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Functional Analyses of Human DNA Repair Proteins Important for Aging and Genomic Stability Using Yeast Genetics

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Abstract

Model systems have been extremely useful for studying various theories of aging. Studies of yeast have been particularly helpful to explore the molecular mechanisms and pathways that affect aging at the cellular level in the simple eukaryote. Although genetic analysis has been useful to interrogate the aging process, there has been both interest and debate over how functionally conserved the mechanisms of aging are between yeast and higher eukaryotes, especially mammalian cells. One area of interest has been the importance of genomic stability for age-related processes, and the potential conservation of proteins and pathways between yeast and human. Translational genetics have been employed to examine the functional roles of mammalian proteins using yeast as a pliable model system. In the current review recent advancements made in this area are discussed, highlighting work which shows that the cellular functions of human proteins in DNA repair and maintenance of genomic stability can be elucidated by genetic rescue experiments performed in yeast.

Keywords

aging; DNA repair; yeast; genetics; genomic stability

1. Theories of Aging Gleaned from Model Genetic Organisms

Understanding the molecular mechanisms of aging and elucidation of the factors that control longevity has attracted great interest. Aging is considered to be a complex process that is controlled by a variety of processes and conditions [1–5]. Caloric intake, accumulation of oxidative DNA damage, decline of DNA repair pathways, mitochondrial dysfunction, and telomere shortening are all widely thought to contribute to aging. Chemical damage to macromolecules such as proteins and lipids, in addition to chromosomal DNA, are proposed to affect aging [6]. For example, accumulation of reactive oxygen species (ROS) may lead to oxidation of proteins, carbohydrates, and lipids that ultimately impairs their structural integrity and function [7]. Increasing the resistance of tissue macromolecules to mitochondrial ROS-mediated oxidative damage may improve longevity. Highly conserved signaling mechanisms affect the onset and degree of age-associated phenotypes. These

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mechanisms, globally referred to as stress response pathways, include (but are not limited to) insulin/ insulin-like growth factor 1 (IGF-1), Target of Rapamycin (TOR), and sirtuin-mediated signaling pathways. For an excellent current review on the topic of aging as it relates to stress response pathways, see [3].

Accumulation of macromolecule damage or perturbation of oxygen and nutrient levels can trigger complex stress response systems that are typically characterized by changes in gene expression that serve to regulate cell homeostasis and maintain genome integrity. Genes which regulate pathways that contribute to aging may be conserved throughout evolution. In some cases, interventions that could slow down or reverse aging and delay onset of age-related diseases and cancer have been investigated based on knowledge gained from model genetic organisms such as nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*), and rodent (*Mus musculus*). In this review, we will focus on budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) as model systems for functional analysis of human DNA repair proteins to understand the molecular-genetic functions and pathways important in the aging process. A focus is placed on the DNA damage response with a special emphasis on DNA repair. For a discussion of eukaryotic signal transduction pathways elicited during the DNA damage response, see [8].

2. Models for the Study of Yeast Aging

With the ease of handling, culturing conditions, genetic manipulations, and availability of mutants, budding yeast represents an important model system to identify and characterize factors that control longevity [9–11]. Models for replicative aging and chronological aging have been used to study yeast aging. The chronological life span (CLS) model deals with the aging of post mitotic cell as determined by the length of time a yeast cell can survive in a non-dividing, quiescent-like state. In addition to dietary restriction (DR), mutations resulting in decreased activity of TOR signaling pathway, nutrient responsive kinase Sch9, adenylate cyclase (Cyr1), or an activator of cyclic AMP-dependent protein kinase A (PKA) pathway accompanied by up-regulation of a highly conserved response to starvation-induced stress has been shown to increase CLS in yeast [12]. Life span extension in *cyr1* mutants is mediated by the stress resistance transcription factors Msn2 and Msn4, which in turn induce the expression of the DNA damage inducible genes *DDR2* and *SOD2* [13, 14]. Reduced activity of these pathways also resulted in an increased replicative life span (RLS) [15], suggesting that the two pathways of aging are interconnected. Decreased activity of TOR and Sch9 kinase orthologs has been shown to extend life span in *Caenorhabditis elegans* [16] and *Drosophila melanogaster* [17] as well. An alternative water-independent and protein:carbohydrate-ratio dependent DR pattern has been proposed for life span extension in *Drosophila melanogaster* [18]. Since DR in mammals includes ad libitum water, the alternative DR regime is more applicable to mammals. Such studies demonstrate that extreme care should be taken to design experimental conditions amongst different species. Deletion of mitochondrial superoxide dismutase *SOD2* in yeast *sch9Δ* eliminated the life span extension and resulted in a reduced survival rate in a *cyr1* mutant [19]. Co-overexpression of both cytosolic SOD1 and mitochondrial SOD2 resulted in extended life span, suggesting roles for these proteins that scavenge free radicals in longevity. Studies in both yeast and mice *sod2* mutants led to the identification of mitochondrial aconitase and succinate dehydrogenase as the primary targets of mitochondrial superoxide [20, 21]. In both organisms, the post-mitotic cells were most severely affected, suggesting that CLS of yeast could be a valuable model to study oxidative stress. The effect of ROS elimination may reduce damage to not only DNA but also other macromolecules (e.g., proteins, lipids), as discussed above.

CLS in yeast can also be affected by the accumulation of acetic acid in the growth medium [22]. Transfer of yeast to water, growth in glycerol, or a high osmolarity medium promotes CLS. These factors promote longevity by either reducing acetic acid accumulation or increasing resistance to acetic acid-induced cell death. Thus while chronologically aged yeast display DNA fragmentation at the end of life, this instability may be due to a programmed cell death response induced by acetic acid build-up. Although it is unlikely that acetic acid toxicity plays a role in mammalian aging, accumulation of deleterious oxidized macromolecule(s) may be involved.

The RLS model involves aging of mitotically active cells in which life span of the yeast mother cell is defined by the number of the daughter cells produced before senescence and is defined in generations. The fidelity with which cells divide asymmetrically to give a small daughter and a large mother cell decreases with age. Daughters of the oldest mother cells have shorter life span than daughters of the young mother cells; however, granddaughters of old mother cells recover a normal life span [23]. This work suggested that the genomes of daughter cells were intact, whereas an intracellular substance accumulated in the old mother cells that caused aging. The extent to which unrepaired macromolecule damage or instability contributes to normal aging in mammals remains unresolved. Extra chromosomal ribosomal DNA (rDNA) circles (ERCs) formed by homologous recombination (HR) within the rDNA may contribute to replicative aging in yeast [24–27]. Mutations that decrease the formation of ERCs have been shown to correlate with increased life span. Deletion of the gene that encodes the rDNA replication fork barrier protein Fob1 resulted in a reduced production of rDNA circles and extension of lifespan of yeast mother cells [28]. On the other hand, deletion of Sir2, an NAD-dependent histone deacetylase necessary for transcriptional silencing near telomeres, HM loci and rDNA, resulted in an increased ERC formation and shortening of the lifespan [29]. Conversely, Sir2 overexpression resulted in the extension of lifespan. Although these studies have demonstrated that ERC accumulation has an effect on the lifespan of a yeast mother cell, the mechanism is still not well understood. It may very well be that rDNA stability is the critical factor in determining longevity in yeast. If so, an understanding of the underlying mechanism would pave the way for investigating if it is conserved in mammalian cells.

Although the yeast models for the study of aging have been informative, aging in multicellular organisms including mammals is likely to involve additional mechanisms. The loss of tissue homeostasis through stem and progenitor cell attrition have been postulated to contribute to aging and DNA damage accumulation that may underlie the reduced capacity of stem cells to mediate a return to homeostasis after exposure to acute stress or injury [30, 31]. Hematopoietic stem cells with deficiencies in nucleotide excision repair (NER) (*XPB^{TTD}*, *XPB^{R722W}*), non-homologous end joining (*Ku80^{-/-}*, *LIG4^{Y288C}*), mismatch repair (MMR) (*Msh2*), homologous recombination (HR) repair (*Brca2*), or telomere maintenance (*mTR^{-/-}*) transplanted into primary mouse recipients were diminished in their ability to self renew, proliferate, or reconstitute multi-lineage cell types, and displayed increased susceptibility to apoptosis [30–33]. Loss of regenerative capacity due to genomic instability might be responsible for many but not all aspects of aging. Quiescent stem cells might serve as a reservoir for multiple mutagenic events underlying oncogenic transformation, prompting studies to understand the importance of DNA damage as a causative factor in normal aging and cancer.

