Cell. Mol. Life Sci. (2012) 69:727–740
DOI 10.1007/s00018-011-0839-7

Cellular and Molecular Life Sciences

REVIEW

Haploinsufficiency in mouse models of DNA repair deficiency: modifiers of penetrance

Diane C. Cabelof

Received: 8 July 2011 / Revised: 8 August 2011 / Accepted: 15 September 2011 / Published online: 28 September 2011

Abstract Mouse models of DNA repair deficiency are useful tools for determining susceptibility to disease. Cancer predisposition and premature aging are commonly impacted by deficiencies in DNA repair, presumably as a function of reduced genomic fitness. In this review, a comprehensive analysis of all DNA repair mutant mouse models has been completed in order to assess the importance of haploinsufficiency for these genes. This analysis brings to light a clear role for haploinsufficiency in disease predisposition. Unfortunately, much of the data on heterozygous models are buried or underinvestigated. In light of a better understanding that the role of DNA repair haploinsufficiency may play in penetrance of other oncogenic or disease causing factors, it may be in the interest of human health and disease prevention to further investigate the phenotypes in many of these mouse models.

Keywords Haploinsufficiency · DNA repair · Heterozygosity · Penetrance

Introduction

Loss of genomic stability increases risk for human diseases. It was the study of human disorders associated with increased cancer risk and premature aging that led to the discovery of many genes responsible for genome maintenance. These discoveries have driven the study of DNA repair, leading to the elucidation of multiple DNA repair pathways. Analysis of rare or familial cancer predisposition and/or aging syndromes has resulted in identification of genes responsible for the following syndromes: xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, severe combined immunodeficiency, Werner syndrome, ataxia telangiectasia, Nijmegen breakage syndrome, Bloom syndrome, Rothmund-Thomson syndrome, breast cancer susceptibility, hereditary non-polyposis colorectal cancer (HNPCC), and Fanconi anemia (reviewed in [1–3]). Many of these syndromes also display phenotypes distinct from cancer and aging. These include photosensitivity, neurodegeneration, growth retardation, immunodeficiency, microcephaly, arteriosclerosis, diabetes mellitus, and skeletal defects [1, 2], demonstrating that repair deficiency increases risk for a variety of human diseases in addition to cancer and aberrant aging. The objective of this review is to evaluate the impact that haploinsufficiency for genes directly associated with DNA repair can exert on disease predisposition. The implications of this are expanded through consideration of conditions of genomic instability not yet directly associated with specific DNA repair pathways, as occurred with the discoveries of the XP and FANC genes. For example, copy number variations are increasingly associated with developmental delay [4], but the underlying source of these structural variations is not well understood. Further investigation into the DNA repair mechanisms that permit formation of and/or tolerance to these structural variations may elucidate additional links between DNA repair and a broad range of developmental diseases.

"DNA repair" is a term used here to describe mechanisms by which the cell responds to DNA damage by either repairing or tolerating DNA damage. In response to DNA damage, some mechanisms function to restore the original DNA sequence (base excision repair, nucleotide excision repair, mismatch repair, and direct reversal of damage); some mechanisms function to resolve DNA strand breaks

D. C. Cabelof (✉)
Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48201, USA
e-mail: d.cabelof@wayne.edu

(non-homologous end-joining, homologous recombination, single strand break repair); and some mechanisms function to permit tolerance to DNA damage and thereby survival. None of the tolerance models display haploinsufficiency and will not be discussed. A working model for the role of DNA repair deficiency in disease predisposition is that persistent DNA damage becomes fixed throughout the genome, promoting genomic instability through altered or reduced gene function. Consistent with this model, loss of DNA repair capacity exerts pleiotropic effects. Total loss of critical DNA repair proteins is often inconsistent with life, but partial loss can result in haploinsufficiency. In mouse models, genetically engineered heterozygosity typically induces phenotypes that are not apparent in the absence of additional disease-modifying factors, characterizing them as conditional haploinsufficiencies. As reviewed in Bartek et al. [5], conditional haploinsufficiency in DNA damage response genes may predispose to malignancy by modifying the penetrance of oncogenic events, primarily as a function of an altered DNA damage threshold. To broaden this concept, haploinsufficiency in DNA repair genes may predispose to human disease by modifying the penetrance of a variety of predisposing factors, including genetics, environmental exposures, and nutrient availability. Consistent with this is the suggestion that even moderate loss of genome maintenance can impact phenotype. Mouse models provide researchers the tools necessary to study the impact of gene dosage on disease risk and DNA damage sensitivity, including chemotherapeutic response.

Mouse models of DNA repair haploinsufficiency

The scope of this review is to evaluate the prevalence of haploinsufficiency in mouse models of DNA repair deficiency. For in-depth explanation of these repair pathways, the reader is referred to comprehensive reviews throughout the text. In addition, the databases listed below provide excellent resources for each repair mechanism. Haploinsufficiency is identified here as any impact of allelic insufficiency on phenotypic expression. In an attempt to identify all heterozygous mouse models of DNA repair deficiency, three primary databases have been used and cross-referenced. The UT Southwestern Medical Center's Mouse Mutation Database (http://pathcuric1.swmed.edu/Research/research.htm) was used to identify mouse models of DNA repair mutants. Mouse mutants for all DNA repair genes within canonical repair pathways were assessed: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), non-homologous end-joining (NHEJ), direct reversal, and genes related to human DNA repair deficiency diseases. As a secondary measure, the "Human DNA Repair Gene" list established by Wood, Mitchell, and Lindahl was used (http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html) to identify human genes and the mouse homologues that may not have been included in the UT Southwestern Medical Center database. Finally, using NCBI's PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), a search for "haploinsufficiency AND [gene name]" for each gene identified by the first two databases was conducted. In total, this search generated 125 gene-targeted DNA repair papers, and 44 of these papers presented data in the heterozygous models. Often when the null mutant is viable the heterozygous model is under-investigated, and therefore phenotypes associated with allelic insufficiency may exist but have not been elucidated. Likewise, data on the heterozygous models are occasionally only available in tissue culture models (mouse embryonic fibroblasts, embryonic stem cells, and lung embryonic fibroblasts, for example), and not in whole animal systems. As such, these data can be used to establish haploinsufficiency, but not to directly establish disease risk. Therefore, this review primarily reports data from whole animal models, but does make reference to some tissue culture data when that is all that is available in the literature. Another complication of these studies is that by virtue of Mendelian birth ratios of heterozygote/heterozygote crosses, heterozygous animals are occasionally used as "wild-type" controls, making it impossible to establish phenotypic differences between heterozygous and wild-type animals. Finally, the distinction between genes directly involved versus indirectly involved in DNA repair can become ambiguous, but an attempt has been made to restrict analysis to those genes directly involved in DNA repair.

For each of the following pathways, summary data can be found in tabular format with further explanation of these data being provided in the text. Mouse mutant papers in which no heterozygous data were presented are not included in this referencing.

Base excision repair

The base excision repair (BER) pathway is responsible for repair of DNA damage arising from spontaneous alkylation, oxidation, deamination, and depurination/depyrimidination events. BER also repairs these same damages that may be induced in response to chemical exposures (reviewed in [6, 7]). Consistent with the predominance of these types of DNA damage, BER is estimated to repair over 1 million nucleotides/cell/day [8]. The overall importance of BER in maintaining genomic stability and preserving embryonic fitness is evidenced in the lethality induced by homozygous deletion of the core BER genes. The BER core genes that are embryonic lethal include: *DNA polymerase β* (*β-pol*), *Apex1*, *Xrcc1*, *Ligase1*, and *Fen1*. Evidence is presented for haploinsufficiency in *β-pol*, *Apex1*, and *Xrcc1* heterozygous models

(Table 1, and discussed below). For *Ligase1* and *Fen1*, no meaningful data on the heterozygous models have been presented in the literature, suggesting that perhaps haploinsufficiency has not been fully investigated in these models. Viable BER homozygous mutant models include: *Parp*, *Myh*, *Aag*, *Polλ*, *Ung*, *Nth1*, and *Ogg1*. For these models, data from the heterozygous models are presented for *Aag*, *Nth1*, *Ogg1*, and *Parp*. Of these there is evidence for haploinsufficiency for *Aag*, *Nth1*, and *Ogg1*. For *Polλ*, *Ung* and *Myh*, no data for the heterozygous models are presented. For *Parp* it appears the heterozygous mouse model presents no phenotype distinct from wild type, though MEFs from the heterozygous mice display haploinsufficiency for Parp activity and chromosomal stability [9]. In total, 26 BER mutant mouse model papers have been analyzed. Of these, 17 demonstrated haploinsufficiency in the following genes: *β-pol*, *Apex1*, *Xrcc1*, *Aag*, *Nth1*, and *Ogg1*.

