Molecular Mechanisms of Photoaging and its Prevention by Retinoic Acid: Ultraviolet Irradiation Induces MAP Kinase Signal Transduction Cascades that Induce Ap-1-Regulated Matrix Metalloproteinases that Degrade Human Skin *In Vivo*

Gary J. Fisher and John J. Voorhees

Department of Dermatology, University of Michigan, Ann Arbor, Michigan, U.S.A.

Ultraviolet radiation from the sun damages human skin, resulting in an old and wrinkled appearance. A substantial amount of circumstantial evidence indicates that photoaging results in part from alterations in the composition, organization, and structure of the collagenous extracellular matrix in the dermis. This paper reviews the authors' investigations into the molecular mechanisms by which ultraviolet irradiation damages the dermal extracellular matrix and provides evidence for prevention of this damage by all-trans retinoic acid in human skin *in vivo*. Based on experimental evidence a working model is proposed whereby ultraviolet irradiation activates growth factor and cytokine receptors on keratinocytes and dermal cells, resulting in downstream signal transduction through activation of MAP kinase pathways. These signaling pathways converge in the nucleus of cells to induce c-Jun, which heterodimerizes with constitutively expressed c-Fos to form activated complexes of the transcription factor AP-1. In the dermis and epidermis, AP-1 induces expression of matrix metalloproteinases collagenase, 92 kDa gelatinase, and stromelysin, which degrade collagen and other proteins that comprise the dermal extracellular matrix. It is hypothesized that dermal breakdown is followed by repair that, like all wound repair, is imperfect. Imperfect repair yields a deficit in the structural integrity of the dermis, a solar scar. Dermal degradation followed by imperfect repair is repeated with each intermittent exposure to ultraviolet irradiation, leading to accumulation of solar scarring, and ultimately visible photoaging. All-trans retinoic acid acts to inhibit induction of c-Jun protein by ultraviolet irradiation, thereby preventing increased matrix metalloproteinases and ensuing dermal damage. *Key words: c-Jun/extracellular/matrix photobiology/collagen/JNJ Kinase/p38 Kinase. Journal of Investigative Dermatology Symposium Proceedings 3:61–68, 1998*

Ultraviolet (UV) radiation from the sun damages human skin cells. Two critical molecular targets of this damage are DNA, which encodes genetic information, and collagen, which constitutes more than 90% of skin connective tissue. UV damage to DNA causes mutations that may lead to skin cancer (Dumaz *et al*, 1993; Berg *et al*, 1996). Damage to collagen reduces the strength and resiliency of the skin, resulting in an aged appearance (photoaging) (Smith *et al*, 1962; Warren *et al*, 1991; Gilchrest and Yaar, 1992). This paper reviews our recent findings on the molecular mechanisms of UV-induced damage to collagen in the extracellular matrix of the dermis. In addition, this paper presents evidence that topically applied all-*trans* retinoic acid (*t*RA), which has been previously shown to stimulate repair of photoaged skin, protects against UV-induced collagen destruction, and may therefore be able to ameliorate the effects of photoaging.

MATRIX METALLOPROTEINASE MODEL FOR THE PATHOPHYSIOLOGY OF PHOTOAGING

Our working model for how UV damages human skin is depicted in **Fig 1**. UV radiation activates cell surface growth factor and cytokine receptors in keratinocytes and fibroblasts (Warmuth *et al*, 1994; Sachsenmaier *et al*, 1994; Huang *et al*, 1996; Rosette and Karin, 1996; Bender *et al*, 1997). Receptor activation results in signal transduction through protein kinase cascades that activate the transcription factor AP-1 in the cell nucleus (Davis, 1993; Karin and Hunter, 1995; Denhardt, 1996). UV-activated AP-1 stimulates transcription of matrix metalloproteinase (MMP) genes encoding collagenase, 92 kDa gelatinase, and stromelysin (Fisher *et al*, 1996). These MMP, which are induced in both the epidermis and the dermis, degrade collagen and other substrates in the dermal extracellular matrix. We propose that this breakdown of the dermal matrix is followed by repair, which is imperfect, yielding flaws in the structure and organization of the collagenous extracellular matrix. We refer to this imperfectly repaired dermal matrix, which is invisible to the naked eye, as solar scarring. With each intermittent UV exposure there is additional solar scarring, with concomitant undermining of the structural integrity of the dermis. Eventually this process manifests itself as wrinkled, photoaged skin (Fisher *et al*, 1997). This model for the pathophysiology of photoaging is largely derived from experiments that have examined molecular responses of human skin to a single UV exposure. As described below, these studies provide experimental evidence to support all of the features of the model through the step of dermal matrix breakdown. Other investigators have demonstrated that collagen fibrils in photodamaged skin are fragmented and disorganized (Bernstein *et al*, 1996). The causal relationships governing collagen breakdown by UV-induced

Reprint requests to: Dr. Gary Fisher, University of Michigan, R6447, Medical Science I, 1150 W. Medical Center Drive, Ann Arbor, MI 48109–0609.

Abbreviations: MMP, matrix metalloproteinase; RAR, retinoic acid receptors.

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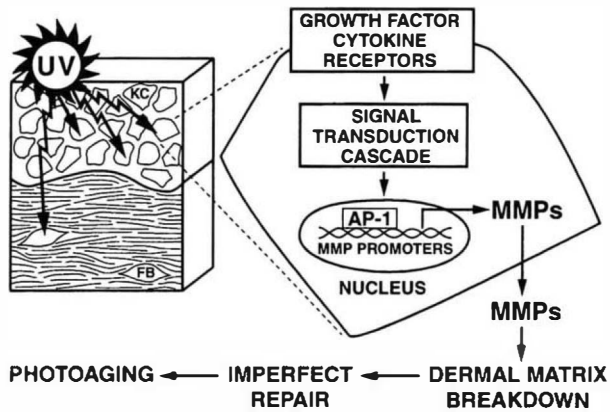

Figure 1. Matrix metalloproteinase model for the pathophysiology of photoaging. Exposure of keratinocytes (KC) or dermal fibroblasts (FB) to UV results in the stimulation of growth factor and cytokine receptors that leads to the activation of protein kinase signal transduction cascades. This results in activation of transcription factor AP-1, which induces expression of MMP. MMP degrade the extracellular matrix in the dermis. Dermal matrix damage is followed by matrix repair, which is imperfect, and thereby results in a deficit in the structure of the dermis. This residual deficit is a solar scar. Accumulation of solar scars, which occurs with repeated intermittent UV exposure, results in visible skin wrinkling.