3. Yeast as an Experimental Model for the Investigation of DNA Damage as a Cause of Age-related Phenotypes

To use yeast as a model system for studying aging, it is helpful to consider what ways single-celled organisms recapitulate aging observed in multicellular organisms and what

ways they do not. Caloric restriction (CR) has been shown to extend longevity in both yeast and multicellular organisms [34, 35]. The effect of CR is proposed to be mediated by a family of NAD⁺ dependent histone deacetylase proteins known as sirtuins in yeast and higher eukaryotes, suggesting a common pathway for lifespan extension [36]. Enhanced expression of genes of the NAD⁺ salvage pathway has been shown to increase yeast lifespan in a SIR2-dependent manner [37]. However, a later study demonstrated the existence of a Sir2- independent pathway for aging that is responsive to CR [38]. Yeast strains that either overexpress SIR2 or harbor *fob1Δ* or *sir2Δ.fob1Δ* mutations in combination with CR displayed an additive increase in lifespan. These studies suggest that Sir2 and CR might act in a parallel pathway to promote longevity in yeast, which may apply to mammals.

There is substantial evidence that mitochondrial respiration that results in the accumulation of ROS may be a unifying mechanism for aging in yeast and mammals. Although sirtuins or caloric restriction promotes longevity in yeast and multicellular organisms, the mechanisms may be different. If so, the processes in yeast that influence aging may not be mirrored in higher eukaryotes. Nonetheless, the wear and tear theory of aging in which cells of an organism are worn down by toxins in the diet and environment seems to apply to organisms of all Kingdoms. Genetic factors would play a major role in helping cells cope with macromolecular damage. Indeed, some genes required for homeostasis in yeast and other life forms are conserved. In this review, we will emphasize genes involved in the DNA damage response, particularly ones involved in maintenance of genomic stability.

A definitive experiment to establish the relationship between DNA damage and aging would be to observe lifespan extension or retardation of specific aging phenotypes in a model organism by lowering the overall level of DNA damage. This may be a challenging task given the varied nature of genomic instability. Caloric restriction is an example of intervention that has been shown to result in lifespan extension in a variety of species such as yeast, nematode, fruitfly and rodent [39]. In rats, dietary restriction (DR) resulted in decreased mitochondrial ROS production and reduced oxidative damage to mitochondrial DNA and proteins [40, 41], emphasizing the importance of endogenous damage for aging. A transgenic mouse that overexpressed human catalase displayed reduced oxidative damage and H₂O₂ production, and extended life span [42]. This work supports the free radical theory of aging linking longevity with anti-oxidant defenses. Further studies to define the targets of anti-oxidant or DNA repair proteins would help to characterize the connections between longevity, oxidative damage, and genomic stability.

Deficiencies in DNA repair pathways that disrupt tissue homeostasis by the accumulation of DNA damage in progenitor cells may be a cause for age-related phenotypes. An excellent example of this principle is that of the *ERCC1*^{-/-} premature aging mouse with a defect in a nucleotide excision repair gene [43]. However, while defects in DNA repair processes are likely to be a prominent factor to shortened lifespan, other factors as discussed above, contribute to natural aging. Moreover, the relationship between the mutational status of a given DNA repair gene and aging may be complex. *ERCC1*^{-/-} mice that display progeroid symptoms are characterized by changes in gene expression that control cell death and anti-oxidant defenses, a shift towards anabolism, and reduced IGF1 signaling [43]. This systemic response was also observed in wild-type mice exposed to chronic stress, undergoing caloric restriction, or with aging. Based on their results, Niedernhofer *et al.* concluded that DNA damage accumulation results in a conserved metabolic response that suppresses the somatroph axis, leading to a shift of resources from growth and proliferation to protective maintenance. This was a significant advance in the field; however, establishing a cause and effect between DNA damage accumulation and aging still remains a challenge to researchers and the use of genetic models is paramount.

Although models for aging in higher eukaryotes have been very useful, a case can also be made that yeast provides an unparalleled genetic system for the study of genomic stability and its relationship to aging. Conserved pathways of DNA replication and repair between yeast and human suggest that yeast provides an ideal heterologous system to study the genetic and cellular roles of human proteins. While a DNA repair-associated phenotype in yeast should not be confused with yeast aging, it may indicate that defects in repair processes may contribute to cell attrition and be relevant to organismal aging. Previously, an excellent review from the Samson lab described a variety of heterologous expression systems to identify novel DNA repair genes and characterize their functions [44]. In this review, we will focus on recent advancements using yeast as a facile model system for genetic studies with human DNA repair proteins.

4. Discovery of Human DNA Repair Genes and Their Functions by Yeast Genetic Complementation Studies

Heterologous expression of human proteins in yeast has enabled researchers to discover new genes, acquire information about cellular DNA damage response pathways, and screen for effects of mutations in DNA repair genes by functional assays (Fig. 1). The discovery and characterization of new mammalian genes that encode proteins which operate in functionally conserved pathways in the lower eukaryote has proven to be very insightful (Table 1). Mutational analyses of human DNA repair genes by yeast genetic complementation studies have also helped to elucidate the functional consequences of disease mutations as well as polymorphic variants that potentially affect DNA repair capacity and influence human health and aging. Moreover, novel functions of human DNA repair proteins have been discovered by genetic rescue experiments in yeast given its tractable nature. These concepts are discussed and examples provided to illustrate the power of yeast genetics to study the functions of human proteins with important roles in DNA repair and maintenance of genomic stability.

4.1 Nucleotide Excision Repair

NER, which is responsible for the removal of bulky DNA lesions (Sancar *et al.*, 2004), represents one of the earliest DNA repair pathways to be studied by genetic complementation experiments using human genes expressed in the corresponding yeast mutant backgrounds. Mutations in the *XPD* gene result in the ultraviolet light (UV)-sensitive skin cancer disorder Xeroderma pigmentosum (XP), and two other genetic diseases known as Cockayne syndrome (CS) and trichothiodystrophy (TTD), which are distinct in their clinical and cellular appearance from one another and XP [45]. CS patients have some phenotypes that resemble accelerated aging and are characterized by neurological dysfunction, but not cancer. TTD has diverse and highly variable clinical symptoms which include photosensitivity, ichthyosis, sulfur-deficient brittle hair, physical and mental impairment. The clinical heterogeneity of *XPD* alleles is likely to reflect multifunctional roles of the protein in DNA repair as well as in RNA polymerase II transcription because it is an integral component of the basal transcription factor TFIIF [46].

Expression of the human *XPD* gene in a *S. cerevisiae rad3* mutant strain rescued the lethality of the yeast *rad3* deletion, suggesting that it could serve as a subunit in the TFIIF complex to substitute for its yeast counterpart Rad3 to perform transcription [47]. To assess the apparently complex involvement of XPD in transcription, genetic complementation studies of the *rad3* mutant yeast strain with site-directed mutant alleles of *XPD* were performed [48]. Replacement of the invariant lysine in the XPD Walker A box with arginine abolished ATPase and helicase activity but did not affect ATP and DNA binding. This ATPase/helicase defective XPD protein expressed in the *rad3* mutant performed its vital

role in transcription. A TTD patient mutation Arg 722 Trp (R722W), which encodes a catalytically active XPD helicase, failed to rescue the viability of the *rad3* mutant, suggesting that the XPD-R722W protein encoded by the mutant allele was defective in its protein interactions which prevented it from performing normally in transcription. In support of this notion, the XPD-R722W protein was indeed found to be defective in its ability to bind the p44 subunit of TFIIH [49]. Although expression of XPD in the *rad3* mutant rescued viability, it failed to complement the excision repair defect [47], suggesting that the human XPD protein was not able to productively interact with other proteins or key DNA intermediates of the NER pathway in yeast.

