Transfection of pseudouridine-modified mRNA encoding CPD-photolyase leads to repair of DNA damage in human keratinocytes: a new approach with future therapeutic potential

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Abbreviations: NHEK – Normal Human Epidermal Keratinocyte; UVB – Ultraviolet B; CPD – Cyclobutane Pyrimidine Dimer; Ψ – Pseudouridine-modified; CPD-PL – Cyclobutane Pyrimidine Dimer-specific photolyase; eGFP – enhanced Green Fluorescent Protein; ELISA – Enzyme Linked Immunosorbent Assay; IL-6 – Interleukin-6; SDHA – Succinate Dehydrogenase Complex, Subunit A; PGK1 – Phosphoglycerate Kinase 1

Abstract

UVB irradiation induces harmful photochemical reactions, including formation of cyclobutane pyrimidine dimers (CPDs) in DNA. Accumulation of unrepaired CPD lesions causes inflammation, premature ageing and skin cancer. Photolyases are DNA repair enzymes that can rapidly restore DNA integrity in a light-dependent process called photoreactivation, but these enzymes are absent in humans. Here, we present a novel mRNA-based gene therapy method that directs synthesis of a marsupial, *Potorous tridactylus*, CPD-photolyase in cultured human keratinocytes. Pseudouridine was incorporated during *in vitro* transcription to make the mRNA non-immunogenic and highly translatable. Keratinocytes transfected with lipofectamine-complexed mRNA expressed photolyase in the nuclei for at least 2 days. Exposing photolyase mRNA-transfected cells to UVB irradiation resulted in significantly less CPD in those cells that were also treated with photoreactivating light, which is required for photolyase activity. The functional photolyase also diminished other UVB-mediated effects, including induction of IL-6 and inhibition of cell proliferation. These results demonstrate that pseudouridine-containing photolyase mRNA is a powerful tool to repair UVB-induced DNA lesions. The pseudouridine-modified mRNA approach has a strong potential to discern cellular effects of CPD in UV-related cell biological studies. The mRNA-based transient expression of proteins offers a number of opportunities for future application in medicine.

Key words: pseudouridine-modified mRNA – mRNA therapy – photolyase – human keratinocyte – UVB – cyclobutane pyrimidine dimers

1. Introduction

Ultraviolet (UV) radiation, especially UVB (290-320 nm), is a common environmental carcinogen that induces harmful photochemical reactions in the skin [1]. UVB photons absorbed by the DNA cause a variety of photoproducts, including cyclobutane pyrimidine dimers (CPD), which is the most frequent and deleterious photolesion. It forms in double-stranded DNA when two adjacent pyrimidine bases in one strand fuse together [2]. Accumulated CPDs induce apoptosis, inflammation, immune suppression, and mutations that may lead to melanoma and other types of skin cancer [3-6]. Timely repair of damaged DNA is critical to prevent these adverse effects. In humans and rodents, photoproducts are removed by nucleotide excision repair (NER), a versatile repair system for removal of bulky DNA lesions. Genetic defects in NER result in photosensitive disorders, such as xeroderma pigmentosum and Cockayne's syndrome, in which patients have UV sensitivity and display skin cancer proneness and/or accelerated aging [7, 8]. NER excises CPDs at a very low rate, requiring hours to days to restore DNA integrity [9-12]. Interestingly, in most organisms from bacteria to marsupials, CPDs are rapidly removed by photolyases that specifically recognize and split these dimers into monomers using energy from visible light [13-15]. Due to an evolutionary loss, photolyases are absent in placental mammals, including humans, which therefore solely rely on NER for removal of photolesions [16-19].

Studies have demonstrated that CPD photolyases from phylogenetically diverse organisms, such as cyanobacteria (*Anacystis nidulans*) [20], yeast [21] or marsupials [22], can function in mammalian cells. Liposome-encapsulated CPD photolyase enzyme from *Anacystis nidulans* has been shown to protect cultured mammalian cells and human skin from the effects of UVB [20, 23-26]. Superior resistance to UVB-induced sunburn, immune suppression and carcinogenesis were demonstrated for transgenic mice ubiquitously expressing CPD photolyase of the rat kangaroo (*Potorous tridactylus*) [22, 27-29]. Mammalian cell lines

stably expressing marsupial CPD photolyases also demonstrated reduced apoptosis and mutation frequencies when exposed to UVB radiation [30-33]. To study sunlight-induced DNA damage and their repair in primary cells, transient adenovirus-mediated CPD photolyase expression was used [34, 35].

