Chapter 11
Genome Stability and Ageing

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Abstract Ageing is defined as the progressive attrition of tissue/organ function resulting in an increased susceptibility to disease and death. The DNA mutation and damage theory of ageing posits that the accrual of genetic damage over time is the underlying cause of ageing. Evidence for this theory stems from the fact that numerous human progeroid syndromes are caused by inherited defects in genome maintenance mechanisms, linking excess genetic damage with accelerated ageing. These diseases have been modelled in mice and other organisms. However, the molecular mechanism by which genomic instability drives ageing is currently not known. The nematode, *C. elegans*, is a genetically tractable, well-studied model organism for investigating mechanisms of ageing and DNA repair pathways identified in mammalian systems are well conserved in the worm. Furthermore, proliferating and post-mitotic cells, which have distinct responses to genomic instability, are clearly delineated in the worm. Thus worms provide an opportunity to study the importance of genomic stability in each of these compartments in the context of a whole organism. Genomic instability can interfere with transcription, trigger apoptosis, attenuate proliferative capacity, and cause metabolic changes. Here, we first examine the DNA repair pathways that are conserved between worms and mammals, with an overview of the spatial and temporal activity of each of these repair pathways. This chapter then explores evidence from studies in the nematode that genomic instability, healthspan and organismal ageing are linked.

Keywords Genomic instability • DNA repair • Ageing

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11.1   DNA Damage and Repair in *C. elegans*

The nuclear and mitochondrial genomes are constantly exposed to damaging agents from endogenous (i.e., spontaneous, e.g., reactive oxygen species) and exogenous (i.e., environmental, e.g., ultraviolet radiation) sources. These agents cause chemical modification of DNA, impacting chromosomal replication and transcription. DNA replication is critical during worm development and in the gonads of adult worms, while transcription is crucial in all cells throughout life. The advent of whole genome sequencing revealed that the mutation frequency in *C. elegans* is ~6.7×10^{-10} per nucleotide per cell division [1] whereas, in humans the mutation rate is 5.0×10^{-11} per nucleotide per cell division [2]. This suggests that even with a short lifespan, worms accumulate a significant number of mutations, analogous to humans. Additionally, single-strand DNA breaks [3] and deletions in the mitochondrial genome [4] increase with age in the worm, which is also comparable to humans (reviewed in [5–7])

The DNA repair pathways that are conserved between mammals and *C. elegans* include the following [8]:

Base excision repair (**BER**): BER identifies and excises subtle lesions that don't distort the helical structure of DNA. The kinds of lesions routinely repaired by BER are abasic (AP) sites, oxidized bases, alkylated bases, deaminated bases and single-strand breaks.

Nucleotide excision repair (**NER**): NER detects and repairs numerous different types of lesions that cause helical distortion, including UV-induced (6-4) photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs), bulky adducts formed by environmental agents such as a by-product of tobacco smoke BP-7,8-diol-9,10-epoxide (BPDE). NER consists of two sub-pathways: Global Genome NER (GG-NER): lesions repaired anywhere in the nuclear genome and Transcription Coupled NER (TC-NER): repair of lesions occurring in the template strand of an actively transcribed gene.

Interstrand cross-link repair (**ICLR**): ICLR or the Fanconi pathway repairs lesions that covalently link both strands of DNA together.

Mismatch repair (**MMR**): MMR is a DNA repair mechanism that is responsible for correcting base-base mismatches, insertion/deletion mismatches and small hairpin structures resulting from misalignment that occurs during DNA replication and recombination.

Homologous recombination (**HR**): HR is used to repair DNA double-strand breaks (DSBs) using a sister chromatid or homologous chromosome as a template to acquire lost sequence information. In addition to double-strand breaks, HR is needed for the repair of interstrand crosslinks (ICL) and for the recovery of stalled replication forks.

Non-homologous end-joining (**NHEJ**): DNA double-strand breaks with two broken ends are repaired by NHEJ via a mechanism by which the two ends are ligated together.

Conventionally, each of these DNA repair mechanisms is thought to tackle a specific type of DNA damage. However, overlap between these pathways is becoming increasingly evident. DNA repair is tightly regulated by DNA damage sensors (proteins that detect damaged DNA) and signal transducers (protein cascades that transmit the damage signal to numerous effector proteins that regulate repair, cell cycle and cell fate) [9]. Translesion synthesis (TLS) is a mechanism by which DNA lesions are tolerated by replicating cells, but not repaired. When the replication machinery is stalled by a DNA lesion, a specialized DNA polymerase can be recruited to enable bypass of the lesion, enabling resumption of replication [10]. TLS is also well conserved between worms and mammals [11] (see Table 11.1).

Table 11.1 List of conserved and absent DNA repair proteins in *C. elegans* [79]

Genome maintenance mechanism	*C. elegans*	Mammalian	Function
Base excision repair(BER)	R09B3.1a (*exo-3*)	APEX1	AP endonuclease
	C29A12.3 (*lig-1*)	LIG3	ATP-dependent DNA ligase
	R10E4.5 (*nth-1*)	NTHL1	DNA N-glycosylase
	Y56A3A.27	NEIL3	
	H23L24.5 (*parg-2*)	PARG	Poly (ADP-ribose) glycohydrolase
	Y71F9AL.18 (*parp-1*)	PARP1/ PARP2	Poly(ADP-ribose) polymerase
	F21D5.5	PNKP	Polynucleotide kinase 3′-phosphatase
	Y56A3A.29 (*ung-1*)	UNG	Uracil-DNA glycosylase
	W03A3.2 (*polq-1*)	POLQ	DNA polymerase
	Y47G6A.8 (*crn-1*)	FEN1	Flap endonuclease
		[b](Missing)	DNA polymerase
		POLB	
		[b]APEX2	Weak apurinic/apyrimidinic (AP) endodeoxyribonuclease
		[b]MBD4	T:G mispair glycosylase
		[b]MPG	3-meA, hypoxanthine glycosylase
		[b]NEIL2	5-hydroxyuracil glycosylase
		[b]OGG1	8-Oxoguanine glycosylase
		[b]SMUG1	Uracil glycosylase (single-strand DNA substrates)
		[b]TDG	T:G mispair glycosylase

