Roles of *Caenorhabditis elegans* WRN Helicase in DNA Damage Responses, and a Comparison with Its Mammalian Homolog: A Mini-Review

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Introduction

Werner syndrome (WS) is a rare genetic disease associated with a rapid acceleration of aging and an increased incidence of cancer [1]. The syndrome has attracted great interest from researchers on aging, since patients’ cells and relevant gene knockouts can be used as model systems for studying the mechanisms of normal aging. One typical phenotype of WS patients’ cells is genome instability in the form of DNA deletions, rearrangements, and isochromatids, and another is an increased oxidative stress [2]. The protein defective in WS patients, WRN, belongs to the RecQ family of DNA helicases [3, 4]. WRN is unique among the five RecQ DNA helicases in having an additional exonuclease activity. WRN, with its two enzymatic activities, is thought to be a DNA repair protein, since the cells of WS patients and other cells with induced WRN defects are hypersensitive to various types of DNA damage. Model organisms such as mice, flies, and worms deficient in WRN homologs have been investigated to understand the physiological results of defects in WRN activity. Premature aging, the most remarkable characteristic of Werner syndrome, is also seen in the mutant mice and worms, and hypersensitivity to DNA damage has been observed in WRN mutants of all three model organisms, pointing to conservation of the functions of WRN. In the nematode *Caenorhabditis elegans*, the WRN homolog contains a helicase domain but no exonuclease domain, so that this animal is very useful for studying the in vivo functions of the helicase without interference from the activity of the exonuclease. Here, we review the current status of investigations of *C. elegans* WRN-1 and discuss its functional differences from the mammalian homologs.
of DNA damage such as double-strand DNA breaks (DSBs) and interstrand DNA crosslinks (ICLs) as well as stalled replication forks (fig. 1). The fact that WRN physically interacts with a number of DNA repair proteins including replication protein A (RPA), RAD51, Ku70/80, and Polβ supports the idea that it is a repair protein [2]. In fact, the protein is mobilized from the nucleolus to the nucleoplasm in response to DNA damage, and then colocalizes in nuclear foci with other repair and checkpoint proteins [5]. WRN as a repair protein is especially important in maintaining telomere DNA of an appropriate length [6]. WRN interacts physically and functionally with TRF2, a telomeric repeat-binding protein, and its absence in organisms and cells leads to progressive shortening of telomere DNA over the generations [7]. The phenotypes of Wrn mutant mice are greatly exacerbated in a telomerase RNA component (Terc) mutant background: premature death, cataract, hair-graying, and osteoporosis [8]. The observations that SIRT6 histone deacetylase modulates telomere chromatin and recruits WRN to telomeres also support the role of WRN in telomere maintenance [9].

Very different roles of WRN from those in DNA damage responses – namely in the regulation of gene expression – have recently been demonstrated [10]. Expression of the microRNA miR-124 is lost in Wrn mutant mice, and it is likewise decreased after knocking down the WRN helicase homolog in Caenorhabditis elegans [11]. The transcriptional activator HIF-1 is stabilized and activated by WRN knockdown, which results in a higher level of reactive oxygen species in mammalian cells [12].

Roles of WRN in the Response to DSBs

WRN appears to be especially important in the repair of DSBs and stalled replication forks, although it has also been suggested to play a role in the repair of modified bases. In spite of extensive studies of the role of WRN in DSB repair and the resumption of replication, it is still not clear how the two enzyme activities participate in these repair processes. Nevertheless, the helicase activity appears to be much more important in DNA repair than exonuclease activity.

WRN is recruited very rapidly to DSBs, within a few seconds or minutes depending on the experimental conditions [13]. It then colocalizes with γ-H2AX, a marker for DSBs, and this colocalization requires NBS1, a component of the MRN complex containing, in addition, Mre11 and Rad50 [5]. The helicase activity of WRN, but not its exonuclease activity, is increased by interaction with the MRN complex [5]. In addition to the MRN complex, 53BP1, a checkpoint protein downstream of ataxia telangiectasia-mutated protein (ATM), colocal-
izes with WRN [14]. In the cells of WS patients in vitro, the growth of γ-H2AX foci is slowed, and its colocalization with the above proteins takes longer than in normal cells, pointing to slower repair kinetics. DSB repair in fact slows gradually as the primary cells multiply in vitro, as well as with aging of the human donors [14]. Even in the absence of exogenous insults, γ-H2AX foci are present in the cells of WS patients, suggesting that the speed of repair is too low to cope with spontaneous DSBs.

