Menage à trois: double strand break repair, V(D)J recombination and DNA-PK

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Summary

All organisms possess mechanisms to repair double strand breaks (dsbs) generated in their DNA by damaging agents. Site-specific dsbs are also introduced during V(D)J recombination. Four complementation groups of radiosensitive rodent mutants are defective in the repair of dsbs, and are unable to carry out V(D)J recombination effectively. The immune defect in Severe Combined Immunodeficient (*scid*) mice also results from an inability to undergo effective V(D)J recombination, and *scid* cell lines display a repair defect and belong to one of these complementation groups. These findings indicate a mechanistic overlap between the processes of DNA repair and V(D)J recombination. Recently, two of the genes defined by these complementation groups have been identified and shown to encode components of DNA-dependent protein kinase (DNA-PK). We review here the three fields which have become linked by these findings, and discuss the involvement of DNA-PK in dsb rejoining and in V(D)J recombination.

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Introduction

About fifteen years ago, an autoantigen termed Ku was identified from the sera of patients with scleroderma-polymyositis overlap syndrome[1]. Ensuing studies showed that Ku interacts with free ends of double stranded (ds) DNA without any sequence specificity, and serves as the DNA binding component of DNA-PK†, a nuclear kinase shown previously to phosphorylate numerous DNA binding proteins[2-4]. A little over a decade ago, a group of radiosensitive hamster mutants were isolated, and subsequently characterised as having defects in the rejoining of DNA ds ends[5-7]. At about the same time, a severe combined immunodefective (*scid*) mouse was identified and shown to have a defect in the process of V(D)J recombination that occurs during B and T cell maturation[8]. In the following years, connections between these diverse areas have emerged. Last year they converged dramatically, resulting in the identification of three mammalian proteins that play a major role in the repair of radiation-induced DNA damage as well as in the development of the immune response and, not least of all, providing a function for the hitherto enigmatic Ku protein[9-13]. In this article we review these distinct fields, the paths leading to their connection, and discuss the implications of these findings.

†Abbreviations: DNA-PK, DNA-dependent protein kinase; DNA-PKcs, catalytic subunit of DNA-PK; V(D)J recombination, variable (diversity) rejoining recombination; scid, severe combined immunodeficiency; ss, single stranded; ds, double stranded; dsbs, double strand breaks; IR, ionising radiation; RSS, recombination signal sequences; TdT, deoxynucleotidyl transferase; TCR, T cell receptor; YACs, yeast artificial chromosomes.

The link was a DNA break

A DNA dsb is the major lethal lesion induced by ionising radiation. Examination of the integrity of DNA following exposure to ionising radiation (IR) demonstrates that all cells possess mechanisms to repair this formidable form of DNA damage. Further evidence for the repair and significance of dsbs was provided by the isolation of dsb repair-defective mutants from bacteria, yeast and mammalian cells and the exquisite radiosensitivity of these mutants[5]. A dsb is also a structure generated in cells during V(D)J rearrangement, which provided a clue to link this process with DNA repair. DNA-PK binds to, and is activated by, DNA ds ends, which led to the suggestion that it might be involved in DNA repair/recombination[3], and finally provided the link to fit this component into the puzzle.

Mammalian mutants defective in dsb repair

At least eleven complementation groups of ionising-radi-

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ation-sensitive mammalian cell mutants have been described[6,7]. These mutants exhibit a range of phenotypes, with some displaying only minor radiosensitivity in contrast to a more dramatic response to other DNA-damaging agents[6], probably reflecting the pleiotropic nature of the damage induced by IR and the range of repair pathways required to deal with the different lesions. However, members of four of these complementation groups (groups 4, 5, 6 and 7; Table 1), show a severe sensitivity to ionising radiation, with little cross-sensitivity to UV, alkylating agents or mitomycin C. These mutants have remarkably similar phenotypes, including a decreased ability to rejoin DNA dsbs and defects in V(D)J recombination (see below). The common impact of these mutations suggested that the gene products that they define might interact in a single pathway of dsb repair.

A range of techniques have been used to examine dsb rejoining in these mutants, and generally similar results have been obtained, which show that the final level of unrejoined breaks is elevated but residual dsb rejoining is detectable[5]. Some reports conclude that the rate of residual dsb rejoining is also decreased, and others that it is unaltered. Additionally the defect in dsb rejoining is dose-dependent, with a smaller defect at lower doses[14,15]. Experiments in which cells are held in a non-dividing state after irradiation from 'potentially lethal damage', a step which enhances survival in wild-type cells, indicate that *xrs* cells cannot slowly repair their breaks and therefore that, under these condition, the defect in dsb rejoining is not 'leaky'[16,17]. Wild-type cells, as well as members of these complementation groups where examined, show reduced sensitivity to radiation in S/G_2 phase[5]. Taken together, these data indicate that these mutants have residual dsb rejoining, which could represent a separate pathway operative at a defined stage (S phase) of the cell cycle.

