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COMMENTARY

Adaptive Response to DNA-Damaging Agents

A REVIEW OF POTENTIAL MECHANISMS

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ABSTRACT. The study of the adaptive response, i.e. a reduced effect from a higher challenging dose of a stressor when a smaller inducing dose had been applied a few hours earlier, has opened many new vistas into the mechanisms by which cells can adapt to hazardous environments. Although the entire chain from the initial event, supposedly the presence of DNA damage, to the end effect, presumably improved DNA repair, has not been fully elucidated, many individual links have been postulated. Initial elements—following the still unknown signal for the presence of radiation damage—are various kinases (protein kinase C and stress-activated protein kinases), which, in turn, induce early response genes whose products initiate a cascade of protein–DNA interactions that regulate gene transcription and ultimately result in specific biological responses. These responses include the activation of later genes that can promote production of growth factors and cytokines, trigger DNA repair, and regulate progress through the cell cycle. Indeed, there appears to be a relation between the induction of the adaptive response and the effects of radiation and cytostatic agents on the cell cycle, although these effects, especially the G_1 delay, occur at much higher doses than the adaptive response, and one may not indiscriminately extrapolate mechanisms responsible for cell cycle changes observed at high doses, e.g. for radiation in the order of grays, to those involved in the adaptive responses at much lower doses, i.e. some tens of milligrays. BIOCHEM PHARMACOL **55**;7:941–951, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. adaptive response; cell cycle modifications; ionizing radiation; cytostatic agents

Cells, tissues, or organisms can often improve their ability to respond to a "challenging" stress when they have been exposed previously to a smaller, "inducing" amount of the same or a similar stress. Such an adaptive response has been noted after exposure to a variety of stressors such as ionizing radiation, radiomimetic chemicals, oxidative agents, alkylating compounds, or heat, but most of the information on the presence and the mechanisms of the adaptive response has been gathered for radiation exposure. Consequently, such studies are prominent in the present review, but this should not detract from the possibility that adaptive responses to drugs acting on DNA might be even more important from a practical point of view and that an understanding of the mechanisms involved has bearing also on problems related to tumor resistance to oxidative stresses acting via free radical mechanisms.

With regard to ionizing radiation, an adaptive response has been observed in single cells as well as in the entire organism. For single cells *in vitro*, adaptive responses have been reported with respect to the induction of chromosome aberrations, sister chromatid exchanges, or micronuclei in human lymphocytes [1–8], in Chinese hamster V79 cells [9], in rabbit lymphocytes [10], in C3H10T1/2 mouse embryo cells [11], and in hepatoma cell lines [12].

An adaptive response to ionizing radiation has also been found for deletional mutations *in vitro* in human T-lymphocytes, in a lymphoblastoid AHH-1 cell line, in a T-cell leukemia line [13–15], and in A_L human–hamster hybrid cells [16]. Other observations were made for neoplastic transformation *in vitro* in C3H10T1/2 cells [11], for cell survival and DNA synthesis in V79 cells [17], for cell survival in U1-Mel and Hep-2 human neoplastic cells *in vitro* [18], lymphocytes *in vitro* [5], and spleen T-lymphocytes *in vivo* [19], for thymic lymphoma incidence in irradiated mice [20], and for dominant lethal mutations in *Drosophila melanogaster* [21]. Doses used for the initiation of an adaptive response are usually in the order of tens of milligrays with challenging doses in the order of grays.

Adaptive responses appear also to occur *in vivo* in bone marrow and germ cells following irradiation of animals with low acute or chronic doses [10, 22–25]. It has also been claimed that fewer dicentrics are induced in lymphocytes exposed to a given dose of X- and γ-rays *in vitro* when these cells originate from people occupationally exposed than when they are collected from people not working with radiation [26].

Other agents besides ionizing radiation, such as radiomimetic chemicals, alkylating agents, or cross-linking agents, can evoke an adaptive response whereby frequently, although not always, ionizing radiation can be replaced by

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chemicals as inducing or challenging agents. Such a substitution of challenging/inducing agents might suggest that some pathways in the reaction chain are common to these agents, although others, such as the DNA lesions caused by these agents, the inducing signals, or even the repair, may not necessarily be so. Cross-reactions have been demonstrated *in vitro* or *in vivo* with mitomycin C, hydrogen peroxide, bleomycin, tritiated thymidine, actinomycin D, and UV B radiation [9, 15, 27–36] but not with ethylmethanesulfonate, methylmethanesulfonate, cisplatin, or cyclophosphamide [9, 28, 35].

