Chapter 11

Genome Stability in *Caenorhabditis elegans*

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Chapter Outline

1. INTRODUCTION

The maintenance of genome integrity is essential for a healthy life of an individual and assures reproductive success of a species. Genomic DNA is constantly threatened by a plethora of environmental and cell-intrinsic genotoxic agents that inflict a variety of DNA lesions, with thousands of events estimated to occur in each individual cell per day [1]. Failure in DNA repair can interrupt or alter gene functions, resulting in cell death, senescence, or cancer; conversely, error-prone repair in the germline is the driving force of genome evolution and intraspecies genome diversity. All organisms have evolved lesion-specific DNA-repair mechanisms to keep the genome in check: helix-distorting lesions, such as ultraviolet (UV) light irradiation-induced 6–4 photoproducts (6–4 PPs) or cyclobutane pyrimidine dimers (CPDs), are repaired by nucleotide excision repair (NER), which either operates genome-wide by instrumenting global genome NER (GG-NER), or upon RNA polymerase II stalling during transcription by employing the transcription-coupled NER (TC-NER). Intrinsic or extrinsic reactive oxygen species (ROS) induce a diverse number of oxidative lesions that are mostly targeted by base excision repair (BER) or mismatch repair (MMR). While oxidative and helix-distorting lesions and single-strand breaks (SSBs) are the most abundant type of damage, double-strand breaks (DSBs) are the most toxic form and are rejoined by the error-prone nonhomologous end joining (NHEJ) or by the high-fidelity homologous recombination (HR) repair. Beyond DNA-repair pathways, specialized mechanisms for DNA-damage signaling have been addressed in great detail, commonly referred to as DNA-damage response (DDR). Upon DNA injury, DNA-damage checkpoint signaling can pause cell cycle progression at various phases, allowing the cell time for repair or, alternatively, induce signaling events that drive the cell into apoptosis. The various DDR mechanisms are well conserved across species and a large body of knowledge rears from studies in bacteria, yeast, and mammalian cell lines.

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Genome Stability. http://dx.doi.org/10.1016/B978-0-12-803309-8.00011-2

2. THE *CAENORHABDITIS ELEGANS* MODEL

The transparent nematode *Caenorhabditis elegans* has proven instrumental in providing insights into the mechanisms underlying numerous cellular and developmental processes, including cell differentiation and apoptosis, organismal aging, host–pathogen interactions, and even molecular aspects of neurodegenerative diseases and tumorigenesis [2–7]. Since the description of *C. elegans* genetics in the 1970s by Sydney Brenner, the nematode was rapidly adopted as a powerful model organism, resulting in a fully sequenced genome, in which 60–80% of the genes have a human counterpart, the affluence of data made available at Wormbase (http://www.wormbase.org/) about gene structure, mutant and RNAi phenotypes, microarray data, transcription factor binding sites, protein–protein interaction networks, and the availability of a vast collection of mutants, for example, at the *Caenorhabditis* Genetics Center (CGC) or the National Bioresource Project for the nematode (NBRP-*C. elegans*) [8]. In 2009, the nematode was also employed as model organism in space biology to study the molecular mechanisms underlying muscle adaptation, space radiation response, and gene expression patterns at zero gravity at the International Space Station (ISS) [9]. In the laboratory, *C. elegans* is easily handled: animals feed on *E. coli* bacteria have a rapid reproductive life cycle of 2.5 days at 22°C, during which they progress through four larval stages to develop into hermaphroditic adults, which have a life span of about 2 weeks and lay 300 eggs (compare Fig. 11.1A). Upon completion of development, adults have 959 postmitotic somatic cells that comprise tissues, such as muscles, intestine, epidermis, and 302 neurons, which form a neuronal network with a fully deciphered wiring plan and largely completed connectome [10]. The dominant sexual form of *C. elegans* is hermaphrodite (XX), but males (XO) can be isolated and used for genetic crosses to produce strains carrying multiple mutations. The adult hermaphrodite reproductive system consists of two U-shaped gonad arms that contain both male and female germ cells, which undergo mitotic and meiotic cell divisions and comprise an immortal and totipotent cell lineage. Hence, *C. elegans* reproduces by self-fertilization, and populations are genetically identical and do not suffer from inbreeding depression. The advantages of a simple body plan, transparency of eggs and cuticle, and the invariance of cell divisions and developmental stages have expedited a highly detailed developmental and anatomical description of the animal, which is well documented in open access resources (eg, http://www.wormatlas.org/ and http://wormbook.org/).

As DDR is highly conserved from worms to man, *C. elegans* serves as a relevant model to study the consequences of DNA-repair deficiency [11,12]. In the nematode, DNA damage induces a vigorous response of DNA repair and signaling pathways in dividing germ cells, which is distinct in mitotic and meiotic compartments of the germline [13]. Conversely, somatic cells are entirely postmitotic and display remarkably high resistance to ionizing radiation (IR)-induced DNA damage [14]. The DDR in germ cells highlights the importance for ensuring the stable passage of genomic material through an immortal germline across generations, while somatic tissues are not important for species-survival after successful reproduction (discussed in Ref. [15]). The disposable soma theory poses that the resources of the organism need to be allocated between the soma and germline to maximize fitness before the soma can be disposed of upon reproduction (summarized in Ref. [16]). In *C. elegans*, the "germline DNA damage–induced systemic stress resistance" (GDISR) illustrates particularly well how somatic maintenance adapts to the requirements of germ cells: GDISR elevates stress resistance in somatic tissues to allow delay of progeny production until DNA damage in germ cells is repaired [17]. Hence, DDR in the nematode can be highly instructive for understanding systemic response mechanisms (discussed in Ref. [18–20]). This chapter presents a comprehensive collection of methodologies that are currently employed to study DNA repair and DDR in the nematode. Further, we provide an overview of the various well-conserved DNA repair mechanisms that are activated in *C. elegans* to counteract genomic instability. Further, we expand on recent reports that exemplify the relevance and advantages of the nematode to the field of systemic DDR during development and aging.

3. POWERFUL GENETIC TOOLS TO EXPLORE DDR DYNAMICS

C. elegans is genetically malleable by various applied methodologies (compiled in WormMethods on http://www.wormbook.org). Most commonly, forward genetic screens are implemented by creating mutant libraries through the use of mutagenic chemicals, such as ethyl methanesulfonate (EMS), or combined treatment with trimethylpsoralen (TMP) and UV irradiation that create random deletion, point mutation, and insertion events throughout the genome [21]. Pioneering studies by Hartman and colleagues in the 1980s led to the genetic identification and characterization of *C. elegans* mutants that show hypersensitivity to various genotoxic agents [22]. The advent of whole genome sequencing allows rapid analysis of single mutants or large mutant libraries and has been successfully employed to define mutation accumulation in DNA-repair-deficient *C. elegans* [12,23]. In 2014, research in the nematode profits from the successful adaptation of the CRISPR/Cas9-based genome engineering system for targeted genomic alterations (summarized in Ref. [24]).

