Defying death after DNA damage

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DNA damage frequently triggers death by apoptosis. The irreversible decision to die can be facilitated or forestalled through integration of a wide variety of stimuli from within and around the cell. Here we address some fundamental questions that arise from this model. Why should DNA damage initiate apoptosis in the first place? In damaged cells, what are the alternatives to death and why should they be selected in some circumstances but not others? What signals register DNA damage and how do they impinge on the effector pathways of apoptosis? Is there a suborganellar apoptosome complex effecting the integration of death signals within the nucleus, just as there is in the cytoplasm? And what are the consequences of failure to initiate apoptosis in response to DNA damage?

With few known exceptions, the terminal apoptotic programme of mammalian cells depends on the activation of intracellular caspases and their modification of protein substrates within the nucleus and cytoplasm (see review in this issue by Hengartner, pages 770–776). Two processes lie immediately upstream of these effector events. The first is the activation of the receptor-mediated death-signalling pathways that ultimately trigger caspase-8 and are exemplified by the interaction of CD95 (Apo-1/Fas) with its ligand (see ref. 1 and review by Krammer, pages 789–795) The second originates from mitochondria, which are central targets for intracellular oxidative stress. Stressed mitochondria release a set of molecules — cytochrome *c*, Apaf-1 and apoptosis-initiating factor — two of which contribute to a suborganellar molecular cluster (the apoptosome), which is then responsible for the activation of caspase-9 (ref. 2). This pathway can be profoundly influenced by both pro-apoptotic and anti-apoptotic members of the Bcl-2 family, which are in turn modified, in response to local survival factors, by phosphoinositide 3-kinase (PI(3)K) and Akt (ref. 3).

This article is concerned with the relation between DNA damage and the terminal apoptotic programme. Because its normal functions demand structural and sequence integrity over many hundreds of millions of non-redundant base pairs, the mammalian genome presents an enormous target to genotoxic agents. Moreover, DNA is highly reactive and is easily altered by cell processes such as oxidation. One estimate is that a mammalian genome undergoes about 100,000 modifications per day, each bearing a finite probability of residual damage[4]. The chromatin proteins in which DNA is embedded might afford some protection from this damage, and powerful repair mechanisms exist to restore DNA structure and sequence once damage has occurred. Nevertheless, the vital processes of replication, transcription and even repair itself require chromatin rearrangement, implying periods during which DNA vulnerability might be enhanced. Apoptosis is numerically important as one possible outcome of such DNA damage. Why is it necessary for cells to adopt this seemingly wasteful strategy alongside repair?

Why should DNA damage initiate apoptosis?

Cells differ hugely in their responses to DNA damage[5]. Whereas splenic lymphocytes in fetus and adult readily initiate apoptosis after exposure to ionizing radiation (which delivers double-strand DNA breaks to all cells), apoptosis forms no part of the response of cardiac myocytes to radiation at any stage of development. Mesenchymal cells of primordial cartilage are sensitive to radiation but become resistant on full differentiation. The post-replicative epithelial cells of the adult intestinal crypt are resistant to apoptosis in response to ionizing radiation and many other DNA-damaging agents, yet replicative cells of the same lineage, just a few hours earlier in their life history, and one cell position deeper in the crypt, are acutely sensitive to both radiation-induced and drug-induced apoptosis[6]. But in the thymic cortex, small $CD4^+/CD8^+$ lymphocytes have completed their last division yet remain sensitive to apoptosis after DNA damage and other stimuli[7,8]. These examples emphasize that apoptosis is not an inevitable consequence of DNA damage. So why should they be coupled at all?

Although apoptosis is uniformly present in metazoans, both as a developmental programme (see review in this issue by Meier *et al.*, pages 796–801) and — in some circumstances — as an injury response, there is still controversy over its existence in unicellular organisms[9]. Certainly, the yeast genome does not encode a protein that, in metazoans, has the capacity to transduce DNA injury stimuli into the apoptotic programme with great efficiency: p53 (ref. 10). Even in mammals, p53 is frequently activated by DNA injury to serve other purposes than the initiation of apoptosis[5]. This raises the possibility that the coupling of DNA damage and apoptosis might be a strategy, adapted from other injury responses, to cope with certain problems of tissue organization.

Metazoan tissues depend absolutely on the ability of their constituent cells to relate to each other. Through cell–cell and cell–matrix communication, the functions of replication, differentiation and movement are orchestrated and topologically constrained. Some of these processes are difficult to reverse or rectify in the event of failure, yet failure is never far away. A half dosage of just one gene — *APC*, which encodes the oncosuppressor protein Adenomatous Polyposis Coli — renders the intestinal epithelium susceptible to the development of cells with inaccurate perceptions of polarity and position, and loss of restraint in replication: the founder cells of adenomas[11]. It is possible that cells in metazoan tissues safeguard all the important phase transitions in their lifespans against injury-induced genetic error by linking them conditionally to a death programme already in use for pruning cellular genealogical trees and sculpting organs during development.

