Detection, Repair and Signalling of DNA Double-Strand Breaks

S. P. Jackson

Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, U.K., and Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, U.K.

Introduction
The cells of our bodies are continually under assault by a wide array of DNA-damaging agents. These include reactive oxygen species generated as a consequence of oxidative metabolism, UV light, ionizing radiation and radio-mimetic chemicals. The forms of damage inflicted are many, and range from base adducts, inter- and intra-strand cross-links, DNA single-strand breaks and DNA double-strand breaks. Since maintaining the integrity of the genome is of the utmost importance, living organisms have evolved a variety of systems that recognize, signal the presence of, and repair the various forms of DNA damage. The important role that such DNA repair systems play is evidenced by the fact that defects in these can result in cell death and hypersensitivity to endogenous or environmental mutagens, and, in multicellular organisms, can initiate events that result in cancer. In this lecture, I will discuss how work in my laboratory has provided some significant insights into one key DNA repair system—the DNA non-homologous end-joining system. I will also describe how this work has impacted on the somewhat related area of DNA damage signalling.

DNA double-strand breaks
DNA double-strand breaks (DSBs) are the principal lethal lesions that are induced by ionizing radiation and radio-mimetic chemicals, and can also be generated during certain site-specific recombination processes and when a DNA polymerase encounters damage on its template. It is of great importance that cells recognize DNA DSBs and act upon them rapidly and efficiently, because major deleterious consequences can result if these are left unrepaired or are repaired inaccurately. Most simply, a DSB can result in the direct inactivation or mutation of a key gene, leading to cell death or impaired cell function. Alternatively, being highly recombinogenic, unrepaired DSBs can lead to chromosomal translocations that generate highly unstable dicentric chromosomes or acentric chromosomal fragments.

In light of the above, eukaryotic cells possess at least two distinct DNA DSB repair systems. One of these, the homologous recombination system, catalyses DNA exchange processes in which the damaged chromosome retrieves genetic information from an undamaged homo-
logue [1]. The other is termed the DNA non-homologous end-joining (NHEJ) system, and brings about the ligation of two DNA DSBs without the requirement for extensive sequence homology between the DNA ends nor synapsis with an undamaged partner molecule. Recent work has revealed that both pathways for DNA DSB repair are highly conserved throughout eukaryotic evolution, although the relative importance of the two pathways differs considerably from one organism to another. Thus, whereas relatively simple eukaryotes, such as the yeast Saccharomyces cerevisiae, rely mainly on homologous recombination to mediate DSB repair, in mammals, the NHEJ pathway predominates under most circumstances [1–3]. Until recently, much more was known about homologous recombination than the NHEJ system. Over the past few years, however, work in the NHEJ area has progressed tremendously and has generated a great deal of excitement. Here, I overview some of these developments and, in particular, point out how biochemical approaches, such as those employed by my own laboratory, have contributed to our current understanding of DNA NHEJ and its physiological roles in repairing ionizing radiation-induced lesions.

DNA-dependent protein kinase

As is frequently the case in science, key findings often arise from an unlikely source — it was through working as a postdoctoral fellow on the control of human gene transcription in the Laboratory of Robert Tjian in Berkeley, California, that I was led into the DNA repair area. This work, which I conducted in 1990 together with an undergraduate colleague, Judy McDonnell, was to characterize the mammalian transcription factor Sp1 and determine how its activity might be regulated by post-translational modification. During the course of these studies, we made the observation that Sp1 protein present within human HeLa cell nuclear extracts becomes phosphorylated when the extract is incubated under conditions suitable for in vitro transcription. Furthermore, during the characterization of this phosphorylation, we made the intriguing discovery that it requires DNA to be present in the reaction and that phosphorylation is most effective if the DNA contains binding sites for Sp1. There were two simple interpretations for these results. One was that DNA binding alters the conformation of Sp1 so that it becomes a better substrate for the kinase. The other was that the kinase itself might be activated by DNA, and the increased phosphorylation in the presence of the Sp1 binding sites was through co-localization of Sp1 and the kinase on the same DNA molecule. In support of the latter, we observed that the addition of non-specific competitor DNA led to reduced Sp1 phosphorylation. Moreover, when I began fractionating the extract to identify the kinase, I found that it bound specifically to chromatographic supports bearing immobilized DNA.