β-Pol heterozygous model

Young, unexposed/unchallenged animals have a minimal phenotype [10], with the exception of spermatogenic cells in which a twofold increase in spontaneous *LacI* [11] and/or lambda cII mutagenicity [12] is observed. Other tissues show no increase in mutagenicity in the absence of a chemical challenge. However, in response to chemical exposure or aging, heterozygous loss of *β-pol* acts as a significant modifier of penetrance: increased mutagenesis in response to DMS [10]; accelerated strand break accumulation in response to oxidative stress [10] and folate depletion [13]; increased aberrant crypt formation (ACF) in response to DMH [14]; increased liver tumors in response to DMH [14]; accelerated spontaneous tumorigenesis (lymphoma and adenocarcinoma) [15]; and an accelerated rate of aging [15]. This model presents an interesting dichotomy with respect to risk (not unlike several of the

Table 1 Base excision repair

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	References
Polβ	Yes [27]	Single strand break accumulation in response to acute oxidative stress; increased *LacI* mutation frequency in response to DMS; increased spontaneous chromosomal aberrations, specifically centromere separation	[10]
		Heterozygosity intensifies the single strand break phenotype induced by folate depletion	[13]
		Accelerated rate of aging; age-dependent lymphoma incidence increased sevenfold; twofold increase in hypoploidy in lymphcytes.	[15]
		Twofold increase in *LacI* mutagenicity in spermatogenic cells	[11]
		Heterozygosity results in two-fold increase in DMH-induced aberrant crypt foci (ACF). When dietary folate is restricted, wild-type mice accumulate ACF, but heterozygous mice appear to be protected. DMH induces liver tumors in the heterozygous mice, not in the wild-type mice, and not in the folate-restricted heterozygous mice. Heterozygous mouse has reduced apoptosis in response to DMH, but when folate is depleted apoptosis in the heterozygous mouse increases fivefold	[14]
		Conditional targeting to delete Polβ in sperm; both heterozygous and homozygous deletions result in increased lambda cII mutation frequencies; G > T and A > T mutations unique to heterozygous mice	[12]
Apex1	Yes [17]	Sensitivity to oxidative stress; reduced survival of pups and embryos; increased incidence of papillary adenocarcinoma and lymphoma	[17]
		Increased LacI mutagenicity in 3 months old liver and spleen and in 9 months old spermatogenic cells	[18]
		Appear to exhibit increased apoptotic response to acute oxidative stress	[19]
		Double mutant Apex1$^{+/-}$/XPC$^{-/-}$: p53-dependent modifier of penetrance for UV induced skin cancer	[20]
XrccI	Yes [28]	Similar to wild-type phenotype in absence of challenge, though there are mild organ abnormalities; increased liver toxicity and ACF formation following AOM exposure, very similar to Polβ phenotype in response to DMH (metabolite of AOM)	[21]
		Hypomorph: Xrcc1 transgene-complemented null mice. Expression of as little as 10% of transgene permitted embryonic survival as well as growth to adulthood and normal fertility. No differential alkylation sensitivity	[29]
		Hypomorphs exhibit 25% reduction in body weight (decreased body fat) with no differential intake; metabolic effect potentially similar to caloric restriction	[22]
Aag	No [30]	Retinal degeneration	[23]
Nth1	No [25, 26]	Sensitivity to acute oxidative stress is very similar between heterozygous and null mutants, as are thymine glycol and urea accumulation; wild-type data not presented for comparison to heterozygous mouse	[25]
		ES cells from heterozygous and null animals demonstrated a gene-dosage effect on Nth1 activity as het exhibits a 35% reduction below wild type in activity and null cells exhibit no activity	[26]
Ogg1	No [24]	8oxoG:T, 8-oxo and faPy cleavage all reduced in manner consistent with gene-dosage	[24]

other models reviewed, below), in that under some conditions (DMH exposure and aging) heterozygosity increases tumor risk and under other conditions it appears to reduce tumor risk. Under normal dietary conditions, *β-pol* heterozygosity increases the number of DMH-induced ACF, but when the B-vitamin folate is limiting in the diet, *β-pol* heterozygosity protects from these tumors. This appears to be due to a robust apoptotic response when folate is depleted in the heterozygous model, suggesting requirement of intact DNA damage responses [14]. This report is not unlike the protective effect of homozygous *Ku80* deletion on APC^{min} tumorigenesis [16], which is a p53-dependent effect. The protective effect of *β-pol* heterozygosity on DMH-induced ACF likewise seems to be dependent on an intact p53 response as well, as the protective effect is lost in a $p53^{+/-}$ background (personal communication, Ahmad Heydari).

Apex1 heterozygous model

In this model, an effect on health in response to heterozygosity is seen early on as increased sensitivity to oxidative stress [17], increased spontaneous mutagenicity at 3 months (liver and spleen) or 9 months (spermatogenic cells) [18]. This is unlike the *β-pol* heterozygous model in which exposure was required to increase mutagenicity (with the exception of spermatogenic cells). Haploinsufficiency is further evidenced by increased development of lymphoma and adenocarcinoma [17] (as seen in the *β-pol* model as well). Evaluation of the oxidative stress response shows an increased apoptotic response to DNA damage [19], suggesting that heterozygous loss of *Apex1* is also a modifier of penetrance. Further evidence for this is seen in the *Xpc*-null background where *Apex1* does act as a modifier of penetrance for UV-induced skin cancer [20]. As described above for *β-pol* and *Ku80*, this effect is p53-dependent.

Xrcc1 heterozygous model

As with *β-pol* and *Apex1*, there is a definite phenotype in the *Xrcc1* heterozygous model, and one remarkably similar to that seen in the *β-pol* model. Perhaps this is not surprising, considering the structural and functional involvement between the two proteins. Mice exposed to AOM (of which DMH is a metabolite) exhibit a twofold increase in ACF formation [21]. This is identical in the phenotype and magnitude of phenotype to that seen in colon of the *β-pol* heterozygous model, demonstrating that Xrcc1 is, likewise, a modifier of penetrance. The hypomorph Xrcc1 model (transgene-complemented null *Xrcc1*, with ~10% Xrcc1 expression) has a very unique phenotype with respect to metabolism. In spite of similar dietary intakes, the hypomorphs display a 25% reduction in body weight, primarily as a decrease in body fat [22]. It's presently unclear what the mechanism for this metabolic abnormality may be, and is also unclear whether this would be a protective or detrimental effect. Experimental conditions that result in smaller sized animals often exert protective effects with respect to both longevity and cancer predisposition. Caloric restriction, for example, reduces body size, extends lifespan, and delays the onset and progression of cancer. As a potential caloric restriction mimetic, one could suggest the possibility of a protective mechanism in the XRCC1 heterozygote, but the increased ACF formation suggests the opposite. Again, it may be that under some conditions, heterozygosity is beneficial, and under others it is detrimental.

Glycosylase heterozygous models

There are not many data in the literature on heterozygous glycosylase models, primarily because the homozygous mutants are viable and are therefore typically the primary models under investigation. There are, however, some intriguing data suggesting haploinsufficiency for three glycosylases: *Aag*, *Ogg1* and *Nth1*. In the *Aag* heterozygous model there is an increase in the rate of retinal degeneration [23], creating a definite link between haploinsufficiency and disease. In the *Ogg1* heterozygous model, no disease-related phenotypes have been reported, but heterozygosity does result in reduced 8oxoG:T and formamidopyrimidine (FaPy) cleavage [24], providing evidence for repair haploinsufficiency as expected based on gene dosage. Though the Ogg1 data provide no direct link to disease, the allelic insufficiency vis-à-vis repair capacity might be anticipated to modify penetrance of diseases associated with oxidative stress (aging, Alzheimer's disease, cancer, etc.). Unfortunately these questions have not been fully investigated. *Nth1* heterozygous mice have DNA damage profiles and sensitivities very similar to the null mutants, though data from the wild-type animals is not shown so it is difficult to interpret the data [25]. In embryonic stem (ES) cells from wild-type, heterozygous and null mice, Nth1 excision activity is reduced 35% below wild-type activity [26] (zero activity in null ES cells). This is potentially interesting within the context of the roughly equal damage profiles between the heterozygous and null animals, suggesting that 65% residual activity in the heterozygous model is not adequate to resolve the DNA damage.