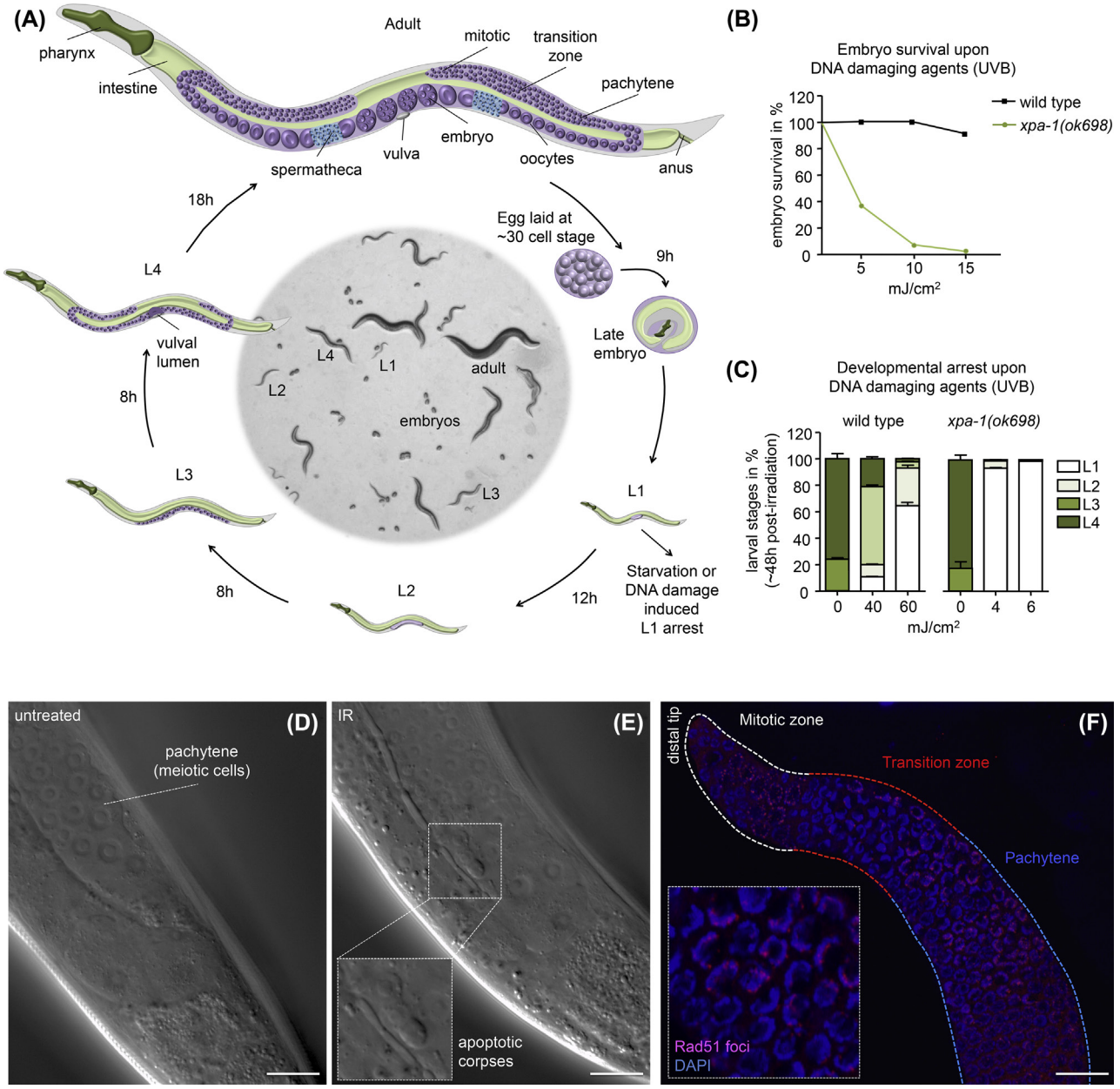

FIGURE 11.1 ***C. elegans* as a model organism to study DNA repair and DDR mechanisms.** All size bars correspond to 25 µm. (A) The *C. elegans* life cycle from fertilized embryo, through the larval stages L1–L4 until fertile adult, is completed in about 2.5 days when grown on bacteria seeded agar plates (22°C). An adult produces about 300 eggs, which require about 9 h of ex utero development before hatching of L1 larvae. Stereoscopic bright field imaging allows clear distinction of mixed *C. elegans* developmental stages on an agar plate (inlay). Detailed descriptions on *C. elegans* development from germ cell to fertile adult are available on http://www.wormbook.org and http://www.wormatlas.org. (B) Representative graph displaying embryo survival of wild type and NER mutant *xpa-1(ok698)* upon different UVB irradiation intensities. Typically, adult animals are exposed to DNA damaging insults and egg-laying and hatching rate are determined. (C) Representative result of larval development 48-h post-irradiation (UVB). Animals are first staged as L1 larvae, and then exposed to DNA damaging insults and developmental stages can be determined 48–72 h later. Note that wild type requires 10-fold UVB intensities as compared to the *xpa-1* mutant to achieve a partial developmental delay or arrest. (D) DIC image of the pachytene region of the meiotic germline in the posterior gonad arm of adult *C. elegans*. Nuclei and outline of germ cells can clearly be distinguished. (E) DIC image of the pachytene region of an animal irradiated with IR resulting in apoptosis induction of meiotic germ cells. Apoptotic corpses are clearly visible as button-like structures (inlay) (D, E, *courtesy of Najmeh Soltanmohammadi*). (F) Fluorescent microscopy image of a dissected germline stained with DAPI (*blue*) and Rad51 antibody (*red*) to visualize DSB events (compare inlay). The germline contains a clearly distinguishable mitotic zone, followed by a transition zone and the meiotic pachytene. The spatiotemporal distribution of mitotic and meiotic germ cells allows the study of distinct DNA repair or checkpoint mechanisms in response to DSB formation (F, *courtesy of Ashley B. Williams*).

Research on the nematode has pioneered the application of RNA interference (RNAi)-mediated gene downregulation, which is achieved by double-stranded DNA (dsDNA) delivery via feeding, soaking, or injection, and resulted in the compilation of genome-wide RNAi libraries [25,26]. Systematic RNAi screens have revealed whole DDR networks that govern genome stability and maintenance [11,27,28]. RNAi is highly efficient in the germline and most somatic tissues with the exception of the neuronal system, which can be overcome by the application of various RNAi hypersensitive transgenic lines or mutants [29]. *C. elegans* transgenes can be rapidly and cost-effectively obtained by genetic transformation through DNA microinjection or DNA-coated microparticle bombardment, allowing the introduction and stable inheritance of exogenous DNA into the genome [30]. A collection of DDR-specific genes can be obtained at the "*C. elegans* TransGeneome" project, a genome-scale transgenic project for fluorescent- and affinity-tagged proteins [31]. Transcriptome and proteome profiling, of whole animals or specific tissues, is standardized in *C. elegans* and has helped to unravel the dynamics of DDR during development and aging in DNA repair deficient mutants or upon DNA-damage induction (eg, see Refs. [32–34]).

4. GENOTOXIC AGENTS FOR DNA DAMAGE INDUCTION

A number of procedures for *C. elegans* are available to evoke genome instability, which in turn mounts a lesion-specific response [35]. UV irradiation is applied to study NER in the soma and the germline, since it results in the formation of bulky photolesions 6–4 PP and CPDs. UV-B (320–290 nm) displays a higher penetrance and thus induces DNA damage throughout the animal as opposed to UV-C (290–100 nm), whose shorter wavelengths are absorbed by water and cellular biopolymers [36]. In addition, UV-induced lesions indirectly give rise to DSB formation, for example, when replication forks break down at unrepaired CPDs and can lead to apoptotic demise of germ cells [37]. UV-A (320–400 nm) mostly damages DNA indirectly through the formation of ROS, which results in the mild induction of germ cell apoptosis and DNA-damage checkpoint activation [38]. Most frequently, IR (X-rays or γ irradiation) is used to cause DSB formation and chromosome rearrangements, which triggers a highly reproducible response of DSB repair, cell cycle arrest, and apoptosis in germ cells (see Figs. 11.1D–F) [39].

Except electromagnetic waves, various chemicals can be used to induce DNA damage in *C. elegans*. Photosensitizers, such as ethidium bromide or bromodeoxyuridine, or the light-sensitive cross-linking agent TMP, enhance the genotoxic havoc caused by UV irradiation [40]. Alkylating agents, such as EMS or MMS, are highly potent mutagens but have been less frequently employed in *C. elegans* to study specific DDR [41–43]. Hydroxyurea (hydroxycarbonate) is known to destabilize the replication fork, thus resulting in DSBs in mitotic germ cells [44]. Illudins are chemical compounds that induce DNA lesions that hamper with transcription and also in the nematode require TC-NER for their removal [45].

Metals, such as silver nanoparticles and cadmium, cause measurable oxidative damage to DNA and result in mitogen-activated protein kinase (MAPK)-dependent germline apoptosis, respectively, though the exact nature of DDR remains to be determined [46,47]. Other prooxidant compounds, including paraquat, sodium azide, or menadione bisulfite, can cause 8-oxo-G formation, which are predominantly cleared by BER [48]. In addition, several studies also show decreased tolerance of NER-deficient animals to oxidative stress [49,50]. In addition, some mitochondrial mutants display hypersensitivity to oxidative stress and accumulate oxidative lesions in their genome [51]. Generally, *C. elegans* exposure to most chemical compounds produces phenotypic readouts that are easily scored, qualifying the nematode as a high-throughput platform for environmental genotoxins [52].

5. METHODS FOR DNA DAMAGE DETECTION

Genome instability in *C. elegans* manifests in various morphological, developmental, and behavioral phenotypes that can be scored in vivo, additionally several in vitro methods are available to directly visualize and quantify various DNA lesions (see Fig. 11.1). The significant differences between repair and response to DNA damage in mitotically and meiotically dividing germ cells as compared to the postmitotic somatic cell types allow the study of tissue-specific as well as systemic DDR.

The germline is easily discerned from somatic tissues by differential interference contrast microscopy (DIC; or Nomarski microscopy) and clearly separated in a distal mitotic zone, where germ stem cells proliferate, and the transition zone, in which germ cells enter meiosis prophase I (Fig. 11.1). Meiotic recombination is completed by late pachytene, before germ cells progress through the diplotene until oocytes arrest in diakenesis, and resume meiosis only upon fertilization in the spermatheca upon which embryogenesis commences in the uterus (see sketch in Fig. 11.1A). Physiological germ cell death occurs in the late pachytene and is enhanced in response to IR or UV, which can be scored via DIC, since apoptotic corpses display a distinct cellular morphology (see Fig. 11.1D–F) [13]. Similarly, cell cycle arrest is easily visualized and

quantified in the mitotic region, since size and number of mitotic germ cells is significantly altered upon DNA damage [53]. DNA-damage checkpoint activity can also be quantified during early embryogenesis by monitoring the asynchronous cell divisions, which are timed during checkpoint activation [42]. Genetic screens based on those phenotypes have contributed to the identification of comprehensive genetic pathways regulating DNA damage–induced apoptosis and checkpoints (summarized in Ref. [54]).

C. elegans lays a defined number of 300 eggs and develops through four well-timed larval stages before reaching adulthood. DNA-damaging insults can significantly reduce offspring number and viability, and delay developmental timing, allowing the distinction between germline and somatic DDR (see Fig. 11.1B,C) [55]. Further, germline development can be genetically suppressed to distinguish between somatic and germline-specific repair [32]. The accumulating effect of DNA damage can be quantified by monitoring animal survival or tissue decline, for example, gross morphology, muscle function in the pharynx or locomotion, in individuals of a defined population from birth to death [56–58].

DNA repair can be directly assessed by immunological methods: antibodies are available for specific lesions (eg, 6-4 PP or CPDs), and can be applied to genomic DNA extracts via slot blot or whole-animal or tissue-specific immunostaining [59]. However, both cuticle and egg shell of the nematode represent barriers for staining that need to be disrupted chemically and/or mechanically. DAPI and BrdU staining to visualize DNA or newly synthesized DNA, respectively, are highly efficient in fixed or alive *C. elegans* [60]. The dissected germline is easily accessible for immunostaining, and DSB-repair processes can be visualized with anti-RAD-51 or anti-CDK-1 phophotyrosine antibodies (see Fig. 11.1F) [61]. Complexes of DNA-repair factors and DNA molecules can be analyzed by electrophoretic mobility shift assays (EMSA) and further resolved by immuno-gold electron microscopy [62]. Fluorescence in situ hybridization (FISH) is readily applied to study chromosome-pairing events upon DNA damage [63].

Several protocols implement quantitative PCR (qPCR) to quantify DNA damage across the genome by exploiting the capacity of many lesions to block or inhibit the progression of DNA polymerases [32,64]. In addition, this method allows the distinction between nuclear and mitochondrial DNA damage, but is not lesion specific [58,65]. The mutation accumulation across many generations is promoted in DNA-repair-deficient animals, which is easily assessed in *C. elegans* due to its rapid reproductive cycle and the availability of transgenes carrying balancer chromosomes that allow for morphological read-outs of mutations [66].

6. EXCISION REPAIR

Three major excision repair pathways are highly conserved between worms and man: NER, BER, and MMR [67]. The importance of NER, BER, or MMR activity for genome stability maintenance has been demonstrated by experiments that follow mutation accumulation over many generations and has revealed that particularly MMR protects the genomes from mutations, followed by NER and eventually by BER [68,69]. However, the most comprehensive studies on single-stranded DNA repair have been performed on *C. elegans* NER and are discussed further in more detail.

