

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/dnarepair

Differential usage of non-homologous end-joining and homologous recombination in double strand break repair

Eiichiro Sonoda, Helfrid Hochegger, Alihossein Saberi,
Yoshihito Taniguchi, Shunichi Takeda*

Radiation Genetics, Graduate School of Medicine, Kyoto University, Konoe Yoshida, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:

Published on line 27 June 2006

Keywords:

DSB

HR

NHEJ

Ku70

DT40

ABSTRACT

Repair of DNA double strand breaks (DSBs) plays a critical role in the maintenance of the genome. DSB arise frequently as a consequence of replication fork stalling and also due to the attack of exogenous agents. Repair of broken DNA is essential for survival. Two major pathways, homologous recombination (HR) and non-homologous end-joining (NHEJ) have evolved to deal with these lesions, and are conserved from yeast to vertebrates. Despite the conservation of these pathways, their relative contribution to DSB repair varies greatly between these two species. HR plays a dominant role in any DSB repair in yeast, whereas NHEJ significantly contributes to DSB repair in vertebrates. This active NHEJ requires a regulatory mechanism to choose HR or NHEJ in vertebrate cells. In this review, we illustrate how HR and NHEJ are differentially regulated depending on the phase of cell cycle and on the nature of the DSB.

© 2006 Elsevier B.V. All rights reserved.

1. Introduction

DSBs are the most critical damage to the cells, as it is believed that a single unrepaired DSB is sufficient for inducing apoptosis [1,2]. DSBs are generated by environmental factors such as ionizing radiation, by cellular metabolic products and as recombination intermediates. In cycling cells, DSBs occur mainly during replication in the following way: Chemical modifications of the genomic DNA, e.g. hydrolysis, oxidation and non-enzymatic methylation of DNA, occur at significant rates *in vivo* [3]. To deal with such chemical insults towards individual bases, multiple pathways have evolved. Covalently modified bases are usually repaired before DNA replication. However, when unrepaired lesions or nicks are encountered by replication forks, replication block at these lesions results in more critical damage, including single-strand gaps and lethal DSBs in the relevant sister-chromatids (Fig. 1A).

The nature of DSBs caused by replication block is quite different from that caused by ionizing radiation. DNA lesions associated with DNA replication can be readily repaired by homologous recombination by using the other intact sister as a template, because the two sisters are localized in close proximity [4]. On the other hand, ionizing radiation results in “accidental” DSB at packed chromatin structure (Fig. 1B). Moreover, such DSB could hardly interact with intact homologous sequences either in homologous chromosomes or even sister-chromatids, because after replication, extensive condensation packs replicated DNA sequences in a highly ordered chromatin structure and thereby significantly separates the two sisters (Fig. 1C and D) [5]. Thus, extensive chromosome condensation may make homology search extremely difficult in the G2 as well as G1 phase in higher eukaryotic cells. Due to this difficulty of homology search, vertebrate cells have to use NHEJ more frequently than yeast, to simply re-ligate the broken ends, even though NHEJ frequently results in errors in

* Corresponding author. Tel.: +81 75 753 4410; fax: +81 75 753 4419.

E-mail address: stakeda@rg.med.kyoto-u.ac.jp (S. Takeda).

1568-7864/\$ – see front matter © 2006 Elsevier B.V. All rights reserved.

doi:10.1016/j.dnarep.2006.05.022

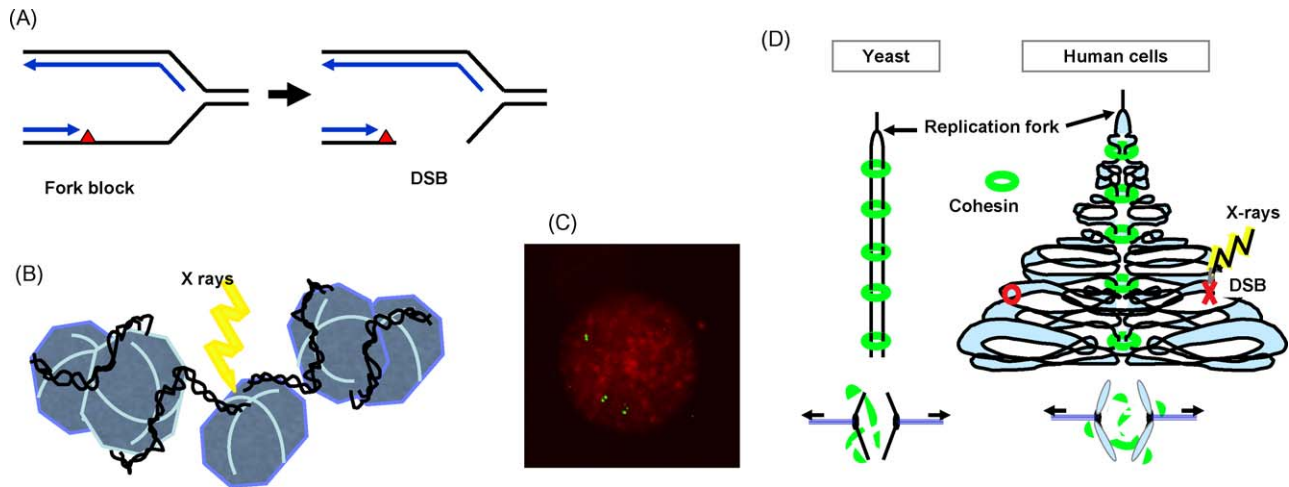


Fig. 1 – Two different types of DSBs. (A) In a DSB associated with replication, the broken ends are in close proximity to a template for repair, i.e. an intact sister-chromatid. **(B)** Ionizing radiation induces DSB in packed chromatin structure, and resulting DSBs hardly access a homologous template for repair. **(C)** In situ hybridization of OVALBUMIN locus in chromosome 2, which is in trisomy in wild-type DT40. Note that three doublet signals in two sisters of chromosome 2 are detectable, indicating that homologous sequences in two sisters are extensively separated by condensation of chromosomes after DNA replication, as illustrated in **(D)**. Red cross and open red circle represent the site of DSB and its homologous region of the intact sister chromatid, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the form of sequence deletions [6,7]. Collectively, the nature of DSB determines the usage of DSB repair pathways, in a way that the DSB caused by replication blockage are repaired by sister homologous recombination, whereas “accidental” DSB in packed chromosomes are frequently repaired by NHEJ.