Table 11.1 (continued)

Genome maintenance mechanism	*C. elegans*	Mammalian	Function
Nucleotide excision repair (NER)	Y49F6B.1	CCNH	Cyclin-dependent protein serine/threonine kinase regulator activity
	T21H3.3 (*cmd-1*)[a]	CETN2	Damage recognition
	F53H4.1 (*csb-1*)	CSB	Required for assembly of the TC-NER machinery
	F10G8.7	ERCC1	Stabilizes *xpf-1*
	K07G5.2 (*xpa-1*)	XPA	DNA damage recognition
	Y66D12A.15	XPB	ATPase and helicase activity; transcription factor II H (TFIIH) subunit
	Y76B12C.2 (*xpc-1*)	XPC	DNA damage recognition
	Y50D7A.2 (*xpd-1*)	XPD	ATPase and helicase activity; transcription factor II H (TFIIH) subunit
	C47D12.8	XPF	3′ side endonuclease
	F57B10.6 (*xpg-1*)	XPG	5′ side endonuclease
	C02F5.7b (*fbxl-1*)	FBXL2	Part of ubiquitin protein ligase complex
	R02D3.3	GTF2H1	Component of the core-TFIIH basal transcription factor
	T16H12.4	GTF2H2	Component of the core-TFIIH basal transcription factor
	ZK1128.4	GTF2H3	Component of the core-TFIIH basal transcription factor
	Y73F8A.24	GTF2H4	Component of the core-TFIIH basal transcription factor
	Y55B1AL.2	GTF2H5	Component of the core-TFIIH basal transcription factor
	F53G2.7 (*mnat-1*)	MT1	CDK-activating kinase assembly factor 1
	ZK20.3	RAD23A/ RAD23B	Ubiquitin binding domain
	F18A1.5 (*rpa-1*)	RPA1	Binds and stabilizes single-stranded DNA intermediates
	M04F3.1 (*rpa-2*)	RPA2	Binds and stabilizes single-stranded DNA intermediates
		[b](Missing) RPA3	Binds and stabilizes single-stranded DNA intermediates

Table 11.1 (continued)

Genome maintenance mechanism	*C. elegans*	Mammalian	Function
Mismatch repair (MMR)	T28A8.7 (*mlh-1*)	MLH1/ MLH3	Component of MutLα
	H26D21.2 (*msh-2*)	MSH2	Component of MutSα
	Y47G6A.11 (*msh-6*)	MSH3/ MSH6	Component of MutSα
	ZK1127.11 (*him-14*)	MSH4	Stabilizes double Holliday junctions and promotes their resolution into crossover products
	F09E8.3 (*msh-5*)	MSH5	Forms a complex with MutS homologue 4
	H12C20.2a (*pms-1*)	PMS1/ PMS2/ PMS2L3/ MS2L4	Component of MutLα
	F45G2.3 (*exo-1*)	EXO1	5–3′ exonuclease
	C29A12.3 (*lig-1*)	LIG1	ATP-Dependent DNA ligase
Homologous recombination (HR)	C36A4.8 (*brc-1*)	BRCA1	Nuclear phosphoprotein resolves double stranded breaks
	F56A6.4 (*eme-1*)	EME1	Essential meiotic structure-specific endonuclease
	C43E11.2a (*mus-81*)	MUS81	Forms a DNA structure-specific endonuclease
	B0041.7 (*xnp-1*)	NBS1	MRN complex
	F10G7.4 (*scc-1*)	RAD21	Part of cohesin complex
	T04H1.4 (*rad-50*)	RAD50	Complex with *mre-11*
	W06D4.6 (*rad-54*)	RAD54B/ RAD54L	Facilitates homologous DNA pairing
	Y119D3B.15 (*dss-1*)	SHFM1	Binds and stabilizes BRCA2
	C23H4.6	SMC6L1	Promotes sister chromatid homologous recombination
	Y43C5A.6a (*rad-51*)	RAD51/ XRCC2/ XRCC3	Required for meiotic recombination
		[b](Missing) BRCA2	Important for assembly of RAD51 onto single-stranded DNA
		[b]GEN1	Flap endonuclease
		[b]RAD52	Annealing of complementary single-stranded DNA and stimulation of the RAD51 recombinase

Table 11.1 (continued)