The *xrs* mutants also show decreased transfection frequencies compared to parental cells, indicating that the protein defective in these mutants may play a role in non-homologous recombination[5,18,19].

Table 1. *Rodent mutants defective in DNA dsb rejoining*

Complementation group	Gene	Mutant	Parent cell line	Chromosomal localisation
4	*XRCC4*	XR-1	CHO-K1	5q13-14
5	*XRCC5/Ku80*	*xrs1-6*	CHO-K1	2q33-35
		XR-V15B	V79	
		XR-V9B	V79	
		sxi-3	CHO-K1	
6	*XRCC6*	sxi-1	CHO-K1	
7	*XRCC7/SCID/ DNA-PKcs*	V-3	AA8	
		scid	BALB-C mice	8q11

V(D)J recombination: a process involving a site-specific dsb

Whilst the above mutants were undergoing extensive analysis for defects in DNA repair, the details of the V(D)J recombination process were also being scrutinised. The results of these studies have been described in several excellent reviews[20-22] and only an overview of the process, relevant to the present context, will be detailed here. Three distinct gene segments, the variable (V), joining (J) and diversity (D) elements, which in germ line cells occur at distinct locations, become reassorted into a contiguous exon during the process of V(D)J rearrangement. One of a number of variable (V) elements can rejoin with any J or D element, providing a mechanism to create a large repertoire of different immunoglobulin and T cell receptor (TCR) genes. Each element containing coding sequences (the V, D or J gene segments) is flanked by recombination signal sequences (RSS), which consist of a conserved palindromic heptamer, a spacer region of 12 or 23 nucleotides and an A/T rich nonamer. The recombination process is initiated by the introduction of a site-specific dsb adjacent to the heptamer at the border between the RSS and its adjacent coding sequence. The two signal ends rejoin to yield precise head-to-head signal joins; the two coding elements also rejoin in a process that, in contrast, is frequently imprecise, involving deletions of 10 bp or less, as well as nucleotide insertions[20,21] (Fig. 1). At some junctions, normally those containing at least one non-truncated coding segment, two or, rarely, three nucleotides complementary to the terminal coding segment nucleotides are present and have been termed P (palindrome) nucleotides[22]. Non-templated insertions of between 2 and 5 non-templated random nucleotides (N nucleotides) also occur as a result of end addition by terminal deoxynucleotidyl transferase (TdT)[20]. This defined imprecision in the rejoining process provides an additional mechanism for diversifying immunoglobulin and TCR genes. Various features of P nucleotides have prompted a model for their formation *via* a hairpin intermediate[23,24], which was proposed to arise directly following cleavage and require resolution by a single-stranded endonuclease[24]. If cutting were random within the loop, then 3′ or 5′ overhangs with short inverted repeats would frequently be generated.

V(D)J recombination occurs only in developing T and B lymphocytes (pre B and T cells). Any cell type, however, can be made competent to undergo V(D)J recombination by the introduction and expression of two adjacent genes, *RAG-1* and *RAG-2*, which act synergistically[25]. Although these genes were identified in 1990 it is still not clear whether they activate the expression of critical genes involved in V(D)J recombination, or act directly in the recombination process. A crucial advance in our understanding of the mechanism of V(D)J recombination was provided by the identification of the severe combined immunodeficient (*scid*) mouse by Bosma *et al.* in 1983[8]. *Scid* mice lack mature T and B lym-

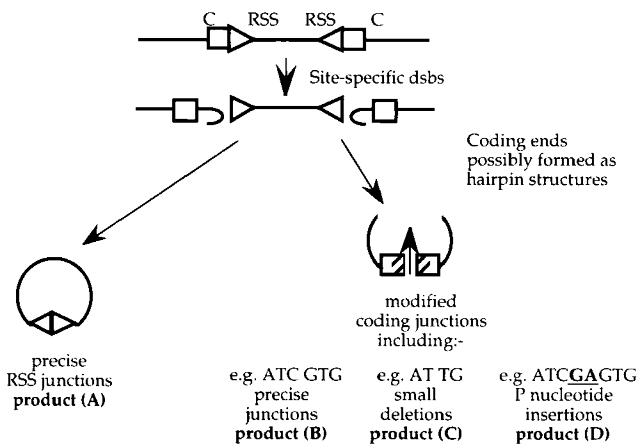


Fig. 1. V(D)J recombination in wild-type cells and IR group 4-7 mutants. RSS, recombination signal sequence; C, coding sequence; P, palindrome.