6.1 Nucleotide Excision Repair

NER removes bulky nucleotide lesions 6–4 PPs, CPDs, and their Dewar valence isomers, which can be induced upon irradiation with UVB and UVC [70]. Already in the 1980s, the analysis of radiation-sensitive (*rad*) mutants revealed a significantly reduced repair capacity for 6–4 PPs and CPDs in *rad-3* mutants [71]. Indeed, almost two decades later, genome sequence analysis for NER homologs and RNAi-mediated gene knock-down revealed that *rad-3* encodes the *C. elegans* homolog of the mammalian xeroderma pigmentosum complementation group A (XPA) [72,73].

Based on studies in *Saccharomyces cerevisiae* and mammalian systems, NER can be divided into four consecutive steps, in which the lesion is first detected, which triggers the recruitment of factors necessary for unwinding the DNA strand, followed by excision of the area containing the damage and finalized by filling the gap through DNA synthesis and ligation. NER is initiated by two distinct mechanisms of DNA-damage detection, which activate the same downstream core machinery to repair the damage: (I) transcription-coupled NER (TC-NER) is activated by stalling of RNA polymerase II during transcription and requires the recruitment of the chromatin remodeling protein Cockayne syndrome protein B (CSB) and Cockayne syndrome protein A (CSA); (II) global genome NER (GG-NER) is initiated upon lesion detection by the UV-damaged DNA-binding protein (UV-DDB) complex and xeroderma pigmentosum group C (XPC), which subsequently recruits several other NER proteins and removes lesions throughout the genome [74]. In humans, inherited mutations in GG-NER genes result in xeroderma pigmentosum (XP), which includes severe UV sensitivity and an increased risk for skin cancer; TC-NER deficiency causes Cockayne syndrome (CS), which is characterized by a variety of neurodevelopmental symptoms and premature aging [75].

Experimental efforts during the last decade have enlarged the group of mammalian NER orthologs in *C. elegans*: a number of mutants for homologs of *CSB, CSA, XPC, XPF, XPG, RAD23,* and *ERCC1* have been isolated, all of them displaying increased sensitivity to UVB [45,55,73,76]. These findings paint the convincing image of a well-conserved NER pathway in *C. elegans*, making the nematode an increasingly important model for studying in vivo NER activity in a time-dependent fashion and in the context of a whole organism [15].

Particularly important is the discovery that the GG-NER and TC-NER sub-pathways perform differential tissue-specific roles in response to UV irradiation: animals carrying mutations in genes of the core NER machinery (*xpa-1*, *xpf-1,* and *xpg-1*) and specifically in the GG-NER sensor *xpc-1* show severely decreased germ cell and embryo survival, and a diminished CPD repair capacity upon UV irradiation [55]. Similar effects are apparent upon loss of RAD-23 that is homologous to HR23A and HR23B, which are responsible for stabilizing and enhancing the binding of XPC in mammalian cells [77]. Conversely, loss of CSB-1 or CSA-1 does not result in germline-specific defects upon UVB irradiation but significantly reduce larval development timing and survival [45,55,56]. However, some studies report partial redundancy for the tissue-specific NER repair function: depending on the UV dose and source applied, CSB-1-deficient animals can display increased levels of germ cell apoptosis, morphological abnormalities, and decreased hatching rates [78]. In summary, GG-NER is the major pathway mediating UV response in early development, germ cells and embryos, while TC-NER mediates somatic repair in juvenile and adult animals [55].

6.1.1 The Role of NER in Development and Aging

In human cell lines, moderate UVB and UVC irradiation (<100 mJ/cm^2 UVB or <1 mJ/cm^2 UVC) result in a transient decrease in cell division and DNA replication activity, whereas higher doses of UV irradiation lead to a permanent arrest of DNA replication [79–81]. UV irradiation of repair-proficient *C. elegans* at L1 larval stage (at which worms can be synchronized to allow assessment of developmental growth on a large scale) causes delayed larval development, while NER deficiency exacerbates UV sensitivity during development (see Fig. 11.1C) [56]. Interestingly, TC-NER is particularly important for withstanding UV-induced lesions during development [55]. Most cell divisions in the development of *C. elegans* occur already during embryogenesis after which differentiated cells mostly grow in size. During active transcription, UV lesions lead to the stalling of RNA pol II and the subsequent degradation of its subunit AMA-1, which is mediated by the putative E3 ubiquitin ligase WWP-1, ortholog of the yeast Rsp5 [73].

TC-NER deficiency in humans leads to the devastating disease of CS that is characterized by developmental growth retardation and a variety of premature ageing symptoms [75]. Mammalian cells respond to DNA lesions that stall RNA polymerase II-mediated transcription by downregulating the growth hormone receptor (GHR) and the insulin-like growth factor-1 receptor (IGF-1R), which in turn promotes IGF-1 resistance and defense to cellular oxidative stress [82]. Both GHR and IGF-1R are regulators not only of postnatal growth, but also of the aging process as mice with reduced GH/IGF-1 signaling show dwarfism and extended life span [83]. Similarly, defects in the IGF-1R homolog *daf-2* lead to greatly extended longevity in *C. elegans* [84]. Ever since the discovery of the *daf-2* mutant longevity, *C. elegans* has served as an important model for the genetics of aging [3].

In 2014, a crosstalk between NER and the insulin/insulin-like growth factor signaling (IIS) in *C. elegans* had become apparent. In the nematode, the conserved IIS pathway is a major regulator of starvation-induced L1 arrest, development, stress resistance, and lifespan [85,86]. Key players of the IIS in the nematode are the insulin/IGF receptor DAF-2 that, upon self-phosphorylation, recruits the phosphatidylinositol 3-kinase (PI3K) subunit AGE-1, which generates phosphatidylinositol-3,4,5-triphosphate (PIP3) molecules that activate kinases of the AKT family. The latter phosphorylates DAF-16, member of the FOXO transcription factor family [87]. DAF-16 remains inactive in its phosphorylated form in the cytoplasm. When the insulin signaling cascade is inactive, DAF-16 is hypophosphorylated and localizes from the cytoplasm to the nucleus, where it governs the transcriptional regulation of a plethora of genes regulating aging, stress response, metabolism, thermotolerance, and pathogen resistance [88]. Thus, knockout of *daf-2* or *age-1* results in the constitutive DAF-16 nuclearization and increased stress resistance and life span (summarized in Ref. [89]).

Transcriptome analysis of UV-treated wild-type and arrested *xpa-1* mutant L1 larvae has revealed that, similar to mammals, IIS is attenuated in response to UV-induced DNA damage. DAF-16 is efficiently activated upon DNA damage during development while its responsiveness, specifically to DNA lesions, declines with aging. Functionally, DAF-16 activity alleviates developmental arrest and enhances somatic tissue functionality in response to UV-induced DNA damage [56]. It was suggested that the longevity assurance factor DAF-16 might thus antagonize DNA damage–driven aging by enhancing tolerance of genotoxic stress [20].

DAF-16 acts in specific tissues to execute differential outputs, which is governed by a number of cofactors and coregulators, including the heat-shock factor HSF-1 and the Nrf-like transcription factor SKN-1 (summarized in Ref. [90]). The

GATA transcription factor EGL-27 genetically interacts with DAF-16 to promote both longevity and stress response [91]. Importantly, upon UV damage induction, DAF-16 functions together with EGL-27 to mount the DDR in *C. elegans*, which does not require HSF-1 or SKN-1 activity [56].

Intriguingly, transcriptomic and proteomic profiling of *xpa-1* mutants shows increased induction of antioxidant defenses and higher ROS levels, in comparison to wild type, which might indicate elevated levels of oxidative DNA damage [34,92]. Surprisingly, loss of *ercc-1* or *xpf-1* prolongs the lifespan of long-lived *daf-2* mutants, which was suggested to rear from an active signaling by DNA damage–detection proteins to implement a hormetic response that promotes survival [57]. An alternative explanation is that meiotic defects in *ercc-1* and *xpf-1* mutants might influence the lifespan of *daf-2* mutants that display compromised egg-laying activity [55,84,93–95].

6.1.2 NER Deficiency in Mitochondrial Diseases

Mitochondria are the powerhouse of the cell and contain their own 16.5 kb genome (mtDNA) that cooperates with the nuclear genome (ncDNA) to encode the proteins of the OXPHOS system [96]. mtDNA is subjected to environmental toxins, and exogenous or endogenous ROS, which is typically repaired by BER (summarized in Ref. [97]). To date there is no conclusive evidence for NER activity in mitochondria leaving them incapable of repairing CDPs or 6-4 PPs in mtDNA and highly susceptible to UV irradiation. Thus, UV-induced lesions potentially persist and stall DNA replication and transcription, and lead to the depletion of mtDNA and mitochondrial proteins, resulting in mitochondrial breakdown [98]. In primary human fibroblasts and in *C. elegans*, UVC-induced lesions in mtDNA are removed by clearing mitochondria via autophagy, mitophagy, and mitochondrial fission and fusion events [99,100]. Findings in 2012 in mice indicate a presence of CSB in mitochondria, where it might act as DNA-damage sensor, signaling the clearance of mitochondria, with damaged genome, by autophagy [101]. Mitochondrial dysfunction is associated with a large number of human neurodegenerative disorders, including the major DNA-repair disorders CS, ataxia–telangiectasia (AT), and XP, which can be qualitatively and quantitatively predicted in silico by specifically designed databases for mitochondrial pathologies [101–103]. Importantly, XPA deficiency results in a well-conserved mitochondrial decline, which is induced by the activation of poly-ADP-ribose polymerase-1 (PARP-1) [104]. PARPs and poly(ADP-ribose)glycohydrolases (PARGs) perform the posttranslational modification poly(ADP-ribosyl)ation upon NAD^+ consumption, which regulates cellular stress responses by mediating DNA repair, chromatin structure, DNA synthesis, apoptosis, and mitochondrial homeostasis [105]. *C. elegans* carries three homologs for PARPs and two homologs for PARGs that maintain a conserved function in DNA repair [106,107]. In addition, studies in cells and the nematode reveal that PARP activity reduces NAD^+ availability, which in turn suppresses the sirtuin-signaling (SIRT1) pathway, a known regulator of proper mitochondrial homeostasis under stress conditions. This intricate mechanism can be attenuated by supplementing PARP inhibitors or NAD^+ precursors, which leads to an activation of the mitochondrial unfolded protein response (UPR^{mt}) and consequently a boost of mitochondrial function resulting in increased health and life span [108]. XPA deficiency in cells, mice, and *C. elegans* trigger a similar pathway: overactivation of the DNA-damage sensor PARP drains cells of NAD^+, suppresses SIRT1, and leads to defective mitophagy, which might explain the neurodegenerative phenotypes in CS, AT, and XP patients [104]. The pathway is analogous in CSB mutant cells, nematodes, and mice: PARP inhibition or treatment with NAD^+ precursors increases SIRT1 expression, rescues the shortened life span of *csb-1* mutant *C. elegans* and restores metabolic, mitochondrial, and transcriptional alterations in *Csb*-deficient mice. In a similar fashion, high fat diet induced by supplementing the ketone β-hydroxybutarate (β-OHB) rescues the reduced life span of *csb-1* mutant nematodes [109].