Lymphocytes from individuals occupationally exposed to ionizing radiation [37] or internally contaminated with ^{137}Cs from the fallout of the Chernobyl accident [38] were reported to also have a decreased sensitivity to an *in vitro* exposure to bleomycin.

Several studies with human lymphocytes *in vitro* suggest that the human population is heterogeneous, perhaps in part on genetic grounds, with respect to the adaptive response to ionizing radiation [39–41]. Other authors have pointed out, however, that the variability in the response observed might not be due to an enhanced cellular radiation resistance, but rather to some factor associated with variations in cell cycle kinetics [42, 43]. In this context, it should be mentioned that an inducible, genetically determined multidrug resistance has been demonstrated for anticancer drugs [44].

Adaptive response and cross-resistance have been reviewed in the past [45]. However, progress in this area has been so rapid that a new review of the mechanisms of the adaptive response appears justified.

MECHANISMS OF THE ADAPTIVE RESPONSE

Several observations are characteristic for the adaptive response:

The adaptive response after an inducing dose is not instantaneous but requires about 4–6 hr to develop full activity against a challenging exposure [9]. The adaptive response recedes during the following hours and, usually, is not detectable after a few days;

The adaptive response can be prevented if, during this latency period, protein synthesis is inhibited by cycloheximide [9, 46]. This protein synthesis is regulated at the transcriptional level as shown by experiments with an inhibitor of RNA synthesis [9]. Newly formed proteins can be detected by electrophoresis in the induced irradiated cells [30, 47]; one of these proteins binds specifically to radiation-damaged DNA [48].

The adaptive response is prevented by 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase; this enzyme is induced in the presence of DNA strand breaks and implicated in DNA repair [9, 49]. Indeed, recent studies on poly(ADP-ribose) polymerase suggest that this system may be involved in a pathway protecting cells from downstream events of DNA damage [50].

Although most of these observations have been obtained for ionizing radiation as an inducing and challenging agent, similar mechanisms are likely to be operative for exposure to other agents including cytostatic agents and those causing oxidative stress. It thus appears that the adaptive response originates from a, still poorly defined, signal due to cellular (DNA) damage, proceeds through a chain of events involving synthesis/activation of certain proteins and genes, and results in a state where repair of DNA damage is, temporarily, improved, presumably by activation of DNA repair enzymes. Thus, as shown recently by Ikushima *et al.* [51], it appears that the adaptive response is due to improved DNA repair, resulting in less residual damage, rather than to protection from initial damage. Several of the reactions involved in the adaptive response seem to be a common feature of the cellular stress response and involve the formation of protective proteins, as does also the response to heat shock [52]. At the present time, several components of this chain of events have been identified, but the elucidation of the entire chain, including the identification of the eliciting signal, remains a major scientific challenge to molecular biology. Indeed, these mechanisms by which cellular integrity can be maintained following stress have bearing for many aspects of health and disease.

Today, a review on the mechanisms of the adaptive response may often appear like an accumulation of unrelated data, since relations between the different changes observed for the adaptive response remain speculative and since the time- and dose-related appearance of the different changes has not been systematically explored. Future research must direct efforts to establishing such links.

EARLY RESPONSE: ACTIVATION OF PROTEIN KINASES

Some of the earliest specific changes observed after an exposure to ionizing radiation and other stressors are the induction of certain protein kinases (Fig. 1) such as:

p44/42 MAPKs†
SAPKs
serine specific protein kinases PK50 and PK55

† *Abbreviations:* AP-1, activator protein 1; CDK, cyclin-dependent kinase; CREB, cAMP-responsive element-binding protein; EGR, early growth factor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; bFGF, basic FGF; FRA, fos-related antigens; GADD, growth arrest and DNA damage-inducible gene; HSP, heat shock protein; *jun*, cell-derived oncogene of ASV 17 (avian sarcoma virus 17), abbreviated from the Japanese ju-nana, the number 17; MAPKs, mitogen-activated protein kinases; MEKs, mitogen-activated protein kinase kinases, protein kinases that phosphorylate MAPK polypeptides at the tyrosine and threonine residues whose phosphorylation is required for activation; MEKKs, MEK kinases (activate the SAPK activator SEK1); NF-κB, nuclear factor-κB; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor (30,000 MW glycoprotein); PKC, protein kinase C; PLDR, potentially lethal damage repair; RAG-1, recombination activating gene; SAPKs, stress-activated protein kinases; SEK, SAPK/ERK kinase protein kinase, which phosphorylates SAPK at Tyr. SEK1 is an upstream activator of the SAPKs; SP1, stimulating protein 1; TNF-α, tumor necrosis factor-α; t-PA, tissue-type plasminogen activator; and XIP, X-ray-induced protein.