Table 2. *V(D)J recombination products formed in IR group 4-7 mutants*

IR group	Mutant	Signal junctions	Coding junctions	
		A	B/C	D
4	XR-1	Decreased frequency; deletions	Decreased frequency; few products rescued	
			Larger and more frequent deletions	Normal
5	*xrs*	Decreased frequency; deletions	Decreased frequency; no products rescued	
6	sxi-1	Decreased frequency; deletions	Detailed analysis of products not known	
7	*scid* V-3	Near normal; small decrease in frequency and accuracy	Decreased frequency	
			Larger and more frequent deletions	Longer p nucleotides

N nucleotides have not been included, since these studies have been carried out on cell lines which do not contain TdT.

phocytes, due to a defect in their ability to carry out V(D)J recombination effectively[8,20-22]. The major manifestation of the *scid* defect is a dramatic decrease (10^2- to 10^3-fold) in coding join formation (Table 2). Furthermore, in the rare junctions that do form, larger deletions and longer P nucleotides are observed. In contrast, signal join formation is only marginally depressed in *scid* cells (twofold) but the joins formed are less precise, with some junctions showing small deletions similar to those observed at coding junctions in normal cells.

Coding sequences in *scid* cells accumulate as hairpin structures, without any evidence of nucleotide loss[24]. These results support the model that a hairpin structure might be an intermediate in the recombination process, an idea that was proposed originally to account for the presence of P nucleotides at coding but not at signal junctions. An alternative explanation, however, is that *scid* cells, rather than being blocked in their ability to resolve a normal hairpin intermediate, have an elevated production of hairpin structures as a result of some other block. Although *scid* cell lines are proficient in their ability to resolve hairpin structures introduced by DNA transfection, they might nevertheless be defective in resolving such structures when they arise as V(D)J recombination intermediates[26].

Links between V(D)J recombination and DNA repair

In procaryotes and lower eucaryotes there is a strong link between dsb repair and recombination: the major pathways of dsb repair occur by recombinational mechanisms, and thus most mutants defective in dsb rejoining are also recombination-defective[5]. The *rad*-52 mutants of *Saccharoymces cerevisiae*, which have a major defect in dsb repair, are unable to undergo mating type switching, providing an example of a pathway involved in DNA repair that also handles a site-specific dsb introduced during a natural developmental process[27]. These links prompted an examination of *scid* cells for a defect in DNA repair, and an examination of the hamster dsb repair-defective mutants for their ability to carry out V(D)J recombination. These studies revealed that all *scid* cell types are profoundly sensitive to IR[28] and are defective in dsb rejoining[29,30]. Furthermore, analysis of the dsb repair-defective mutants using a transient transfection assay involving the introduction of a reporter substrate carrying coding and signal gene sequences and expression of the *RAG1/2* genes[31], revealed that they were unable to carry out V(D)J recombination effectively[32-34]. This defect is not a feature common to all radiosensitive mutants, but is specific to those mutants defective in dsb repair[32-35]. Significantly, the dsb repair-defective mutants differ in the specificities of their defect. The groups 4 and 5 mutants display the most dramatic defect, since coding junctions have not been isolated from *xrs* cells (group 5) and only rarely from XR-1 (group 4) cells (Table 2). Signal join formation is also dramatically reduced, and the junctions that do form bear large deletions[33]. In contrast to the above, murine *scid* and hamster V-3 cells, which fall into the same complementation group[34], display a major defect in coding join formation with only a modest change in signal join formation[32,34] (Table 2). Taken together, these results suggest that signal and coding join formation differ mechanistically, and indicate that V(D)J recombination, a process specific to T and B cells, recruits the dsb repair machinery, which is expressed ubiquitously. Moreover, the existence of three, and more recently a fourth, complementation groups[36] with these overlapping defects suggests strongly that a multi-subunit complex or pathway is employed in both processes.

