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Homologous recombination as a mechanism for genome rearrangements: environmental and genetic effects

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Novel findings over the last 2 years have led to an increased emphasis on homologous recombination (HR) as both a pathway for DNA repair and a cause for genomic rearrangements. Indeed, environmental carcinogens increase the frequency of HR, as can be observed when two copies of a duplicated sequence recombine to delete the intervening sequences. Such HR events between dispersed homologous sequences may result in not only deletions, but also gene duplications or translocations. These types of genomic rearrangement have been observed to be the cause of several different genetic diseases, including cancer. In reflection of this, several genes have been identified that, when mutant, predispose an individual to an increased frequency of cancer. These genes have been shown to be either directly or indirectly involved in HR. In addition, HR is induced by a wide variety of carcinogens, preferentially in proliferating cells. This fits the most current models of recombination and its involvement in reinitiating stalled replication forks. Thus, 'correct' HR repair may act with high fidelity, an important issue for proliferating cells, but in the context of alternative homologous partner sequences, 'aberrant' HR can cause genomic rearrangements with dire consequences.

INTRODUCTION

It is commonly considered that homologous recombination (HR) is downregulated in mammalian cells in preference to another recombination pathway, namely non-homologous end-joining (NHEJ) (1). The reason for this is 2-fold: first, the highly repetitive nature of mammalian genomes, leading to the belief that HR would be detrimental to genetic integrity and, secondly, the experimental observation that gene targeting is very inefficient in mammalian cells (2). Despite this, there has been mounting evidence over the last 10 years that HR is actually fairly frequent, in particular following a variety of different types of DNA damage including those classically thought to be repaired by other DNA repair pathways. The last 2 years in this field have been particularly exciting as several tumor suppressor genes have been demonstrated to be involved in recombination. For instance, a direct connection with recombination has been established for BRCA1 (3,4) with elements known to be involved in recombination and indirectly for ATM (5–8), which is responsible for activating the p53 cell cycle damage response pathway (9,10). Here we will describe the evidence that HR, including errant HR, is prevalent in mammalian systems. In addition, HR is induced by a variety of DNA damages. We believe that induction is usually limited to dividing cells, and that this fits current models of recombination and carcinogenesis.

THE GOOD OF HR

HR is classically thought to be a mechanism for promoting genetic diversity. For example, in a diploid cell, meiotic recombination switches allele combinations along a linear chromosome, thereby producing a novel haploid for the subsequent generation. In yeast, a double strand break (DSB) is thought to initiate meiotic HR (11), and it is thought that the mechanics of HR are conserved through to mammals (12,13). HR also occurs in mitotically dividing cells and is stimulated in yeast, plant and mammalian cells by a site-specific DSB (14–20). In fact, a recent investigation with a model mammalian system with a site-specific endonuclease has demonstrated that 30–50% of the breaks created are repaired by HR (20). In the same way, HR can be stimulated by a variety of DNA-damaging agents; some of these are discussed in more detail below. Thus, HR can act as a repair mechanism, and as such it can repair a region of DNA with high fidelity. This is especially true when the HR event is a gene conversion, copying information from an allele on either a sister or homologous chromosome. Having such good fidelity is important in proliferating cells as, by virtue of its state of proliferation, there will be subsequent generations of cells that must be genetically functional.

DNA DSBs are considered to be the most potent substrates for recombination repair. This follows the idea that a DSB, produced by a controlled endonuclease reaction, initiates meiotic recombination. The most widely accepted model proposed to explain the mechanics of HR is the DSB model (21), with alternatives including gap repair (22), single strand annealing (23) and synthesis-dependent strand annealing (13,24). Most recently there have been a number of papers ascribing a new function to recombination: re-initiation of a stalled replication fork (25,26). These events have been proposed to be the result of either replication intermediates that

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resemble recombination intermediates or replication of single strand breaks (SSBs) to effectively produce a DSB, which is then recombinagenic. This again supports the idea that recombination, and probably HR repair, is more prevalent in actively dividing cells than in arrested cells.