WRN mediates end resection of DSBs by physically interacting with Dna2 and RPA [15]. During the end resection process, WRN unwinds the DNA duplex, and Dna2 catalyzes the degradation of nucleotides from the 5′ end of DNA (fig. 2). WRN has 3′→5′ DNA helicase and 3′→5′ exonuclease activities and thus preferentially binds to DNA molecules with a single-stranded 3′ overhang. After binding to the single-stranded overhang of a duplex DNA molecule, WRN moves in a 3′→5′ direction, unwinding the duplex DNA. If the exonuclease of WRN worked on the same DNA substrate, it would chew up the 3′ end of the single-stranded DNA, generating a 5′ overhang that could not initiate the strand invasion of homologous recombination (HR). Instead of WRN, Dna2 degrades the 5′ end of the opposite DNA strand, producing a 3′ overhang. Although Dna2 has 5′→3′ he-
licase and nuclease activities [16], only its nuclease activity is required for the end resection of DSBs. Dna2 moves in a 5′→3′ direction on the strand that is degraded by its exonuclease activity while being attached to WRN moving in a 3′→5′ direction on the opposite DNA strand. The DNA helicase activity of WRN and the 5′→3′ exonuclease activity of Dna2 are promoted by RPA in both mammals and *Xenopus laevis* [17]. The WRN-Dna2-RPA complex can work on DNA molecules with 3′ overhangs, but not on ones with blunt ends. Prior to the action of the WRN-Dna2-RPA complex, the MRN complex, assisted by CtIP, produces a 3′ overhang from blunt ends of DNA. The MRN complex is thought to make an incision away from a blunt end and then remove nucleotides from the incision site towards the end, using the 3′→5′ exonuclease activity of Mre11 [18, 19]. Although the major pathway in which WRN participates to repair DSBs is HR, it is probably involved in other DSB repair pathways such as nonhomologous end joining (NHEJ) and single-strand annealing (fig. 2). It physically interacts with Ku70/80 and X4L4 (XRCC4-Lig4) in NHEJ, and its exonuclease but not helicase activity is stimulated by these interactions [20]. Conversely, WRN exonuclease promotes end joining by X4L4 through prior processing of blunt DNA ends [20].

**Roles of WRN at Stalled Replication Forks**

In the cells of WS patients and cells depleted of WRN, replication fork progression and, consequently, cell cycling are slower than normal. The replication problem in WRN-deficient cells is further aggravated under replication stress induced by aphidicolin and hydroxyurea (HU), which inhibit DNA polymerase-α and ribonucleotide reductase, respectively [21]. After replication forks stall, single-stranded DNA is exposed and binds RPA. RPA in turn activates cell cycle checkpoint proteins such as the Ser/Thr kinase ataxia telangiectasia and Rad3-related protein (ATR) and its downstream effector, Chk1 (fig. 3). WRN depletion attenuates Chk1 phosphorylation by ATR, but helicase activity is not essential for Chk1 phosphorylation [22], though it is needed to resume the progression of replication forks. The checkpoint activation allows stalled replication forks to be stabilized before resuming progression after the replication inhibitors have been removed. If the checkpoint is not properly activated, replication forks collapse and produce single-ended DSBs (fig. 3). Mus81 endonuclease makes an incision at stalled replication forks, and the newly generated DSBs initiate HR to permit replication forks to recover [23]. When WRN is deficient, DSBs accumulate, probably due to re-
duced checkpoint activation after DNA replication inhibition [23]. The role of the WRN helicase in the resumption of replication has been demonstrated in yeast cells that are hypersensitive to methylmethanesulfonate and DSBs due to mutation in rad50 [24]. The hypersensitivity of rad50 mutant cells to methylmethanesulfonate but not to DSBs is rescued by overexpression of WRN, suggesting that WRN is important in resistance to replication inhibition but not to DSBs. The helicase activity of WRN is required in yeast cells for the rescue of stalled replication forks, as in mammalian cells. WRN is thought to promote the cleavage of stalled replication forks by Exo1 in the rescue of rad50 yeast cells [24]. The critical role of WRN in replication fork progression is clearly evident at common fragile sites. These are chromosomal regions where the progression of replication forks is often blocked, and which are seen as very thin regions in metaphase chromosomes. The helicase activity of WRN – not its exonuclease activity – is important for the stability of common fragile sites [25]. When replication is first inhibited in mammalian cells, WRN is phosphorylated by ATR, whereas later it is phosphorylated by ATM, a Ser/Thr kinase activated by DSBs [26]. The phosphorylation of WRN by ATR is needed for colocalization of WRN with RPA, while that by ATM allows recruitment of RAD51.