Genome maintenance mechanism	C. elegans	Mammalian	Function
Non-homologous end joining (NHEJ)	C07H6.1	LIG4	Ligates single strand ends together
	R11A8.4 (*sir-2.1*)	SIRT1	Deacetylation of Ku70
	R07E5.8 (*cku-80*)	KU80	ATP-Dependent DNA Helicase II
	Y47D3A.4 (*cku-70*)	KU70	ATP-Dependent DNA Helicase II
		[b](Missing) Artemis	Resects DNA ends
		[b]DNA-PK	Ser/Thr kinase- DDR cascade
		[b]XRCC4	Enhances the joining activity of LIG4
Translesion synthesis (TLS)	C35B1.1 (*ubc-1*)	UBE2A/ UBE2B	E2 ubiquitin-conjugating enzyme
	Y54G2A.31 (*ubc-13*)	UBC13	E2 ubiquitin-conjugating enzyme
	F39B2.2 (*uev-1*)	MMS2	Ubiquitin-conjugating enzyme
	Y37B11A.2	REV3L	TLS DNA polymerase
	ZK675.2	REV1	TLS DNA polymerase
	F22B7.6 (*polk-1*)	POLK	TLS DNA polymerase
	F53A3.2 (*polh-1*)	POLH	TLS DNA polymerase
		[b](Missing) RAD18	E3 ubiquitin ligase
		[b]POLI	DNA polymerase

[a]Active
[b]Only active in the absence of the cannonical pathway

An important consideration when studying DNA damage and its role in ageing is that repair mechanisms are differentially utilized in tissues (reviewed in [12]). For instance, germ cells respond more strongly to DNA damage than somatic cells. Therefore, post-mitotic adult worms are relatively resistant to ionizing radiation, whereas germ cells are extremely sensitive. Proliferating and meiotic germ cells repair DSBs by HR, whereas, post-mitotic somatic cells utilize NHEJ. Similarly, GG-NER, BER and ICLR maintain DNA stability in the mitotic germ cell compartment. TLS is highly active during early embryonic growth, contributing to resistance to genotoxic stress during this phase of development. During development, somatic cell genome maintenance requires HR and NHEJ, whereas the post-mitotic adult is mostly dependent on TC-NER. These observations reveal complex spatial and temporal regulation of DNA repair mechanisms (Fig. 11.1).

Notably, there are a few key proteins involved in genome maintenance that appear to be lacking in the worm. γH2Ax, MDC1 and RNF8 DNA damage signalling proteins have not been identified in *C. elegans*. Similarly, some regulators of NHEJ and ICL repair pathway do not appear to be present in the worm (see Table 11.1).
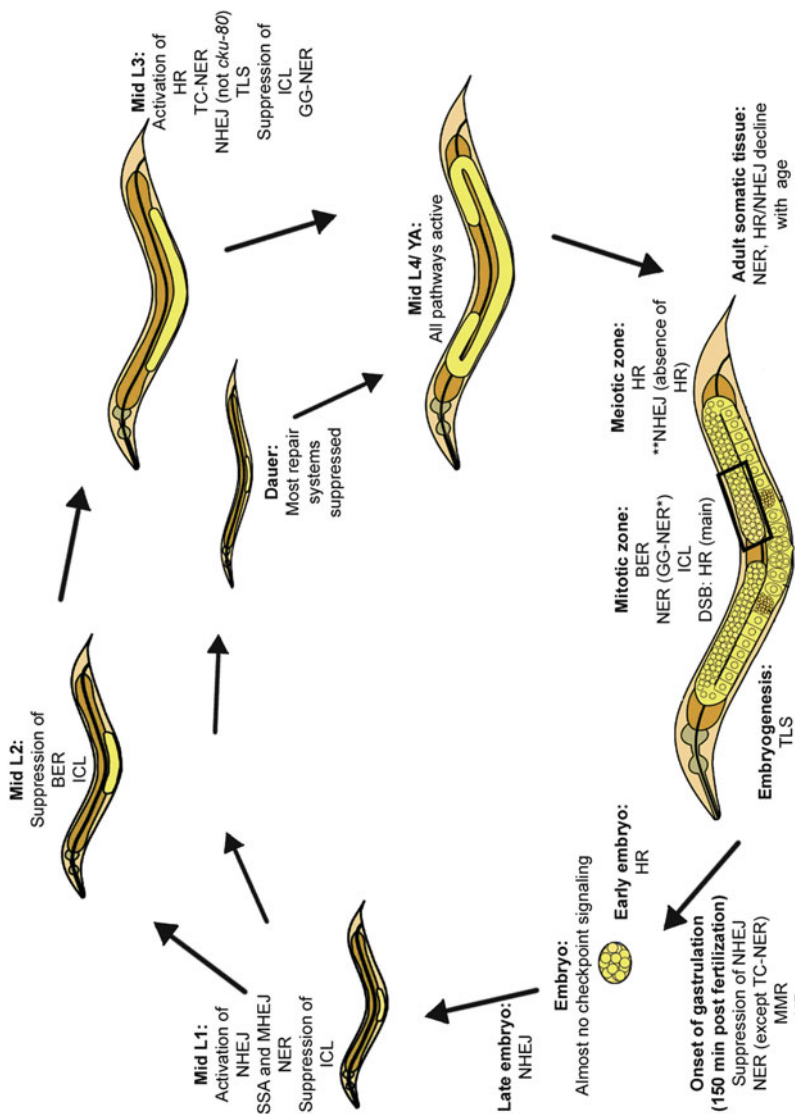
Fig. 11.1 DNA repair pathways utilized during different stages of *C. elegans* development and ageing

Another point to consider is that in higher organisms DNA damage can induce cellular senescence [13], which has been shown to drive ageing [14]. However, it is currently unclear if *C. elegans* have a cellular senescence programme [15]. Nonetheless, the importance of DNA repair mechanisms to genome stability and organismal lifespan is well documented in the worm. We focus here on each of the genome maintenance pathways and their relationship to ageing.