THE BAD OF HR

Approximately 25% of the mammalian genome consists of repetitive sequences (27). These include dispersed elements such as SINES, LINES, microsatellites and minisatellites. There are also structural elements of chromosomes: centromeres, telomeres and origins of replication. Ribosomal DNA sequences for the 18S and 28S rRNAs are present in many copies. Further, there are duplicated genes such as the globins, genes that are very closely related at the sequence level, categorized into families or superfamilies (e.g. the immunoglobulin superfamily) and even pseudogenes. If HR was prevalent, it would be expected that recombination between these different repetitive elements would produce adverse gross rearrangements, loss of genetic information and general genetic instability. This is in fact the case; for instance, it has been shown that regions enriched in *Alu* repeat sequences display genomic instability (28).

Loss of genetic information is a common event. Cancer usually results from a mutation in a tumor suppressor gene, be it inherited or acquired, which is revealed when the functional counterpart to the mutated gene is lost. The major mechanisms by which such a loss of heterozygosity occurs are chromosomal loss, gene conversion and deletion (29). Though chromosome loss is not likely to be due to a recombination event, both gene conversion and deletion are. Gene conversion is undisputedly an HR event, where genetic information is copied from one locus to another. Deletions, though, can occur by illegitimate recombination (NHEJ) or by HR. In fact, many of the deletion events that have been mapped appear to have been mediated by regions of homology; some of these are outlined below.

Deletions are usually the result of either intrachromosomal events or unequal interchromosomal events. These events can be mediated by regions of homology and may result in the removal of very large regions of DNA. Such deletions have been identified as the cause of several diseases which include X-linked ichthyosis, in which 1.9 Mb of DNA are deleted mediated by flanking S232 elements (30,31); hereditary neuropathy with liability to pressure palsies in which 1.5 Mb are deleted mediated by CMT1A-REP (32–34); as well as Prader–Willi syndrome (35), DiGeorge syndrome (36) and hypercholesterolemia (37). Alternatively, deletions may be due to an interchromatid mechanism such as unequal crossing over between misaligned homologous regions on sister chromatids or homologous chromosomes. The deletion product of these mechanisms is often indistinguishable, though it is interesting to note that Charcot–Marie–Tooth syndrome type 1A occurs from a duplication of the same region as is deleted in hereditary neuropathy with liability to pressure palsies (34,38,39). Similarly, a tandem duplication within the *ALL-1* gene is mediated by *Alu* recombination and results in acute myeloid leukemia (40). These duplications suggest an interchromatid crossing-over mechanism in these events.

It should also be noted that HR between ectopic regions of homology would result in a translocation. Mapping the breakpoints of common translocation breakpoints has again revealed regions of shared homology. For example, Philadelphia chromosome (chromosome 9/22 translocation), which is often found in chronic myelogenous leukemia patients, has breakpoints that map to *Alu* sequences (41,42).

DNA DAMAGE INDUCES HR

DNA damage comes in many forms and can affect any aspect of the DNA molecule. To cope with the large variety of DNA damage, several different repair reactions have evolved, these include base excision repair, nucleotide excision repair, mismatch repair and recombination. Each of these repair reactions demonstrates particular substrate specificity, but there has been some evidence that there is overlap (43). Here we will present some of the carcinogenic agents that are known to result in HR repair, including some that are considered to cause damage that is mainly repaired by one of the other repair reactions. These results are compared with the Ames *Salmonella* assay, which is the most widely used assay in predictive carcinogenesis to determine genotoxicity based on the induction of point mutations.

