

Light-induced Resistance of the Keratin Network to the Filament-disrupting Tyrosine Phosphatase Inhibitor Orthovanadate

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Epidermal keratinocytes respond to low-dose light irradiation by inducing signaling cascades that lead to long-term effects on gene transcription thereby protecting cells against damage. In contrast, little is known about immediate light-induced alterations of structural proteins. We have made the intriguing observation that light produces fundamental changes in the properties of the keratin filament system of cultured epidermoid A-431 cells. A short light exposure (1–10 min) causes the keratin cytoskeleton to become immediately resistant to the tyrosine phosphatase inhibitor orthovanadate, which otherwise disrupts the keratin filament network completely in just a few minutes. This protective effect is inducible throughout the entire visible spectrum and is elicited by normal room light (<200 Lux). Exposure of cells to monochromatic light

of various wavelengths is therefore equally effective. In addition, the acquisition of orthovanadate resistance has been directly monitored in living cells; a partially disrupted keratin cytoskeleton recovers to a completely filamentous network in half an hour. Finally, the protective light effect is largely reversed in 2 h and can be mimicked by preincubation with the p38 kinase inhibitor SB203580. In contrast, the mitogen-activated protein kinase inhibitor PD98059 and epidermal growth factor inhibit orthovanadate action to a lesser extent. Taken together, these observations suggest a stabilizing function of light on the keratin filament network; this may be of relevance to the treatment of skin diseases with reduced keratin stability. *Key words:* cytokeratin/intermediate filament/phosphorylation/ultraviolet light/vanadate. *J Invest Dermatol* 120:198–203, 2003

Keratins are hallmark features of epithelial differentiation and represent the majority of cellular protein in suprabasal epidermal keratinocytes (Moll, 1998; Coulombe and Omary, 2002). They are part of the 8–12 nm epithelial intermediate filament (IF) system that provides, together with the interacting desmosomal adhesion sites, a stabilizing transcellular network (Coulombe and Omary, 2002). The importance of keratin integrity for the maintenance of this scaffolding and epithelial resilience is evidenced by a number of hereditary skin diseases of the epidermolysis bullosa-type and epidermolytic hyperkeratosis-type, which are caused by point mutations in keratin genes and lead to blister formation (Fuchs and Weber, 1994; Korge and Krieg, 1996; Irvine and McLean, 1999).

Keratins are obligatory heteropolymers contributing, in stoichiometrically equal amounts of type I and type II polypeptides, to the formation of keratin filaments (KF) (Fuchs and Weber, 1994; Herrmann and Aebi, 2000; Coulombe and Omary, 2002). Although KF form spontaneously and rapidly *in vitro* (Herrmann and Aebi, 2000), the much slower keratin network formation and organization *in vivo* must be subject to

extensive regulation in order to allow fine tuning according to specific cellular requirements. Various levels of regulation have been recognized. First, the approximately 50 keratin polypeptides, which differ with respect to their assembly kinetics, their influence on cell proliferation, and their contribution to the cellular stress response, are expressed in a differentiation-dependent manner (Moll, 1998; Hesse *et al*, 2001; Coulombe and Omary, 2002). Secondly, association of KF with IF-associated proteins (IFAP) confers specific properties, thereby affecting, for example, the equilibrium between the filamentous *vs* the nonfilamentous state or the re-assembly potential (Liao and Omary, 1996; Coulombe and Omary, 2002; Strnad *et al*, 2002). Thirdly, probably the most important and dynamic means for regulating keratin organization are post-translational modifications, especially phosphorylation (Omary *et al*, 1998; Coulombe and Omary, 2002). Thus, hyperphosphorylation and increased keratin solubility occur concomitantly, e.g., during mitosis and meiosis (cf. Omary *et al*, 1998; Coulombe and Omary, 2002). Furthermore, drug-induced hyperphosphorylation results in KF network reorganization (e.g., Baribault *et al*, 1989; Kasahara *et al*, 1993; Baricault *et al*, 1994; Toivola *et al*, 1997; Stumppner *et al*, 2000; Strnad *et al*, 2001). Most notably, we have recently observed that treatment of cells with the tyrosine phosphatase inhibitor orthovanadate leads to the rapid and reversible disassembly of the KF network and is accompanied by the transient formation of granular keratin aggregates (Strnad *et al*, 2002). We have therefore previously suggested that the use of orthovanadate could serve as a relevant paradigm for the investigation of temporo-spatially restricted modes of KF network reorganization as it occurs during the normal life cycle of each epithelial cell (Strnad *et al*, 2002).

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A movie is accessible at [http://www.uni-mainz.de/FB/Medizin/Anatomic/Leube/after acceptance of the manuscript](http://www.uni-mainz.de/FB/Medizin/Anatomic/Leube/after%20acceptance%20of%20the%20manuscript).

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Abbreviations: IF, intermediate filament; IFAP, intermediate filament-associated protein; KF, keratin filament.

Epidermal keratinocytes are directly exposed to environmental light, which is known to elicit various cellular responses resulting in the immediate induction of signaling cascades and long-term alterations of gene transcription and cellular differentiation (Bender *et al.*, 1997; Herrlich *et al.*, 1999). Therefore, we wanted to know whether and in which way light modulates the plasticity of the KF system. So far, studies have mostly concentrated on long-term alterations of the IF cytoskeleton as signified by changes in keratin expression patterns in response to ultraviolet (UV) light (reviewed in Moll *et al.*, 1994). The spectrum of keratin expression has been shown to shift from the differentiated phenotype to a reactive, hyperproliferative phenotype as evidenced by the induction of K17 in suprabasal cells, the increase of K6 and K16 in all epidermal cell layers, the low level production of K18 in cultured keratinocytes, and the repression of K1/10 (Kartasova *et al.*, 1987; Moll *et al.*, 1994; Bayerl *et al.*, 1995; Sesto *et al.*, 2002). Little is known, however, about the direct effects of light on keratin network stability and dynamics (for long-term effects see, e.g., Zamansky *et al.*, 1992). As a first indication of such a close relationship, we now show that light leads to the rapid acquisition of resistance to orthovanadate-induced KF network disassembly, thereby suggesting a way in which sunlight-exposed epidermal keratinocytes generate a particularly stable cytoskeleton.