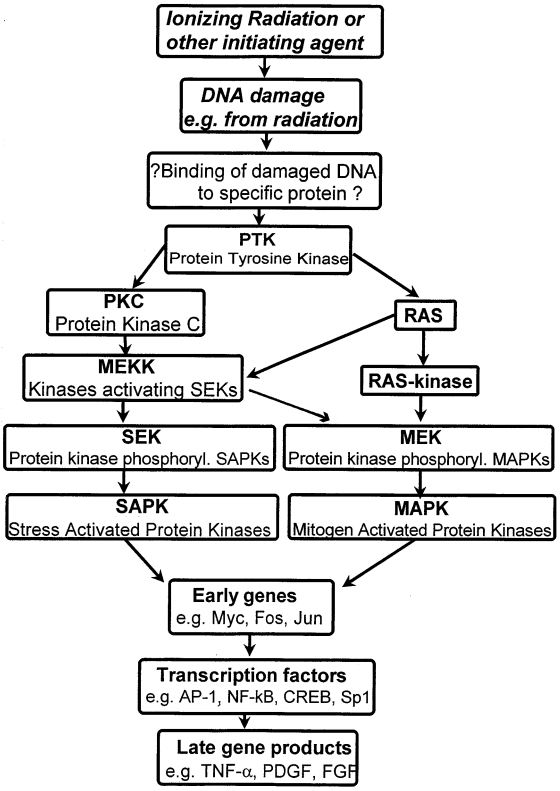

FIG. 1. Putative scheme of the activation of kinases, early and late gene products by ionizing radiation or other inducing agents.

Exposure to ionizing radiation causes an activation of both p44/42 MAPKs (e.g. p42 or ERK-2, p44 or ERK-1, p45, p55, and p80) and SAPKs (also known as JNKs or $p54^{MAPK}$); these kinases pass the signals induced by damage to the nucleus and regulate the expression of early response genes such as *jun*, *fos*, and *egr* that code for the corresponding transcription factors. SAPKs belong to a family of p54/p46 serine/threonine kinases related to the MAPKs [53]. Although both MAPKs and SAPKs are induced following exposure to ionizing radiation, MAPKs seem to be the ones preferentially activated by certain growth factors or phorbol esters [54], whereas SAPKs are induced by a variety of stressors such as UV light, ionizing radiation, heat shock, or protein synthesis inhibitors. It appears that, following a stimulation, SAPKs are more active than MAPKs in phosphorylating c-Jun. In addition to the well-studied effect on the c-*jun* gene, the activation of these kinases probably also regulates several other, still unknown, substrates.

An important hint as to the role of signals from radiation damage upstream of SAPKs comes from observations [55–58] showing that radiation-induced activation of SAPK is missing or reduced in ataxia-telangiectasia cells, i.e. cells that are more sensitive to DNA damage and bear a genetic defect in a gene homologous with phosphatidylinositol-3′-kinase. It is, however, uncertain whether the activation of SAPK represents a direct response to DNA damage or occurs later among the events leading to DNA repair [53].

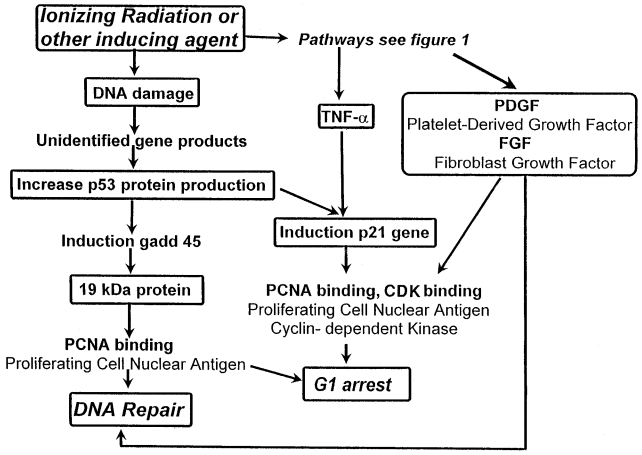

FIG. 2. Putative pathways leading to cell cycle arrest in G_1 phase after exposure to ionizing radiation.