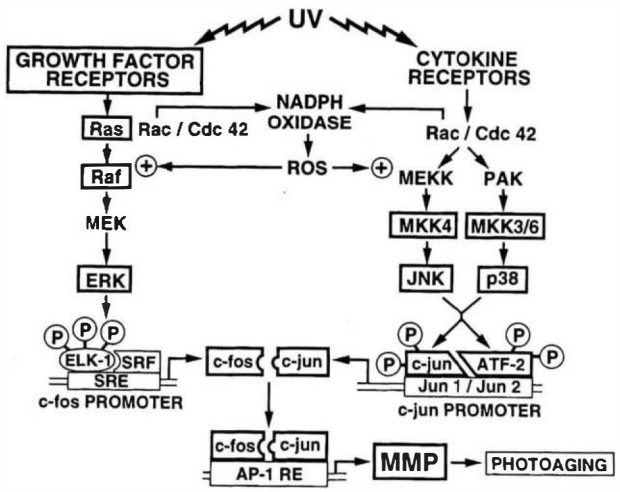

Figure 2. UV induces MAP kinase signal transduction pathways in human skin *in vivo*. UV activation of growth factor or cytokine receptors leads to the activation of MAP kinases ERK, JNK, and p38. Activation of NADPH oxidase, via Rac, results in production of reactive oxygen species (ROS), which are required for MAP kinase activation. Activation of ERK and JNK/p38 results in phosphorylation (P) of the transcription factors ELK-1 and c-Jun/ATF-2, respectively. This leads to increased expression of the transcription factor AP-1 (c-fos/c-jun), which in turn results in upregulation of metalloproteinases (MMP). Those molecules shown to be activated by UV in human skin *in vivo* are boxed in thick lines.

MMP, abnormal dermal collagen in photoaged skin, and skin wrinkling have not been experimentally determined, and therefore these aspects of the model are, at present, speculative.

UV INDUCES MAP KINASE SIGNAL TRANSDUCTION PATHWAYS IN HUMAN SKIN *IN VIVO*

Figure 2 illustrates signal transduction pathways leading from activation of growth factor and cytokine receptors at the cell surface to activation of transcription factor AP-1 in the cell nucleus. Components of the signal transduction pathways that we have shown to be activated in UV-irradiated human skin *in vivo* are outlined in bold. One of the earliest effects of UV irradiation is activation of cell surface receptors (Warmuth *et al*, 1994; Sachsenmaier *et al*, 1994; Huang *et al*, 1996; Rosette and Karin, 1996; Bender *et al*, 1997). We have examined

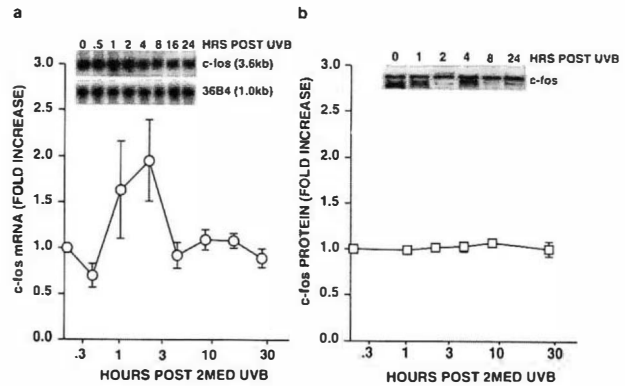

Figure 3. c-Fos mRNA and protein levels are not altered by UV. (*a*) Total RNA was isolated from skin biopsies taken at the indicated times following UV exposure (2 MED). Northern analysis was performed with randomly primed [^{32}P]cDNA probes for c-Fos or 36B4 (a ribosomal protein used as an internal control for quantitation). A representative northern blot is shown in the inset. Data are expressed as means ± SEM (n = 8–17). Differences between c-Fos mRNA levels in nonirradiated and UV-irradiated skin were not statistically significant at all time points. (*b*) c-Fos protein expression, in nuclear extracts of skin biopsies taken at the indicated times following UV exposure, was measured by western analysis. A representative western blot is shown in the inset. Data are expressed as means ± SEM (n = 6–8).

activation of the epidermal growth factor receptor by UV in human skin. Activation of the epidermal growth factor receptor, as demonstrated by autophosphorylation of specific tyrosine residues, occurs within 15 min of UV irradiation (2 MED), and remains elevated for at least 2 h (Fisher *et al*, 1998). Although we have not yet examined other cell surface receptors, prior investigators have demonstrated that UV irradiation activates interleukin-1 and tumor necrosis factor-α receptors, in addition to epidermal growth factor receptors, in cultured cells (Sachsenmaier *et al*, 1994; Huang *et al*, 1996; Rosette and Karin, 1996). It is likely that UV irradiation activates most, if not all, growth factor and cytokine receptors. Because cellular physiology is largely regulated by hormones, growth factors, and cytokines acting through their specific cell surface receptors, activation of cell surface receptors by UV irradiation must be a critical mechanism through which it exerts its effects on cellular function.

Activation of growth factor and cytokine cell surface receptors results in assembly of protein complexes with the cytoplasmic tails of the receptors. These complexes function to transduce signals from the cell surface to the nucleus. Assembly of signaling complexes results in activation of small, GTP-binding regulatory proteins, including family members Ras, Rac, and Cdc42 (Ullrich and Schlessinger, 1990; Boguski and McCormick, 1993; Denhardt, 1996). These proteins act as molecular switches that, when in the active GTP-bound state, stimulate MAP kinase signal transduction pathways (Coso *et al*, 1995; Minden *et al*, 1995). In human skin, Ras is activated within 30 min of UV exposure and remains activated for at least 24 h (Fisher *et al*, 1998). At present, it is not technically possible to measure activation of Rac or Cdc42 in cells or tissue directly. It has been demonstrated, however, that activated Rac directly stimulates the NADPH oxidase enzyme complex in cells (Abo *et al*, 1991; Heyworth *et al*, 1993). In human skin *in vivo*, UV irradiation stimulates NADPH oxidase activity 2-fold within 15 min (data not shown), consistent with activation of Rac. In addition to serving as a marker for Rac activation, stimulation of NADPH oxidase results in increased levels of two reactive oxygen species, superoxide anion and hydrogen peroxide (Jones, 1994). These reactive oxygen species appear to be critical for MAP kinase-mediated signal transduction (Lo and Cruz, 1995; Whisler *et al*, 1995). The mechanism through which reactive oxygen species participate in signal transduction, however, is not clear.