Recombination between repeated sequences has been examined in bacteria (44), yeast (45), *Drosophila* (46) and mammals (47). In mouse cells, it was observed that deletions between such sequences occur both spontaneously and following exposure to DNA-damaging agents (47,48). In our laboratory we have expanded on these studies over the last decade using a variety of similar systems. These include *his3* mutation alleles in the yeast *Saccharomyces cerevisiae* (45), an internal duplication of exons 2 and 3 of the human *HPRT* gene (49) and a duplication within the *p* gene (the p^{un} mutation) of the mouse (50). Intrachromosomal HR events result in specific, measurable deletions (DEL events) in all three systems and more frequently after treatment with many carcinogens including carcinogens that are missed by other assays (49–58) (Table 1). For instance, the *in vivo* mouse assay has shown inducibility not only with carcinogens such as ionizing radiation, alkylating agents, including ethylmethane sulfonate, methylmethane sulfonate and ethylnitrosourea, and benzo(*a*)pyrene, which produces DNA adducts, but also carcinogens that are negative in the *Salmonella* assay such as trichloroethylene, benzene, sodium arsenate, TCDD and PCBs (50,57,58). In addition, the mouse assay is also inducible following exposure to cigarette smoke (56).

Studies in our yeast system determined that *Salmonella* test-negative carcinogens induce DNA deletions with an apparent threshold. We found the same effect to be true for oxidative mutagens (59). Considering the need for more information about the mechanism of toxicity of these *Salmonella* test-negative carcinogens, we initiated a study to determine the relationship between these two types of agent. In particular we were interested to determine whether *Salmonella*-negative, DEL-positive carcinogens, including the human carcinogens cadmium and benzene, generate free radical species intracellularly (52,53,60–62). The toxicity and the recombinagenic activity of many *Salmonella*-negative, but not of *Salmonella*-positive, carcinogens was significantly reduced in the presence of the free radical scavenger *N*-acetylcysteine. In support of

Table 1. Response of carcinogens and non-carcinogens in the DEL recombination assays with yeast, human cells and in the mouse and the Ames (*Salmonella*) assay

Compound	Carcinogen	Response in assay		References
		DEL	*Salmonella*	
Safrole	+	+	–	51,54,55,86,87
Methyl eugenol	+	+	–	55,87
Ethionine	+	+	–	54,86
Urethane	+	+	–	54,86
Auramine O	+	+	–	54,86
Methylene chloride	+	+	–	54,86
Carbon tetrachloride	+	+	–	54,86
Cadmium chloride	+	+	–	54,86
Cadmium sulfate	+	+	–	54,86
3-amino-1,2,4-triazole	+	+	–	54,86
Acetamide	+	+	–	54,86
Thioacetamide	+	+	–	54,86
Thiourea[a]	+	+	–	54,86
DDE	+	+	–	50,57,58
Ethylenethiourea	+	+	–	54,86
Aniline	+	+	–	54,86
o-toluidine	+	+	–	51
o-anisidine	+	+	–	
Hexamethyl				
Phosphoramide	+	+	–	51
Acrylonitrile	+	+	–	51
Benzene[a,b]	+	+	–	51
Arsenate[b,c]	+	+	–	50,57,58
Aroclor 1221 (PCB)[a,b]	+	+	–	57
Arochlor 1260 (PCB)[b]	+	+	–	57
TCDD[b,c]	+	+	–	57
UV irradiation[a]	+	+	+	54,86
Ionizing radiation[a,b]	+	+	+	54,86
4-NQO	+	+	+	54,86
Benzo(a)pyrene[b,c]	+	+	+	50,57,58
ENU[b,c]	+	+	+	50,57,58
MMS[a,b]	+	+	+	54,86
EMS[b]	+	+	+	54,86
Nitrogen mustard	+	+	+	54,86
Epichlorohydrin	+	+	+	54,86
Aflatoxin B1	+	+	+	54,86
Ethylene dibromide	+	+	+	54,86
Dimethylhydrazine	+	+	+	54,86
Cyclophosphamide	+	+	+	51
Formaldehyde	+	+	+	54,86
Ethylene oxide	+	+	+	88
Propylene oxide	+	+	+	88
2,4-diaminotoluene	+	+	+	60
TPA	+	–	–	54,86
Diethylstilbestrol	+	–	–	51
Peroxysome proliferators	+	–	–	89
Diethylhexylphthalate	+	–	–	51

continued overleaf

Table 1. *Continued*

Compound	Carcinogen	Response in assay		References
		DEL	*Salmonella*	
Phenobarbital	+	–	–	51
2,6-diaminotoluene	–	–	+	60
Hydroxylamine HCl	–	–	+	54,86
Sodium azide	–	–	+	54,86
5-bromouracil	–	–	+	54,86
2-aminopurine	–	+	+	54,86
Ethidium bromide	–	+	+	54,86
Benzoin	–	–	–	51
Methionine	–	–	–	54,86
Ethanol	–	–	–	54,86
Caprolactam	–	+	–	51