Modes of death that are less proactive than apoptosis are intolerably disruptive to tissue organization. Furthermore,

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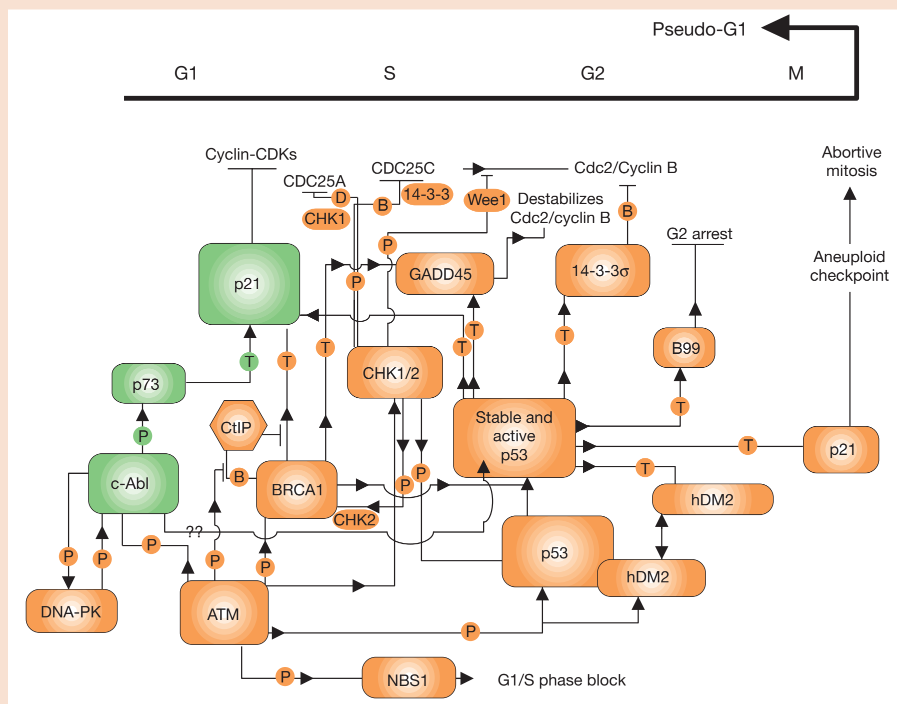

Figure 1 ATM, checkpoints and the cell cycle. DNA damaged by ionizing radiation can be sensed by ATM, triggering a cascade of downstream pathways to arrest the cell cycle. ATM-proximal events are phosphorylation reactions (denoted by P) that can lead to downstream transactivation events (T), degradations (D) or inhibitory blockades (B). p21 can block the G1/S transition and prevents aneuploidy. Multiple proteins transactivated by p53 block the S/G2 transition. Green boxes mark an auxiliary pathway that uses p73 to activate p21. S-phase blocking is also achieved by the phosphorylation of CDC25C by CHK, resulting in its cytoplasmic sequestration (by 14-3-3). Similarly, Cdc2 and cyclin B are inhibited by 14-3-3σ.

the presence of free DNA ends in a cell that retains a capacity for DNA repair leads to the activation of poly(ADP-ribose) polymerase (PARP) and the consequent exhaustion of cellular energy supplies[12]. The resulting clusters of dead cells would distort the critical ongoing cell–cell and cell–matrix signalling of a metazoan tissue. In contrast, apoptosis is designed to delete cells from tissues rapidly, tagging them for phagocytosis and recycling their constituent molecules, while neatly delaying energy exhaustion by uncoupling (through caspase activation) the catalytic and DNA-binding domains of PARP. By implication, the threshold for the activation of apoptosis in response to DNA damage can be set low: tissue stem cells and their immediate descendants can be deleted by apoptosis in response to damage stimuli much less severe than those required to kill other members of the same lineage, if indeed the damage is intrinsically lethal to such cells at all[6]. The *Drosophila* gene *reaper* is a good example of threshold setting: in its absence, the resistance of *Drosophila* embryos to cell death after ionizing radiation is enhanced about 1,000-fold[13]. Indeed the general suicidal tendency of injured stem cells is a testament to the extreme measures adopted to counter the threat posed by progenitors that might have acquired a flawed genome. Failure to initiate apoptosis in response to DNA injury of various types is associated with the appearance of cells with a mutation prevalence one or two orders of magnitude above background[14,15]. How, then, is DNA damage identified and linked to the apoptosis programme?