The ERK MAP kinase module consists of Raf, MEK, and the MAP kinase, ERK. The JNK MAP kinase module is comprised of MEKK, MKK4, and the MAP kinase, JNK. The p38 MAP kinase module consists of PAK (or a closely related kinase), MKK3 and 6, and the

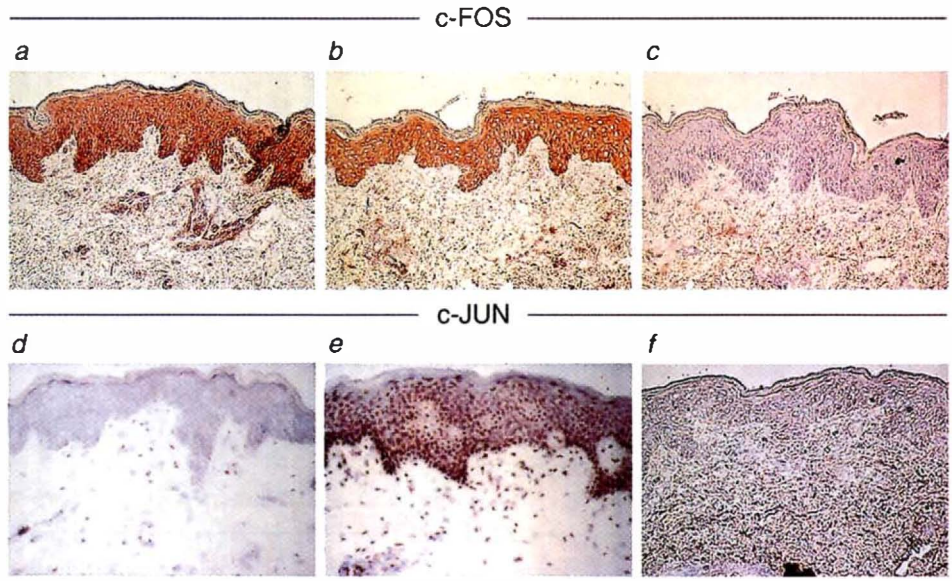

Figure 4. UV induces expression of c-Jun, but not constitutively expressed c-Fos, in human skin *in vivo*. Biopsies were taken from untreated human skin (*a*, *d*) or UV-irradiated skin 8 h post-exposure to 2 MED UV (*b*, *c*, *e*, *f*). c-Fos (*a*, *b*) and c-Jun (*d*, *e*) protein expression was localized by immunohistology using appropriate isotype controls (*c*, *f*). Reddish-brown color indicates the presence of c-Fos protein (*a*, *b*) or c-Jun (*e*). Results are for a single individual and are representative of results for six individuals.

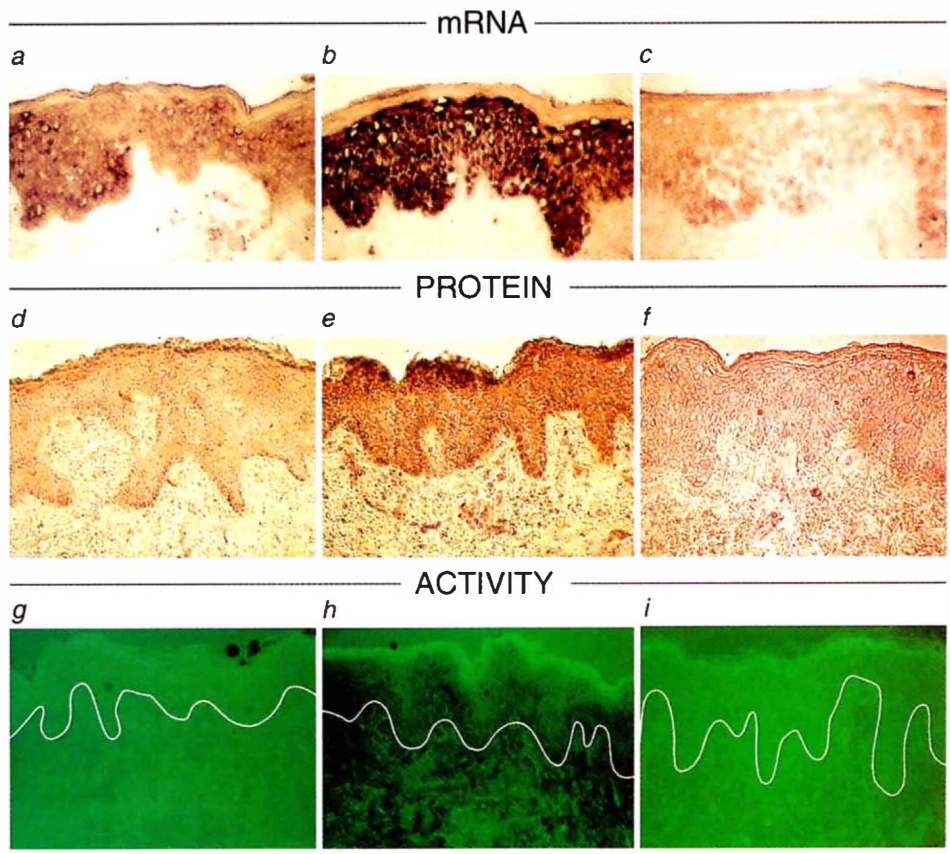

Figure 5. UV irradiation induces 92 kDa gelatinase mRNA, protein, and activity in human skin *in vivo*. 92 kDa gelatinase mRNA was localized by *in situ* hybridization, using digoxigenin-labeled anti-sense riboprobe, in tissue sections from untreated skin (*a*) and UV-irradiated (24 h post-2 MED) skin (*b*). Hybridization of tissue sections from UV-irradiated skin with digoxigenin-labeled sense riboprobe served as control for background and specificity (*c*). Purple staining indicates the presence of 92 kDa gelatinase mRNA. 92 kDa gelatinase protein was localized by peroxidase immunohistology in tissue sections from nonirradiated (*d*) and UV-irradiated (*e*) skin. Control immunoglobulin showed low background staining with UV-irradiated skin (*f*). Reddish-brown color indicates the presence of 92 kDa gelatinase protein. 92 kDa gelatinase activity was determined by *in situ* zymography in tissue sections taken from nonirradiated (*g*) and UV-irradiated (*h*) skin. Substrate hydrolysis by UV-irradiated skin sections was inhibited by exogenous TIMP-1 (*i*). The green color is fluorescene-labeled gelatin, which was coated onto glass slides. A 5 μm skin section was placed on top of the gelatin and incubated for 24 h at 37°C. Darkened areas show the degradation of the fluorescene-coated substrate and indicate the presence of 92 kDa gelatinase. The solid white line (*g*, *h*, *i*) demarcates the epidermal (upper)/dermal (lower) boundary. Results are for a single individual and are representative of results for six (mRNA and protein) or five (activity) individuals.