11.2   Changes in DNA Damage Levels and Mutation Frequency with Age

One of the earliest studies measuring DNA damage over the lifespan of worms was reported by Klass et al. in the 1980s. The authors observed a 34-fold increase in single-strand DNA breaks in day 15 adults compared with young, day 5 animals, using an *Escherichia coli* DNA polymerase I assay. Additionally, 5-methylcytosine (an epigenetic marker regulated to some extent by DNA repair) was also exponentially increased in older worms compared to larvae and young adults. These changes were accompanied by reduced transcription [3]. These data are consistent with the notion that incomplete repair of DNA damage leads to damage accumulation with age.

Similarly, the oxidative DNA lesion 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) was measured in the short-lived mutant, *mev-1* (a gene that encodes a subunit of complex II in the mitochondrial electron transport chain) [16] causing accumulation of dysfunctional mitochondria, reduced mitochondrial membrane potential [17], increased ROS, and hypersensitivity to oxidative stress [18]. A significant increase in adducts is detected in *mev-1* mutants compared to wild-type worms by high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC). *mev-1* mutants also have a five to ten-fold higher mutation frequency than WT worms, based on a *fem-3* mutation assay that detects loss-of-function mutations by measuring reversal of temperature sensitive sterility [19]. These studies are consistent with the idea that mitochondrial ROS contributes to nuclear genomic instability and mutagenesis, and promotes ageing. However, longitudinal studies in worms measuring DNA damage accumulation over the organism's lifespan have yet to be reported.

11.3   DNA Repair Capacity with Age

Radiation-sensitive mutant strains (*rad*) were first isolated in the 1980s, based on their sensitivity to ultraviolet light (UV). These mutants are also sensitive to other DNA damaging agents such as methyl methane sulphonate (MMS) and ionizing radiation (IR) [20]. In this study, no significant differences in lifespan were observed in the *rad* mutants compared to wild-type worms. Five of the seven *rad* mutants (except *rad-4* and *rad-7*) have slightly shortened lifespans after exposure to IR, but

this is dependent on the dose of IR used. An intermediate dose of radiation (10–30 krad) leads to increased mean lifespans in all strains (significant in WT (N2) and *rad-4*), while a higher dose (>100 krad) shortens lifespan (significant in WT (N2) and *rad-3*). This can be attributed to hormesis, where low levels of damage induce stress responses that are beneficial [21]. However, higher levels of stress lead to irreversible, persistent damage that has detrimental effects.

Hartman *et. al.* reported that there is no correlation between lifespan and sensitivity to genotoxic stress in several inbred strains of worms that have lifespans ranging from 13 to 30.9 days [22]. In addition, there is no correlation between excision of UV induced lesions (a direct measure of NER) and lifespan, based on measurement of (6–4) photoproducts and CPDs by radioimmunoassay in UV irradiated worms. It is important to note that these experiments were performed 24–48 h after egg lay (small differences in NER between strains were observed at 24 h but not at 48 h), which may not accurately portray DNA repair capacity of adult worms.

In contrast, several studies show that most long-lived mutant strains are resistant to multiple stressors including UV irradiation. In mammals, there is evidence, although not overwhelmingly convincing, that long-lived animals have higher DNA repair capacity compared to short-lived species [23, 24]. To perform similar studies in *C. elegans*, Hyun et al. examined DNA repair capacity of WT and long-lived strains by measuring the number of pyrimidine dimers in a target gene, *vps-45* of UV irradiated worms, using T4 endonuclease V (T4 endo V). T4 endo V incises DNA specifically at sites of pyrimidine dimers, which can be quantified by Southern blot. Using this technique in WT worms, the majority of repair is completed within 8 h and repair plateaus by 12 h (~67 % of lesions are repaired). However, in long-lived mutants, 85 % pyrimidine dimer repair is complete within 4 h and plateaus at 90 % by 12 h. This suggests that DNA repair capacity (at least NER) is faster in several long-lived mutants including *age-1 fer-15*, *age-1* and *daf-2*. Similarly, UV irradiation of adults (post-reproduction stage) significantly decreases lifespan in both WT and *daf-16* worms (37.2 %) and in long-lived (~16.4 %) mutants, suggesting that failure to repair UV-induced adducts, does shorten lifespan [25]. Although these experiments were done with an exogenous source of DNA damage that may cause damage to other macromolecules, they do suggest that DNA repair capacity impacts lifespan.

11.4   DNA Repair Pathways and Ageing

One line of evidence that supports the theory that decreased repair capacity drives ageing comes from humans with genetically inherited defects in DNA repair pathways (reviewed in [26, 27]). These defects lead to hypersensitivity to DNA damaging agents, accumulation of DNA damage and accelerated ageing of one or more tissues. The identification of such progeroid syndromes in humans led to the design of mutant *C. elegans* strains that have defects in DNA repair mechanisms. Below, we examine each DNA repair pathway and evidence that links it to ageing.

11.4.1 Base Excision Repair (BER)

C. elegans possess two AP endonucleases, EXO-3 (exo III family) and APN-1 (endo IV family) [28]. EXO-3 (R09B3.1a) is an endonuclease required for BER that nicks DNA 5′ to an AP site. *exo-3* mRNA levels decline 45 % by day 5 of adulthood and are maintained at low levels as worms age beyond that point [29]. RNAi depletion of *exo-3* increases ROS and mitochondrial genome deletions, which are characteristics of aged worms. Knockdown of *exo-3* also leads to other common ageing features such as neuronal damage and reduced motility.

