The Werner syndrome gene

the molecular basis of RecQ helicase-deficiency diseases

Werner syndrome (WS) is an autosomal recessive genetic disorder that is manifested by genetic instability and premature onset of age-related diseases, including atherosclerosis and cancer. The gene that is mutated in WS cells (*WRN*) has been identified recently. Characterizations of the *WRN* gene product indicate that *WRN* encodes both a 3′→5′ DNA helicase, belonging to the *Escherichia coli* RecQ helicase family, and a 3′→5′ DNA exonuclease. Studies to define the molecular mechanism of WRN–DNA transactions are currently underway in many laboratories. Preliminary results indicate that WRN functions as a key factor in resolving aberrant DNA structures that arise from DNA metabolic processes such as replication, recombination and/or repair, to preserve the genetic integrity in cells.

Discovered by Otto Werner in 1904 in a family displaying symptoms similar to premature aging[1], Werner syndrome (WS) is an uncommon autosomal recessive disorder characterized by early onset of age-related diseases including atherosclerosis, osteoporosis, type II diabetes mellitus, cataracts and rare soft-tissue sarcomas[2]. In culture, cells from WS patients exhibit a shortened life-span[3] and a prolonged S-phase of the cell cycle[4]. WS cells are genetically unstable, as discovered initially by the finding of nonclonal translocations, termed 'variegated translocation mosaicism'[5]. Genetic instability is also manifested in cell culture by the generation of mutations that consist mainly of extensive deletions[6]. Thus, Werner syndrome, like Bloom syndrome, xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anemia, can be classified as an inherited disease of genomic instability[7].

The gene responsible for Werner syndrome (*WRN*) has recently been identified, cloned and expressed *in vitro*[8–10]. The recombinant protein, WRN, has been characterized biochemically, allowing the formulation and construction of *in vitro* models to investigate the cellular function of WRN[11–19]. In addition, transgenic mice with mutations in *WRN* are being analyzed to gain insight into the physiological function(s) of WRN and the pathologies associated with WS[20]. In this review, we focus on recent biochemical characterizations of the helicase and exonuclease activities of WRN, and discuss the possible roles of WRN in cells and the WRN-interacting proteins that have been identified. Several models that postulate a function for WRN helicase and/or exonuclease in pathway(s) of DNA biosynthesis, including the intriguing mouse model for WS, are considered. We hope that this discussion will foster new insights into the molecular bases of WS and other RecQ helicase-deficiency diseases.

WRN is a human DNA helicase homologous to *E. coli* RecQ

In 1992, the Werner syndrome gene (*WRN*) was mapped to chromosome 8 at 8p12 (Ref. 21). The cDNA sequence of the entire gene was deciphered by positional cloning and extensive DNA sequencing, and was reported in 1996 to encode a DNA helicase homologous to *E. coli* RecQ (Fig. 1)[8]. Interestingly, the cDNA sequence of the Bloom syndrome gene (*BLM*) was published just a year earlier and reported to be homologous to RecQ (Ref. 22). Considering the differences in clinical pathology, WS being a disease associated with premature aging that manifests in post-pubescent individuals, and BS being a disease associated with malignancies, principally leukemias, that manifests in children, it was initially surprising to learn that *WRN* is also a RecQ helicase family member.

To demonstrate that *WRN* encodes an active DNA helicase, many researchers attempted to express the gene in a variety of systems but were unable to obtain unequivocal evidence of a helicase activity in yeast, *E. coli* or in an *in vitro*, reticulocyte-based protein-synthesizing system. Only expression of WRN in insect cells transfected with *WRN*-bearing baculovirus vectors provided evidence of DNA helicase activity[9,10]. Biochemical characterization of the recombinant WRN protein in a DNA strand-displacement assay clearly indicated WRN to be a DNA helicase[9,10] (Table 1.) WRN helicase similar to *E. coli* RecQ, unwinds duplex DNA in a 3′→5′ direction and is dependent on the hydrolysis of ATP. The ATPase is DNA-dependent, and, as

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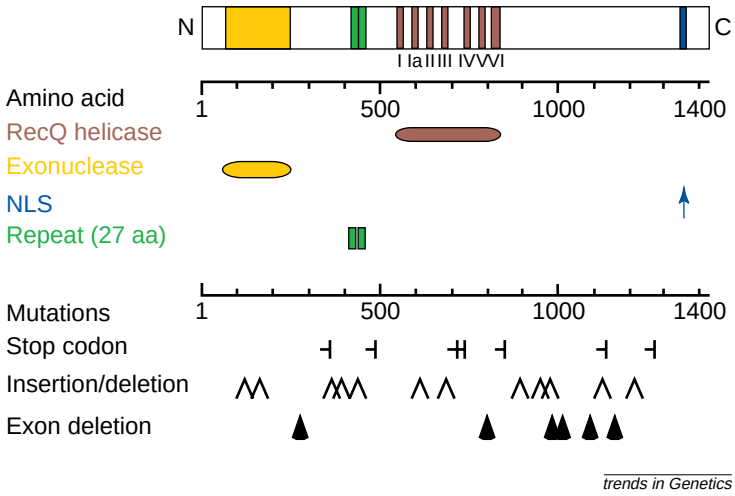
is the case for other DNA helicases, both the ATPase and helicase activities are abolished by an amino-acid substitution in the Walker A motif (K577M)[9]. In addition, WRN helicase is able to unwind an RNA–DNA hetero-duplex[10]. WRN also preferentially uses ATP and dATP and, to a lesser extent, CTP and dCTP as substrates[11].