MATERIALS AND METHODS

Cell culture Human vulva carcinoma-derived A-431 cells (line E₃; Leube *et al.*, 1988) and cDNA-transfected subclones thereof expressing fluorescent K13 chimera HK13-EGFP have been previously described in detail (Windoffer and Leube, 1999, 2001; Strnad *et al.*, 2001). These cell lines were propagated in 10% (v/v) fetal bovine serum (Invitrogen, Karlsruhe, Germany) in Dulbecco minimal Eagle's medium (PAA Laboratories, Cölbe, Germany) at 37°C with 5% CO₂. Cells were split at a 1 : 3 to 1 : 6 ratio twice weekly. For fluorescence microscopy, cells were seeded on 12 mm diameter glass coverslips and grown to near confluence. For time-lapse fluorescence microscopy, cells were cultivated on γ -irradiated Petri dishes with a glass bottom (MatTek Corporation, Ashland, MA).

Light exposure and orthovanadate incubation Hank's medium [containing Hank's salt solution, 25 mM HEPES, minimum essential medium nonessential amino acid solution, minimum essential medium amino acid solution, 100 U per ml penicillin, 100 μ g streptomycin per ml, 5% fetal bovine serum (all from Invitrogen)], 4.8 mM *N*-acetyl-L-cysteine (Sigma, St Louis, MO; pH 7.4) was used instead of Dulbecco minimal Eagle's medium for light exposure and orthovanadate treatment. Cells were kept continuously in the dark after splitting. In some irradiation experiments, cell cultures were exposed to normal room light [<200 Lux as determined by a digital luxmeter (Mavolux 5032B, Gossen Foto- und Lichtmeßtechnik GmbH, Nürnberg, Germany)] for various periods of time (1–10 min) after removal of the lid of the culture dish. In other experiments, monochromatic light of 390 nm, 440 nm, and 482 nm was produced from a Xenon short arc lamp (type UXL-SI50M0; Ushio Incorporation, Tokyo, Japan) in combination with a monochromator (TILL-Photonics, Gräfelfing, Germany) and was applied through optical fibers on to cells. The light intensity at all three wavelengths was less than 4 mW per cm² according to the data provided by the manufacturer. To examine the effect of infrared light, a darkroom lamp (type E27PF712E, Philips, Eindhoven, The Netherlands) was used that emitted light of >600 nm. A setup usually employed for the detection of ethidium bromide-stained DNA in agarose gels was used for short wavelength UV irradiation (illumination at 366 nm; type NU-72; Merck Eurolab, Frankfurt, Germany). In this case, Petri dishes were placed directly on the surface of the apparatus for up to 10 min. To minimize light exposure during handling, all procedures were carried out in the dark (less than 0.2 Lux).

Sodium orthovanadate was obtained from Aldrich Chemical Corporation (Milwaukee, WI) and was dissolved in distilled water immediately prior to use. In some experiments, cells were pretreated for 1 h either with epidermal growth factor (EGF) (5 ng per ml; Sigma), the mitogen-activated protein kinase (MAPK) inhibitor PD98059 (100 μ M; Sigma), or the p38 kinase inhibitor SB203580 (20 μ M; Sigma).

Fluorescence microscopy For fluorescence microscopy, cells were fixed with methanol (-20°C , 5 min) and acetone (-20°C , 15 s). Cells expressing

fluorescent keratins were immediately embedded in elvanol [solution of 1 g Mowiol 4–88 (Calbiochem, Frankfurt, Germany) in 4 ml distilled water and 2 ml glycerol] supplemented with Hoechst 33258 (Sigma) at 0.1 μ g per ml for DNA staining and were examined by epifluorescence microscopy (Axiophot, Zeiss, Jena, Germany). Pictures were recorded with a CCD camera (Hamamatsu 4742-95, Hamamatsu, Herrsching, Germany) and edited by means of Photoshop software (Adobe Photoshop, version 5.0). For indirect immunofluorescence microscopy, methanol-acetone-fixed cells were first incubated with either polyclonal broad-reactive keratin antibodies from guinea pig (Progen Biotechnics, Heidelberg, Germany), polyclonal antibodies from rabbit against K5 (kind gift of Drs Regina Reichelt and Thomas Magin, Department of Biochemistry, Bonn University, Germany; cf. Peters *et al.*, 2001), or monoclonal antibodies L2A1 reacting with K8/18 (generously provided by Drs Bishr Omary and Nam-On Ku, Stanford University, Palo Alto, CA; cf. Chou *et al.*, 1993). Subsequently Cy3-, or Cy2-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) were applied, as described previously (Strnad *et al.*, 2001).

For the statistical analysis of KF network breakdown, areas were selected at random on the basis of nuclear staining without prior knowledge of the keratin fluorescence. Subsequently, the keratin distribution was recorded, and the pattern was analyzed in 100 cells in each experiment (see below).

