BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **245,** 1–10 (1998)
ARTICLE NO. RC988257

BREAKTHROUGHS AND VIEWS

Cellular Responses to DNA Damage in the Absence of Poly(ADP-ribose) Polymerase

Yves Le Rhun,* J. B. Kirkland,† and Girish M. Shah*,[1]

Laboratory for Skin Cancer Research, Hospital Research Center for University Laval, CHUL Research Center of CHUQ, Québec, Québec, Canada G1V 4G2; and †Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Received February 6, 1998

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme which is catalytically activated by DNA strand interruptions. The involvement of PARP has been implicated in different cellular responses to genotoxic damage, including cell survival, DNA repair, transformation, and cell death. However, the exact contribution of PARP polypeptide or its enzymatic product has remained ill defined. Recent studies with two different PARP knock out mice have demonstrated the beneficial role of PARP in maintaining genomic integrity and in survival responses after exposure to whole body γ-irradiation. Other studies have demonstrated the instrumental role of PARP in death of the neuronal cells after ischemia-reperfusion injury. The recombination inhibiting function of PARP at DNA strand breaks was more evident in a model system deficient in activities of two major DNA strand break binding proteins, PARP and DNA-dependent protein kinase. The present review summarizes similarities and differences obtained with the two PARP knock out mice and reanalyzes the role of PARP in various cellular responses to DNA damage.

POLY(ADP-RIBOSYL)ATION REACTION IN RESPONSE TO DNA DAMAGE

Among the earliest responses of a mammalian cell to DNA strand interruptions is activation of the nuclear enzyme, poly(ADP-ribose) polymerase (PARP) (Fig. 1). PARP, dubbed "a molecular nick sensor" (1) recognizes and rapidly binds to DNA single or double strand breaks though its N-terminal DNA binding domain (DBD) (Fig. 1, Step 1). The DNA-bound, catalytically activated PARP utilizes NAD to synthesize poly(ADP-ribose) (pADPr) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Step 2). Most polymer-modified proteins transiently lose their normal functions due to a large increase in the negative charge. Automodified PARP loses its ability to bind DNA strand breaks due to electrostatic repulsion between pADPr and DNA (Step 3), exposing the DNA strand breaks to proteins involved in the repair process (step R-1). PARP and other pADPr-modified proteins are eventually restored to their native state through the action of poly(ADP-ribose) glycohydrolase (Step 4). A variety of experimental approaches have been used to understand the physiological role of pADPr synthesis in response to DNA damage. These approaches ranged from chemical inhibition of the catalytic activity of PARP (Fig. 1, approach A) (2, 3), transdominant inhibition of PARP by overexpression of its DBD (Fig. 1, approach B) (1, 4-8) to other methods, such as anti-sense PARP expression (9) and cell lines with reduced (10, 11) or increased (12, 13) expression of PARP. From these studies, strong arguments were made in favour of PARP participating in a variety of DNA-related functions, including gene amplification, gene expression, cell division, differentiation, transformation, and most critically, in DNA base excision repair (reviewed in (1, 3, 14-17)).

Three additional lines of reasoning support the argument that rapid activation of PARP is of major importance to the perturbed cell: a) PARP-activation precedes many other stress-responses, such as DNA repair, activation of DNA-dependent protein kinase and induction of p53; b) PARP is a constitutively expressed

[1] To whom correspondence should be addressed at Laboratory for Skin Cancer Research, CHUL Research Center, RC-9700, 2705, Laurier Boulevard, Québec, Québec, Canada G1V 4G2. Fax: (418) 654-2159. E-mail: girish.shah@crchul.ulaval.ca.

Abbreviations used: BER, base excision repair; DNA-PK, DNA dependent protein kinase; DBD, DNA binding domain of PARP; KO-1, PARP knock out-1: mice from the group of Dr. Z.Q. Wang; KO-2, PARP knock out-2: mice from the group of Dr. G. de Murcia; MNU, *N*-methyl-*N*-nitrosourea; PARP, poly(ADP-ribose) polymerase; pADPr, polymer of ADP-ribose; SCID, severe combined immunodeficiency; SCE, sister chromatid exchange; V(D)J, variable, diversity and joining segments.