The structural and functional placement of TTD mutations in XPD provided by the recently solved crystal structures of archaea XPD proteins suggested that TTD mutations would affect the structural integrity of XPD and thus its protein interactions [50–52]. Presumably, the same principal would apply to the R722W substitution which resides in a C-terminal portion of XPD that is absent in the archaea XPD proteins. Depending on its location, certain *XPD* alleles may lead to diverse clinical symptoms by causing changes in the protein that interfere with the catalytic functions of XPD, its protein interactions, or its role as a structural component of the TFIIH complex necessary for efficient transcription [46]. The initial genetic studies of the *XPD-R722W* mutant allele in yeast provided the first hint of evidence to support the idea that a TTD patient mutation may interfere with the normal role of XPD in transcription.

4.2 Base Excision Repair

The accumulation of oxidative DNA damage leads to mutagenesis and genome destabilization, and is proposed to be an important contributing factor to aging and carcinogenesis [53]. A primary line of defense against oxidative base damage is its correction via base excision repair (BER) [54]. BER plays an essential role in removing the small, non-helix distorting base lesions that arise spontaneously from endogenous cellular biochemical processes or from certain environmental agents. DNA glycosylases are enzymes that initiate BER by recognizing and cleaving the glycosidic bond between the sugar and damaged base. The human homolog of *S. cerevisiae* DNA glycosylase Ogg1 was discovered through a sequence homology search and genetic complementation studies in yeast [55]. Human OGG1 (*hOGG1*) was found to have significant similarity both in terms of sequence as well as substrate specificity with its *S. cerevisiae* homolog. The ability of human *OGG1* to suppress the spontaneous mutator phenotype of *S. cerevisiae* *ogg1*, its DNA substrate specificity for lyase activity on 8-oxoG/C base pairs, and its product formation elucidated the functional human homolog of yeast *OGG1*.

Although yeast has proven useful for the functional analysis of human BER enzymes, a slight imbalance of BER can be deleterious as exemplified by a study in which the human 3-methyladenine DNA glycosylase (MAG) expressed in yeast led to increased spontaneous mutation [56]. This highlights an important note of caution when using the yeast system to evaluate the function of human DNA repair proteins that the level of protein expression can affect cellular phenotypes. Therefore, quantitative determination of the amount of endogenous or exogenously expressed protein should be performed. A finely tunable expression system for yeast genetic complementation studies is advantageous to achieve a physiological level of expression of the protein under investigation. Rather than plasmid-based expression of a DNA repair protein, it may be more appropriate to integrate the gene into the yeast genome under the control of its endogenous promoter.

DNA glycosylases cleave the *N*-glycosidic bond at the altered base, creating an apurinic/aprimidine (AP) site which is incised by an AP endonuclease. AP endonucleases can also incise sites of spontaneous base loss. Wilson *et al.* (1995) performed trans-complementation

studies in yeast to evaluate the functional roles of a human AP endonuclease known as Ape1. Overexpression of Ape1 in an *apn1* deficient yeast strain reduced its spontaneous mutation rate comparable to the wild-type, confirming the mutagenic potential of unrepaired AP sites *in vivo* [57]. Ape1 expression in yeast also complemented the methylmethanesulfonate (MMS) sensitivity of the *apn1* mutant; however, H₂O₂ resistance was not restored, consistent with the weak 3'-repair diesterase activity of human Ape1.

Additional yeast AP endonucleases were not thought to exist until the late 1990's [58]. A search of the yeast genome revealed an open reading frame with homology to exonuclease III. Sequence alignment placed the predicted Eth1 protein in the exonuclease III grouping. *ETH1* mRNA expression in yeast was induced in response to DNA damage. Unlike the first identified AP endonuclease identified in yeast (known as Apn1) that plays a role in both alkylation and UV damage, Eth1 was implicated only in the response to alkylation damage as evidenced by the significantly increased sensitivity of the *apn1 eth1* mutant to MMS, but not UV light, compared to the wild-type strain or *eth1* single mutant [58]. Analysis of spontaneous mutation rate demonstrated that Eth1 functions in the maintenance of genomic stability. The identification of the second form (*ETH1*) in yeast suggested that mammalian genomes are likely to have multiple AP endonucleases with specialized functions. Indeed, a second human protein (designated Ape2) bearing sequence homology with *E. coli* Exonuclease III and human Ape1 was identified, but the partially purified Ape2 was found to have only a weak incision activity on DNA substrates with an abasic site [59]. Moreover, Ape2, unlike Ape1, only weakly complemented the *apn1* yeast mutant for MMS resistance, suggesting a divergent role in DNA repair from its human counterpart Ape1. Another human AP endonuclease, designated PNK and APTX-like FHA protein (PALF), was more recently identified that has endo- and exonuclease activities against abasic site and other forms of base damage [60]. PALF accumulates rapidly at the sites of single-stranded or double-stranded DNA breaks; however, its precise role in the mammalian DNA damage response remains to be elucidated.

The benefit of yeast as a model genetic system is highlighted by its usefulness to decipher the roles of certain enzymes in known pathways such as DNA repair, when expressed in their dominant negative forms. The Sweazy lab demonstrated a role of rat DNA polymerase β (Pol β) in BER by expressing dominant negative mutants of Pol β in *S. cerevisiae*. Expression of dominant negative mutant Pol β rendered cells sensitive to MMS but not UV, suggesting that the mutant protein interfered with the alkylation repair pathway but not NER [61]. Epistatic analysis in *S. cerevisiae* demonstrated that rat Pol β mutants function in the same pathway as Apn1 and MAG, but not O⁶-methylguanine DNA methyltransferase (MGMT) [62]. These studies further substantiated the ability of specific Pol β mutants to interfere with the BER pathway.

O⁶-alkyl-guanine is the major carcinogenic lesion induced by alkylating agents and is removed by the repair protein O⁶-methylguanine DNA methyltransferase (MGMT). The MGMT enzyme prevents the formation of transition mutations (G:C to A:T) in DNA by transferring the alkyl group from the O⁶-guanine in DNA to an active cysteine within its own sequence [63]. Historically, the role of the human *MGMT* gene in the protection against DNA alkylation damage was difficult to study because human cell lines deficient in the enzyme were characterized by down-regulation of the *MGMT* gene and other genes, as opposed to mutations in the *MGMT* gene itself. In this scenario, it was advantageous to study the human protein in the yeast system. An approach was taken in which a yeast *mgt1* mutant strain was created to perform genetic complementation with the human gene [64]. In a regulatable manner, expression of human *MGMT* protected the yeast *mgt1* cells from alkylation-induced DNA damage and mutation [64]. This work helped to establish a role of

MGMT in the repair of alkylated DNA bases which if left uncorrected lead to base mismatches.

MGMT limits the effectiveness of alkylating chemotherapies; therefore, it is a target for anti-cancer therapy [65]. A greater understanding of structural and mechanistic aspects of *MGMT* and other O⁶-alkyl-guanine transferases has been insightful for its biological roles and implications for cancer therapy. The yeast *mgt1* mutant transformed with human *MGMT* may serve as a useful system for biological screens of small molecule inhibitors of *MGMT*. The discovery of small molecule inhibitors of DNA repair proteins that could be anti-cancer targets could be facilitated by high throughput screens of appropriate yeast genetic backgrounds with functional assays.

The identification and characterization of human BER genes using yeast genetics has helped to advance aging studies because of the importance of this pathway for the response to oxidative stress. Animal models strongly support the importance of caloric restriction as a means to reduce free radical production to protect against age-related phenotypes. Furthermore, genetic studies in yeast demonstrated that BER is important to attain a full chronological lifespan [66]. Since many of the BER proteins have human orthologs, it follows that BER defects in humans will also affect aging and the acquisition of age-related diseases [67]. Indeed, defects in BER are associated with a number of human diseases, particularly those associated with neurodegeneration and cancer.

4.3 Recombinational Repair and Interstrand Cross-link Repair

Double-strand breaks (DSBs) that arise from ionizing radiation (IR) or blocked replication forks at sites of alkylation DNA damage must be corrected to prevent genomic instability. Two pathways of DSB repair exist: error-prone nonhomologous end-joining or HR repair of DNA that is replicated during S phase [68, 69]. In yeast, mutants belonging to the *RAD52* epistasis group are implicated in DSB repair through HR. Among the proteins of the *RAD52* pathway, *RAD54* encodes a motor ATPase that is involved in chromatin remodeling, a process that is presumably critical for recombinational repair. Human *RAD54* could partially complement the MMS-sensitive phenotype of *rad54* mutant cells, indicating conservation of function [70]. Mutation of the invariant lysine residue in the Walker A box of human Rad54 that renders the mutant protein inactive as an ATPase abolished its ability to confer MMS resistance. This early work provided important evidence that the DNA repair function of Rad54, and by inference other HR proteins, is conserved from yeast to humans, suggesting a common pathway in eukaryotes.