For time-lapse fluorescence microscopy, phenol-red-free Hank's medium was used and images were recorded by epifluorescence microscopy by inverse optics (Olympus, Hamburg, Germany) and an attached IMAGO slow scan CCD camera (TILL-Photonics). The microscope was placed in a closed chamber in which the temperature was kept constant at 37°C. The whole system was controlled by TILLvisION software (TILL-Photonics). Excitation with monochromatic light of 496 nm was accomplished with a monochromator. Images were imported into Image-Pro Plus 4.5 (Media Cybernetics) and converted into movies (QuickTime 5.0, Apple).

RESULTS

To examine the effect of light on the organization of the epithelial KF network, we used vulva carcinoma-derived A-431 cells, which synthesize abundant keratin polypeptides (cf. Leube *et al.*, 1988). These cells were exposed to visible light of defined wavelength (390 nm, 440 nm, 482 nm) for times up to 10 min at <4 mW per cm². As expected, no significant alterations in cellular morphology were noted, although minor retractions occurred occasionally. This indicated that doses of less than 24,000 J per m² at these wavelengths were not cytotoxic in contrast to the delayed cytotoxicity observed in A-431 cells subjected to 120–2400 J per m² UVB (Straface *et al.*, 1995). Furthermore, no changes of the KF network organization was detected after light exposure in indirect immunofluorescence analyses (not shown).

To determine whether more discrete changes were induced by our protocol, the recently established A-431 subline AK13-1, which stably expresses fluorescent K13 chimeras, was used for further analyses, as we had previously shown that these chimeric polypeptides were reliable reporters for the continuous monitoring of KF organization and dynamics under various conditions (Windoffer and Leube, 1999, 2001; Strnad *et al.*, 2001, 2002). As seen in wild-type A-431 cells, 1–10 min irradiation at 390 nm, 440 nm, and 482 nm did not significantly affect the overall distribution of KF in individual cells during or after light exposure. We also found no differences in the motility of KF in irradiated AK13-1 cells.

To determine whether light altered the properties of the KF system in some other way, we decided to combine light exposure with treatments that affected KF network organization and stability. As an ideal agent for this purpose, we chose the phosphotyrosine phosphatase inhibitor orthovanadate, which leads to rapid and reversible changes of the KF network (Strnad *et al.*, 2002). When AK13-1 cells were exposed to 20 mM orthovanadate for 10 min, the KF network was completely disassembled, and multiple granular aggregates were formed in the rounding cells (Fig 1B); however, a largely intact KF network remained when cells were subjected to monochromatic light (440 nm, <4 mW per cm²) for 3 min immediately prior to orthovanadate incubation (Fig 1C). Furthermore, cells did not round up and preserved their flat arrangement. Generally, the KF network

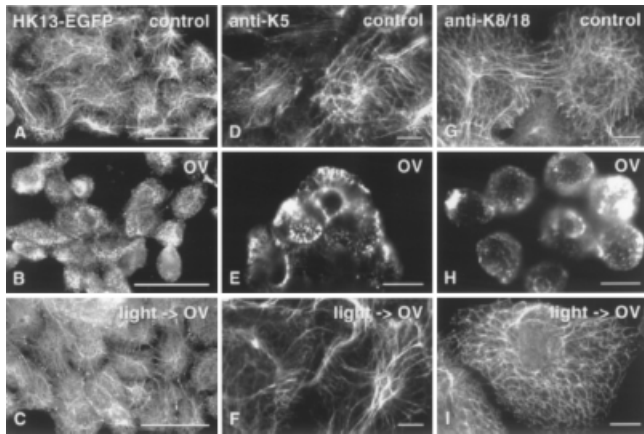


Figure 1. Exposure of epithelial cells to light prevents orthovanadate-induced disruption of the KF network. (A–C) The KF network of A-431 clone AK 13-1 is labeled by fluorescent keratin 13 chimera HK13-EGFP. (A) fluorescence microscopy detecting HK13-EGFP in untreated control cells; (B) fluorescence pattern of HK13-EGFP 10 min after addition of 20 mM orthovanadate; (C) fluorescence micrograph of AK13-1 cells that were irradiated (440 nm, <math><4\text{ mW per cm}^2</math>, 3 min) prior to incubation with 20 mM orthovanadate for 10 min (D–I) The immunofluorescence micrographs show the distribution of K5 (D–F) and K8/18 (G–I) in wild-type A-431 cells without any treatment (D,G), 10 min after addition of 20 mM orthovanadate (E,H), and after exposure to room light (<math><200\text{ Lux}</math>) for 5 min followed by treatment with 20 mM orthovanadate for 10 min (F,I). Note the similarity in reactivity to that shown in A–C for keratin 13 chimera HK13-EGFP. Scale bars: (A–C) 50 μm ; (D–I) 10 μm .

appeared to be finer after this treatment, a finding reminiscent of the reassembled and rather delicate KF network formed after transient orthovanadate treatment (Strnad *et al*, 2002). After light exposition, only a few keratin aggregates were seen in some scattered cells. Remarkably, the same protective effect was observed when AK13-1 cells were simply exposed to normal room light in Hank's medium for 3–5 min (<math><200\text{ Lux}</math>). Similar light-induced resistance of the KF system to orthovanadate was also noted in wild-type A-431 cells by indirect immunofluorescence (Fig 1D–I) thereby excluding the possibility that green fluorescent protein-induced fluorescence is the reason for the observed protection and/or that it is a specific property of the fluorescent keratin fusion protein. Furthermore, the protective effect of light was not restricted to specific keratins but affected the entire KF system of A-431 and AK13-1 cells (Fig 1 and data not shown).

