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DAMAGE TO DNA BY UV LIGHT AND ACTIVATION OF TRANSCRIPTION FACTORS

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Abstract—Bacteria react to irradiation with short wave length UV (UVC) by mounting a rescuing response which involves the synthesis of proteins engaged in DNA repair, replication and mutagenesis. We analyse here an analogous response shown by mammalian cells in culture and present experimental evidence for the chain of events induced by UV irradiation that leads to enhanced gene expression. Available results suggest that the UV induced signal cascade depends on damage to DNA and also involves components located at the plasma membrane, such as src, ras and raf. These components, upon activation by UV, signal into the cell's nucleus, thereby activating transcription factors which control the activity of UV responsive genes.

With continuous use of chlorofluorocarbons, the stratospheric ozone layer appears to be reduced over time [1]. In certain areas of the world the thinner ozone layer is expected to lead to an increased dose of UVC† reaching the earth's surface and consequently to an appreciable increase in skin cancer. Two UVC-induced processes may contribute to carcinogenesis. UVC suppresses parts of the immune system of the affected organism [2, 3] and it induces mutations, some of which may change the structure of regulatory genes (proto-oncogenes, suppressor genes). The accumulation of such mutations may lead to the formation of fully transformed cells which are not recognized by the immunocompromized organism and thus can develop into aggressive skin cancers.

With the idea in mind that for both UV induced endpoints, the suppression of the immune system and cell transformation, cellular reactions occurring in all or many cells are obligatory intermediates, we investigated such cellular reactions. The most impressive UVC induced process detectable in all cells examined, is the increased synthesis of certain proteins. Affected proteins include the transcription factor subunits c-Fos and c-Jun, stress associated proteins such as metallothionein IIA, differentiation-associated proteins such as the MHC class II associated invariant chain, several secreted proteases such as collagenase I and plasminogen activator, and several growth factors (e.g. interleukin 1α and basic fibroblast growth factor). Also several viral genomes are activated in UV irradiated mammalian cells (e.g. HIV-1 and SV40; for a more complete list of UV induced proteins see Ref. 4).

UV induced protein synthesis depends on UV induced accumulation of the respective RNAs and occurs maximally at UV doses which a person would receive during a 1 hour walk on a sunny day in the mountains (altitude 6000 feet; Ref. 5). The accumulation of several mRNAs is drastic and rapid: for instance in HeLa cells c-jun RNA accumulates over 200-fold-upon irradiation with 30 J/m^2 within 60 min [6, 7], and also c-fos RNA is rapidly induced [8]. In contrast collagenase and metallothionein IIA RNA accumulate with much slower kinetics [9]. The instantaneous accumulation of c-fos and c-jun RNA upon UV irradiation does not depend on the synthesis of regulatory proteins and is entirely mediated by pre-existing cellular factors. In contrast, UV induced accumulation of collagenase, plasminogen activator and metallothionein IIA mRNAs depends largely on the prior synthesis of regulatory proteins [10], presumably transcription factors.

Short wave length UV irradiation induces the accumulation of RNAs by increasing the rate of transcription of the genes, which code for the respective RNAs. This has been shown by nuclear "run on" analyses and by showing that recombinant genes containing the promoters of endogenous UV responsive genes linked to reporter genes respond to UV after transient or stable transfection into cells in culture (for review see Ref. 4). UV induces gene transcription by eliciting a signal, much in the same way as do phorbol ester tumor promoters or growth factors, and not indirectly, e.g. by first inducing mutations, gene rearrangements or gene amplification. This is supported by the fact that UV induced transcription of for instance the c-jun gene occurs in minutes and that the protein accumulates in all cells (Fig. 1).

Using the methods that led to the understanding of growth factor induced gene transcription, it should be possible to define the chain of events occurring after UV irradiation and ending with enhanced gene transcription. We will describe the UV induced

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† Abbreviations: UVC, short wave length ultraviolet irradiation; MHC, major histocompatibility complex; SV40, simian virus 40; AP-1, activator protein 1; HIV-1, human immunodeficiency virus 1; NFκB, nuclear factor κB; MAP-2, mitogen activated protein kinase 2; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; CAT, chloramphenicol acetyltransferase; URE, UV responsive element; ROI, reactive oxygen intermediate; MEK, MAP kinase/ERK-activating kinase.