0006-291X/98 $25.00

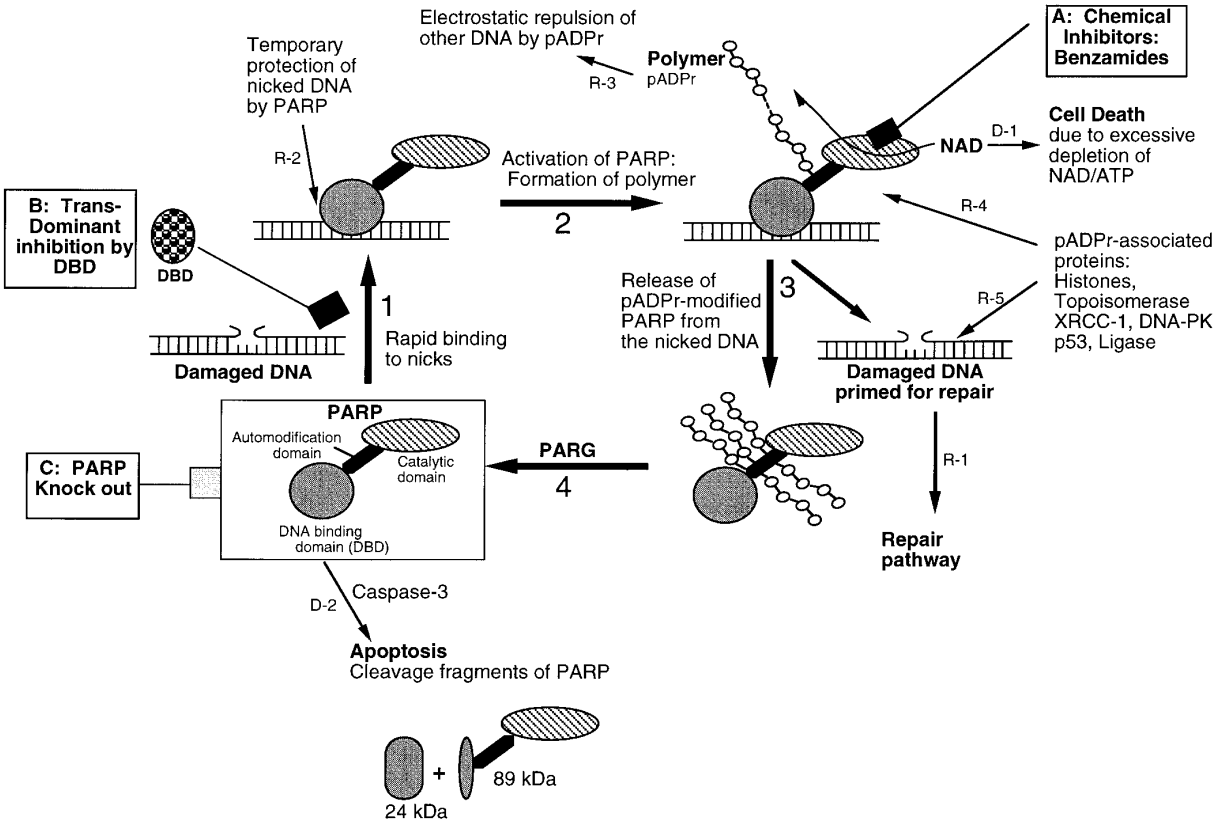

FIG. 1. Physiological role of PARP in response to DNA damage.

and abundant protein, suggesting that it must function within a time frame which is too short to allow for transcriptional regulation; and finally c) PARP, evolutionarily absent in prokaryotes and some lower eukaryotes, is conserved among higher eukaryotes, despite the high energy cost of its metabolism.

The role of PARP in cell death is still being debated and the answer depends on the experimental model of cell death. Activation of PARP in the presence of large numbers of DNA strand breaks and consequent depletion of NAD and ATP were earlier suggested to contribute to cell death (Fig. 1, step D-1) (18). The most consistent support for this model was obtained during the death of brain cells following ischemia or treatment with nitric oxide (reviewed in (19)). However, in other models of cell death, PARP may not remain functionally active long enough to deplete NAD and ATP. During apoptosis, PARP is one of the earliest targets for cleavage by the apoptotic caspase 3, resulting in formation of 24 and 89 kDa fragments (Fig. 1, step D-2) (20-23).

To provide definitive answers about the physiological role of PARP in different cellular responses, results from the PARP knock out (KO) mice were eagerly awaited (Fig. 1, approach C). The first PARP knock out mouse line (KO-1) reported two years ago by Wang et al. (24) belied many of the expectations about the functions of PARP. The very existence of the KO-1 mouse suggested that PARP is dispensable during embryogenesis. Cells derived from the KO-1 mouse had a marginally slower proliferation rate with or without DNA damage. These cells could carry out apparently normal DNA base or nucleotide excision repair of damaged plasmids. A somewhat unusual observation was the development of skin hyperplasia in ~1/3 of the older KO-1 mice (24). While some of these results, such as a lack of effect on DNA excision repair, could be expected from earlier studies, it was apparent that the subtle roles of PARP in perturbed cells may be brought out only if an appropriate experimental model is employed. This was evident in subsequent studies with the KO-1 mice or the cells derived from these mice (25-31). Meanwhile, the group of G. de Murcia independently created a second PARP knock out mouse (KO-2) that showed an extreme sensitivity to γ-radiation and *N*-methyl-*N*-nitrosourea (MNU) (32, 33). A third independent PARP knock out mouse (KO-3) has also been created by the group of T. Sugimura and results from this mouse are eagerly awaited (T. Sugimura, personal communication). Recent results with the first two knock out mice have been summarized in the Table I and a reevaluation for the role of PARP in some of the critical cellular responses to DNA damage is described in this review. The knock out studies have clearly

TABLE I

Comparison of Two PARP Knock Out Mice

Effect	KO-1 (Wang et al.)	KO-2 (de Murcia et al.)
References	(24-31)	(32, 33)
Disruption of PARP gene at	part of 2nd exon and 2nd intron	4th exon
Truncated peptide (if made)	From N-terminus to part of finger F I	From N-terminus to part of finger F II
Proliferation-parameters		
(a) Cell growth	Slower before or after stress	NA (not available)
(b) Normal cell cycle profile	Normal	Normal
(c) Cell cycle after DNA damage	Normal, no accumulation in any phase	G2/M phase accumulation
(d) Body weight and litter size	Normal	Smaller in each case
Skin abnormalities	Spontaneous skin lesions in ~30% mice	No effect
Survival of KO mice after		
(a) γ-Irradiation (8 Gy)	As sensitive as KO-2	Extreme sensitivity
(b) MNU	NA	Extreme sensitivity
DNA repair	Normal NER and BER of damaged DNA	NA
Cell death parameters		
(a) Ischemic-brain death	Resistant	NA
(b) Drug-induced inflammation	Resistant	NA
(c) Apoptosis	No effect for most cell types, except resistance in pancreatic cells	Increased susceptibility of splenocytes
Genomic stability parameters		
(a) Frequency of SCE	High both before and after DNA damage	High both before and after DNA damage
(b) Micronuclei	High only after DNA damage	NA
(c) Chromatid breaks, rejoining	NA	High only after DNA damage
p53-related parameters	Low basal level, defective post-damage induction, but normal transactivation	Normal or higher induction of p53 after apoptosis-inducing DNA damage
DNA-PK related parameters		
(a) V(D)J recombination in KO	Normal	NA
(b) PARP KO added to SCID	More mature T-cells: increased V(D)J recombination and Lymphoma	NA

established the role of PARP in the maintenance of genomic integrity, in the initial survival responses of mice after severe genotoxic exposure, and in ischemic death of brain cells. The rest of the results from knock out studies were apparently as ambivalent as those obtained with earlier experimental approaches, confirming that the precise physiological role of PARP in response to DNA damage will be a subject of many more studies.