Pharmacological suppression of ROS in *exo-3* deficient worms inhibits neuronal damage and increases motility, suggesting that ROS is a key cause of morbidity in the mutant worms [29]. In accordance, *exo-3* RNAi leads to a reduction in both mean (20 %) and maximum (10 %) lifespan of *C. elegans*. Interestingly, suppression of *cep-1*, ortholog of the tumour suppressor p53, rescues ageing phenotypes of *exo-3* RNAi mutants. One possible explanation is that *cep-1* is known to increase oxidative stress by inducing expression of pro-oxidant genes and repressing antioxidant genes, in response to cellular stress including genotoxic stress [29]. Thus deleting *cep-1* should reduce ROS and oxidative DNA damage in the *exo-3* mutant worms. Interestingly, in WT nematodes, suppression of *cep-1* leads to upregulation of *exo-3* and preserves healthspan (neuronal integrity and motility). This suggests that *cep-1* and *exo-3* coordinately respond to oxidative or genotoxic stress and this influences age-related decline.

Kato et al. further characterized the *exo-3* deletion mutant (*tm4374*) and confirmed a reduced lifespan [30]. The short lifespan of *exo-3* mutant is also suppressed by deletion of *ung-1*, a monofunctional uracil DNA glycosylase. UNG-1 acts upstream of EXO-3 in BER to remove uracil from DNA (caused by spontaneous hydrolysis of cytosine) creating an AP site. This reveals that AP sites are more deleterious than uracil lesions. Additionally, the authors reported a surprising difference between somatic versus germline cells (post-mitotic vs proliferating). In the germline, *exo-3* is highly expressed and loss of EXO-3 leads to a reduced brood size (reflecting the proliferative capacity of germ cells). Interestingly, the impact of *exo-3* on brood size requires the presence of *nth-1*, a second DNA glycosylase that removes oxidized pyrimidines [31]. This suggests that oxidative DNA lesions are a major substrate of BER in germ cells, whereas deamination products are more important in somatic cells.

Although one might predict that increased levels of oxidative DNA lesions would promote ageing, *nth-1* null mutants show a normal mean and maximum lifespan [32]. Surprisingly, QPCR studies reveal that the rate of removal of damage caused by oxidative and alkylating agents in the WT and *nth-1* adult worms is similar [33]. This could imply that there are redundant mechanisms for removing oxidized purines in *nth-1* deficient somatic cells and that it is unlikely that *nth-1* depletion induces ROS as occurs in *exo-3* mutants. It is also possible that oxidative DNA lesions may not be a major determinant of lifespan in somatic cells. Collectively, these genetic studies help reveal what endogenous DNA lesions are apt to contribute to ageing and lifespan [30].

Suppression of the other AP endonuclease, *apn-1*, causes classic phenotypes associated with a DNA repair defect, including increased mutation frequency and sensitivity to DNA damaging agents [34]. Additionally, knock down of *apn-1* causes a delay in the division of the P1 blastomere, typical of worms with increased DNA damage. However, unlike *exo-3* mutants that have a shortened lifespan, *apn-1* RNAi does not reduce the lifespan of worms unless they are treated with tert-butyl hydroperoxide (tert-BH) or MMS [34]. This suggests that EXO-3 may play a redundant role for APN-1 and is the major AP endonuclease in somatic maintenance. Collectively, these studies show that BER is required for normal lifespan of worms, implicating endogenous DNA damage as a driver of ageing.

11.4.2   Nucleotide Excision Repair (NER)

Inherited mutations affecting NER are responsible for several progeroid syndromes in humans including Xeroderma pigmentosum (XP), Cockayne syndrome (CS), trichothiodystrophy (TTD) and XFE progeroid syndrome [26]. These syndromes are all characterized by accelerated age-related decline of several tissues and the premature onset of diseases associated with old age. Many of these progeroid syndromes have been recapitulated in mice, often by single DNA repair gene mutations. These human syndromes fuelled several studies to interrogate whether NER promotes healths and longevity in worms.

In *C. elegans*, the mechanism of repair of UV-induced DNA lesions (i.e., NER) is very similar to humans [35]. There are several lines of evidence suggesting that DNA lesions that are substrates for NER promote ageing in worms. Expression of NER proteins is significantly lower in non-gravid adults (older adults) compared to gravid adults [35], indicating that NER is important for replicative longevity. *glp-1* mutants have an arrested germline and therefore enable measurement of DNA repair exclusively in post-mitotic animals. Repair of UV lesions is slower in ageing *glp-1* adults compared to young worms. This diminished repair in somatic cells however, is not because of decreased expression of DNA repair proteins (at least at the mRNA level). This could mean that protein translation, subcellular localization or post-translational modification of DNA repair proteins is affected with age [35]. These studies contribute evidence that DNA repair capacity decreases with age.

XP Complementation Group A (XPA) is required for GG-NER and TC-NER and plays a key role before damage excision. As in humans and mice [36], *rad-3/xpa-1* worms are hypersensitive to UV irradiation and have an increased mutation frequency in response to UV. Steady state levels of the oxidative lesions formamidopyrimidines (FapyGua and FapyAde) and 8-hydroxyadenine are significantly increased in *xpa-1* (*ok698*) mutants [37]. Human XP-A lymphoblasts also show an accumulation of these oxidative lesions, suggesting the importance of NER in repairing these endogenous lesions [38]. These adducts block both replication and transcription, and are increased in several age-related diseases such as Alzheimer's and cancer [39].