Both NHEJ and HR are carried out by multiple step reactions. NHEJ is initiated by DNA end-binding proteins Ku70 and Ku80, which rapidly associate with exposed DNA breaks, followed by the recruitment of the catalytic subunit of the DNA-dependent

protein kinase (DNA-PKcs) [8]. The Ku/DNA-PKcs complex ultimately recruits ligase IV, which completes the repair of the break [9]. HR dependent DSB repair involves strand resection of DSB, and forming 3’ single-strand (ss) overhangs (Fig. 2A). 3’ Overhangs associate with Rad52 and subsequently with polymerized Rad51, a key enzyme in HR [10]. The complex of Rad51 and ssDNA invades intact homologous sequences to form heteroduplexes with a help of Rad54 (reviewed in Ref. [11]). Despite the high degree of conservation of the HR effectors, the

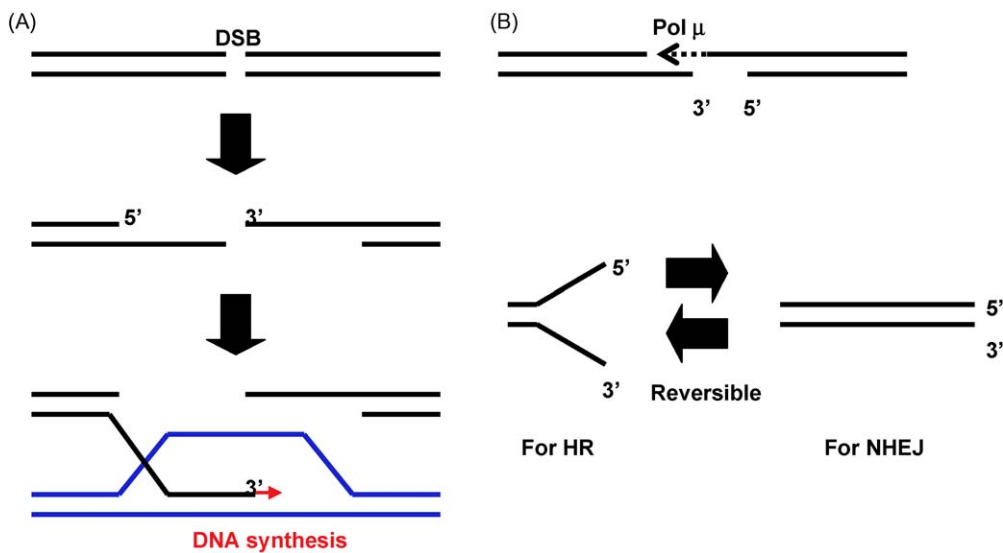


Fig. 2 – Resection of DSB determines the choice of HR and NHEJ. (A) In yeast, 3’ overhang formation at DSB precedes subsequent homology search in HR dependent DSB repair. **(B)** In vertebrates, 3’ overhang can be repaired by Polμ in NHEJ [30]. Alternatively, resection does not occur, and instead, reversible unwinding of DSB allows parallel actions of HR and NHEJ.

contribution of each ortholog protein to the HR reaction seems to have dramatically changed during evolution. For example, yeast mutants in Rad51, Rad52 and Rad54 exhibit similar phenotypes. In marked contrast, in vertebrate cells, the relative contribution of the three major HR factors is significantly different, in the manner that the depletion of Rad51 results in cellular lethality, whereas RAD52 and RAD54 gene disrupted mice exhibit no developmental abnormality and are even proficient in meiosis [12,13].

The relative contribution of the NHEJ and HR pathways varies greatly between budding yeast and mammalian cells. In yeast, HR plays a dominant role in DSB repair following ionizing radiation in the G1 as well as G2 phase, and NHEJ plays only a minor role [14,15]. In contrast, NHEJ repairs over 60% of exogenously induced DSBs in mouse ES cells [6]. Furthermore, genes involved in HR, e.g. RAD51 or RAD54, are not expressed in resting mammalian cells even after exogenous genotoxic stresses [16]. Thus, a majority of the “accidental” DSBs are repaired by NHEJ in vertebrate cells. In yeast, the first step of DSB repair appears to determine which pathway is chosen for repair, because 3′ overhang formation correlates with the usage of HR [17]. However, it remains to be elucidated whether the resection of DSB should be the decisive event that channels the subsequent steps into either HR or NHEJ, because 3′ overhang formation has not yet been demonstrated in mammalian cells (Fig. 2B). Taken together these observations lead to several questions: how do cells differentially employ HR and NHEJ, and control the balance between them? What are the molecular mechanisms of this control? If such control mechanisms exist, what is the consequence of their abrogation?

In this review, we illustrate how research over the past decade has begun to address these questions. We will focus on genetic experiments in yeast and DT40 cells, as these two model systems have been used for pioneering studies, and will integrate these findings with genetic data from mammalian model systems.

2. Complementary and competitive roles of HR and NHEJ in DSB repair

2.1. Cell cycle phase specific usage of repair for ionizing radiation-induced DSB

A genetic study published by our laboratory several years ago, first demonstrated that the stage of the cell cycle is a decisive factor in the control of DSB repair [18]. In this study, we generated chicken DT40 cells lacking Rad54 or Ku70. In $\Delta rad54$ cells, HR is only moderately impaired, and the mutant is able to proliferate [19], whereas in $\Delta ku70$ cells, NHEJ is defective. As shown in Fig. 3A, both $\Delta rad54$ cells and $\Delta ku70$ cells are sensitive to killing by ionizing radiation, suggesting that both pathways contribute to the repair of DSB. However, the phase specific sensitivity profile to ionizing radiation of these mutants clearly shows that HR and NHEJ are differentially employed during the cell cycle (Fig. 3B). DT40 wild-type as well as mammalian cells acquire ionizing radiation-resistance as they proceed through S-phase. In contrast, $\Delta rad54$ mutant, which shows a relatively flat IR sensitivity pattern, is ionizing radiation-sensitive only during the late S to G2 phases. This corresponds well with studies of HR mutants in mammalian cell lines [20]. Hence, an increase in the radio-resistance of wild-type cells is most likely a consequence of the increased employment of HR after the appearance of sister-chromatids. In addition, although Rad54 is dispensable for HR dependent DSB repair at replication blockage, Rad54 appears to be essential for HR dependent repair of ionizing radiation-induced DSB presumably by acting as a chromatin-remodeling factor.