E. coli RecQ is the prototype of the RecQ family of DNA helicases, members of which have been found in various species, including yeast, *Caenorhabditis elegans*, *Drosophila*, *Xenopus*, mouse and humans[23] (Box 1). All the RecQ homologs contain a characteristic seven-motif domain with the typical DEXH helicase box (Fig. 1). RecQ helicases are thought to retain a conserved function in governing DNA transactions, and genetic and biochemical studies in various organisms should lead to a better understanding of the function of the RecQ family as a whole, including WRN.

FIGURE 1. The Werner syndrome protein (WRN) and mutations observed in affected individuals



The Werner syndrome protein (WRN) is a large, 1432-amino-acid polypeptide homologous to *Escherichia coli* RecQ. A conserved 7-motif RecQ helicase domain is located centrally, and contains the Walker A ATPase consensus sequence in motif 1. An exonuclease domain, unique among known RecQ helicases, resides in the N-terminal region. A nuclear localization signal (NLS) is located at the C-terminus (amino acids 1370–1375) and a direct repeat of 27 amino acids is situated between the exonuclease and helicase domains. Mutations in Werner syndrome patients are located over the entire gene and include stop codons, insertions/deletions and exon deletions; no missense mutations have been reported to date. Figure courtesy of Dr Junko Oshima, University of Washington.

Human RecQ helicases and genetic stability

Werner syndrome, as a genetic instability disease, is characterized by reciprocal chromosomal translocations[5] and extensive genomic deletions[6]. The two other human RecQ-deficiency diseases Bloom syndrome[22] and Rothmund–Thomson syndrome[24] are also characterized by genetic instability (Box 1). In Bloom syndrome, genetic instability is manifested as an increase in sister chromatid exchange, which is indicative of a hyper-recombination phenotype. In the Rothmund–Thomson syndrome, the instability manifests as chromosomal rearrangements that result in somatic mosaicism. Thus, elucidating the molecular mechanisms that underlie the genetic aberrations associated with RecQ-deficiency diseases, including WS, will aid in understanding the role of RecQ helicases in preserving the genomic integrity of human cells.

Possible roles of WRN helicase in cells

Resolving aberrant DNA structures?

Owing to the genetic instability of phenotypes of WS cells (Table 2), wild-type WRN is believed to function in maintaining the integrity of genetic information. But how, at the molecular level, does the DNA helicase activity of WRN play such a role? The finding that WRN can unwind partially duplexed DNA *in vitro* does not establish that this is the pathogenically significant DNA structure that WRN resolves in cells. For example, it has been demonstrated recently that WRN can also unwind a tetraplex DNA structure (a tetrahelical DNA stabilized by guanine–guanine non-Watson–Crick hydrogen bonds; also called G4 quadruplex DNA) *in vitro*. This tetraplex is composed of $d(CGG)_n$ repeats that are expanded in the human Fragile X syndrome[13] (Table 1). This observation suggests that WRN helicase unwinds aberrant DNA structures that might impede DNA biosynthesis. Sgs1 and BLM helicases (Box 1) are also able to unwind G4 quadruplex DNA[25,26] (Table 1). It remains to be determined whether different alternate DNA structures would be unwound by different DNA helicases and whether deficits in unwinding these structures are of pathological significance.

A suppressor of illegitimate recombination?

E. coli RecQ helicase acts in the RecF pathway of homologous recombination[27]. Although its biological role has yet to be fully established, it has been suggested that *E. coli* RecQ functions as a suppressor of illegitimate recombination[28]. RecQ, functioning in concert with RecA

TABLE 1. Activities of WRN and other RecQ helicases

Property	WRN	BLM	SGS1	RecQ
Amino acids	1432	1417	1447	610
Directionality of helicase	3′→5′	3′→5′	3′→5′	3′→5′
ATPase activity	Yes	Yes	Yes	Yes
DNA substrates	3′-overhang dsDNA Forked DNA[10]	3′-overhang dsDNA Forked DNA[69]	3′-overhang dsDNA Forked DNA[70]	3′-overhang dsDNA Forked DNA Blunt-ended dsDNA Closed circular DNA[29,50]
Alternate DNA substrates	Tetraplex[13] RNA–DNA hybrid[10]	Tetraplex[26] 4-way junctions[69]	Tetraplex[25] 3- or 4-way junctions[70] RNA–DNA hybrid[70] Nicked or grapped DNA[70]	3- or 4-way junctions[29]
Interacting proteins	RPA[11,12] p53[45,46]	ND	Topo II[48] Topo III[47]	ND

Abbreviations: RPA, replication protein A; p53, gene product of the tumor suppressor gene *p53*; Topo II, DNA topoisomerase II; Topo III, DNA topoisomerase III; ND, not determined.