GENERATION OF PARP KNOCK OUT MICE

PARP is a single copy gene on chromosome 1q in human and mouse cells and it is composed of 23 exons (15, 24). The two different knock out mice were created by interruption of exon 2 (KO-1) (24) or exon 4 (KO-2) (32) of the PARP gene in mice. Both of the groups have carefully demonstrated an absence of full length PARP (by Western or activity blot) and a lack of production of pADPr in the cells from these mice. However, there has been no categorical demonstration that a truncated protein product is not formed from the interrupted PARP gene. This may be a crucial point for the eventual outcome of these studies, because the N-terminal DNA binding domain (DBD) of PARP extending up to exon 7 has two DNA strand break binding zinc fingers, and the DBD has been successfully used as a trans-dominant inhibitor of PARP (4, 8). Even in absence of full size PARP, a partial fragment could have a significant impact on DNA repair by binding to strand breaks and preventing access by DNA repair proteins. The KO-1 cells were shown to make a truncated 1.2 kb transcript (24) that could generate a fragment of PARP extending through part of the first Zn finger. Due to lack of complete expression of the first zinc finger, it is unlikely that Zn coordination or DNA binding would occur with this structure. In contrast, interruption within exon 4 of KO-2 (full finger I and part of finger II) suggests that an intact finger I may be produced. Finger I is also known to be sufficient for binding to DNA double as well as single strand breaks (34). However, neither the truncated transcript nor the truncated protein has been reported in the KO-2 mice (32). It may be useful to carry out additional analyses of cells from both KO-1 and KO-2 for possible formation of the truncated PARP protein using antibodies shown to recognize the expected polypeptide fragments.

MINOR ABNORMALITIES IN THE PARP KNOCK OUT ANIMALS

The fibroblasts from KO-1 mice had a marginally slower growth rate in the absence of additional genotoxic

stress (24) or after heat shock (27). During the growth of a chimeric embryo composed of an equal number of normal and KO-1 cells, the contribution of mutant cells in different organs was less than that of normal cells, thus confirming a growth disadvantage for KO-1 cells (27). Thymocyte proliferation was also delayed after exposure of KO-1 mice to sublethal γ-irradiation (24). Surprisingly, this growth disadvantage of KO-1 cells could not be correlated with other growth-parameters, e.g., distribution of KO-1 cells in each phase of the cell cycle was identical to the control cells, before or after the DNA damage (28), and the KO-1 mice had normal body weight and litter size (24, 27). In contrast, KO-2 animals had a smaller average litter size (4.5 versus 7.4) and smaller average body weight (19 versus 23 g) as compared to the controls. Fibroblasts cultured from KO-2 mice displayed a cell cycle distribution similar to controls under optimal conditions, but they exhibited an accumulation of cells in the G_2/M phase after DNA damage (32). Further studies would be needed with different knock out animals to understand the role of PARP in cell cycle regulation and growth before and after different types of DNA damage.

About one-third of the older KO-1 mice developed skin abnormalities, i.e., in the area of lesions, there was thickening of all layers of the epidermis, active proliferation of keratinocytes, intracellular edema and inflammatory responses (24). Since nearly 70% of KO-1 mice remained free of these skin problems, it is difficult to correlate these skin abnormalities with the absence of PARP, especially since the KO-2 mice were reportedly completely free of any skin pathologies (32). The skin of aged animals is subjected to cumulative environmental stresses and that may be one of the reasons for some of the KO-1 mice developing skin problems (24). More studies are necessary to determine if PARP has a role in the response of skin to DNA damaging agents.

ACUTE SENSITIVITY OF PARP KNOCK OUT MICE TO DNA DAMAGE

The group of de Murcia were the first to report an extreme sensitivity of PARP knock out mice to whole body γ-radiation, using the KO-2 model (32), and this was subsequently confirmed in the KO-1 mice by Wang et al. (27). Exposure of KO-2 mice to 8 Gy of γ-irradiation resulted in a very short half-life of 4 days and 100 % lethality by 8 days, as compared to 50 % lethality observed in control mice from 14-20 days (32). Although some effects of radiation toxicity, such as reduction in size of the thymus and spleen, were identical among the wild type and KO-2 mice, it was the shortening of vili and extensive necrosis occurring in the epithelial lining of small intestine that appeared to be the cause of precocious lethality (32). The KO-2 line was also observed to be extremely sensitive to intraperitoneal administration of 75 mg/kg MNU, i.e., 80% lethality in the first week for KO-2 mice as compared to 40% lethality until 8th week for the control (32). The protective effect of PARP was most evident in the first week after exposure to the toxic agents, especially in the rapidly proliferating tissues, such as intestinal epithelium. However, the exact function of PARP during this initial survival response was not defined in these studies. Since exposure to γ-radiation or MNU induces synthesis of pADPr in the cell and the DNA single strand breaks produced by these agents would require base excision repair, it may be tempting to speculate that defective DNA base excision repair in the absence of PARP would be the cause of lethality. However, this does not correspond with the normal DNA base excision repair reported in KO-1 cells (24).

The acute gut toxicity following γ-irradiation in the KO-2 line was comparable to that observed in mice deficient in *atm*, a gene implicated in double strand break repair, meiotic recombination and cell cycle control (35). Therefore, it is possible that these two proteins could be part of the same pathway involved in radiation resistance in the gut. It is known that the *atm*-dependent pathway does not involve p53, because *atm*/*p53*-double knock out mice remain as sensitive to radiation induced gut toxicity as the *atm*-deficient mice (36). It would be interesting to observe if the deletion of p53 could rescue PARP knock out mice from radiation toxicity. Further studies are also necessary to determine if the PARP knock out mice are sensitive only to those DNA damaging agents that activate PARP, and not to other agents that do not activate PARP or to other non-DNA damaging toxic agents.

DNA REPAIR IN THE ABSENCE OF PARP

PARP is believed to play an indirect role in DNA base excision repair (BER) (reviewed in (17) and see all the R-steps in Fig. 1). The presence of NAD was shown to stimulate repair, PARP was automodified during BER, and chemical inhibitors of PARP were shown to decrease repair. However, cell extracts depleted of PARP were fully competent to carry out the repair, and repair lost its dependence on NAD (17). Thus, PARP is not thought to be an absolute requirement for the process of DNA repair, but it is certainly an unavoidable co-incident process in damaged cells. In light of these results and the fact that prokaryotes and some lower eukaryotes devoid of PARP carry out efficient DNA repair, it was not surprising that embryonal fibroblasts from PARP knock out KO-1 mice were observed to be efficient in DNA base excision and nucleotide excision repair of transfected plasmids damaged by monofunctional alkylating agent or ultraviolet C, respectively (24). It is important to note that this simple repair assay does not give any indication about

the long term effects of PARP absence in all the events associated with DNA repair.