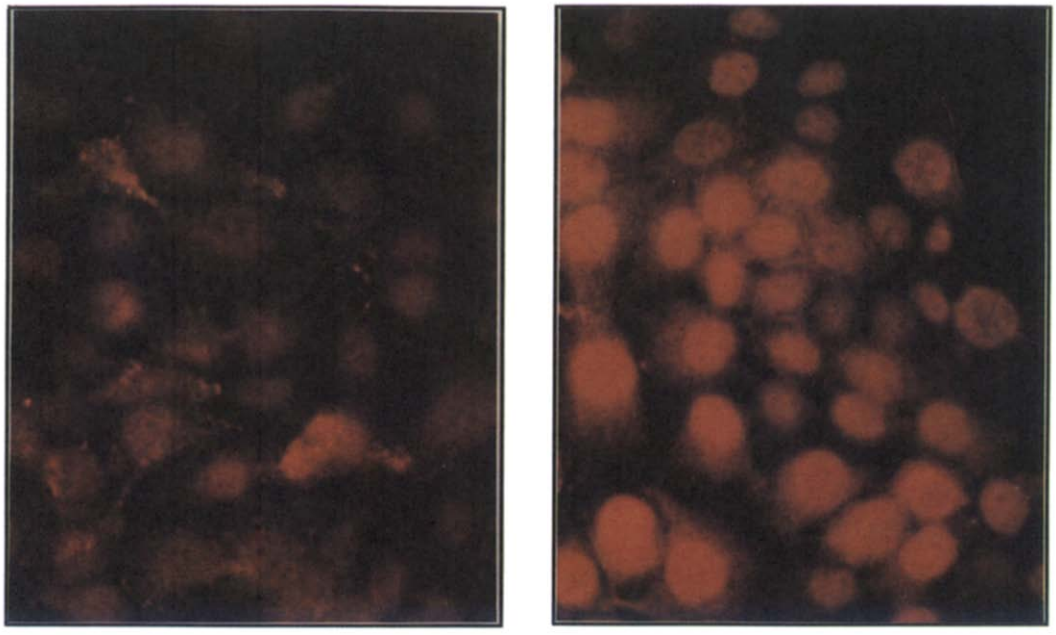

Fig. 1. UV-induced accumulation of c-Jun protein in keratinocytes. c-Jun protein was visualized in UV irradiated (UV) and mock treated (control) human immortalized keratinocytes (HaCaT, Ref. 43), harvested at 3 hr after irradiation. Fixed cells were incubated with a polyclonal anti c-Jun antibody (Ab-1, *Oncogene Sci*), followed by application of a rhodamine-conjugated anti rabbit IgG antibody and detection by fluorescence emission at 529 nm.

pathway of signal transduction, discussing in subsequent sections where the signal chain ends, where the signal could be generated and which essential components mediate the UV induced signal cascade.

(1) *UVC responsive genes possess enhancers controlled by transcription factors which are post-translationally activated by UVC*

With the technology available to isolate eukaryotic genes, to reintroduce them into cells and to analyse their expression independently of the expression of the respective endogenous genes, it was possible to answer the question what distinguishes UVC inducible genes from genes that do not respond to UVC. In all cases so far analysed, the nucleotide sequence (UREs) recruiting genes to the UV response, maps to the promoter/enhancer of the responsive genes. In contrast to the situation with e.g. glucocorticoids or heavy metals, the UREs differ from one gene to the other and bind different transcription factors. Moreover, in all cases examined, the enhancers which are addressed by UVC, are also addressed by growth factors and by phorbol ester tumor promoters. Thus UVC makes, at least in part, use of existing signal chains used by physiologic modulators of cell growth and differentiation. Like these more physiologic agents, UV modulates the activity of several transcription factors.