MAP kinase, p38 (Denhardt, 1996). The three MAP kinases ERK, JNK, and p38 are activated through dual phosphorylation of threonine and tyrosine catalyzed by their upstream activators MEK, MKK4, and MKK3 and 6, respectively (Denhardt, 1996). The MAP kinase activators MEK, MKK4, and MKK3 and 6 are activated by the serine/threonine kinases Raf, MEKK, and PAK, respectively (Fanger *et al*, 1997; Robinson and Cobb, 1997). UV irradiation activates each of the three MAP kinase modules with similar kinetics. ERK, JNK, and p38 are activated within 1 h of UV irradiation, maximally activated 4 h after UV irradiation, and return to baseline within 24 h after UV exposure (Fisher *et al*, 1998).

UV IRRADIATION INDUCES C-JUN AND ACTIVATES AP-1 IN HUMAN SKIN *IN VIVO*

It has been demonstrated in cultured cells that activation of ERK, JNK, and p38 MAP kinases leads to increased expression of the transcription factors c-Fos and c-Jun. c-Fos and c-Jun form stable heterodimers, and, together with other protein factors, form the

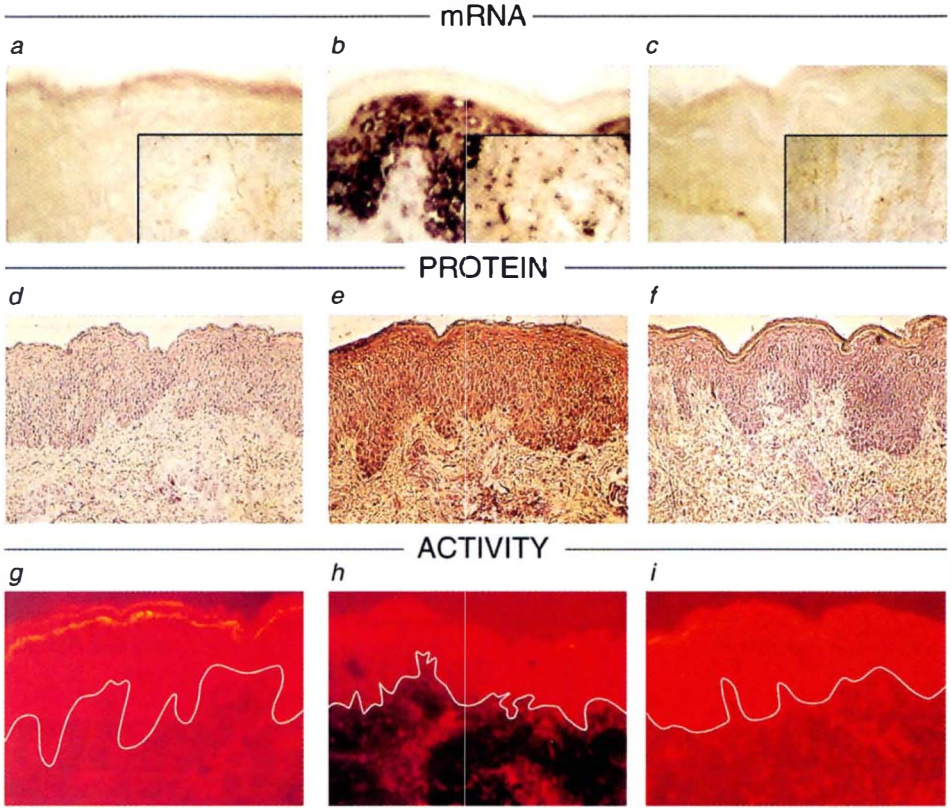

Figure 6. UV induces stromelysin mRNA, protein, and activity in human skin *in vivo*. Stromelysin mRNA was detected with a digoxigenin-labeled anti-sense riboprobe by *in situ* hybridization in tissue sections from nonirradiated skin (*a*) and UV-irradiated skin (24 h post-2 MED) (*b*). Tissue sections from UV-irradiated skin (*c*) were also hybridized with a digoxigenin-labeled sense riboprobe as a control for specificity. Purple staining indicates the presence of stromelysin mRNA. Stromelysin protein was localized by peroxidase immunohistology in tissue sections from nonirradiated (*d*) and UV-irradiated (*e*) skin. Normal sheep IgG showed low background staining with tissue sections from UV-irradiated skin (*f*). Reddish-brown color indicates the presence of collagenase protein. Stromelysin activity was determined by *in situ* zymography in tissue sections from nonirradiated (*g*) and UV-irradiated (*h*) skin. Substrate hydrolysis by UV-irradiated skin sections was inhibited by exogenous TIMP-1 (*i*). The red color is resorufin-conjugated casein, which was coated onto glass slides. A 5 μm skin section was placed on top of the casein and incubated for 24 h at 37°C. Darkened areas show the degradation of the casein-resorufin. The solid white line (*g*, *h*, *i*) demarcates the epidermal (upper)/dermal (lower) boundary. Results are for a single individual and are representative of results for six (mRNA and protein) or five (activity) individuals.

transcription factor AP-1 (Karin and Hunter, 1995; Whitmarsh and Davis, 1996; Claret *et al*, 1996). AP-1 regulates expression of many genes involved in the regulation of cellular growth and differentiation. The genes for several members of the MMP family are strongly regulated by AP-1 (Angel *et al*, 1987; Angel and Karin, 1992; Sato and Seiki, 1993). In nonirradiated human skin, c-Jun mRNA and protein levels are minimally detectable (Fisher *et al*, 1998). In contrast, c-Fos mRNA and protein are readily detectable in untreated, normal human skin (**Fig 3*a*, *b***). c-Fos protein is expressed throughout all the layers of the epidermis and in dermal cells, and its level is not affected by UV irradiation (**Fig 4*a–c***).

In contrast, c-Jun mRNA and protein are rapidly induced by UV irradiation. c-Jun mRNA is induced within 2 h of UV irradiation, and then slowly declines over the next 24 h. c-Jun protein is maximally induced 8 h after UV irradiation and remains maximally induced for at least 24 h (Fisher *et al*, 1998). c-Jun protein is induced throughout the epidermis and in dermal cells (**Fig 4*d–f***). These data indicate that, in human skin *in vivo*, c-Fos is constitutively expressed, and therefore AP-1 activation is primarily dependent on induction of c-Jun.