DNA-dependent protein kinase and Ku autoantigen: a complex recognising DNA ds ends

The Ku protein and DNA-PK have also been the subject of considerable investigation during the past ten years. In human cells, Ku is an abundant nuclear protein, composed of two polypeptides of approximately 70 and 80 kDa (Ku70 and Ku80, respectively), that was identified originally as an autoantigen recognised by the sera of an autoimmune patient with the initials K.U.[1]. Ku binds to ds DNA ends without apparent sequence specificity[4]. Ku has also been reported to recognise ss-to-ds transitions in DNA, and to bind to a variety of additional structures including gapped and nicked molecules, and closed DNA hairpins[37,38]. More recently, Ku was shown to correspond to the DNA-binding component of DNA-dependent protein kinase (DNA-PK); this linkage was initially suggested by the common nucleic acid binding properties of Ku and DNA-PK[2,3]. The other component of DNA-PK is a large polypeptide (DNA-PK_{CS}) of approximately 460 kDa, which contains a serine-threonine kinase domain[4]. The kinase activity is expressed only when the enzyme is bound to DNA. DNA-PK is able to phosphorylate many DNA-binding proteins *in vitro*, including transcription factors such as Sp1, c-Jun and p53[39]. Interestingly, these substrates are only phosphorylated effectively when they are bound to the same DNA molecule as DNA-PK, suggesting that DNA-PK activity *in vivo* might be restricted to proteins relatively close to the site of DNA-PK activation[3]. Additionally, it has been demonstrated that many Ku proteins can bind to a single DNA molecule, leading to the suggestion that it enters the DNA at an end and can then translocate along the molecule to internal positions[40]. cDNAs for Ku70 and 80 have been cloned and sequenced, and contain no significant homologies to other proteins[41,42]. DNA-PK_{CS} is a large gene encoding a 14 kb mRNA, and has a kinase domain at its carboxy-terminal end.

Menage à trois; dsb repair, V(D)J recombination and DNA-PK

Direct evidence linking dsb repair, V(D)J recombination and DNA-PK came from two directions. Members of IR group 5 mutants were shown to lack a DNA end-binding activity that corresponded to Ku[43,44]. Parallel studies, aimed at cloning the gene defective in *xrs* cells, localised a complementing human gene, designated *XRCC5*, to the region 2q33-35, which included the Ku80 gene[45,46]. The ability of Ku80 cDNA to complement the radiosensitivity and V(D)J recombination defects of *xrs* mutants provided confirmation that Ku80 is the product of *XRCC5*[9,10,47]. *xrs* mutants were also shown to lack DNA-PK activity, which was restored in *xrs* cells bearing the Ku80 gene, consistent with the notion that Ku represents the predominant, if not only, mechanism for activation of DNA-PK[48]. These results implicated an involvement of Ku80 in dsb rejoining and V(D)J recombination, and suggested that Ku70 and DNA-PK_{CS} might also be involved in these processes. In line with this, the recently identified IR group 6 mutants were also found to lack end-binding activity, raising the possibility that they could be defective in Ku70[36] (E. A. Hendrickson, personal communication). Group 4 and 7 mutants, in contrast, contained normal levels of Ku-dependent end-binding activity[43,44]. Several lines of evidence have now demonstrated that two group 7 mutants, V-3 and *scid* cells, are defective in DNA-PK_{CS}. Firstly, both mutants lack DNA-PK activity, and are depleted in a protein that cross-reacts with DNA-PK_{CS}[11-13]. Secondly, the DNA-PK_{CS} gene and the *XRCC7* gene, a human gene complementing *scid* cells, map to the same region of human chromosome 8[11,49]. Finally, yeast artificial chromosomes (YACs) encoding the DNA-PK_{CS} gene can correct the defects in dsb repair, V(D)J recombination and DNA-PK activity of V-3 cells[11]. A radiosensitive human tumour cell line with a defect in DNA-PK_{CS} has also recently been identified[50]. Taken together, these studies have identified two gene products, Ku80 and DNA-PK_{CS}, involved jointly in dsb repair and V(D)J recombination. The involvement of a third gene product, Ku70, seems likely but remains to be verified. Confirmation that the mutants actually carry mutations in these genes will have to await sequencing of the rodent genes in wild-type and mutant cells, a particularly formidable task for the gene encoding the 14 kb DNA-PK_{CS} mRNA.

Differences between the repair of site-specific breaks and radiation-induced breaks

DNA-PK clearly functions in the repair of the site-specific breaks introduced during V(D)J recombination, in the repair of radiation-induced dsbs, and in the repair of breaks generated by restriction enzymes introduced into cells by permeabilisation or electroporation[51,15]. Despite the essential similarity of these processes, there are informative differences.