The following examples should serve to illustrate the above issue. The major UVC responsive element in the collagenase promoter is the AP-1 (c-Fos/c-Jun) binding site located between positions −72 and −66. Other elements located 5′ of this site contribute to UVC induction of the gene, but they are inactive in the absence of the AP-1 binding site [11, 12]. The AP-1 binding site also mediates phorbol ester and growth factor induced collagenase gene transcription [13]. UV, phorbol esters and several growth factors (e.g. interleukin 1α) induce the promoter of the c-jun gene through two AP-1 like binding sites located between positions −71 and −64 and −190 and −183 [7, 14, 15, I. Herr *et al.*, in preparation]. Both sites bind AP-1 complexes composed of c-Jun/ATF-2 or c-Jun/ATFa [15, M. Duyndam, unpublished]. The induction of the HIV-1 long terminal repeat by UVC and phorbol esters depends critically on the binding of the cytoplasmic transcription factor NFκB [11, 16, 17]; and the activation of the c-fos gene by serum, phorbol esters and UVC is mediated by the dyad symmetry element located between positions −300 and −320 and binding the serum response factor (p67) and p62^{TCF} [8, 18–21]. In all cases the UREs are not only necessary for the induction of the genes which they control, but they are sufficient

to mediate the induction process; for instance the AP-1 binding element of the collagenase gene cloned in front of a TATA box and a reporter responds to UVC. The response is fast and does not require the synthesis of regulatory proteins, showing that the delayed induction of the endogenous collagenase gene which, as described above, requires protein synthesis, depends on regions of the gene that modulate the activity of the AP-1 binding site [12, H. P. Auer, unpublished].

UVC irradiation causes increased activity of the pre-existing transcription factors that control the UVC responsive enhancers. Since it has been shown that c-Jun/c-Jun homodimers transactivate the AP-1 binding site of the collagenase gene also in the absence of c-Fos [22], we examined first the fate of c-Jun protein in UV irradiated (in comparison with the fate in phorbol ester treated) cells. In order to examine pre-existing c-Jun protein exclusively, we labeled the cells before with either ^{35}S-methionine or inorganic ^{32}P and isolated the c-Jun protein within minutes after treatment of the cells by precipitation with antibodies directed against c-Jun. UVC irradiation induces increased phosphorylation of serines 73 and 63 and of a third amino acid in the transactivation domain of c-Jun and dephosphorylation(s) near the DNA binding domain of c-Jun [12, 23]. Both hyperphosphorylation in the transactivating domain and dephosphorylation in the DNA binding domain have also been seen in phorbol ester and growth factor treated cells and in cells in which oncogenes have been overexpressed [24–29]. The modifications of the c-Jun transactivating domain after UVC irradiation of cells are necessary intermediates in the UVC induced signal chain to c-Jun dependent genes, since mutations in serines 63 and 73 abolish UVC induced activation of the c-Jun protein [12, 23]. Dephosphorylations at threonine and serine sites near the DNA binding domain of c-Jun can be mimicked by mutation to alanine. The mutations increase the transactivating capability of c-Jun [30].

We suspect that transcription factors that control the response of other genes to UVC, are also activated by post-translational modification.

(2) *Site of UVC induced signalling: for and against a role of the cell nucleus as primary site of UVC absorption*

An important and currently debated question addresses the molecule absorbing UVC. Absorption would alter the structure of the molecule which then would either be recognized by a signal-generating protein or itself form a messenger. UVC could be absorbed by many different cellular components. Several lines of experimental evidence are best compatible with the hypothesis that nuclear DNA absorbs the energy and that subsequent UVC induced DNA damage is recognized by a protein that generates the decisive signal. Cells unable to repair UVC induced DNA damage (such as *Xeroderma pigmentosum* cells) are induced to transcribe responsive genes by lower UV doses than repair proficient cells. This has been reported for plasminogen activator [31], collagenase, metallothionein IIA and HIV-1 [11] determined at 48, 36, 36 and 42 hr, respectively, after UVC irradiation. The earlier the time point of harvest the less difference was observed between *Xeroderma pigmentosum* and wild type cells (see c-fos in Fig. 2). The observation could be interpreted to indicate a need for DNA damage for the UVC induced initiation and/or maintenance of transcription. DNA lesion density is likely to be identical between wild type and