Although the idea of a possible DNA-activated protein kinase was new to me, a search through the literature revealed that a kinase with such properties had been described previously; in 1985, Carl Anderson and colleagues [4] at Cambridge University reported an activity in extracts of clam eggs that phosphorylated the protein hsp90 in a DNA-dependent fashion. Upon contacting Carl, now at the Brookhaven National Laboratory, U.S.A., I learned that he and his collaborator Susan Lees-Miller, and the group of Tim Carter at St John’s University in New York, had both independently partially purified this enzyme as a single large polypeptide of >300 kDa. Through exchanging kinase preparations, we concluded that the Sp1 kinase we had identified and the kinase identified by the Anderson laboratory were identical. Later that year, three papers were published on this kinase [5–7], now known as DNA-dependent protein kinase, or more simply ‘DNA-PK’.

DNA-PK is activated by DNA ends

In 1991, I relocated to the U.K. to set up my own laboratory in the Wellcome/CRC Institute, Cambridge. At this time, the best characterized in vitro substrates for DNA-PK were all transcription factors, suggesting that its role might be to regulate transcription. However, despite intensive efforts by myself and my first graduate student, Tanya Gottlieb, we could find no effect of DNA-PK on Sp1-mediated transcription. In the course of these experiments, however, we found by DNase I footprinting that an activity present within our purified DNA-PK preparations binds with high selectivity to DNA ends. Moreover, and consistent with earlier findings [5,6], we found that DNA-PK is activated strongly by linear but not by supercoiled plasmid DNA. Further analyses revealed that this effect is due to a requirement for DNA ends. We therefore concluded
that DNA-PK binds to and is activated by DNA ends [8].

**DNA-PK is a multi-protein complex**

At that time, besides DNA-PK itself, one of the very few proteins that had been shown to bind to DNA ends was Ku, a human autoimmune antigen whose function was obscure. Previous work had shown that Ku comprises a tightly associated heterodimer of polypeptides of approximately 70 and 80 kDa (Ku70 and Ku80 respectively). Notably, when we tested for Ku in our DNA-PK preparations, we found that it was present in all cases. Furthermore, through a variety of biochemical and immunological approaches, we established that Ku is actually a key component of DNA-PK, and that optimal DNA-PK activity is only exhibited when Ku is present together with fractions containing the large subunit, now known as the DNA-PK catalytic subunit (DNA-PKcs) [8]. Essentially at the same time, biochemical analyses of DNA-PK by William Dynan and colleagues led to the same conclusion that the DNA-PK holoenzyme it composed of Ku and a separate catalytic subunit [9]. Taken together, these studies yielded a model in which Ku binds to DNA DSBs then recruits DNA-PKcs to such structures, activating its catalytic potential in the process (Figure 1).

**Ku functions in DNA DSB repair**

The above work suggested that DNA-PK might be involved in recognizing DNA ends in vivo, and thus raised the exciting possibility that it functions in DNA repair and/or recombination. Specifically, since the DNA-PK holoenzyme binds to and is activated most efficiently by DNA DSBs, it suggested that DNA-PK might play a role in the repair or signalling of DNA ends generated as a consequence of exposure to ionizing radiation, and raised the idea that DNA-PK-deficient cells might exhibit hypersensitivity to ionizing radiation.