Gene therapy for the replacement of defective genes or for the expression of therapeutic proteins has made great progress in the last decade. After exploring numerous systems for expressing the encoded proteins [36], *in vitro* transcribed mRNA seems to be the most suitable tool for transient protein expression [37]. It has many features that make mRNA-mediated gene transfer especially valuable for functional characterization of encoded protein. The transfected mRNA is translated with high efficiency in any cell, including primary and non-dividing mammalian cells [37]. Importantly, when mRNA is delivered to the cell, only the encoded protein of interest is generated, unlike other vectors, such as plasmids that contain sequences for additional proteins, or viral vectors that not only code for but also carry viral proteins into the cell. In the last several years, mRNA-mediated transfection technology has improved tremendously [38]. It is now well documented that incorporation of pseudouridine (Ψ), a naturally-occurring modified nucleoside, into mRNA makes it less immunogenic by avoiding the activation of RNA sensors [39-41]. *In vitro*-transcribed mRNA containing pseudouridine (Ψ-mRNA) is also translated more efficiently than mRNA containing unmodified nucleosides [42]. We have demonstrated that the presence of Ψ nucleosides improved the stability of RNA [43] and diminished both activation of RNA-dependent protein kinase (PKR) and inhibition of translation [44]. A newly established preparative HPLC purification procedure was critical to obtain Ψ-mRNA that is free of aberrant transcription products, resulting in superior translational potential and no immunogenicity [45, 46].

Here, we present a novel mRNA-based gene therapy method that directs functional

photolyase synthesis in human keratinocytes. Our study demonstrates that delivery of HPLC-purified, Ψ-mRNA encoding CPD-photolyase into keratinocytes leads to the rapid repair of UVB-induced CPDs and suggest that such mRNA has therapeutic potential to repair damage caused by exposure to the sun and other sources of UV radiation.

2. Material and methods

2.1 RNA synthesis

A codon-optimized photolyase gene from *Potorous tridactylus* (rat kangaroo) containing GC-rich codons for superior translation was synthesized by Entelechon (Bad Abbach, Germany). The optimization increased the GC-content of the photolyase coding sequence (Accession: D26020) from 51.8% to 65.0%. Messenger RNAs encoding CPD-photolyase (CPD-PL Ψ-mRNA) and enhanced green fluorescent protein (eGFP Ψ-mRNA) were transcribed as previously described [42] from linearized plasmids (pTEV-CPD-PL-A101 and pTEVeGFP-A101), using the Megascript T7 RNA polymerase kit (Ambion, Austin, TX) in which UTP was replaced with pseudouridine triphosphate (TriLink, San Diego, CA). Subsequently, the mRNA was HPLC purified as described [45], and provided with a 5' cap using capping enzyme and 2'-O-methyltransferase according to the manufacturer (CellScript, Madison, WI). The RNA was transcribed to contain an encoded 101-nt long 3' poly(A) tail, which was extended with ~ 300 nucleotides using poly(A) polymerase (USB, Cleveland OH). RNA samples were analyzed by agarose gel electrophoresis for quality assurance. The mRNAs were shown to be free of dsRNA contaminants using dsRNA-specific J2 mAb (English & Scientific Consulting, Budapest, Hungary) in a dot-blot assay [45]. The mRNAs were stored in siliconized tubes at -20°C.

2.2 Cell cultures

The human keratinocyte cell line HaCaT was obtained from the ATCC and grown in high glucose DMEM (PAA, Traun, Austria) supplemented with 2 mM L-glutamine (PAA), 10%

heat-inactivated fetal bovine serum (Lonza, Verviers, Belgium) and 0.5% antibiotic/antimycotic solution (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a 5% CO_2 atmosphere. Normal human epidermal keratinocytes (NHEK) were isolated from healthy adult skin derived from plastic surgery and cultured in EpiLife serum-free, complete keratinocyte growth medium (Life Technologies, Carlsblad, CA, USA). Ethics approval was received from the National Scientific and Research Ethics Commission. HaCaT cells and second passage NHEK were used at 70-80% confluency in each experiment.