Molecular anatomy of a DNA injury response

The eukaryotic strategy to deal with damaged DNA can be split into three components: the recognition of injured DNA, a period of damage assessment (enforced by checkpoints), and the implementation of the appropriate response (DNA repair or cell death). These procedures are not activated in a simple linear fashion, because damage recognition elicits multiple synchronous signals that can trigger both repair and apoptotic processes. Checkpoints have a critical role in the damage response system as they provide an opportunity to monitor the appropriateness of suicide over repair. Checkpoints establish relations between cellular processes so that the execution of one process is contingent on the successful completion of an earlier unrelated activity[16]. The checkpoint to oversee the accurate replication of the genome before allowing cell division is an example. In the context of DNA damage, checkpoints erect barriers to prevent the perpetuation of injured genomes. These can be lifted once the cell has recovered. Occasionally, mutations affect the checkpoint genes themselves. The consequent loss of synchronous quality control can have disastrous results, as seen in the destabilized genomes that are characteristic of cancer[17]. This was the threat that early metazoans countered by weaving the apoptosis programme into the web of their checkpoint controls. The existence of multiple points of contact between the apoptotic and checkpoint programmes might explain the heterogeneity of downstream events in the DNA damage response. These mixed signals might compel a cell to die even though DNA repair machines have been successfully engaged[18].

The figure in Box 1 illustrates the major DNA repair options for a mammalian cell. In some cases large complexes of proteins must sequentially assemble over the lesion. This raises the critical question of how DNA damage detectors should be distributed in a manner that allows them to survey the entire genome. Although the 'active' nucleotide excision repair (NER) repairosome can tether itself to complexes that naturally navigate the DNA thread, not all repair processes are tied to transcription or replication. An attractive solution would be to corral repair proteins at various nuclear foci for release under conditions of genotoxic stress. One example of this in simple eukaryotes is the discharge of a damage repair protein and chromatin modifiers from yeast telomeres after genotoxic treatment[19]. Telomeres are repetitive DNA sequences protected by densely compact chromatin and are particularly appropriate sites in which to sequester detection and repair proteins. Tethered to nuclear pore complexes, yeast telomeres maintain a pool of repair proteins just beneath the nuclear envelope[20]. A damage-induced flux of repair proteins from them might even provide a useful gauge for the severity of a particular DNA injury.

In a striking correlation, the protein components of mammalian telomeres also include DNA repair proteins[21]. A unifying explanation for the tendency of repair proteins to dock at telomeres could be that they regard the ends of the chromosome as a double-strand break (DSB), albeit a naturally occurring one[22]. Other, naturally occurring, 'benign' DSBs use DNA repair proteins for processes such as immune gene *V*(*D*)*J* recombination[23]. Similarly, hoarding of repair proteins at telomeres might represent a shrewd mechanism for optimizing

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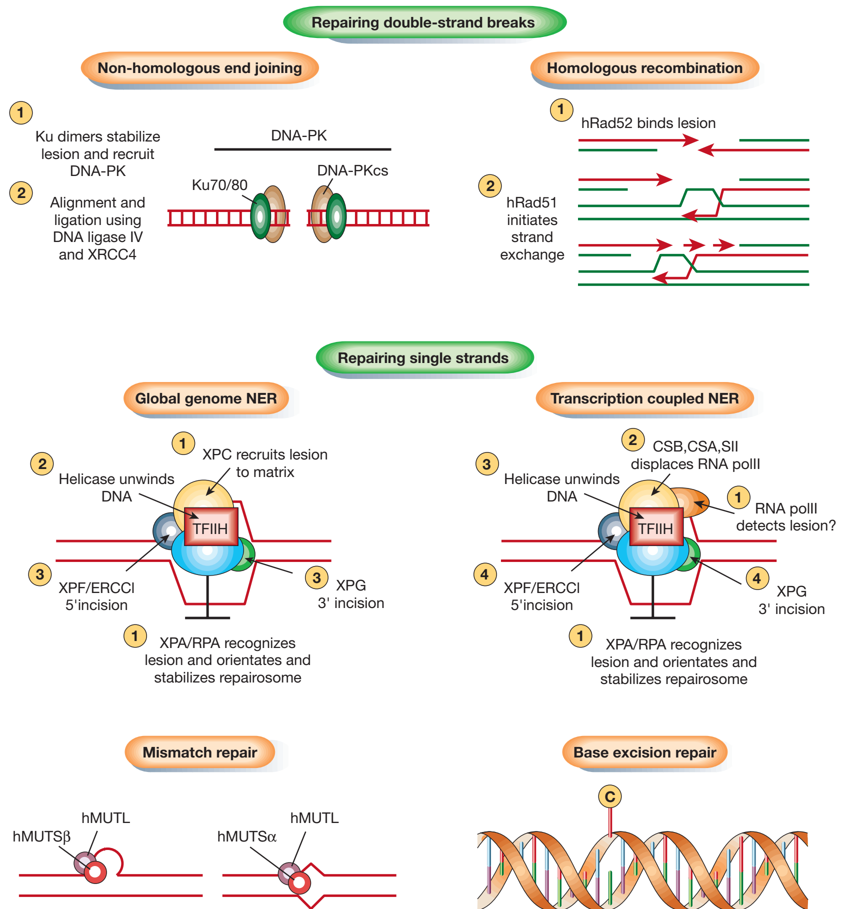
Box 1
DNA repair mechanisms