In early 1994, I was fortunate to receive a phone call from Penny Jeggo at the MRC Cell Mutation Unit in Brighton. For a number of years, Penny’s laboratory and several others had been working on mammalian cell mutants, many of which were originally derived from the Chinese Hamster Ovary cell line (for reviews, see [10,11]). Notably, a subset of these mutants displays the phenotypes of hypersensitivity to ionizing radiation and a deficiency in the repair of ionizing radiation-induced DNA DSBs. Furthermore, these cells were also shown to be deficient in the resolution of DNA DSBs that arise during V(D)J recombination, the site-specific recombination process that helps generate the mature genes for immunoglobulin and T-cell receptor proteins in the developing mammalian immune system (Figure 2; for reviews, see [10,12,13]). Analyses of V(D)J recombination in wild-type and mutant cells had revealed that this reaction proceeds via a cut-and-ligation process that involves DNA DSBs as intermediates. Furthermore, and providing a clear link to DSB repair processes, it was shown that it is not the cutting but the ligation step of V(D)J recombination that is impaired in the ionizing radiation-sensitive cell mutants (Figure 3).
Work in several laboratories had established that the DNA DSB repair-deficient mutant cells could be assigned into three distinct complementation groups, termed IR4, IR5 and IR7, which thus defined three genes whose products play key roles in DNA DSB repair and V(D)J recombination (for reviews, see [10,13]). One goal of Penny’s group was to clone the human XRCC5 gene that complements the IR5 defect and, in the previous year, her group had mapped the location of this gene to human chromosomal interval 2q33-35 [14]. The reason why Penny had called me was that she was aware of our work on Ku and DNA-PK, and had just read a manuscript reporting that the gene for Ku80 maps to human chromosome 2q33-35 [15]. The question was, could Ku80 be the product of XRCC5?

To answer the above question, Nick Finnie and Tanya Gottlieb in my laboratory carried out a series of biochemical assays and cell-based radiosensitivity studies together with Penny Jeggo and colleagues, and we also collaborated with Guillermo Taccioi and Fred Alt in Boston, U.S.A., who conducted studies in parallel on V(D)J recombination. Our work, and similar work performed by other laboratories, appeared in a flurry of papers published in 1994 and early 1995 [16-21]. These studies revealed that cells of IR5 indeed lack Ku DNA binding function and DNA-PK activity, and that their radiosensitivity and V(D)J recombination defects can be complemented by expression of the Ku80 cDNA. Taken together, these studies revealed that Ku80 plays a key role in DNA DSB repair and V(D)J recombination, a conclusion supported by the subsequent demonstration that mice disrupted for Ku80 function are radiosensitive and are unable to perform V(D)J recombination effectively [22,23]. More recent work has revealed that similar phenotypes are observed for mice or mouse cells deficient in Ku70 [24-26], indicating that both components of the Ku heterodimer function in DSB repair. Cells deficient in Ku70 have thus been assigned to complementation group IR6, and the gene for human Ku70 accordingly designated XRCC6.

**Figure 3**

Overall mechanism of V(D)J recombination

In the first step, the V(D)J recombination apparatus generates DNA DSBs between the signal and coding DNA segments to yield recombination intermediates. In the second step, DNA DSB repair components mediate DNA processing and ligation events to yield signal and coding joins. Cells of IR4, 5, and 6 are deficient in both coding and signal join formation, whereas rodent cells of IR7 are impaired severely for coding join formation only.

**DNA-PKcs functions in DNA DSB repair**

In light of the involvement of the two Ku subunits in DNA DSB repair and the biochemical interactions between Ku and DNA-PKcs, we and others were intrigued by the possibility that the defects in cells of IR4 or IR7 might be caused by loss of DNA-PKcs activity. Consistent with this idea, we discovered that, although cells of IR4 are DNA-PKcs proficient, extracts of cells from IR7 lack detectable DNA-PK catalytic activity, and this activity can be restored biochemically by addition of purified DNA-PKcs but not by Ku [27]. Furthermore, collaborative work we performed with the Jeggo and Alt laboratories indicated that the gene for DNA-PKcs can complement both the radiosensitivity and V(D)J recombination defects of IR7 cells [27]. Similar conclusions on cells of IR7 were also reached in parallel studies conducted by the groups of David Chen and Martin Brown [28,29]. In addition, a collaboration between the groups of Susan Lees-Miller and Joan Allalunis-Turner identified a radiosensitive human cell line that is defective in DNA-PKcs [30]. Taken together, these results therefore establish that, like Ku, DNA-PKcs is an important component of the mammalian DNA DSB repair apparatus.
The severe-combined-immune-deficient (scid) mouse is mutated in the gene for DNA-PKcs