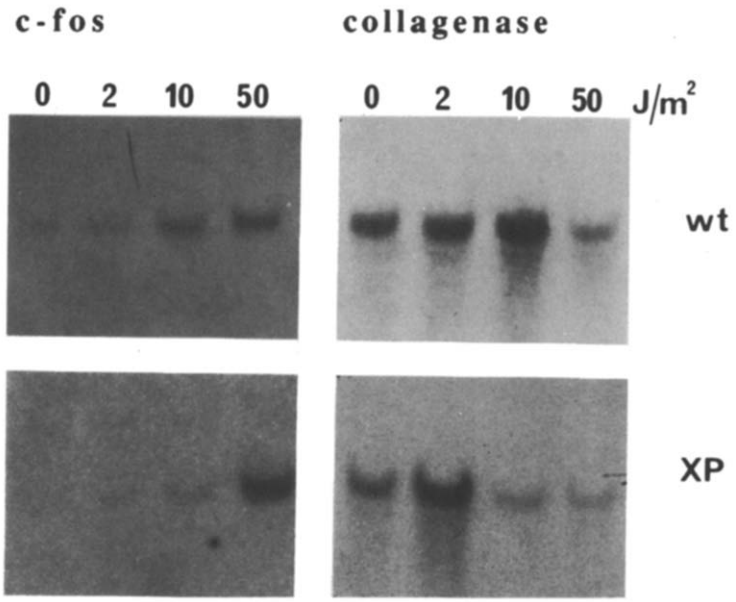

Fig. 2. UV-induced accumulation of c-fos and collagenase mRNA. Primary human fibroblasts derived from a normal donor and from a *Xeroderma pigmentosum* group A patient (GM2994) were irradiated with short wave length UV and the indicated doses. RNAs were prepared 45 min (c-fos) and 36 hr after UV irradiation (collagenase 1) and analysed by northern blot analysis with the probes indicated (for experimental details see Ref. 11).

Xeroderma pigmentosum cells in the first minutes after UV irradiation, but diverges with ongoing repair. Thus in order to determine whether DNA damage is also a prerequisite for the early cellular reactions, other experimental approaches are necessary. An interesting aspect has been introduced by cell fusion experiments. Cells that did not contain a responsive gene (the globin genes) in active conformation, were irradiated and then fused to non-irradiated erythroleukemia cells [32]. These responded by expressing hemoglobin in a dose-dependent manner (dose to the fusion partner). The irradiated cell must have carried a "message" molecule sufficiently long-lived. This could be damaged DNA. A more telling approach would be to co-transfect, or co-inject, together with UV responsive gene constructs, UV damaged DNA into cells and to determine, whether damaged DNA provides a signal for enhanced transcription of the reporter constructs. Attempts in our laboratory yielded variable results, not allowing clear conclusions. Further attempts are ongoing. Compatible with DNA as the primary and necessary UVC target is the spectrum of UV induced gene expression. The spectrum resembles that of UV induced cell killing. Both cell killing and enhanced gene transcription may therefore be mediated by the same primary event, namely damage to DNA [11]. Also experiments with enucleated cells seem to support a need for the cell nucleus in induction. The experiments were made possible by the finding that UVC activates several protein kinases, such as c-src, c-raf and the mitogen activated protein kinase (MAP-2). We found that cytoplasts and platelets do not respond to UVC with the activation of MAP-2 kinase (while responding to growth factors and to phorbol esters), suggesting that the cell's nucleus plays an obligatory role also in the very early reactions to UV irradiation. Thus, in order to stick to a coherent minimal mechanism, one may assume that DNA lesions are decisive (limiting) events for both initial and late steps in transcriptional induction.

It has been argued that UV induced reactive oxygen species and radical induced membrane damage may be the signals triggering all subsequent steps of the UVC induced signal cascade. Radical scavengers indeed interfere severely with the UVC response [23, our own unpublished observations].

Table 1. Activation of cytoplasmic compounds by UV

src-	Tyrosine kinase activity
fyn-	Tyrosine kinase activity
ras-	GTP binding
raf-	Serine/threonine kinase activity
MAP-2-	Serine/threonine kinase activity
NFκB-	Cytoplasmic transcription factor

Refs. 11, 12 and 23.