DNA interstrand cross links (ICLs) represents a class of cytotoxic lesions that involve covalent tethering of both strands of DNA duplex thereby preventing the progression of processes such as replication and transcription. The mechanism by which ICLs are repaired in mammalian cells is not yet fully understood and the yeast system has been useful to characterize genes involved in conferring resistance to DNA cross-linking agents. The McHugh lab investigated the potential functional overlap of the human SNM1/Pso2 family of nucleases SNM1A, SNM1B/Apollo, and SNM1C/Artemis with a budding yeast paralog known as Pso2 nuclease that is implicated in ICL repair [71]. They demonstrated that human *SNM1A*, but not *SNM1B* or *SNM1C*, was able to efficiently rescue the sensitivity of the *pso2* mutant to the DNA ICL agent nitrogen mustard. Human *SNM1A* was also able to complement the ICL-associated DSB repair defect of the *pso2* mutant and the spontaneous HR phenotype of *pso2 msh2* double mutants. Thus SNM1A is likely to be the functional homolog of budding yeast Pso2. A greater understanding of how Pso2 or SNM1A operates in the resection of incised ICL intermediates may lead to mechanistic insight to the downstream events of HR and translesion synthesis. For an in-depth recent review on this topic, see [72].

4.4 Mismatch Repair

MMR is responsible for recognizing and repairing erroneous insertions, deletions, or base mismatches that arise during cellular DNA replication, recombination, or repair (for review, see [73]). The MMR pathway is initiated by binding of Msh2 to mismatched DNA in complex with Msh6 (Mut α) or with Msh3 (Mut β). A heterodimer of the mismatch repair proteins Mlh1-Pms2 interacts with the mismatch recognition complex to recruit other DNA repair factors to initiate and complete MMR. Several pieces of evidence support the hypothesis that accumulation of mutations and genomic instability due to decline of MMR is relevant to the aging process. Microsatellite instability, a hallmark of mismatch repair deficiency, is observed to increase with aging in humans. A compromised DNA damage response, thought to be associated with aging, is prevalent in mismatch repair deficient cells. Furthermore, defective mismatch repair mediated DNA damage signaling results in the activation of cell cycle checkpoints, interruption of cell cycle progression, and apoptosis that lead to loss of the stem cell population, poor tissue homeostasis, and functional decline.

A decline in DNA repair capacity due to an accumulation of mutations and genome rearrangements, de-regulation of transcription, and an impaired stress response ultimately leads to more mutations which may contribute to aging or cancer, depending on a host of genetic and environmental factors. A challenge in the field has been to understand the role of the DNA damage response mediated by MMR and other pathways in delicately balancing the scale between cancer and aging. Since age is the greatest risk factor for the majority of cancers, a greater understanding of the dual role of DNA repair pathways in the suppression of aging or cancer is needed. To address this question, yeast can provide an advantage when the functions of DNA repair factors are conserved with human proteins. Due to the genetic complexity of aging (and cancer) in eukaryotes, it is helpful to evaluate the activity of DNA repair proteins encoded by human alleles in a uniform genetic background provided by the yeast model. This approach enables the investigator to study effects of genetic mutations in a manner that excludes 'private' genetic diversity which may influence variability in the rates of aging [74] or accumulation of age-related phenotypes (*e.g.*, cancer) between humans. Thus, yeast can provide a functional system to assay missense polymorphisms that do not segregate with a disease according to Mendelian genetics. In a uniform yeast genetic background, factors such as disease penetrance, epigenetic modifications, and heterogeneity in genetic background of the human cell/tissue type can be excluded from the analysis.

In addition to the reasons described above, yeast has been a highly popular system to study the effects of mutations in certain mismatch repair genes because domains and amino acid residues of the proteins are conserved between yeast and human. The clinical importance of MMR is perhaps best exemplified by the evidence that germline missense mutations frequently detected in hereditary non-polyposis colorectal cancer (HNPCC) patients are found mainly in the MMR genes *MLH1* and *MSH2* [75]. HNPCC tumor cells are marked by microsatellite instability as a consequence of the MMR defect. Genetic studies in the late 1990's provided a functional analysis of the human Mut α and Mut β complexes in yeast [76]. Although expression of human *MSH2*, *MSH3* and *MSH6* alone or in combination failed to reduce the high mutation rates of a *S. cerevisiae msh2* strain, co-expression of human *MSH2/MSH6* or human *MSH2/MSH3* produced a mutator phenotype attributed to the ability of the human mismatch protein complex to effectively bind mismatched DNA and prevent correction of replication slippage errors in yeast. A missense mutation R524P in human *MSH2* which reduces mismatch binding abolished the mutator effect. The significance of this work lies in the fact that the R524P mutation, associated with microsatellite instability, is found in HNPCC patients. Thus yeast provided a valuable approach to show that a clinically relevant disease allele of the MMR pathway was perturbed in its function *in vivo*.

Development of functional assays to serve as genetic tests for HNPCC is a high priority. Ectopically expressed human MMR proteins in yeast can be assayed for their mutator effect by analysis of forward mutations at the canavanine (*CAN*) locus to confer *CAN* resistance or mutations in homopolymeric constructs to restore prototrophy. The efficacy of yeast as a model system to investigate the functional consequences of mutations in MMR genes that may be pathogenic, partial loss of function, or silent polymorphisms was demonstrated by a functional analysis of human *MLH1* and *MSH2* variants in a *S. cerevisiae* screen [77]. Quantitative *in vivo* DNA MMR assays which measure microsatellite instability of a (GT)₁₆ tract in yeast were performed to evaluate amino acid substitutions found in the human population. The missense mutations were introduced at the homologous residue in yeast *MLH1* or *MSH2* genes. The authors were also able to successfully construct a human-yeast hybrid *MLH1* gene which was able to complement the *mlh1* null strain by substantially reducing mutation frequency. This approach was used to evaluate amino acid substitutions of the residues in human genes that are not conserved in yeast. The results from the genetic assays in yeast and available human clinical data indicated a good correlation; those mutant alleles show to be dysfunctional in the yeast genetic assays were found to segregate with disease in families. A yeast screen which used a hybrid human-yeast *MLH1* gene in which the yeast ATPase domain was replaced by the human ATPase domain provided further validation of the approach [78]. It was proposed that the yeast trans-complementation system may be valuable to enhance the interpretation of *MLH1* genetic tests.

Of the several hundred known human *MLH1* mutations, approximately one third of these correspond to missense variants. To assess the effects of some of these alleles on *MLH1* function, the Alani lab introduced missense mutations associated with HNPCC into *S. cerevisiae* *MLH1*, and the corresponding plasmids were used for genetic complementation assays in an *mlh1* deletion strain to determine MMR proficiency [79]. Of the 28 alleles analyzed, 15 showed significant MMR defects, which were in agreement with previous functional assays and clinical data that suggested their pathogenicity. The yeast system afforded another level of analysis to assess the *mlh1* polymorphisms. The authors assessed the functional effects of the *mlh1* alleles in two different yeast strains, and found that genetic background plays a role in pathogenicity. Thus, atypical inheritance of disease such as colorectal cancer due to polymorphic variation in MMR genes may be due to differences in genetic background.

Recently, a novel system to assess the pathogenicity of *MLH1* variants was developed in which the yeast *MLH1* and *PMS2* genes were replaced by human orthologs directly on yeast chromosomes by HR [80]. The resulting yeast strain displayed a mutation rate equivalent to wild-type yeast. Cancer-related *MLH1* missense variants were tested for their effect on the MMR pathway and a good correlation with clinical data was observed for five of the seven variants. This system is unique in that a single copy of the MMR gene was introduced per cell which was placed under the control of the yeast promoter, providing uniformity in the genetic analysis.