In the course of the above work, we also made an impact on an issue that had remained enigmatic to the immunology field for over a decade: the molecular nature of the mouse scid mutation. The scid mouse was first reported in 1983 by Bosma and colleagues as a spontaneously arising immune-deficient mouse. Subsequent studies had revealed that the scid mouse virtually lacks mature B- or T-lymphocytes. Furthermore, it had been shown that the scid defect is reflected at the level of V(D)J recombination and that scid mice are also radiosensitive - their defect falling into complementation group IR7 (for review, see [31]). Consistent with the scid locus corresponding to the gene for mouse DNA-PKcs, our collaborative work with the laboratories of Penny Jeggo, Roy Riblet and Carl Anderson indicated that these do indeed coincide genetically [27,32,33]. Furthermore, Martin Brown and colleagues established that the scid defect can be corrected at the cellular level by the gene for human DNA-PKcs. Finally, and most persuasively, through analyses of both the gene and the cDNA for DNA-PKcs, we and others have shown that the scid mutation generates a termination codon in the DNA-PKcs open-reading frame, leading to an 83-amino-acid residue C-terminal truncation of DNA-PKcs that disrupts the kinase domain and results in an unstable polypeptide [34-36].

XRCC4 encodes a protein that interacts with DNA ligase IV

As mentioned above, cells of IR4 have very similar defects to those of IR5, IR6 and IR7; namely hypersensitivity to ionizing radiation, DSB repair defects and an inability to mediate V(D)J recombination effectively. This therefore implies that the gene product defective in these cells functions in a common pathway with DNA-PKcs and Ku. In 1995, Fred Alt and colleagues [37] cloned the cDNA for the human XRCC4 gene, which complements the hamster XR-1 cell line that defines the IR4 group, and showed that the hamster homologue of XRCC4 is deleted in XR-1. Furthermore, analysis of the XRCC4 coding region revealed that it encodes a protein of 334 amino acid residues. Unfortunately, this did not in itself provide major insights into XRCC4 function, because it did not reveal significant homologies between XRCC4 and other characterized proteins.

Recently, however, biochemical investigations by my laboratory and the laboratory of Michael Lieber at the University of Southern California have increased our understanding of how XRCC4 functions [38,39]. The studies in my laboratory, which were conducted by Susan Critchlow, were conducted in collaboration with Richard Bowater of the ICRF Clare Hall Laboratories, South Mimms. This work initiated with the observation that XRCC4 in crude mammalian extracts migrates on gel-filtration chromatography as a much larger entity than the recombinant protein. Subsequent analyses revealed that this is because XRCC4 exists in an extremely tight and very specific complex with a polypeptide of approximately 100 kDa that corresponds to mammalian DNA ligase IV [38]. Notably, ligase IV had already been identified biochemically and its cDNA had been isolated some 2 years earlier [40]. However, no physiological function for this protein had been defined. Further studies in my laboratory revealed that the interaction between XRCC4 and ligase IV is mediated by the non-catalytic C-terminal region of ligase IV that contains two tandem copies of the BRCT (BRCA1 carboxy-terminus) domain, which has been identified in a variety of nuclear proteins [41,42]. In addition, we and others have found that the XRCC4/ligase IV complex interacts directly or indirectly with the DNA-PK holoenzyme [38,43], and Michael Lieber and colleagues have reported that ligase IV activity is stimulated by its association with XRCC4 [39]. Taken together, these results suggest that XRCC4 might serve to activate ligase IV and target it to the DNA-PK-containing DNA NHEJ complex (see below).

DNA-PK is a potent repressor of transcription by RNA polymerase I

Although our work has not established any major effects of DNA-PK on transcription mediated by RNA polymerase II, collaborative work between Tanya Gottlieb in my laboratory and Anne Kuhn from the laboratory of Ingrid Grummt in Heidelberg has established that DNA-PK is able to repress transcription by RNA polymerase I - the polymerase that synthesizes the precursor to the large ribosomal RNAs. Thus, we found that addition of DNA-PK to a reconstituted in vitro transcription system leads to a dramatic reduction in polymerase I transcription. Furthermore, this
inhibition is dependent on ATP hydrolysis and appears to be via DNA-PK phosphorylating and inactivating a key transcription component [44]. Similar results have also been reported by the laboratory of Paul Labhart [45]. Although other possibilities exist, our current model is that this effect on transcription may be to potentiate DNA repair in certain physiological settings (see below).