To quantify the inhibitory effect of light on orthovanadate-induced KF disruption, morphometric analyses were performed. The results of a representative experiment are shown in Fig 2. They confirmed that incubation of AK13-1 cells with orthovanadate alone led to complete KF disruption and formation of granular aggregates in almost all cells. This granular phenotype was distinguished from a mixed morphotype that was also characterized by significant amounts of keratin aggregates but presented largely intact KF. The latter was only found in single cells. A granule-free KF network was not observed in any cell (filamentous morphotype). On the other hand, light treatment effectively prevented granule formation and KF disruption (lower panel in Fig 2). In the experiment shown, the majority of cells presented a normal-appearing KF network. In each instance, the mixed morphotype was more frequent than the granular morphotype; this might even have been an overestimate, as mitotic cells, which also exhibit a granular phenotype (cf. Windoffer and Leube, 1999, 2001), were included in the count.

In the next set of experiments, we tried to discover whether the induction of orthovanadate resistance was wavelength dependent. Wavelengths of 390 nm, 440 nm, and 482 nm were selected

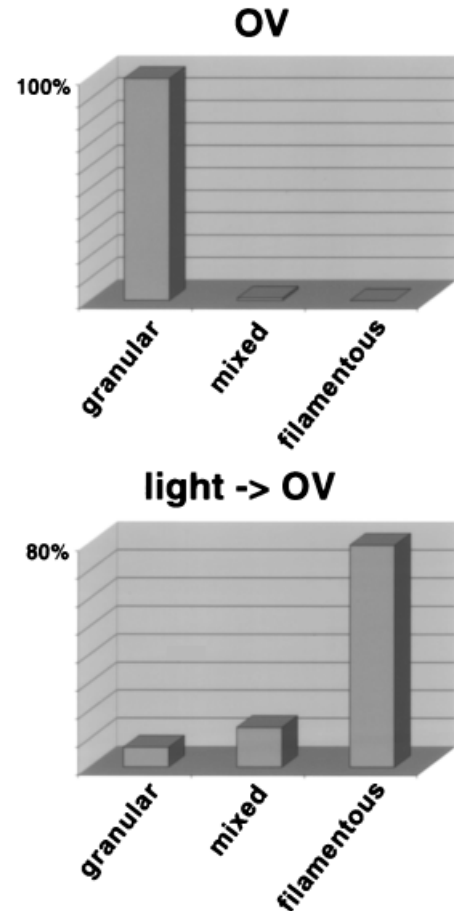


Figure 2. Quantification of the inhibitory effect of light on orthovanadate-induced KF network disruption and keratin aggregate formation in AK13-1 cells. The histograms show an experiment in which cells were either treated for 10 min with 20 mM orthovanadate only (top panel) or were first subjected to irradiation (440 nm, <math><4\text{ mW per cm}^2</math>, 5 min) prior to orthovanadate exposure (20 mM, 10 min; bottom panel). One hundred cells were selected at random, and the amount of keratin aggregate formation was assessed by visual inspection of the fluorescence pattern. Three categories were distinguished: predominant granular keratin fluorescence (granular), significant amount of keratin aggregates but largely intact KF (mixed), and normal-appearing extended KF network with almost no granules (filamentous).

on the basis of the comparable resulting light intensity of approximately 4 mW per cm^2 in our system. In each situation, a strong inhibition of KF network disruption was observed (Fig 3C–E), and no statistically relevant differences in the number of protected cells were noted. Additional experiments in the infrared and long wavelength UV range yielded similar strong protective effects (not shown).

Further, to examine dynamic aspects of the light-induced protection of the KF network, orthovanadate treatment was combined with simultaneous light exposition. This situation is encountered during time-lapse fluorescence microscopy when cells are subjected to short pulses of excitation irradiation (496 nm) at regular intervals. In most recordings, orthovanadate-induced KF network disruption was either considerably slowed down or did not proceed to completion. In the latter case, an extended KF network was usually re-established in approximately 30 min. Figure 4 and the corresponding movie (<http://www.uni-mainz.de/FB/Medizin/Anatomie/Leube/depict>) a typical sequence. Multiple cytoplasmic granules appeared within a few minutes reaching a maximum after 10 min, whereas a significant number of filaments remained. Subsequently, the granular

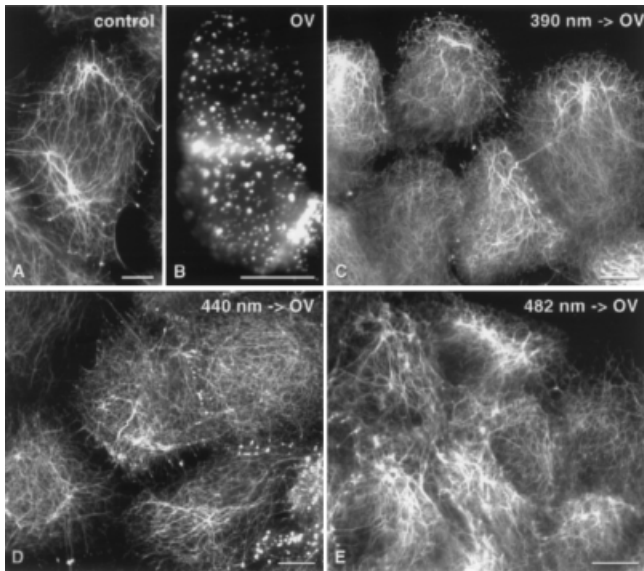


Figure 3. The inhibitory effect of light on orthovanadate-induced KF network disruption and keratin aggregate formation is wavelength independent. Fluorescence micrographs of AK13-1 cells without treatment (A), after incubation with 20 mM orthovanadate for 10 min (B), or after irradiation with either 390 nm, 440 nm, or 482 nm monochromatic light at approximately 4 mW per cm² for 5 min and subsequent treatment with 20 mM orthovanadate for 10 min (C, D, and E, respectively). Note, in C–E, the persistence of an extended KF network, which however, exhibits a smaller mesh size than that in control cells (A). Bars = 10 μ m.