DDE, 2,2-bis[4-chlorophenyl]-1,1-dichloroethylene; 4-NQO, 4-nitroquinoline *N*-oxide; ENU, 1-ethyl-1-nitrosourea; MMS, methyl methanesulfonate; EMS, ethyl methanesulfonate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PCB, polychlorinated biphenyl; TCDD, 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin.

Data for carcinogenicity and results with the *Salmonella* assay of the agents can be found in the Carcinogenesis Potency Database (http://potency.berkeley.edu/chemicalsummary.html).

[a]These agents have also been used in the mammalian cell culture DEL assay (49).

[b]These agents have also been used in the mouse DEL assay (50,57,58).

[c]These agents have not been used in the yeast DEL assay.

this observation, a strain deficient in the antioxidant enzyme superoxide dismutase was found to be more sensitive to the toxicity of many *Salmonella*-negative carcinogens. Intracellular oxidation of a free radical-sensitive reporter compound was increased in cultures exposed to *Salmonella*-negative but not to *Salmonella*-positive carcinogens. These data indicate a role for oxidative stress in the toxic and recombinagenic activity of *Salmonella*-negative carcinogens, giving a plausible mechanistic rationale. Oxidative stress causes DNA SSBs (63) which on DNA replication (see below) may turn into DSBs that induce DNA deletions.

CELL CYCLE AND HR

A current model for recombination that has gained much attention over the past year is the re-initiation of a stalled replication fork by recombination. DNA damage may act to block replication (64). For example, an SSB represents an end of contiguous template, and replication to the end of this broken template produces a DSB, again, a highly recombinagenic substrate. Thus, it would be expected that DNA-damaging agents that cause different forms of DNA lesion will have differing abilities to cause HR repair due to replication. To further our understanding of the mechanism by which differently acting DNA-damaging agents induce deletion, we have examined the effects of cell cycle. In these studies we determined the ability of carcinogens to induce DNA deletions when exposed cells were arrested at the G_1 or G_2 cell cycle stage or were actively dividing (65,66). These experiments revealed that ionizing radiation induced deletions in G_1- or G_2-arrested cells, events that probably result from the direct induction of DSBs between the duplicated sequences. In contrast, other DNA damages, such as SSBs, UV irradiation or alkylation of DNA, need replication to turn those DNA damages into recombinagenic substrates. Therefore, HR repair is probably a secondary effect of the DNA damage that initially results in the stalling of a replication fork (Fig. 1).

DISEASES WITH INCREASED FREQUENCY OF HR

Assuming that genome rearrangements and deletion events cause a significant proportion of cancers, then there should be a correlation between those mutations that result in a higher recombination frequency and cancer predisposition. In fact, there are several genetic diseases that have a genetic instability phenotype and indeed have a high frequency of carcinogenesis. These include ataxia telangiectasia (AT) (67), Li–Fraumeni syndrome (68), Bloom's syndrome (69), Werner's syndrome (70), Cockayne's syndrome, Fanconi's anaemia, Lynch syndromes I and II, Wiscott–Aldrich syndrome and xeroderma pigmentosum (71).