2.3 Transient transfection and treatments

HaCaT cells and NHEK were seeded into 96-well plates at a density of 2×10^4 cells per well one day prior to transfection. Aliquots of RNA samples (0.25 µg) were complexed with 0.8 µl Lipofectamine LTX-PLUS (Life Technologies) in a final volume of 100 µl of EpiLife and the complexed RNA was added to each well. After one hour the lipofectamine-RNA complex was replaced with 100 µl culture medium. At 20 h post transfection, cells covered with 50 µl Dulbecco's Phosphate Buffer Saline (DPBS) (Life Technologies) were subjected to 20 mJ/cm^2 UVB using two TL-20W/12 bulbs (Philips). Immediately after UVB treatment, cells were either exposed to visible light using a F18W Daylight fluorescent bulb (Sylvania,) at a distance of 16 cm and using a 4 mm thick glass filter as a shield or kept in the dark for one hour. Cells were further cultured in complete medium for the indicated times. Cell viability was determined by the EZ4U assay (Biomedica Gruppe, Vienna, Austria), according to the manufacturer.

2.4 Western blot analysis

Cells were lysed in RIPA buffer (R0278, Sigma-Aldrich) containing protease inhibitor cocktail (Sigma-Aldrich). Proteins were separated on 10% polyacrylamide gels and transferred to nitrocellulose membranes. The membrane was blocked in 5% non-fat dry milk for 1 h and incubated overnight at 4°C with primary CPD-photolyase antibody [27] diluted

(1:500) in PBGTNa made of PBS containing 0.5% bovine serum albumin (BSA, Amresco, Solon, OH, USA), 0.05% gelatin (Sigma-Aldrich), 0.05% Tween-20 (Amresco) and 300 mM NaCl (VWR, San Diego, CA, USA). HRP-conjugated anti-rabbit IgG was used as a secondary antibody (1:3000; 1 h, at room temperature, BioRad, Berkeley, CA, USA). Proteins were visualized with the Pierce ECL Plus Western blotting detection system (Thermo Fisher Scientific, Rockford, IL, USA).

2.5 Fluorescence microscopy

Expression of eGFP in HaCaT cells was documented using an epifluorescent Zeiss Axiovert 100 microscope mounted with a Zeiss AxioCam MRc 5 digital camera using AxioVision 4.8 and 0.4-second exposure time.

In the expression studies, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) and permeabilized with 1% Triton X-100 (Amresco). Before immunolabeling, cells were blocked in 20% FBS and then probed overnight at 4°C with CPD-photolyase-specific antibody diluted (1:100) in PBGTNa. FITC-conjugated secondary antibody (1:2000; anti-rabbit IgG; Vectashield, Irvine, CA, USA) was added for 2 h at room temperature.

For the analysis of CPDs, experiments were carried out as described above, except, before blocking, cellular DNA was denatured in 2 M HCl (VWR) at room temperature for 30 min and then probed with primary anti-CPD antibody (TDM-2, Cosmo Bio, Tokyo, Japan) diluted (1:1500) in PBS containing 5% FBS for 30 min. Alexa Fluor 568-conjugated anti-mouse IgG was used as secondary antibody (1:2000; Life Technologies). In both studies, nuclei were stained with DAPI (Vectashield) and cells were analyzed by fluorescence microscopy.

2.6 Enzyme-linked immunosorbent assay (ELISA)

Genomic DNA was extracted by the QIAamp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer. Flat-bottom 96-well plate were precoated with 0.003% protamine sulfate (50 µl/well, Sigma-Aldrich) and incubated overnight with denatured DNA

(15 ng/well) at 37°C. The plates were washed with PBS containing 0.05% Tween-20 and incubated with 2% FBS at 37°C for 30 min to prevent non-specific binding of the antibody and washed again. Anti-CPD monoclonal antibody (TDM2) diluted in PBS (1:1000) was used as primary antibody at 37°C for 30 min. The plates were washed and then incubated with HRP conjugated anti-mouse IgG secondary antibody (1:3000, BioRad) at 37°C for 30 min. After washing, samples were equilibrated with citrate-phosphate buffer (pH 5.0; 150 µl/well), then substrate solution was added to each well (100 µl/well) containing 0.04% o-phenylenediamine (Sigma-Aldrich) and 0.0063% H_2O_2 in citrate-phosphate buffer. Samples were incubated at 37°C for 15 min, and 2 M sulfuric acid (VWR) was added (50 µl/well) to stop the reaction and absorbance was measured at 492 nm using an Anthos 2020 microplate reader (Biochrom Ltd; Cambridge; UK).