$\Delta ku70$ cells are extremely sensitive in the G1 and early S-phases (Fig. 3B). This indicates that NHEJ is the sole machinery for DSB repair in the G1 phase, while HR starts to be employed in addition to NHEJ in the late S to G2 phases. Given that HR needs a homologous template for repair, this differential usage

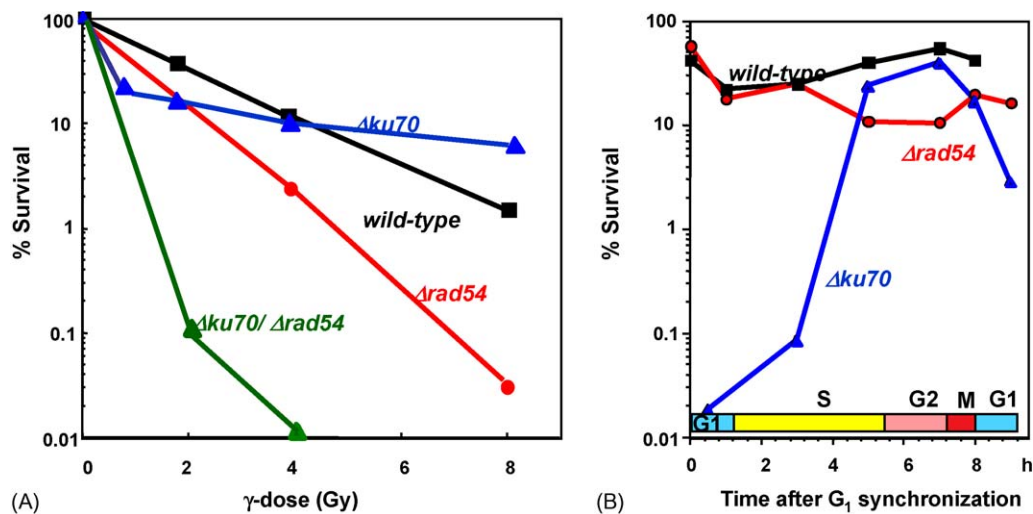


Fig. 3 – HR and NHEJ are complementary to each other in the repair of X-ray induced DSBs. (A) $\Delta ku70$ and $\Delta rad54$ double mutation cause a synergistic increase in sensitivity to ionizing radiation. (B) Cell cycle phase specific usage of the repair of DSB induced by ionizing radiation; $\Delta ku70$ is sensitive in the G₁ to early S-phases, while $\Delta rad54$ is sensitive in the late S to G₂ phases. Each mutant cells were synchronized in the G₁ phase of the cell cycle by elutriation. An enriched G₁ cell fraction was released into cell cycle progression, γ -irradiated at the indicated time points, and subjected to colony survival assay.

of the two pathways makes sense. In a diploid organism, the only available template for HR during the G1 phase is the other homologous chromosome. Employment of HR under such circumstances, would lead to a gradual loss of heterozygosity [21]. As replication proceeds, sister-chromatids become available as suitable homology donors. Thus, with the progression of S-phase, HR increasingly becomes the pathway of choice for DSB repair.

Having established a cell cycle regulation of DSB repair, the question arises what constitutes the molecular mechanism of this phenomenon. Transitions between cell cycle phases are controlled by a group of protein kinases, termed CDK (cyclin dependent kinase), and their regulatory cyclin subunits [22,23]. Each cell cycle phase is characterized by the distinct activity of different cyclin CDK complexes. A straightforward model to explain the cell cycle control of DSB repair would thus imply that a component of NHEJ or HR is either activated or inhibited by CDKs. Accordingly, two studies from *S. pombe* first pointed to a direct involvement of CDKs in this cell cycle control of DSB repair [24,25]. Subsequently, Ira et al. [17] firmly established a role for *cdc28* (the budding yeast CDK that is active during the S and G2 phases) in activating HR, using the well established HO-meganucleotide recognition endonuclease system. According to Ira et al.'s results, CDK facilitates the resection stage of the HR reaction (Fig. 2A). However, the downstream targets of *Cdc28* in this pathway remain to be established. In budding yeast, the resection of DSB appears to prevent NHEJ, which could not simply ligate 3' overhang ends. It is unclear whether the resection determines the selective usage of HR and NHEJ in vertebrates (Fig. 2B). The reduction of Rad51 foci formation in the G1 phase [26,27] and by the CDK inhibitor roscovitine in human cells [28] does, however, point to an important role of CDKs in the vertebrate HR reaction.

2.2. Functional overlap between HR and NHEJ for ionizing radiation-induced DSB repair in the late S–G2 phase

Although the choice between HR and NHEJ is determined by the processing of DSB ends in yeast, the two DSB repair pathways appear to act simultaneously in the G2 phase in higher eukaryotic cells, as suggested by the following experiment. In DT40 cells, HR is preferentially used over NHEJ in the G2 phase, as shown by X-ray hypersensitivity of $\Delta rad54$ cells but not $\Delta ku70$ cells at this stage (Fig. 3B). Interestingly, a significant increase in ionizing radiation-sensitivity is observed in $\Delta ku70/\Delta rad54$ DT40 cells in comparison with $\Delta rad54$ cells, indicating that NHEJ can substitute for lack of Rad54 in DSB repair in the G2 phase. This is surprising, because in the absence of Rad54, HR can be initiated and Rad51 foci, a hallmark of successful strand resection, form normally, but the HR reaction is blocked at a later stage [29]. This suggests that even after the initiation of HR, there is a way back to NHEJ, possibly by either removal of the 3' overhang, or Pol μ -dependent DNA synthesis from the 3' overhang using a template from the other end of the DSB [30] (Fig. 2B). Alternatively, DSB ends are unwound by a DNA helicase without being resected, so that NHEJ and HR could act in parallel.

Similarly, the prior binding of Ku might not be an unsurpassable hindrance for the HR machinery [27]. Collectively,

even after the repair reaction is initiated by either HR or NHEJ, intermediate molecules could be taken over by the other DSB repair pathway, which may account for the synergistic increase in the X-ray sensitivity in cells deficient in both Rad54 and Ku (Fig. 3B).