It has been suggested that PARP activation during DNA repair may have other functions important for survival of the damaged cell (Steps R in Fig. 1). The immediate physical occupation of the nicked site by PARP could protect the nicked site from nucleases and other DNA binding proteins (Fig. 1, step R-2). The negatively charged pADPr formed on PARP may also electrostatically repel other DNA molecules in the vicinity of the breaks and prevent recombination events before repair (Fig. 1, step R-3) (17). PARP, pADPr-modified PARP or free pADPr could also facilitate survival responses of the damaged cell by association with other critical cellular components at the damaged site, such as XRCC1, DNA ligase III, DNA polymerase β (33, 37), p53 (38, 39) and DNA-dependent protein kinase (40) (Fig. 1, step R-4). Thus, PARP could be acting as a beacon for recruiting other proteins at the site of damage, and some of these proteins could carry out the DNA repair process (Fig. 1, step R-5) or bring about cell cycle changes, etc. The extreme susceptibility of the knock out mice to γ-irradiation and MNU also supports this argument that other events coordinated by PARP during the repair of DNA damage may have long term consequences for the survival of cells.

DIFFERENT TYPES OF CELL DEATH IN THE ABSENCE OF PARP

PARP could be participating in different varieties of cell death at two possible levels: i) the catalytic function of PARP could deplete NAD and ATP resulting in cell death (Fig. 1, step D-1) (18); or ii) PARP could serve as one of the substrates for the apoptotic caspases (Fig. 1, step D-2) (20-23), and early cleavage of PARP was suggested to conserve energy that would be required for the apoptotic process. Recent studies suggest that these two events could occur at different times during apoptosis, i.e., the catalytic function of PARP could precede its cleavage by the caspases (41), and formation of pADPr has been visualized in apoptotic nuclei (42). Recently, suppression of catalytic activation of PARP during the early phase of apoptosis was shown to inhibit apoptotic body formation and nuclear fragmentation during the final execution phase (43). It is quite likely that sheer abundance of PARP in the nucleus makes it the most accessible target for the caspases, but there were suggestions that its rapid cleavage could either prevent the cell from returning back to normalcy or even facilitate the apoptotic process (reviewed in (44, 45)).

If the catalytic action of PARP and NAD depletion are critical in any model of cell death, the absence of PARP could have a strong effect in preventing cell death. However, if PARP is only a passive substrate for the caspases, then an absence of PARP would have no influence on apoptosis, because caspases would continue to act on other substrates and achieve the goal of apoptosis. The use of PARP knock out mice and cells have provided some very clear and other ambiguous answers about the role of PARP in different forms of cell death.

(a) Resistance to Ischemia Induced Neuronal Death in the Absence of PARP

It has been suggested that PARP activation plays a key role in neuronal cell death after ischemia-reperfusion injury (e.g. (19, 46, 47)). There is sufficient experimental evidence to suggest that overexcited neurons release glutamate which binds to and activates specific N-methyl-D-aspartate receptors, resulting in activation of nitric oxide synthetase and formation of oxidant molecules that activate PARP. The resulting formation of pADPr has been demonstrated in the nuclei of these neurons (47), and depletion of NAD by activated PARP is believed to cause neuronal cell death (19). Chemical inhibitors of PARP were shown to suppress neuronal cell death, but doubts remained concerning the side effects of these inhibitors contributing towards the protection. Two recent studies carried out with KO-1 mice (29, 30) clearly show that PARP is a perpetrator of the ischemic brain injury (48). Using brain cortical cells from KO-1 mice, it was shown that the absence of PARP confers protection from oxidant-induced cell death. In the animal model of ischemic injury, blood supply to the brain is physically interrupted for a few hours. Several hours after resumption of the blood flow, injury can be measured as an infarct in the brain. In KO-1 mice, the brain infarct volume and size were significantly reduced compared to control animals. There has been a complete agreement between results obtained with PARP knock out and chemical inhibition approaches. This is further corroborated by the protection against oxidant-mediated apoptosis offered by an increase in brain NAD levels (49). Therefore, the mechanism of death following ischemia-reperfusion in brain cells appears to involve activation of PARP and subsequent depletion of NAD. In this model, however, some associated questions need to resolved. It is important to note that ischemic brain is suggested to undergo two types of cell death: necrotic death in the core of ischemic injury and apoptotic death in the periphery (50). The reduction in infarct volume could arise due to reduction in both the core or the peripheral damage. Therefore, the absence of PARP could be preventing both necrotic or the apoptotic death of neurons. It is also possible that PARP could be playing a major role only in preventing the core necrotic death and may have no influence on the peripheral apoptotic events, because the absence of PARP does not prevent activation of the apoptotic DNA degradation events (29). The oligonucleosomal fragmentation of DNA and TUNEL stain-

ing was identical in ischemic brain cells from both mutant and control animals (29). Study of the activation of the apoptotic caspases in the core and the periphery of the brain infarct of the mutant animals would provide a clue as to whether the absence of a dominant substrate for caspases has any influence on the effectiveness of these death proteases. Further studies with this model of ischemic death in knock out mice should provide a better understanding of the role of PARP in apoptosis and necrosis.

(b) Inhibition of Zymosan Induced Inflammation in the Absence of PARP

Szabo and coworkers have been studying the role of PARP in the inflammatory response initiated by intraperitoneal injection of zymosan (31). This is a complex model consisting of several stages, including initial damage to nearby tissues, activation of macrophages, release of chemotactic cytokines and recruitment and activation of neutrophils. Neutrophil-induced damage is a major component of the eventual cell death and tissue pathology (multi organ failure). Using both chemical inhibition of PARP and the KO-1 mouse model, the authors have shown that a loss of PARP function decreases the extent of tissue damage and the degree of neutrophil infiltration (31). The authors propose that early cell injury (in the presence of activated macrophages or early neutrophils) is caused by PARP activation and NAD depletion. PARP inhibition or deletion could protect cellular NAD pools, minimize cytotoxicity and prevent amplification of the inflammatory response. It is important to remember that PARP inhibition or deletion is not likely to have a direct effect on neutrophil function, as PARP is completely downregulated in mature neutrophils (51). The involvement of PARP in this model of inflammation appears similar to its role in the models of ischemic brain injury and pancreatic cell death described above. All of these models are initiated by significant levels of DNA damage and appear to be dependent on NAD depletion. PARP may play a very different role in apoptosis initiated by non-genotoxic signals.

