey bars) or they were illuminated with GL from underneath (30 min afore, then 5 min concomitantly with UV from above) (light grey bars). Following irradiation, cells were diluted in sterile water and spread on YPD plates. Colony forming units (CFU) were counted after three days incubation at 28 °C in the dark. Cell viability was calculated relatively to non-irradiated cells from the same strain. GL (527 nm, 14.64 mW/cm²); UV (254 nm, 10 mW/cm²).

and $pmr1\varDelta$ cells were subjected to the GL/30, (GL + UV)/5 irradiation scheme, and their viability was subsequently determined. It was noted that GL had a protective effect on $cch1\varDelta$, $mid1\varDelta$, and $yvc1\varDelta$, similarly to the wild type cells, but not on $pmc1\varDelta$ cells (Fig. 6). Cch1p and Mid1p are ion channels necessary for the high affinity calcium influx across the plasma membrane and elevation of cytosolic calcium, while Yvc1p is the vacuolar cation channel which mediates release of Ca^{2+} from the vacuole [23]. Altogether, the lack of the channels responsible for cytosolic Ca^{2+} bursts keeps a low calcium profile, benefic for the GL photoprotective capacity. In this line of evidence, the GL protection was lower on $pmc1\varDelta$ cells lacking the vacuolar Ca^{2+} ATPase involved in depleting cytosol of Ca^{2+} ions (Fig. 6). Special attention must be paid to the $pmr1\varDelta$ cells, which were the most responsive to GL protection (Fig. 6). Pmr1p encodes the High affinity Ca^{2+}/Mn^{2+} P-type ATPase required for Ca^{2+} and Mn^{2+} transport into Golgi whose deletion was reported to induce UPR [28]. And as GL protection against UV irradiation is favoured by UPR pre-activation, the $pmr1\varDelta$ cells are likely to be more prepared for the ravaging effects of UV irradiation.

4. Discussions

Recently, the interaction of light with matter generated a new scientific domain known in physics as optical manipulation, with the new concepts of optical matter and optical force. Using optics to determine biological forces or to manipulate cells and organelles by optical tweezers and stretchers [37,38] or to explore the action of individual molecules within living cells [39] are just a few examples of optical manipulation potential. While generating controlled optical forces is feasible, the interaction between light and biologic systems is still difficult to assess, due to the heterogeneity and optical imperfections of the latter. In this study, we optically manipulated populations of yeast cells and we obtained reproducible responses of UV-irradiated cells to high density green photons. Using a chemo-genetic approach, we revealed that the protection offered by the GL requires a healthy, intact UPR pathway (Fig. 3A), that UPR chemical inducers act in synergy with GL (Fig. 2A and B), and so do mutations which induce the UPR, such as PMR1 deletion (Fig. 6). While the necessity of an intact and active UPR pathway seems obvious (UV exposure randomly damages

biomolecules, including proteins, triggering defense responses such as the UPR) we were intrigued by the putative role of calcium in the process. The fact that the GL photoprotective effect against the UV-induced damages could not be seen in $cnb1\varDelta$ but was evident in $crz1\varDelta$ cells suggested that this effect depends on calcium cellular level rather than on calcium-mediated signaling. The $cnb1\varDelta$ cells are notorious for accumulating intracellular Ca^{2+}, which may block the GL from photoprotecting the cells. The fact that BAPTA restored the GL protection (Fig. 5B) and boosted the UPR response in $cnb1\varDelta$ cells (Fig. 5C) strongly supports this hypothesis.

In the end, the observation that the protective action of GL could be recorded only when UV irradiation was concomitant with GL illumination preceded by GL exposure is worthwhile mentioning. Although no satisfactory explanation is available, it is possible that the long-range interaction between the living cells and the polarization effects of the high density green photons induce a metastable state which renders the cells fit for survival, a state which is short-lived and ends soon after the GL is switched off.

The mechanism by which GL offers protection to the cells against UV irradiation is still elusive and remains to be investigated. Nevertheless, the synergy between GL and UPR inducers is noteworthy and may have important scientific and technological implications.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.09.008.

References

[1] Karu, T.I., Tiphlova, O.A. and Fedoseyeva, G.E. (1984) Biostimulating action of low intensity monochromatic visible light: is it possible? Laser Chem. 5, 19–25.

[2] Kolyakov, S.F., Pyatibrat, L.V., Mikhalov, E.L., Konpanets, O.N. and Karu, T.I. (2001) Changes in the circular dichroism spectra of a suspension of live cells exposed to low intensity laser radiation ($\lambda = 820$ nm). Dokl. Biochem. Biophys. 377, 128–131.

[3] Babynin, E.V., Nuretdinov, I.A., Gubskaya, V.P. and Barabanshchikov, B.I. (2002) Study of mutagenic activity of fullerene and some of its derivatives using His⁺ reversions of Salmonella typhimurium as an example. Russ. J. Genet. 38, 359–363.

[4] Triessheijn, M., Baas, P.M., Schellens, J.H. and Stewart, F.A. (2006) Photodynamic therapy in oncology. Oncologist 11, 1034–1044.

[5] Kelly, K.L. (1943) Color designations for lights. J. Opt. Soc. Am. 33, 627–632.

[6] Comorosan, S., Kappel, W., Constantinescu, I., Gheorghe, M., Ionescu, E., et al. (2009) Green light effects on biological systems: a new biophysical phenomenon. J. Biol. Phys. 35, 265–277.

[7] Comorosan, S., Polosan, S., Popescu, I., Ionescu, E., Mitrica, R., et al. (2010) Structural studies on serum albumins under green light irradiation. Eur. Biophys. J. 39, 1483–1491.