Models for DNA DSB repair
Investigations into the DNA substrates that can be utilized by the DNA-PK-associated DNA repair system, together with analyses of the repair products generated, have revealed that this system catalyses a pathway of DNA non-homologous end-joining (NHEJ). Thus, the system can mediate the ligation of DNA ends that do not bear extensive homology with one another, and does not require the presence of an undamaged DNA molecule to act as a template. In light of the results that I have described, it is now possible to start to build up a picture of how DNA-PK, XRCC4 and other NHEJ components may function in DNA repair (Figure 4; for more extensive reviews, see [3,13]). Firstly, given the biochemical properties of DNA-PK, it seems likely that the enzyme will assemble at sites of DNA damage in vivo and will then function directly or indirectly in potentiating DNA DSB repair. One possibility is that DNA-PK could serve to hold the two broken chromosome ends together to facilitate their ligation. Indeed, support for this model has recently been provided by electron microscopy studies from the group of David Chen, in which single Ku molecules have been visualized to bridge two DNA ends [46]. Another possibility is that DNA-PK protects the broken DNA ends from exonucleases, a possibility that is consistent with the fact that the rare V(D)J recombination products that are generated in DNA-PK-negative cells are usually found to have suffered deletions. Alternatively, or in addition, the Ku/DNA-PKcs complex could prevent the DNA ends from engaging in recombination reactions with other DNA molecules, thus preventing the generation of undesirable chromosomal rearrangements.

Another possibility is that DNA-PK represses processes that might otherwise interfere with DNA repair, such as DNA replication or transcription. In this regard, it is tempting to speculate that the observed inhibition of transcription by RNA polymerase I in vitro may be of importance — when DNA DSBs occur in the rDNA, perhaps it is necessary to prevent the high levels of transcription through this locus from interfering with the assembly of the repair apparatus. In addition, it is possible that DNA-PK functions directly or indirectly in modulating chromatin structure in the vicinity of DNA damage to potentiate its repair. It is also possible that DNA-PK activation by DNA DSBs triggers DNA damage signalling pathways. For example, DNA-PK has been shown recently to play a role in triggering induction of the p53 protein in response to exposure to ionizing radiation [47,48]. A role of DNA-PK in DNA damage signalling is also supported by analyses of the DNA-PKcs polypeptide sequence (see below).

The available data suggest that DNA-PK might also function by recruiting other components of the DNA repair machinery to sites of DNA damage (Figure 5). This idea is particularly attractive in light of the fact that we have obtained evidence for interactions between DNA-PK and the XRCC4/ligase IV complex. In such a scenario, the role of XRCC4 could be to act as an adaptor to target ligase IV to this type of repair complex. Other potential interaction partners for the DNA-PK/XRCC4/ligase IV complex include nucleases and DNA polymerases, whose concerted actions may often be required to 'clean up' damaged DNA ends before they can be repaired. In this regard, it is noteworthy that work in yeast has identified two nucleases (Rad50 and Mre11) which function in the DNA NHEJ process (see below). Another possibility is that
DNA-PK may recruit other proteins to the site of the DNA DSB

In this model, Ku binds to DNA ends and recruits/activates DNA-PKcs. The ligase IV/XRCC4 complex is then recruited, either by direct interactions with DNA-PK or indirectly via other DSB repair components, such as Rad50, Mre11 or NBS1.

phosphorylation of DNA repair components by DNA-PK activates them at sites of DNA damage or inactivates them to promote their disassembly from the DNA repair complex after their job is completed. In this regard, we have discovered that XRCC4 is a very effective substrate for DNA-PK in vitro [38].