The following kinases were shown to be induced after exposure to ionizing radiation:

Stress-activated phosphoprotein kinase p53 is the product of a tumor suppressor gene involved in cell cycle control. Levels of p53 increase rapidly in response to DNA damage induced by ionizing radiation (1–5 Gy). Interestingly, cells from ataxia-telangiectasia patients lack the radiation-induced increase in p53 levels [59]. p53 functions as a transcription factor for several genes, and increased nuclear p53 levels are required to initiate the G_1 block, suggesting that p53 is a component of the signal transduction pathway leading to an arrest in the G_1 phase. Indeed, activation of p53 correlates with an activation of *GADD45* and the expression of the *p21* gene, and these factors have been found activated after exposure to ionizing radiation [60] (Fig. 2). This observation, together with the known DNA-binding properties of p53, suggests that p53 could act as a transcriptional factor influencing critical gene expression controlling cell cycle progression. Increased synthesis and binding activity of p53 to its consensus DNA sequence have been demonstrated after exposure to several DNA damaging agents (ionizing radiation, mitomycin C, actinomycin D, hydrogen peroxide) but not to the alkylating agent iodoacetamide. p53 is also induced by UV radiation but with a different kinetics than after X-irradiation [61]. In this context, it should be noted that an exposure of mice to low doses (0.25 to 0.5 Gy) of X-rays results in an organ-specific accumulation of p53 protein [62].

It appears that agents causing DNA strand breaks can activate a homeostatic cellular mechanism that results in increased levels of wild-type p53 bound to its specific DNA sites in the genome. This binding could serve to activate transcription of growth arrest genes and/or repress cell cycle genes and thereby halt DNA replication. Indeed, a block in progression through the cell cycle, such as from G_1 to S phase and from G_2 to mitosis, following DNA damage may

have the purpose of allowing time for repair. Indeed, it has been shown [63, 64] that irradiation causes a decrease in the number of S-phase ML-1 cells due to cells arrested in both G_1 and G_2/M. In this study, G_1 arrest predominates at low doses (<1 Gy), and G_2 arrest predominates at higher doses regardless of p53 status. Cells that are in S-phase appear to continue to progress to G_2/M, whereas cells in G_1 do not enter S-phase.

p53 also interacts with transcription factors that participate in nucleotide excision repair. Thus, in addition to transactivating genes involved in cell cycle progression, p53 could interact directly with factors required for the repair of DNA lesions [65]. From observations on the recognition and interaction of p53 with damaged DNA, Reed *et al.* [66] conclude that the action of p53 as a key transcription factor is not the only mechanism by which p53 participates in repair pathways. These authors demonstrated that the C-terminus of p53 is necessary and sufficient to recognize DNA damage and to transfer strand fragments between complementary DNA molecules. Thus, p53 could be a guard against genetic instability by sensing DNA damage and initiating the cascade of events culminating in cell cycle arrest and repair of DNA lesions (Fig. 2).

PKC, a protein of 77 kDa, is activated within 1 hr following exposure to ionizing radiation. The activation of this kinase seems to precede the activation of nuclear signal transducers of the early response genes that subsequently participate in the transactivation of late genes resulting eventually in the adaptive response to ionizing radiation [67, 68]. In turn, PKC is activated by, as yet unidentified, protein tyrosine kinases that are probably sensitive to reactive oxygen intermediates [69]. PKC is known to play a central role in cellular signal transduction as part of a general cellular response in many circumstances. Several *in vitro* experiments have shown that PKC is also associated with an adaptive response following exposure to 0.05 Gy ionizing radiation [17, 47] and that irradiating the whole body of mice with 0.075 Gy causes an increase in PKC activity [70].

The activity of serine specific protein kinases PK50 and PK55, which depend on PKC [69], is also enhanced 15 min after exposure to 2 Gy ionizing radiation.

A specific DNA-binding protein with different sized forms of 70 kDa and 43–47 kDa has been described in human cells exposed to 10 Gy of ionizing radiation. While the 70 kDa protein binds most DNA in unirradiated cytoplasmic extracts [71], the 43–47 kDa band binds DNA preferentially in crude nuclear extracts from irradiated cells [72]. Radiation causes the translocation of the binding activity of a precursor form from the cytoplasm to the 43–47 kDa species present in the nucleus. The appearance of this protein in irradiated cell nuclei is not due to a *de novo* synthesis but rather to an activation of a pre-existing protein. Ionizing radiation and radioimetic agents but not UV-irradiation or heat shock could induce this process, which is of a transient nature and disappears 9 hr postirradiation. This period correlates approximately with the time needed for repair of most radiation-induced DNA lesions and the recovery of DNA synthesis [72]. The envisaged function of this protein is the regulation of gene expression.