Double-strand break (DSB) repair. A DSB is potentially lethal. Two competing repair processes called homologous recombination and non-homologous end-joining (NHEJ) target DSBs[75]. Homologous recombination uses a sister chromatid or homologue to patch up the damage, whereas NHEJ is less accurate and simply joins DNA ends together. Variations of each process exist, most importantly in the use of conservative or non-conservative homologous recombination, which, as the name suggests, have different mutagenic potentials. NHEJ and homologous recombination are often described as the dominant repair pathways for mammals and yeast respectively. Despite its inaccuracy, mammals seem to favour NHEJ as their repeat-ridden genomes make sequence alignment tricky. But it is now known that vertebrates are also proficient at homologous recombination[76], prompting a major reassessment of the value of this process to mammalian repair. The mechanics of NHEJ entails the binding of Ku heterodimers to DNA breaks, protecting them from degradation and stabilizing the lesion. Ku then recruits the catalytic subunit of DNA-PK (DNA-PKcs) to activate the DNA-PK holoenzyme. The formation of this activated nucleoprotein complex promotes rejoining by a DNA ligase IV–XRCC4 heterodimer (XRCC4 denotes the X-ray cross-complementation group containing a deletion of the XRCC4 gene product). For the particular case of homologous recombination shown, hRAD52 is recruited to the DNA break, followed by invasion of the intact sister chromatid by hRAD51 to generate a recombination intermediate. As the sister chromatid acts as a template, repair must take place in late S or the G2 phase of the cell cycle. The breast-cancer susceptibility gene *BRCA1* product co-localizes with hRAD51 and promotes homologous recombination, perhaps to discourage the less accurate NHEJ process. There is also considerable interest in the Mre11–Rad50–NBS1 complex, which performs the nucleolytic processing of DSBs and is also implicated in cell-cycle checkpoints through ATM.

Single-strand repair. Single-strand repair is determined by the site and nature of the break. Nucleotide excision repair (NER) is used to excise bulky lesions, such as pyrimidine dimers, that distort the DNA helix. Two NER machines repair the inactive (the global mechanism) and active portion of the genome; RNA polymerases have a major role in the latter. For either complex, lesion recognition is followed by excision of the damaged DNA (steps 1–3 and 1–4 in the respective panels) so that re-replication can occur. Mismatch repair (MMR) detects several types of single-base mismatches in addition to more complicated loops or deletions[77]. Current interest in this process derives from the identification of defective MMR genes as the causative agents of hereditary non-polyposis cancer. Various combinations of hMUTS and hMUTL heterodimers recognize each class of lesion to recruit repairosomes. ATP hydrolysis facilitates either translocation/looping of the DNA or the conversion of hMUTS to a sliding clamp that activates and recruits repair proteins including hMUTL complexes, polymerases-δ/ϵ, exonucleases and replication factors. Finally, base excision repair removes small lesions such as alkylated and methylated bases. This is an ancient repair process that counteracts the natural instabilities of DNA as well as those posed by environmental genotoxins[78]. In the example shown, the damaged base is literally swung out of the helix and into the 'pocket' of a correcting enzyme (yellow ball), which snips it from the helix. The abasic site can be processed by APE1 endonuclease before DNA polymerase-β inserts the correct nucleotide and XCC1/ligase III seals the nick. These proteins may be orientated on PARP. 'Short patch repair' is used in this instance, although 'long patch repair' is available for gaps of two to eight nucleotides.

telomeric maintenance. As telomeres shorten with age, the subsequent exposure of chromosome ends can trigger their end-to-end ligation, which is a catastrophic outcome for the cell and its progeny. A checkpoint that forces cells to senesce or undergo apoptosis when telomeres become critically short is required to prevent this occurrence[24]. One such checkpoint activator, sensitive to the presence of free double-stranded DNA ends, is ATM (for ataxia telangiectasia mutated)[25].

The ATM family of DNA damage sensors

ATM is one of a remarkable group of PI(3)K-related kinases that also includes DNA-PKcs (the catalytic subunit of DNA-dependent protein kinase)[26] and ATR (ataxia telangiectasia Rad3 related)[27]. These proteins are all crucial in detecting the most lethal type of DNA damage, the DSB. *ATM* encodes a protein with a relative molecular mass of ~350,000 (M_r 350K)containing a DNA-binding domain and a PI(3)K catalytic domain. Atomic force micrographs provide compelling evidence that ATM and DNA-PK bind directly to free DNA ends[28]. Having done so, these kinases catalyse phosphorylation cascades to transmit damage signals to checkpoints and repair proteins. With the appropriate kinetics, such cascades can operate as sensitive molecular switches[29]. Exploring this issue further, it is predicted that instabilities in phosphorylation–dephosphorylation cycles could provide the core mechanism of a G2/M checkpoint[30]. The attractiveness of this model is its inherent ability to ratify each component of the system before proceeding, one of

