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The Molecular Basis for the UV Response of Cultured Human Cells

Progress Report

During the past year we have analyzed the mechanism by which UV and another DNA damaging agent, H_2O_2, increase the activity of transcription factor AP-1 in HeLa cells. Previous work implicated AP-1 as a transcription factor responsible for induction of several target genes during the mammalian UV response (1,2). AP-1 is a dimeric protein complex composed of the *jun* and *fos* gene products (3,4). We found that upon UV irradiation of HeLa cells or treatment with H_2O_2 the *c-jun* gene is rapidly and dramatically induced, at least 50-fold. Expression of the two other *jun* genes, *junB* and *junD*, is not affected by UV or H_2O_2. These results indicate that the major contributor to increased AP-1 activity during the UV response is the *c-jun* gene (5). Therefore, we analyzed the *c-jun* promoter to identify its UV response element. This analysis indicated that the induction occurs through the previously identified AP-1 site in the c-jun promoter (6). Mutagenesis of this site eliminates the response of the *c-jun* gene to UV (5). Other work done in our laboratory indicated that the positive autoregulation of *c-jun* expression is mediated by post-translational modification of pre-existing AP-1 complexes that are composed mostly of cJun (7,8). In support of this notion we find that UV and H_2O_2 lead to rapid increase in AP-1 activity that is not prevented by protein synthesis inhibitors (5). We have previously shown that cJun is a phosphoprotein that undergoes complex changes in its phosphorylation in response to phorbol esters, growth factors and transforming oncogenes and that these changes modulate its ability to bind DNA and activate transcription (7-9). To examine whether UV irradiation affects cJun phosphorylation we isolated cJun

protein from ^{32}P labelled cells before and after exposure to UV. We find that UV irradiation leads to increased phosphorylation of cJun (unpublished results).

We have also been trying to determine what is the common signal by which DNA damaging agents lead to activation of the UV response. One likely mechanism could involve either damaged DNA or a by-product of DNA damage as the primary signal leading to activation of the UV response. We tested this possibility by cotransfecting *jun*-CAT and AP-1-CAT reporter constructs into HeLa cells together with non-damaged and damaged (UV irradiated) plasmid or phage DNA. Despite many attempts neither of these reporters, whose expression is induced by UV or H_2O_2, was activated by cotransfection with damaged DNA. This suggested to us that DNA damage itself may not be the primary signal eliciting the UV response. A common denominator of many DNA damaging agents, including UV, H_2O_2 and ionizing radiation, is the induction of oxidative stress within the cell. A change in the cellular redox potential can therefore be the signal that elicits the UV response. We tested this possibility by examining the effect of N-acetyl-cysteine (NAC), a reducing agent, on the induction of AP-1 activity by UV and H_2O_2. We found that NAC completely inhibits AP-1 induction by both of these agents (unpublished results). These findings support the hypothesis that oxidative stress may serve as a signal for AP-1 induction during the UV response.

The other part of the sponsored project concerns the purification and characterization of UVIC, a factor secreted by UV-irradiated cells that protects non-irradiated cells from radiation damage. We have shown that either UVIC or a similar factor is also induced by treatment of HeLa cells with H_2O_2, another type of DNA damaging agent. Our previous results indicate that UVIC application can protect HeLa and rat embryo fibroblast cells against UV induced killing. During the past year we also tested the ability of UVIC to protect HeLa cells against the lethal effect of UV irradiation using a clonogenic assay. We found that UVIC leads to at least 10-fold increase in cell survival. We have also

performed small scale purification of UVIC and were able to identify it as a 17 kd polypeptide that retains UVIC activity after elution from an SDS-polyacrylamide gel and renaturation. By labelling cells with ^{35}S-methionine we found that UV irradiation induced the secretion into the culture medium of polypeptide of an identical size. We are now in the process of scaling up our UVIC production and purification efforts.

New Publications Supported by DE-FG03-86ER60429

1. Boyle, W.J., Smeal, T., Defize, L.H.K., Angel, P., Woodgett, J.R., Karin, M. and Hunter, T. (1991). Activation of protein kinase C decreases phosphorylation of cJun at sites that negatively regulate its DNA binding activity. Cell 64:573-584.
2. Devari, Y., Gottlieb, R., Lau, L.F. and Karin, M. (1991). Rapid and preferential activation of the *c-jun* gene during the mammalian UV response. Mol. Cell. Biol. 11:2804-2811.
3. Smeal, T., Binetruy, B., Mercola, D.A. and Karin, M. Oncoprotein mediated signalling cascade potentiates the transcriptional activity of cJun by stimulating phosphorylation of serines 63 and 73. Submitted for publication.
4. Devari, Y., Meinkopf, J. and Karin, M. Induction of a novel cytostatic and protective factor by DNA-damaging agents. In preparation.

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