EARLY RESPONSE: ACTIVATION OF EARLY RESPONSE GENES

Several early response genes are activated by exposure to ionizing radiation (1–10 Gy) and other DNA-damaging agents (Fig. 1). These genes, which encode a variety of transcription factors in response to the type and size of the upstream signal, belong to the *jun* family: c-jun (39 kDa), jun B (40 kDa), and jun D (40 kDa); the *EGR* family: EGR-1, -2, -3, and -4; and the *fos* family: c-fos (55 kDa), Fra1 (35 kDa), Fra2 (46 kDa), and fosB (45 kDa) [68, 73, 74]. AP-1 is an example of a transcription factor regulated by the *jun* family. The members of the *jun* family differ in their ability to activate AP-1 transcription, and certain specific combinations of members of the *fos* and *jun* family can also transactivate AP-1 as reported in Ref. 75. The regulatory mechanisms governing AP-1-dependent transcription thus involve a delicate network of different regulating factors.

In irradiated cells, c-jun transcripts are degraded post-transcriptionally by a labile protein, suggesting that the increase in c-jun RNA observed after radiation exposure is mediated also, at least in part, by post-transcriptional mechanisms, i.e. the synthesis of a protein that affects the turnover of c-jun RNA. Many target genes activated by these transcriptional factors, however, remain unknown. Most likely, their activation is involved in the mechanism transducing the signal that initiates cellular responses to radiation exposure such as DNA repair, transformation, and inhibition of cell cycle progression.

Other genes also seem to participate in this early response. Thus, Liu [70] observed an activation of the c-*fos* gene and an expression of Fos protein in immune tissues of mice 1 hr after whole body exposure to 75 mGy.

Radiation exposure at low dose rates results, in general, in a greater cell survival and a lower rate of malignant transformed cells than that at high dose rates. Conversely, the low dose rate causes a more marked induction of c-*jun*, and this may explain the more efficient error-free repair under these circumstances. Perhaps, the product of the c-*jun* gene initiates a cascade of DNA repair genes that enhance cell survival and reduce malignant transformation. However, the mechanism and significance of increased transcription of c-*jun* at lower doses are as yet unknown [73].

It was demonstrated that mRNA levels of transcription factors Egr1 and Jun increase following exposure of human cell lines to ionizing radiation in the absence of *de novo* protein synthesis and that the induction of these genes is regulated by a PKC-dependent pathway [68]. Irradiation (4.5 Gy) of the radioresistant human melanoma (U1-Mel)

cell line causes an induction of the binding activity of the transcription factors CREB, SP1, and NF-κB to their consensus sequences [76]. This induction appears to occur independently of transcription or translation processes and suggests that the binding of DNA to pre-existing proteins proceeds via post-translational mechanisms. In this respect, an up-regulation of the binding factors NF-κB and CREB in immune tissues of mice exposed to 75 mGy is noteworthy [70].

p21 (also known as WAF1 or Cip1) mediates G_1 arrest, at least in part, by binding to and inhibiting a variety of CDKs that are involved in cell cycle progression [77, 78]. p53 is responsible for its induction after exposure to ionizing radiation. El-Deiry *et al.* [79] demonstrated that the p53 induction of p21 probably represents a direct effect. These authors pointed out that the fact that binding of p53 to DNA occurs near the p21 promoter region investigated in their study may be coincidental, and p21 may be induced by another pathway. Indeed, Shiohara *et al.* [80] observed that p21 expression is also induced through a p53-independent pathway involving TNF-α.