Nucleotide excision repair

In contrast to BER, nucleotide excision repair (NER) removes helix-distorting lesions from DNA (reviewed in [31, 32]). Much of the damage repaired by NER is induced by ultraviolet (UV) radiation or other environmental toxins

that induce similar bulky lesions in the DNA. Human diseases traced to genetic defects in the ability to repair these types of DNA damage include Xeroderma pigmentosum, Cockayne syndrome and trichothiodytsrophy, and the genes involved in this repair are the complement of NER genes. The NER genes whose loss results in embryonic lethality include: *Xpd*, *Ddb1*, and *Hr23b* (90% of embryos die in utero). *Xpa*, *Xpc*, *Csa*, *Csb*, *Ercc1*, *Xpg*, and *Xpf* are all viable homozygous mutant models, though *Ercc1*, *Xpg*, and *Xpf* homozygotes die before weaning. In total, 15 NER mutant mouse model papers representing 11 NER genes have been analyzed. In ten of these papers the heterozygous models failed to exhibit any phenotype. In five of these papers representing three genes (*Xpc*, *Xpa*, and *Ercc1*) a phenotype was observed for the heterozygous models (Table 2).

Xpc heterozygous model

Xpc heterozygosity increases UVB radiation-induced skin cancers in a gene-dosage manner. Median survival following UVB irradiaion for the null model, heterozygous model and wild-type model is 25, 50, and nearly 100 weeks, respectively [33]. In addition, *Xpc* heterozygosity slightly accelerates UVB irradiation-induced skin cancers in $p53^{+/-}$ mice and does so without promoting loss of heterozygosity (LOH) [33]. Oddly, *Apex* heterozygosity appears to modify this Xpc effect on skin tumors, once again suggesting that the specific experimental and genetic conditions are critical factors determining disease risk [33]. Uehara et al. investigated *LacZ* mutagenesis in liver, spleen, heart, and lung of the *Xpc* null and heterozygous models, and found that in liver and spleen there is a definite impact of heterozygosity on spontaneous, age-dependent mutagenesis [34]. While at weaning mutation levels are essentially identical in all three *Xpc* models, over time the differences from wild type grow such that by 24 months of age the mutation frequency in the heterozygous *Xpc* mice is three- to fourfold higher in spleen and two- to threefold higher in liver as compared to wild type. Thus, data presented for the *Xpc* heterozygote demonstrate not only a phenotype of increased disease risk (skin cancer), but also point to mechanism (mutation accumulation). This is not an unanticipated finding, but is rare in that only infrequently are heterozygous phenotypes investigated when the homozygous model is viable.

Xpa heterozygous model

The case for haploinsufficiency for *Xpa* is not very strong, but there is some evidence of a mild phenotype in the heterozygote. While unscheduled DNA synthesis (UDS) is not different between wild-type and heterozygous genotypes, mouse embryonic fibroblasts (MEFs) from these animals do exhibit very slight differences in UV sensitivity at high doses (wild-type and heterozygous survival diverges at 6 J m^{-2}) [35]. Contrarily, survival in response to DMBA does not differ between the genotypes. In spite of no effect of genotype on survival in response to DMBA, there does appear to be a $\sim$10% difference in DMBA-induced papilloma incidence between the wild-type and heterozygous genotypes [35].

Ercc1 heterozygous model

McWhir et al. show that MEFs from the *Ercc1* heterozygous model have a slight decrease in survival following UV exposure, but nothing compared to the dramatic impact of homozygous loss on UV sensitivity. Interestingly, at low doses of UV exposure, wild-type and heterozygous MEFs

Table 2 Nucleotide excision repair

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	Referencees
Xpc	No [38]	Increased predisposition to UVB radiation-induced skin cancers; heterozygosity accelerates p53-dependent cancers	[33]
		Increased *LacZ* mutagenesis in liver and spleen, intermediate between null and wild type; with age, mutagenicity increases three to fourfold more in heterozygous than in wild type in spleen and two to threefold more in heterozygous than in wild type in liver	[34]
Xpa	No [35]	Appears to be a slight differences in survival in response to UV (but not DMBA) and an approximate 10% difference in the number of papillomas induced following DMBA as compared to wild type (in null mutant, it is a 60% difference from wild type)	[35]
Ercc1	No, but died before weaning [36]	Following UV exposure there appears to be a slight decrease in survival in the heterozygous mouse, though very mild compared to effect in null mutant. With increasing doses of UV, the heterozygous mice show a slight attenuation in incision activity (null mutants have essentially no incision acitivity)	[36]
		Ercc1$^{-/m}$: one knockout allele, one mutant allele. This extends lifespan of the null mutant approximately 6 months; *LacZ* mutagenicity approximately threefold higher in liver compared to wild type	[37]

exhibit similar incision activity (i.e., repair response), but as the UV dose increases, the heterozygous MEFs are not able to maintain the level of repair induced in wild-type MEFs [36]. In spite of this repair haploinsufficiency, there does not appear to be a difference in tumor formation between wild-type and heterozygous models in response to UVB irradiation [36]. These data suggest that the repair insufficiency may not extend to the critical disease phenotype. However, in the *Ercc1* model in which one allele is disrupted and the other is mutated (*Ercc1*$^{-/m}$), *LacZ* mutation frequency is increased approximately threefold as compared to wild type [37], supporting the likelihood of haploinsufficiency for *Ercc1* and a role for its haploinsufficiency in tumorigenesis.

Mismatch repair

Mismatch repair is the cell's mechanism for resolving replication errors (reviewed in [39, 40]). Loss of the ability to repair mismatches results in a phenotype of microsatellite instability (MSI), strongly associated with HNPCC (hereditary non-polyposis colorectal cancer). In total, 19 mismatch repair knockout papers have been analyzed covering the following genes: *Msh2, Mlh1, Pms1, Pms2, Msh6, Msh3, Msh4, and Msh5*. The null mutant for each of these genes is viable. As such, several papers present no data at all on the heterozygous models, including *Msh3, Msh4, Msh5*, and *Pms1*. However six papers, representing *Msh2, Mlh1, Pms2*, and *Msh6* do provide data on the heterozygous model and, accordingly support a role for haploinsufficiency within these genes (Table 3).

Msh 2 heterozygous model

De Wind et al. show no effect of *Msh2* heterozygosity on tolerance to MNNG, suggesting that the heterozygous model might exhibit a wild-type phenotype [41]. In their later work, however, they demonstrate clearly that Msh2 is a strong modifier of penetrance in the APC^{min} model of intestinal tumorigenesis. While there was no difference in survival between the heterozygous and wild-type models in an APC^{min} background, approximately 40% more tumors developed in the *Msh2* heterozygous animals as compared to the wild-type animals [42]. Further, only ~10% of these tumors exhibited loss of residual MMR [42], suggesting that loss of heterozygosity (LOH) was not the mechanism by which tumor incidence was accelerated by *Msh2* heterozygosity. These data strongly link allelic insufficiency for Msh2 with tumor incidence. These two reports reinforce the issue that baseline phenotypic differences between wild-type and heterozygous models are not always present, but that when challenged the loss of repair capacity can increase damage loads, mutagenesis, and tumorigenesis.

Mlh1 heterozygous model

Edelmann et al. clearly demonstrate that heterozygous loss of *Mlh1* results in a loss of repair activity that follows a gene-dosage response. They show that G:G mismatch is repaired 50% less efficiently in the heterozygous animal than in the wild-type and twofold more than in the null mutant [43]. In a follow-up analysis of survival, they show a survival curve in which survival likewise follows gene-dosage, with a mean lifespan at approximately 6, 16, and over 24 months in the null, heterozygous and wild-type models, respectively [44]. This lifespan study did not continue on to evaluate maximum lifespan in the heterozygous and wild-type models (it was ~13 months in the null mutant), but the curves strongly suggest a significant difference in maximum lifespan as well. Further, there are more gastrointestinal and extra-gastrointestinal tumors in the heterozygous animals than in the wild-type animals.