[8] Comorosan, S., Polosan, S., Jipa, S., Popescu, I., Marton, G., et al. (2011) Green light radiation effects on free radicals inhibition in cellular and chemical systems. J. Photochem. Photobiol., B 102, 39–44.

[9] Fröhlich, H. (1970) Long range coherence, the activity of enzymes. Nature 228, 1093–1099.

[10] Comorosan, S. (1976) Progress in Theoretical Biology, Biological Observables, Academic Press, New York.

[11] Comorosan, S., Polosan, S., Popescu, I., Stamatin, I., et al. (2013) Optical manipulation of complex molecular systems by high density green photons: experimental and theoretical evidence. Eur. Phys. J. B 86, 232.

[12] Kohno, K. (2007) How transmembrane proteins sense endoplasmic reticulum stress. Antioxid. Redox Signalling 9, 2295–2303.

[13] Mori, K. (2009) Signalling pathways in the unfolded protein response: development from yeast to mammals. J. Biochem. 146, 743–750.

[14] Walter, P. and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334, 1081–1086.

[15] Kohno, K. (2010) Stress-sensing mechanisms in the unfolded protein response: similarities and differences between yeast and mammals. J. Biochem. 147, 27–33.

[16] Cocs, J.S. and Walter, P. (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell 87, 391–404.

[17] Kawahara, T., Yanagi, H., Yura, T. and Mori, K. (1997) Endoplasmic reticulum stress-induced mRNA splicing permits synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response. Mol. Biol. Cell 8, 1845–1862.

[18] Mori, K., Sant, A., Kohno, K., Normington, K., Gething, M.J., et al. (1992) A 22 bp *cis*-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins. EMBO J. 11, 2583–2593.

[19] Matsumoto, T.K., Ellsmore, A.J., Cessna, S.G., Low, P.S., Pardo, J.M., et al. (2002) An osmotically induced cytosolic Ca^{2+} transient activates calcineurin signaling to mediate ion homeostasis and salt tolerance of *Saccharomyces cerevisiae*. J. Biol. Chem. 277, 33075–33080.

[20] Viladevall, L., Serrano, R., Ruiz, A., Domenech, G., Giraldo, J., et al. (2004) Characterization of the calcium-mediated response to alkaline stress in *Saccharomyces cerevisiae*. J. Biol. Chem. 279, 43614–43624.

[21] Popa, C.V., Dumitru, I., Ruta, L.L., Danet, A.F. and Farcasanu, I.C. (2010) Exogenous oxidative stress induces Ca^{2+} release in the yeast *Saccharomyces cerevisiae*. FEBS J. 277, 4027–4038.

[22] Cyert, M. (2003) Calcineurin signaling in *Saccharomyces cerevisiae*: how yeast go crazy in response to stress. Biochem. Biophys. Res. Commun. 311, 1143–1150.

[23] Cunningham, K.W. (2011) Acidic calcium stores of *Saccharomyces cerevisiae*. Cell Calcium 50, 129–138.

[24] Stathopoulos, A.M. and Cyert, M.S. (1997) Calcineurin acts through the CRZ1/TCN1-encoded transcription factor to regulate gene expression in yeast. Genes Dev. 11, 3432–3444.

[25] Yoshimoto, H., Saltsman, K., Gasch, A., Li, H., Ogawa, N., et al. (2002) Genomewide analysis of gene expression regulated by the calcineurin/Crz1p signaling pathway in *Saccharomyces cerevisiae*. J. Biol. Chem. 277, 31079–31088.

[26] Stathopoulos-Gerontides, A., Guo, J. and Cyert, M. (1999) Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev. 13, 798–803.

[27] Cebulski, J., Malouin, J., Pinches, N., Cascio, V. and Austriaco, N. (2011) Yeast Bax inhibitor, Bxi1p, is an ER-localized protein that links the unfolded protein response and programmed cell death in *Saccharomyces cerevisiae*. PLoS One 6, e20882.

[28] Bonilla, M., Nastase, K.K. and Cunningham, K.W. (2002) Essential role of calcineurin in response to endoplasmic reticulum stress. EMBO J. 21, 2343–2353.

[29] Bodvard, K., Jörhov, A., Blomberg, A., Molin, M. and Käll, M. (2013) The yeast transcription factor Crz1 is activated by light in a Ca^{2+}/calcineurin-dependent and PKA-independent manner. PLoS One 8, 1–13.

[30] Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.

[31] Sherman, F., Fink, G.R. and Hicks, J.B. (1986) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

[32] Schiestl, R. and Gietz, R.D. (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acid as carrier. Curr. Genet. 16, 339–346.

[33] Amberg, D.C., Burke, D.J. and Strathern, J.N. (2005) Methods in Yeast Genetics, A Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA;. pp. 135–139.

[34] Promlek, T., Ishiwata-Kimata, Y., Shido, M., Sakuramoto, M., Kohno, K., et al. (2011) Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Mol. Biol. Cell 22, 3520–3532.

[35] Prakash, S. and Prakash, L. (2000) Nucleotide excision repair in yeast. Mutat. Res. 451, 13–24.

[36] Prakash, S., Sung, P. and Prakash, L. (1993) DNA repair genes and proteins of *Saccharomyces cerevisiae*. Annu. Rev. Genet. 27, 33–70.

[37] Grier, D.G. (2003) A revolution in optical manipulation. Nature 424, 810–816.

[38] Kuo, S.C. (2001) Using optics to measure biological forces and mechanics. Traffic 2, 757–763.

[39] Difato, F., Pinato, G. and Cojoc, D. (2013) Cell signaling experiments driven by optical manipulation. Int. J. Mol. Sci. 14, 8963–8984.