2.3. Competition between HR and NHEJ for ionizing radiation-induced DSB repair during S and G2 phase

Although the two DSB repair pathways could act in parallel as discussed above, the initial binding of repair factors to the DNA break may affect the choice of HR or NHEJ. Given that both HR and NHEJ are active in the S and G2 phases of the cell cycle [31], it is conceivable that early players are competing to gain access to the DSB. Upon induction of DSB, the Ku70/Ku80 heterodimer is recruited to DSBs more quickly than HR factors [27], implying that NHEJ could interfere with HR but not necessarily vice versa (Fig. 4A). The Ku complex is abundant in the nucleus and appears to form a pore that glides onto a broken DNA end [32], followed by the recruitment of DNA-PKcs, XRCC4 and ligase IV to complete NHEJ [33]. As shown Fig. 4A, the binding of the Ku proteins to DSBs may interfere with HR. If this hypothesis is correct, deletion of Ku should result in higher HR efficacy. Deletion of other NHEJ factor, on the other hand, should not diminish the competitive effects of Ku and should not result in more efficient HR. To test this model, we assessed the competition between Ku and HR, by studying ionizing radiation-sensitivity in the late S to G2 phases and recombination induced by a rare cutting endonuclease *I-SceI* in cells lacking either Ku70, DNA-PKcs or ligase IV [34]. As shown in Fig. 4B, Ku70 deficiency confers ionizing radiation-resistance to the cells in the G2 phase. A radio-resistant fraction is also present in murine ES cells deficient in Ku [35]. The increased radio tolerance is likely caused by more efficient usage of HR, because Ku70 deficiency up-regulates the efficiency of DSB repair induced by the restriction endonuclease *I-SceI*. On the other hand, deletion of DNA-PKcs or ligase IV does not confer ionizing radiation-resistance or higher HR efficacy in DT40 cells [36] (Fig. 4B and unpublished data). Collectively, binding of the Ku proteins, but not other components of NHEJ, to DSBs may moderately interfere with the initiation of HR at ionizing radiation-induced DSBs or *I-SceI* mediated DSBs. A similar competition between HR and Ku has also been documented in mouse ES cells [37] and correlates with *in vitro* studies [38].

3. Repair of DSB at stalled replication forks

HR-deficient mutants generally exhibit a prominent increase in spontaneously arising chromosomal breaks [39,40] and gene disruption mice of a number of HR factors are embryonic lethal [41]. In particular, $\Delta rad51$ mutant cells are not viable even at a cellular level, and display extensive spontaneous chromosomal breaks [42]. In contrast, cells deficient in NHEJ are viable and exhibit only few spontaneous chromosomal breaks. Given that NHEJ repairs the majority of exogenously induced DSB in mammals, this requirement of HR but not NHEJ for cellular survival is surprising. Conceivably, "accidental" DSBs, such as ionizing radiation-induced DSBs (Fig. 1B), occur only occasionally. HR, on the other hand, seems to be

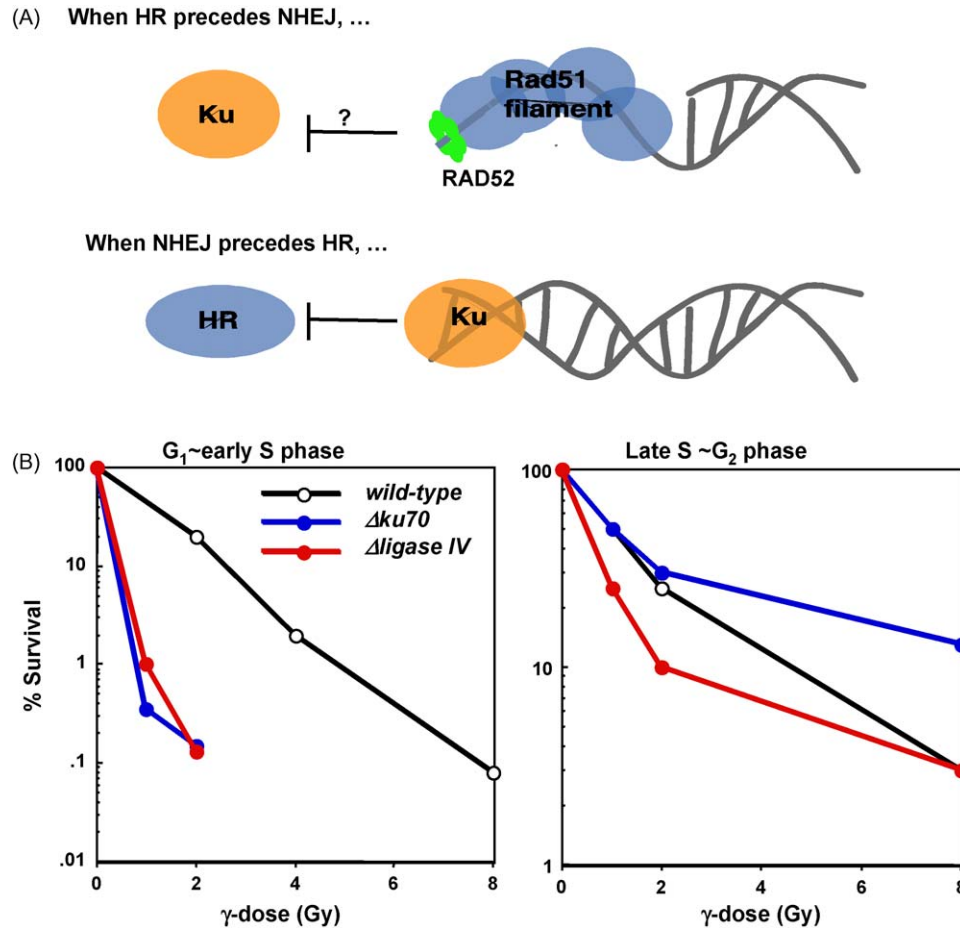


Fig. 4 – Competition between HR and NHEJ. (A) It was assumed that after the occurrence of a DSB, the initial events decide which pathway is chosen for repair. If HR is initiated, Rad51 and Rad52 cover the 3' overhang, leading to inhibition of Ku's access to the DSB (top). However, this model has not been substantiated by genetic data. On the other hand, when Ku has gained access to the DSB, execution of HR is partially suppressed (bottom). (B) $\Delta ku70$ is sensitive to ionizing radiation in the G₁ phase but exhibits more resistance than wild-type cells in the late S to G₂ phases. $\Delta ligIV$ cells do not show such resistance in the G₂ phase, presumably due to the partial inhibition of HR dependent DSB repair by the Ku proteins associated with DSBs.

essential for the repair of another type of DSB that occurs much more frequently during each cell cycle. These DSB are most likely the result of replication fork stalling that arises frequently during each S-phase (Fig. 1A). Accordingly, HR factors form spontaneous foci during S-phase in both budding yeast and HeLa cells [43,44], and HR repairs the majority of DSB at collapsed replication forks [45,46]. A role for NHEJ in genome maintenance has been implicated during mouse development from the following finding. *Xrcc4* and *ligase IV* gene disruption mice are embryonic lethal, and fibroblasts derived from these embryos exhibit retarded growth and marked genomic instability, including chromosomal translocations [47,48]. However, these findings should be carefully interpreted, because this severe phenotype is caused not only by defective NHEJ but also by suppression of HR by the Ku proteins, as shown by the partial suppression of *ligase IV* deficient phenotype by additional mutation of *Ku70* in DT40 [36]. The virtually exclusive usage of HR over NHEJ at stalled replication suggests that another regulatory mechanism is required for the choice of HR and NHEJ, as discussed in the following paragraph.