Table 3 Mismatch repair

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	References
Msh2	No [41]	Double mutant: Msh/APC^{min}. Survival is similar between heterozygous and wild-type mice, but ~40% more tumors develop in the heterozygous model. Further, only about 10% of these display loss of residual MMR activity, suggesting LOH of the wild-type allele is not responsible for tumorigenesis in the heterozygous mice	[42]
Mlh1	No [43, 48]	Mismatch repair activity follows gene dosage: G:G mismatch repaired 50% less in heterozygote than in wild type but twofold greater in heterozygote compared to null mutant	[43]
		Gene dosage effect on both mean and maximum lifespan, with heterozygote falling between wild-type and null mutant; increased incidence of tumors (both gastrointestinal and extra-gastrointestinal) over wild type	[44]
		MNU-induced tumor data analyzed on Mgmt null background; Mlh1 heterozygous mice have fewer tumors than Mlh1 null mice; no Mlh1 wild-type data presented in order to determine whether Mlh1 heterozygosity is a modifier of penetrance	[45]
Pms2	No [49]	Increased intestinal adenomas in APCmin mouse in a gene dosage manner	[46]
Msh6	No [47]	Increased tumor incidence; haploinsufficiency for repair of 5′G:G, 3′C:A mispairs, and 1 and 4 nucleotide ins/del mismatches	[47]

Interestingly, addition of the APC mutation did not impact the tumor profile of the *Mlh1* heterozygous mouse, so it does not modify the penetrance of APC mutations [44]. Takagi et al. used lung embryonic fibroblasts from null and heterozygous *Mlh1* animals and found that loss of Mlh1 rescued MNU killing in *Mgmt* mutant cells [45], another of the counterintuitive findings. The authors also investigated mutant frequencies and tumor formation in response to MNU on an *Mgmt* null background, but no *Mlh1* wild-type controls were used, so the ability of Mlh1 to modify the impact of MNU under these conditions cannot be evaluated.

Pms2 heterozygous model

Baker et al. analyzed the impact of Pms2 on tumor formation in the APC^{min} model. They found that the number of intestinal ademonas in the APC^{min} mouse was 72, 90, abd 270 in the *Pms2* wild-type, heterozygous and null models, respectively [46]. These data support haploinsufficiency for *Pms2*, again as a modifier of penetrance.

Msh6 heterozygous model

Edelmann et al. established an Msh6 mouse model in which they evaluated the impact of Msh6 gene dosage on repair capacity, survival, and tumor formation. There is clear haploinsufficiency for repair of G:G mismatches with the strand-specifying nick 5′ of the lesion, C:A mismatches with the nick 3′ of the lesion, and 1 and 4 nucleotide insertion/deletion mismatches [47]. In addition, survival curves suggest there may be subtle differences between wild-type and heterozygous models with respect to survival, but the curves were only extended to 16 months. This was well short of both the mean and maximum lifespans for wild-type and heterozygous mice, so no conclusions about lifespan in the heterozygous model can be drawn. However, there is a clear impact of heterozygosity on tumor development. By 20 months of age, no tumors had been identified in the wild-type animals. In the heterozygous animals NHL, carcinomas, microadenomas, B cell lymphomas, and dermatofibromas were identified, and had developed starting at about 8 months of age [47]. Clearly, heterozygous loss of *Msh6* induces a cancer phenotype, supporting a role for Msh6 haploinsufficiency in disease risk.

Homologous recombination

Canonical homologous recombination (HR) is a pathway for repair of DNA double strand breaks that utilizes either the homologous chromosome or the sister chromatid to promote essentially error-free repair (reviewed in [50]). Permutations of HR such as non-allelic homologous recombination and/or break-induced replication may promote survival in response to double strand breaks at the expense of fidelity, and require further investigation in mammalian models. Gene-targeted mutants for core genes within the HR pathway have been developed for *Rad52, Rad52, Rad51l1, Rad51l3, Rad50, Xrcc2,* and *Rad21*. Biallelic disruption of *Rad51, Rad51l1, Rad51l3, Rad50, Xrcc2,* and *Rad21* is embryonic lethal. The original papers describing *Rad51, Rad52, and Rad50* do not present any data on the heterozygous models. The original papers describing *Rad51l1* and *Rad51l3* present data on the heterozygous models, but identify no phenotypes associated with monoallelic disruption of the genes. Only *Rad21* and *Xrcc2* mutations generated heterozygous data consistent with haploinsufficiency (Table 4). In total, 14 papers were analyzed, and only 2 of these papers support haploinsufficiency for HR, keeping in mind that phenotypes in heterozygous models were not investigated for most of the HR mutants (Table 5).

Rad21 heterozygous mutant

Homozygous loss of *Rad21* is lethal [51], but the heterozygous model has been well characterized. The heterozygous model exhibits reduced HR and SCE (sister chromatid exchange) [51], demonstrating haploinsufficiency for repair capacity and suggesting increased disease risk in response to exposures that induce DNA double strand breaks. In support, the heterozygous mice are more sensitive to whole body irradiation, and they experience gastrointestinal hypersensitivity in response to ionizing radiation [51]. Not only do these data suggest increased cancer risk, they also point to important clinical considerations for haploinsufficient individuals undergoing radiation treatment. These animals also display impaired bone marrow stem cell regeneration [51].

Xrcc2 heterozygous mutant

Deans et al. established ES cells from *Xrcc2* wild-type, heterozygous and homozygous embryos, and found no difference between wild-type and heterozygous cells with respect to either gamma-irradiation sensitivity or chromosomal aberrations [52], suggesting a lack of haploinsufficiency for *Xrcc2*. However, when crossed with the APCmin mouse model, Haines et al. found that Xrcc2 modifies the impact of APC mutagenesis [53]. These data are interesting, though not inconsistent with the above described studies, in that the effect of *Xrcc2* gene dosage (i.e., protective or detrimental) depends upon the specific experimental conditions. *Xrcc2* heterozygosity protects from the spontaneous intestinal tumorigenesis induced by the APC mutation, but enhances IR-induced tumorigenicity in this same APC model [53]. It is difficult to reconcile such data without further experimental evidence. It is important to note that heterozygosity does increase LOH, so

Table 4 Homologous recombination

Gene	$^{-/-}$lethal?	Heterozygous phenotype	References
Rad21	Yes [51]	Reduced homologous recombination and sister chromatid exchange (SCE); increased whole body IR sensitivity; gastrointestinal hypersensitivity to IR; bone marrow stem cell clonogenic regeneration reduced	[51]
Xrcc2	Yes [52]	Double mutant APC^{min}/Xrcc2. Xrcc2 heterozygosity protects from spontaneous tumorigenesis but increases IR-induced tumors; heterozygosity increases LOH	[53]

Table 5 Non-homologous end-joining

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	References
Ku70	No [55, 57]	Data in mouse embryonic fibroblasts (MEFs) only. Looks like there may be a trend towards an impact of heterozygosity on senescence and cell cycle regulation	[55]
DNA-PKcs	No [56, 58]	Data in MEFs only. Looks like there may be a slight increase in IR sensitivity	[57]

the phenotypes observed may not be due to haploinsufficiency.

Non-homologous end-joining

Non-homologous end-joining (NHEJ) and HR work in coordination through the cell cycle to resolve DNA double strand breaks (reviewed in [50]). Loss of NHEJ results in immunodeficiency as a function of its critical role in V(D)J recombination (reviewed in [54]). NHEJ functions to repair DNA double strand breaks arising in response to exposure during resting and early synthesis phases of the cell cycle (when sister chromatids are not available for use as repair templates), and during maturation of B and T lymphocytes. Mice with targeted deletions in *Ku80, Ku70, Ligase IV, Xrcc4*, and *DNA-PKcs* have been developed, as well as a combined *Ku80/Ku70* model. Of these, *Ligase IV* and *Xrcc4* are embryonic lethal. The null models exhibit the following phenotypes: severe immune deficiency, exhibiting aberrant formation of coding joints and/or signal joint formation, inability to form mature B cells, and/or inability to conduct V(D)J recombination; double strand break repair deficiency; and severely runted size. In nearly all these models the heterozygous models are used as the control against the null mutants. This makes it impossible to identify phenotypes in the heterozygous model distinct from wild-type models. In total, 12 gene-targeting papers have been analyzed, and in only 2 of these studies is there even a slight indication that the heterozygous models may exhibit a phenotype. At that, these data are only presented for the MEFs derived from the Ku70 and DNA-PKcs embryos. The data in these papers suggest, at best, only a very mild phenotype in the heterozygous cells.