BOX 1. The RecQ family

RecQ	Syndrome	Construct (Exonuclease / Helicase)	Amino acids	Refs
Human WRN	Werner		1432	8
Human BLM	Bloom		1417	22
Human RecQL			649	64, 65
Human RecQ4	Rothmund–Thomson		1208	24, 66
Human RecQ5			410	66
Saccharomyces cerevisiae Sgs1			1447	47, 48
Schizosaccharomyces pombe Rqh1			1328	67
Escherichia coli RecQ			610	68

and single-stranded DNA binding protein (SSB) in an *in vitro* model system, can both initiate and disrupt DNA recombination[29], suggesting two roles for the enzyme *in vivo*: (1) as an initiator of homologous recombination and (2) as a disrupter of joint molecules formed by illegitimate recombination. It is possible that WRN serves a similar function. Genetic studies in *Saccharomyces cerevisiae* have shown that *WRN* (and *BLM*) can suppress the hyper-recombination (both homologous and illegitimate) phenotype exhibited by an *sgs1* mutant that lacks the sole endogenous RecQ homolog[30]. Thus WRN might belong to a class of proteins, whose function is to promote the regularity of DNA recombination and hence prevent alterations in genetic information.

A participant in replication restart?

A recent study has strengthened the association between certain *E. coli* proteins in the RecF recombination pathway and recovery of DNA replication following UV-induced arrest[31,32]. RecF and RecR were found to be essential both for preventing extensive degradation of newly synthesized DNA following UV-irradiation and for resuming replication[31]. Unlike *recF* and *recR* mutants, *recQ* cells are not hypersensitive to UV. Nonetheless, additional work indicated that RecQ, together with RecJ, a $5'\rightarrow3'$ exonuclease that hydrolyzes single-stranded DNA, selectively degrades nascent lagging strand DNA following UV-induced fork disruption[32]. Courcelle and Hanawalt propose a model[32] in which lagging strand degradation by RecQ and RecJ enlarges a region of single-stranded DNA at the disrupted fork (Fig. 2). In this model, RecF and RecR function together with RecA to promote formation of a triple-stranded structure, consisting of newly replicated, duplex leading strand and unreplicated lagging strand. This stabilization of fork DNA would allow time for the repair of the downstream, UV-induced photoadduct, and would permit subsequent replication restart at the original site of fork disruption, without strand breakage and recombination.

WS cells exhibit prolonged S-phase[4] and other abnormalities in DNA replication[33,34]. It is thus possible that WRN helicase, like *E. coli* RecQ, functions to restore DNA replication after replication-fork disruption caused by DNA lesions or aberrant DNA structures. Notably, like *E. coli recQ* cells, WS cells are also insensitive to UV (Table 2).

Based on the model of Courcelle and Hanawalt[32], we suggest that WRN helicase displaces Okazaki fragments on the lagging strand at stalled replication forks. By associating with an as yet unidentified $5'\rightarrow3'$ exonuclease (functionally homologous to *E. coli* RecJ) or FEN-1 endonuclease[35] or a proposed intrinsic exonuclease activity of WRN[17], WRN might promote degradation or cleavage of the displaced DNA strand. Selective degradation of the lagging strand would permit the replication fork to be stabilized within a triple-stranded structure, perhaps involving Rad51 (a human RecA homolog). Hence, replication would be resumed at the site of disruption without strand breakage or recombination, after repair of the downstream blocking lesion (Fig. 2).

Involvement in recombination repair?