(c) Apoptosis in the Absence of PARP

The role of PARP in apoptosis has been a subject of debate ever since PARP-cleavage by the apoptotic caspases was described by the groups of Kaufmann, Poirier, and Earnshaw (20, 21). If PARP were to be simply a passive substrate for the caspases with no other impact on apoptosis, inhibition of PARP or the absence of PARP would have no influence on the apoptotic events. In contrast, if NAD depletion or PARP-cleavage or PARP-cleavage products were to actively participate in apoptosis (reviewed in (45)), the absence of PARP could alter the progression of apoptotic events. A variety of PARP knock out cells were subjected to diverse apoptosis-inducing treatments, and the following three categories of results have added more confusion concerning the role of PARP or PARP-cleavage in apoptosis.

(i) Resistance to apoptosis. Pancreatic islet cells from KO-1 mice were found to be resistant to oxidant-mediated cell death (25). These results are in agreement with the protective effects observed in oxidant-mediated death of ischemic neuronal cells, described earlier. These two models appear to represent a specialized type of apoptosis which is initiated by NAD depletion caused by PARP activation. Because of this, the effects of PARP inhibition or deletion may be more dramatic or even opposite to those observed in most models of apoptosis, as described below.

(ii) No effect on apoptosis. Two separate studies using different cells from KO-1 mice clearly showed that the absence of PARP has no impact on apoptotic death (26, 27). In these studies, different death-inducing agents were used for each of the cell-types, e.g., primary embryonal fibroblasts were treated with TNF or anti-Fas antibody (27), thymocytes were treated with dexamethasone, γ-irradiation (27), or etoposide, ionomycin and ceramide (26), hepatocytes were treated with TNF or CD-95 (26), and cerebellar neurons were treated with K^+-withdrawal, colchicine, staurosporin, and oxidants (26). These studies support the argument that PARP does not influence upstream events, such as activation of caspases or independent events such as apoptotic DNA fragmentation.

Some of these results are in disagreement with results obtained by other groups under comparable conditions. While there was no effect of PARP absence on the peroxynitrite-induced apoptosis of primary neuronal cells from the cerebellum (26), this was in sharp contrast to the protective effects observed during oxidant-mediated death of primary cerebral cortical cells (30). Since both the studies were carried out with brain cells isolated from KO-1 mice and nearly identical DNA fragmentation assays were used, a likely reason for the opposite results could be the source of the cells from different regions of the brain.

(iii) Increased susceptibility to undergo apoptosis. The splenocytes from KO-2 mice were reported to undergo more rapid apoptosis either spontaneously or in response to MNU exposure (32). There was an interesting suggestion that DNA damage which requires base excision repair is likely to lead to apoptosis, presumably because of malfunctioning of DNA repair-associated events in the absence of PARP (33). Acute sensitivity of both the knock out mice to γ-radiation and MNU indirectly supports this argument. However, additional studies are needed to clarify this issue, because the response of a cell to DNA damage may depend not only on the type of damaging agent, but also on the cell type. For example, γ-radiation and oxidants both activate

PARP, but γ-irradiation-induced death of KO-1 thymocytes was unchanged (27), while as discussed earlier, oxidant-induced death of KO-1 brain cells was decreased in the absence of PARP (29, 30).

These studies also demonstrate that the absence of PARP has no influence on activation of the caspases in the apoptotic hepatocytes (26), or on activation of DNA degradation events in the ischemic brain cells (29). However, a caution needs to be exercised in generalization of these observations to all the models of apoptosis. It may be more informative to study in one model of cell death, all the three parameters, i.e., PARP activation/ NAD depletion, activation of the caspases and DNA fragmentation.

GENOMIC STABILITY IN THE ABSENCE OF PARP

The role of PARP in maintenance of genomic stability and in preventing recombination appears to be the only subject on which there is a full agreement, not only within the results from two knock out mice but also with earlier models. The ability of PARP to quickly seize the DNA strand breaks suggests its possible influence on various subsequent events, such as homologous or non-homologous recombination, chromosome breakage, antibody class switching, etc. One of the consistent demonstrations of this property of PARP was an increase in the frequency of sister chromatid exchange (SCE) by chemical or trans-dominant inhibitors of PARP (e.g., (3, 8, 52)). For the PARP knock out studies, the frequency of SCE was analyzed before or after DNA damage either in the intact KO-2 mice (32) or in the splenocytes isolated from KO-1 mice (27). In both of these studies, knock out cells exhibited a 2-5 fold higher level of SCE either before or after DNA damage. PARP could act as an anti-recombinogenic factor either by a) binding to the strand breaks and preventing the action of exonucleases (Fig. 1, step R-2), which could generate recombination intermediates, or b) forming pADPr which could repel other DNA molecules from the vicinity of the strand breaks (Fig. 1, step R-3) (17). Inhibitors of the catalytic function of PARP could act by the latter mechanism, while the absence of PARP could act by either mechanism, although this needs to be experimentally confirmed.

An unstable genome after DNA damage can also be detected by parameters that detect fragmentation and exchange within the chromosome. The role of PARP in the maintenance of genomic integrity after DNA damage was confirmed by a higher frequency of micronuclei formation in KO-1 cells (27), and a larger number of chromatid breaks and chromatid exchanges in KO-2 cells (32). While the SCE frequency was higher both before and after DNA damage, the micronuclei and chromatid breaks were higher only after DNA damage, suggesting more definitive involvement of PARP in the stability of genome after DNA damage.

It is important to know if the decrease in chromosomal stability causes an increase in spontaneous carcinogenesis or cancer incidence in the PARP knock out mice following low doses of chemical carcinogens. Wang et al. (27) report a lack of spontaneous carcinogenesis in KO-1 mice, but the data is not shown. This is a very important question which requires a significant number of animals, careful postmortem analysis and rigorous statistics. Long term carcinogenesis studies are essential to determine if PARP plays a role in chromosomal stability during exposure to low levels of DNA damage in cells which are not undergoing a severe stress response.

p53-DEPENDENT REACTIONS IN THE ABSENCE OF PARP

The tumor suppressor protein p53, like PARP, has been implicated in the response to DNA-damage and induction of apoptosis, suggesting that both proteins could be part of some common pathways. Various studies have demonstrated three types of interaction between PARP and p53: a) PARP poly(ADP-ribosyl)ates p53 (38), and there are suggestions that pADPr or automodified PARP can interact with p53 (53); b) PARP and p53 interact with each other in vitro and ex vivo in at least one cell type (39), and finally, c) PARP and p53 together can bind to DNA (39). The influence of PARP on p53 or p53-dependent reactions was shown earlier by different technical approaches, e.g., V79 Chinese hamster cells selected for low PARP levels exhibited reduced capacity to accumulate p53 in response to etoposide, as well as defective induction of apoptosis (54). Also chemical inhibition of PARP was shown to decrease p53-dependent increase in levels of p21 or mdm2, and to delay senescence (39).