A simple yeast assay was developed to detect and evaluate pathogenic mutations in *MLH1* by suppression of the dominant mutator effect of human *MLH1* expressed in *S. cerevisiae* [81]. Heterozygous loss of function missense mutations in human *MLH1* were also identified by the assay. In a subsequent study by the same group, 101 *MLH1* variants were examined for the dominant mutator effect *in vivo* by three independent yeast-based assays and *in vitro* by a plasmid-based MMR assay using extracts. A diverse range of activity was determined for the *MLH1* variants with the majority of functionally inactive variants located in the putative ATP-binding pocket of the amino-terminal domain or a carboxyl-terminal domain of the protein [82]. Using this type of approach, the development of a functional database for a large number of *MLH1* variants may be informative to evaluate cancer risk in

individuals or families, and to understand the molecular defects of proteins encoded by the associated mutant *MLH1* alleles. For an additional perspective of using yeast to identify HNPCC alleles and the limitations of modeling human MMR repair in yeast, see [83]. Recent progress in this area has enabled researchers to identify weak alleles of MMR genes and MMR gene polymorphisms that can interact with other weak alleles of MMR to confer strong polygenic MMR defects [84]. This is an exciting area of research and further illustrates the value of yeast genetics to probe more subtle mutation interaction networks. Yeast-based screens are likely to provide insight for screening of biomarkers in HNPCC and additional clinical presentations including Muir-Torre syndrome and Turcot syndrome that arise from defective MMR genes [85].

It should be pointed out that there are several limitations of the yeast system to assay human MMR alleles. For one, yeast can only be used to assess the impact of mutations in conserved domains/residues of the MMR protein. Secondly, mutations that affect mRNA splicing in human cells cannot be evaluated in yeast because of differences in splicing consensus sequences/mechanisms. Thirdly, yeast genetic backgrounds may influence the functional consequence of a gene polymorphism, as discussed above. Although there are limitations in using yeast to study the effect of human alleles, and it is generally agreed that understanding the function of a human protein in its biological context (human cells) is preferable, a case can be made for genetic analysis of single human polymorphisms in a uniform genetic background provided by the yeast system. Perhaps most importantly, yeast provides facile functional assays for MMR function in cells that are advantageous over *in vitro* MMR assessments. Functional assays can be valuable for uncharacterized alleles in MMR genes, particularly those in which there are only limited clinical data. Advances in functional analysis of germline mutations in MMR genes associated with HNPCC, provided by yeast genetics, suggest an opportunity for the study of other cancers and genetically inherited age-associated diseases.

5. Tumor Suppressors BRCA1 and p53

In addition to DNA repair, yeast has provided a wealth of information about mammalian tumor suppressor proteins important in the maintenance of genomic stability, cell cycle regulation, and the DNA damage response. We will provide examples of yeast genetic studies that have advanced our understanding of two tumor suppressor molecules, BRCA1 and p53.

Germline mutations in the tumor suppressor gene *BRCA1* lead to an increased lifetime risk of breast and/or ovarian cancer [86]. BRCA1 is required for the maintenance of chromosomal stability and a proper DNA damage response (for review see [87, 88]). Humphrey *et al.* sought to develop an approach to screen *BRCA1* missense mutations for interference of biological function and made the observation that expression of *BRCA1* in *S. cerevisiae* inhibited cell growth, and the effect was localized to the tandem C-terminal BRCT motifs, a conserved protein sequence found in a large number of DNA damage-response proteins [89]. Growth suppression was diminished by disease-associated missense mutations in the C-terminal domain of BRCA1, consistent with the structural and functional importance of the BRCT domain. A yeast small colony phenotype assay was later employed to characterize *BRCA1* missense mutations throughout a large (~300 amino acid) C-terminal region of BRCA1 [90]. Only inactivating mutations within the conserved BRCT repeat domain could be identified by the yeast small colony assay. Later, a yeast HR assay was developed which showed that tumor-derived *BRCA1* missense mutations outside the conserved BRCT domain can induce elevated HR [91].

Lethality caused by *BRCA1* expression in yeast can be rescued by deletion of *DHH1* which encodes a member of the DEAD/H box family of helicases [92]. Curiously, yeast *DHH1* shares greatest homology with a human DEAD box RNA helicase (DDX6), and significantly less homology with the BRCA1 Associated C-terminal Helicase (BACH1) [93]. BACH1 was later identified to be FANCI, which is mutated in Fanconi Anemia (FA), a disease characterized by progressive bone marrow failure (for review, see [94, 95]). Evidence from the yeast study supported a model in which *DHH1* functions to activate the G1/S checkpoint after DNA damage or *BRCA1* expression [92]. A genome-wide screen to identify mutants that permit growth in the presence of *BRCA1* established a connection between *BRCA1* and transcription complexes stalled by DNA damage [96]. A function for RNA Polymerase II carboxyl terminal domain cleavage was implicated in the role of *BRCA1* as a tumor suppressor. However, a complete understanding of *BRCA1* phenotypes is still emerging as another study by the Skibbens lab identified a role of chromosome segregation genes in *BRCA1*-dependent lethality in yeast [97]. Based on the genes identified from the screen, it was suggested that *BRCA1* may alter kinetochore and cohesion pathways, ultimately affecting sister chromatid pairing. Clearly, studies of *BRCA1* expression in yeast indicate that the tumor suppressor has pleiotropic roles in the maintenance of genomic stability. The application of yeast as a screening tool to interrogate differences between *BRCA1* mutations has its value; however, the disadvantage is that the effect of amino acid substitutions in the BRCT domain (or other domains) of *BRCA1* on protein interactions occurring in yeast may not necessarily reflect effects on protein interactions in human cells. Results from yeast genetic complementation assays with human proteins can most easily be interpreted when homologs exist, and are functionally interchangeable.

The most recent use of yeast to study the molecular functions of *BRCA1* has employed microarray analyses. *BRCA1* affects gene expression both globally and at specific loci, which contribute to adverse effects on cell growth and genomic stability [98]. Characterization of gene expression profiles in yeast expressing five different *BRCA1* missense variants in comparison with wild-type *BRCA1* showed changes in genes involved in a variety of pathways including transcription elongation, DNA replication and repair [96]. These effects are presumably mediated by the interactions of exogenously expressed *BRCA1* with the yeast genome and interacting proteins. Although the microarray analysis revealed differences for certain *BRCA1* variants from wild-type, extrapolations of the findings to human cells should be confirmed in human cells since yeast lacks a *BRCA1* homolog.

The tumor suppressor p53 is widely considered a master regulator gene that controls the differential expression of its target genes in a sequence-dependent manner [99]. p53 plays a critical role in the DNA damage and replication stress response to activate checkpoint control in mammalian cells. Loss of p53 function results in genomic instability, a key feature of carcinogenesis. Many genes implicated in diverse processes are regulated by p53 including (but not limited to) apoptosis, DNA repair, replication, and cell cycle progression. As a transcription factor, p53 binds to a consensus sequence in the promoter response element of its target gene and participates in transactivation in a highly coordinated fashion. The majority of p53 mutations result in single amino acid substitutions, and their molecular effects on the transactivation function of p53 have been of considerable interest. In work dating back to the 1990's, a yeast assay was developed in which human p53 was constitutively expressed in a strain that contains a p53-regulated reporter gene [100–102]. A number of p53 tumor mutations were analyzed and found to be defective in transactivation in the yeast-based assay. The Resnick lab developed an inducible and regulatable p53 expression system in yeast that enabled them to identify p53 mutants that enhanced transactivation and perturbed promoter selectivity compared to wild-type p53 [103]. Certain

novel p53 mutations that enhance transactivation of specific promoters could reactivate tumor-derived p53 mutants [104].

Additional studies provided further proof of principle that the yeast p53 system is a viable approach to acquire an analysis of the effects of p53 mutations on transcriptional activation [105, 106], genetic and epigenetic factors that influence their expression [107], and the impact of genetic variation in promoter response elements of p53 target genes [108–111]. Recent work suggests that p53 missense mutations associated with breast cancers have subtle effects on transactivation which can be detected by the sensitive yeast assay [112]. It was proposed that p53 missense mutations which vary in their effects on functionality may delineate tumors in their clinical behavior and outcome.