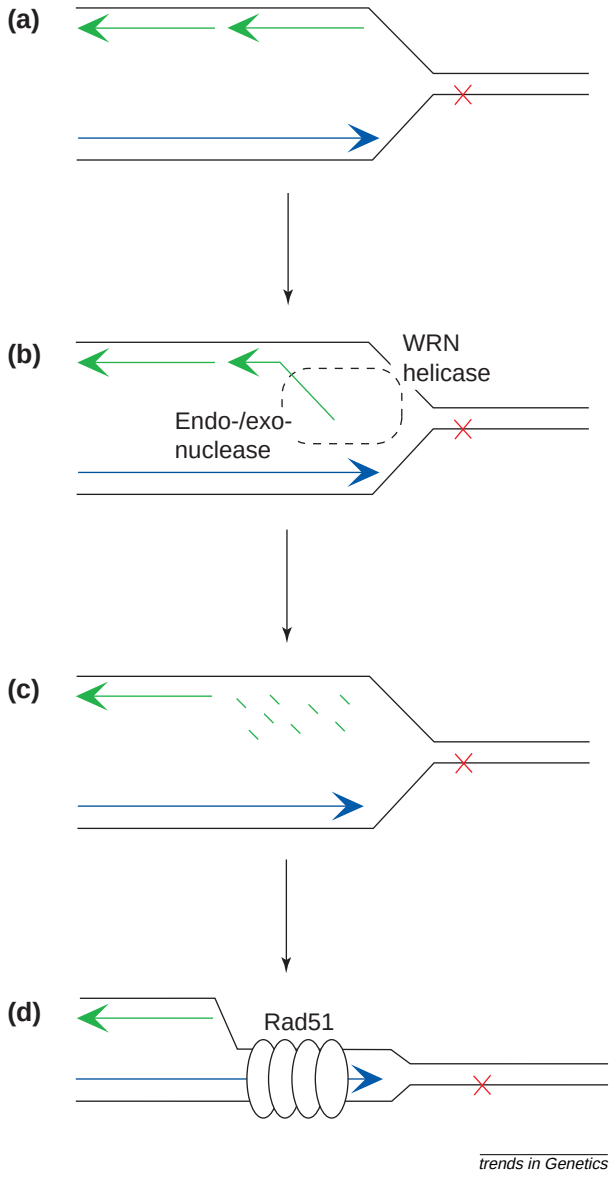
The RecF pathway (which includes the *recF*, *recO*, *recR*, *recQ* and *recJ* genes) in *E. coli* is required not only for homologous recombination but also for recombinational repair in a *recBCsbcBC* background—where the RecF pathway replaces the inactive, major recombinational pathway RecBCD (Ref. 27). Models for recombinational repair at a stalled replication fork have been proposed elsewhere[36–39]. The involvement of RecFOR proteins (gene products of *recF*, *recO* and *recR*) in stalled fork-mediated recombination events has been observed, although the role of RecQ in this process(es) has yet to be established. Nonetheless, the work

TABLE 2. Phenotypes of Werner syndrome cells

Phenotypes		Refs
Cell culture	Poor growth, shortened life span	3, 4
Cytogenetics	Variegated translocational mosaicism	5
Genetics	Extensive genomic deletion	6
UV sensitivity	No	71
X-ray sensitivity	No	71
Drug sensitivity		
4-NQO	Yes	56, 72
Camptothecin	Yes	49
Bleomycin	No	72
Hydroxyurea	No	73
Alkylating agents (e.g. MMS[a] and MNNG[b])	No	73

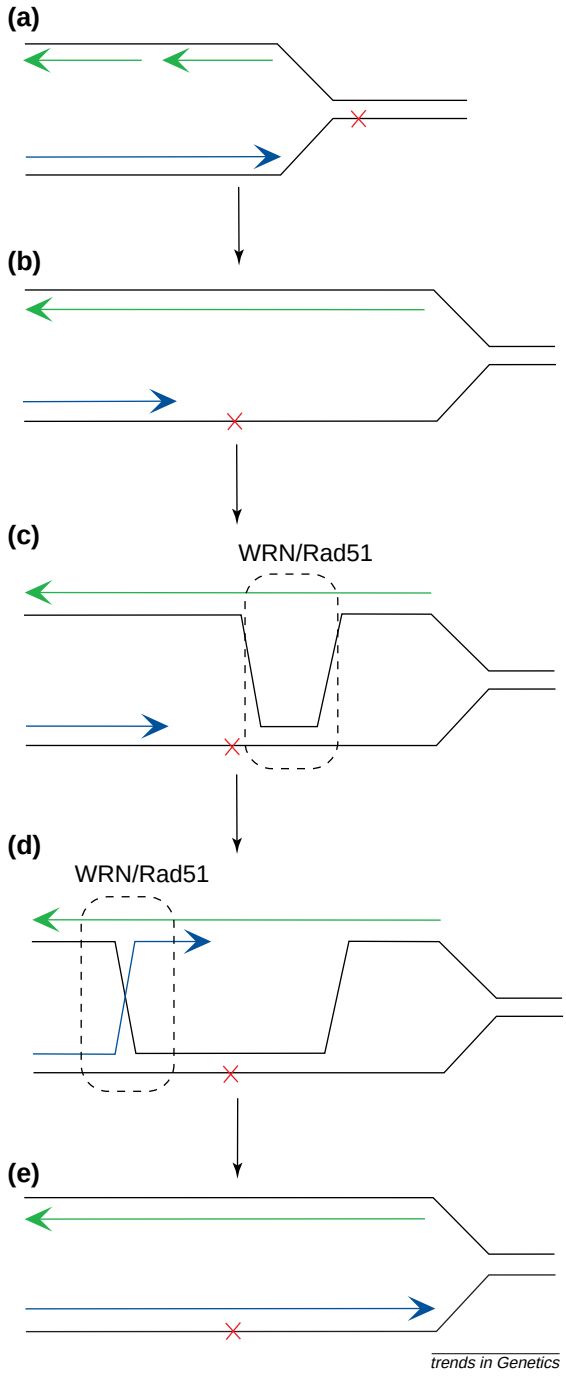
[a]MMS: methyl methanesulfonate.
[b]MNNG: 1-methyl-3-nitro-1-nitrosoguanidine.