Firstly, V(D)J recombination involves the rejoining of four DNA ends resulting from two dsbs, and therefore differs from the repair of damage-induced breaks which involve the rejoining of just two ends. In addition to the formation of junctions between two coding or two signal ends, hybrid junctions between signal and coding ends are occasionally observed[22], and the coding ends of these hybrid joins exhibit the nucleotide additions and deletions characteristically found at coding junctions. These data argue that the four ends are still associated at the stage when modification of the coding ends takes place. The coding ends which accumulate as hairpin intermediates in *scid* cells do not contain deletions[24], suggesting that end modification takes place downstream of DNA-PK_{CS} binding, and therefore that the four ends are still associated at a time when the DNA-PK_{CS} component is normally bound. A second characteristic feature of V(D)J recombination is the presence of specific

end modifications at the coding junction termini, a feature designed to enhance genetic diversity within the immune system. Although deletions have been observed following the repair of radiation induced breaks, there is no evidence suggesting that P and N nucleotide additions occur frequently[52]. Moreover, the deletions are detected following the selection of radiation-induced mutants, and thus probably represent rare mis-repair events. Intuitively, it appears unlikely that a cell would utilise a mechanism enhancing genetic diversity to repair its double strand breaks. Therefore, we consider it likely that end modification is a feature unique to coding junctions formed during V(D)J recombination. The suggestion that P nucleotides arise from the processing of hairpin intermediates therefore indicates that these too occur uniquely at coding junctions.

Finally, there is one aspect in which signal join formation contrasts strikingly with the other types of end joining events. The group 5 mutants are markedly defective in all the processes of end joining that we have discussed. In contrast, V-3 and *scid* cells, although displaying hypersensitivity to radiation comparable to that of group 5 mutants and having a major defect in coding join formation, exhibit only a modest defect in signal join formation[20,21,32,34]. These data support a model in which Ku is essential for all these end-joining processes, whereas signal join formation is a unique event which can occur independently of DNA-PK_{CS}. The features of signal join formation, namely the lack of end modifications and their independence of DNA-PK_{CS}, are unlikely to be merely the result of rapid ligation, since unmodified signal ends can be detected with ease in normal cells whereas coding ends cannot be detected in normal cells either as free ends or as hairpin intermediates[53,24].

A model for the action of DNA-PK

To accommodate these features of V(D)J recombination, we suggest the existence of a V(D)J specific complex (Fig. 2). Specificity of this complex could be provided by proteins recognising the RSS elements. The phenotype of the group 5 mutants suggests that Ku binds to both signal and coding ends in the V(D)J intermediate, as well as to ends formed by DNA damaging agents. Significantly, Ku can bind to hairpin structures, which have been suggested to arise directly at coding ends following endonucleolytic cleavage. We suggest that Ku binding is an early event, possibly serving to stabilise the ends and prevent nucleolytic degradation. DNA-PK_{CS} then rapidly associates with DNA-bound Ku, and undergoes a conformational change activating its kinase function. DNA-PK_{CS}, however, does not appear to be required for the rejoining of signal ends. One possibility is that the large catalytic subunit is precluded from binding to signal sequences due to the presence of the V(D)J specific recognition enzyme or associated proteins. Alternatively, an essential function of the DNA-PK complex may require its translocation along the DNA molecule, which might be