Ku70 heterozygous model

While the null mutant MEFs exhibit a robust early senescence phenotype, data in the heterozygous MEFs suggest a mild senescence phenotype relative to the wild-type cells [55]. There may also be subtle differences in cell cycle regulation in the heterozygous cells, but it is not possible to quantify or evaluate statistical significance. This does not rule out the possibility of haploinsufficiency for Ku70, but does indicate that more robust assessment would be necessary to establish this impact.

DNA-PKcs heterozygous model

As with the Ku70 MEFs, there is no dominant phenotype apparent in the DNA-PKcs heterozygous cells. There may be a slightly increased IR sensitivity [56], but as above, it is not possible to evaluate the statistical significance of this observation.

Direct reversal

Direct reversal is a mechanism for repairing DNA damage without compromising the phosphodiester backbone (reviewed in [59]). In direct reversal a suicide enzyme transfers the alkyl group (i.e., the damage) on the DNA base to its own cysteine residue, all without disrupting the DNA structure. There are very few genes with these specificities, and they include *O^6-methylguanine DNA-methyltransferase* (*Mgmt*) and the *AlkB* homologues. Mgmt removes alkylation damage to guanine and thymine, thus preventing mispairing and mutation fixation that could arise during replication over the damaged bases. The *AlkB* homologues directly remove 1-methyladenine and 3-methylcytosine from the DNA. There are four mouse models for direct reversal, and biallelic loss is tolerated in all four (*Mgmt, mAlkb1, mAlkb2, mAlkb3*), though the *mAlkb1* model exhibits skewed Mendelian ratios. Two of the four papers presented no data on the heterozygous models, while two present evidence of haploinsufficiency for both *Mgmt* and *mAlkb1* (Table 6).

Table 6 Direct reversal

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	References
Mgmt	No [61]	MEFs only. Methyltransferase activity consistent with gene dosage; sensitivity to BCNU, MNNG, STZ, and temozolomide follow gene dosage, with heterozygous mutant cells intermediate between wild-type and null mutant sensitivities	[59]
Alkb1	No [62]	One female born to every 3-4 male pups in heterozygous and null mutants; both heterozygous and null mutants display exencephaly, but phenotype in heterozygote is milder	[60]

Mgmt heterozygous model

Data presented from liver tissue, ES cells, and MEFs from the wild-type, heterozygous, and null *Mgmt* models clearly demonstrate a gene dosage effect on methyltransferase activity [59], establishing haploinsufficiency for repair capacity. In addition, the MEFs are sensitive to BCNU, MNNG, STZ, and temozolomide in a gene-dosage manner [59], such that allelic insufficiency for *Mgmt* would be anticipated to modify risk in response to environmental exposure.

mAlkb1 heterozygous model

Both heterozygous and null mAlkb1 models are born at reduced Mendelian ratios with a strong sex selection against females [60]. Both mutants exhibit exencephaly, evident at E14.5, but the extent of exencephaly and the extent of runting in the null model are substantially more severe than in the heterozygous model [60]. This establishes that haploinsufficiency exists and that gene dosage has an important impact on severity of phenotype.

DNA repair genes associated with human disease

Several genes have been identified as repair genes by virtue of their association with DNA damage intolerance as a phenotype of specific human diseases. These include the BRCA genes, the Fanconi anemia genes, the helicases associated with Werner, Bloom, and Rothmund-Thomson syndromes, as well as genes associated with ataxia telengiectasia. Thirty-seven papers reporting on gene-targeted mutations for the following genes were reviewed*: Brca1, Brca2, Fanca, Fancc, Fancd2, Fancg, Fancl, Fancm, Fancn, Fancp, Fanca/c, Wrn, Blm, Atm, and Atr*. Ten of these papers present data consistent with haploinsufficiency for *Brca1, Brca2, Fancd2, Fancm, Fancp, Wrn, Blm*, and *Atm* (Table 7).

Table 7 Human disease-related DNA repair genes

Gene	$^{-/-}$Lethal?	Heterozygous phenotype	References
Wrn	No [79]	Heterozygous data in ES cells only. Sensitivity to 6-thioguanine, etoposide and camptothecin follows gene dosage	[79]
Blm	Yes [80]	Heterozygous data in MEFs only. Growth of MEFs intermediate between wild-type and null mutant. SCE not different between wild-type and heterozygote	[80]
Atm	No [81]	The number of thymocytes in heterozygotes is intermediate between wild-type and null mutant	[81]
Atr	Yes [83]	Heterozygous Atr mice do not display phenotypes distinct from wild type; but on a Mlh null background, Atr haploinsufficiency accelerates development of tumors	[82]
Fancd2	No [75]	Some tumor incidence with age, but does not appear to be statistically different from age-dependent tumors in wild-type mice	[75]
Fancm	No [76]	SCE in response to MNNG is similar between heterozygous and wild-type mice, and endpoint survival data are not statistically different; slopes of overall and tumor-free survival appear very different between wild-type and heterozygotes	[76]
Fancp	No, but born at sub-mendelian ratios [77]	AKA: Slx4/Btbd12. Body weight effect of heterozygosity consistent with gene dosage effect. Heterozygous mice intermediate in weight between wild-type and null mutant	[77]
Brca1	Yes [66, 67]	Shortened lifespan; 70% tumor incidence; tumors retain wild-type allele; increased IR sensitivity	[70]
		MEFs only: increased radiation-induced transformation and increased apoptosis	[84]
Brca2	Yes [68, 69]	MEFs only, truncated exon11 mutant. Appears to be slight increase in sensitivity to UV, x-ray, and MMS. No apparent proliferation phenotype	[71]

Haploinsufficiency in BRCA genes

The BRCA genes were discovered through their association with inherited breast cancer. Through interaction with Rad51 (a homologous recombination gene), the BRCA genes have been linked to DNA repair, and BRCA1- and BRCA2-deficient cells have been shown to be HR deficient [63, 64]. In human breast and ovarian cancer predisposition, it has generally been understood that loss of heterozygosity (LOH) of the wild-type allele was required for expression of the cancer phenotype. However, King et al. [65] have demonstrated that heterozygosity of either BRCA1 or BRCA2 may be sufficient to increase breast cancer risk. In mouse, biallelic disruption of both *Brca1* [66, 67] and *Brca2* [68, 69] is embryonic lethal. The *Brca1* heterozygous mice appear normal and are fertile up to at least 10 months, but female heterozygous animals exhibit a shortened lifespan and a 70% tumor incidence [70]. Importantly these tumors did not undergo LOH. The heterozygous animals exhibited increased IR sensitivity [70]. This is highly relevant with respect to development of secondary cancers following radiation treatment for BRCA carriers treated for breast cancer. In MEFs derived from the heterozygous animals, increased radiation-induced transformation and increased apoptosis were observed.

For *Brca2*, no mouse model studies have been published. However in MEFs, a slight increase in sensitivity to UV, X-ray, and MMS is observed [71]. And in a human study of transformed lymphocytes from familial heterozygous carriers, a significant impact of heterozygosity is seen with respect to SCE [72], demonstrating haploinsufficiency in a relevant human model of *BRCA2* allelic insufficiency.

Haploinsufficiency in Fanc genes

In the example of Fanconi anemia, the clinical features of the disorder suggested a DNA repair defect. Not all the Fanconi anemia (FANC) genes are directly associated with DNA repair, but in general the pathway plays a role in interstrand crosslink (ICL) repair and perhaps in HR through interaction with BRCA genes (reviewed in [73]). Biallelic disruption of the Fanc genes is generally tolerated, with the exception of *Fancn* (*Palb2*), which is lethal [74]. Additionally, *Fancp* is viable, but homozygous mutants are born at sub-Mendelian ratios. Only three of the gene-targeting papers for the Fanc genes report a phenotype for the heterozygous mutants: *Fancd2, Fancm, and Fancp*. Some age-dependent tumors are observed in the *Fancd2* heterozygous animals, but it does not seem that incidence in the heterzygotes differs from that in the wild-type animals [75]. So, while there are some data in the heterozygotes, the case for haploinsufficiency is weak. In the *Fancm* model, SCE rates are similar between wild-type and heterozygous animals, but survival curves in response to MNNG are very different between the genotypes [76]. Though the endpoint survival data are not significantly different between these genotypes, there is a definite trend toward reduced survival in the heterozygous animals. Further, early on in survival the heterozygous animals appear to be protected, resulting in survival curves with very different slopes [76]. These data suggest there is a potentially important difference in the mortality rate between the genotypes, similar to what is observed in the β-pol heterozygous model. The *Fancp* heterozygous animals express phenotypes with respect to body weight and hydrocephalus such that each is intermediate in severity between the wild-type and homozygous genotypes [77], consistent with gene dosage response.