FIGURE 2. A schematic model for Werner syndrome protein (WRN) function at a stalled replication fork



(a) A replication fork stalls after encountering a block, such as a premutagenic DNA adduct or an aberrant DNA structure (shown as a red cross). To enable replication to resume, (b) WRN helicase might displace Okazaki fragments on the lagging strand and associate with FEN-1 endonuclease and/or a 5′→3′ exonuclease to (c) cleave or degrade the displaced DNA strand. (d) This editing process would permit the replication fork to be stabilized within a triple-stranded structure, perhaps by Rad51 protein (a human RecA homolog), to allow enzymatic removal of the block. Following removal, replication will resume without strand breakage or recombination. This model was originally presented by Courcelle and Hanawalt[32].

FIGURE 3. A model for the function of Werner syndrome protein (WRN) in recombination repair



(a) When a replication fork encounters a block (shown as a cross), (b) a single-strand gap forms in the stalled fork. (c) This single-stranded region can be used for Rad51-mediated strand exchange and D-loop formation to initiate (d) a recombination event. WRN associates with Rad51 to facilitate strand exchange and D-loop formation, and might also be involved in the ensuing branch migration and/or joint molecule resolution. (e) After recombinational DNA synthesis and Holliday junction resolution, the DNA lesion resides in a duplex structure that is the preferred substrate for repair enzymes.

of Harmon and Kowalczykowski[29], showing that RecQ can initiate and disrupt homologous DNA pairing in concert with RecA and SSB, indicates that RecQ might be involved. Moreover, RecQ can unwind 3- or 4-way DNA junctions, further supporting the hypothesis that RecQ is involved in D-loop formation and/or joint molecular resolution. It is therefore proposed that WRN serves in recombination-mediated gap repair after replication-fork stalling (Fig. 3). In this model, WRN associates with Rad51 and RPA (human replication protein A) to facilitate strand exchange and/or joint molecular resolution. After recombination, the single-strand gap is filled and repair enzymes can remove the lesion.

A replication origin protein?

Initiation of DNA replication in eukaryotic nuclei occurs at a discrete number of foci that contain the single-strand

binding protein replication protein A (RPA). In 1995, a 170-kDa protein that promotes the formation of replication foci on sperm chromatin was purified from *Xenopus laevis* egg extracts[40]. This protein, foci-forming activity 1 (FFA-1), was required for the ATP-dependent assembly of RPA into foci. Biochemical characterization revealed that FFA-1 is an ATP-dependent DNA helicase[41]. Surprisingly, sequencing of the cDNA showed that *Xenopus* FFA-1 is a homolog of human WRN; the two proteins share 66% similarity and 50% identity. This finding raises basic questions. Does human WRN provide foci-forming activity at replication origins in human cells? Is human WRN a replication protein and, if so, what is its molecular function? These questions can be approached by searching for WRN-interacting proteins in replication foci; *Xenopus* foci contain neither DNA topoisomerase II nor the large subunit of DNA polymerase α (Ref. 40), two potential WRN partners.

WRN helicase-interacting proteins

Replication protein A (RPA)

Identification of a protein's functional partners provides an approach to understanding its biological function. The first WRN-interacting protein to be recognized was RPA[11] (Table 1). In an assay to compare the effects of single-stranded DNA binding proteins (SSBs) from *E. coli*, T_4 phage and human on the helicase activity of WRN, maximum stimulation with *E. coli* and T_4 phage SSBs was observed at concentrations that were stoichiometric with the amount of DNA in the assay, whereas stimulation by human RPA occurred at a much lower concentration. This suggested that human RPA enhanced the function of WRN helicase by binding to the displaced DNA strand, thus preventing it from re-annealing, but some other mechanism might also be involved, such as one involving the direct interaction of RPA and WRN.

The physical interaction between RPA and WRN was first confirmed by their co-immunoprecipitation[12]. Their interaction *in vitro* is consistent with the observation that binding of FFA-1, a *Xenopus* homolog of WRN, to RPA might be involved in the formation of replication foci[41]. Association of RPA with another mammalian RecQ helicase has been reported; mouse BLM co-localizes with RPA in meiotic prophase nuclei of mouse spermatocytes[42]. RPA is a multifunctional protein that interacts with proteins in almost every known DNA metabolic pathway, including replication, recombination, repair and probably transcription[43], and thus its interaction with WRN is not diagnostic of the function of WRN in cells.