The *GADD45* gene is induced by ionizing radiation (0.5 to 20 Gy) as shown by an increase in mRNA levels as early as 30 min after irradiation. The product of this gene is a protein of 19 kDa, which seems to play a role in the delay of the cell cycle during the G_1 phase. In contrast to other genes (early response genes) activated after irradiation, its induction is not mediated by PKC but by another protein kinase(s) [81], such as p53 [82]. Gadd45 was found to bind to PCNA. This antigen is a normal component of CDK complexes and represents a protein that is also involved in DNA replication and repair. Gadd45 was shown to stimulate DNA excision repair *in vitro* and to inhibit entry of cells into S phase; thus, it might link the p53-dependent cell cycle checkpoint and DNA repair [83]. The likely role of cyclin–PCNA complexes in the adaptive response and the regulation of the cell cycle and DNA repair is also underlined by the observation of Boothman *et al.* [84] that the adaptive survival response of cells correlates well with constitutionally elevated levels of PCNA and cyclin D1 as well as with the inducibility of cyclin in different cells.

ACTIVATION OF LATER-RESPONSE GENES AND PRE-EXISTING PROTEINS

Transcription factors formed during the early response to ionizing radiation can bind to promoter regions of several genes and, thereby, increase the synthesis of new transcripts. For example, SP1 target genes, such as those for thymidine kinase, t-PA, and DT diaphorase, are induced by ionizing radiation [85]. Other transcription factors such as CREB are also induced by ionizing radiation, but their target genes are still unknown. The following products have been studied most intensively (Fig. 1):

The gene for TNF-α (17.5 kDa) is one of the target genes for NF-κB and thus could originate from the regulation of gene transcription by early response genes. Indeed, the response to noxious and stressful stimuli in the mammalian organism is mediated and integrated by means of inflammatory cytokines such as TNF-α and interleukin-1β. TNF-α is released from cells following irradiation with 2–50 Gy, and this increase is associated with transcriptional activation [86]. TNF-α has a direct effect on human cancer cells *in vitro* that results in growth inhibition and death, whereas it stimulates growth in certain normal cells. TNF-α activates SAPKs [87] and mediates the release of PDGF-like molecules in various cell types [88, 89]. TNF-α can also participate in the increase of p21 levels via a post-transcriptional stabilization of the mRNA [80]. However, TNF-α plays a role in many cellular processes; thus, its induction probably represents a general response to cell injury rather than a specific one to ionizing radiation.

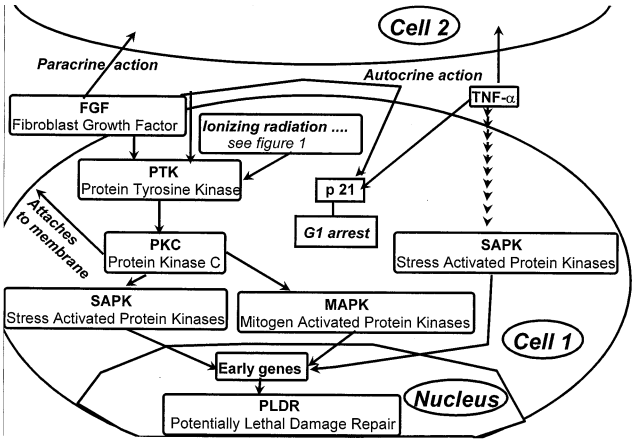

FIG. 3. Putative scheme of potential extracellular (autocrine or paracrine) transmission of an adaptive response.

Mitogenic factors [PDGF-like molecules (MW 30,000) and the FGF] are released into the medium after exposure of cultured endothelial cells to ionizing radiation (1.25 to 20 Gy) [90, 91]. These growth factors might also originate from an activation of later genes in response to early response genes [92]. They were shown to induce p21 protein by a p53-independent mechanism [93]. The secreted FGF could play a role in the repair of radiation-induced damage in endothelial cells. Indeed, further experiments on endothelial cells suggested that bFGF (isoforms of 18, 22, and 24 kDa) [94] activates the PLDR via an interaction of extracellular bFGF with receptors located on the plasma membrane of the cell. This cycle is initiated by DNA damage and followed by an increase in bFGF-specific mRNA, bFGF synthesis, and its secretion into the medium. The newly synthesized FGF appears to induce the PLDR pathway via an extracellular autocrine loop (Fig. 3) that can be inhibited by specific anti-bFGF antibodies [93]. Since many types of cells do not express receptors to bFGF, the authors suggest that the capacity to stimulate the PLDR pathway is probably not specific to bFGF and that other cell types may

respond to radiation-induced DNA damage and different stressors by means of other inducers of PLDR coming from the same cell or released from neighbouring cells, i.e. by auto- or paracrine pathways. Thus, several cell types that express bFGF *in vitro* and *in vivo*, such as tumor cells of different origin, macrophages, and T-lymphocytes, may activate PLDR in other cells by releasing bFGF [94].