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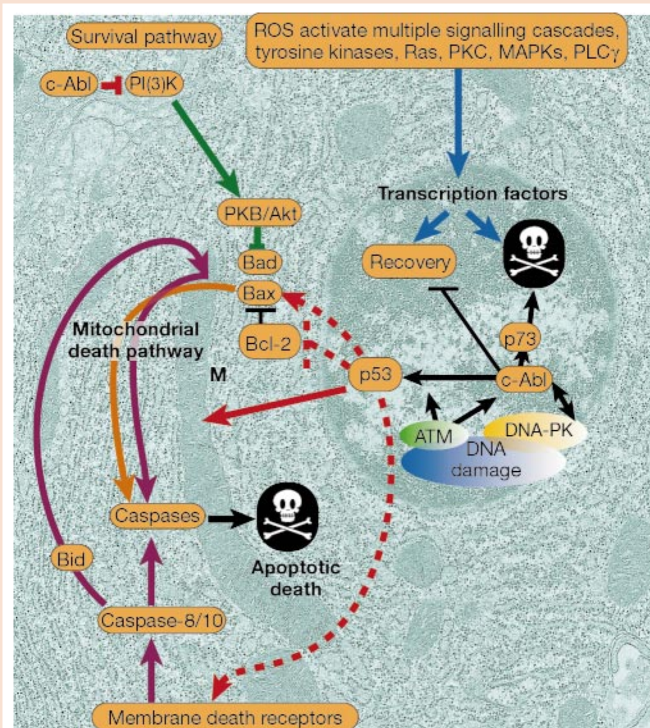

Figure 2 Apoptotic and survival pathways. Radiation damage triggers multiple stress and apoptotic pathways dependent on the cell type involved. Stress signals generated outside the nucleus include activated mitogen-activated protein kinase (MAPK) cascades (extracellular signal-regulated protein kinase (ERK), JNK and p38) and protein kinase C (PKC) (blue arrow). Transcription factors are an important target of MAPK cascades: ERK activation tends to favour survival, whereas JNK activation assists cell death. The redox-sensitive transcription factor NF-κB also translocates to the nucleus after its activation by reactive oxygen species (ROS). The activity of this transcription factor is generally associated with protection from apoptosis. Expression levels of several membrane death receptors might be augmented by stabilized p53 (red dashes). These outcompete decoy receptors, leading to the activation of caspases and an additional caspase-dependent pathway that loops through the mitochondrion (M) via Bid. Stabilized p53 also increases the concentration of Bax while diminishing the level of Bcl-2, thus favouring the disruption of mitochondrial membranes and, ultimately, the activation of caspases. The non-receptor tyrosine kinase, c-Abl, has dual roles in the cytoplasm and in the nucleus. The nuclear version is activated by ATM and can stabilize p53. Several pro-apoptotic activities have been suggested for c-Abl, although it is probably fair to say that many of these are still speculative. An important survival pathway (green arrow) is the protein-kinase-B-mediated inactivation of Bad, which is inhibited by cytoplasmic c-Abl.

the central tenets of checkpoint control. In a startling estimate for the sensitivity of these damage detection systems it has been calculated that a single DSB can trigger the arrest of the cell cycle[31]. But why are such large kinases, each with a M_r >250K, required to detect DNA damage? One possibility is that these proteins could provide a platform over which other detectors and repair proteins can assemble.

ATM, ATR and DNA-PK act as checkpoint sensors that signal to both cell-cycle and apoptosis machines. Figure 1 outlines the various cell-cycle arrests that can be instigated after the activation of ATM. Given that DNA can be damaged at any point of the cell cycle, multiple checkpoints are required to ensure a comprehensive arrest strategy for each phase[32]. However, the flaw in this system is that the overall control of these pathways rests in the hands of relatively few sensor molecules so that a single protein often polices multiple phase transitions. For example, p21 can arrest the cell at G1/S or in situations of abortive mitosis[33]. Similarly, ATM can signal to checkpoint arrests throughout the cell cycle[34]. Tracking from G1 through the cell cycle (Fig. 1) it can be seen that p21, a potent inhibitor of cyclin-dependent kinases, is transactivated by p53 and p73, although p73 has not yet been shown to be a genuine tumour suppressor[35]. Damage incurred while DNA is replicating during S phase prevents fresh origins of replication from being fired. At this stage the crucial components are the mammalian CHK1 and CHK2 proteins, which, after phosphorylation by ATM, can inhibit the phosphatases required for G1/S and G2/M progression[32]. The checkpoint strategy used at the G2/M phase of the cycle provides a textbook example of how the separation of an enzyme from its substrate can block unwanted activity. In this instance the CDC25C phosphatase is prevented from activating the cdc2–cyclin B complex that is required for entry to mitosis[36]. The recent demonstration that ATM links p95/Nbs1 to an S-phase checkpoint[37] indicates that the checkpoints described here might represent only a subset of those that operate through ATM. They do, however, illustrate how checkpoint molecules that detect DNA damage can force injured cells to engage in cycle arrest and repair their DNA. There are already examples of other, equally specific molecules (for example, the mismatch repair protein MSH-2) that can detect very different types of DNA damage (nucleotide mismatch or inappropriate methylation) to force similar outcomes[14]. However, although the proteins involved in the initial recognition and repair of DNA damage have been known for some time, the means by which they induce the terminal events of apoptosis are not yet clear.