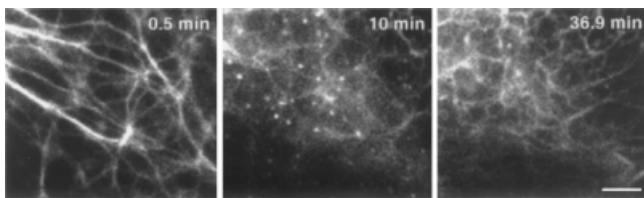


Figure 4. Orthovanadate-mediated KF network disruption is reversed by simultaneous irradiation. The fluorescence micrographs are taken from a time-lapse recording (<http://www.uni-mainz.de/FB/Medizin/Anatomic/Leube>). The recording was started 0.5 min after the addition of orthovanadate (10 mM), and pictures were taken at high frequency. KF network disruption is maximal at about 10 min with multiple granular keratin aggregates. By 36.9 min, most granules have disappeared, and a fine network has reassembled. Scale bar = 2 μ m.

material was re-integrated into the filament network. These observations suggested that the effect of orthovanadate on the KF system was undone by light exposure, even at time points when the KF network disruption was in progress.

Next, we wanted to determine the duration of the light-induced orthovanadate resistance. To this end, cells were first subjected to 3 min of normal room light (<200 Lux) and were subsequently incubated in the dark for various time intervals (30 min to 5 h) prior to orthovanadate treatment. Considerable recovery of orthovanadate sensitivity was apparent after 2 h (Fig 5). We also examined whether culture density was of importance as transcellular signaling could enhance the protective effect of light. A comparison of cultures grown to various degrees of confluence, however, did not reveal any significant differences.

As EGF-mediated signaling has been described as being induced by UV light in A-431 cells (Sachsenmaier *et al*, 1994; Kitagawa *et al*, 2002), we wanted to test whether EGF could also antagonize orthovanadate-mediated KF network disassembly. EGF pretreatment, however, produced little inhibition of granule

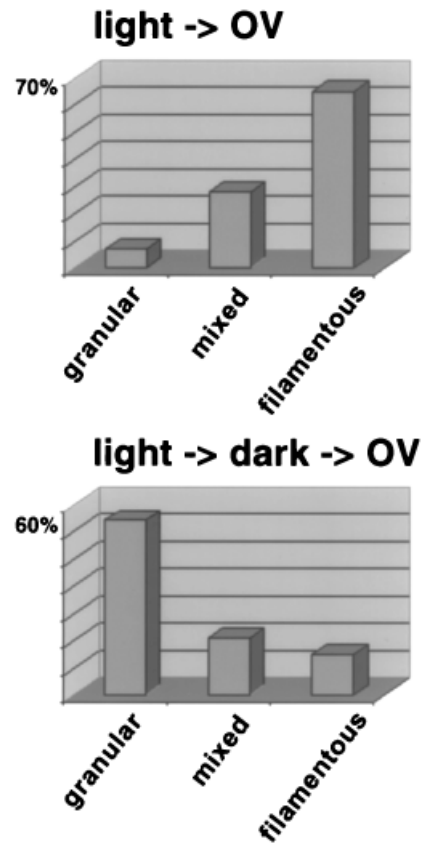


Figure 5. Light-induced protection of the KF network against orthovanadate-mediated disruption is reversible. AK13-1 cells were exposed to normal room light for 3 min and were subsequently either treated immediately with 10 mM orthovanadate (upper panel) or first incubated in the dark for 2 h prior to orthovanadate incubation (lower panel). In each instance, cells were fixed, and keratin aggregate formation was assessed in 100 randomly picked cells. Three categories were distinguished: predominant granular keratin fluorescence (granular), significant amount of keratin aggregates but largely intact KF (mixed), and normal-appearing extended KF network with almost no granules (filamentous).

formation, although a minor protective effect was still discernible (Fig 6).

Among the various keratin phosphorylation sites, S73 of K8 has received particular attention given its on/off properties and its induction during mitosis, cell stress, and apoptosis (Liao *et al*, 1997). The regulation of this phosphorylation has been shown to be p38 dependent and was therefore inhibited by the p38 kinase inhibitor SB203580 but not by the MAPK kinase inhibitor PD98059 (Feng *et al*, 1999; Ku *et al*, 2002). To ascertain whether p38 was able to prevent the orthovanadate-mediated KF disruption, AK13-1 cells were preincubated with SB203580 and, as a control, with PD98059 prior to orthovanadate treatment. All procedures were carried out in the dark (<0.2 Lux). Remarkably, SB203580 inhibited orthovanadate activity efficiently in over two-thirds of cells, whereas PD98059 was much less effective (Fig 6). Interestingly, the degree of protection by SB203580 was comparable with that observed after light exposure (compare Fig 6 with Fig 2).