6. DNA Replication and Repair Nuclease FEN-1

In addition to its usefulness for characterizing clinically relevant mutations in DNA repair genes such as *XPB* (NER) or *MLH1* (MMR), yeast has been used to study conserved DNA nucleases and helicases that are important in cellular DNA replication and the response to DNA damage or replication stress. Since DNA nucleases and helicases play critical roles in the maintenance of genomic stability, the functions of these proteins help to suppress gradual cell attrition associated with aging and age-related diseases. A good example of a structure-specific nuclease necessary for genomic stability is Flap Endonuclease 1 (FEN-1), an enzyme that is implicated in Okazaki fragment processing during lagging strand DNA synthesis. FEN-1 is also important for 5' flap processing during long patch BER.

Genetic complementation assays involving human FEN-1 expressed in yeast began with UV resistance assays in *S. pombe*. The *rad2* mutant of *S. pombe* is sensitive to UV light and defective in repairing UV-induced DNA damage [113]. In addition to the UV sensitive phenotypes, *rad2* mutants display a high level of chromosome loss/nondisjunction. To identify the equivalent human gene, the Murray lab employed a PCR-based strategy with human cDNA and degenerate primers to highly conserved regions of the *rad2* gene found in both *S. pombe* and *S. cerevisiae*. The corresponding amplified fragments were used to identify a full-length human cDNA with a single open reading frame. The human cDNA cloned into a *S. pombe* expression vector was able to complement the UV sensitivity of the *rad2* null mutant [113]. Expression of the human gene which encoded FEN-1 not only restored UV resistance but also chromosomal stability of the yeast *rad2* mutant. Biochemical analysis of the *S. pombe* Rad2 protein later confirmed that Rad2 possesses 5'-flap endonuclease and exonuclease activities similar to that of the human enzyme [114]. Thus yeast can serve as a model genetic organism to characterize the functions of proteins encoded by human genes that share sequence homology with yeast genes, a theme that prevails in other DNA repair pathways. More recently, yeast genetics and biochemical studies suggested that FEN-1 helps to process stalled DNA replications forks that arise due to bulky DNA damage induced by UV light [115].

The observation that the human homolog of *S. pombe* *RAD2* gene product was important for the fidelity of chromosome segregation in yeast suggested a novel function for the structure-specific nuclease. Although FEN-1 itself has not been implicated in chromosome segregation in human cells, the sequence-related Gen1 protein belonging to the Rad2/XPG family of nucleases was isolated from human cell extracts and found to efficiently resolve Holliday junctions (HJs), an activity that is necessary for proper chromosome segregation [116]. Further characterization of Gen1 revealed that it dimerizes on HJs, which provides the two symmetrically aligned active sites for junction resolution [117]. Evidence that Gen1 resolves HJs *in vivo* was obtained from studies in which human *GEN1* was ectopically expressed in a fission yeast *mus81* mutant [118]. Here it was shown that human *GEN1*

rescued the severe chromosome segregation defect and meiotic lethality of a *S. pombe mus81* mutant. Thus human *GEN1* can substitute for yeast Mus81-Eme1 to promote meiotic crossover formation through its ability to resolve HJs. To our knowledge, this is the strongest evidence that Gen1 promotes HJ resolution *in vivo*.

An increasing number of human genetic diseases are characterized by genomic instability of repeat tract elements [119]. For example, expansion of DNA trinucleotide repeats is responsible for myotonic dystrophy, Huntington's disease, several ataxias, and fragile × syndrome. Evidence supports a model in which the trinucleotide repeats form hairpin structures that cause replication stalling, which contributes to replication slippage and ultimately expansion or contraction of the repeats. These vulnerable expandable repeats in the human genome are fragile sites that are prone to chromosomal breakage. The Zakian lab provided early evidence that deletion of the *S. cerevisiae* gene *RAD27*, which shares 85% similarity with human FEN-1, resulted in length-dependent destabilization of CTG repeats and an increase in expansion frequency [120]. Evidence supports a role of FEN-1 to prevent 5' flaps from becoming overly long and forming structures that are resistant to cleavage [121].

Yeast has served as an attractive model for studying *in vivo* the molecular functions of FEN-1. The Shen laboratory pioneered a genetics approach to study FEN-1, beginning in the late 1990's. In their initial paper, they characterized active site residues of human FEN-1 through biochemical studies of the purified recombinant proteins as well as yeast genetics complementation assays in the *rad27* null mutant strain [122]. The conserved glutamic acid (E) at position 160 in human FEN-1 that coordinates the Mg²⁺ center implicated in nucleolytic cleavage was changed to an alanine (A) or aspartic acid (D). The E160A mutant failed to complement the sensitivity of *rad27* mutant cells to a restrictive temperature shift or exposure to the DNA damaging agent MMS as measured by survival assays, consistent with its biochemical defects in cleavage efficiency determined from *in vitro* assays. In contrast, a moderately defective E160D mutant partially complemented the biological functions of Rad27. This work provided an important proof of principle that the yeast system could be used to perform structure-function studies of human FEN-1 proteins.

In addition to temperature sensitive lethality and mutagen sensitivity, the Resnick group examined the ability of human FEN-1 to rescue the genomic instability of a *rad27* mutant strain and synthetic lethal interactions of a *rad27 rad51* and *rad27 pol3-01* mutant [123]. The *rad51* strain is defective in a vital strand exchange recombinase protein implicated in HR repair. The *pol3-01* mutant is defective in the proofreading function of the replicative DNA polymerase delta. Human FEN-1 expressed in either double mutant background could rescue synthetic lethality. Human FEN-1 also complemented the genomic instability of a yeast *rad27* strain as judged by three independent assays which measured chromosomal events or ectopic interchromosomal recombination. This work, together with the studies from the Shen lab, should provide a foundation for future studies of FEN-1 polymorphisms derived from epidemiological studies. Given the interest in the linkage of FEN-1 to cancer [124], knowledge obtained from functional assays in yeast should help to understand the functional consequences of human mutations.

The genomic instability, elevated spontaneous mutation rate, and instability of micro- and mini-satellite sequences in *rad27* mutants raised the question if Rad27 (FEN-1) might have a role in the repair of DSBs in addition to its role in Okazaki fragment processing during cellular DNA replication. A search for nucleases that prevent recombination between prevalent short (less than 300 base pairs) repetitive DNA sequences revealed a role for Rad27 and its human homolog FEN-1 [125]. The elevated short sequence recombination phenotypes of a *rad27* mutant strain could be suppressed by expression of catalytically

active human FEN-1, whereas a FEN-1 mutant protein with partial endonuclease activity did not fully complement the elevated recombination phenotype. These studies advanced the model that FEN-1 helps to process recombining DSBs by endonucleolytically incising DNA intermediates that accumulate during short sequence recombination.

In addition to its nuclease domain and proliferating cell nuclear antigen (PCNA) interaction motif, a nuclear localization signal (NLS) motif resides in the C-terminus of FEN-1 and its yeast counterpart Rad27. Nuclear localization of FEN-1, mediated by the NLS sequence, was demonstrated to be cell cycle dependent and DNA damage inducible [126]. Human FEN-1 protein with site-directed mutations in the NLS failed to rescue the temperature or DNA damage sensitivity of a *rad27* mutant strain, providing evidence that nuclear localization is critical for FEN-1 to function in DNA replication and repair. In more recent work, FEN-1 was observed to accumulate in the nucleoli where rDNA replication takes place [127]. In this location, FEN-1 is proposed to resolve stalled replication forks at sites of natural replication fork barriers. This idea is interesting in light of observations that yeast lifespan correlates with rDNA stability [128]. Furthermore, it was shown that replicative age induces rDNA mitotic recombination in *S. cerevisiae* [129]. It will be important to understand the potential role of FEN-1 to suppress the mutator phenotype associated with replicative age in yeast, and if this function is conserved in mammalian cells. Although a number of studies have established a linkage of FEN-1 to cancer [124], a connection to aging has not been determined.

Yeast genetic complementation analysis suggests that FEN-1 phosphorylation controls nucleolar accumulation of FEN-1 [126]. Understanding how FEN-1 functions with its protein partners in human cells to mediate processing of replication, repair, and recombination intermediates of DNA sequences prone to be genetically unstable is an important area of ongoing study. A subsequent section in this review will discuss the use of yeast genetics to provide *in vivo* evidence that the Werner syndrome (WS) protein (WRN) is an interacting partner of FEN-1, [130, 131]. WS patients suffer from premature aging, and WRN, like its yeast homolog Sgs1, is important for the maintenance of genomic stability [132, 133].