Tumor suppressor gene product p53

p53 (also known as TP53) binds to xeroderma pigmentosum complementation group B and D helicases and modulates their activity in the transcription factor IIH complex[44]. This interaction could explain the observation that fibroblasts from xeroderma pigmentosum complementation group B or D patients fail to undergo p53-mediated apoptosis. Attenuation of p53-mediated apoptosis was observed recently in several WS fibroblast lines versus normal fibroblasts, and could be restored by expression of *WRN* (Refs 45, 46). Co-immunoprecipitation of p53 and WRN[45,46] indicated their physical interaction (Table 1). Thus, WRN probably functions downstream of the p53-mediated apoptotic pathway, and its activity might be modulated by p53 by direct physical interaction.

DNA topoisomerase(s)?

Important candidate WRN-interacting proteins include DNA topoisomerases. The link between topoisomerases and RecQ helicases was first revealed by discovery of the *S. cerevisiae* mutant *sgs1*, which is a suppressor of the slow-growth phenotype of *top3* mutants deficient in DNA topoisomerase III (Ref. 47). Interestingly, *top3* mutants also exhibit genomic instability, manifested as hyper-recombination between repetitive DNA elements, and this phenotype can also be suppressed by the *sgs1* allele. As Sgs1 and Top3 bind to one another[47] (Table 1), Sgs1 might generate topologically altered DNA structures that Top3 functions in concert to resolve. Sgs1 also physically interacts with topoisomerase II (Table 1), and is required for faithful chromosome segregation[48]. To date, a suspected WRN–topoisomerase binding has not been reported. However, there is indirect evidence to support a functional association between WRN helicase and topoisomerase. WS cells are sensitive to camptothecin-induced apoptosis in S-phase[49]; camptothecin is an inhibitor of type I DNA topoisomerases (Table 2). Moreover, homozygous mutant mouse embryonic stem cells harboring two copies of a *WRN* allele that contain a disrupted helicase domain are sensitive both to camptothecin and to etoposide (a topoisomerase II inhibitor)[20], suggesting that WRN helicase and topoisomerase(s) might associate functionally to preserve genomic integrity.

Recently, Harmon *et al.*[50] used *E. coli* RecQ and wheat germ topoisomerase I (wheat germ Topo I) in a topological assay to demonstrate the unwinding of relaxed, closed circular dsDNA by RecQ. In the presence of SSB and wheat germ Topo I, RecQ unwound the closed circular plasmids (Table 1), changing the linking number and transforming the plasmids from a relaxed to a negatively supercoiled form. The effects of RecQ on the function of Topo III acting on negatively supercoiled DNA was also studied[50]. Surprisingly, RecQ stimulated Topo III to catenate dsDNA molecules, apparently by exerting its unusual unwinding activity to create a single-stranded binding site on the covalently closed dsDNA. *S. cerevisiae* Topo III, *E. coli* RecQ and *S. cerevisiae* SSB (yRPA) were also found to produce catenated molecules similar to those observed with the bacterial enzymes. These findings suggest a concerted function of RecQ helicase and topoisomerase in the regulation of DNA recombination, particularly in the enhancement of strand passage to resolve topological intermediates produced during recombination. So far, direct evidence for the interaction of WRN with any topoisomerase is lacking.

WRN is also a DNA exonuclease

Soon after the identification of WRN helicase, advanced sequence alignment analysis revealed a putative exonuclease domain near the N-terminus of WRN[51,52] (Fig. 1). This domain contains three conserved motifs that resemble those in the proofreading exonuclease domain of *E. coli* DNA polymerase I and in *E. coli* RNaseD (Table 3).

The predicted exonuclease activity of WRN was verified in biochemical studies that demonstrated that recombinant WRN purified from a baculovirus expression system exhibited 3′→5′ exonucleolytic activity on a 3′-recessed dsDNA substrate[14,15] (Table 3). Single mutations at two conserved catalytic residues in the predicted exonuclease domain (D82A and E84A) eliminated exonuclease activity, whereas helicase activity was

TABLE 3. Properties of WRN exonuclease

Properties		Refs
Sequence homology	Proofreading exonuclease of [a]*E. coli* DNA polymerase I	51, 52
	E. coli RNaseD	51, 52
Directionality	3′→5′	14, 15
	5′→3′	17
DNA substrates	3′-recessed dsDNA	14, 15
	3′-terminally mismatched DNA	16
	3′-PO_4 dsDNA	16
	5′-recessed dsDNA	17
RNA substrate	RNA in an RNA–DNA duplex	17
Metal ions	Mg^{2+}	16, 17
	Mn^{2+}	17
ATP dependence	Yes	16, 17

[a]*Escherichia coli*

retained by both mutant proteins[14]. The putative exonuclease domain was expressed separately and its exonuclease activity was comparable to that of the full-length protein[14,15]; deletion of this exonuclease domain abolished exonuclease but not helicase activity[15]. Moreover, the mutant WRN protein K577M that lacks helicase activity was shown to exhibit exonucleolytic activity characteristic of wild-type WRN (Refs 14, 15).