Reports on lifespan of *xpa-1* (*ok698*) mutants vary. Hyun et al. report a ~20 % reduction in mean lifespan [25]. Lans et al. find no lifespan shortening when looking only at a population of healthy adults (but observe a shortened lifespan in an unbiased population consisting of developmentally delayed mutants) [40, 41]. Fensgård et al. see a reduction in mean lifespan but not in maximum lifespan, when strains are grown on standard *E. coli* OP50 bacteria [32]. Interestingly, *nth-1* deletion (BER-see above) restores lifespan of *xpa-1* mutants. Furthermore, transcription of several DNA damage response genes is attenuated in the double mutant (*nth-1*;*xpa-1* compared to *xpa-1* alone) [32]. Taken together, these data suggest that upon loss of *xpa-1*, *nth-1* tries to process the lesions usually repaired by NER. However, NTH-1 and BER is apparently unable to resolve this damage through BER and instead causes an increased genome stress signal that culminates in a shortened lifespan. One possible interpretation of these data is that it is not the accumulation of DNA lesions itself that affects healthspan and lifespan but the damage-associated stress signal that is detrimental.

Many long-lived mutants, such as *daf-2* and *age-1*, require the FOXO transcription factor, *daf-16* for their extended lifespan [42, 43] (see Chap. 4). In the absence of cellular stress (or presence of insulin and IGF-1) DAF-16 is hyperphosphorylated by AKT and maintained in the cytoplasm under basal conditions. Upon stress, such as starvation, DAF-16 phosphorylation is attenuated and this allows for nuclear translocation and induction of several downstream target genes, including ROS scavengers and detoxifying enzymes. DAF-16 is predominantly in the nucleus in response to DNA damage (UV and in the *xpa-1* mutant), and is required for growth and development in the presence of genotoxic stress. As worms age, DAF-16 nuclear translocation in response to UV radiation diminishes [44]. This would suggest that with age the responsiveness of DNA damage-associated stress-protective genes is attenuated. The ability to respond to stress and longevity has long been proposed to go hand-in-hand. Thus the loss of stress responses upon genomic instability may explain the shortened lifespan in some DNA repair mutants.

To determine if DNA repair and genomic stability is necessary for the increased lifespan of the longevity mutants, *xpa-1* was knocked-down in *age-1* mutants. Although *age-1* mutants live ~1.6-fold times longer than N2, the lifespan of *xpa-1* (RNAi);*age-1* is similar to that of WT worms [25], suggesting that NER is critical for longevity. However, in stark contrast, knock-down of ERCC-1/XPF-1 expression further extends the life span of *daf-2* mutants. This is puzzling since ERCC-1/XPF-1 functions downstream of XPA-1. However, interpretation of these results is complicated by the fact that ERCC-1/XPF-1 plays a role in several DNA repair pathways including DSB repair and ICL. Further studies, with suppression of different NER proteins in long-lived mutants, is required to resolve this conundrum.

11.4.3   *Homologous Recombination (HR)*

In humans, Bloom syndrome, caused by mutations in *BLM*, is characterized by genomic instability, chromosomal breaks and gross chromosomal rearrangements, growth retardation, facial erythema, impaired fertility and an elevated risk of cancer. HIM-6 is the *C. elegans* ortholog of human BLM RecQ helicase, a class of enzymes that play an integral role in HR. *him-6* mutants exhibit several phenotypes that are characteristic of genomic instability [45]. For instance, *him-6* worms have increased apoptosis and heightened sensitivity to ionizing radiation, a known inducer of double-strand breaks, as well as an increased frequency of small insertions and deletions. This is in accordance with human cells that lack BLM and have short deletions and duplications and an elevated number of sister-chromatid exchanges [46]. Although, Bloom patients do not display signs of classical premature ageing they do succumb to early onset of cancer. *him-6* (*ok412*) mutant worms display a slight but significant decrease in lifespan [47]. Other healthspan measurements, such as motility or neuron maintenance have not been carefully examined in *him-6* mutants.

DNA-2 helicase/endonuclease is involved in DNA replication and repair. It is recruited by BLM to cleave 5′ ssDNA during double-strand break repair. The worm ortholog CeDNA-2, protein is highly expressed in proliferative germ cells and during the early stages of embryo development, consistent with a role in a DNA replication [48]. *dna-2* mutants display embryonic lethality (consistent with a role in replication and repair) and shortening of lifespan, which is more pronounced with each successive generation [49]. This result is similar to other DNA repair mutants such as *mre-11*, required for both homologous and non-homologous repair of double-strand breaks, RecQ5 in humans promotes DNA double-strand break repair by strand annealing [50, 51] and its loss is mainly associated with promoting cancer. In *C. elegans*, RCQ-5 is highly expressed in gonads, embryos and the intestine of adult worms. *rcq-5* RNAi leads to increased sensitivity to ionizing radiation, consistent with a role in HR. Inhibition of *rcq-5* leads to a 13 % decrease in lifespan at 20 °C and 37 % decrease at 25 °C [52]. This warrants further studies to investigate how temperature affects genomic instability in worms. An interesting analogy in humans is the exacerbation of the disease phenotypes in trichothiodystrophy patients with a defect in TC-NER when they have a fever [53].

11.4.4   *Non-homologous End Joining (NHEJ)*

Careful studies using a transgenic knock-in GFP-based NHEJ reporter mouse observed a significant decline in repair capacity in several tissues with age [54]. NHEJ deficiency leads to gross chromosomal rearrangements. Also, mice lacking Ku70, Ku80 or both, display signs of premature ageing including kyphosis, alopecia, osteoporosis, skin atrophy, and early onset of cancer [55]. Consistent with a role in NHEJ, RNAi of *cku-70* or *cku-80* (*C. elegans* orthologs of Ku70 and Ku80

respectively) show sensitivity to radiomimetics and MMS, but not to UV [56]. Interestingly, suppression of *cku-70* both in WT and in long-lived *daf-2* worms significantly increases thermotolerance (resistance to heat stress), in a *daf-16* dependent manner. In WT worms, there is no concomitant increase in lifespan. In contrast, knockdown of *cku-70* in an RNAi sensitive strain *rrf-3* (*pkl426*) and in *daf-2* mutants increases mean lifespan by 14 % and 35 %, respectively. One explanation is that the lifespan extension observed in *daf-2* mutants maybe due to an HSF-1 dependent increase in multiple stress resistance. Interestingly, these longevity phenotypes are independent of germline signals, since *glp-4*;*daf-2* mutants that lack germ cells also show an increase in lifespan (~9 %) when *cku-70* expression is knocked-down.