p53 signals to apoptosis effector pathways

p53 provides one well-worked example of how the decision between apoptosis and other fates can be made at checkpoints activated by DNA damage[38]. Checkpoint activation, involving ATM and other recognition molecules, leads to p53 phosphorylation, which alters its conformation and greatly increases its stability. Several amino-terminal serines are consistently phosphorylated after radiation-induced DNA damage, and there is some specificity of mechanism. For example, phosphorylation by ATM preferentially occurs at Ser 15, whereas DNA-PK modifies Ser 15 and Ser 37 (details of p53 modifications are reviewed in depth elsewhere[39]). For most replicative cell populations, p53 levels increase within minutes of DNA damage and the first apoptotic events occur within a few hours. No early death is seen within tissues engineered to have no p53 (refs 40, 41). How, then, does the activation of p53 by DNA damage lead to the initiation of apoptosis? Several cell-cycle regulators are induced by p53, for example p21, GADD45 and members of the 14-3-3 family. Other induced proteins include Bax, CD95, DR5 (a receptor for the death ligand TRAIL)[42] and (in *Drosophila*) Rpr (ref. 43), which are all classical members of the core apoptosis pathways (red dashes in Fig. 2). However, the significance of these inductions remains somewhat obscure, as some cells from $bax^{-/-}$ and *gld* (CD95-inactive) mice show normal radiation sensitivity[44]. Moreover, CD95 induction is dependent on a p53-response element in the first intron (reassuringly conserved between mammalian species) that is activated equally by wild-type p53 and point mutants that are inactive in initiating apoptosis[45]. A further important p53-induced protein is MDM2. This escorts p53 from the nucleus and targets it for proteasomal degradation, thus ensuring that the p53 signal is transient and carefully controlled.

The advent of microarray and other genome-wide technologies has drawn attention to scores of newly transcribed molecules that might transmit the p53 signal to the apoptotic machinery[46]. One convincing newcomer is PERP, a four-span plasma membrane protein with similarity to the PMP-22/Gas3 family[47]. This transcript is associated exclusively with the apoptotic rather than the cycle-arrest

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functions of p53. A separate mechanism is suggested by the induction of MIC-1 (a secreted transforming growth factor-β-like cytokine)[48] and IGF-BP3 (a secreted binding protein for the survival factor IGF-1). These proteins could conceivably promote apoptosis through alteration of the cellular microenvironment. Perhaps the most remarkable challenge to conventional paradigms is the observation that a proportion of stabilized p53 finds its way on to mitochondrial membranes (solid red line in Fig. 2)[49]. Mitochondrial targeting seems to occur only in the context of cells within which the induction of p53 promotes death rather than cell-cycle arrest. Moreover, variants and wild-type p53 engineered to target mitochondria in the absence of any nuclear signal can induce apoptosis. p53 also binds to centrosomes and other components of the mitotic spindle[50]. This invites the question of whether these sites also nucleate primed apoptosomes and, if so, whether they are activated by binding p53.

E2F-1 activity and apoptosis

A second candidate linking DNA damage to apoptosis is the transcription factor E2F-1. This protein is released from the pocket of Rb as it becomes phosphorylated during cell-cycle progression through G1. Concomitant with the induction of the immediate early genes of DNA replication (including, among many others, the proto-oncogene c-*myc*), E2F-1 heterodimerizes with DP-1 (ref. 51). It is now known that both E2F-1 and p53 lie within a DNA damage pathway[52] and become stabilized after exposure to ionizing radiation or ultraviolet C radiation. Like p53, E2F-1 is bound and inactivated by hDM2 (the human version of MDM2), at the same time releasing DP-1 to the nucleus. Moreover, the expression of E2F-1 can initiate apoptosis, even in a p53-null background. Thus, hDM2 can act as a survival factor, independently of its interaction with p53, through its ability to bind and destabilize E2F-1. In a new development, two groups now place E2F-1 and p73 in an apoptosis pathway, providing a mechanism for the E2F-1-mediated killing that can occur in the absence of p53 (refs 53, 54). One protocol exploited receptor-mediated 'hyper' stimulation to kill T cells. Death coincided with the induction of p73 and the peak of E2F-1's transcriptional activity — which is at the late G1 or S phase. Given appropriate circumstances, the association of both E2F-1 (ref. 55) and c-Myc (ref. 56) with apoptosis rather than cell proliferation suggests that entry to a replicative (or pre-replicative) state is somehow necessary to initiate apoptosis in cells bearing damage to DNA. This concept would readily fit with long-established observations on the role of the Rb-binding adenoviral protein E1A in the response of fibroblasts to ionizing radiation. The irradiation of primary fibroblasts leads to pre-replicative cell-cycle arrest, but in fibroblasts transfected with E1A, the Rb protein is silenced and E2F is released from its pocket so that the cells respond to an identical injury by entering apoptosis[57].