The following two arguments seem to speak against the hypothesis: (1) radiation qualities such as long wave length UV and X-rays, which produce reactive oxygen intermediates much more efficiently as compared to short wave length UV [33, 34] induce the UV response much less efficiently than UVC [11, Blattner, Litfin and Rahmsdorf, unpublished]. (2) The difference in dose dependence between wild type and *Xeroderma pigmentosum* cells (which can only be detected with "late" reactions to UV irradiation, see above) could not be explained on this basis, since these two cell types do not differ in the repair of DNA damage induced by ROIs (except for one type of damage, which has not yet been defined, Ref. 35). Thus at the moment a reasonable hypothesis is, that radical scavengers lower the endogenous cellular levels of radicals and that the UV response needs a high level of radicals in order to proceed efficiently.

(3) *The UV response involves cytoplasmic and possibly membranal components*

With UV lesions in DNA playing a role and responding transcription factors such as the nuclear transcription factor AP-1 waiting in the nucleus one could argue that the UV response is a totally "intranuclear affair". That this may not be the case has been noticed very early. UV induces systemic effects in organisms, such as the suppression of parts of the immune system; UVC irradiation of cells in culture gives rise to the secretion of growth factors into the culture medium [36–38]; UV irradiation induces the activation of the cytoplasmic transcription factor NFκB [11]. With the identification of the growth factors released from UVC irradiated HeLa

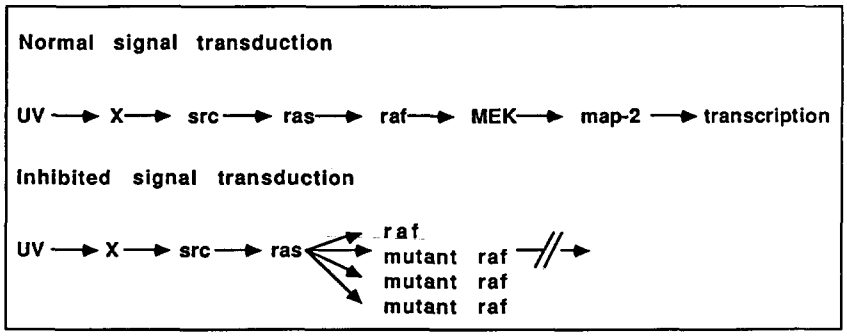

Fig. 3. Interruption of signal transduction by transfection of plasmids coding for mutant proteins.