PARP knock out cells offer a better opportunity to analyze the role of PARP in p53 induction and in p53-dependent reactions. The embryonal fibroblasts from the KO-1 mouse had a 50 % reduction in the basal level of p53, and there was decreased induction of p53 after DNA damage or nucleotide depletion (28). However, the p53-dependent activation of p21 and the G_1 checkpoint were unaffected in KO-1 cells (28). Since p53 regulates genomic stability, cells with normal p53 can not multiply and form colonies in the presence of N-(phosphonacetyl)-L-aspartate (PALA), an inhibitor of UMP synthesis. In contrast, p53 knock out cells form PALA-resistant colonies. PARP KO-1 fibroblasts were incapable of forming PALA-resistant colonies (28), suggesting that the absence of PARP does not decrease the levels of p53 to a point where it could impede its "guardian of genome" function.

In contrast to defective induction of p53 and normal apoptosis observed with KO-1 fibroblasts (26-28), the splenocytes from KO-2 mice exhibit a normal or even elevated p53 induction in parallel with increased sus-

ceptibility to apoptosis in response to MNU (32). A possible explanation for these differences could be the characteristic response of these cell types to DNA damage. The fibroblasts often respond to DNA damage by p53 activation and cell cycle arrest, DNA repair etc., while rapidly dividing cells often undergo p53-dependent induction of apoptosis. Additional studies would be required with different cell types from the PARP knock out mice to resolve the action of PARP in p53 induction and activation. Since the overall colony forming ability of PARP KO-1 fibroblasts or p53 knock out fibroblasts after exposure to γ-irradiation was not different from control cells, it was suggested that neither PARP nor p53 regulate the long term effects of survival after DNA damage (28). However, acute radiosensitivity of both the knock out mice suggest that PARP is involved in post-irradiation survival responses in rapidly dividing cells (32).

PARP AND DNA-DEPENDENT PROTEIN KINASE

Severe combined immunodeficiency (SCID) mice with an inactivating mutation in the catalytic subunit of DNA-dependent protein kinase (DNA-PK), present acute radiosensitivity (55) similar to the PARP knock out mice. Since DNA-PK and PARP are two nuclear enzymes that bind to and are activated in presence of DNA strand breaks, the most recent study of SCID/PARP(−/−) mice (56) is an excellent experimental approach to probe the possible redundancy of action at the site of DNA damage. It is generally accepted that DNA-PK and PARP have selective affinity for double and single strand breaks, respectively, although it is known that each enzyme does have some affinity for the other type of strand breaks (see recent discussion in (40, 56)). Therefore, DNA-PK and PARP may be competing for binding to DNA strand breaks formed due to exposure to genotoxic agents or occurring normally in the cell during recombination events. This led to the suggestion by Lindahl et al. (17) that DNA-PK could substitute for some of the functions of PARP in PARP knock out cells.

SCID mice are defective in processing and rejoining of the V(D)J recombination intermediates during developments of immunoglobulins and T-cell receptors, leading to immature B and T cells (57-59). Since the absence of PARP increases recombination events at strand breaks and also increases the frequency of SCE, deletion of PARP expression in the SCID mice could correct the recombination defect of SCID mice. Therefore, a double knock out mice with SCID/PARP(−/−) phenotype was created to determine the interaction between these two proteins (56). Three quarters of the SCID/PARP(−/−) animals died just after birth, but among the survivors, the block in T cell development was partially reversed (56). While SCID mice showed low thymic cellularity (90-fold less than control) and very immature thymocytes lacking CD4/CD8 markers, the double knock out mice showed a nine fold increase in total thymic cellularity relative to SCID controls. Moreover, the thymocytes were more mature than the ones in SCID mice, as 10-15 % of these cells were positive for CD4/CD8 receptors. Nonetheless, the development was not complete, as these T-cells lacked the CD3 marker and T-cell receptor (56). In contrast to the thymic T lymphocytes, the peripheral T lymphocytes isolated from lymph nodes exhibited full maturity with CD4, CD8 as well as CD3 markers and T-cell receptor. The V(D)J recombination was also restored in the double knock out mice, although the pattern of rejoining appeared to be different from controls (56). As compared to T cells, B cell development was as deficient in the double knock out as in the SCID mice. Thus, an absence of PARP partially rescues T lymphocyte development but not that of B-lymphocytes. The mechanism for increased V(D)J recombination in SCID/PARP(−/−) double knock out mice appears to be unclear, because deficiency of PARP alone (KO-1 cells) was shown to have no effect on the V(D)J recombination (27). However, V(D)J recombination was increased in PARP replete normal cells in the presence of chemical inhibitors of PARP (60). One of the possible explanations offered by the authors was that an increase in DNA repair activity or decrease in p53-dependent apoptosis could alter V(D)J recombination activity among the T cells (56). However, the absence of PARP (KO-1 cells) was shown to influence neither the DNA repair activity (27) nor the capacity to undergo apoptosis (26). Therefore, it was suggested that a general increase in recombination activity in the absence of PARP could be the mechanism to resolve the V(D)J recombination intermediates (56). It is also likely that the absence of two proteins, PARP and DNA-PK, which can bind to DNA strand breaks and which also serve as substrates for apoptotic caspases may have more than additive effects observed due to absence of individual proteins.

A most interesting development was the early occurrence (11-20 weeks) of massive T-cell lymphoma in 70 % of the SCID/PARP(−/−) mice, compared to 15 % incidence in SCID mice and no lymphomas in PARP KO-1 mice (56). Therefore, lymphoma development in T cells of the SCID/PARP(−/−) mice could be due to anomalous V(D)J recombination resulting in chromosomal abnormalities. The SCID/PARP(−/−) mice had no abnormalities in B cell maturity and developed no B cell lymphomas. This is in contrast to the T and some B cell lymphomas observed in the SCID/p53(−/−) mice (61, 62). Therefore, DNA-PK appears to be the real tumor suppressor while a lack of DNA-PK and PARP activities appear to cooperate in tumorigenesis.