4. Competition between HR and NHEJ for replication block induced DSB repair

In principle, the structure of DNA breaks at replication forks should not differ from other types of DSB, and should be recognized by the initiating factors of both NHEJ and HR. This raises the question to what extent NHEJ competes with HR for replication block induced DSBs. To address this question Adachi et al. analyzed the effect of Camptothecin (CPT), a topoisomerase I inhibitor on various DT40 mutants in NHEJ genes [49]. Topoisomerase-I is covalently linked to nicked DNA, and CPT blocks the release of the enzyme from DNA. Replication fork stalling at Topoisomerase-I associating single-strand breaks results in the formation of DSBs, which are subsequently repaired by HR, using the intact sister-chromatid as a template. Consequently, yeast HR mutants in the *RAD52* epistasis group are sensitive to CPT [50], as are several DT40 cell lines defective in HR genes (Takeda lab. unpublished results) [51]. Interestingly, Adachi and coworkers found that $\Delta ku70$

cells, are more resistant to CPT than wild-type. Competition between Ku and HR for DSB binding, as discussed above, is a straightforward explanation of this finding. Indeed, CPT-induced HR is enhanced in $\Delta ku70$ cells as judged by increased levels of sister-chromatid exchange (SCE) (Takeda lab. unpublished results), which reflects HR mediated repair associated with replication [52]. However, this hypothesis does not fully explain several other observations. Although only Δku mutation, but not $\Delta ligIV$ deficiency, confers ionizing radiation-resistance to the cells in the G2 phase, $\Delta ligIV$ show a similar increase in CPT resistance as does Δku mutation. Thus, upon DSB following replication blockage, NHEJ dependent ligation, rather than binding of Ku to DSB, is toxic to the cells. The question remains, why the execution of NHEJ at CPT induced DSBs has a negative impact on cell survival, and which repair pathway is affected by NHEJ. Possible candidates for this pathway are the replication bypass via HR and template switch [53]. Such bypass is achieved either by using the other sister as a template or by a hypothetical structure, termed “chicken foot”, which ends in a DSB [54]. If such DSB like structures were religated by NHEJ, the cell would end up in an irresolvable mess. However, this hypothesis has to date not been explored and remains speculation.

5. The inhibitory effect of Ku on HR is controlled by PARP

So far we have discussed the control of HR during the cell cycle, and the competition between HR and NHEJ. In summary, a model emerges, that suggests that HR activity starts during

S-phase and competes with NHEJ for access to DSBs. The ratio between HR and NHEJ should consequently depend on the affinity of the initiation factors for the DSB. Alternatively, the process of initiation could be subjected to additional controls. One could imagine an activity that suppresses the binding of one initiation factor in favor of the other. For example, certain factors might inhibit the access of Ku to DNA and thus allow unperturbed initiation of HR. This hypothetical factor should be activated shortly after DSB formation and biochemically interact with Ku.

Poly[ADP-ribosylation], the covalent attachment of ADP-ribose moieties derived from NAD to target proteins, is one of the earliest cellular responses to DNA strand breaks [55]. Biochemical studies revealed a physical interaction between Parp and the Ku/DNA-PKcs complex, which is involved in the NHEJ pathway of DSB repair [56–58]. These studies also implied that Ku and DNA-PKcs are substrates of Parp, and Li et al.’s study suggests that Parp could decrease the affinity of Ku to DSB. PARP is thus a good candidate for the activity that limits the access of Ku to DSB in favor of HR. If this was true, deletion of PARP should result in decreased HR efficacy, which should be normalized by concomitant deletion of Ku. In order to confirm this hypothesis genetically, we have deleted chicken PARP-1 in DT40 wild-type and $\Delta ku70$ cells, and analyzed the HR efficacy in these cell lines [59]. Note that another paralog, PARP-2 gene [60] is not present in the chicken genome. Thus, DT40 $\Delta parp-1$ cells should be equivalent of mammalian $\Delta parp-1/2$ double mutants, which are embryonic lethal despite normal development of $\Delta parp-1$ and $\Delta parp-2$ deficient mice. Surprisingly, our observations match the theoretical predictions, discussed above (Fig. 5A). DT40 $\Delta parp-1$ cells show reduced levels