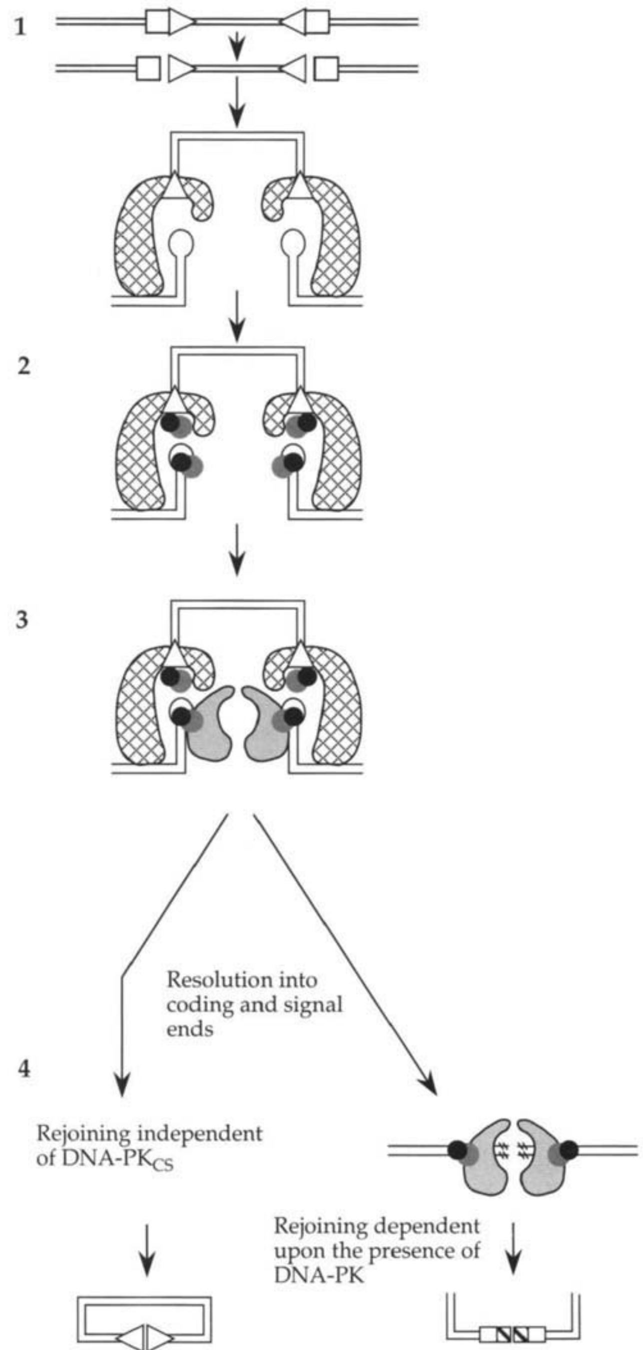


Fig. 2. Model for DNA-PK involvement in V(D)J recombination. Step 1. Site-specific dsbs are introduced. A V(D)J specific complex (possibly involving RAG1/2) could serve to hold the four ends together. Hairpin intermediates are formed at the coding ends. Step 2. Ku binding. Ku binds to the DNA ends and prevents nucleolytic degradation. Step 3. DNA-PK_{CS} binds to Ku. (i) DNA-PK_{CS} binds to Ku at the coding ends but is possibly excluded from binding to the signal ends due to the presence of a V(D)J specific complex. (ii) A complex involving V(D)J enzymes and the DNA-PK holoenzyme stabilises Ku at the signal ends. (iii) Kinase activity is induced (for possible roles of the kinase activity, see discussion). Step 4. Resolution and rejoining. The complex resolves into signal ends associated with V(D)J-specific enzymes and coding ends associated with DNA-PK. DNA-PK_{CS} may act as a scaffold around which other proteins can bind.

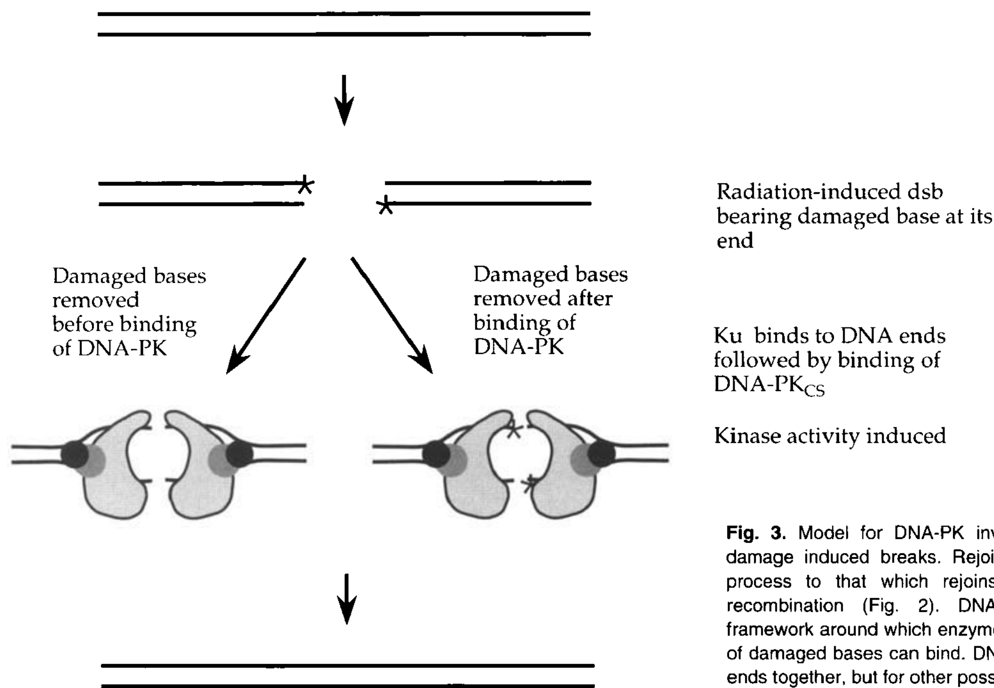


Fig. 3. Model for DNA-PK involvement in rejoining of damage induced breaks. Rejoining occurs by a similar process to that which rejoins coding ends in V(D)J recombination (Fig. 2). DNA-PK$_{CS}$ may act as a framework around which enzymes involved in the removal of damaged bases can bind. DNA-PK may act to hold the ends together, but for other possibilities see Fig. 4.