In summary, TC-NER deficiency leads to neurodegenerative phenotypes that are linked to mitochondrial dysfunction and involve conserved mechanisms. *C. elegans* is a highly versatile system and suitable for high-throughput drug screenings, which will be a relevant feature for future therapeutic target identification in NER-deficiency disorders.

6.2 Base Excision Repair

The recognition and excision of oxidized nucleotides in BER is executed by DNA glycosylases that cleave the *N*-glycosolic bond between the DNA base and the sugar phosphate backbone. Subsequently, the baseless site (apurinic/apyrimidinic site or AP site) is transformed into an SSB by the activity of an AP endonuclease, leaving a 3′-hydroxyl and 5′-phosphate gap that is filled by the DNA polymerase β (Pol β) (summarized in Ref. [110]). To date, 11 human damage-specific DNA glycosylases are known and a branchy BER system in mammals is resolved in great detail [111].

In *C. elegans*, only two DNA glycosylases have been identified, the uracil-DNA glycosylase homolog UNG-1 and the DNA N-glycosylase homolog NTH-1 [112,113]. Transcriptome analysis revealed that NTH-1 deficiency activates oxidative stress response and lowers IIS activity, which does not result in a clear phenotype related to oxidative DNA damage, such as resistance to oxidative stress or altered life span [92]. Activity measurements of UNG-1 in *C. elegans* embryonic

protein extract demonstrate its capability to specifically cleave U:G mispairing, which requires the Mg^{2+}-dependent hydrolytic AP endonuclease EXO-3 [114,115]. There are two AP endonucleases homologs in the nematode, EXO-3 and APN-1, and cross-species complementation studies show the rescue of DNA-repairdeficiency in yeast [115,116]. Both AP endonucleases are known to differentially function in the MMR pathway to induce toxicity in response to the cancer therapeutic 5-Fluorouracil (5-FU): EXO-3 is required for RPA-1 filament formation, indicating its requirement for MMR activation, while APN-1 acts in checkpoint activation [117]. Furthermore, APN-1, but not EXO-3, is specifically required for resistance to a variety of DNA-damaging agents, including UVC, oxidative stressors *tert*-butylhydroperoxide (*tert*-BH) and H_2O_2, and the methylating agent MMS [118].

In the *C. elegans* genome, no homolog for Pol β could be identified but BER is finalized by the gap-filling activity of a Pol β polymerase homolog, which acts as a lesion bypass polymerase and a backup BER polymerase in vertebrate systems [119]. Thus far, the *C. elegans* BER mechanism appears to be of much lower complexity as compared to mammalian systems, and BER deficiency generally results in relatively mild stress response or age-related phenotypes [120].

7. MISMATCH REPAIR

Major advances in defining MMR activity come from studies in *E. coli*, but the mechanisms of this repair machinery are conserved between bacteria and eukaryotes. MMR removes mismatched base pairs, mainly resulting from replication errors, and is therefore critical for DNA replication accuracy and genome stability maintenance across generations, which is highlighted by increased cancer occurrence in MMR-deficient humans [121].

C. elegans expresses orthologs of the central human MMR factors MSH2, MSH6, MLH1, and PMS2. MMR deficiency results in elevated somatic DNA instability and germline mutagenesis, when monitored across 40 generations in transgenes carrying a heat shock-promoter-driven frame-shifting DNA repeat that interrupts *LacZ* expression, which upon mutation events, can shift back in frame, resulting in a *LacZ*-positive readout [27,66]. Similar in-depth approaches relying on PCR-based genome analysis confirmed this observation [68,122].

Thus far, with the exception of the above-mentioned crosstalk between BER and MMR, the nematode has been less intensely utilized to elucidate mechanistic information on MMR activity [117]. However, 2015 results demonstrate that MLH-1-deficient animals display decreased meiotic cell death in the germline upon DNA damage, and an elevated resistance to alkylating and oxidizing agents. Further, MMR induces autophagy-mediated cell death of nondividing adult somatic cells independently of the checkpoint signaling factor ATL-1 [123].

8. DOUBLE-STRAND BREAK REPAIR IN *C. ELEGANS*

DNA DSBs are considered to be the most toxic form of DNA damage: in yeast, a single DSB can lead to cell cycle arrest or cell death, if left unrepaired [124]. The impact of DSB-repair deficiency in humans is highlighted by the appearance of disorders including cancer predisposition and infertility [125]. DSB repair is of high importance for the production of germ cells which must carry the correct genetic material to the next generation. Uncontrolled DSB repair can lead to tumorigenesis, previously reported in mice and humans, or serious inborn diseases due to chromosomal aberrations [54,126].

Cells employ different DNA damage–response (DDR) mechanisms depending on the nature of DSBs: breaks in the DNA can be caused upon exposure to IR or stalling of DNA replication forks [127]. DSBs most commonly occur during the production of gametes in the process of meiotic recombination (compare Fig. 11.1F). Depending on the cause leading to DSBs, different intermediates and substrates are formed, which are target of a delicately balanced array of repair proteins [128].

The two major mechanisms for DSB repair are homologous recombination (HR) and nonhomologous end joining (NHEJ). HR is characterized as an error-free method, which uses the sister chromatid or homologous chromosome as an undamaged template for repair [126,129,130]. In *C. elegans*, HR functions in DSB repair in proliferating somatic cells during early embryogenesis and remains active in germ cells during adulthood [129]. NHEJ is known for its efficient, yet error-prone repair that joins damaged DNA ends regardless of homology, which can result in the addition or removal of nucleotides [126,129–131]. NHEJ functions predominantly in somatic cells in *C. elegans* starting from late embryogenesis [15,129]. Various studies report that NHEJ and HR can cooperate, compete or act in parallel to repair DSBs, as evidenced by experiments where HR is impaired in germ cells or somatic cells, but not after IR [11,132–137].

8.1 Homologous Recombination

HR repair in *C. elegans* has been extensively studied during meiotic recombination, which takes place in the transition zone of the gonad [15]. Fig. 11.2 summarizes the HR-repair mechanism and functions of known homologs in *C. elegans*.

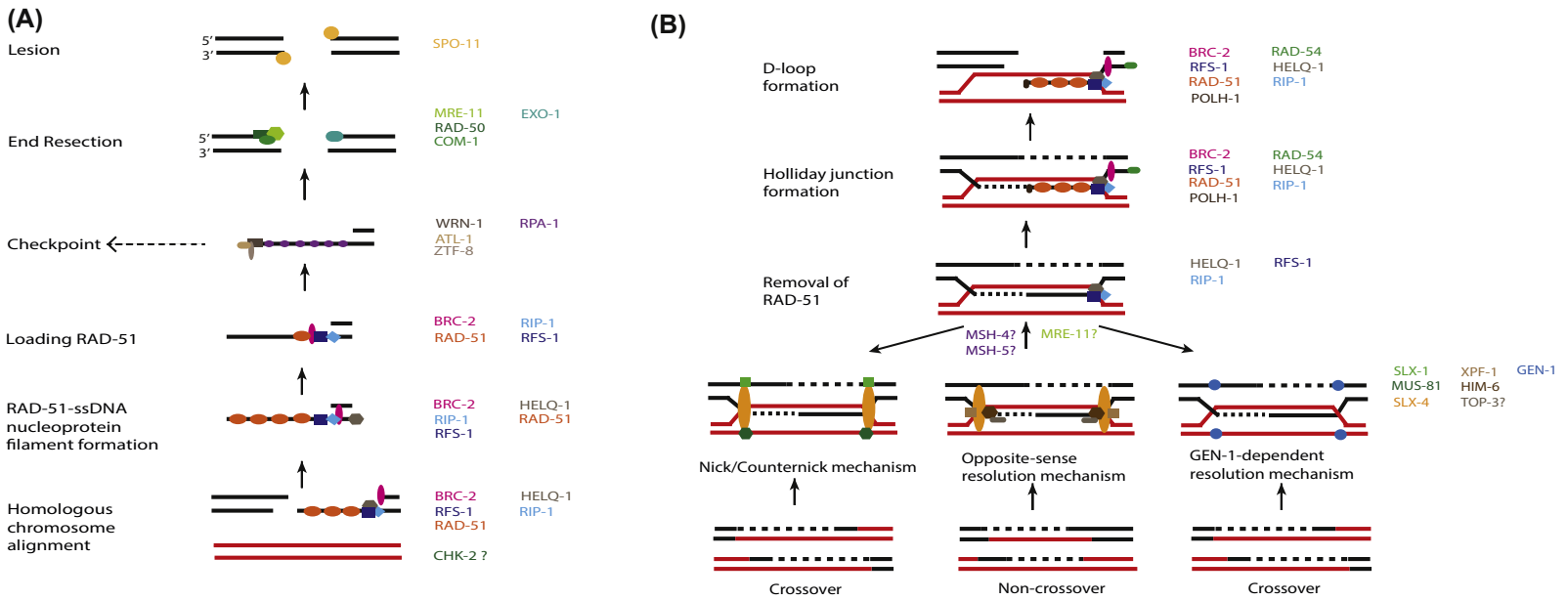

FIGURE 11.2 Schematic diagram of the *C. elegans* homologous recombination pathway. All colors of the proteins match the colors of the names of the proteins (A) Homologous recombination starts when the protein SPO-11 introduces breaks around the double-stranded DNA damage. The MRN complex composed of MRE-11, RAD-50, and COM-1, and the nuclease EXO-1 resect the DNA producing an area of ssDNA. The ssDNA is bound by the replication protein A (RPA), which is later replaced by RAD-51 with the help of BRC-2. Proteins, such as WRN-1, ATL-1, and ZTF-8 also act in the HR pathway; however, they have been found to function at the divergence point between checkpoint repair and DSB repair. Once the RAD-51-ssDNA nucleoprotein filament is formed, CHK-2 is speculated to mediate the alignment of the damaged chromosome with a template chromosome. (B) Strand invasion is mediated by a number of proteins: the helicase HELQ-1, the complex formed by RFS-1 and RIP-1, the translesion synthesis polymerase POLH-1, and the recombination protein RAD-54. After strand invasion, a D-loop is formed, followed by a Holliday junction structure. Once new DNA has been synthesized, the DNA is marked by MSH-4 and MSH-5 for resolution by nucleases. The Holliday junction is then resolved to form CO and NCO products. Three different mechanisms have been found at this step: the nick/counternick mechanism involves the scaffold protein SLX-4 acting as a platform for the interactions of SLX-1 and MUS-81, and produces CO in the chromosomes. The opposite-sense resolution mechanism involves, again, SLX-4, as a platform for the interactions of XPF-1 and HIM-6, and the topoisomerase TOP-3 also plays a role, although its functions have still to be elucidated. This mechanism results in chromosomes showing no CO events. The GEN-1-dependent resolution mechanism is known to produce CO events independent of SLX-4. MRE-11 is also involved in the next step of chromosomal CO, although its function is still unclear.