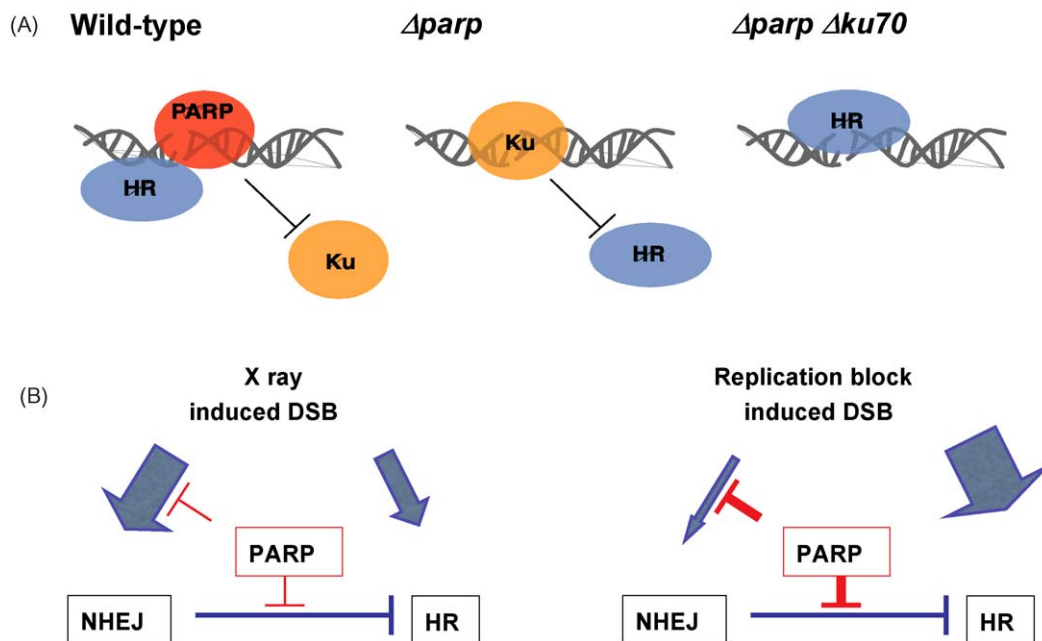


Fig. 5 – Poly[ADP ribose]polymerase controls the balance between HR and NHEJ. (A) This model is derived from genetic experiments in DT40 cells. In wild-type cells (left), Parp is rapidly and transiently activated by the DSB. This inhibits binding of Ku and allows access of HR factors. In the absence of Parp (middle), the affinity of Ku for DNA increases and access of HR is suppressed. This leads to a reduction in HR efficacy. When Ku is deleted in DT40 $\Delta parp$ mutants (right), HR can again be initiated effectively. **(B)** The relative usage of HR and NHEJ for repair of ionizing radiation-induced DSBs (left) and for DSB repair at replication block (right). Parp and Rad18 may limit access of NHEJ factors to DSBs particularly at replication block.

of HR, and this defect depends entirely on the presence of Ku, because $\Delta parp-1/\Delta ku70$ cells show increased levels of HR. Chicken PARP-1 also seems to be essential for suppressing NHEJ during replication stress, because $\Delta parp-1$ but not $\Delta parp-1/\Delta ku70$ and $\Delta parp-1/\Delta ligIV$ cells, are highly sensitive to CPT. We have also observed a similar interaction between PARP and NHEJ in human cells, using PARP inhibitors. We therefore conclude that PARP limits the competitive effects of Ku on HR. Moreover, another regulatory mechanism involving Rad18, which is essential for all post replicational repair pathways in budding yeast, also seems to suppress NHEJ at replication blocks in HeLa cells as well as DT40 (manuscript in preparation). Conceivably, suppression of NHEJ at replication blockage by PARP and possibly by Rad18 may be crucial in higher eukaryotes, because their relative usage of NHEJ in comparison to HR is significantly higher than that of yeast, while NHEJ is frequently associated with deletion and mis-pairing (Fig. 5B).

6. Variation in the balance between HR and NHEJ in different model systems

The balance between NHEJ and HR in DSB repair seems to vary considerably between yeast and vertebrate cells. In the budding yeast, HR plays a dominant role in virtually every type of DSB repair. On the other hand, the contribution of NHEJ is far greater in vertebrate cells in comparison with yeast. This active NHEJ in the vertebrates raises the following two problems, (1) whether NHEJ may interfere with HR dependent DSB repair at replication block and (2) how the two DSB repair pathways operate at ionizing radiation-induced DSBs (Fig. 5B). At replication block, higher eukaryotic cells seem to evolve new mechanisms involving PARP and probably Rad18, in order to minimize the toxic effect of NHEJ and thereby facilitate sister HR to accurately repair DSBs using the other intact sister as a template (Fig. 2A). To solve the second problem, budding yeast and higher eukaryotic cells appear to employ different strategies. In yeast, prior to resection of DSBs, NHEJ can play a role, whereas relatively quick 3' single-strand tail formation allows only HR to execute DSB repair. In contrast with yeast, a significant increase in the X-ray sensitivity of $\Delta ku70/\Delta rad54$ DT40 cells when compared with $\Delta rad54$ cells (Fig. 3A) supports the notion that, even when Rad54 dependent HR fails to complete DSB repair, Ku dependent NHEJ could operate as a back-up for the HR in DSB repair. Furthermore, vertebrate cells might be able to undergo homology search for HR and attempt NHEJ simultaneously. The molecular mechanism that governs the choice between HR and NHEJ remains to be elucidated.

The relative usage of the two DSB repair pathways is distinctly different even between different cell lines from the same species. Mouse ES cells tend towards HR, while primary cells tend towards NHEJ. DT40 appears to possess significantly higher HR efficiency than any mammalian cell line, partly because G1 phase constitutes only less than 20% of the whole cell cycle. Besides, other unknown factors may also contribute to the up regulation of HR in DT40. The special features of DT40 cells, for example, may underscore a defect of HR and concurrently underestimate a defect of NHEJ. Taken this into

consideration, DT40 is a unique system to comprehensively analyze the role for NHEJ, HR and their regulatory mechanisms in DSB repair in different circumstances, as a panel of isogenic repair mutants are available [61]. In general, both complementation and competition between the two DSB repair pathways are also likely to take place in all cells.

7. Concluding remarks

Over the past decade the molecular mechanism of individual repair pathways have been intensively analyzed. Both the considerable increase in knowledge and more and more sophisticated techniques allow us to move on with this analysis to a more integral picture of DNA repair. As we seem to have entered an age of systems biology [62], the focus of DNA repair research is likely to shift more and more to the study of how different players and pathways overlap and interact, and how they are coordinated within the living cell. The case of NHEJ and HR, as discussed in this review, is a relatively simple example of how different repair pathways are differentially employed, and are competing as well as collaborating for the same lesion. A comprehensive genetic analysis has revealed redundant, as well as essential roles in each DSB repair pathway in different circumstances. This knowledge could help us to manipulate their balance to our own ends. Thus, a clear understanding of the control of NHEJ and HR could benefit the development of novel chemotherapeutic treatments, and genetic technologies such as gene therapy using gene targeting.

Acknowledgement

We regret being unable to cite all relevant references because of the restricted length of the review.

REFERENCES

- [1] D.C. van Gent, J.H. Hoeijmakers, R. Kanaar, Chromosomal stability and the DNA double-stranded break connection, *Nat. Rev. Genet.* 2 (2001) 196–206.
- [2] T. Rich, R.L. Allen, A.H. Wyllie, Defying death after DNA damage, *Nature* 407 (2000) 777–783.
- [3] T. Lindahl, Instability and decay of the primary structure of DNA, *Nature* 362 (1993) 709–715.
- [4] H. Hohegger, E. Sonoda, S. Takeda, Post-replication repair in DT40 cells: translesion polymerases versus recombinases, *Bioessays* 26 (2004) 151–158.
- [5] E. Sonoda, T. Matsusaka, C. Morrison, P. Vagnarelli, O. Hoshi, T. Ushiki, K. Nojima, T. Fukagawa, I.C. Waizenegger, J.M. Peters, W.C. Earnshaw, S. Takeda, *Scc1/Rad21/Mcd1* is required for sister chromatid cohesion and kinetochore function in vertebrate cells, *Dev. Cell* 1 (2001) 759–770.
- [6] F. Liang, M. Han, P.J. Romanienko, M. Jasin, Homology-directed repair is a major double-strand break repair pathway in mammalian cells, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1998) 5172–5177.
- [7] E. Heidenreich, R. Novotny, B. Kneidinger, V. Holzmann, U. Wintersberger, Non-homologous end joining as an important mutagenic process in cell cycle-arrested cells, *EMBO. J.* 22 (2003) 2274–2283.