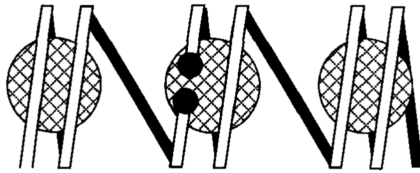
blocked at the signal sequences by V(D)J specific proteins. At this point, coding junctions and radiation-induced breaks may resemble one another in having the DNA-PK holoenzyme bound at the break, but may differ in their terminal structures with coding ends bearing hairpins, and radiation-induced ends having damaged bases at their termini (Figs 2 and 3). The large DNA-PK complex may serve to facilitate the resolution, modification and repair of damaged bases, in addition to functioning in the latter stages of the rejoining. Its function may include acting as a structural framework around which other enzymes involved in these processes bind, or itself providing additional enzyme activities. In this context, there is evidence that Ku possesses an ATP-dependent helicase activity[54], which may serve to unwind the DNA in the vicinity of the break where enzymes, possibly associated with DNA-PK, are ready to act. Also, localised Ku-associated unwinding may potentially help to facilitate cleavage of hairpin intermediates, as well as the repair of damaged DNA termini. Rejoining of the coding junctions by enzymes associated with the DNA-PK holoenzyme might then release the signal ends still associated with the V(D)J complex and Ku. The rejoining of these ends could then ensue by a DNA-PK$_{CS}$ independent mechanism.

The accumulation of hairpin intermediates that contain unmodifed coding ends in *scid* cells has led to the suggestion that DNA-PK either directly, or indirectly *via* its kinase activity, facilitates their resolution[55,24]. We should stress however, that while this may be one function of DNA-PK, it is unlikely to be the sole function, since damage-induced ends, which apparently do not contain hairpin intermediates, are not efficiently rejoined in *scid* cells.

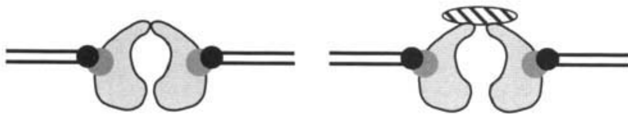
Mechanism for rejoining of damage induced breaks

Despite the differences between the rejoining of the site-specific dsbs introduced during V(D)J *recombination and* damage-induced dsbs, the overlap in enzymatic machinery between these two processes suggests that they occur by similar mechanisms. The rejoining of both signal and coding ends involves direct end-joining without any requirement for sequence homology. We therefore suggest that DNA-PK-dependent repair of damage-induced dsbs also occurs by a similar mechanism, and indeed there is circumstantial evidence for this[18,19,56]. If homology is not utilised then how are damage-induced breaks maintained in close association and how is faithful rejoining ensured? Although the defect in V(D)J recombination in group 4-7 mutants is detected using a plasmid-based assay, defects in the repair of damaged plasmids are generally not observed in these mutants[15,18,57]. One possibility, therefore, is that damage-induced ends rely on chromatin structure to hold them in close association (Fig. 4A). A second possibility is that this function is provided, either directly or indirectly, by a DNA-PK-associated complex. Although there is at present no evidence for dimer formation between DNA-PK molecules, for the binding of two Ku molecules to a single catalytic subunit, or for the binding of one holoenzyme to two DNA ends, it is possible is that an additional component recruited by DNA-PK$_{CS}$ serves as an alignment protein to retain an association between the broken molecules (Fig. 4B). Evidence for an alignment protein has been observed in extracts from Xenopus eggs[58]. A third mechanism might be the use of short regions of sequence homology to join the ends (Fig. 4C)[56]. Significantly, sequence analysis of mutations aris-

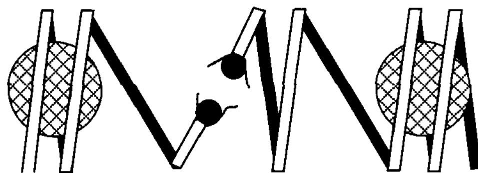
(A) Use of chromatin structure



(B) DNA-PK holoenzyme or an alignment protein holds the ends together



(C) Use of short direct repeat sequences



Ku protects the ends. Chromatin structure is destroyed and short direct repeat sequences are utilised for rejoining. Results in deleted bases and mutations

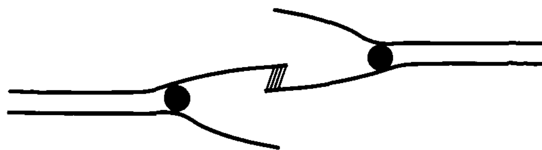


Fig. 4. Mechanisms of rejoining of a damage-induced dsb.

ing after DNA end-joining indicates that short direct repeat sequences are commonly utilised[59]. Although this mechanism might appear unsatisfactory, since it will inevitably result in deletions and/or mutations in the DNA, this type of event might be tolerated in more complex genomes with little deleterious effect due to the large number of repetitive and redundant sequences.