In the past 2 years AT has been the focus of much intensive research and has proven to be an exciting link between recombination and the cell cycle damage response. AT is an autosomal recessive syndrome with a mutation in *ATM*. Among the phenotypes of this syndrome, patients display chromosomal instability, radiosensitivity and a predisposition to lymphoid cancer in childhood. Cytogenetic analysis reveals a higher spontaneous incidence of chromosome breaks, chromosome gaps, acentric fragments, dicentric chromosomes and aneuploidy. In addition, T lymphocytes of these patients have an elevated frequency of translocations with breakpoints mapping to the T cell antigen receptor genes and the Ig heavy chain genes (reviewed in ref. 72). Following exposure to ionizing radiation

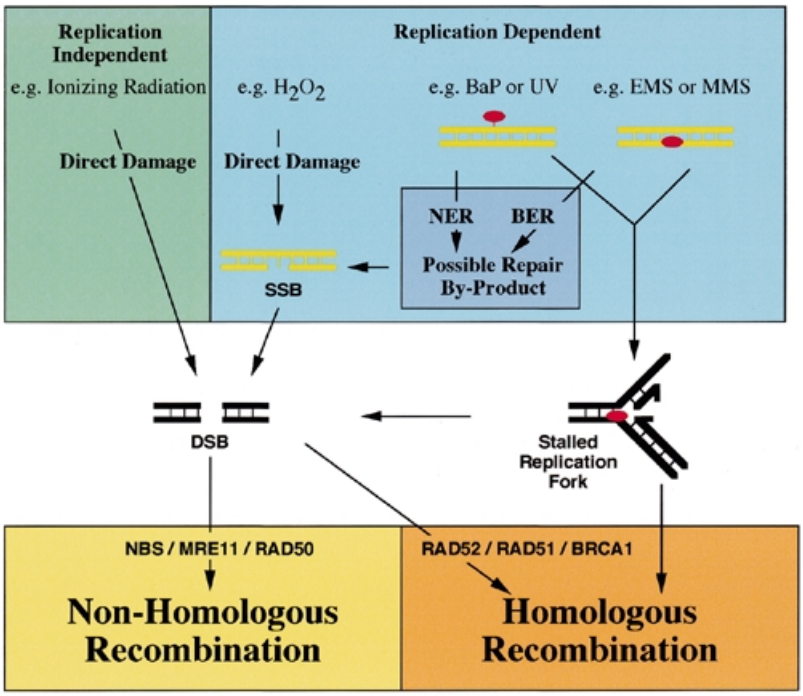

Figure 1. DNA damage may result in recombination. Direct DSB formation by agents such as ionizing radiation does not require replication to form a recombinagenic substrate. SSBs may be formed as a direct result of agents such as hydrogen peroxide (H_2O_2) or indirectly as an incomplete product of DNA damage repair [e.g. benzo(*a*)pyrene (BaP) or UV irradiation forming nucleotide excision repair (NER) substrates, or ethylmethane sulfonate (EMS) or methylmethane sulfonate (MMS) forming base excision repair (BER) substrates]. Replication of these lesions may result in the formation of a DSB. Alternatively, DNA damage may result directly in replication fork stalling, which can be processed into a DSB or may be reconfigured into a Holliday junction structure and processed directly as a recombination substrate. DSBs, however formed, are potent substrates for the competing reactions of non-HR and HR.

or radiomimetic agents, cells from AT patients have an increased frequency of chromosomal aberrations compared with normal cells (reviewed in refs 72,73). The knockout mouse model of this disease also displays genetic instability; in particular, it has an increased frequency of spontaneous deletion by HR (8). In contrast though, studies with the same knockout mouse model using the APRT mutation detection system found no increase in the frequency of mutation (74). This is highly reminiscent of the *Salmonella*-negative, DEL-positive carcinogens that demonstrated an oxidative stress effect. One current hypothesis is that cells from AT patients are oxidatively stressed, with mounting evidence that this is in fact the case (75). The increased levels of genetic instability observed in AT patient cells may be the result of an increased frequency of HR stimulated by endogenous oxidative damage in proliferating cells. Alternatively, these AT cells may be processing or responding to exogenous environmental genotoxic agents with reduced efficiency, as exemplified by the exquisite ionizing radiation sensitivity of AT patients. In either scenario, an increased amount of damaged DNA will result in more aberrant HR possibly being the cause of cancer in these patients.