DISCUSSION

The importance of light for skin physiology has been recognized for a long time, and both its beneficial and its toxic effects have been the subject of intense research. To improve epidermal disease states and skin function various types of irradiation regimens have

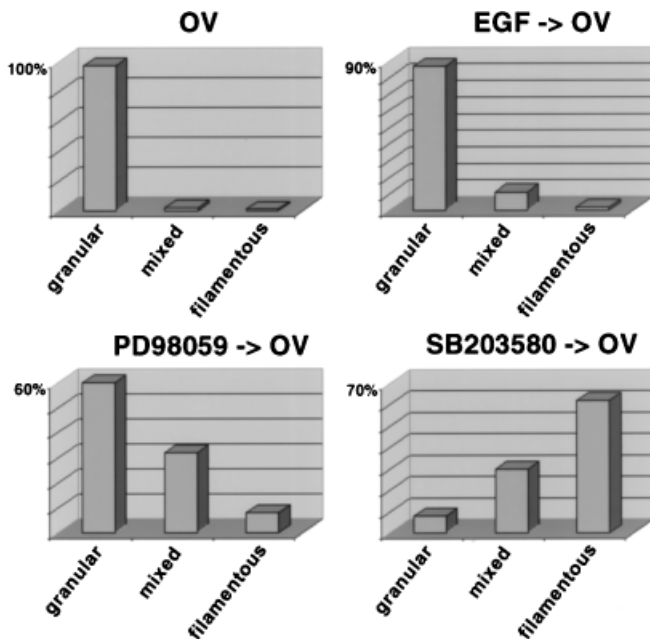


Figure 6. Orthovanadate (OV)-mediated KF network disruption is efficiently antagonized by the p38 kinase inhibitor SB203580 but to a much lesser degree by EGF and the MAPK kinase inhibitor PD98059. In the control experiment, cells were treated solely with OV. The histograms show the results of experiments in which AK13-1 cells were either pretreated in the dark with 5 ng EGF per ml (EGF → OV), 100 μ M PD98059 (PD98059 → OV), or 20 μ M SB203580 (SB203580 → OV), each for 1 h prior to addition of OV (10 mM). In each experiment, cells were fixed, and 100 cells were selected at random for the determination of keratin aggregate formation. Three categories were distinguished: predominant granular keratin fluorescence (granular), significant amount of keratin aggregates but largely intact KF (mixed), and normal-appearing extended KF network with almost no granules (filamentous).

been designed in which the beneficial effects are exploited. These are considered to represent a productive response that protects cells against further insult. The details of the beneficial effects on structural cellular components are only poorly understood. Therefore, our detection of dramatic changes in the keratin cytoskeleton responsiveness to the filament-disrupting agent orthovanadate by mere short-term irradiation with normal room light adds an entirely novel piece of information. Although our results were obtained in cells that are derived from an epithelium that physiologically is not sun exposed, it is attractive to assume that the observed light-dependent induction of KF network resilience may be a basic protective mechanism to counterbalance cytotoxic effects on keratinocytes that are directly subjected to environmental irradiation and various toxic agents. In this way, the continuous maintenance of a more resistant cytoskeleton might be conferred on light-exposed cells. This would prevent KF reorganization in response to short-term regulatory cues such as phosphorylation. Such increased stability would be of particular benefit to the postmitotic suprabasal cells in skin, cells that are a major component of the skin barrier and need to preserve their abundant and densely packed KF bundles.

Probably the most remarkable features of the detected acquisition of orthovanadate resistance are the reversibility and speed of the process, which requires only a little over 1 min. We conclude therefore that gene transcription and protein translation cannot be involved and that, instead, post-translational alterations are responsible for the protection. Similarly fast reactions have been observed *in vitro* during the UV response, while using intensities comparable with the light intensities employed in this study (review in Bender *et al*, 1997; Herrlich *et al*, 1999). In the case of UV

irradiation, ligand-independent activation of receptor tyrosine kinases by autophosphorylation has been observed to occur within seconds, being caused by the inactivation of receptor-directed tyrosine phosphatases (Sachsenmaier *et al*, 1994; Knebel *et al*, 1996; Groß *et al*, 1999; Herrlich *et al*, 1999) and/or by receptor dimerization and clustering (Rosette and Karin, 1996; Kato *et al*, 2000). Although the EGF receptor has been shown to participate in the UV response in A-431 cells (Sachsenmaier *et al*, 1994; Kitagawa *et al*, 2002), we have found little evidence for the involvement of EGF signaling in our system. EGF signaling is mediated through MAPK pathways and is known to lead to keratin hyperphosphorylation and KF reorganization (Aoyagi *et al*, 1985; Baribault *et al*, 1989; Ku and Omary, 1997; Wan *et al*, 2001). Thus, the MAPK kinase inhibitor PD98059 has been shown to inhibit EGF-induced keratin phosphorylation, most notably of S431 of K8 (Feng *et al*, 1999). In our experiments, however, we have found only a minor antagonistic effect of PD98059 on orthovanadate activity indicating that, in contrast to the UV response, EGF-mediated pathways are not of major importance in our system. Furthermore, the protection against orthovanadate is operative throughout the visible spectrum, extending even to infrared wavelengths and thereby clearly demonstrating that it is not dependent on UV light. Therefore, this property in combination with the comparatively low-dose requirements (normal room light) are strong indications of the physiologic relevance of the observed phenomenon for the reactivity of the cytoskeleton under low-light intensity conditions that occur in everyday life.

Two principal mechanisms may be responsible for the rapid light-dependent keratin network stabilization, namely post-translational modification of keratins and/or altered interaction with IFAP. The first alternative is supported by the observation that the p38 kinase inhibitor SB203580, which is known to prevent pervanadate-dependent tyrosine phosphorylation of K8 and K19 (Feng *et al*, 1999) and p38-dependent phosphorylation of S73 of K8 (Ku *et al*, 2002), inhibits orthovanadate-mediated KF network breakdown to a similar degree as light. At this point, however, the characterization of phosphorylation sites, their functional interrelationship, and their contribution to KF organization need further investigation. Therefore, it is premature to link light-induced KF alterations to specific signaling pathways, although it is noteworthy that the MAPK kinase inhibitor PD98059 and EGF compete much less efficiently than SB203580 with orthovanadate (this study). It will therefore be of particular interest to examine the relevance of p38 inhibitors for strengthening the epithelial IF cytoskeleton in other situations of increased stress. In this regard, it is of note that clinical studies have been initiated by using p38 inhibitors for the treatment of psoriasis (English and Cobb, 2002), a disease that is also responsive to a combined psoralen and UVA treatment.