c-Abl activity and apoptosis

A third substrate of ATM phosphorylation after DNA injury is the proto-oncoprotein c-Abl. c-Abl is a Src-like tyrosine kinase with an unusual carboxy-terminal domain that contains nuclear localization signals and DNA-binding sites[58,59]. In accordance with its distribution to both the nucleus and the cytoplasm, immunoprecipitation data suggest that it binds DNA-PK, ATM, Rad51, Rb, p53, p73 and perhaps other proteins[60]. After damage to DNA by ionizing radiation, c-Abl might be activated by phosphorylation through an ATM-dependent mechanism to enhance its kinase activity. DNA-PK also phosphorylates c-Abl, which in turn phosphorylates DNA-PKcs in a feedback mechanism that causes it to dissociate from Ku (ref. 59). Theoretically, therefore, c-Abl activation also contributes to turning off a signal at the heart of damage detection. The cycle arrest and apoptosis that are normally induced by ionizing radiation are prevented in cells that lack c-Abl or possess only a kinase-dead mutant. Although c-Abl is known to be an ATM substrate and can interact with many of the nucleoproteins concerned with the cellular response to DNA injury, the significance of most of its reactions is not yet clear[59].

The question arises of why the signals that couple DNA damage to the apoptosis machinery need to be so redundant and complex. One possible answer derives from the observation that many of the signals favouring death can be overridden. Presumably the many stimuli arriving in the injured cell define a threshold for apoptosis that can vary with time. The final decision to initiate apoptosis rather than cell-cycle arrest or a failure to respond by either route is likely to be conditioned by the magnitude and duration of the damage stimulus. It will also reflect the damaged cell's replicative status, its recent history as demonstrated by the availability of MDM2 or CD95, and even its position, because the local growth factor environment expresses proximity to neighbouring cells and to basement membrane.

A nuclear apoptosome

Jeffrey Nickerson in 1998 remarked: "There are are, however, two properties of tumors that are fundamental and that define some tumors as malignant. These are, first, alterations in the architecture of cells and tissues and, second, genetic instability. Both of these

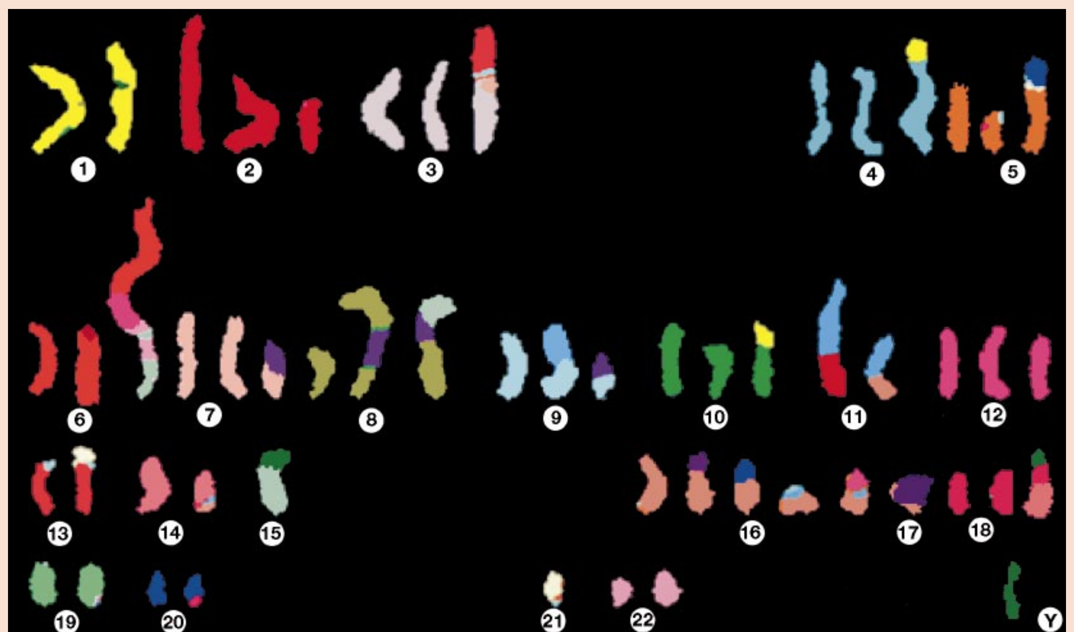

Figure 3 Spectral karyotyping. This metaphase image of the breast cancer cell line MDA-MB-361 (courtesy of J. Davidson, Department of Pathology, University of Cambridge) was obtained by 24-colour fluorescence *in situ* hybridization, with spectral imaging as described by Schrock *et al.*[72]. Each chromosome is labelled with a different combination of the final fluorescent dyes and the final image is interpreted by software that colours each pixel to show which chromosome is most likely to be present at that point.