- [8] M. O'Driscoll, P.A. Jeggo, The role of double-strand break repair—insights from human genetics, *Nat. Rev. Genet.* 7 (2006) 45–54.
- [9] U. Grawunder, M. Wilm, X. Wu, P. Kulesza, T.E. Wilson, M. Mann, M.R. Lieber, Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells, *Nature* 388 (1997) 492–495.
- [10] A. Shinohara, H. Ogawa, Y. Matsuda, N. Ushio, K. Ikeo, T. Ogawa, Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA, *Nat. Genet.* 4 (1993) 239–243.
- [11] L.S. Symington, Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair, *Microbiol. Mol. Biol. Rev.* 66 (2002) 630–670.
- [12] J. Essers, R.W. Hendriks, S.M. Swagemakers, C. Troelstra, J. de Wit, D. Bootsma, J.H. Hoeijmakers, R. Kanaar, Disruption of mouse RAD54 reduces 14 ionizing radiation resistance and homologous recombination, *Cell* 89 (1997) 195–204.
- [13] T.L. Tan, R. Kanaar, C. Wyman, Rad54, a Jack of all trades in homologous recombination, *DNA Rep. (Amst)* 2 (2003) 787–794.
- [14] F. Paques, J.E. Haber, Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*, *Microbiol. Mol. Biol. Rev.* 63 (1999) 349–404.
- [15] Y. Aylon, M. Kupiec, New insights into the mechanism of homologous recombination in yeast, *Mutat. Res.* 566 (2004) 231–248.
- [16] T.L. Tan, J. Essers, E. Citterio, S.M. Swagemakers, J. de Wit, F.E. Benson, J.H. Hoeijmakers, R. Kanaar, Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation, *Curr. Biol.* 9 (1999) 325–328.
- [17] G. Ira, A. Pelliccioli, A. Balijja, X. Wang, S. Fiorani, W. Carotenuto, G. Liberi, D. Bressan, L. Wan, N.M. Hollingsworth, J.E. Haber, M. Foiani, DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1, *Nature* 431 (2004) 1011–1017.
- [18] M. Takata, M.S. Sasaki, E. Sonoda, C. Morrison, M. Hashimoto, H. Utsumi, Y. Yamaguchi-Iwai, A. Shinohara, S. Takeda, Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells, *EMBO J.* 17 (1998) 5497–5508.
- [19] O. Bezzubova, A. Silbergleit, Y. Yamaguchi-Iwai, S. Takeda, J.M. Buerstedde, Reduced X-ray resistance and homologous recombination frequencies in a RAD54^{-/-} mutant of the chicken DT40 cell line, *Cell* 89 (1997) 185–193.
- [20] N. Cheong, X. Wang, Y. Wang, G. Iliakis, Loss of S-phase-dependent radioresistance in *irs-1* cells exposed to X-rays, *Mutat. Res.* 314 (1994) 77–85.
- [21] J.M. Stark, M. Jasin, Extensive loss of heterozygosity is suppressed during homologous repair of chromosomal breaks, *Mol. Cell. Biol.* 23 (2003) 733–743.
- [22] M. Doree, T. Hunt, From Cdc2 to Cdk1: when did the cell cycle kinase join its cyclin partner? *J. Cell Sci.* 115 (2002) 2461–2464.
- [23] P. Nurse, Regulation of the eukaryotic cell cycle, *Eur. J. Cancer* 33 (1997) 1002–1004.
- [24] M.G. Ferreira, J.P. Cooper, The fission yeast Taz1 protein protects chromosomes from Ku-dependent end-to-end fusions, *Mol. Cell* 7 (2001) 55–63.
- [25] T. Caspari, J.M. Murray, A.M. Carr, Cdc2-cyclin B kinase activity links Crb2 and Rqh1-topoisomerase III, *Genes Dev.* 16 (2002) 1195–1208.
- [26] D.K. Bishop, U. Ear, A. Bhattacharyya, C. Calderone, M. Beckett, R.R. Weichselbaum, A. Shinohara, Xrcc3 is required for assembly of Rad51 complexes in vivo, *J. Biol. Chem.* 273 (1998) 21482–21488.
- [27] J.S. Kim, T.B. Krasieva, H. Kurumizaka, D.J. Chen, A.M. Taylor, K. Yokomori, Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells, *J. Cell. Biol.* 170 (2005) 341–347.
- [28] A. Jazayeri, J. Falck, C. Lukas, J. Bartek, G.C. Smith, J. Lukas, S.P. Jackson, ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks, *Nat. Cell Biol.* 8 (2006) 37–45.
- [29] N. Sugawara, X. Wang, J.E. Haber, In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination, *Mol. Cell* 12 (2003) 209–219.
- [30] S.A. Nick McElhinny, J.M. Havener, M. Garcia-Diaz, R. Juarez, K. Bebenek, B.L. Kee, L. Blanco, T.A. Kunkel, D.A. Ramsden, A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining, *Mol. Cell* 19 (2005) 357–366.
- [31] M. Schwartz, E. Zlotorynski, M. Goldberg, E. Ozeri, A. Rahat, C. le Sage, B.P. Chen, D.J. Chen, R. Agami, B. Kerem, Homologous recombination and nonhomologous end-joining repair pathways regulate fragile site stability, *Genes Dev.* 19 (2005) 2715–2726.
- [32] J.A. Downs, S.P. Jackson, A means to a DNA end: the many roles of Ku, *Nat. Rev. Mol. Cell. Biol.* 5 (2004) 367–378.
- [33] M.R. Lieber, Y. Ma, U. Pannicke, K. Schwarz, The mechanism of vertebrate nonhomologous DNA end joining and its role in V(D)J recombination, *DNA Rep. (Amst)* 3 (2004) 817–826.
- [34] T. Fukushima, M. Takata, C. Morrison, R. Araki, A. Fujimori, M. Abe, K. Tatsumi, M. Jasin, P.K. Dhar, E. Sonoda, T. Chiba, S. Takeda, Genetic analysis of the DNA-dependent protein kinase reveals an inhibitory role of Ku in late S-G2 phase DNA double-strand break repair, *J. Biol. Chem.* 276 (2001) 44413–44418.
- [35] Y. Gao, J. Chaudhuri, C. Zhu, L. Davidson, D.T. Weaver, F.W. Alt, A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination, *Immunity* 9 (1998) 367–376.
- [36] N. Adachi, T. Ishino, Y. Ishii, S. Takeda, H. Koyama, DNA ligase IV-deficient cells are more resistant to ionizing radiation in the absence of Ku70: implications for DNA double-strand break repair, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 12109–12113.
- [37] A.J. Pierce, P. Hu, M. Han, N. Ellis, M. Jasin, Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells, *Genes Dev.* 15 (2001) 3237–3242.
- [38] M. Frank-Vaillant, S. Marcand, Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination, *Mol. Cell* 10 (2002) 1189–1199.
- [39] E. Sonoda, M. Takata, Y.M. Yamashita, C. Morrison, S. Takeda, Homologous DNA recombination in vertebrate cells, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 8388–8394.
- [40] M. Yamazoe, E. Sonoda, H. Hochegger, S. Takeda, Reverse genetic studies of the DNA damage response in the chicken B lymphocyte line DT40, *DNA Rep. (Amst)* 3 (2004) 1175–1185.
- [41] J. Thacker, M.Z. Zdzienicka, The XRCC genes: expanding roles in DNA double-strand break repair, *DNA Rep. (Amst)* 3 (2004) 1081–1090.
- [42] E. Sonoda, M.S. Sasaki, J.M. Buerstedde, O. Bezzubova, A. Shinohara, H. Ogawa, M. Takata, Y. Yamaguchi-Iwai, S. Takeda, Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death, *EMBO J.* 17 (1998) 598–608.
- [43] M. Tarsounas, D. Davies, S.C. West, BRCA2-dependent and independent formation of RAD51 nuclear foci, *Oncogene* 22 (2003) 1115–1123.