The second mechanism leading to alterations in the properties of the KF network may be determined by IFAP, which are also subject to phosphorylation-dependent regulation (Foisner *et al*, 1996; Weinreb *et al*, 2001). In preliminary analyses, we have found no indication that light affects the distribution of IFAP, although we have recently observed that orthovanadate treatment results in significantly increased colocalization with the cytoskeletal cross-linker plectin (Strnad *et al*, 2002).

Taken together, the identified interrelationship of light and the phosphatase inhibitor orthovanadate provides the first experimental tool to enable us to examine the naturally occurring plasticity and regulation of the KF network. The speed and reversibility of the process demonstrate the suitability of the revealed phenomena to facilitate the required cytoskeletal fine tuning. Finally, the detection of light-induced KF stabilization is of potential consequence for disease management and the prevention of keratin aggregate formation in skin diseases with reduced keratin network stability, e.g., in hereditary diseases of the epidermolysis bullosa simplex type (Anton-Lamprecht, 1983; Coulombe *et al*, 1991). Future studies are needed to address these fascinating possibilities more systematically.

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REFERENCES

- Anton-Lamprecht I: Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolysis: pathogenesis heterogeneity fetal manifestation, and prenatal diagnosis. *J Invest Dermatol* 81(Suppl. 1): 149s-156s, 1983
- Aoyagi T, Suya H, Umeda K, Kato N, Nemoto O, Kobayashi H, Miura Y: Epidermal growth factor stimulates tyrosine phosphorylation of pig epidermal fibrous keratin. *J Invest Dermatol* 84:118-121, 1985
- Baribault H, Blouin R, Bourgon L, Marceau N: Epidermal growth factor-induced selective phosphorylation of cultured rat hepatocyte 55-kD cyokeratin before filament reorganization and DNA synthesis. *J Cell Biol* 109:1665-1676, 1989
- Baricault L, de Nechaud B, Sapin C, Codogno P, Denoulet P, Trugnan G: The network organization and the phosphorylation of cyokeratins are concomitantly modified by forskolin in the enterocyte-like differentiated Caco-2 cell line. *J Cell Sci* 107:2909-2918, 1994
- Bayerl C, Taake S, Moll I, Jung EG: Characterization of sunburn cells after exposure to ultraviolet light. *Photodermatol Photoimmunol Photomed* 11:149-154, 1995
- Bender K, Blattner C, Knebel A, Jordanov M, Herrlich P, Rahmsdorf HJ: UV-induced signal transduction. *J Photochem Photobiol* 37:1-17, 1997
- Chou CF, Riopel CL, Rott LS, Omary MB: A significant soluble keratin fraction in "simple" epithelial cells. Lack of an apparent phosphorylation and glycosylation role in keratin solubility. *J Cell Sci* 105:433-444, 1993
- Coulombe PA, Hutton ME, Letai A, Herbert A, Paller AS, Fuchs E: Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. *Cell* 66:1301-1311, 1991
- Coulombe PA, Omary MB: "Hard" and "soft" principles defining the structure, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* 14:110-122, 2002
- English JM, Cobb MH: Pharmacological inhibitors of MAPK pathways. *Trends Pharmacol Sci* 123:40-45, 2002
- Feng L, Zhou X, Liao J, Omary MB: Pervanadate-mediated tyrosine phosphorylation of keratins 8 and 19 via a p38 mitogen-activated protein kinase-dependent pathway. *J Cell Sci* 112:2081-2090, 1999
- Foisner R, Malecz N, Dressel N, Stadler C, Wiche G: M-phase-specific phosphorylation and structural rearrangement of the cytoplasmic cross-linking protein plectin involve p34^{cdc2} kinase. *Mol Biol Cell* 7:273-288, 1996
- Fuchs E, Weber K: Intermediate filaments structure, dynamics, function, and disease. *Annu Rev Biochem* 63:345-382, 1994
- Groß S, Knebel A, Tenev T, Neininger A, Gaestel M, Herrlich P, Böhmer FD: Inactivation of protein-tyrosine phosphatases as mechanism of UV-induced signal transduction. *J Biol Chem* 274:26378-26386, 1999
- Herrlich P, Bender K, Knebel A, et al: Radiation-induced signal transduction. Mechanisms and consequences. *C R Acad Sci* 322:121-125, 1999
- Herrmann H, Aebi U: Intermediate filaments and their associates: multitasking structural elements specifying cytoarchitecture and cytodynamics. *Curr Opin Cell Biol* 12:79-90, 2000
- Hesse M, Magin TM, Weber K: Genes for intermediate filament proteins and the draft sequence of the human genome: novel keratin genes and a surprisingly high number of pseudogenes related to keratin genes 8 and 18. *J Cell Sci* 114:2569-2575, 2001
- Irvine AD, McLean WH: Human keratin diseases: the increasing spectrum of disease and subtlety of the phenotype-genotype correlation. *Br J Dermatol* 140:815-828, 1999
- Kartasova T, Cornelissen BJC, Belt P, van de Putte P: Effects of UV, 4-NQO and TPA on gene expression in cultured human epidermal keratinocytes. *Nucleic Acids Res* 15:5945-5962, 1987