The SCID/PARP knock out study by Morrison et al. (56) has shown that, in normal cells, PARP and DNA-PK both interact at the site of the strand breaks, and

an absence of one protein may be compensated by the other. Since PARP knock out mice with normal DNA-PK activity displayed far fewer phenotypic defects, as compared to SCID mice with normal PARP activity, DNA-PK seems to be the dominant player at strand breaks, as assessed by current endpoints. PARP may be a more critical player in situations where single strand breaks predominate or when there is an absence of DNA-PK activity. These studies should be explored further to determine the mechanism by which PARP acts as an anti-recombinogenic factor at sites of damaged DNA. Does PARP act alone at the strand breaks or does it recruit other factors through the intermediacy of poly(ADP-ribosyl)ation reaction?

CONCLUSION

The crucial question to ask is why PARP, which carries out energy intensive metabolism, is an abundant and ubiquitous protein in the cells of higher eukaryotes. The lack of any obvious phenotype observed in the PARP KO-1 mouse may have been disappointing in the initial analysis, but subsequent studies with two different PARP knock out mice have amply demonstrated that PARP is an important player in cells responding to DNA damage. The effect of PARP deletion could be manifested differently depending on three major variables: a) the tendency of the given cell type to respond to DNA damage, e.g., fibroblasts may opt for prolonged cell cycle arrest and DNA repair while rapidly proliferating marrow or blood or intestinal cells may opt for apoptosis; b) the capacity of the damaging agent to activate PARP; and c) the role of NAD depletion in the response. A likely scenario for PARP action following DNA damage would include the following basic steps, and depending on the pathway followed by a given cell to DNA damage, different end results may be observed. An abundance of PARP in the nucleus would ensure that PARP binds to and temporarily protects the site of DNA damage. Activation of PARP would result in formation pADPr from NAD. In a heavily damaged cell with massive activation of PARP, depletion of NAD could have death-inducing consequences, while in a mildly damaged cell, NAD depletion will not be significant to cause death. On the contrary, in these cells, PARP could recruit a variety of factors either by direct association or through the intermediacy of pADPr. These factors could then rally DNA repair or other cellular responses to DNA damage with or without further participation of PARP. Development of PARP knock out mice has offered an unique opportunity to discover the subtle roles of PARP and the challenge lies in devising the right physiological or genetic models for study, using appropriate organs or appropriate cell-types from the knock out mice.

ACKNOWLEDGMENTS

This work was supported by a grant from Natural Sciences and Engineering Research Council of Canada (G.M.S.). We are very thankful to Dr. Z.-Q. Wang and Dr. G. de Murcia for providing critical comments and for generously providing the manuscripts of their current work.

REFERENCES

1. de Murcia, G., and Menissier-de Murcia, J. (1994) *Tr. Biochem. Sci.* **19,** 172–176.
2. Shall, S. (1984) *Adv. Rad. Biol.* **11,** 1–69.
3. Althaus, F. R., and Richter, C. (1987) ADP-Ribosylation of Proteins, Springer Verlag.
4. Kupper, J. H., de Murcia, G., and Burkle, A. (1990) *J. Biol. Chem.* **265,** 18721–18724.
5. Kupper, J. H., Muller, M., Jacobson, M. K., Tatsumi Miyajima, J., Coyle, D. L., Jacobson, E. L., and Burkle, A. (1995) *Mol. Cell. Biol.* **15,** 3154–3163.
6. Kupper, J. H., Muller, M., and Burkle, A. (1996) *Cancer Res.* **56,** 2715–2717.
7. Molinete, M., Vermeulen, W., Burkle, A., Menissier-de Murcia, J., Kupper, J. H., Hoeijmakers, J. H., and de Murcia, G. (1993) *EMBO J.* **12,** 2109–2117.
8. Schreiber, V., Hunting, D., Trucco, C., Gowans, B., Grunwald, D., de Murcia, G., and Menissier-de Murcia, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4753–4757.
9. Ding, R., and Smulson, M. (1994) *Cancer Res.* **54,** 4627–4634.
10. MacLaren, R. A., Witner, M. V., Richardson, E., and Stamato, T. D. (1990) *Mutat. Res.* **231,** 265–274.
11. Chatterjee, S., and Berger, N. A. (1994) *Mol. Cell. Biochem.* **138,** 61–69.
12. Vangool, L., Meyer, R., Tobiasch, E., Cziepluch, C., Jauniaux, J. C., Mincheva, A., Lichter, P., Poirier, G. G., Burkle, A., and Kupper, J. H. (1997) *Eur. J. Biochem.* **244,** 15–20.
13. Bernges, F., Burkle, A., Kupper, J. H., and Zeller, W. J. (1997) *Carcinogenesis* **18,** 663–668.
14. Cleaver, J. E., and Morgan, W. F. (1991) *Mutat. Res.* **257,** 1–18.
15. Lautier, D., Lagueux, J., Thibodeau, J., Menard, L., and Poirier, G. G. (1993) *Mol. Cell. Biochem.* **122,** 171–193.
16. Shall, S. (1995) *Biochimie* **77,** 313–318.
17. Lindahl, T., Satoh, M. S., Poirier, G. G., and Klungland, A. (1995) *Tr. Biochem. Sci.* **20,** 405–411.
18. Berger, N. A. (1985) *Radiation Res.* **101,** 4–15.
19. Szabo, C. (1997) *Biochem. Soc. Trans.* **25,** 919–924.
20. Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E., and Poirier, G. G. (1993) *Cancer Res.* **53,** 3976–3985.
21. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C. (1994) *Nature* **371,** 346–347.
22. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Munday, N. A., Raju, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1995) *Nature* **376,** 37–43.
23. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) *Cell* **81,** 801–809.
24. Wang, Z. Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M., and Wagner, E. F. (1995) *Genes Dev.* **9,** 509–520.