This conclusion is further supported by measurement of AP-1 complexes, and by gel shift analyses, in nonirradiated and UV-irradiated human skin. In nonirradiated human skin, AP-1 complexes contain predominantly c-Fos and Jun D, which like c-Fos is constitutively expressed, and not induced by UV irradiation (Fisher *et al*, 1998). Eight hours after UV irradiation, AP-1 levels are increased and AP-1 complexes are enriched in c-Jun, relative to nonirradiated human skin (Fisher *et al*, 1998). These data indicate that the c-Jun induced by UV irradiation forms functional complexes with constitutively expressed c-Fos, and that the c-Jun cannot dimerize with Jun D. In addition, western analyses indicate that c-Jun induced by UV irradiation is phosphorylated on its N-terminal transactivation domain (serines 63 and 73) (Fisher *et al*, 1998). This phosphorylation is catalyzed by the MAP kinases JNK and p38, which are induced by UV irradiation as described above, and enhances c-Jun's ability to stimulate gene transcription. UV activation of AP-1, measured by gel shift analyses, is induced at very low levels of UV irradiation. Half-maximal induction is observed at 0.1 MED and maximal induction occurs at 1 MED (Fisher *et al*, 1996). Taken together, the above data indicate that UV irradiation induces increased levels of activated AP-1 complexes, composed of c-Jun and c-Fos, in human skin *in vivo*. Activated AP-1 would be expected to stimulate transcription of MMP target genes.

UV IRRADIATION INDUCES MMP THAT DEGRADE THE DERMAL EXTRACELLULAR MATRIX IN HUMAN SKIN *IN VIVO*.

MMP are a family of zinc-requiring enzymes that specifically break down proteins that comprise connective tissue. Transcription of at least the following four members of the MMP family is strongly regulated by AP-1 (Angel *et al*, 1987; Angel and Karin, 1992; Sato and Seiki, 1993; Quinones *et al*, 1994): collagenase, which cleaves fibrillar type I and III collagens; 92 kDa gelatinase, which further breaks down collagen subsequent to collagenase cleavage, and also degrades Type IV collagen and elastin; stromelysin, which degrades Type IV collagen, elastin, and other extracellular matrix molecules, including proteoglycans and laminins; and metalloelastase, which cleaves elastin fibers (Matrisian and Hogan, 1990; Birkedal-Hansen *et al*, 1993). UV irradiation induces expression of these four MMP genes in human skin *in vivo*, consistent with UV activation of AP-1. Collagenase, 92 kDa gelatinase, and stromelysin mRNA levels are increased within 8–16 h following UV irradiation and remain elevated for more than 24 h. Interestingly, 72 kDa gelatinase, an MMP that is not regulated by AP-1, is not induced in human skin by UV irradiation (Fisher *et al*, 1996). These data are consistent with the notion that UV activation of AP-1 drives MMP induction in human skin *in vivo*. This notion is further supported by UV dose dependency data on induction of collagenase, 92 kDa gelatinase, and stromelysin, which closely resembles induction of AP-1. Protein levels and enzyme activities for each of the three MMP are significantly induced at 0.1 MED and fully induced at 1 MED (Fisher *et al*, 1996).

Damage to the extracellular matrix of the dermis from sunlight becomes histologically and clinically observable only after it accumulates

with multiple sun exposures (Warren *et al*, 1991; Gilchrest and Yaar, 1992). As stated above, a single UV exposure causes acute induction of MMP, which return to basal levels within 48–72 h after exposure. If MMP induction by UV irradiation is responsible for dermal damage in photoaging, then MMP induction would be expected to remain responsive to multiple UV exposures. To examine this possibility, subjects were irradiated four times at 48 h intervals over a 7 d period. As expected, 24 h following a single UV exposure, both collagenase and 92 kDa gelatinase were maximally induced. These maximally induced levels were maintained for 7 d as a result of exposure to UV every other day (Fisher *et al*, 1997). Thus, there appears to be no attenuation of MMP induction with multiple UV exposures. As repeated exposures over many decades may produce a different outcome, however, our findings in this context warrant further investigation.

In order for UV-induced MMP to cause the dermal damage known as photoaging, they must be localized in the dermis. We utilized *in situ* hybridization, immunohistology, and *in situ* zymography (Galis *et al*, 1995) to localize expression and activity of MMP in nonirradiated and UV-irradiated human skin. In nonirradiated human skin, mRNA, proteins, and activities of collagenase (Fisher *et al*, 1997), 92 kDa gelatinase (**Fig 5*a*, *d*, *g***) and stromelysin (**Fig 6*a*, *d*, *g***) were minimally detectable with these techniques. In contrast, 24 h following UV irradiation, mRNA and activities of these MMP were readily detectable, consistent with the biochemical data presented above. Collagenase (Fisher *et al*, 1997), 92 kDa gelatinase (**Fig 5*b***), and stromelysin (**Fig 6*b***) mRNA were induced throughout all of the layers of the epidermis. Collagenase (Fisher *et al*, 1997) and stromelysin (**Fig 6*b***, *insert*) mRNA were also induced in dermal cells. In contrast, expression of 92 kDa gelatinase mRNA was not detected in the dermis (**Fig 5*b***). Collagenase (Fisher *et al*, 1997), 92 kDa gelatinase (**Fig 5*e***), and stromelysin (**Fig 6*e***) proteins were elevated throughout the epidermis and in the dermis. *In situ* zymography revealed that each of the three MMP proteins was enzymatically active in both the epidermis and the dermis (Fisher *et al*, 1997) (**Figs 5*h*, 6*h***). These data indicate that collagenase, 92 kDa gelatinase, and stromelysin are substantially elevated and active in the dermis of human skin following UV irradiation.

Together, collagenase, 92 kDa gelatinase, and stromelysin have the capacity to degrade all of the protein components of the dermal extracellular matrix (Birkedal-Hansen, 1987; Matrisian and Hogan, 1990; Matrisian, 1992; Birkedal-Hansen *et al*, 1993; Fisher *et al*, 1997). To obtain direct evidence for collagen breakdown following acute UV exposure, we quantitated soluble breakdown products of type I collagen in nonirradiated and UV-irradiated human skin. To do this, we used a radioimmunoassay to measure cross-linked telopeptides that are released from mature fibrillar collagen by MMP-catalyzed digestion. Released telopeptides were elevated 60% 24 h following UV irradiation, demonstrating that the MMP induced by UV irradiation, and localized in the dermis, degrade endogenous fibrillar collagen in human skin *in vivo* (Fisher *et al*, 1997).