Role of the kinase activity

Above we have suggested a structural or 'framework' role for DNA-PK$_{CS}$, a possibility compatible with the large size of this protein. Alternatively, or in addition, the kinase activity of this enzyme, which is induced specifically following strand breakage, might play an important role in dsb repair and V(D)J recombination. In this context, it is pertinent to note that DNA-PK most effectively phosphorylates proteins bound to DNA in *cis*[3]. One possibility is that the kinase phosphorylates and activates proteins involved in the repair/recombination process, but if so, such proteins would probably have to be localised in the vicinity of the break. Autophosphorylation of the DNA-PK$_{CS}$ and Ku components has been observed[39] and the ATPase activity, which has recently been associated with Ku, is reported to be activated by autophosphorylation of Ku[60]. An attractive scenario therefore is that autophosphorylation may be important for the repair function of DNA-PK, or for regulating its activity. A second possibility is that the kinase activity serves to alert the cell to the presence of a dsb by triggering a signal transduction mechanism. The available evidence suggests that DNA-PK-defective cell lines arrest normally in G_2 and S phases of the cell cycle following DNA damage[5], although arrest at the G_1/S boundary has not been well investigated and may be particularly significant since V(D)J recombination occurs in the G_1 phase of the cell cycle[53,61]. The lack of obvious cell cycle checkpoint defects in DNA-PK-deficient cells, however, does not necessarily mean that this enzyme lacks a checkpoint function since mammalian cells may have multiple, partially redundant, mechanisms for detecting DNA damage. A final possibility is that DNA-PK kinase activity enhances repair through effecting localised inhibition of transcription. Recent observations have shown that DNA-PK is able to arrest transcription *in vitro* by phosphorylating components of the transcription apparatus[62]. *In vivo*, repression of transcription in the vicinity of a dsb may prevent the transcription apparatus from interfering with the assembly of the DNA repair machinery[4]. Such a model is attractive since it is compatible with the localised phosphorylation capacity of DNA-PK.

Evolution

Recently, a Ku70 homologue of yeast cells has been isolated[63]. A deletion mutant, however, shows no detectable gamma-ray hypersensitivity (unpublished observations of S. P. Jackson, S. Boulton and A. R. Lehmann) indicating that, at least in yeast cells, it does not function in the major, *RAD52*-dependent, mechanism of dsb rejoining. On closer examination, this phenotype is to be expected, since mating type switching, a process which relies on the *RAD52* pathway for repairing site specific dsbs, actually bears little resemblance mechanistically to V(D)J recombination. Overall therefore, it appears that, in lower organisms, the majority of dsb rejoining occurs by a *RAD52*-dependent, Ku-independent mechanism involving homologous recombination, whilst in mammalian cells another mechanism, dependent upon DNA-PK but probably not requiring extensive homology, dominates. Whether yeast cells contain functionally

equivalent kinase activity remains to be determined. Since none of the mammalian mutants is completely deficient in rejoining of dsbs, it appears that mammalian cells possess a DNA-PK-independent mechanism of dsb rejoining, and it is tempting to speculate that this might correspond to a *RAD52*-dependent mechanism involving homologous recombination.

Mechanistically, transposition shares some similarity to V(D)J recombination, and it has been suggested that the original immunoglobulin V domain has been separated into sub-exon segments by the insertion of a transposable element[64]. A signal join would therefore represent the joining of two transposon ends. It has also been suggested that a hairpin might be an intermediate in some transposition processes[65]. Furthermore, a Drosophila homologue of Ku70 interacts with P element-associated inverted repeats, possibly implicating a role in transposition[66]. As more becomes known about the various DNA repair and transposition pathways, it will be of interest to see how extensively these processes are linked evolutionarily and mechanistically.

Links to the future

The identification of a protein complex (DNA-PK) involved in V(D)J recombination and DNA repair provides an important stepping stone to determine the exact molecular mechanisms of these processes. Could the interaction of this machinery with transcription emerge as an important future link? Overlapping association between the excision repair and transcriptional apparatus has emerged during the past year[67]. Evidence for the involvement of DNA-PK and/or the Ku component in transcriptional regulation has been reported consistently during the history of these proteins. In addition, it will be of considerable importance to assess linkages between these events and cell cycle control mechanisms. Now that mutants defective in these proteins are known, it should be possible to identify the significance of these potential links.

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