These data concerning the induction of extracellular factors acting via a paracrine mechanism raise further questions with regard to the implications of communicating molecules in the adaptive response; indeed, an induced extracellular factor (extracellular protein inducing factor) was shown to be capable of transmitting the UV-induced response of irradiated cells to non-irradiated ones [95]. Adaptation to an alkylating agent implicates poly(ADP-ribose) polymerase, an enzyme that recognizes DNA nicks, and that can also be activated by a factor that can be transferred via conditioned medium [50]. Although Cai and Liu [52] failed to detect the protective effect of supernatant from pre-irradiated adapted lymphocyte cultures on other cultured cells, further investigations in this field seem warranted.

XIPs (XIP126, XIP135, XIP138, XIP141, XIP145, XIP147, XIP269, and XIP275) [96] expressed by X-irradiated (1.5 to 2.5 Gy) radioresistant human malignant melanoma cells (U1-Mel) merit particular interest because they are specifically induced by ionizing radiation but not by heat shock, hypoxia, and alkylating agents. Synthesis of XIP145 and XIP269 after X-irradiation was also found in a wide range of normal and tumor cells with different repair capacities. The XIP269 (269 kDa) polypeptide was detected regularly in 6 of 7 normal cell types but was completely absent in cells from patients with Bloom's syndrome or ataxia telangiectasia. XIPs started to appear 3 hr after exposure and were maximally expressed at 4 hr. The expression of this XIP269 was induced by as little as 0.05 Gy and correlated well with the capacity to repair potentially lethal damage. Its expression was inhibited by cycloheximide under conditions in which both PLDR and subsequent adaptive survival responses were prevented [18]. No function has yet been ascribed to the other induced proteins. In another experiment, Boothman *et al.* [97] demonstrated the X-ray induction of another protein of 175 kDa ($XIP_{bp}175$), which selectively binds to damaged DNA.

Several transcripts from genes (*xip*1 to *xip*12) induced by low doses of X-rays (0.05 Gy) in the U1-Mel cell line within 3 hr after radiation were isolated by cDNA cloning in an attempt to identify the entire spectrum of such genes [85]. Although ionizing radiation provided the greatest stimulus, some xips were also regulated by other agents (UV and phorbol ester). Only xip4, -7, and -12 were specific for ionizing radiation. In checking for identities and homologies to known genes, it was found that some xips are likely to intervene in:

Cell growth and recombination: xip5 is a clone homologous to human growth hormone genes; xip7 is a clone homologous to an RAG-1, which might be involved in PLDR [85, 98] and xip11 is a thymidine kinase;

Metastasis: xip6 is the human t-PA, a serine glycoprotease of approximately 70 kDa, and xip12 is a clone homologous to the human angiogenesis factor;

Cellular defense mechanisms: xip1 is homologous to a cyt P-450 gene, and xip3 is a DT diaphorase.

Other proteins of 35 kDa and 14–18 kDa size that bind to damaged DNA have also been reported to be induced or produced in increased amounts in human lymphocytes exposed to very low doses of X-rays (0.005 to 0.2 Gy) [48]. The binding of this protein to DNA occurs as early as 1 hr after irradiation and reaches its maximum by 6 hr. A similar experiment on *in vitro* irradiated human lymphocytes (0.05 Gy) revealed the appearance of proteins of 25, 167, 168, and 174 kDa 4 hr after exposure [99]. Nevertheless, some doubts exist as to the relevance, for the adaptive response, of the binding of transcription factors to DNA because, recently, Boothman *et al.* [100] could not demonstrate such a binding after low doses of radiation. The melanoma cell strains used are rather radioresistant, however, and only 2 of 8 strains studied showed an adaptive survival response.

INDUCTION OF HSPS

HSPs are a class of proteins that are preferentially synthesized following exposure to a variety of stressors. They perform important cellular functions under both stressed and non-stressed conditions, act as so-called molecular chaperones, and play important roles in cellular transportation, assembly/degradation, and cell survival. The HSP 70 (HSP 72, HSP 73, Grp75, Grp78), HSP 60, and HSP 90 families of HSPs have been studied most extensively [101, 102]. They are constituents of normal, unstressed cells and assure the acquired final biologically active conformation of proteins. These HSPs are induced by denatured proteins or by agents denaturing proteins. In addition, HSP 72 (an inducible isoform of HSP 70) is induced by *in vitro* UV C radiation due to the presence of residual thymine dimers [103] and by *in vivo* exposure of mice to low doses of ionizing radiation, possibly as a result of damaged DNA [104]. Thus, HSPs are not specific for a certain type of cellular stress since either UV, ionizing radiation, or heat shock can activate them.