**WRN in C. elegans**

A WRN homolog has been identified in *C. elegans* based on its close homology with mammalian WRN; however, it has only a helicase domain, not an exonuclease domain. Besides WRN, three other RecQ family DNA helicases are present in *C. elegans*: the open reading frame T04A11.6, homologous with mammalian RecQL; HIM-6, equivalent to BLM, and RCQ-5, corresponding to RecQ5. No homolog of mammalian RecQ4 has been found. The WRN helicase in *C. elegans*, WRN-1, has 43% amino acid identity with human WRN in the helicase domain (fig. 4a) and has been shown to be a functional homolog; when it is knocked down, worms have a reduced life span (from 13.6 to 11.0 days at 25 °C) and accumulate lipofuscin faster (fig. 4b). A similar reduction of life span in worms (from 9.0 to 7.0 days at 25 °C) was also induced by a *wrn-1* mutation [28]. The protein has helicase activity in vitro, and the activity is stimulated by RPA [29]. One unique phenotype of *wrn-1* knockdown is the acceleration of embryonic cell divisions and of larval growth [27]. In early embryos, the S-phase is shorter after *wrn-1* knockdown, which contrasts with the extended S-phase in the cells of WS patients. A reduced S-phase is also observed upon *chk-1* knockdown, implying that WRN-1 functions in the DNA replication checkpoint (fig. 4b). Indeed, mitotically proliferating germ cells in the gonad of adult worms do not show proper cell cycle arrest when DNA replication is inhibited by HU. Based on an analysis of CHK-1 phosphorylation, and nuclear accumulation of RPA-1 (RPA70 homolog), WRN-1 has been proposed to function upstream of CHK-1 and to promote RPA-1 recruitment to stalled replication forks [30]. WRN was also observed to function in S-phase arrest after treating mammalian cells with HU, camptothecin (DNA topoisomerase I inhibitor), or PUVA (psoralen plus UV-A radiation producing ICLs) [31]. The drugs HU, camptothecin, and PUVA all block the progression of replication forks, after which the stalled replication forks are cleaved to produce DSBs. Interestingly, WRN is required for ATM activation after the formation of ICLs in mammalian cells [31].

*C. elegans* WRN-1 functions as a checkpoint protein in response to DSBs as well as to DNA replication inhibition [27], whereas WRN has a very limited role in checkpoint activation in mammals, where it only acts after replication inhibition [31]. When wild-type *C. elegans* larvae are exposed to a low dose of γ-rays, their growth is markedly retarded, but this does not happen in *wrn-1*-depleted or -mutated worms, implying a checkpoint function of WRN-1 in response to DSBs. This checkpoint function was confirmed by scoring the number of germ cells in the mitotically proliferating region of the gonad [30]. As is the case after DNA replication inhibition, accumulation of RPA-1, as well as that of ATM-1 (ATM homolog), was attenuated in *wrn-1* mutant worms after DSB formation (fig. 4b), suggesting that WRN-1 is upstream of RPA-1 and ATM-1. Since RPA-1 was reported to function upstream of ATL-1 (ATR homolog) in response to DSBs, WRN-1 is upstream of both ATM-1 and ATL-1 in *C. elegans* [30, 32]. How WRN-1 promotes the accumulation of RPA-1 at DSBs is not yet clear. One possible role of WRN-1 at DSBs is to mediate the end resection at DSBs to produce a 3′ overhang. Such a role of WRN, in a complex with Dna2, in end resection has been proposed in mammalian and *Xenopus* cells. In mammalian cells, the initial end resection is mediated by the MRN complex together with CtIP, and this is followed by more extensive resection involving several kilobases [19]. The later end resection is mediated by two parallel pathways: one involves Exo1, and the other requires Dna2 in combination with WRN or BLM. In *C. elegans*, the NBS1 unit of the MRN complex is absent in the genome, whereas the Mre11 and Rad50 homologs are present. In addition,
**C. elegans** does not have the variant form of histone H2A (H2AX), which interferes with detection of DSBs using antibodies to the phosphorylated form of H2AX. The CtIP homolog in *C. elegans*, COM-1, functions during HR in meiotic cells, but not in mitotically proliferating germ cells. The fact that NBS1 and CtIP do not function in mitotically proliferating germ cells of *C. elegans* could be the reason for the high activity of WRN-1 in checkpoint activation in response to DSBs; in the absence of these proteins, WRN-1 may play a critical role in end resection, generating single-stranded DNA for RPA-1 binding and subsequent checkpoint activation. The helicase activity of WRN-1 is probably essential for the accumulation of RPA-1 at DSBs and the subsequent checkpoint activation. Nevertheless, the helicase activity may not be required for checkpoint activation after inhibition.
of replication in *C. elegans*, as observed for WRN in mammalian cells [22]. In connection with the role of WRN-1 in checkpoint activation in response to stalled replication forks and DSBs, very little work has been done on the role of *C. elegans* WRN-1 in DNA repair (fig. 4c).