The concentration of IL-6 in the supernatants was determined by the human IL-6 Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer.

2.7 RNA extraction and quantitative RT-PCR

Total RNA was isolated using TRI reagent (MRC, Cincinnati, OH, USA) followed by DNase I treatment (Fermentas, St. Leon-Rot, Germany). To quantitate IL-6, SDHA, and PGK1 mRNAs, reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies), according to the manufacturer. Quantitative PCR was performed on the ABI 7900 HT Sequence Detection System (Life Technologies) using a reaction mixture containing 1x PCR buffer, 3 mM $MgCl_2$ and 0.1 mM dNTP mix (Fermentas), 1x TaqMan Gene Expression assay (Life Technologies), 1x ROX reference dye (Life Technologies), 0.0125 U Taq DNA polymerase (Fermentas) and 12.5 ng cDNA template in a total volume of 10 µl. qPCR was performed in 384-well optical plates (Life Technologies) and the PCR program used for amplification was: 94°C 1 min, followed by 40 cycles of 94°C for 12 sec and 60°C for 45 sec. The following TaqMan Gene Expression

assays were used: IL-6 (Hs00985639_m1), SDHA (Hs00188166_m1) and PGK1 (Hs00943178_g1), the sequences are proprietary and not released by the company. SDHA and PGK1 mRNA levels were used for normalization [47].

2.8 Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Values were compared between photoreactivated and non-photoreactivated samples using two-tailed, paired *t*-test. A *p* value of less than 0.05 was considered as statistically significant.

3. Results

3.1 Synthesis of CPD-specific photolyase in human keratinocytes transfected with the encoding pseudouridine-modified mRNA

To test whether pseudouridine-modified mRNA (Ψ-mRNA) is translated in human keratinocytes, eGFP-encoding Ψ-mRNA was complexed with lipofectamine LTX and delivered to the cells. The expression of eGFP was monitored by fluorescence microscopy at different time points after transfection. eGFP was detectable as early as 1 h, peaked between 6 and 20 h, and its level gradually declined, but remained detectable even at 48 h after mRNA transfection (Fig. 1a). This eGFP mRNA served as a control for photolyase experiments. Using western blotting, high levels of CPD-photolyase synthesized from the transfected CPD-PL Ψ-mRNA could be detected in both HaCaT cells and normal human epidermal keratinocytes (NHEK) at 20 h post transfection (Fig. 1b and c). The photolyase migrated with the expected size of 62 kDa (Fig. 1c) and localized in the nuclei of keratinocytes, as demonstrated by immunofluorescence imaging (Fig. 1d).

3.2 Enhanced repair of UVB-induced CPDs in keratinocytes is mediated by CPD-photolyase translated from the encoding Ψ-mRNA

To investigate whether CPD-photolyase translated from the encoding Ψ-mRNA is functional and capable of cleaving CPDs, HaCaT cells were first transfected with lipofectamine-complexed CPD-PL Ψ-mRNA. Twenty hours later, the transfected cells were exposed to 20 mJ/cm^2 UVB. Immediately thereafter, cells were either exposed to photoreactivating light, or left in the dark for 1 hour. Using CPD-specific antibodies, efficient repair of these photolesions could be detected in CPD-PL Ψ-mRNA-transfected cells that were subjected to photoreactivating light, while those deprived from this energy source or transfected with eGFP Ψ-mRNA contained high levels of CPDs, when it was analyzed immediately after the photoreactivation or incubation in the dark (Fig. 2).

To quantitate the amount of CPDs removed from the genomic DNA of the CPD-PL Ψ-mRNA-transfected keratinocytes immediately after exposure to visible light, a CPD-specific ELISA was used. We determined that photoreactivation of UVB-irradiated, CPD-PL Ψ-mRNA-transfected cells eliminated more than 60% of the CPDs, as compared to cells kept in the dark or transfected with control eGFP Ψ-mRNA (Fig. 3, left panel).

UVB treatment of keratinocytes results in the induction of apoptosis and reduction in cell proliferation. Cell viability, determined 24 h after UVB irradiation, demonstrated that photoreactivation significantly increased cell survival of CPD-PL Ψ-mRNA transfected keratinocytes compared to those that were kept in the dark or transfected with eGFP Ψ-mRNA (Fig. 3, right panel).