AN ATTEMPT TO INTEGRATE PRESENT INFORMATION

It is, to say the least, difficult at this moment to speculate on the ways and means by which the adaptive response is effected. Researchers are using different type of cells in different cell cycle stages and exposing them to a variety of doses of ionizing radiation at various intervals thereafter. No comparison of the different observations under the same

conditions (cell line, dose of radiation) and in dependence on the post-exposure time has been carried out thus far. Nevertheless, all of the various studies indicate that mammalian cells respond to radiation-induced injury by the up-regulation of proteins involved in cell signalling and by an increased pleiotropic expression of genes associated with growth control and DNA repair. Initial elements—following the still unknown signal for the presence of radiation damage—are various kinases (PKC and SAPKs) which, in turn, induce early response genes, whose products initiate a cascade of protein–DNA interactions that regulate gene transcription and ultimately result in specific biological responses. These responses include the activation of later genes and can promote production of growth factors and cytokines, trigger DNA repair, and regulate progress through the cell cycle. Indeed, several mechanisms of the induction of the adaptive response (perhaps also of apoptosis) parallel those involved in the effects of radiation on the cell cycle, although it should be emphasized that the former are prominent at considerably lower doses than the latter, and that an adaptive response can proceed without changes in the cell cycle [105]. Nevertheless, it appears useful in this context to briefly summarize some of the most marked features of the mechanisms of the delay of the cell cycle (see reviews in Refs. 106–110).

Delay at different phases of the cell cycle, mainly at G_1 (or the resting phase G_0) and G_2, represents responses common to different types of DNA damage that are actively mediated by various genes. The delay in transition from the G_1 to the S phase is not universal, depends on the status of the cell, arrives after relatively high doses, and does not occur in all cells. However, the transition of G_1 to S is a critical breakpoint in the life cycle of the cell, since a damaged cell either might be discarded by initiating apoptosis or might be given additional time to effect DNA repair. It appears that the tumor suppressor p53 has an important function, and the accumulation of p53 coincides with the induction of p21 and gadd45 involved in cell cycle arrest after DNA damage. In addition to transactivating genes involved in regulating cell cycle progression, interactions between p53 and DNA as well as with repair proteins implicate p53 in the repair process itself. The arrest in G_2 phase prior to mitosis occurs after smaller doses and is universal, although its mechanisms of action might not be the same in all cells. Some of the mechanisms of G_2 delay have also been studied in some detail. Protein complexes consisting of cyclin-dependent protein kinase as catalytic subunits and cyclin molecules as regulatory subunits play a major role in the regulation of the phosphorylation of CDK, the turnover of cyclin, and binding to inhibitors, and a signal of "DNA damage" or "uncompleted DNA synthesis" inhibits further progress.

With regard to the adaptive response, it is now clear that the induction process stimulates repair of potentially lethal damage probably via several polypeptides and growth factors acting through autocrine/paracrine transmission. The expression of specific proteins such as XIP269 and the induction of RAG-1 transcripts as well as the activation of specific DNA-binding proteins are important steps in this process. It appears that these studies now begin to approach the crucial question as to what makes the adaptive response after ionizing radiation a specific feature of DNA damage, at least with respect to the final effect of improving DNA repair. As regards the initial signal that sets in motion the process of the adaptive response, recent studies by Wolff [111] show that DNA double-strand breaks *per se* can initiate an adaptive response. It seems likely that damaged DNA is then bound in a specific way to certain proteins, but one should not forget that, in contrast to the higher doses causing cell cycle arrest, double-strand breaks will be very rare at doses (10 mSv) capable of initiating the adaptive response, although a substantial number of single-strand breaks would be present in the cell. The step by which the various phosphokinases become activated in the presence of such minute amounts of damaged DNA, bound or not to proteins, also remains an enigma. With regard to the further chain of events, links exist that are common to the adaptive response to radiation and the general response to other stressors and that either directly lead to DNA repair or branch out towards other mechanisms. This is shown, among others, by the role of PKC and PKC-dependent kinases, which modulate early genes playing manifold roles in the regulation of cell function and proliferation, as well as by the activation of HSPs [82] by radiation as well as by the other stressors.

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