Haploinsufficiency in Wrn, Blm, Atm, and ATR

RecQ helicases are involved in Bloom, Werner, and Rothmund-Thomson syndromes (reviewed in [1]). A role for these genes in DNA repair was suggested by the chromosomal instability observed in these syndromes. Only the helicases responsible for Bloom and Werner (Blm and Wrn) show evidence of haploinsufficiency. In a distinct human disease, ataxia telangiectasia (AT), severe chromosomal instability and cancer predisposition are likewise observed (reviewed in [78]). While cells from AT patients exhibit signs of DNA repair deficiency, the role in DNA repair may be more indirect, as sensors of DNA damage more than effectors. Historically, however, ATM and ATR have been considered within the context of DNA repair (by virtue perhaps of radioresistant DNA synthesis) and have been included here. In addition, heterozygous mutations in human ATR (ataxia telangiectasia and Rad3-related) have been observed in tumors displaying microsatellite instability, the type of tumors observed in HNPCC (discussed above). In total, eight papers have been analyzed (1 Wrn, 2 Blm, 1 Recql4, 3 Atm, and 1 Atr). Of these, only four presented data consistent with possible haploinsufficiency for *Wrn, Blm*, and *Atm*. Often, as we saw above, the heterozygous animals were grouped with the wild type and used as control against the null mutants. With respect to viability, homozygous deletion of *Wrn* results in reduced embryonic survival [79]; *Blm* is lethal [80]; and Atm nullizygous animals are viable [81]. The case for haploinsufficiency for these genes is not particularly strong. For *Blm* [80] and *Atm* [81] the heterozygous animals exhibit some mild phenotypes intermediate between wild-type and null genotypes. But the critical phenotypes (SCE for *Blm* and double strand breaks for *Atm*) do not seem to be impacted by heterozygosity. The case for haploinsufficiency for *Wrn* is stronger, as ES cells from the heterozygous animals show intermediate sensitivity to

6-thioguanine, etoposide, and camptothecin [79], suggesting an increased susceptibility in heterozygous genotypes. Atr, which is embryonic lethal for the nullizygous mutants, does not display haploinsufficiency, but the heterozygotes do accelerate development of tumors on an Mlh-null background [82]. These data are consistent with a modification of penetrance, which could be more fully investigated in the heterozygous model.

Summary

This collection of data on heterozygous mutants demonstrates a clear role for haploinsufficiency in DNA damage sensitivity and genomic instability—phenotypes that can be expected to modify penetrance of exposure and/or additional genotype effects. From this analysis, 27 genes across DNA repair pathways have been identified as exhibiting some phenotype in the heterozygous animals. The association between haploinsufficiency in these genes and disease is summarized in Table 8. Haploinsufficiency in the following ten genes showed increased tumorigenesis: *β-pol, Apex1, Xpc, Xpa, Msh2, Mlh1, Pms2, Msh6, Xrcc2*, and *Brca1*. For essentially all cases of haploinsufficiency, evidence for genomic instability exists (and phenotypes consistent with increased cancer predisposition), but studies were not always carried out to investigate tumorigenesis. For the 27 genes demonstrating haploinsufficiency, 21 clearly demonstrate an impact of heterozygosity on damage sensitivity and/or genomic instability. While the impact of increased DNA damage sensitivity on cancer predisposition and aging is obvious, these phenotypes can impact many other factors impacting health. Much research is currently underway investigating the role of DNA repair capacity on drug sensitivity and chemotherapeutic response. Much of this work has implicated

Table 8 Disease phenotypes in DNA repair haploinsufficient mouse models

Pathway	Gene	Cancer (specified by type)	Aging (accelerated)	Other disease	Modifier of penetrance
BER	*β*-pol	Lymphoma, adenocarcinoma [15]	X [15]	–	–
	Apex1	Lymphoma, adenocarcinoma [17]	–	–	Modifies XPC-dependent UV-induced skin cancer [20]
	Aag	–	–	Retinal degeneration [23]	–
NER	Xpc	UVB-induced skin cancer [33]	–	–	Modifies p53-dependent UV-induced skin cancer [33]
	Xpa	DMBA-induced papillomas [35]	–	–	–
MMR	Msh2	–	–	–	Modifies tumorigenesis in APC^{min} model [42]
	Mlh1	Gastrointestinal and non-gastrointestinal tumors [44]	X [44]	–	–
	Pms2	–	–	–	Modifies tumorigenesis in APC^{min} model [46]
	Msh6	Carcinoma, lymphoma [47]	–	–	–
HR	Rad21	–	–	Gastrointestinal sensitivity following irradiation [51]	–
	Xrcc2	–	–	–	Increases tumorigenesis in response to IR; decreases tumroigenesis in APC^{min} spontantous tumorigenesis [53]
DR	mAlkb1	–	–	Exancephaly [60]	–
BRCA	Brca1	Ovarian tumors [70]	X [70]	–	–
FANC	Fancm	–	X [76]	–	–
	Fancp	–	–	Hydrocephalus [77]	–

BER base excision repair, *NER* nucleotide excision repair, *MMR* mismatch repair, *HR* homologous recombination, *DR* direct reversal

A check mark is used to indicate whether there is any phenotype of accelerated aging

– indicates that no phenotype has been reported in the heterozygous mouse model. Modifier of penetrance is only marked here if a disease is associated with the haploinsufficiency

DNA repair as a therapeutic target, suggesting that repair capacity (i.e., haploinsufficiency in repair) should be a critical factor in determining treatment options. Perhaps one of the most useful observations made in this analysis is that the impact of allelic insufficiency may depend upon external factors, such as other genetic predispositions, exposures and/or nutritional status. There is not always an absolute effect of haploinsufficiency, not even within the same gene. Rather, allelic insufficiency is able to modify the penetrance of other predisposing factors.

Acknowldgements This work is supported by a grant from the Ellison Medical Foundation [DCC].