In addition to inducing AP-1-regulated MMP, UV irradiation induces an endogenous MMP inhibitor, TIMP-1 (Fisher *et al*, 1997). Whereas UV induction of TIMP-1 mRNA peaks at 24 h post-2 MED UV and thereafter declines (**Fig 7*a***), UV induction of TIMP-1 protein remains elevated at 72 h post-2 MED UV (**Fig 7*b***). TIMP-1 mRNA (**Fig 7*a***) and protein (**Fig 7*b***) are induced with similar kinetics and UV dose dependence as the MMP. This coinduction of MMP and TIMP-1 acts to inhibit MMP activity, and thereby prevent excessive connective tissue breakdown (Logan *et al*, 1996; Borden and Heller, 1997). Data presented above, however, suggest that UV-induced MMP activities exceed the inhibitory capacity of TIMP-1, and therefore some dermal extracellular matrix destruction occurs.

*t*RA PRETREATMENT INHIBITS UV INDUCTION OF MMP IN HUMAN SKIN *IN VIVO*

The above data indicate that UV irradiation induces AP-1, which stimulates expression of MMP that degrade collagen in the dermis of human skin *in vivo*. In cultured cells, *t*RA has been shown to antagonize AP-1 through a mechanism termed "transrepression." Transrepression

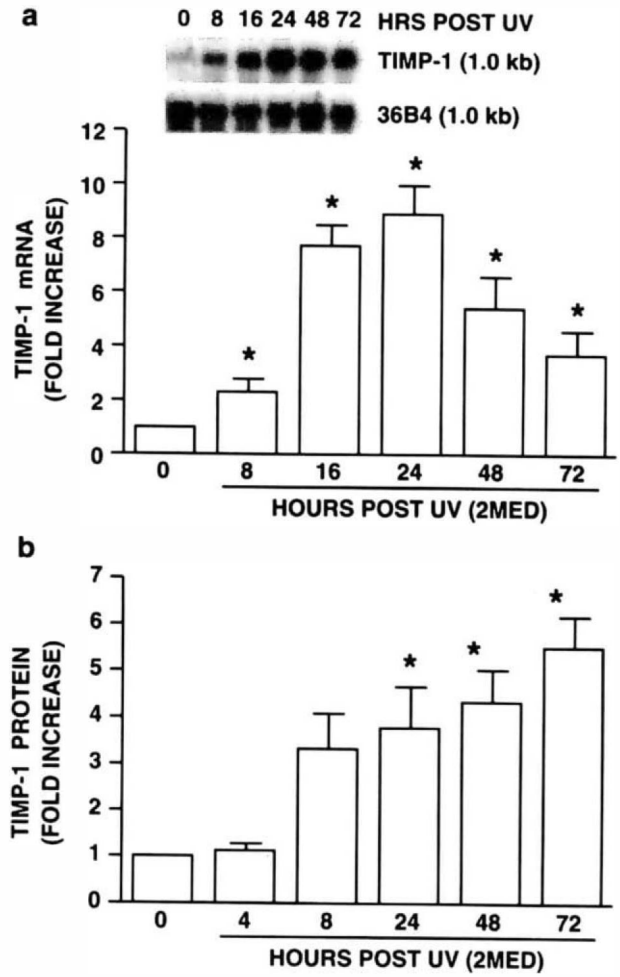

Figure 7. UV induces TIMP-1 mRNA and protein in human skin *in vivo*. (*a*) Total RNA was isolated from skin biopsies taken at the indicated times following UV exposure (2 MED). Northern analysis was performed with randomly primed [^{32}P] cDNA probes for human TIMP-1 or 36B4 (a ribosomal protein used as an internal control for quantitation). A representative northern blot is shown in the inset. Data are expressed as means ± SEM (n = 6). *p < 0.05 *versus* nonirradiated control skin. (*b*) Cell lysates were prepared from skin biopsies obtained at the indicated times following UV (2 MED) irradiation. TIMP-1 protein was quantitated by ELISA. Data are expressed as means ± SEM (n = 4–8).

of AP-1 by *t*RA is mediated by nuclear retinoic acid receptors (RAR) (Schule *et al*, 1991; Pfahl, 1993; Chen *et al*, 1995). RAR are ligand-activated transcription factors that are members of the steroid/thyroid hormone/vitamin D receptor superfamily (Chambon, 1994; Saurat, 1995). Evidence suggests that transrepression of AP-1 by RAR, which are activated when they bind *t*RA, results from binding of protein factors required for AP-1 activity to activated (i.e., *t*RA ligated) RAR (Schule *et al*, 1991; Pfahl, 1993; Saurat, 1995). We therefore examined the effects of *t*RA on UV induction of MMP in human skin *in vivo*. Pretreatment of human skin with *t*RA resulted in significant inhibition of UV induction of collagenase, 92 kDa gelatinase, and stromelysin mRNA and proteins. UV induction of mRNA levels was inhibited 50%–60%, whereas induction of MMP proteins was inhibited 60%–80% (Fisher *et al*, 1996). Immunohistology revealed that *t*RA inhibited UV induction of MMP proteins throughout the epidermis and dermis (**Fig 8**). *In situ* zymography also revealed that pretreatment with *t*RA inhibited UV-induced MMP activities in the epidermis and dermis (Fisher *et al*, 1997). Interestingly, *t*RA did not inhibit UV induction of TIMP-1 (Fisher *et al*, 1997). Therefore, *t*RA pretreatment increased the ratio of TIMP-1 to MMP in human skin following UV irradiation. This excess TIMP-1 would effectively inhibit most, if not all, of the residual MMP activities induced by UV in *t*RA-pretreated skin. This