Like mammalian WRN, *C. elegans* WRN-1 has a role in regulating gene expression as well as in DNA damage responses. Mutation of WRN-1, or its depletion, affects the transcription of a number of genes, including those involved in the metabolism of lipids, ketones, and organic acids, as well as in DNA repair and cell cycling [28]. Interestingly, the expression of miR-124 microRNA is reduced in *wrn-1* mutants, as observed in WRN helicase mutant mice [11]. The loss of miR-124 in *C. elegans* leads to an increased level of reactive oxygen species, the accumulation of lipofuscin, and thus to a reduced life span (fig. 4d). This close relationship between the *wrn-1* and *mir-124* phenotypes suggests that one important role of WRN could be that of promoting the expression of *mir-124*, which in turn would repress the posttranscriptional expression of many target genes.

So far, no homolog of the WRN exonuclease domain has been identified in *C. elegans*, although MUT-7 is a candidate. However, MUT-7 is primarily involved in RNA interference and gene silencing, and thus is not likely to be a functional homolog of the WRN exonuclease [33]. Nevertheless, *mut-7* mutations increase mutation rates in *C. elegans*, raising the possibility that MUT-7 is involved in DNA damage responses. It is noteworthy that *Drosophila* possesses a homolog of the WRN exonuclease domain (WRNexo) [34], whereas no counterpart of the WRN helicase domain has been found [34]. Reduced expression of WRNexo or a missense mutation critical for the exonuclease activity leads to hyperrecombination, suggesting a role in DNA repair [35, 36]. However, the sensitivity of *Drosophila* to replication inhibition is increased by deletion of WRNexo but not by inactivation of the exonuclease activity, implying that the WRNexo protein, but not its enzymatic activity, is required at stalled replication forks [34].

### Conclusions

The *C. elegans* WS protein WRN-1 contains a helicase domain without an exonuclease domain and has been an excellent model system for investigating the functions of the helicase domain. Using this model, the checkpoint function of WRN-1 in response to replication inhibition and DSBs was easily detected. Mammalian WRN is similar to WRN-1 in its checkpoint activity in response to replication inhibition, but it does not have this role in response to DSBs, probably because of differences in the checkpoint pathways that respond to DSBs in the two systems [31]. One of these differences is that RPA is upstream of the ATM and ATR homologs in *C. elegans*, whereas activation of ATM does not require RPA in mammals. The helicase domain of WRN-1 promotes the accumulation of RPA at DSBs, probably by physical interaction with RPA or by stimulating the end resection of DSBs to generate single-stranded DNA for RPA binding. Nevertheless, the two WRN homologs share the ability to promote the activation of ATM, which occurs at ICLs and stalled replication forks in mammals, and at DSBs in *C. elegans*. The checkpoint function of WRN-1 at replication forks may not require its helicase activity, as is the case for mammalian WRN [22]. However, it is very likely that the helicase activity is needed to promote the accumulation of RPA at DSBs. Besides its role in response to DNA damage, WRN-1 has been shown to increase the level of miR-124, which opposes aging in *C. elegans* [28]. Therefore, the regulation of gene expression by WRN-1 is thought to be important in protection against aging, as well as in resistance to DNA damage.

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