During meiosis I, before recombination, homologous chromosomes must first align with their pairs and associate via the synaptonemal complex (SC) [138]. Subsequently, the topoisomerase II-like enzyme SPO-11 introduces around 11–12 meiotic DSBs per nucleus in a programmed fashion [138,139]. The area around the DSBs is then resected by an endonuclease activity of the MRN complex, which causes the release of SPO-11 and the formation of a 3′-single-stranded DNA (ssDNA) tail [140,141]. This ssDNA tail is bound by replication protein A (RPA) that is later replaced with RAD-51 proteins, which are recombinases with individually weak activity [142,143].

The protein complex consisting of MRE11, RAD50, and NSB1 (MRN complex) in mammals, or Mre11, Rad50, and Xrs2 (MRX complex) in yeast, derives its name from the proteins required for meiotic cell division. Presently, *C. elegans* homologs for MRE-11, RAD-50, and a putative candidate that shares significant homology to the human NBS1 protein have been identified [129,138,140]. Apart from initiating DSB repair by resecting the damaged DNA strand, MRE-11 is involved in downstream processes of repair, which are crucial for chromosomal crossover. In addition, MRE-11 plays a role in NHEJ [144]. In 2013, Lemmens and colleagues identified the 5′ to 3′ acting nuclease EXO-1 to be involved in DSB repair [126]. The proposed model predicts that bidirectional DNA end resection takes place with MRE-11 starting resection in a 3′ to 5′ direction and allowing the efficient recruitment of EXO-1, which facilitates resection from 5′ to 3′. The MRN complex only aids in the recruitment of EXO-1 during the early prophase I of meiosis, and not in later stages of cell division [130].

Further, the model suggests that MRE-11 causes the release of SPO-11 and, together with the meiotic recombination factor COM-1, triggers the release or blockage of the protein complex Ku, which consists of CKU-70, an ortholog of human XRCC6, and CKU-80, an ortholog of human XRCC5. This mechanism allows HR to repair up to 97% of all the meiotic DSBs and suppresses compensatory NHEJ activation [126,130]. Ku proteins have been suggested to be toxic when unbound, as they can cause chromosomal aggregates and cause a decrease of crossing-over events and thus of meiotic recombination. Despite Ku protein toxicity, germline cells in *C. elegans* have a bias of at least one hundred-fold toward HR compared to NHEJ repair [126].

The *C. elegans* gene rdh-1/rad-51 codes for RAD-51 protein monomers, which bind the damaged DNA and form a nucleoprotein filament that is responsible for two functions: finding a homologous template and invading the homologous DNA strand [138,140,141,145]. RAD-51 activity requires BRC-2, the *C. elegans* homolog of the human breast cancer type 2 susceptibility protein BRCA2 [137]. BRC-2 transports RAD-51 from the cytoplasm to the nucleus, where it is recruited to the sites of DSBs, and loaded onto the ssDNA. RAD-51 function depends on BRC-2 activity but not vice versa. BRC-2 has been shown to interact with the RAD-51 paralog RFS-1, which mediates binding the DSB and displacing RPA, before recruiting RAD-51 onto the DNA [146].

Upon binding, RAD-51 proteins convey the ssDNA to invade the homologous double-strand DNA (dsDNA), which will be used as a template for synthesis of new DNA on the resected end of the invading ssDNA. This invasion causes the formation of a D-loop structure, which is mediated by BRC-2 and the translesion synthesis polymerase POLH-1 [147]. RAD-51 is also known to catalyze ATP hydrolysis, which leads to the depolymerization of nucleoprotein filaments. This process is suppressed by BRC-2 activity [137,140,141]. After completion of DSB repair, the RAD-51-like protein RFS-1 acts together with the helicase HELQ-1 to release RAD-51 from the DNA by directly interacting with the DNA via distinct mechanisms [146].

RFS-1 and HELQ-1 display additional functions: RFS-1 has the prorecombinogenic role of loading RAD-51 onto ssDNA, while HELQ-1 stabilizes the ssDNA-RAD-51 filaments prior to strand invasion [146]. In addition, RFS-1 stabilizes the HR mechanism in replication fork barriers during S-phase by mediating the loading of RAD-51 to one-ended DSBs, that occur upon replication fork regression, and to ssDNA stalled replication forks [128]. RFS-1 activity requires complex formation with RIP-1 (RFS-1 Interacting Protein), which is crucial for optimal HR function in *C. elegans*. In addition, this complex stimulates the recombinase activity of RAD-51, which in turn remodels and stabilizes RAD-51-ssDNA filaments to take a more flexible conformation. Thereby, RFS-1/RIP-1 facilitates the search for a homologous DNA template and strand exchange with the template via displacement loop (D-loop) formation [62]. In yeast, RAD-51-mediated strand exchange, cross-bridging double-stranded DNA, and remodeling the chromatin to facilitate for HR repair, is aided by RAD-54, which is proposed to be conserved in *C. elegans* [146]. Subsequently, the D-loop structure induces a so-called “double Holliday junction” (dHJ or HJ), and later a crossover (CO) that is visible as a chiasmata and needed for the continuation of meiosis. Studies in *C. elegans* show that interhomolog crossovers are tightly regulated, limiting the occurrence to one crossover event between homologous chromosomes, while the other DSBs are repaired as non-crossovers [93,148].

Both HJ and CO formation are dependent on meiosis-specific members of the MutS homolog family, namely HIM-14/MSH-4 and MSH-5 proteins in the nematode [140]. Some evidence shows that MSH-4 and MSH-5 mark DSBs for resolution by nucleases but their exact functions have not been elucidated [148]. Another important element in DSB repair is

the checkpoint kinase CHK-2, which is speculated to mediate alignment of homologous chromosomes and act epistatic to RAD-51, thus influencing the formation of the chiasmata [63].

COs can form via the opposite-sense resolution of double Holliday junctions or via a "nick/counternick" mechanism [149]. COs at collapsed replication forks in mitotic and meiotic nuclei are resolved by a protein complex consisting of the scaffold protein SLX-4 (other name: HIM-18), which acts as a platform for the coordination of multiple enzymes involved in processing recombinant intermediates [95,149]. SLX-4 either interacts with the endonucleases SLX-1 and MUS-81 involved in the "nick/counternick" mechanism, or the ATP-dependent helicase HIM-6 and the endonuclease XPF-1, the *C. elegans* ortholog of ERCC4, involved in the "opposite-sense resolution" of HJs [93,95]. SLX-1 and MUS-81 have a different substrate preference as compared to XPF-1 [148]. According to the "nick/counternick" model, SLX-4 coordinates a symmetrical cleavage, in which SLX-1 nicks HJs that are subsequently processed by MUS-81 endonuclease [93]. Conversely, the "opposite-sense resolution" pathway employs HIM-6 to unwind thermodynamically unstable HJs, while XPF-1 acts as an HJ-nicking enzyme [150]. In addition, HIM-6 is important for recombination initiation, for which it requires interaction with the nuclease DNA-2 [95].

Consistently, MUS-81 acts redundantly to HIM-6 in limiting the accumulation of double HJs during early meiosis, and to XPF-1 in the production of interhomolog crossovers. MUS-81 also has a nonredundant but overlapping role with SLX-4 in processing recombinant intermediates, such as dHJs. Several studies show that SLX-1 regulates CO distribution along the chromosomes by locally suppressing the formation of COs at the center of the chromosomes via "same sense resolution" of HJs to produce non-crossover products, or via synthesis-dependent strand annealing [93,148–150].

In a parallel pathway, the *C. elegans* homolog of the human HJ resolving enzyme GEN1 is involved in the repair of DSBs upon IR-induced DNA damage by producing same sense HJ nicks at later steps of the repair [127,149]. GEN1-mediated DSB repair is the major pathway to resolve HJs and form COs upon DNA damage in mammals and flies [151–153].

Consequently, several DSB repair factors are involved in DNA-damage checkpoint signaling in response to IR, including GEN-1 and HIM-6 [127]. Here, HIM-6 has a crucial role in ensuring normal mitotic function and processes 80–90% of all meiotic recombination intermediates, generating non-crossover products [95]. To this end, HIM-6 acts partially redundant when compared with the topoisomerase TOP-3 downstream of SPO-11 and RAD-51, preventing a toxic accumulation of recombination intermediates [154]. TOP-3 is required for meiotic recombination and interacts both genetically and physically to process DNA damage during normal mitotic germ cell divisions and to form non-crossovers [93,154].

HIM-6 physically interacts with the *C. elegans* ATR homolog ATL-1 during DNA damage–checkpoint responses, while their exact role in DSB repair is not known [154]. However, several studies indicate that ATL-1 functions in DSB repair requires the simultaneous activation of CHK-1 by the helicase WRN-1, ortholog of human Werner's syndrome factor WRN. Studies in *C. elegans* reveal that in response to IR-induced DSBs, WRN-1 functions upstream of ATL-1 and ATM-1 to trigger cell cycle arrest, by regulating proper RPA-1 stabilization [140,155]. Further, WRN-1 causes nuclear accumulation of ATM-1 and takes the role of the human regulatory partner of ATR, ATRIP, in recruiting both ATL-1 and ATM-1 to the replication fork [156,157].