Although AT has been identified to be the result of a mutation in the *ATM* gene, two other mutations result in syndromes that were originally mistaken for AT. These variants of AT are caused by mutations in NBS (the syndrome is presently called Nijmengen break syndrome) (76) and MRE11 (77), presenting similar phenotypes, including genetic instability. NBS, MRE11 and RAD50 form a complex that NBS modulates once it is phosphorylated by ATM in response to DNA damage (78–80). NHEJ can repair DSBs and competes with HR. RAD50 and MRE11 are involved in NHEJ in yeast (81–83). Assuming that the mammalian homologs of these genes are also involved in NHEJ, it seems plausible that a deficiency in AT also results in a slight deficiency in NHEJ. Therefore, damage would be channeled into HR as an alternative pathway, possibly explaining the hyper-recombination phenotype that we found in ATM-deficient mice (8).

ATM is responsible for activating the p53 cell cycle damage response pathway following ionizing radiation by phosphorylating p53 and MDM2 (9,10). Another protein that is phosphorylated by ATM in response to DNA damage is BRCA1. Inactivation of BRCA1 in mouse mammary epithelial cells results in genetic instability such as aneuploidy and chromosomal rearrangements (84). BRCA1 is known to play a role in HR; in its absence homologous repair is defective (4). It has been reported that BRCA1 forms foci with BRCA2 and RAD51 following DNA damage in an ATM-dependent manner (7). How these foci relate to HR is still unknown. In addition, BRCA1 may be part of a super complex with BRCA2, MSH2, MLH1, ATM, BLM and the RAD50–MRE11–NBS complex (85). The actual function of BRCA1 is still being elucidated, and should reveal more insight into the breadth of response to damage mounted by ATM in the cell (Fig. 2).

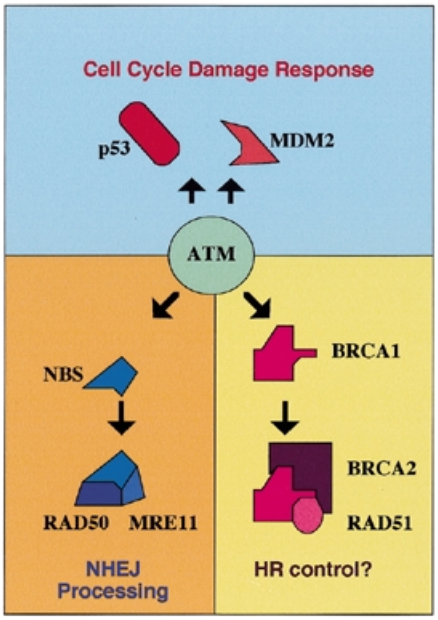

Figure 2. ATM is a multifunctional protein that is activated by damage, in particular ionizing radiation. Once activated, ATM phosphorylates MDM2 and p53 (9,10), thus activating the p53 cell cycle damage response pathway, NBS (78–80), which then modulates the activity of the RAD50–MRE11 complex in NHEJ, as well as BRCA1 (7), which is known to then form foci with BRCA2 and RAD51. The function of these foci is not known, although RAD51 is a strand exchange protein central in the RAD52 HR epistasis group and BRCA1-deficient cells have a deficiency in HR (4).

CONCLUSION

In summary, HR is an important pathway in mammalian cells, which is important for DNA repair and meiosis, but is also emerging as a mechanism for genome rearrangements, inherited disease and cancer. Here we have represented HR repair involved in maintaining the genomic integrity unless initiated in sequences with non-allelic homologous sequences. Initiation of HR leading to deletions appears to be inducible by a number of different types of damage, though only those that cause DSBs appear to be independent of replication. This presents a case for proliferating cells having a higher level of HR, and therefore being most likely to display genetic instability. Thus, it follows that cell cycle control in response to damage is important, though its relationship to HR-induced repair is still only poorly understood. Future work in this field is of paramount importance, both for being able to understand patients where the control of HR is disturbed and in understanding the full implications of exposure to various carcinogens.

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