The Ku-associated DNA NHEJ system exists in yeast

As mentioned earlier, yeast cells use homologous recombination as their principal mechanism for DNA DSB rejoining. However, it has recently become clear that yeasts also possess a NHEJ pathway which is highly related to that in mammalian cells. For example, we and others have identified *S. cerevisiae* homologues of mammalian Ku70 and Ku80 (termed Yku70p or Hdf1p, and Yku80p or Hdf2p, respectively). Furthermore, studies by Simon Boulton from my laboratory, and by workers elsewhere, have established that disruption of *YKU70* or *YKU80* leads to impaired DNA NHEJ [49–54]. Notably, because NHEJ is not the predominant DNA DSB repair system in yeast, loss of Ku function does not lead to significant hypersensitivity to ionizing radiation. This is in contrast to the significantly increased radiosensitivity of *rad52* mutant strains, which are debilitated in homologous recombination. However, loss of Ku function does further sensitize *rad52* mutant strains to ionizing radiation, indicating that yeast Ku functions in a repair pathway that is involved in the repair of ionizing radiation-induced DNA damage and that this pathway is distinct from that of homologous recombination (Figure 6).

The *S. cerevisiae* genome is now fully sequenced [55], and this has already proved to be an enormously useful resource. Notably, when Soo-Hwang Teo in my laboratory searched for potential DNA ligases in the yeast genome, she came across an open-reading frame that encoded a protein with strong homology to mammalian DNA ligase IV. In light of the linkage that was emerging at that stage between ligase IV and XRCC4, we decided to see whether the yeast protein, now known as Lig4p or DnHp, functions in DNA NHEJ. Strikingly, these studies revealed that Lig4p functions in the NHEJ pathway and, furthermore, does not appear to be essential for any other characterized yeast DNA repair process [56]. Thus, disruption of *LIG4* further sensitizes *rad52* mutant yeasts to ionizing radiation and does this in an epistatic manner with Ku (Figure 6). In addition, a role for *LIG4* in the Ku pathway for NHEJ can be observed using an *in vivo* plasmid repair assay (Figure 7). Similar results have also been obtained by the groups of Tomas Lindahl and Michael Lieber [57,58]. Most recently, a yeast homologue of XRCC4 has been identified and shown to function together with Lig4p in DNA NHEJ ([59]; S.-H. Teo and S. P. Jackson, unpublished work). These studies therefore reinforce the conclusion that mammalian ligase IV is involved in DNA DSB repair, and
again indicate that the yeast and human DNA NHEJ pathways are fundamentally homologous. Database searches reveal that S. cerevisiae does not contain a clear homologue of DNA-PKcs. However, yeast does possess the DNA-PKcs relatives Tel1p and Mec1p, raising the possibility that these perform DNA-PKcs-related functions.

Yeast molecular genetics has also been instrumental in identifying additional components of the DNA NHEJ apparatus. Thus, we and others have established an important role for the factors Rad50p, Mre11p and Xrs2p in the Ku pathway of DNA NHEJ (60) and references therein; Figure 8). Although the function(s) of these proteins is not fully clear, it is noteworthy that Rad50p and Mre11p share homology with the Escherichia coli ATP-dependent exo nucleases SbcC and SbcD respectively [61]. This raises the possibility that Rad50p and Mre11p function in the processing of radiation-induced DNA ends before their ligation. Consistent with the NHEJ apparatus being conserved from yeast to man, human homologues of Rad50p and Mre11p have been identified [62,63] and appear to function in ionizing radiation-induced DNA repair responses [64]. Furthermore, a functional homologue of Xrs2p has recently been identified as the product of the NBS1 gene. Notably, a deficiency in NBS1 leads to the human genetic disease Nijmegen breakage syndrome (NBS), which is characterized by developmental abnormalities, radiosensitivity and chromosomal instability [65–68]. In addition to establishing further links between NHEJ in the yeast and human systems, these studies are exciting because they show that deficiencies in this DNA repair pathway can lead to significant human pathologies. It is thus tempting to speculate that defects in other NHEJ components will be associated with human disease states.