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hallmarks of cancer may be addressed by examination of the nuclear structure."[61] In fact, it seems that both are intimately connected. Repair systems must contend with the complex topology of DNA, probably by anchoring it to the nuclear matrix. In addition, massive nuclear complexes are known to choreograph multiple nuclear functions. Indeed, there is accumulating evidence that the nucleus is a burgeoning mass of these supercomplexes, several of which are heavily implicated in apoptosis and DNA repair[62–64]. One is the PML body, which takes its name from the cancer (promyelocytic leukaemia) that disrupts its structure[65,66]. PML bodies (also called promyelocytic oncogenic domains) are nucleated by multimers of the PML protein. PML procures a large number of nucleoproteins, crucial to almost the entire range of nuclear functions, and stores them in PML bodies. The mode of this recruitment is largely unknown, although modification by the ubiquitin-related modifier (SUMO-1) seems to be one mechanism[66]. PML bodies contain the Nijmegen breakage syndrome disease protein (p95/Nbs1), which assists in the repair of DSBs[67]. PML might also act in concert with DAXX (a transcriptional repressor) to potentiate apoptosis[68], a theory supported by the resistance observed in PML-deficient systems from multiple apoptotic stimuli[63]. Sorties of damage and repair proteins from PML bodies are likely to occur when these proteins are in demand, and the natural fluctuations in the size and number of PML bodies could reflect their servicing of various nuclear machines.

The sequestration of molecules by scaffold proteins is a familiar concept in the field of protein signalling and imposes order and substrate specificity on proteins that are common to several pathways. The existence of such regulatory supercomplexes within the nucleus would be especially prudent given that crucial repair nucleases cannot be allowed to diffuse freely. A supercomplex of tumour suppressors and DNA damage and repair proteins called BASC (for 'BRCA1-associated genome surveillance complex') has recently been described[62]. BRCA1 is another enormous protein (1,863 residues) that, by virtue of expressing a BRCT (BRCA1 C-terminal) domain, is part of a superfamily of DNA damage and cell-cycle checkpoint proteins. BASC bodies might be multiple aggregates of repairosomes that can be remodelled (perhaps by ubiquitinating BRCA1's RING finger domains) to suit each type of damage detected. It will be interesting to determine whether BASC complexes act as distributors or as platforms for DNA repair engines and to examine the importance of BRCA1 as a scaffold for these complexes. BRCA1's latest incarnation as part of the human version of the SWI/SNF complex (which remodels nucleosomes) is equally provocative and might explain how BRCA1 regulates transcription[69]. Significantly, many of the cancer-associated exon 11 deletions of BRCA1 also negate its ability to associate with the SWI/SNF complex.

Cancer is associated with gross alterations to the organization of the nuclear matrix and is therefore likely to affect both DNA metabolism and subnuclear organization. In fact, many cancers are typified by complex recombinations, often involving three or more chromosomes. Chromosomes reside in distinct territories of the interphase nucleus and we might expect recombination events to be more likely between adjacent chromosomes. The loss of $p21^{WAF/CIP}$ alone is sufficient to reorganize these domains[70], so it is possible that a cell lacking a critical checkpoint gene might have shuffled the position of its chromosome territories to favour such events. As the signature lesions of cancer are often rapidly immersed in successive waves of mutations, sometimes as many as 10^3–10^5 per tumour cell, it can be extremely difficult to identify the founder lesions[71]. The spectral karyotyping technique (Fig. 3) might help us to uncover the recurrent patterns of chromosomal aberrations that characterize these lesions[72] and the 'master genes' that control them.

Future directions

DNA damage and apoptosis are both fast-growing fields and our current knowledge might well be only scratching the surface of what awaits. However, a growing understanding of these processes is already paying dividends. During preparation of this manuscript, the success of the first phase II clinical trial to combat cancer by using a modified virus (Onyx015) was announced. Onyx015 can only replicate in, and ultimately kill, p53-null cells[73]. Early concerns about replication in p53-positive tumours were allayed when it emerged that such tumours often lack other elements in the p53 response pathway. Away from the clinic, there have been considerable successes in our understanding of the cell biology of DNA damage responses. Many of these have arisen from fresh initiatives to study nuclear organization. Confocal and other imaging techniques have been trained on this most enigmatic organelle in a renewed attempt to understand its organization. The reward is that we can now appreciate some of the unique stratagems devised by the nucleus to coordinate its work. Given that compartmentation and supercomplexes provide some of the answer, it seems only a matter of time before a nuclear apoptosome is described. A fresh impetus to these studies comes from the revelation that PML bodies might also control the entry to cellular senescence by regulating p53 acetylation[74]. We should also ask about the repair lesion itself: How is chromatin remodelled during repair? Does it re-organize to stabilize the lesion or to block checkpoint signals[22]? Does chromatin exclude some proteins and attract others to repair sites? These are all crucial questions because close cooperation must exist between nucleosome remodelling and repair proteins to ensure access of the latter and facilitate repair. Delays might allow the levels of stabilized p53 to creep up and increases the chance of activating apoptosis. This leads us to the central problem of how this cell fate is sometimes sanctioned over any other. Identifying new damage-induced transcripts in cultured cells will go some way to answering this, but at some point we shall have to resolve the responses of cells in their authentic tissue microenvironment. □

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