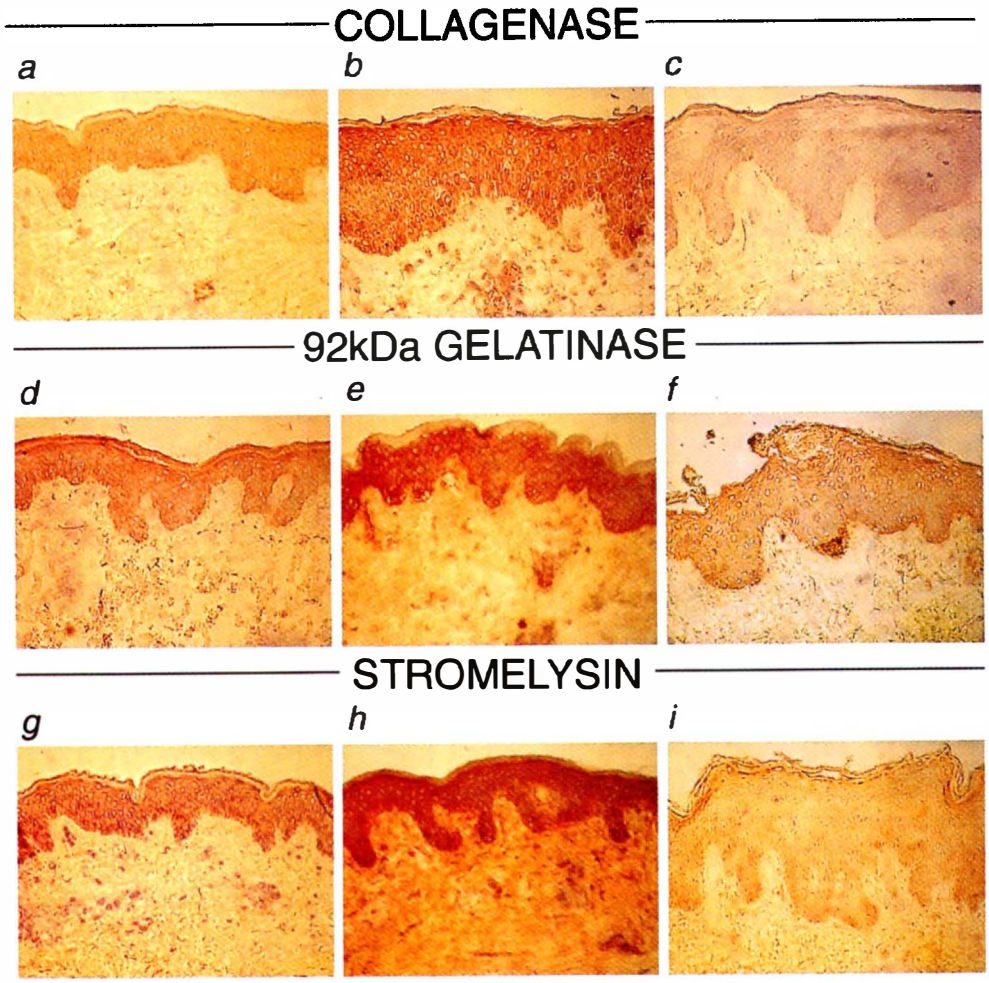

Figure 8. *t*RA blocks UV induction of collagenase, 92 kDa gelatinase, and stromelysin proteins in human skin *in vivo*. Adult human skin was pretreated with vehicle (*a, b, d, e, g, h*) or 0.1% *t*RA (*c, f, i*) for 48 h. Vehicle-treated sites were either left untreated (*a, d, g*) or were exposed to 2 MED UV (*b, e, h*). *t*RA-treated sites were also exposed to 2 MED UV (*c, f, i*). Skin biopsies were obtained 8 h post-UV, and collagenase (*a, b, c*), 92 kDa gelatinase (*d, e, f*), and stromelysin (*g, h, i*) proteins were localized by immunohistology. The presence of MMP protein is indicated by the reddish-brown staining. Results are representative of six experiments.

state of affairs may explain why *t*RA pretreatment appears to inhibit UV induction of MMP activities completely, as measured by *in situ* zymography. These data raise the possibility that *t*RA, which has previously been shown to improve the appearance of photodamaged skin, may also prevent photodamage from occurring.

*t*RA INHIBITS UV INDUCTION OF C-JUN PROTEIN IN HUMAN SKIN *IN VIVO*

As UV induction of AP-1 appears to be critical for increased MMP expression, we examined the effect of *t*RA on AP-1 levels. Pretreatment of human skin with *t*RA for 48 h resulted in 70% inhibition of UV-induced AP-1 DNA binding. In marked contrast, 8 h of *t*RA pretreatment had no effect on UV-induced AP-1 DNA binding (Fisher *et al*, 1998).

We next examined the effect of 48 h of *t*RA pretreatment on levels of c-Fos and c-Jun in nonirradiated and UV-irradiated human skin. Pretreatment with *t*RA for 48 h did not alter the levels of c-Fos mRNA (**Fig 9*a***) or c-Jun mRNA or protein (Fisher *et al*, 1998) in nonirradiated human skin. Similarly, 48 h of *t*RA pretreatment did not alter c-Fos mRNA (**Fig 9*a***) or protein levels (**Fig 9*b***) in UV-irradiated skin. As described above, UV irradiation induces c-Jun mRNA and protein. Pretreatment with *t*RA for 48 h did not inhibit UV induction of c-Jun mRNA. In contrast, however, 48 h of *t*RA pretreatment inhibited UV induction of c-Jun protein by 70% (Fisher *et al*, 1998). Interestingly, no inhibition of UV induction of c-Jun protein was observed with 8 h of *t*RA pretreatment (Fisher *et al*, 1998). Immunohistology revealed that pretreatment with *t*RA for 48 h inhibited the induction of c-Jun protein throughout the epidermis and dermis (Fisher *et al*, 1998). Taken together, these data suggest a mechanism whereby *t*RA, acting through its nuclear receptors, induces expression of an inhibitor of c-Jun protein translation, or an activator of c-Jun degradation. We hypothesize that the inhibition of UV induction of c-Jun protein is responsible for *t*RA inhibition of MMP expression (**Fig 10**).

Recent evidence indicates that the ubiquitin proteasome pathway represents a major route for c-Jun degradation (Treier *et al*, 1994). N-terminal phosphorylation of c-Jun by JNK inhibits c-Jun ubiquitination and thereby prolongs the half-life of c-Jun (Musti *et al*, 1997). As described above, UV irradiation induces JNK-catalyzed c-Jun phosphorylation and elevates c-Jun protein levels for at least 24 h. It is tempting to speculate that *t*RA, acting through RAR, inhibits c-Jun phosphorylation by inducing an inhibitor of c-Jun kinase or an activator of c-Jun phosphatase. Under these circumstances, nonphosphorylated c-Jun would be degraded, and therefore could not accumulate. We are currently investigating this possibility.