- [44] M. Lisby, R. Rothstein, U.H. Mortensen, Rad52 forms DNA repair and recombination centers during S phase, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 8276–8282.
- [45] N. Saleh-Gohari, H.E. Bryant, N. Schultz, K.M. Parker, T.N. Cassel, T. Helleday, Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks, *Mol. Cell. Biol.* 25 (2005) 7158–7169.
- [46] M. Lomonosov, S. Anand, M. Sangrithi, R. Davies, A.R. Venkitaraman, Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein, *Genes Dev.* 17 (2003) 3017–3022.
- [47] N.E. Sharpless, D.O. Ferguson, R.C. O'Hagan, D.H. Castrillon, C. Lee, P.A. Farazi, S. Alson, J. Fleming, C.C. Morton, K. Frank, L. Chin, F.W. Alt, R.A. DePinho, Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions, *Mol. Cell* 8 (2001) 1187–1196.
- [48] Y. Gao, D.O. Ferguson, W. Xie, J.P. Manis, J. Sekiguchi, K.M. Frank, J. Chaudhuri, J. Horner, R.A. DePinho, F.W. Alt, Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development, *Nature* 404 (2000) 897–900.
- [49] N. Adachi, S. So, H. Koyama, Loss of nonhomologous end joining confers camptothecin resistance in DT40 cells. Implications for the repair of topoisomerase I-mediated DNA damage, *J. Biol. Chem.* 279 (2004) 37343–37348.
- [50] Y. Pommier, C. Redon, V.A. Rao, J.A. Seiler, O. Sordet, H. Takemura, S. Antony, L. Meng, Z. Liao, G. Kohlhausen, H. Zhang, K.W. Kohn, Repair of and checkpoint response to topoisomerase I-mediated DNA damage, *Mutat. Res.* 532 (2003) 173–203.
- [51] J. Thacker, A.N. Ganesh, DNA-break repair, radioresistance of DNA synthesis, and camptothecin sensitivity in the radiation-sensitive *irs* mutants: comparisons to ataxia-telangiectasia cells, *Mutat. Res.* 235 (1990) 49–58.
- [52] E. Sonoda, M.S. Sasaki, C. Morrison, Y. Yamaguchi-Iwai, M. Takata, S. Takeda, Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells, *Mol. Cell. Biol.* 19 (1999) 5166–5169.
- [53] N.P. Higgins, K. Kato, B. Strauss, A model for replication repair in mammalian cells, *J. Mol. Biol.* 101 (1976) 417–425.
- [54] J.M. Sogo, M. Lopes, M. Foiani, Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects, *Science* 297 (2002) 599–602.
- [55] J.C. Ame, C. Spenlehauer, G. de Murcia, The PARP superfamily, *Bioessays* 26 (2004) 882–893.
- [56] S. Galande, T. Kohwi-Shigematsu, Poly(ADP-ribose) polymerase and Ku autoantigen form a complex and synergistically bind to matrix attachment sequences, *J. Biol. Chem.* 274 (1999) 20521–20528.
- [57] B. Li, S. Navarro, N. Kasahara, L. Comai, Identification and biochemical characterization of a Werner's syndrome protein complex with Ku70/80 and poly(ADP-ribose) polymerase-1, *J. Biol. Chem.* 279 (2004) 13659–13667.
- [58] Y. Ariumi, M. Masutani, T.D. Copeland, T. Mimori, T. Sugimura, K. Shimotohno, K. Ueda, M. Hatanaka, M. Noda, Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase *in vitro*, *Oncogene* 18 (1999) 4616–4625.
- [59] H. Hohegger, D. Dejsuphong, T. Fukushima, C. Morrison, E. Sonoda, V. Schreiber, G.Y. Zhao, A. Saberi, M. Masutani, N. Adachi, H. Koyama, G.d. Murcia, S. Takeda, Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells, *EMBO J.* 25 (2006) 1305–1314.
- [60] J.C. Ame, V. Rolli, V. Schreiber, C. Niedergang, F. Apiou, P. Decker, S. Muller, T. Hoger, J. Menissier-de Murcia, G. de Murcia, PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase, *J. Biol. Chem.* 274 (1999) 17860–17868.
- [61] K. Nojima, H. Hohegger, A. Saberi, T. Fukushima, K. Kikuchi, M. Yoshimura, B.J. Orelli, D.K. Bishop, S. Hirano, M. Ohzeki, M. Ishiai, K. Yamamoto, M. Takata, H. Arakawa, J.M. Buerstedde, M. Yamazoe, T. Kawamoto, K. Araki, J.A. Takahashi, N. Hashimoto, S. Takeda, E. Sonoda, Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells, *Cancer Res.* 65 (2005) 11704–11711.
- [62] M.W. Kirschner, The meaning of systems biology, *Cell* 121 (2005) 503–504.