Cloning of the cDNA for DNA-PKcs reveals links with proteins involved in DNA damage signalling

Through purifying DNA-PKcs and determining the sequences of several peptides derived from it, we were able to isolate a partial cDNA clone for DNA-PKcs. Subsequent work by Kathy Hartley and David Gell in my laboratory led to the isolation and sequencing of clones spanning the entire DNA-PKcs cDNA [69]. Analysis of this cDNA sequence and those of partial cDNA clones identified through other routes [34,70,71]
revealed that DNA-PKcs is a polypeptide of approximately 470 kDa. The portion comprising the N-terminal ~3500 residues does not appear to be significantly related to any other characterized proteins. This region of the protein does, however, possess a leucine-zipper motif, and Ugur Yavuzer from my laboratory has recently implicated this in interacting with the protein CID, which is a DNA binding component of the nuclear matrix [72]. In addition, the DNA-PKcs polypeptide contains a kinase catalytic domain at its C-terminus. Strikingly, this kinase domain is very different from those of most protein serine/threonine kinases and, instead, falls into the phosphatidylinositol (PI) 3-kinase family of enzymes. Some members of the PI 3-kinase family function in signal transduction by phosphorylating inositol phospholipids [73]. A well-characterized example of these is mammalian PI 3-kinase, which phosphorylates PI at the 3-position of the inositol ring in response to a variety of stimuli, including the activation of various growth factor receptors. Although our findings initially suggested that DNA-PK may also be a lipid kinase, collaborative work between Graeme Smith in my laboratory and Nullin Divecha of the Babraham Institute, Cambridge, has been unable to ascribe such a function to the enzyme [69,74]. Thus, despite being in the ‘PI 3-kinase family’, DNA-PKcs appears to be a protein kinase, not a lipid kinase. In regard to this, it is worth noting that the PI 3-kinase homology region is considerably similar in sequence to the catalytic domains of classical serine/threonine protein kinases [69,75,76], particularly at residues which, in classical protein kinases, function in ATP binding and phosphate transfer [77]. Furthermore, several PI 3-kinase family members, such as PI 3-kinase itself, are able to phosphorylate both lipid and protein targets [78]. It therefore appears that PI 3-kinases and classical protein serine/threonine kinases share a common evolutionary origin, and that members of the PI 3-kinase family can have protein or lipid kinase activity, or both.

Sequence comparisons and phylogenetic analyses of PI 3-kinase family members reveal that the family can be subdivided into two distinct subgroups [69,75,76]. One of these contains mammalian PI 3-kinase and other lipid kinases; the other contains DNA-PKcs and a series of other large (>250 kDa) proteins which are involved in controlling cell cycle progression and/or functioning in DNA repair and DNA damage signalling (Figure 9). One of the best characterized of these DNA-PKcs-related proteins is ATM, which was identified in 1995 by an international consortium of researchers by way of the fact that its deficiency leads to the human autosomal recessive syndrome ataxia telangiectasia (A-T) [75,76,79,80]. The most obvious features of this disease, which has a worldwide incidence of around 1 in 100,000, are an unsteady posture (ataxia), which is caused by the degeneration of Purkinje cells in the brain cerebellum, and the presence of dilated blood vessels (telangiectases) in the eyes and often in the facial skin. In addition, A-T is characterized by a variety of other

![Figure 9](image-url)
symptoms, including progressive neuronal degeneration, aspects of premature ageing, growth retardation, immune deficiencies, and a greatly elevated incidence of cancer. Furthermore, A-T heterozygotes, which comprise around 1% of the population, have been reported to exhibit a higher incidence of breast cancer, although this conclusion is highly controversial [81]. At the cellular level, A-T is characterized by a high degree of chromosomal instability and hypersensitivity to ionizing radiation and radio-mimetic drugs. In addition, A-T cells are defective in a variety of radiation-induced cell cycle checkpoint processes (see below).