WRN exonuclease activity is stimulated markedly by ATP, dATP or CTP (Ref. 16), consistent with the nucleotide substrate preference of the helicase[11], and can excise 3′-phosphoryl termini as efficiently as 3′-hydroxyl termini in 3′-recessed dsDNA (Ref. 16; Table 3). Interestingly, WRN exonuclease exhibits similarities to the proofreading activity of DNA polymerases in removing a 3′-terminal mismatch[16] (Table 3), in agreement with sequence homology predictions that WRN exonuclease is similar to the proofreading exonuclease of *E. coli* DNA polymerase I (Refs 51, 52; Table 3). However, WRN lacks at least one hallmark of proofreading exonucleases: the ability to hydrolyze single-stranded DNA. Thus, WRN seems to possess three catalytic activities[53]: ATPase, helicase and exonuclease.

Although the observed 3′→5′ directionality of WRN exonuclease is consistent with the sequence-homology prediction[51,52], a 5′→3′ exonuclease activity that acts on 3′-overhang dsDNA has been reported in full-length recombinant WRN proteins expressed by the baculovirus system; deletion of the putative exonuclease domain abolished this activity but retained the helicase activity[17]. The proposed 5′→3′ exonuclease activity of WRN is also dependent on ATP hydrolysis, leading to a tentative model that allows coordination of the exonuclease and helicase activities in WRN (Ref. 17)[17]. Accordingly, the exonuclease could excise the displaced DNA strand 5′→3′ after the helicase unwinds a 3′-overhang dsDNA. This model solves the existing opposite-direction problem raised by previous observations in which the two activities work simultaneously on a linear dsDNA substrate[14–16]. Additional studies could establish the directionality and substrate specificity of WRN exonuclease.

Possible roles of WRN exonuclease in cells

Possession of both exonuclease and helicase activities distinguishes WRN from other members of the RecQ helicase family, including BLM, and could account for some of the phenotypic differences between Werner and Bloom syndromes. Knowledge of the biological role of WRN exonuclease and of the mechanisms that control coordination between the exonuclease and helicase activities is limited. Known mutations in WS patients are predicted to produce truncated proteins that contain a disrupted helicase but intact exonuclease domain[54] (Fig. 1). However, the presence of an intact exonuclease domain does not establish that the exonuclease is expressed or functional in WS cells. There is insufficient evidence at present to conclude that loss of helicase activity alone is responsible for WS phenotypes, and the loss of exonuclease activity might be paramount. This caveat is reinforced by the presence of a nuclear localization signal (NLS) in the C-terminal amino acids 1370–1375 (Ref. 55; Fig. 1). C-terminally truncated WRN proteins do not enter the nucleus[55], thus loss of WRN function(s) owing to failure of nuclear localization is a possible cause of WS. Expression of the exonuclease and helicase portions of WRN in WS cells and analysis of the respective phenotypes might be informative in this regard; the approach seems feasible as the catalytic activities are separable and function alone *in vitro*.

WRN exonuclease can remove a 3′-phosphorylated nucleotide from 3′-recessed dsDNA, like *E. coli* ExoIII, suggesting that the exonuclease is involved in DNA repair. WS cells are peculiarly sensitive to 4-NQO (4-nitroquinoline 1-oxide)[56], despite their reported insensitivity to UV and most alkylating agents (Table 2). Among the DNA alterations caused by 4-NQO are guanine adducts[57]; these lesions are probably removed by the nucleotide-excision repair (NER) pathway[58], which is unaffected in WS cells. DNA glycosylases might hydrolyze these altered guanosine residues to lead to base excision repair (BER)[59]. However, WRN might not contact 4-NQO adducts directly[18]. 4-NQO is also a potential redox agent and can generate reactive oxygen species[60] that produce single- and double-strand breaks with a variety of termini, including 3′-phosphate termini. Whether WRN exonuclease is involved in repairing this type of 4-NQO damage has not been established, although WS cells are insensitive to other agents that produce reactive oxygen species, such as X-rays or H_2O_2 (Table 2).

The involvement of WRN in maintaining the fidelity of DNA synthesis needs to be considered, in the light of its 3′→5′ *E. coli* DNA polymerase I-like exonuclease activity. Alternatively, the observed 5′→3′ exonuclease activity of WRN (Ref. 17) might function similarly to *E. coli* RecJ, by excising the DNA strand displaced by RecQ helicase in processing an arrested replication fork[32]. Should this be true, it agrees with the model in which the lagging strand is removed in the 5′→3′ direction (Fig. 2).

The mouse model for human Werner syndrome

The mouse *Wrn* gene on chromosome 8A4 is syntenic to the human *WRN* gene on chromosome 8p (Refs 21, 61) and its cDNA sequence shares >70% homology with the human gene[61]. The structural similarities of mouse and human proteins suggest that mice could provide a model in which to study human WS, although mouse WRN is located diffusely throughout the nucleus compared with the confined nucleolar localization of human WRN[62].