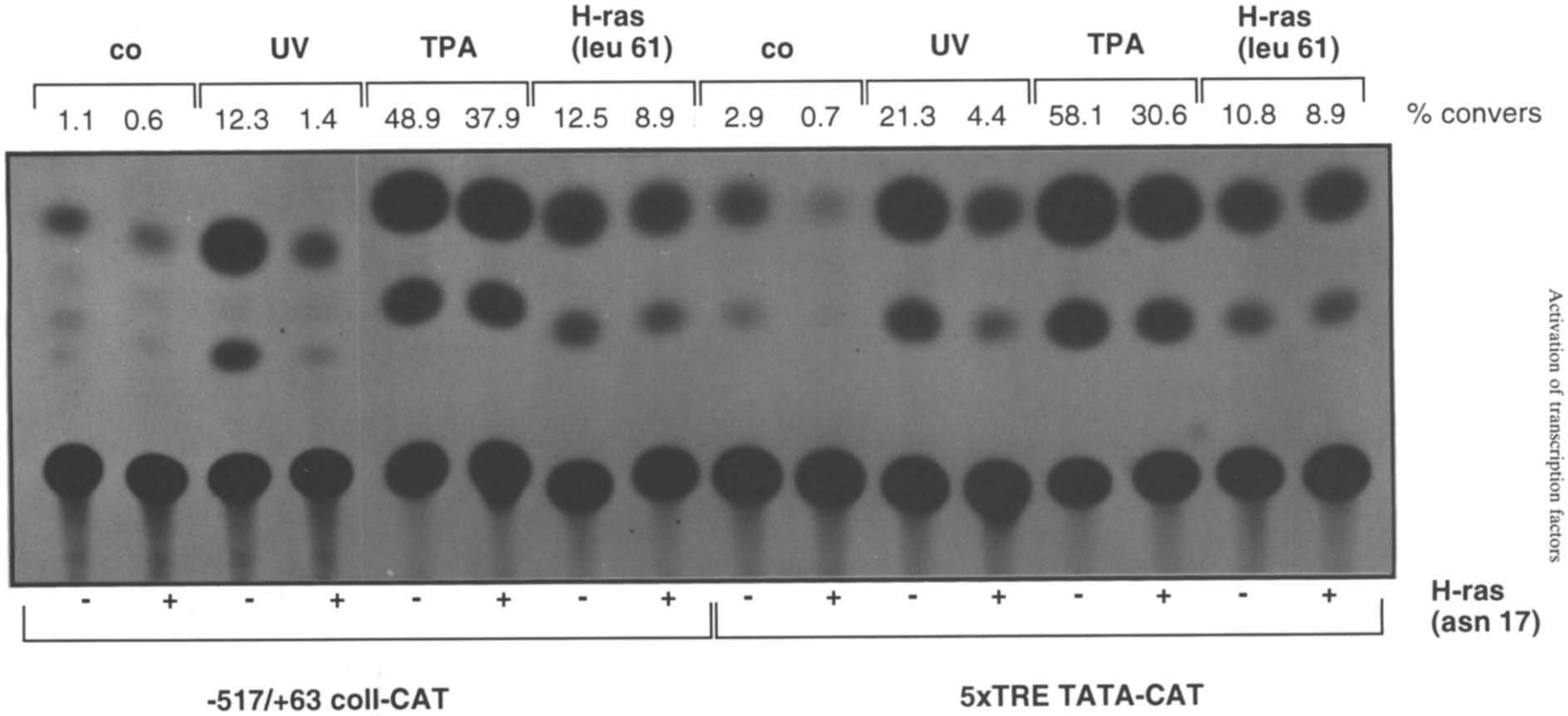

Fig. 4. A dominant-negative ras mutant inhibits UV, but not phorbol ester induced expression of AP-1 dependent promoter constructs. HeLa tk^- cells were transiently transfected with either a promoter fragment of the human collagenase gene (+517/+63 coll-CAT) or a pentameric fusion of the minimal AP-1 binding site (5xTRE TATA-CAT) fused to the chloramphenicol-acetyltransferase (CAT) reporter gene [44]. Where indicated, cells were co-transfected with an empty expression vector (−) or expression vectors coding for H-ras (asn 17, a dominant negative ras mutant, e.g. Ref. 42) (+) or H-ras (leu 61, oncogenic ras, e.g. Ref. 45). After transfection (24 hr), cells were treated with UV (30 J/m^2) or phorbol esters (TPA, 60 ng/mL) and CAT-enzyme activity was determined after an additional 24 hr (Ref. 12).

cells, as interleukin 1α and basic fibroblast growth factor [39], it was possible to ask, whether the secretion of these growth factors is a necessary event in UVC induced collagenase mRNA accumulation. Antibodies directed against interleukin 1α and basic fibroblast growth factor, if given to the culture medium immediately after UV irradiation, inhibited most of UV induced collagenase mRNA accumulation, suggesting that at least part of this UV induced response was mediated through the growth factors [39]. The same experimental approach did, however, not substantially inhibit the early responses to UV irradiation, such as UV induced transcription of c-fos and c-jun (Sachsenmaier, unpublished), suggesting that growth factors were not responsible for the initial phase of the UV response.

Interestingly, however, the early cellular reactions to UVC can still be blocked at the cell surface. Suramin, an inhibitor of growth factor – growth factor receptor interactions [40], does not only inhibit efficiently and specifically UV induced collagenase mRNA accumulation [39], but also the early reactions to UV, such as induced transcription of c-fos and c-jun, induced c-jun phosphorylation and activation of cytoplasmic protein kinases (Radler-Pohl and Sachsenmaier, unpublished). Suramin does not interfere with early or late phorbol ester induced processes, suggesting that suramin does not cross the cell membrane or does not interact with protein kinase C.