DSBs can arise during a break-induced recombination pathway that acts to restart stalled replication forks. The single-stranded gaps that accumulate at stalled replication forks may be targeted by endonucleases to generate DSBs. The Shen lab investigated the hypothesis that FEN-1 plays a role in processing stalled replication forks [115]. Using model DNA substrates that resemble synthetic replication fork structures, they were able to show that FEN-1 possesses a novel gap endonuclease activity on such structures. Expression of an engineered FEN-1 mutant protein that specifically lacks the gap endonuclease activity but retains endonucleolytic activity on conventional 5' flap structures failed to restore the resistance of a *rad27* null strain to UV or camptothecin, which introduce DNA damage that blocks replication fork progression. From these results, the authors suggested that FEN-1 participates in break-induced recombination by processing stalled replication forks.

To better understand the role of FEN-1 to prevent the expansion or contraction of repeat tract elements, a yeast model system was devised by the Nicolas lab to examine genetic factors that regulate stability of a human minisatellite CEB1 sequence composed of 36- to 43-bp repeat arrays [134]. A 42 repeat CEB1 minisatellite was inserted into the *S. cerevisiae* genome mutated in *rad27* and the rearrangements that accumulated within a single generation were analyzed. The complexity of genomic rearrangements could be suppressed by expression of human FEN-1 in the *rad27* mutant background. The authors suggested that the accumulation of unresolved flap structures during replication was responsible for homology-driven rearrangements. Importantly, this work using the yeast system provided

evidence that FEN-1 plays a critically important role in maintaining genomic stability of repeat elements derived from human sequence that are known to be at risk for expansion or contraction.

The elegant use of yeast as a model system to investigate the molecular functions and pathways of a DNA replication/repair protein is perhaps best exemplified from the human FEN-1 studies in the *rad27* null background. Yeast genetics have provided valuable insight to understanding the protective roles of FEN-1 to maintain genomic stability. The knowledge gained from the FEN-1 yeast genetic studies provides a foundation for the study of human genetic diseases that arise from instability of genomic repeat elements.

7. RecQ Helicases

The RecQ family of DNA helicases shares an ATPase/helicase core domain conserved throughout evolution from bacteria to humans [133]. A single RecQ family member exists in bacteria and budding or fission yeast, whereas there are generally multiple representatives in higher eukaryotes. The human genome encodes five RecQ homologs, three of which have been associated with rare genetic disorders characterized by chromosomal instability [132]. The human RecQ genes *BLM*, *WRN* and *RECQL4* are mutated in Bloom's syndrome (BS), Werner syndrome (WS) and Rothmund-Thomson syndrome (RTS) respectively. *RECQL4* mutations can also lead to RAPADILINO syndrome or Baller-Gerold syndrome. The most dramatic feature of WS is the early onset of age-related diseases including cardiovascular disease, diabetes mellitus (Type II), and osteoporosis. BS is associated with an early incidence of most types of cancers that are found in the normal population. Both WS and BS cells show sensitivity to certain DNA damaging agents and display replication defects; however, their cellular phenotypes, like their clinical characteristics, are distinct from one another. A major hallmark of BS is elevated sister chromatid exchange which is not observed in WS. There has been much interest in the development of functional assays for *WRN* and *BLM* helicases *in vivo* to understand the molecular basis for these genetic disorders.

Genetic complementation studies of the *S. cerevisiae* RecQ *sgs1* and other relevant mutants with the human genes *WRN* and *BLM* have provided some insights to the conserved roles of these helicases in chromosomal DNA metabolism (Table 2). Mutation of the *sgs1* gene in yeast was observed to result in premature aging as assessed by shortened life-span, aging induced phenotype of sterility, and redistribution of Sir3 proteins from telomeres to nucleoli [135].

The *sgs1* mutants also displayed enlarged and fragmented nucleoli, hyperrecombination in rDNA loci and enhanced illegitimate recombination [27, 136]. It was suggested that old yeast cells accumulate ERCs that arise from recombinational events in the rDNA. Subsequent studies demonstrated that age-matched *sgs1* mutants displayed similar levels of ERCs compared to wild-type cells [24], suggesting that the relationship between aging and ERC accumulation may be complex and additional factors are involved. Life span analysis of *sgs1* and/or *srs2* mutants show two distinct components: early generation and late generation [25]. Late-generation *sgs1* and/or *srs2* cells senesce similarly as wild-type cells. The extension of life span observed upon deletion of *FOB1* or overexpression of *SIR2* suggested that late generation cells were aging in a manner similar to the wild-type cells. The early generation cells ceased dividing stochastically as large budded cells and displayed age-independent mitotic arrest. It was suggested that the short life span was due to an age-independent premature mitotic arrest attributable to promiscuous HR. Loss of a component of the RNA polymerase II complex, Hrp1p, also reduced life span and increased rDNA

recombination, which was not accompanied by ERC accumulation [26], suggesting a novel pathway of reduced life span.

Genetic regulation of aging in yeast may be an attractive model to explore mammalian aging. The existence of multiple human RecQ helicases, but only the singular Sgs1 yeast homolog, suggests a division of labor in the higher eukaryote; therefore yeast as a genetic system for understanding the functions of human RecQ helicases may have its limitations. Cells derived from BS and WS patients display genomic instability as shown by increased rate of chromosomal losses and deletions, suggesting elevated rates of illegitimate recombination. Both the human *WRN* and *BLM* genes could suppress the enhanced illegitimate and homologous recombination of *sgs1* [137]. However, a delineation of *WRN* and *BLM* function was suggested by observations that *BLM*, but not *WRN*, restored resistance of *sgs1* to the replication inhibitor hydroxyurea (HU) [137] and suppressed the premature aging and hyper-recombination in rDNA loci caused by *sgs1* mutation [24]. Expression of *BLM* was also found to suppress growth in the *sgs1 top3* double mutant background [137]. Recently our lab demonstrated that *WRN* could also restore the *top3* slow growth phenotype in an *sgs1 top3* background; however, *WRN* failed to complement the *sgs1* HU sensitivity [138]. *WRN* helicase, but not exonuclease activity, was genetically required for restoration of *top3* growth phenotype, demonstrating separation of function of *WRN* catalytic activities. Collectively, the yeast genetic complementation studies with human RecQ helicases suggest that they may have some overlapping but also distinct functions. For a detailed description of the yeast system used for *WRN* genetic complementation and functional assays, see [139].

Genetic and biochemical studies from a number of groups have provided evidence that Sgs1 and *BLM* helicases are involved in DSB end resection with other proteins including the Mre11-Rad50-NBS1(XRS2) complex, Dna2 nuclease, Replication Protein A (RPA), Top3-Rmi1, and Exonuclease 1 (EXO-1) [140–145]. In addition, *BLM* (in human cells) or Sgs1 (in yeast) forms a tight complex with Top3 and Rmi1, and this complex has been shown to be involved in dissolution of double HJs, functioning as a anti-recombinase to suppress the hyper-recombination characteristic of BS and *sgs1* cells [146, 147]. The Brill lab identified an N-terminal domain of Sgs1 responsible for strand exchange that is required for suppression of hyper-recombination and synthetic lethality and mediates heteroduplex rejection [148]. The highly divergent strand exchange domain of *BLM* was shown to function in yeast as well [148], suggesting that strand exchange catalyzed by either RecQ helicase mediates the strand passage events catalyzed by Top3-Rmi1.

In addition to their more general role in recombination, RecQ helicases play a role in telomere maintenance. The *S. cerevisiae* Sgs1 helicase participates in a Rad52-dependent alternative lengthening of telomeres (ALT) pathway that operates in telomerase negative mutants [149]. Expression of catalytically active human *BLM* helicase rescued the telomere defects in telomerase-negative *sgs1* [150], suggesting that *BLM* and Sgs1 have conserved functions *in vivo* to unwind similar DNA structures in recombination-mediated telomere lengthening. Mouse *WRN* could also partially suppress the telomere defect in the ALT pathway [151], leaving doubt as to the specificity of human RecQ helicase function at telomeres, as assayed in yeast.