The checkpoint kinases of the phosphatidylinositol-3 kinase-related kinases (PIKK) family, ATM-1 and ATL-1, regulate CO interference and are involved in the initial steps of DSB recognition in mitotic and meiotic germ cells, triggering checkpoint response and cell cycle arrest, or apoptosis [54]. ATM-1 is involved in IR-induced DSB response independent of HR and NHEJ [140]. Upon activation, via autophosphorylation, ATM-1 gets recruited to the DNA-damage site by interacting with the MRN complex factor MRE-11 and RPA-1. UV/IR induces replication fork stalling and DSB formation, causing the recruitment of ATL-1, by RPA-1-bound-ssDNA, to the damaged site and activation by WRN-1 activity. ATL-1 requires MRE-11 to bind to the resected ssDNA–RPA-1 complex. This preprocessing step, before ATL-1 recruitment, is apparently required upon IR-induced DSBs but not observed during replication stress [140].

The presence of ATL-1 and ATM-1 in mitotic and meiotic germline cells with DSBs is of importance for the factor ZTF-8, a functional homolog of the mammalian RHINO, which is involved in DDR and cell cycle regulation [158]. ZTF-8 is a player of meiotic recombination as it is involved in processing stalled replication forks and has a supportive function in intersister repair when a homologous chromosome is not available. ZTF-8 also aids in recruiting the 9-1-1 complex and other proteins to the site of DSB. 9-1-1 is formed by the proteins HPR-9, HUS-1, and MRT-2 (RAD9, HUS1, and RAD1 in mammals) and is commonly known as the cell cycle checkpoint complex. Both MRT-2 and HUS-1 has been found to directly interact with ZTF-8, highlighting its importance in the checkpoint response, specifically in DNA-damage checkpoint-induced apoptosis. ZTF-8 activation and localization, as well as interaction with ATL-1 and ATM-1, require SUMOylation. ZTF-8 is proposed to act upstream of the divergence point of the DSB repair and the checkpoint repair pathways, suggesting that the 9-1-1 complex is required to interact with ZTF-8 for either of the repair pathways to take place [159].

8.2 Nonhomologous End Joining

During meiosis I and II, the spindles pull apart the DNA strands, which can tear apart HJs, by force, leading to DSBs that are commonly repaired by NHEJ [93]. NHEJ depends on the conserved Ku proteins CKU-70 and CKU-80, which form heterodimers at the damaged site to protect the DNA from HR-mediated resection. NHEJ is a highly error-prone repair mechanism that is suppressed in germ cells by the protein COM-1, the *C. elegans* homolog for the human tumor-suppressor CtIP, to ensure maintenance of the genetic material for the next generation. During meiosis, COM-1 blocks the toxic effects of CKU-70 and CKU-80 by misplacing them from the damaged DNA. COM-1-deficient animals are able to repair SPO-11-induced DSBs via HR and NHEJ, indicating that COM-1 is not required for meiotic recombination per se, but for DNA end resection and CO formation [54,126].

In addition, DNA repair of induced meiotic DSBs, in the absence of the MRN complex, takes place in the middle to late-prophase phases of meiosis I and is not carried out via HR but via NHEJ. This indicates that the MRN complex is essential for HR activity but can be bypassed by EXO-1-dependent resection [130].

8.3 Other Conserved DSB-Repair Mechanisms

Alternatively to HR and NHEJ, DSBs can be repaired by single-strand annealing (SSA) and alternative end joining (alt-EJ), which is also termed microhomology-mediated end joining (MMEJ). Both act in somatic cells and can make use of a large stretch of homology (30–400 bp) or a very small stretch of homology (5–15 bp) between damaged and template strands [54]. SSA employs a genetic network overlapping with HR, including XPF-1 activity and RPA binding to the resected ends of the damaged ssDNA. Subsequently, BRC-2 binds to the ssDNA to displace RPA from the DSB [160]. In *C. elegans*, no clear mechanism for alt-EJ has been identified yet [131].

Meiotic recombination can also make use of a sister chromatid instead of a homologous chromosome as a template, and not lead to CO. This mechanism consists of a sub-repair route of HR that is called synthesis-dependent strand annealing (SDSA) or intersister HR. SDSA occurs early in meiosis, and is independent of recombinant intermediates. It takes place after the disassembly of the D-loop when the ssDNA has been resected, and interacts with the other broken DNA end [93].

During *C. elegans* meiosis, only one CO event per chromosome can be observed, while at least two DSBs occur, indicating a second level of DSB repair mediated by non-crossover events (NCO) [161]. During SDSA, RTEL-1, the homolog of the human regulator of telomere elongation helicase 1, is employed to limit CO formation by dissociating strand-invasion events [162]. The elongated invading strand is then annealed to the complementary ssDNA tail on the other side of the DSB, the single-stranded gaps are filled and the nicks ligated, which bypasses CO product formation [54].

Intersister HR is crucial to repair a number of meiotic DSBs to ensure genomic integrity. This sub-pathway is of major importance at the arm regions of the chromosomes where most DSBs are converted into NCOs in an RTEL-1-dependent manner [148]. Intersister HR involves the *C. elegans* homolog of the breast cancer tumor suppressor BRCA1, which interacts with SMC-5 and SMC-6, the homologs of the structural maintenance of chromosome proteins in humans. Single mutants of *smc-5* and *smc-6* show phenotypes similar to *brc-1* mutant, the proteins localize to condensed nuclear chromosomes and also appear to be involved in meiotic intersister HR [163].

Alt-EJ (or MMEJ) acts independently of other DSB-repair pathways, highlighting its importance in avoiding developmental arrest of the animal [131]. Both SSA and alt-EJ require DNA replication and/or cell cycle progression occurrence for their function and they produce similar end products.

Interstrand crosslink (ICL) is another type of DNA damage characterized by interlinking the two strands of the DNA double helix, which blocks replication, and interrupts the translocation of crucial proteins along the DNA required for transcription. Nucleolytic processing of ICL lesions has been shown to lead to DSBs, which creates a substrate for HR. However, ICL repair involves factors from various repair pathways including the Fanconi anemia (FA) proteins and members of the NER pathway [164]. The ubiquitin ligase RNF-113 is important for the repair of ICL-induced DSBs: it acts epistatically to RFS-1 and mediates RAD-51 binding to ssDNA. RFS-1 binds to the ssDNA together with RPA-1. RNF-113 ubiquitinates an unknown factor, most likely RFS-1 or RPA-1, causing the release of RPA-1 from the DNA and subsequent RAD-51 binding to the ssDNA, which initiates strand invasion and the HR pathway [165]. Once RAD-51 is loaded onto the ssDNA, the putative histone demethylase JMJD-1.1 modulates the chromatin structure and influences RAD-51 dissociation from the single strand. The exact mode of action of JMJD-1.1 is not known, although some synergism with RAD-54 in the HR regulation has been demonstrated [166]. The endonucleases MUS-81 and XFP-1 generate ICL-induced DSBs by causing dual incisions around the damage site in order to separate the covalently bound DNA strands [149].

The ICL-specific repair pathway, known as Fanconi anemia (FA), employs the DNA helicase HEL-308 that is known to act in HR [164]. Further, FA involves FCD-2, the homolog of human FANCD, which is known to guide DSBs to HR

repair, instead of NHEJ [165]. FCD-2 binds to the DNA together with BRC-2 and RAD-51. HEL-308 partly contributes to the loading and stabilization of RAD-51 on the ssDNA [167].

The NER machinery cooperates with HR damage repair of UV-C lesions in the germ line: the current hypothesis proposes that damage is either directly repaired via HR activity and, in parallel, the NER pathway produces repair intermediates which are subsequently processed and repaired by GG-NER. Alternatively, the NER machinery activates the well-conserved core HR factors RPA-1, MRE-11, and RAD-54, and employs the 9-1-1 complex to induce p53/CEP-1-mediated germ cell apoptosis [37].

9. DNA-DAMAGE CHECKPOINTS

DNA-damage checkpoint is a signal transduction pathway that halts cell cycle progression upon detection of different DNA lesions. Various sensors recognize DNA damage, including the ring structure complex Rad9, Hus1, and Rad1 (9-1-1 complex) or the ataxia telangiectasia (AT) mutated (ATM) protein, and the ATM and Rad3-related (ATR) response complex. Depending on the quality and extent of the DNA damage, specific signal transducer proteins mediate the activation of effector protein networks that respond by arresting the cell cycle, triggering DNA repair, or leading to apoptotic cell death. Genetic networks in eukaryotes that define DNA-damage checkpoint and apoptotic response to DNA damage are evolutionarily conserved and well defined in nematodes, flies, and mammals [13,168–171].

DNA damage–checkpoint responses during mitosis and meiosis are genetically distinct: somatic cells arrest proliferation to allow time for DNA repair, while meiotic germ cells that carry DNA-damage or -display asynapsis are removed by apoptosis to ensure genomic stability across generations [61]. The *C. elegans* germline is specifically suitable to study both mitotic and meiotic checkpoint mechanisms, since cell cycle arrest and apoptosis are spatially defined, easily quantified, and controlled by a well-known developmental gene network [13]. Cell cycle arrest in the mitotic germline can occur during G1/S, S, and G2/M checkpoint phases of cell cycle progression in order to allow repair before DNA replication or cell division [171–174]. The importance of apoptotic events in maintaining genomic stability across generations is emphasized in two waves of cell death during *C. elegans* development. The first wave occurs during embryogenesis, where a sequence of well-defined apoptosis events in somatic cells determines tissue development and shapes the organism. The second wave occurs during oogenesis in the adult germline to eliminate cells that could compromise the transfer of genetic material to the offspring. Several reviews summarize the genetic pathway of programmed cell death in *C. elegans* [175,176]. Apoptosis events in the germline occur to maintain tissue homeostasis and can be triggered in response to bacterial pathogens or genotoxic stresses, in each case employing genetically distinct pathways.

9.1 Sensors of the DNA Damage Response

The major checkpoint sensor complex 9-1-1 is well conserved in *C. elegans*: homologs of the yeast Rad9, Hus1, and Rad1 proteins are HPR-9, HUS-1, and MRT-2 in the nematode [44,177]. The 9-1-1 scans the chromatin and senses DNA damage– or unrepaired-– recombination intermediates, for example, produced during the DSB-repair procedure [44]. The complex acts as a recruitment platform for the translesion synthesis (TLS) machinery to act on stalled replication forks. TLS repair is able to replicate the DNA amid unrepaired lesions [158,178,179].