3.3 Photorepair of CPDs in keratinocytes transfected with CPD-photolyase Ψ-mRNA reduces UVB-induced IL-6 expression

To determine whether repair of CPDs in photolyase mRNA-transfected cells can ameliorate UVB-triggered cytokine induction, we measured the expression of IL-6 mRNA and protein using qRT-PCR and ELISA. We found that the repair of CPDs in CPD-PL Ψ-mRNA transfected keratinocytes significantly decreased the levels of IL-6 mRNA measured 24 h

after UVB irradiation (Fig. 4). Accordingly, the levels of IL-6 protein in the supernatants of photolyase mRNA-transfected keratinocytes were also significantly reduced at 6 and 24 h (Fig. 4).

4. Discussion

Intracellular delivery of therapeutic proteins has been performed using many different viral and non-viral techniques. Recent advancements in the use of mRNA for expressing the encoded proteins in mammalian cells made the present study possible. Here, we demonstrate that HPLC-purified, lipofectamine-complexed mRNAs containing pseudouridine are efficiently taken up and translated by primary human keratinocytes and an established keratinocyte cell line, with greater than 95% of the cells expressing the protein encoded by the mRNA (eGFP or photolyase). The CPD-photolyase of *Potorous tridactylus* encoded by the mRNA is efficiently translated into a functional protein that correctly localized to the nucleus. The CPD-photolyase mediated rapid repair of UVB-induced cyclobutane pyrimidine dimers. This led to a significant survival advantage and reduction in IL-6 mRNA and protein secretion.

This is the first report of transient expression of functional CPD-photolyase in human cells from transfected mRNA. Several studies have reported successful protection from UVB-induced damage using topical application of liposomes-encapsulating recombinant CPD-photolyase protein, however, direct evidence for nuclear localization of this cyanobacteria-derived protein has not been provided [20, 23, 26, 48-51]. Here, we found that nucleoside-modified mRNA encoding CPD-photolyase correctly located to the nucleus and removed more than 60% of CPDs within the first hour of photoreactivation. In placental mammals that lack a functional CPD-photolyase, DNA repair of CPDs is performed by the NER complex that in mammals contains 9 major proteins and over 20 associated genes. In UVB-irradiated primary keratinocytes, NER-mediated repair removed only 30% of the CPDs in 24 hours [52].

If a cell containing CPD lesions divides before the repair can be completed, an alteration to the DNA, i.e. mutation, can occur in the daughter cell and all of its progeny [6]. Such mutations are the initial steps in the development of sun-induced skin cancers. As shown for *Potorous tridactylus* CPD photolyase-expressing transgenic mice [27-29], very rapid clearance of CPDs by photolyase produced from the transfected mRNA should result in a large reduction of DNA mutations and other CPD-driven deleterious effects of UVB.

It is well established that UVB irradiation induces cell cycle arrest and inhibits cell proliferation, which is partly mediated by CPD photolesions [53, 54]. Removal of CPDs after photoreactivation of keratinocytes transfected with photolyase mRNA increased survival, confirming that CPDs are major contributors to cell death after UVB irradiation [22, 31, 55, 56] and demonstrating that CPD-photolyase mRNA transfection is an effective tool for photorepair.

UVB irradiation also induces erythema in the skin [57] and the involvement of the proinflammatory cytokine IL-6 has been considered [58, 59]. Consistent with these observations, we demonstrate that enhanced repair of CPDs in photolyase mRNA-transfected keratinocytes reduced UVB-induced IL-6 expression when cells were subjected to photoreactivation compared to those kept in dark. CPD-photolyase mediated repair of UVB-induced damage did not return IL-6 levels to non-irradiated levels likely due to the presence of modified host RNA acting through TLR3 [60] and other UVB mediated effects, including the induction of reactive oxygen species, protein–DNA crosslinking, oxidative base damage (e.g. 8-oxo-7,8-dihydroxyguanine), single-strand breaks and a 6-4 photoproduct (6-4PP). Thus, these results demonstrate that CPDs play an important but not exclusive role in triggering IL-6 release from keratinocytes, and transfection of Ψ-mRNA encoding CPD-photolyase reduces production of IL-6 by UVB irradiated keratinocytes and supports their survival.