In contrast to *cku-70*, knockdown of *cku-80* does not confer thermotolerance and lifespan extension, suggesting possible divergent functions of these proteins that are thought to exist primarily as a complex [56]. *Ku86* knock-out mice (*cku-80* ortholog in mammals) display progeroid symptoms, premature senescence and a shortened lifespan [57]. Future studies to resolve this discrepancy between mice and worms are important.

Werner syndrome is a rare autosomal recessive disorder in humans, characterized by accelerated ageing. WRN belongs to the RecQ family of proteins and possesses an unusual exonuclease domain with 3–5′ activity. WRN plays a role in telomere maintenance, replication and interacts with Ku80/70 to facilitate NHEJ [58–60]. In the nematode, WRN-1 displays 43 % identity in protein sequence with human WRN. However, it lacks the exonuclease domain [61]. WRN-1 is expressed in larval stages, as well as in the hypodermis, intestine and germ cells of the adult, and protein expression decreases with age [61]. Loss of *wrn-1* significantly reduces brood size and leads to increased growth arrest at larval stages. Additionally, *wrn-1* mutants display increased lipofuscin accumulation, tissue deterioration in the head, and have shortened lifespans [61, 62]. Whether the role of WRN-1 in NHEJ is required for its role in lifespan maintenance needs to be further examined. Genetic studies to place the NHEJ proteins in pathways associated with ageing are key to further understanding its role in healthspan and longevity.

11.5   DNA Damage Response (DDR) and Ageing

The DNA damage response (DDR) is an integral part of damage recognition, recruitment of DNA repair proteins and maintenance of genomic stability (reviewed in [9]). The most upstream DDR kinases are: (1) ataxia telangiectasia mutated (ATM/ATM-1) and (2) ataxia telangiectasia and RAD3-related protein (ATR/ATL-1). ATM-1 usually recognizes double-strand breaks, such as those caused by IR and crosslinking drugs like mitomycin C (MMC), whereas ATL-1 responds to single-strand breaks and bulky adducts caused by agents such as UV [63]. These serine-threonine protein kinases, ATM-1 and ATL-1, phosphorylate a number of targets including the transcription factor p53/CEP-1, either directly or by first phosphorylating and activating checkpoint kinase 2 (CHK2). Additionally, ATR

phosphorylates checkpoint kinase 1 (CHK1) effector protein, which in turn phosphorylates the dual-specificity phosphatase CDC25C, thus arresting cells prior to mitosis and affording time for repair. These signalling pathways are well conserved in the nematode. Loss of these checkpoint proteins causes severe disease in humans including ataxia-telangiectasia and related syndromes and predisposition to cancer [64]. Since *C. elegans* is not prone to cancer, it is easier to examine the role of DNA damage response proteins in maintaining healthspan and lifespan.

Poly ADP-ribose polymerase (PARP), is a family of proteins that detects single-strand breaks, binds to DNA, and begins the synthesis of a poly-ADP-ribose chain (PAR) that acts as a recruitment signal for other repair proteins [65]. PARP also directly binds and activates ATL-1, to recruit other repair proteins [66]. PARylation increases markedly in mice and nematodes with ageing, suggesting that DNA damage increases with age. Accordingly, *xpa-1* (*ok698*) mutants have significantly higher levels of PAR than WT worms [67]. PARP activity requires NAD^+. In a *C. elegans pme-1* mutant, the worm PARP-1 homologue, both PARylation and NAD^+ consumption is attenuated, implying that *pme-1* is a major consumer of NAD^+. Notably, NAD+ levels decline with age across species [68], indirectly supporting a rise in DNA damage with age.

Ageing-associated lipid peroxidation and lipofuscin accumulation is substantially reduced in *pme-1* mutant worms, and suppression of *pme-1* increases levels of NAD^+ and leads to an extension of mean lifespan. NAD^+ consumption by *pme-1* leads to suppression of *sir2.1* activity, which also requires NAD^+ as a coactivator. *sir2.1* in turn regulates mitochondrial unfolded protein response (UPR^{mt}) and the *daf-16*-dependent antioxidant response needed to maintain mitochondrial homeostasis. This ties together nuclear DNA damage and repair mechanisms with mitochondrial function. Importantly, these signalling pathways are conserved in mammals [68].

Sirtuins also play a role in mitochondrial biogenesis by regulating PGC1α [69, 70] in mammals. Increased DNA damage (e.g., in *xpa-1* mutants), leads to hyperactivation of PARP-1 and attenuation of the NAD^+-SIRT1-PGC1α axis. In turn, mitophagy is compromised and defective mitochondria accumulate. Thus treatment of short-lived *xpa-1* (*ok698*) mutants with a PARP inhibitor AZD2281 or NAD^+ precursors (nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN)) rescues lifespan of these DNA repair deficient worms [67]. This reveals a complex relationship between DNA damage response pathways and metabolic homeostasis, necessary for maintaining healthspan and lifespan.