References

1. Singh DK, Ahn B, Bohr VA (2009) Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. Biogerontology 10:235–252
2. Cleaver JE, Revet I (2008) Clinical implications of the basic defects in Cockayne syndrome and xeroderma pigmentosum and the DNA lesions responsible for cancer, neurodegeneration and aging. Mech Ageing Dev 129:492–497
3. Tischkowitz M, Winqvist R (2011) Using mouse models to investigate the biological and physiological consequences of defects in the Fanconi anaemia/breast cancer DNA repair signalling pathway. J Pathol 224:301–305
4. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif B, Shaffer LG, Eichler EE (2011) A copy number variation morbidity map of developmental delay. Nat Genet 43:838–846
5. Bartek J, Lukas J, Bartkova J (2007) DNA damage response as an anti-cancer barrier: damage threshold and the concept of 'conditional haploinsufficiency'. Cell Cycle 6:2344–2347
6. Wilson SH, Kunkel TA (2000) Passing the baton in base excision repair. Nat Str Biol 7:176–178
7. Barnes DE, Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu Rev Genet 38:445–476
8. Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci USA 96:13300–13305
9. Kanai M, Tong W-M, Want Z-Q, Miwa M (2007) Haploinsufficiency of poly(ADP-ribose) polymerase-1-mediated poly(ADP-ribosyl)ation for centrosome duplication. Biochem Biophys Res Comm 359:426–430
10. Cabelof DC, Guo Z, Raffoul JJ, Sobol RW, Wilson SH, Richardson A, Heydari AR (2003) Base excision repair deficiency caused by polymerase beta haploinsufficiency: accelerated DNA damage and increased mutational response to carcinogens. Cancer Res 63:5799–5807
11. Allen D, Herbert DC, McMahan CA, Rotrekl V, Sobol RW, Wilson SH, Walter CA (2008) Mutagenesis is elevated in male germ cells obtained from DNA polymerase-beta heterozygous mice. Biol Reprod 79:824–831
12. Kidane D, Dalal S, Keh A, Liu Y, Zelterman D, Sweasy JB (2011) DNA polymerase beta is critical for genomic stability of sperm cells. DNA Repair (Amst) 10:390–397
13. Cabelof DC, Raffoul JJ, Nakamura J, Kapoor D, Abdalla H, Heydari AR (2004) Imbalanced base excision repair in response to folate deficiency is accelerated by polymerase beta haploinsufficiency. J Biol Chem 279:36504–36513
14. Ventrella-Lucente LF, Unnikrishnan A, Pilling AB, Patel HV, Kushwaha D, Dombkowski AA, Schmelz EM, Cabelof DC, Heydari AR (2010) Folate deficiency provides protection against colon carcinogenesis in DNA polymerase beta haploinsufficient mice. J Biol Chem 285:19246–19258
15. Cabelof DC, Ikeno Y, Nyska A, Busuttil RA, Anyangwe N, Vijg J, Matherly LH, Tucker JD, Wilson SH, Richardson A, Heydari AR (2006) Haploinsufficiency in DNA polymerase beta increases cancer risk with age and alters mortality rate. Cancer Res 66:7460–7465
16. Holcomb VB, Rodier F, Choi Y, Busuttil RA, Vogel H, Vijg J, Campisi J, Hasty P (2008) Ku80 deletion suppresses spontaneous tumors and induces a p53-mediated DNA damage response. Cancer Res 68:9497–9502
17. Meira LB, Devaraj S, Kisby GE, Burns DK, Daniel RL, Hammer RE, Grundy S, Jialal I, Friedberg EC (2001) Heterozygosity for the mouse Apex gene results in phenotypes associated with oxidative stress. Cancer Res 61:5552–5557
18. Huamani J, McMahan CA, Herbert DC, Reddick R, McCarrey JR, MacInnes MI, Chen DJ, Walter CA (2004) Spontaneous mutagenesis is enhanced in Apex heterozygous mice. Mol Cell Biol 24:8145–8153
19. Unnikrishnan A, Prychitko TM, Patel HV, Chowdhury ME, Pilling AB, Ventrella-Lucente LF, Papakonstantinou EV, Cabelof DC, Heydari AR (2011) Folate deficiency regulates expression of DNA polymerase β in response to oxidative stress. Free Radic Biol Med 50:270–280
20. Meira LB, Cheo DL, Hammer RE, Burns DK, Reis A, Friedberg EC (1997) Genetic interaction between HAP1/REF-1 and p53. Nat Genet 17:145
21. McNeill DR, Lin P-C, Miller MG, Pistell PJ, de Souza-Pinto NC, Fishbein KW, Spencer RG, Liu Y, Pettan-Brewer C, Ladiges WC, Wilson DM III (2011) XRCC1 haploinsufficiency in mice has little effect on aging, but adversely modifies exposure-dependent susceptibility. Nucleic Acids Res (in press)
22. Ladiges WC (2006) Mouse models of XRCC1 DNA repair polymorphisms and cancer. Oncogene 25:1612–1619
23. Meira LB, Moroski-Erkul CA, Green SL, Calvo JA, Bronson RT, Shah D, Samson LD (2009) Aag-initiated base excision repair drives alkylation-induced retinal degeneration in mice. Proc Natl Acad Sci USA 106:888–893
24. Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci USA 96:13300–13305
25. Takao M, Kanno S, Shiromoto T, Hasegawa R, Ide H, Ikeda S, Sarker AH, Seki S, Xing JZ, Le XC, Weinfeld M, Kobayashi K, Miyazaki J, Muijtjens M, Hoeijmakers JH, van der Horst G, Yasui A (2002) Novel nuclear and mitochondrial glycosylases revealed by disruption of the mouse Nth1 gene encoding an endonuclease III homolog for repair of thymine glycols. EMBO J 21:3486–3493
26. Ocampo MT, Chaung W, Marenstein DR, Chan MK, Altamirano A, Basu AK, Boorstein RJ, Cunningham RP, Teebor GW (2002) Targeted deletion of mNth1 reveals a novel DNA repair enzyme activity. Mol Cell Biol 22:6111–6121
27. Sobol RW, Horton JK, Kühn R, Gu H, Singhal RK, Prasad R, Rajewsky K, Wilson SH (1996) Requirement of mammalian

DNA polymerase-beta in base-excision repair. Nature 379:183–186
28. Tebbs RS, Flannery ML, Meneses JJ, Hartmann A, Tucker JD, Thompson LH, Cleaver JE, Pedersen RA (1999) Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. Dev Biol 208:513–529
29. Tebbs RS, Thompson LH, Cleaver JE (2003) Rescue of Xrcc1 knockout mouse embryo. DNA Repair (Amst) 2:1405–1417
30. Engelward BP, Weeda G, Wyatt MD, Broekhof JL, de Wit J, Donker I, Allan JM, Gold B, Hoeijmakers JH, Samson LD (1997) Base excision repair deficient mice lacking the Aag alkyladenine DNA glycosylase. Proc Natl Acad Sci USA 94:13087–13092
31. Friedberg EC (2001) How nucleotide excision repair protects against cancer. Nat Rev 1:22–33
32. Wood RW (2010) Nucleotide excision repair proteins and interstrand crosslink repair. Environ Mol Mutagen 51:520–526
33. Cheo DL, Ruven HJ, Meira LB, Hammer RE, Burns DK, Tappe NJ, van Zeeland AA, Mullenders LH, Friedberg EC (1997) Characterization of defective nucleotide excision repair in XPC mutant mice. Mutat Res 374:1–9
34. Uehara Y, Ikehata H, Furuya M, Kobayashi S, He D, Chen Y, Komura J, Ohtani H, Shimokawa I, Ono T (2009) XPC is involved in genome maintenance through multiple pathways in different tissues. Mutat Res 670:24–31
35. de Vries A, van Oostrom CT, Hofhuis FM, Dortant PM, Berg RJ, de Gruijl FR, Wester PW, van Kreijl CF, Capel PJ, van Steeg H et al (1995) Increased susceptibility to ultraviolet-B and carcinogens of mice lacking the DNA excision repair gene XPA. Nature 377:169–173
36. McWhir J, Selfridge J, Harrison DJ, Squires S, Melton DW (1993) Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning. Nat Genet 5:217–224
37. Dollé ME, Busuttil RA, Garcia AM, Wijnhoven S, van Drunen E, Niedernhofer LJ, van der Horst G, Hoeijmakers JH, van Steeg H, Vijg J (2006) Increased genomic instability is not a prerequisite for shortened lifespan in DNA repair deficient mice. Mutat Res 596:22–35
38. Cheo DL, Meira LB, Burns DK, Reis AM, Issac T, Friedberg EC (2000) Ultraviolet B radiation-induced skin cancer in mice defective in the Xpc, Trp53, and Apex (HAP1) genes: genotype-specific effects on cancer predisposition and pathology of tumors. Cancer Res 60:1580–1584
39. Helnen CD, Schmutte C, Fishel R (2002) DNA repair and tumorigenesis. Cancer Biol Therapy 1:477–485
40. Modrich P (2006) Mechanisms in eukaryotic mismatch repair. J Biol Chem 281:30305–30309
41. de Wind N, Dekker M, Berns A, Radman M, te Riele H (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell 82:321–330
42. de Wind N, Dekker M, van Rossum A, van der Valk M, te Riele H (1998) Mouse models for hereditary nonpolyposis colorectal cancer. Cancer Res 58:248–255
43. Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Kolodner RD, Kucherlapati R (1996) Meiotic pachytene arrest in MLH1-deficient mice. Cell 85:1125–1134
44. Edelmann W, Yang K, Kuraguchi M, Heyer J, Lia M, Kneitz B, Fan K, Brown AM, Lipkin M, Kucherlapati R (2003) Tumorigenesis in Mlh1 and Mlh1/Apc1638 N mutant mice. Cancer Res 59:1301–1307
45. Takagi Y, Takahashi M, Sanada M, Ito R, Yamaizumi M, Sekiguchi M (2003) Roles of MGMT and MLH1 proteins in alkylation-induced apoptosis and mutagenesis. DNA Repair (Amst) 2:1135–1146
46. Baker SM, Harris AC, Tsao JL, Flath TJ, Bronner CE, Gordon M, Shibata D, Liskay RM (1998) Enhanced intestinal adenomatous polyp formation in Pms2-/-;Min mice. Cancer Res 58:1087–1089
47. Edelmann W, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF, Pollard JW, Kunkel T, Lipkin M, Kolodner R, Kucherlapati R (1997) Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. Cell 91:467–477
48. Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13:336–342
49. Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM (1995) Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. Cell 82:309–319
50. Kass EM, Jasin M (2010) Collaboration and competition between DNA double-strand break repair pathways. FEBS Lett 584:3703–3708
51. Xu H, Balakrishnan K, Malaterre J, Beasley M, Yan Y, Essers J, Appeldoorn E, Tomaszewski JM, Vazquez M, Verschoor S, Lavin MF, Bertoncello I, Ramsay RG, McKay MJ (2010) Rad21-cohesin haploinsufficiency impedes DNA repair and enhances gastrointestinal radiosensitivity in mice. PLoS One 5:e12112
52. Deans B, Griffin CS, Maconochie M, Thacker J (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. EMBO J 19:6675–6685
53. Haines JW, Coster MR, Adam J, Cheeseman M, Ainsbury EA, Thacker J, Bouffler SD (2010) Xrcc2 modulates spontaneous and radiation-induced tumorigenesis in Apcmin/+mice. Mol Cancer Res 8:1227–1233
54. Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Gyader G, Abramowske V, Revy P, de Villartay JP (2007) V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. Oncogene 26:7780–7791
55. Gu Y, Seidl KJ, Rathbun GA, Zhu C, Manis JP, van der Stoep N, Davidson L, Cheng HL, Sekiguchi JM, Frank K, Stanhope-Baker P, Schlissel MS, Roth DB, Alt FW (1997) Growth retardation and leaky SCID phenotype of Ku70-deficient mice. Immunity 7:653–665
56. Taccioli GE, Amatucci AG, Beamish HJ, Gell D, Xiang XH, Torres Arzayus MI, Priestley A, Jackson SP, Marshak Rothstein A, Jeggo PA, Herrera VL (1998) Targeted disruption of the catalytic subunit of the DNA-PK gene in mice confers severe combined immunodeficiency and radiosensitivity. Immunity 9:355–366
57. Ouyang H, Nussenzweig A, Kurimasa A, Soares VC, Li X, Cordon-Cardo C, Li W, Cheong N, Nussenzweig M, Iliakis G, Chen DJ, Li GC (1997) Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination In vivo. J Exp Med 186:921–929
58. Gao Y, Chaudhuri J, Zhu C, Davidson L, Weaver DT, Alt FW (1998) A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. Immunity 9:367–376
59. Glassner BJ, Weeda G, Allan JM, Broekhof JL, Carls NH, Donker I, Engelward BP, Hampson RJ, Hersmus R, Hickman MJ, Roth RB, Warren HB, Wu MM, Hoeijmakers JH, Samson LD (1999) DNA repair methyltransferase (Mgmt) knockout mice are sensitive to the lethal effects of chemotherapeutic alkylating agents. Mutagenesis 14:339–347
60. Nordstrand LM, Svärd J, Larsen E, Nilsen A, Ougland R, Furu K, Lien GF, Rognes T, Namekawa SH, Lee JT, Klungland A (2010)