8. Functional Consequences of Protein Interactions between RecQ Helicases and Rad2 Nucleases Revealed by Genetic Rescue Studies in Yeast

RecQ helicases are believed to maintain genomic stability by suppressing recombination events when replication or other DNA metabolic processes are perturbed by DNA damage, alternate DNA structures, or compromised DNA synthesis. Given the evidence that at least one function of RecQ helicases is to enable DNA replication to proceed smoothly and without errors, we became interested in the hypothesis that WRN (and perhaps other RecQ helicases) might interact with FEN-1 to facilitate its function in processing DNA replication and repair intermediates. Indeed biochemical studies from our lab and others have demonstrated that WRN [130, 131, 152, 153], BLM [154, 155], and other RecQ helicases [156, 157] interact with FEN-1 or the sequence related EXO-1 to stimulate nucleolytic processing of key DNA repair and replication intermediates (for review, see [158]). Yeast has proven to be a valuable system to explore the functional interactions of human RecQ helicases with Rad2 nucleases *in vivo* (Table 2).

To test the hypothesis that the WRN: FEN-1 interaction might be important during DNA replication *in vivo*, we used *S. cerevisiae* as a model genetic system based on previous work that yeast and human FEN-1 share common roles (see earlier section). To simulate a condition of replication stress, we evaluated WRN function in a *dna2* mutant that is characterized by a defect in processing of DNA replication intermediates [159]. We hypothesized that WRN might stimulate yeast FEN-1 cleavage *in vivo* to rescue the DNA replication and repair phenotypes of the *dna2* mutant. It was found that human WRN expressed in a yeast *dna2-1* mutant background rescued the associated replication and repair phenotypes; moreover, a non-catalytic C-terminal domain of WRN that mediates the physical interaction with FEN-1 was sufficient for genetic rescue, demonstrating that WRN helicase activity is not required to rescue the mutant cellular phenotypes associated with the *dna2-1* mutant [131]. Based on genetic and biochemical results, the explanation for this finding was that WRN rescued the *dna2-1* mutant by interacting with endogenous yeast FEN-1 and stimulating its nuclease activity, thereby bypassing the requirement for wild-type Dna2 nuclease to process DNA replication and repair intermediates. In a study from the Campbell lab, it was reported that expression of human BLM also complemented the *dna2-1* temperature sensitive growth phenotype [160]. Thus, it is plausible that WRN and BLM may act in the same cellular pathway(s) by using a conserved domain of the proteins to interact with common DNA intermediates and protein partners. However, the observation that a BLM-K695T ATPase mutant failed to complement the *dna2-1* cellular phenotypes [160] whereas a noncatalytic domain of WRN rescued *dna2-1* [131] suggests that WRN and BLM may act by related yet distinct mechanisms in their functional interactions with FEN-1.

Although WRN is likely to play an important role with FEN-1 in DNA processing during replication stress, it has been proposed that WRN has pleiotropic roles in DNA metabolism that are dictated by its interactions with multiple protein partners and possibly the genetic background. Previously, we reported that WRN stimulates the endonucleolytic and exonucleolytic incision activities of EXO-1 through a direct protein interaction domain of WRN that is also responsible for the FEN-1 interaction [152]. Both RECQ1 and BLM were also reported to interact with EXO-1 [154, 156]. Since overexpression of EXO-1 in yeast can compensate for the DNA processing deficiency in the Rad50-Mre11-Xrs2 complex responsible for DSB detection and signaling [161], we reasoned that WRN might stimulate the functionally conserved yeast EXO-1 to help cells deal with DNA damage or replication stress. WRN partially restored MMS resistance of *rad50* in an Exo-1-dependent manner, but failed to rescue IR sensitivity [162]. Expression of human BLM on the other hand partially

restored IR but not MMS resistance, suggesting separate roles of the two human RecQ helicases in the *rad50* mutant background [162]. According to the model, WRN in collaboration with EXO-1 acts at stalled or regressed replication fork structures, whereas BLM functions with EXO-1 to perform strand resection at the DSB [163]. It will be important to determine if this delineation of function for the WRN and BLM helicases also applies to human cells and is dependent on genetic background.

9. Summary

Yeast has served as an excellent system to study the pathways that are required for genomic stability and DNA repair. Processes that influence longevity in yeast may be conserved in higher eukaryotes and important for homeostasis. In this review, we have discussed examples of studies in which mammalian (mostly human) DNA repair genes expressed in defined yeast genetic mutant backgrounds have provided new insights to their roles in conserved eukaryotic pathways. Yeast has enabled researchers to discover and characterize the functions of human genes in classic DNA repair pathways, maintenance of genomic stability, and cellular DNA replication. Although much of this work involved genetic complementation of yeast mutants with human genes sharing sequence homology with their yeast counterparts, more recent work has focused on genetic screens to characterize potentially deleterious alleles and genetic rescue experiments which have helped to elucidate the roles of DNA repair proteins through their protein interaction domains and catalytic functions. Although the field of mammalian genetics has advanced considerably, the advantages of yeast models are still apparent and continue to provide answers to challenging questions in DNA repair that are highly relevant to aging, cancer, and age-related diseases.

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Figure 1.
Scope of using yeast as a heterologous host to study the functions of human DNA repair proteins and genome stability factors.

Table 1

Complementation of mutant yeast phenotypes by human homologs

DNA repair pathway	Yeast homolog		Human homolog	Reference
	<i>S. cerevisiae</i>	<i>S.pombe</i>		
Nucleotide excision repair	<i>RAD3</i>	<i>RAD15</i>	<i>XPB</i>	Sung <i>et al.</i> , 1993
	<i>RAD2</i>	<i>RAD13</i>	<i>XPG</i>	Murray <i>et al.</i> , 1994
Base excision repair	<i>OGG1</i>	ND	<i>hOGG1</i>	Radicella <i>et al.</i> , 1997
	<i>APN1</i>	<i>APN1</i>	<i>APE1</i>	Wilson, III <i>et al.</i> , 1995
	<i>MGT1</i>	ND	<i>MGMT</i>	Xiao and Fontanie, 1995
Recombinational repair	<i>RAD54</i>	<i>rhp54</i>	<i>RAD54</i>	Kanaar <i>et al.</i> , 1996
	<i>YEN1</i>	ND	<i>GEN1</i>	Lorenz <i>et al.</i> , 2010
Mismatch repair	<i>MSH2</i>	<i>MSH2</i>	<i>MSH2</i>	Clark <i>et al.</i> , 1999
	<i>MLH1</i>	<i>MLH1</i>	<i>MLH1</i>	Ellison <i>et al.</i> , 2001; 2004

See text for details

ND not found

Table 2

Yeast genetic rescue studies with WRN and BLM helicases

<i>S. cerevisiae</i>	Mutant yeast phenotypes	Phenotypic rescue by		Reference
		WRN	BLM	
<i>sgs1</i>	Illegitimate recombination	√	√	Yamagata <i>et al.</i> , 1998
	Hydroxyurea sensitivity	×	√	Yamagata <i>et al.</i> , 1998
	Premature aging	×	√	Heo <i>et al.</i> , 1999
	rDNA hyper-recombination	×	√	Heo <i>et al.</i> , 1999
<i>sgs1 top3</i>	<i>top3</i> phenotypes	√ ^a	√ ^b	^a Aggarwal and Brosh, 2000 ^b Yamagata <i>et al.</i> , 1998
<i>sgs1Δ slx4</i>	Synthetic lethality	ND ^φ	√ [*]	[*] Chen & Brill, 2010
<i>dna2-1</i>	Temperature sensitive growth	√ ^c	√ ^d	^c Sharma <i>et al.</i> , 2004b ^d Imamura & Campbell, 2003
	Hydroxyurea and Methylmethane sulfonate sensitivity	√ ^c	√ ^d	^c Sharma <i>et al.</i> , 2004b ^d Imamura & Campbell, 2003
	Cell cycle arrest	√ ^c	√ ^d	^c Sharma <i>et al.</i> , 2004b ^d Imamura & Campbell, 2003
<i>rad50</i>	Methylmethane sulfonate sensitivity	√	×	Aggarwal <i>et al.</i> , 2010
	Ionizing radiation sensitivity	×	√	Aggarwal <i>et al.</i> , 2010
<i>sgs1 est2</i>	Telomere defects	√ ^e #	√ ^f	^e Cohen & Sinclair., 2001 ^f Lillard & Wetherell <i>et al.</i> , 2005

See text for details

^φ not determined^{*} Sgs1-BLM95–300[#] mouse WRN