25. Heller, B., Wang, Z. Q., Wagner, E. F., Radons, J., Burkle, A., Fehsel, K., Burkart, V., and Kolb, H. (1995) *J. Biol. Chem.* **270,** 11176–11180.
26. Leist, M., Single, B., Kunstle, G., Volbracht, C., Hentze, H., and Nicotera, P. (1997) *Biochem. Biophys. Res. Commun.* **233,** 518–522.
27. Wang, Z.-Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schultz-Osthoff, K., and Wagner, E. F. (1997) *Genes Dev.* **11,** 2347–2358.
28. Agarwal, M. L., Agarwal, A., Taylor, W. R., Wang, Z. Q., Wagner, E. F., and Stark, G. R. (1997) *Oncogene* **15,** 1035–1041.
29. Endres, M., Wang, Z.-Q., Namura, S., Waeber, C., and Moskowitz, M. A. (1997) *J. Cerebral Blood Flow Metab.*, in press.
30. Eliasson, M. J. L., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., Pieper, A., Wang, Z. Q., Dawson, T. M., Snyder, S. H., and Dawson, V. L. (1997) *Nature Med.* **3,** 1089–1095.
31. Szabo, C., Lim, L. H. K., Cuzzocrea, S., Getting, S. J., Zingarelli, B., Flower, R. J., Salzman, A. L., and Perretti, M. (1997) *J. Exp. Med.* **186,** 1041–1049.
32. Menissier-de Murcia, J., Niedergang, C., Trucco, C., Ricoul, M., Dutrillaux, B., Mark, M., F., J.-O., Masson, M., Dierich, A., LeMeur, M., Walztinger, C., Chambon, P., and de Murcia, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7303–7307.
33. Trucco, C., Rolli, V., Olivier, F. J., Flatter, E., Masson, M., Dantzer, F., Niedergang, C., Dutrillaux, B., Menissier-de Murcia, J., and de Murcia, G. (1997) *Mol. Cell. Biochem.*, in press.
34. Ikejima, M., Noguchi, S., Yamashita, R., Ogura, T., Sugimura, T., Gill, D. M., and Miwa, M. (1990) *J. Biol. Chem.* **265,** 21907–21913.
35. Barlow, C., Hirotsune, S., Paylor, R., Liyanage, M., Eckhaus, M., Collins, F., Shiloh, Y., Crawley, J. N., Ried, T., Tagle, D., and Wynshaw-Boris, A. (1996) *Cell* **86,** 159–171.
36. Westphal, C. H., Rowan, S., Schmaltz, C., Elson, A., Fisher, D. E., and Leder, P. (1997) *Nature Gent.* **16,** 397–401.
37. Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. (1996) *Nucl. Acid Res.* **24,** 4387–4394.
38. Wesierska-Gadek, J., Schmid, G., and Cerni, C. (1996) *Biochem. Biophys. Res. Commun.* **224,** 96–102.
39. Vaziri, H., West, M. D., Allsopp, R. C., Davison, T. S., Wu, Y. S., Arrowsmith, C. H., Poirier, G. G., and Benchimol, S. (1997) *EMBO J.* **16,** 6018–6033.
40. Weinfeld, M., Chaudhry, M. A., D'Amours, D., Pelletier, J. D., Poirier, G. G., Povirk, L. F., and Lees-Miller, S. P. (1997) *Radiation Res.* **148,** 22–28.
41. Rosenthal, D. S., Ding, R., Simbulan-Rosenthal, C. M. G., Vaillancourt, J. P., Nicholson, D. W., and Smulson, M. E. (1997) *Exp. Cell Res.* **232,** 313–321.
42. Negri, C., Donzelli, M., Bernardi, R., Rossi, L., Burkle, A., and Scovassi, A. I. (1997) *Exp. Cell Res.* **234,** 174–177.
43. Shiokawa, D., Maruta, H., and Tanuma, S.-I. (1997) *FEBS Lett.* **413,** 99–103.
44. Rosen, A., and Casciola-Rosen, L. (1997) *J. Cell. Biochem.* **64,** 50–54.
45. Duriez, P. J., and Shah, G. M. (1997) *Biochem. Cell Biol.* **75,** 337–349.
46. Zhang, J., Dawson, V. L., Dawson, T. M., and Snyder, S. H. (1994) *Science* **263,** 687–689.
47. Zhang, J., Pieper, A., and Snyder, S. H. (1995) *J. Neurochem.* **65,** 1411–1414.
48. Choi, D. W. (1997) *Nature Med.* **3,** 1073–1074.
49. Mukherjee, S. K., Klaidman, L. K., Yasharel, R., and Adams, J. D. J. (1997) *Eur. J. Pharmacol.* **330,** 27–34.
50. Leist, M., and Nicotera, P. (1997) *Biochem. Biophys. Res. Commun.* **236,** 1–9.
51. Bhatia, M., Kirkland, J. B., and Meckling-Gill, K. A. (1995) *Biochem. J.* **308,** 131–137.
52. Caria, H., Quintas, A., Chaveca, T., and Rueff, J. (1997) *Mutat. Res.* **377,** 269–277.
53. Althaus, F. R., Kleczowska, H. E., Malanga, M., Muntener, M., and Pleschke, J. M. (1997) *in* The 12th International Symposium on ADP-Ribosylation Reactions: From Bacterial Pathogenesis to Cancer , pp. 90, Alvarez-Gonzales, A., Cancun, Mexico.
54. Whitacre, C. M., Hashimoto, H., Tsai, M. L., Chatterjee, S., Berger, S. J., and Berger, N. A. (1995) *Cancer. Res.* **55,** 3697–3701.
55. Biederman, K. A., Sun, J., Giaccia, A. J., Tosto, L. M., and Brown, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1394–1397.
56. Morrison, C., Smith, G. C. M., Sting, L., Jackson, S. P., Wagner, E. F., and Wang, Z.-Q. (1997) *Nature Genet.* **17,** 479–482.
57. Danska, J. S., Holland, D. P., Mariathasan, S., Williams, K. M., and Guidos, C. J. (1996) *Mol. Cell. Biol.* **16,** 5507–5517.
58. Blunt, T., Finnie, N. J., Taccioli, G. E., Smith, G. C. M., Demengeot, J., T.M., G., Mizuta, R., Verghese, A. J., Alt, F. W., Jeggo, P. A., and Jackson, S. P. (1995) *Cell* **80,** 813–823.
59. Blunt, T., Gell, D., Fox, M., Taccioli, G. E., Lehman, A. R., Jackson, S. P., and Jeggo, P. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10285–10290.
60. Shockett, P., and Stavnezer, J. (1993) *J. Immunol.* **151,** 6962–6976.
61. Nacht, M., Strasser, A., Chan, Y. R., Harris, A. W., Schlissel, M., Bronson, R. T., and Jacks, T. (1996) *Genes Dev.* **10,** 2055–2066.
62. Bogue, M. A., Zhu, C., Aguilar-Cordova, E., Donehower, L. A., and Roth, D. B. (1996) *Genes Dev.* **10,** 553–565.