CONCLUDING REMARKS

The above data present a framework for understanding how solar UV radiation damages the dermis in human skin. In essence, our results suggest that UV radiation acts as a universal ligand to activate cell surface growth factor and cytokine receptors. Once activated, these receptors engage downstream signaling pathways that regulate the activity of many different transcription factors, thereby altering expression of many different genes. Among the genes that are induced by UV irradiation in human skin are the subset of MMP family members that are regulated by AP-1. These UV-induced MMP are induced throughout the epidermis and dermis, where they degrade collagen and other proteins that comprise the dermal extracellular matrix. Matrix degradation is followed by new synthesis of matrix proteins. We speculate that the repair process is imperfect and we refer to the product of this imperfect repair as solar scarring. We speculate that the process of dermal damage followed by imperfect repair occurs after each separate UV exposure, leading to an accumulation of solar scarring

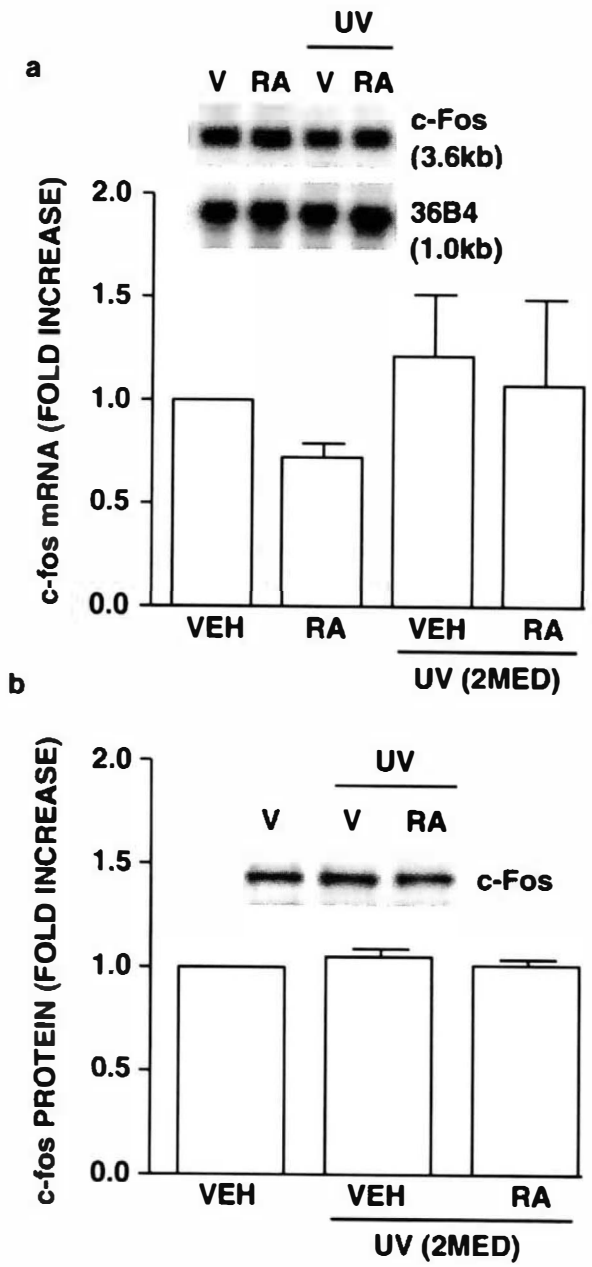

Figure 9. c-Fos mRNA and protein levels are not altered in UV-irradiated skin pretreated with tRA. Adult human skin was pretreated with vehicle or 0.1% tRA for 48 h. Vehicle-treated sites were either left untreated or exposed to 2 MED UV. tRA-treated sites were also exposed to 2 MED UV. Skin biopsies were obtained 8 h post-UV. c-Fos mRNA (*a*) was measured by northern analysis, performed with 36B4 (a ribosomal protein used as an internal control for quantitation). A representative northern blot is shown in the inset. c-Fos protein expression (*b*) was measured by western analysis. A representative western blot is shown in the inset. Data are expressed as means ± SEM (n = 6).

that eventually manifests itself as wrinkling of skin. It is important to note that, based on our experimental evidence, solar scar formation is likely to occur even at low doses of UV irradiation that do not induce skin reddening (Fisher *et al*, 1996). For these reasons, minimal sun exposure over many years would be expected to lead to photoaging. This prediction is consistent with common experience.

In addition to providing a framework for understanding how UV damages skin, the data presented here also provide a theoretical foundation for the development of new clinical strategies to prevent photoaging. Such strategies could act to block UV-induced responses at the level of cell surface receptors, protein kinase cascades, transcription factors, or MMP. In this context, it is worth noting that the molecular

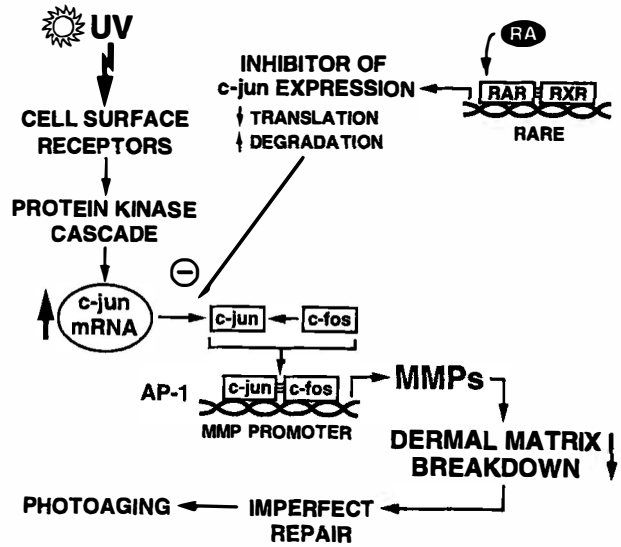

Figure 10. Model for mechanism of inhibition by tRA of UV induction of MMP. Retinoic acid, acting through nuclear retinoid receptors (RAR and RXR), induces expression of an inhibitor of c-Jun protein translation or an activator of c-Jun protein degradation. This inhibition of UV induction of c-Jun protein prevents activation of transcription factor AP-1, which is required for MMP expression. Inhibition of MMP induction is postulated to spare the extracellular matrix in the dermis from MMP-mediated degradation, and ensuing dermal damage that leads to photoaging.

machinery that is engaged inappropriately by UV irradiation to bring about skin damage is not unique to skin, or to photoaging. Inappropriate activation of cell surface receptors, signal transduction pathways, transcription factors, and MMP occurs in a variety of pathologic conditions characterized by hyperproliferation, malignant transformation, inflammation, and connective tissue damage. It follows that understanding the pathophysiology of photoaging, and identifying new pharmacologic agents to prevent it, may also provide insight into a wide range of human diseases.

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