The first *Wrn* knockout mouse was created by the homozygous deletion of exons that encode helicase motifs III and IV[20] (see Fig. 1). This deletion abolishes WRN helicase activity but the exonuclease domain is

retained and might still function in the mutant protein. It would be interesting to examine the exonuclease activity of the mutant WRN and to look for phenotypic, as well as genetic, changes in cells that harbor the helicase-deficient alleles. The WS mice appear normal during their first year of life[20]. However, embryonic stem cells of homozygous WS mice exhibit increased sensitivity to certain mutagens compared to wild-type cells[20]. Moreover, embryo fibroblasts derived from homozygous WS mice show premature loss of proliferative capacity[20], as seen in cells cultured from WS patients. Many questions, such as those relating to the coordinating mechanism of both activities of WRN in cells and to the pathology of WS, could be addressed using this mouse model.

Summary

The Werner syndrome protein WRN has gained increasing attention because of its potential to illuminate mechanisms by which cells maintain their genetic integrity. Although the exact biological role(s) of WRN is not yet clear, it seems that this disease-associated DNA helicase/exonuclease might deal with alternate DNA structures. In particular, the ability of WRN helicase to resolve a tetrahelical DNA structure suggests that it might function together with other proteins to resolve DNA biosynthetic intermediates, in a manner similar to that demonstrated for *E. coli* RecQ, SSB and Topo III[50]. WRN helicase might also facilitate the reconstruction of genomic structure after DNA damage and/or biosynthetic disruptions; the unique 4-NQO sensitivity of WS cells and the roles of *E. coli* RecQ in re-initiation of arrested replication, in particular, suggest such a function.

The 3′→5′ (or 5′→3′) exonuclease activity of WRN, unique among RecQ helicases, could be central to understanding the biochemical function of WRN and the pathogenesis of premature aging in WS. If the helicase activity of WRN is involved in resolving alternative DNA structures during DNA synthesis, then the exonuclease might also function in a similar capacity and work in concert with DNA polymerases. Accordingly, an attractive role for the exonuclease is to excise altered nucleotides during DNA synthesis. In conjunction with its helicase activity, WRN exonuclease might function as a DNA-processing or editing enzyme that aids cells in restoring DNA structures after biosynthetic alterations or mutagenic insults.

The coordination of helicase and exonuclease activities residing in the same polypeptide presents an interesting issue. Do these activities function simultaneously or separately? Are regulatory molecules or proteins required to control the timing of the two activities? These questions remain to be addressed, and could be important for understanding the function of WRN. If the two activities occur simultaneously, possible multimeric structures of WRN (e.g. BLM has been shown to be a hexamer[63]) and complex DNA substrates (e.g. joint molecules during strand invasion and branch migration) might need to be considered.

It is intriguing that a single mutation in *WRN* can cause genetic instability and a spectrum of diverse pathologies associated with aging. Until recently, WS could be studied only by observing afflicted humans and the behavior of cultured cells. Cloning the gene and establishing the biochemical activities of WRN have yielded many diverse insights. The transgenic mouse model with a deletion in the WRN helicase domain[20] might enable specific mutations to be related to Werner syndrome-associated pathologies. There are many questions to be answered, and it is certain that new data will be forthcoming in the near future. We are close to revealing the molecular basis of Werner syndrome, and other human RecQ helicase-deficient diseases, but we are still not there.

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Two new WRN-interacting proteins have been identified recently. Proliferating cell nuclear antigen (PCNA) and DNA topoisomerase I co-immunoprecipitate with WRN[1]. In addition, WRN co-purified with the characterized 17 S DNA replication protein complex through serial steps of centrifuge, chromatography and sucrose gradient from cell lysates, suggesting that WRN interacts with components of the DNA-replication machinery[1].

A new line of transgenic mice expressing mutant human WRN proteins (K577M in the Walker A motif) has been created recently[2]. The K577M mutation in WRN protein results in the inactivation of the helicase activity but the retention of the exonuclease activity *in vitro*. A similar substitution in *Escherichia coli* UvrD helicase results in dominant-negative mutation. Fibroblast cultures derived from the K577M-WRN mice that harbor this putative dominant-negative human *WRN* gene show the characteristics of the WS phenotype, including reduced replicative potential, reduced expression of endogenous mouse WRN protein and hypersensitivity to 4-nitroquinoline 1-oxide (4-NQO).

Telomere shortening triggers the senescence of normal human fibroblasts. Although WS fibroblasts display accelerated senescence in culture, it is generally believed to be due to the accumulation of DNA damage. However, forced expression of telomerase (hTERT) in WS cells has been shown to confer extended cellular lifespan and probably immortality[3]. This finding suggests that *WRN* mutations also accelerate normal telomere-driven replicative senescence.

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