During checkpoint activation, HPR-9 and MRT-2 interact to achieve proper nuclear localization of the HUS-1 and the 9-1-1 complex [44,172]. The 9-1-1 complex interacts with the transducer factor ZTF-8, homolog of the mammalian protein RHINO, to resolve DNA damage that leads to replication fork stalling, meiotic checkpoint activation, as well as the repair of meiotic and mitotic DSBs. To that end, transducer activity of the checkpoint kinase members of the PI3K superfamily, ATM-1 and ATL-1, homologous to the mammalian ATM and ATR proteins, phosphorylate ZTF-8 at the chromatin or in the nucleolus. ZTF-8 acts as a TLS platform during S-phase and is required for the optimal regulation of the DNA damage–induced apoptotic pathway [158].

RAD-5 (also called CLK-2), ortholog of the *S. cerevisiae* telomere length–regulating protein Tel2p, acts independently of 9-1-1 as a checkpoint-sensing factor in response to endogenous and exogenous DNA damage [171]. This pathway is absent in yeast, suggesting that it developed as a novel pathway during evolution in metazoans [172]. Studies in the early 2000s localized CLK-2/RAD-5 at DNA-damage sites, either as a primary sensor for damage or as a repair protein, affecting both the DNA-damage checkpoint as well as the S-phase replication checkpoint downstream of ATL-1 [168,169].

9.2 Checkpoint Sensor Proteins in Telomere Length Maintenance

The 9-1-1 factors HUS-1 and MRT-2 are implicated in telomere length maintenance, by facilitating telomerase-mediated telomere replication and acting to prevent telomere shortening. This role of 9-1-1 appears to be an evolutionary adaptation

as it is presently undiscovered in yeast but highly conserved in multicellular organisms. Several studies show that HUS-1 and MRT-2 recognize telomeres either during replication fork stalling at telomeres, or during S phase when unfolding of chromatin or telomere-binding proteins at the T-loop can lead to a structure similar to a recombination intermediate. Subsequent to recognition, checkpoint proteins are recruited [180,181]. Upon telomere binding, the 9-1-1 complex and the *C. elegans* Rad17 RFC clamp loader homolog, HPR-17, stimulate the recruitment of telomerase. To mediate repair, telomeric chromatin is kept in an open conformation by the activity of PME-5 (also called TANK-1), which contains a C-terminal PARP regulatory and catalytic domain, which is upregulated by HUS-1 [182].

9.3 Effectors of DNA-Damage Checkpoints

After DNA-damage detection the signal has to be passed from the transducers to the effectors. To this end, transducers often amplify and diversify the signal via phosphorylation of multiple effectors. Two main transducers in *C. elegans* are ATM-1 and ATL-1. One effector that is being phosphorylated is CHK-2, which is a member of the Cds1/Chk2 checkpoint kinase family that acts at the checkpoints G1/S, G2/M, and S phase by transforming information detected by the sensors and translated by the transducers, into specific biological responses. CHK-2 is required for pairing and spatial reorganization of homologous chromosomes during early meiotic prophase. To this end, CHK-2 induces the co-localization of homolog sister chromatids and organizes the chromatin into a form more receptive to pairing. Further, it regulates the length of the premeiotic S phase and mediates completion of replication and pairing of chromosomes [63].

Another effector phosphorylated by ATL-1 is CHK-1, a CHK1-like serine threonine protein kinase, which has a conserved role from basal metazoans to humans mediating cell cycle arrest at the S/M checkpoint during early embryogenesis and in the postembryonic germline cell cycles [183,184].

ATM-1 and ATL-1 are also known to connect the checkpoint pathway to the apoptosis stimulation in the germline: as discussed earlier, DNA damage induced by gamma irradiation leads to the recruitment of HUS-1 and ZTF-8.

DNA damage–checkpoint activation in meiotic pachytene cells leads to activation of the *C. elegans* p53 homolog CEP-1. Prior to late pachytene, CEP-1/p53 is kept at bay by translational repression through GLD-1, thus preventing the DSBs from triggering apoptosis aberrantly during normal meiotic recombination [185]. Once activated, CEP-1/p53 induces the expression of the proapoptotic gene *egl-1* and *ced-13* [44,186]. Both of those BH3-only domain proteins trigger the apoptotic demise of meiotic pachytene cells by removing the Bcl2 homolog CED-9 from the Apaf-1 homolog CED-4, which in turn activates the CED-3 caspase [187–192].

Germ cell survival and suppression of apoptosis upon minor DNA-damage events caused by environmental factors or meiotic recombination is mediated by the anti-apoptotic factor ABL-1 that negatively regulates CEP-1, thereby allowing the system to distinguish between different types of DNA damage, such as DSBs and DNA adducts [193].

9.4 Cytokinesis Checkpoint

APC/C, the anaphase-promoting complex/cyclosome, is the major regulator of chromosome segregation in eukaryotes. It is an E3 ubiquitin ligase, which facilitates polyubiquitination of its substrates for degradation via the ubiquitin–proteasome system [194]. During the metaphase-to-anaphase transition APC/C acts to degrade the protein IFY-1 (interactor with FZY-1), a *C. elegans* securin, which inhibits the activity of the separase SEP-1 [195]. Separases are enzymes important for cleaving cohesin, which is the complex holding the sister chromatids together [196,197]. Cohesin produces a tension in the spindle microtubles, opposite to the pull produced by the spindle pole on microtubules attached to the kinetochore of each sister chromatid. Upon faulty attachment of kinetochores or the absence of tension in the spindle, APC activity is inhibited via sequestration of FZY-1, the ortholog of the *S. cerevisiae* Cdc20p [195]. This inhibition causes a delay in the onset of anaphase and is called the spindle assembly checkpoint (SAC). Only when the spindle is correctly assembled, the cell continues through its cycle [198–200].

FZY-1 is inhibited via the involvement of the kinetochore-bound MDF-1–MDF-2 complex interfering with free MDF-2 proteins and changing their conformation, from an open to a closed MDF-2 form, which in turn inhibits FZY-1 [201]. This complex, however, is not the sole inhibitor of FZY-1. The kinase BUB-1 has also been suggested to either bind and phosphorylate FZY-1 or function via the MDF-1–MDF-2 complex to inhibit FZY-1, as well as regulate kinetochore function and chromatin cohesion [199–201]. Besides BUB-1, the kinetochore scaffold protein KNL-1 regulates two other components of checkpoint activation: the NDC-80 complex and the RZZ (Rod/Zwilch/Zw10) complex, of which only the kinetochore components ROD-1 and CZW-1, homologs of Rod and Zwilch, respectively, have been described in *C. elegans*. BUB-1 and KNL-1 interact with HCP-3, homolog of the centromere CENP-A protein, and with HCP-4, homolog of the centromere CENP-C protein, to mediate the localization of HCP-1, a centromere CENP-F protein homolog, and of HCP-2, an ortholog

of the human CAGE1 protein. The functions of HCP-1 and HCP-2 proteins are not fully elucidated but, similarly to the spindle checkpoint component BUB-1, they overlap in spindle checkpoint regulation by interacting with the SAC proteins SAN-1 and MDF-2 to facilitate the correct pairing of chromosomes and their segregation [198,201].

HCP-1 and HCP-2 regulate the levels of free MDF-2, which is rate limiting for the folding of MDF-2 in its closed conformation, thus inhibiting checkpoint. The rate-limiting levels allow for the integration of yet another branch of SAC, in which SAN-1 and BUB-3 interact in the cytoplasm to inhibit APC/C. Both checkpoint branches involving MDF-1/MDF-2 and SAN-1/BUB-3 are not sufficient to cause cell cycle arrest on their own [201].

The APC/C pathway for chromosomal alignment and segregation only takes place in meiosis. Alignment and segregation of the chromosomes generally can differ during meiosis and mitosis. However, the aurora kinases, also known as chromosomal passengers, act in both mitosis and meiosis [202]. *C. elegans* expresses two aurora kinases AIR-1 and AIR-2, but only AIR-2 acts in SAC. AIR-2 is a kinase involved in chromosome alignment during metaphase I in mitosis, where it localizes to the point of contact between sister chromatids [203]. During metaphase II of meiosis, AIR-2 is involved in chromosomal separation, where it localizes to chromosome arms distal to the chiasmata [204]. AIR-2 moves along the microtubules from the chromosomes to the midzone microtubules during division and is required for mechanistically similar processes, such as polar body extrusion and stabilization and completion of cytokinesis. During this process, AIR-2 promotes proper localization of other midbody microtubule components, such as ZEN-4, an MKLP-1-related kinesin. AIR-2 acts upstream of ZEN-4 and physically interacts with ZEN-4, allowing association with the spindle midzone and aiding in polar body extrusion and cytokinesis [205].

AIR-2 also regulates proper localization of BMK-1, a BimC kinesin. The same study shows that AIR-2 kinase activity and movement depends on ICP-1, a chromosomal passenger protein, that mediates physical interaction between AIR-2 and BMK-1 via phosphorylation events at three residues, causing BMK-1 relocalization from the kinetochore microtubules to the midzone microtubules [205].

For chromosomal segregation to take place during meiosis AIR-2 must phosphorylate the meiosis cohesin REC-8. Phosphorylation of this cohesin by AIR-2 is negatively regulated by CeGLC-7 α/β phosphatases. These phosphatases antagonize AIR-2 by blocking its localization, thereby inhibiting AIR-REC-8 dephosphorylation [202]. Although AIR-2 phosphorylates cohesin during meiosis, it remains unclear whether it also executes this role during mitosis. Instead, during mitosis in embryonic and postembryonic development, AIR-2 promotes the bi-orientation of sister kinetochores, the association of condensin to chromosomes, and the function of some condensins, such as chromosomal organization [204,206].

10. CONCLUDING REMARKS

The nematode model has been firmly established as an important model system for studying DNA repair. *C. elegans* has played a major role as a model organism for a large variety of biological processes, including programmed cell death, neurobiology, RNA interference, development, and aging. The traceable genetics and host of methodologies have made the worm also a crucial system for investigating DDR ranging from mechanistic discoveries of DSB repair, also in the context of meiosis, to the systemic responses, on the organismal level. In many ways, *C. elegans* has closed the gap between the traditional "work horses" of genome stability research, the powerful genetic yeast system and the mouse as a disease model. The vibrant and ever-expanding community of *C. elegans* research will continue to gain new and unexpected insights into cellular and organismal mechanisms of genome stability.