- Kasahara K, Kartasova T, Ren X, Ikuata T, Chida K, Kuroki T: Hyperphosphorylation of keratins by treatment with okadaic acid of BALB/MK-2 mouse keratinocytes. *J Biol Chem* 269:23531-23537, 1993
- Kato M, Iwashita T, Takeda K, et al: Ultraviolet light induces redox reaction-mediated dimerization and superactivation of oncogenic Ret tyrosine kinases. *Mol Biol Cell* 11:93-101, 2000
- Kitagawa D, Tanemura S, Obata S, et al: Activation of extracellular signal-regulated kinase by ultraviolet is mediated through Src-dependent epidermal growth factor receptor phosphorylation. Its implication in an anti-apoptotic function. *J Biol Chem* 277:366-371, 2002
- Knebel A, Rahmsdorf HJ, Ullrich A, Herrlich P: Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. *EMBO J* 15:5314-5325, 1996
- Korge BP, Krieg T: The molecular basis for inherited bullous diseases. *J Mol Med* 74:59-70, 1996
- Ku N-O, Omary MB: Phosphorylation of human keratin 8 *in vivo* at conserved head domain serine 23 and at epidermal growth factor-stimulated tail domain serine 431. *J Biol Chem* 272:7556-7564, 1997
- Ku N-O, Azhar S, Omary MB: Keratin 8 phosphorylation by p38 kinase regulates cellular keratin filament reorganization: modulation by a keratin 1-like disease-causing mutation. *J Biol Chem* 277:10775-10782, 2002
- Leube RE, Bader BL, Bosch FX, Zimbelmann R, Achtstaetter T, Franke WW: Molecular characterization and expression of the stratification-related cyokeratin 4 and 15. *J Cell Biol* 106:1249-1261, 1988
- Liao J, Omary MB: 14-3-3 proteins associate with phosphorylated simple epithelial keratins during cell cycle progression and act as a solubility cofactor. *J Cell Biol* 133:345-357, 1996
- Liao J, Ku N-O, Omary MB: Stress, apoptosis, and mitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. *J Biol Chem* 272:17565-17573, 1997
- Moll R: Cyokeratins as markers of differentiation in the diagnosis of epithelial tumors. *Subcell Biochem* 31:319-362, 1998
- Moll I, Bohnert E, Treib U, Jung EG: Effects of ultraviolet B radiation on cytoskeletal and adhesion molecules in human epidermis. *Photodermatol Photoimmunol Photomed* 10:26-32, 1994
- Omary MB, Ku N-O, Liao J, Price D: Keratin modifications and solubility properties in epithelial cells and *in vitro*. *Subcell Biochem* 31:105-140, 1998
- Peters B, Kirfel J, Bussow H, Vidal M, Magin TM: Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. *Mol Biol* 12:1775-1789, 2001
- Rosette C, Karin M: Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 274:1194-1197, 1996
- Sachsenmaier C, Radler-Pohl A, Zinck R, Nordheim A, Herrlich P, Rahmsdorf HJ: Involvement of growth factor receptors in the mammalian UVC response. *Cell* 78:963-972, 1994
- Sesto A, Navarro M, Burslem F, Jorcano JL: Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc Natl Acad Sci USA* 99:2965-2970, 2002
- Straface E, Santini MT, Donelli G, Giacomoni PU, Malorni W: Vitamin E prevents UVB-induced cell blebbing and cell death in A431 epidermoid cells. *Int J Radiat Biol* 68:579-587, 1995
- Strnad P, Windoffer R, Leube RE: *In vivo* detection of cyokeratin filament network breakdown in cells treated with the phosphatase inhibitor okadaic acid. *Cell Tissue Res* 306:277-293, 2001
- Strnad P, Windoffer R, Leube RE: Induction of rapid and reversible cyokeratin filament network remodeling by inhibition of tyrosine phosphatases. *J Cell Sci* 115:4133-4148, 2002
- Stumptner C, Omary MB, Fickert P, Denk H, Zatloukal K: Hepatocyte cyokeratins are hyperphosphorylated at multiple sites in human alcoholic hepatitis and in a Mallory body mouse model. *Am J Pathol* 156:77-90, 2000
- Toivola DM, Goldman RD, Garrod DR, Eriksson JE: Protein phosphatases maintain the organization and structural interactions of hepatic keratin intermediate filaments. *J Cell Sci* 110:23-33, 1997
- Wan YS, Wang ZQ, Voorhees J, Fisher G: EGF receptor crosstalks with cytokine receptors leading to the activation of c-Jun kinase response to UV irradiation in human keratinocytes. *Cell Signal* 13:139-144, 2001
- Weinreb O, Dovrat A, Dunia I, Benedetti EL, Bloemendal H: UV-A-related alterations of young and adult lens water-insoluble α -crystallin, plasma membranous and cytoskeletal proteins. *Eur J Biochem* 268:536-543, 2001
- Windoffer R, Leube RE: Detection of cyokeratin dynamics by time-lapse fluorescence microscopy in living cells. *J Cell Sci* 112:521-4534, 1999
- Windoffer R, Leube RE: De novo formation of cyokeratin filament networks originates from the cell cortex in A-431 cells. *Cell Motil Cytoskeleton* 50:33-44, 2001
- Zamansky GB, Nguyen U, Chou I-N: An immunofluorescence study of effects of ultraviolet radiation on the organization of microfilaments, keratin intermediate filaments, and microtubules in human keratinocytes. *Cell Motil Cytoskeleton* 22:296-306, 1992