It has been know for some time that treatment of cells with several inducers of cell multiplication is followed by a period of non-responsiveness. For instance, cells pretreated with phorbol esters, are thought to lose protein kinase C. Reactivity only reappears after resynthesis of the enzyme. Also cells irradiated with UVC do not respond to a second treatment with UVC for 24 hr after the initial irradiation [8]. Non-responsiveness could help to identify components deactivated after UVC treatment and, in turn, could define whether other agents deactivate components needed for UVC induction. Pretreatment of cells with several growth factors, such as epidermal growth factor, interleukin 1α or basic fibroblast growth factor interferes severely with the early UV induced processes such as induced transcription and protein kinase activation. Although other explanations are possible, the simplest hypothesis is, that pretreatment with growth factors leads to the internalization and/or down-regulation of the respective receptors, and that the UV induced signal chain involves the activation of these growth factor receptors (which are not any more available in cells pretreated with growth factors). Alternatively, a receptor dependent membrane proximal step could be used by both growth factors and UVC.

If UV indeed worked through the activation of growth factor receptors, than the components of the signal cascade which are activated by growth factors [e.g. Ref. 41] should also be activated by UVC. This is indeed the case. In minutes after UV irradiation of cells in culture the membrane-associated tyrosine kinases src and fyn are activated, the GTP-bound form of ras is increased, and the cytoplasmic protein kinases raf and MAP-2 are activated [12, 23; Table 1]. A way to show that the UV induced activation of these regulatory proteins is an obligatory step in the UV induced signal cascade to responsive genes is to block the function of the endogenous proteins by co-transfecting, with UV responsive reporters, gene constructs coding for mutated negative-dominant proteins, which still receive but cannot forward the upstream signal (Fig. 3). Using such mutants it was shown, that src, ras and raf are obligatory intermediates in the UV induced signal chain [12, 23]. Figure 4 shows for instance that a dominant negative ras mutant (H-ras [asn17], Ref. 42) inhibits efficiently the induction of two AP-1 dependent reporter constructs (−300/+63 coll-CAT; 5xTRE TATA-CAT) by UVC, while only poorly interfering with the induction of these same gene constructs by phorbol esters or constitutively active ras. Induction of the two reporters by oncogenic ras (H-ras, leu 61) is not inhibited by the negatively acting ras mutant because the oncogenic form is independent of incoming signals. Since dominant negative raf-mutants inhibit both, UV and phorbol-ester induced transcription of the reporters [12], our results suggest, that in HeLa cells UV, in contrast to phorbol esters, works through ras, and that the UV and phorbol ester induced signal chains meet at the level of raf kinase. Activation of raf kinase then leads, probably through MAP kinase-kinase (MEK) and MAP-kinase, to the activation of the transcription factor c-Jun.

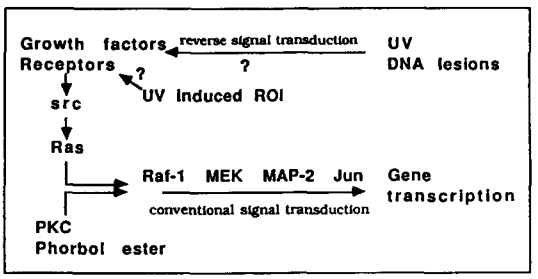

Fig. 5. Hypothetical scheme of UV induced signal transduction.

Conclusion

The experimental work of the last decade revealed a completely new aspect of UVC action in eukaryotic cells; a similar type of action has only previously been detected in bacteria. UVC induced DNA damage not only leads to cell killing and to mutations in a minority of the affected cells, but works as a signalling molecule, just as do growth factors or hormones for the induction of transcription. It is debated whether the initial phase of induction is triggered by reactive oxygen intermediates or by a combination of DNA damage and reactive oxygen intermediates. The signalling cascade induced by UV appears to be complex (Fig. 5). Wherever generated, the signal seems to reach the cell membrane and to activate growth factor receptors or other membrane associated protein kinases. This leads to ras activation and to the activation of the

cytoplasmic protein kinases raf and MAP-2. Depending on the cytoplasmic components nuclear transcription factors are activated which regulate the transcription of UV responsive genes.

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