GLOSSARY

6-4 photoproducts The consequence of a covalent bond formation of carbons at position six and four between adjacent thymine bases upon exposure to UV. The resulting distortion of the DNA helix can be removed by NER mechanism.

9-1-1 complex A ring structure complex formed by Rad9, Hus1, and Rad1 (gene names taken from yeast) which act as a sensor complex for recognizing DNA damage.

Alternative end joining Also known as microhomology-mediated end joining, is a type of double-strand break repair which is found only in somatic cells and can use as many as 30–400 bp or as little as 5–15 bp of homology between the damaged and the template strand for repair. No clear mechanism for this type of repair has been identified in *C. elegans* yet.

Anaphase-promoting complex/cyclosome An E3 ubiquitin ligase, which facilitates polyubiquitination of its substrates for degradation via the ubiquitin–proteosome system. It is the major regulator of chromosome segregation in eukaryotes, and acts by tagging specific proteins for degradation, such as the protein IFY-1, in *C. elegans*.

Ataxia telangiectasia A rare inherited neurodegenerative disease that is defined by impaired coordination and small, dilated blood vessels. Patients display uncoordinated movements, a weakened immune system, and DNA-repair deficiency of double-strand breaks based on defects in the ATM gene.

Cockayne syndrome A rare autosomal recessive neurodegenerative disorder underlying DNA-repair defect that includes devastating characteristics, such as growth failure, misdevelopment of the nervous system, high sensitivity to sunlight, and premature aging, but no cancer predisposition.

Crossover An event that occurs after Holliday junction formation. While the newly synthesized DNA strand and the template DNA strand cross over, genetic information can be exchanged, producing recombinant chromosomes. This exchange of genetic material is called crossover.

Cyclobutane pyrimidine dimers Arise upon UV irradiation that causes the coupling of C=C double bonds of pyrimidines in thymine or cytosine. The resulting four-membered ring structure leads to distortion of the DNA helix structure that is primarily repaired by NER in eukaryotic cells.

Displacement loop The structure formed by the damaged strand of DNA and the template DNA being used for repair. It forms after strand invasion has taken place, and refers to the shape the template DNA strand forms.

DNA double-strand breaks A type of DNA damage that cause both DNA strands to break. It is considered to be the most toxic form of DNA damage and can be repaired by a variety of different ways, all of which involve the simultaneous repair of both strands at the same time.

DNA-damage response The mechanisms in which each organism detects the DNA damage and initiates its repair.

DNA-damage checkpoint Is a type of DNA-damage response which causes the cell to halt its cell cycle progression, and trigger either DNA repair, or apoptotic cell death.

DNA glycosylase Mediates the repair of single damaged bases in DNA in base excision repair by flipping the damaged base out of the double helix and subsequently cleave the N-glycosidic bond. This creates an apurinic/apyrimidinic site and leaves the sugar-phosphate backbone intact.

Fanconi anemia A genetic disorder that causes bone marrow failure.

Holliday junction The structure formed consecutive to the displacement loop, after new DNA has been synthesized using the template DNA strand and two points are formed where the newly synthesized DNA strand and the template DNA strand cross over.

Homologous recombination A major type of double-strand break repair that is characterized as an error-free method, and uses either the sister chromatid or homologous chromosomes as undamaged templates for repair.

Ionizing radiation Majorly gamma rays, X-rays, and to some extent UV radiation that carry enough energy to free electrons from atoms or molecules, thereby resulting in their ionization. Causes a broad range of damage to DNA, including double-strand breaks that are repaired by a variety of DNA-repair mechanisms.

Intersister homologous recombination A type of double-strand break repair which uses a sister chromatid instead of a homologous chromosome as a template. It does not lead to crossover events. It is also called synthesis-dependent strand annealing.

Interstrand crosslink Another type of DNA damage characterized by interlinking the two strands of the DNA double helix, thus blocking replication and interrupting the translocation of crucial proteins along the DNA required for transcription.

Microhomology-mediated end joining Also known as alternative end joining, is a type of double-strand break repair which is found only in somatic cells and can use as many as 30–400 bp or as little as 5–15 bp of homology between the damaged and the template strand for repair. No clear mechanism for this type of repair has been identified in *C. elegans* yet.

MRN complex Named after the proteins that for this complex are found in mammals, subsequently MRE11, RAD50, and NSB1. It has also been found in yeast, this time named as the MRX complex, after the proteins Mre11, Rad50, and Xrs2. This complex is important during the initiation of the repair of double-strand breaks.

Non-crossover The situation in which the Holliday junction is resolved and does not result in a crossover event. Since crossover events only occur once per chromosome, non-crossover events are common.

Nonhomologous end joining A major type of double-strand break repair that is known for its efficient, yet error-prone repair which joins damaged DNA ends regardless of their homology, leading to the addition or removal of nucleotides.

Nick/counternick mechanism A mechanism used by cells to resolve the Holliday junction structure and can lead to the occurrence of crossovers. The mechanism involves the proteins SLX-1 and MUS-81 that act by nicking the Holliday junction twice, one after the other, in a symmetrical manner.

Opposite-sense resolution A mechanism used by cells to resolve the Holliday junction structure and can lead to the occurrence of crossovers. The mechanism involves the proteins HIM-6 and XPF-1, which unwind thermodynamically unstable Holliday junctions, and nick the Holliday junction, respectively.

Poly-ADP-ribose polymerase An enzyme that mediates single-strand DNA break repair and programmed cell death and requires NAD^+. Upon single-strand break detection, PARP binds to the DNA and synthesizes a poly ADP-ribose (PAR) chain to signal a DNA repair mechanism involving XRCC1. Upon repair, PAR chains are degraded by Poly(ADP)-ribose) glycohydrolase.

Synaptonemal complex The protein structure that forms between homologous chromosomes during meiosis. This complex is important for chromosome alignment and pairing, synapsis and recombination.

Single-strand annealing A type of double-strand break repair which is found only in somatic cells and can use as many as 30–400 bp or as little as 5–15 bp of homology between the damaged and the template strand for repair.

Synthesis-dependent strand annealing A type of double-strand break repair which uses a sister chromatid instead of a homologous chromosome as a template. It does not lead to crossover events. It is also called intersister homologous recombination.

Spindle assembly checkpoint A type of cell cycle halt which causes a delay in the onset of anaphase. It occurs in mitosis and ensures the fidelity of chromosome segregation, since only when the spindle is correctly assembled is the cell allowed to continue through its cycle. Upon faulty attachment of kinetochores or the absence of tension in the spindles, the spindle assembly checkpoint is activated.

Sister chromatids Identical chromatids that were produced by replication of only one copy, and can be found together bound by a centromere.

Sumoylation A posttranslational modification, similar to ubiquitylation, however with the addition of SUMOs (small ubiquitin-like modifiers) instead of ubiquitin. This modification can affect both protein structure and its subcellular localization.

Translesion synthesis A type of DNA damage–repair mechanism that is able to replicate the DNA amid unrepaired lesions. This type of repair uses more specialized translesion polymerases which are able to insert new bases next to damaged nucleotides.

Xeroderma pigmentosum An autosomal recessive genetic disorder in which the ability to repair DNA damage, including pyrimidine dimers and 6-4 photoproducts, caused by UV light is deficient. Patients early on develop basal cell carcinomas and most commonly die upon the occurrence of metastatic malignant melanomas and squamous cell carcinoma.

LIST OF ABBREVIATIONS

53BP1 p53 binding protein 1
6-4PP 6-4 photoproducts
alt-EJ Alternative end joining
AP site Apurinic/apyrimidinic site
AT Ataxia-telangiectasia
BER Base excision repair
C. elegans *Caenorhabditis elegans*
CO Crossover
CPDs Cyclobutane pyrimidine dimers
CS Cockayne syndrome
CSA Cockayne syndrome protein A
CSB Cockayne syndrome protein B
DDR DNA-damage response
D-loop Displacement loop
DNA Deoxyribonucleic acid
DSBs Double-strand breaks
dsDNA Double-stranded DNA
ERCC1 Excision repair cross complementation group 1e
FA Fanconi anemia
GG-NER Global genome NER
HJ Holliday junction
HR Homologous recombination
ICL Interstrand crosslink
IFY-1 Interactor with FZY-1
IIS Insulin/insulin-like growth factor signaling
IR Ionizing radiation
MMEJ Microhomology-mediated end joining
MMR Mismatch repair
MMS Methyl methanesulfonate
mtDNA Mitochondrial DNA
ncDNA Nuclear DNA
NCO Non-crossover
NER Nucleotide excision repair
NHEJ Nonhomologous end joining
OXPHOS Oxidative phosphorylation
PARGs Poly(ADP-ribose)glycohydrolases
PARP Poly(ADP-ribose) polymerase
PARP-1 Poly-ADP-ribose polymerase-1
PI3K Phosphatidylinositol 3-kinase
PIKK Phosphatidylinositol-3 kinase-related kinases
PIP3 Phosphatidylinositol--3,4,5-triphosphate
Pol β DNA polymerase β
Pol θ DNA polymerase θ
RZZ Rod/Zwilch/Zw10 complex
SAC Spindle assembly checkpoint
SC Synaptonemal complex
SDSA Synthesis-dependent strand annealing
SSA Single-strand annealing

SSB Single-strand break
ssDNA Single-stranded DNA
TC-NER Transcription-coupled NER
TLS Translesion synthesis
TTD Trichothiodystrophy
UV Ultraviolet light
UVB Ultraviolet light type B
UVC Ultraviolet light type C
UV-DDB UV-damaged DNA-binding protein
XP Xeroderma pigmentosum
XPA Xeroderma pigmentosum complementation group A
XPC Xeroderma pigmentosum complementation group A
XPF Xeroderma pigmentosum complementation group F
XPG Xeroderma pigmentosum complementation group F

ACKNOWLEDGMENTS

We thank Najmeh Soltanmohammadi and Ashley B. Williams for providing images. MR is supported by FP7 ITN CodeAge 316354, AFCL by FP7 ITN MARRIAGE 316964, BS acknowledges funding from the Deutsche Forschungsgemeinschaft (CECAD, SFB 829, SFB 670, and KFO 286), the European Research Council (ERC Starting grant 260383), Marie Curie (FP7 ITN CodeAge 316354, aDDRess 316390, MARRIAGE 316964), the German-Israeli Foundation (GIF 1104–68.11/2010), the Deutsche Krebshilfe (109453), and the Bundesministerium fu¨r Forschung und Bildung (Sybacol FKZ0315893A-B).

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