Mice lacking Alkbh1 display sex-ratio distortion and unilateral eye defects. PLoS One 5:e13827
61. Tsuzuki T, Sakumi K, Shiraishi A, Kawate H, Igarashi H, Iwakuma T, Tominaga Y, Zhang S, Shimizu S, Ishikawa T et al (1996) Targeted disruption of the DNA repair methyltransferase gene renders mice hypersensitive to alkylating agent. Carcinogenesis 17:1215–1220
62. Ringvoll J, Nordstrand LM, Vågbø CB, Talstad V, Reite K, Aas PA, Lauritzen KH, Liabakk NB, Bjørk A, Doughty RW, Falnes PØ, Krokan HE, Klungland A (2006) Repair deficient mice reveal mABH2 as the primary oxidative demethylase for repairing 1meA and 3meC lesions in DNA. EMBO J 25:2189–2198
63. Moynahan ME, Chiu JW, Koller BH, Jasin M (1999) Brca1 controls homology-directed repair. Mol Cell 4:511–518
64. Moynahan ME, Pierce AJ, Jasin M (2001) BRCA2 is required for homology-directed repair of chromosomal breaks. Mol Cell 7:263–272
65. King TA, Li W, Brogi E, Yee CJ, Gemignani ML, Olvera N, Levine DA, Norton L, Robson ME, Offit K, Borgen PI, Boyd J (2007) Heterogenic loss of the wild-type BRCA allele in human breast tumorigenesis. Ann Surgical Onc 14:2510–2518
66. Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH (1996) Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. Genes Dev 10:1835–1843
67. Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH (1996) Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. Nat Genet 12:191–194
68. Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature 386:804–810
69. Suzuki A, de la Pompa JL, Hakem R, Elia A, Yoshida R, Mo R, Nishina H, Chuang T, Wakeham A, Itie A, Koo W, Billia P, Ho A, Fukumoto M, Hui CC, Mak TW (1997) Brca2 is required for embryonic cellular proliferation in the mouse. Genes Dev 11:1242–1252
70. Jeng YM, Cai-Ng S, Li A, Furuta S, Chew H, Chen PL, Lee EY, Lee WH (2007) Brca1 heterozygous mice have shortened life span and are prone to ovarian tumorigenesis with haploinsufficiency upon ionizing irradiation. Oncogene 26:6160–6166
71. Patel KJ, Yu VP, Lee H, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS, Ponder BA, Venkitaraman AR (1998) Involvement of Brca2 in DNA repair. Mol Cell 1:347–357
72. Kim M-K, Zitzmann S, Westermann F, Arnold K, Brouwers S, Schwab M, Savelyeva L (2004) Increased rates of spontaneous sister chromatid exchange in lymphocytes of BRCA2+/− carriers of familial breast cancer clusters. Cancer Lett 210:85–94
73. Kee Y, D'Andrea AD (2010) Expanded roles of the Fanconi anemia pathway in preserving genomic stability. Genes Dev 24:1680–1694
74. Rantakari P, Nikkila J, Jokela H, Ola R, Pylkas K, Lagerbohm H, Sainio K, Poutanen M, Winqvist R (2010) Inactivation of Palb2 gene leads to mesoderm differentiation defect and early embryonic lethality in mice. Hum Mol Genet 19:3021–3029
75. Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Meyn MS, Grompe M (2003) Epithelial cancer in Fanconi anemia complementation group D2 (Fancd2) knockout mice. Genes Dev 17:2021–2035
76. Bakker ST, van de Vrugt HJ, Rooimans MA, Oostra AB, Steltenpool J, Delzenne-Goette E, van der Wal A, van der Valk M, Joenje H, te Riele H, de Winter JP (2009) Fancm-deficient mice reveal unique features of Fanconi anemia complementation group M. Hum Mol Genet 18:3484–3495
77. Crossan GP, van der Weyden L, Rosado IV, Langevin F, Gaillard PH, McIntyre RE, Gallagher F, Kettunen MI, Lewis DY, Brindle K, Arends MJ, Adams DJ, Patel KJ (2011) Disruption of mouse Slx4, a regulator of structure-specific nucleases, phenocopies Fanconi anemia. Nat Genet 43:147–152
78. Bensimon A, Aebersold R, Shiloh Y (2011) Beyone ATM: The protein kinase landscape of the DNA damage response. Febs Lett 585:1625–1639
79. Lebel M, Leder P (1998) A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. Proc Natl Acad Sci USA 95:13097–13102
80. Chester N, Kuo F, Kozak C, O'Hara CD, Leder P (1998) Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. Genes Dev 12:3382–3393
81. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, Baltimore D (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev 10:2411–2422
82. Fang Y, Tsao C–C, Goodman BK, Furumai R, Tirado CA, Abraham RT, Wang X-F (2004) ATR runctions as a gene dosage-dependent tumor suppressor on a mismatch repair-deficient background. EMBO J 23:3164–3174
83. Brown EJ, Baltimore D (2000) ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 14:397–402
84. Su F, Smilenov LB, Ludwig T, Zhou L, Zhu J, Zhou G, Hall EJ (2010) Hemizygosity for Atm and Brca1 influence the balance between cell transformation and apoptosis. Radiat Oncol 5:15