In summary, we demonstrate that *in vitro*-synthesized, pseudouridine-containing mRNA encoding *Potourus* photolyase is a powerful tool to rapidly repair UVB-induced CPD lesions in human keratinocytes. This novel mRNA-based gene therapy method efficiently directs functional protein synthesis providing number of opportunities for future dermatological applications if the nucleic acid delivery to intact epidermis has been resolved. Concerning photolyase, thus it could be used in UV-sensitive diseases [7, 8] and in the prevention of skin cancer and tanning, which requires CPD formation to increase the production of melanin [61]. Ψ-mRNA encoding CPD-photolyase is also a new model system that uses primary human keratinocytes to investigate the cellular and molecular effects of UVB irradiation with the ability to distinguish between CPD-dependent and CPD-independent events and the potential to deliver and study any protein of interest.

Conflict of interests

Katalin Karikó and Drew Weissman hold a patent for the use of nucleoside modified mRNA as a therapeutic protein delivery platform. They have also formed a small biotech company, RNARx, that receives funding from the National Institutes of Health (R42HL87688) to explore the use of nucleoside-modified mRNA for gene therapy.

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Figure captions

Fig. 1. Translation of CPD-photolyase-encoding, Ψ-modified mRNA in keratinocytes. HaCaT cells or normal human epidermal keratinocytes (NHEK) were transfected with lipofectamine-complexed Ψ-mRNA encoding eGFP or CPD-photolyase. Translated eGFP was detected at the indicated times post transfection in HaCaT cells transfected with eGFP Ψ-mRNA by fluorescence microscopy (panel a) (original magnification ×20). Cells were lysed

at the indicated times (panel b) or at 20 h post transfection (panel c) and analyzed by western blot. Arrows mark positions of the 62 kDa CPD-photolyase and the loading reference, β-actin. CPD-photolyase protein was observed in NHEK transfected with CPD-PL Ψ-mRNA using immunofluorescent detection (panel d). Nuclei were visualized by DAPI staining (original magnification ×40).

Fig. 2. Photorepair of CPDs in HaCaT cells transfected with CPD-PL Ψ-mRNA. HaCaT cells were transfected with lipofectamine-complexed Ψ-mRNA encoding CPD-photolyase or eGFP, or left untransfected (none). Twenty hours later, cells were subjected to 20 mJ/cm^2 UVB and immediately exposed to photoreactivating light or left in the dark for 1 h. Immediately after photoreactivation or incubation in the dark, CPDs were detected by immunofluorescent labeling with CPD-specific antibody and Alexa-conjugated secondary antibody (red). Nuclei were visualized by DAPI staining. Images are representatives of three independently performed experiments (original magnification ×20).

Fig. 3. Reduced levels of CPDs and improved cell viability in photolyase mRNA-transfected keratinocytes exposed to UVB and photoreactivating light. HaCaT cells and NHEK were irradiated with a physiological dose of UVB (20 mJ/cm^2) 20 h after delivery of Ψ-mRNAs and immediately exposed to photoreactivating light (+Phr) or left in the dark (-Phr) for 1 h. After photoreactivation or incubation in the dark, the amount of CPDs in the DNA of keratinocytes transfected with Ψ-mRNA encoding CPD-PL or eGFP was determined by ELISA (left panel). The OD values obtained were in the linear range of the ELISA. Cell viability was determined by EZ4U assay at 24 h after UVB irradiation (right panel). The values were calculated relative to those obtained with cells that were not UVB irradiated. Significance was assessed by paired *t*-test, asterisk, $p<0.05$. Error bars represent the standard error of the mean. The results are means of three independent experiments.

Fig. 4. Photorepair of CPDs reduces UVB-induced IL-6 expression in HaCaT cells. Cells were transfected with lipofectamine-complexed CPD-PL Ψ-mRNA, 20 h later irradiated with 20 mJ/cm^2 UVB and then immediately exposed to photoreactivating light (+Phr) or left in the dark (-Phr) for 1 h. Total RNA was isolated and supernatants were collected 6 and 24 h after UVB irradiation. Levels of IL-6 mRNA were determined by qRT-PCR. Values measured in UVB irradiated –Phr or +Phr cells were normalized to those measured in the Ψ-mRNA-transfected, non-UVB irradiated control cells. IL-6 was quantitated in the supernatant by ELISA. Asterisks indicate significant differences (paired t-test; $p<0.05$) between photoreactivated (+Phr) and non-photoreactivated (-Phr) samples. Error bars represent the standard error of the mean. Three independent experiments were performed, each in triplicate.