CID-1 (caffeine induced death-1) shares homology with poly(A)+ polymerase domain proteins, which play a role in the S phase to mitosis checkpoint [71, 72]. Hydroxyurea (HU) works as a ribonucleotide reductase (RNR) inhibitor and depletes dNTP causing developmental arrest [73]. In contrast, *cid-1* suppression (RNAi and mutant) permits normal development upon exposure to HU, suggesting a failure to induce checkpoint signalling that leads to cell cycle arrest or apoptosis. Loss of *cid-1* leads to *hsp-4* induction, increases thermotolerance and significantly increases lifespan. Although, *hsp-4* is induced in *cid-1* mutant worms, this is not accompanied by *hsp-16* activation. This suggests a role for *hsp-4* in stress resistance

that is independent from the *hsp-16*-dependent unfolded protein response in the endoplasmic reticulum (UPR^{ER}) [73].

Other checkpoint proteins, such as *cdc-25.1* (orthologue of CDC25C) and *chk-1* (orthologue of CHK-1), also influence longevity [73]. Inactivation of *cdc-25.1*, *cdc25.2*, *cdc-25.3* (but not *cdc-25.4*), results in stress resistance and an increase in lifespan. *cdc-25.1* gain-of function mutants are thermosensitive and short-lived. Suppression of *chk-1* leads to resistance to thermal stress (confirmed using the *chk-1* inhibitor UCN-01), extends lifespan (~15 to 25 %) and induces *hsp-4*. Surprisingly, stress resistance upon *chk-1* inhibition is not dependent on DAF-16 and DAF-12, as no change in nuclear localization of these proteins is observed. Consistently, loss of *chk-1* increases lifespan in *daf-16*, *daf-12* and in the long-lived *daf-2* mutants [73]. This data suggests that these checkpoint proteins promote somatic maintenance in post-mitotic cells, clearly through processes that are different from their well-established role germline and IIS pathways.

C. elegans p53 ortholog, *cep-1*, shares homology with mammalian p53, both in form and in function. Upon DNA damage, CEP-1, a transcription factor translocates to the nucleus and regulates the DNA damage response [74]. Suppression of CEP-1 leads to an increase in chromosomal nondisjunction events [75]. In humans, p53 is a well-established tumour suppressor, mutation of which causes the cancer predisposition syndrome Li-Fraumeni. The importance of tight regulation of p53 is documented by the fact that mutations causing chronic activation of p53 leads to segmental progeria in mice, and decreases both median (23 %) and maximum (21 %) lifespan [76]. Deletion or loss-of-function mutations of p53 leads to increased cancer incidence. Although, *C. elegans* is a largely post-mitotic organism, *cep-1* still seems to play a role in the DNA damage response and repair in the worm.

Suppression of *cep-1*, using RNAi or the deletion mutant (*gk138*), does not confer resistance to heat, high oxygen levels or UV, but does lead to increased mean lifespan. *cep-1* requires *daf-16* to exert its effects on lifespan, but this is not through differential nuclear localization of *daf-16*, suggesting that CEP-1 is not biochemically upstream of DAF-16. Of other proteins known to be involved in DNA repair *clk-2* (*qm37*), *rad-5* (*mn159*), *him-7* (*e1480*), *ced-4* (*n1162*), *egl-1* (*n487*), *msh-2* (*ev679*::*Tc1*) and *hus-1*), only suppression of *hus-1*, a checkpoint protein required for genomic stability, exhibits an increase in lifespan (~11 %). *cep-1* RNAi in the *hus-1* mutant does not further alter longevity, suggesting they both influence lifespan by the same pathway(s) [77].

11.6   Conclusions and Future Perspectives

Despite strong evidence in humans that DNA damage increases with age and is associated with several age-related diseases, whether it plays a causal role in driving ageing remains contentious. *C. elegans* as a model system has provided critical insights on the DNA damage theory of ageing. It is undeniable that several DNA repair pathways examined in the worm have an effect on lifespan. However, the

mechanism remains unclear. Does chronic activation of the DNA damage response or multi-stress response mechanisms play a role? Or is it mutagenesis caused largely by transcription drive ageing?

Mutation accumulation has been measured in 24 different regions of the genome to compare the relative importance of BER vs. NER vs. MMR in protecting genomic stability [78]. This revealed that loss of MMR led to 48-fold increase in mutations, while NER mutants caused a 28-fold increase and BER deficient worms had a 17-fold increase compared to WT worms. In contrast, whole genome next generation sequencing (NGS) in WT and 17 different DNA repair-deficient mutants revealed no significant increase in mutation rate in the absence of various DNA repair mechanisms [1]. These differences could stem from the number of generations examined. However, both of these studies do not reveal any significant accumulation of mutations during one lifespan, suggesting minimal role of mutation accumulation on lifespan.

Likewise, another question that can be answered in the nematode, is the relative importance of DNA repair in proliferating versus post-mitotic cells and its effect on lifespan. Additionally, *C. elegans* does not seem to have the traditional cellular senescence and senescence associated secretory phenotype (SASP) [13]. This could be an advantage in understanding primary mechanisms that respond to DNA damage and impact cellular programming finally impacting ageing. Last, but not the least, the one question that plagues the field is whether improving DNA repair efficiency leads to longevity? This poses a challenging problem, since DNA damage recognition and repair processes are extremely complex. However, with the advent of newer techniques such CRISPR, generating transgenic overexpression lines in the worm is feasible, timely and cost-effective.

In conclusion, using the strengths of *C. elegans* to elucidate the role of DNA damage in maintaining healthspan and lifespan is key to understanding how evolutionary adaptations has led to lifespan differences in species.

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