Although it is possible that ATM plays direct roles in DNA repair, the available evidence indicates that its prime role is in DNA damage signalling. Most normal proliferating cells temporarily arrest cell cycle progression at the G1–S, S or G2–M cell cycle 'checkpoints' in response to DNA damage. Arrest in G1 or within S-phase prevents the replication of damaged DNA, which would otherwise fix mutations or lead to the generation of DNA strand breaks. On the other hand, cell cycle checkpoint arrest in G2 prevents mitosis until DNA damage is repaired, thus precluding the generation of unstableacentric chromosomal fragments. Notably, A-T cells are deficient in the ionizing-radiation-induced G1–S, S and G2–M cell cycle checkpoints due, at least in part, to defective induction of the transcription factor p53 in response to ionizing radiation [82–88]. Consequently, p53-mediated transcriptional activation of p21/Waf1/Cip1 and the subsequent inhibition of G1 cyclin-dependent kinases after exposure to ionizing radiation, are also defective in A-T cells [89,90]. Recent work with the human protein ATR, which is another DNA-PKcs and ATM relative, reveals that this protein is also involved in DNA damage signalling and cell cycle checkpoint control processes [91–93].

Taken together with the DNA-PK paradigm, the above data suggest models in which ATM and ATR function as part of DNA damage sensing complexes. Thus, activation of these proteins by various forms of DNA damage might trigger phosphorylation cascades that impinge on the transcription, cell cycle and apoptotic machineries. Very recently, we have obtained evidence in support of such models by conducting biochemical analyses of the ATM and ATR proteins. For example, Graeme Smith and Nick Lakin in my laboratory, in collaboration with D. Chen and R. Cary, have shown that, like DNA-PKcs, purified ATM protein binds selectively to DNA DSBs. Furthermore, Nick has recently purified ATR from human cell extracts and has demonstrated that it displays a DNA-stimulated protein kinase activity that targets the N-terminal region of p53. Since this region of p53 has been shown to function in transcription and in controlling p53 protein stability, this work suggests that ATR acts directly upstream of p53 in a DNA damage-inducing signalling pathway. Given that DNA-PKcs and ATM have also been shown to function in p53-dependent DNA damage signalling, the available data therefore support models in which DNA-PKcs, ATM and ATR function in distinct but possibly overlapping DNA damage detection pathways.

Conclusions and future prospects
I have described how recent work in my laboratory and elsewhere has helped in the identification and characterization of a series of proteins that function in DNA NHEJ and V(D)J recombination. In addition, I have shown how this work has provided significant insights into DNA damage signalling pathways. These advances have opened up a plethora of additional exciting avenues for further investigation. One prominent issue for future studies will be to determine exactly how the DNA-PK, ATM and ATR systems are activated in response to DNA damage or by other perturbations in cellular integrity. Furthermore, it will be of great interest to identify additional components of these systems. Another key goal will be to identify the physiological targets for DNA-PKcs, ATM and ATR, and determine the consequences of phosphorylation on the activities of these target molecules. Given that deficiencies in ATM and NBS1 lead to human disease states, it will also be of interest to see whether this is the case for other components of the pathways that I have discussed. Finally, recent developments have provided the exciting opportunity for developing drugs that inhibit the DNA-PK, ATM and ATR systems. Such drugs, in conjunction with our increased comprehension of DNA repair and DNA damage signalling, might yield significant improvements in radiotherapeutic and chemotherapeutic treatments for cancer. Given the prominent role that biochemistry has played in bringing about recent developments in the DNA repair arena, it seems clear that biochemical
approaches will be instrumental in achieving further progress.

None of our progress would have been possible without the efforts of the hard-working, enormously talented and highly interactive group of individuals that I have been lucky to recruit to my laboratory over the past 7 years. I apologize to colleagues in my lab and elsewhere whose work I have not been able to mention due to time constraints. Thanks also to my many other colleagues for their advice and encouragement, particularly Robert Tjian, Carl Anderson, Penny Jeggo, Alan Lehmann, Thomas Lindahl, Peter Rigby and Ron Laskey. Finally, I thank the Cancer Research Campaign for their support.

37 Li, Z., Otevrel, T., Gao, Y., Cheng, H. L., Seed, B., 1999
Received 2 September 1998