

MUTATION UPDATE

WRN Mutations in Werner Syndrome

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Communicated by R.G.H. Cotton

Werner syndrome (WS) is one of a group of human genetic diseases that have recently been linked to deficits in cellular helicase function. We review the spectrum of WS-associated WRN mutations, the organization and potential functions of the WRN protein, and potential mechanistic links between the loss of WRN function and pathogenesis of the WS clinical and cellular phenotypes. *Hum Mutat* 13:271-279, 1999. © 1999 Wiley-Liss, Inc.

INTRODUCTION

Werner syndrome (WS; MIM# 277700) is an uncommon autosomal recessive disease whose phenotype mimics premature aging. WS patients develop, as young adults, many of the phenotypic changes associated with aging. These changes include the graying and loss of hair, bilateral cataract formation, osteoporosis, atherosclerosis, hypogonadism, diabetes, and scleroderma-like skin changes with ulceration. The most conspicuous laboratory finding is markedly elevated urinary hyaluronic acid secretion [Epstein et al., 1966; Salk et al., 1985b; Goto, 1997]. Patients with WS also have an elevated risk of cancer. Five neoplasms predominate among WS patients: soft tissue sarcomas, thyroid carcinoma, osteosarcoma, acral lentiginous melanoma, and meningioma [Epstein et al., 1966; Goto et al., 1996b]. The most common causes of death are cancer and atherosclerotic cardiovascular disease, with an age at death of ~46 years [Epstein et al., 1966; Goto, 1997]. The phenotypic overlap between WS and normal aging is extensive, though not complete. Thus WS is often considered a "segmental" progeroid syndrome that may share mechanistic or pathogenetic overlap with normal aging [Martin, 1978, 1997].

Three cellular phenotypes have been identified in cells or cell lines from WS patients. First, fibroblasts and lymphocytes from WS patients have a limited capacity to proliferate in culture, as measured by cumulative population doubling or clonal growth assays [Martin et al., 1970; Tollefsbol and Cohen, 1984]. Second, the presence of cytogenetic and/or molecular genetic instability has been identified in at least three different cell lineages of WS

patients. Evidence for genetic instability includes (1) clonal chromosomal rearrangements in WS fibroblast and lymphocyte cultures [Hoehn et al., 1975; Salk et al., 1985a; Melaragno et al., 1995]; (2) a deletion mutator phenotype in SV40-transformed fibroblast cell lines from WS patients [Fukuchi et al., 1989]; (3) an elevated frequency of 6-thioguanine-resistant peripheral blood lymphocytes from WS patients [Fukuchi et al., 1990]; (4) altered telomere dynamics in WS lymphoblastoid cell lines and in primary WS fibroblasts [Schultz et al., 1996; Tahara et al., 1997]; and (5) an elevated frequency of red blood cell glyophorin-A variants in WS patients [Kyoizumi et al., 1998; M.J. Moser et al., submitted].

A third cellular phenotype that may be related to limited growth and spontaneous genetic instability is the selective sensitivity of WS cells and cell lines to 4-nitroquinoline-1-oxide (4NQO) [Gebhart et al., 1988; Ogburn et al., 1997] and to the topoisomerase I-specific inhibitor camptothecin [Okada et al., 1998; Poot et al., 1999]. These selective sensitivities are particularly intriguing, as WS cells are not sensitive to other DNA damaging agents and do not have rate-limiting deficits in any of the major DNA repair pathways (e.g., nucleotide or base excision

Received 5 September 1998; accepted revised manuscript 2 December 1998.

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Grant sponsor: National Cancer Institute; Grant sponsor: National Institute on Aging; Grant number: T32 AG00057; Grant number: R24 CA78088; Grant number: RO1 AG14446.

repair; reviewed in Monnat [1992]). There have been several other reports of abnormal DNA metabolism and/or repair in WS cells [Poot et al., 1992; R nger et al., 1994; Weirich-Schwaiger et al., 1994; Kruk et al., 1995; Webb et al., 1996; Brooks-Wilson et al., 1997; Bennett et al., 1997]. It is unclear which of the reported differences represent direct effects of the loss of WRN function, and which are secondary consequences of genetic instability with mutation accumulation.

WRN STRUCTURE AND EXPRESSION

The WRN locus was localized to the short arm of chromosome 8 by linkage analysis [Goto et al., 1992; Schellenberg et al., 1992]. The position of WRN was further refined by meiotic and homozygosity mapping and linkage disequilibrium analyses to a 1.2- to 1.4-Mb region of 8p. This region of 8p11–12 was screened for expressed sequences and potential open reading frames, and the WRN locus identified on the basis of mutations found in WS patients [Yu et al., 1996]. The WRN locus consists of 35 exons in a 165-kb region of chromosome 8p11–12. Two WRN mRNAs, of 5.8 and 8.1 kb, have been detected and the major 5.8-kb species is ubiquitously expressed at different levels in different tissues or organs [Yu et al., 1996]. Sequence data and published protocols that provide the basis for mutation delineation include a WRN cDNA sequence of 5,189 bp (GenBank Accession # L76937); sequences of WRN intron–exon junctions and of primers for cDNA and exon-specific amplification [Yu et al., 1996, 1997; Oshima et al., 1996b; Matsumoto et al., 1997b]; and the sequence of the human WRN promoter [Wang et al., 1998; Yamabe et al., 1998]. Thus far, mutation identification and characterization have depended on the sequencing of the open reading frame as an reverse transcription-polymerase chain reaction (RT-PCR) product or as individual, amplified exons. This approach has allowed the identification of both mutations and polymorphisms, and will remain the “gold standard” until more rapid detection protocols can be developed and validated.

MUTATIONAL SPECTRUM IN WS PATIENTS

WRN mutations have been characterized in greater than 100 WS patients from several different geographic areas and ethnic groups, with the greatest number from Japan [Yu et al., 1996, 1997; Oshima et al., 1996b; Goto et al., 1996a; Matsumoto et al., 1997b; Meisslitz et al., 1997]. Nineteen different WS-associated WRN mutations are described in Table 1. These mutations are described using the

WRN cDNA as a point of reference and follow recommendations of the Nomenclature Working Group [Antonarakis and Nomenclature Working Group, 1998]. In addition to one new mutation, Table 1 includes updated or corrected information on several previously published mutations. These mutational data are available as a locus-specific database as part of the HUGO Mutation Database Initiative (The WRN Mutational Database; <http://www.pathology.washington.edu/werner/>).

WRN mutations in WS patients are distributed across the WRN open reading frame, with a higher proportion (63%) falling in the C-terminal half of the predicted WRN protein (Fig. 1). For example, only one mutation falls in the N-terminal nuclease domain, while 6 of 19 mutations affect exons encoding the C-terminal HRDC domain [Mushegian et al., 1997; Morozov et al., 1997; see below]. One-half of the WRN mutations (11 of 19) found in association with WS are base substitutions, with most of the remainder consisting of small deletions or insertions (6 of 19). One large genomic rearrangement, the deletion of ~15 kb spanning exons 19–23, has been identified. The remaining mutation is a 105-bp insertion between exons 18 and 19 that is not obviously intron 18-derived and is incompletely characterized at the genomic level [Oshima et al., 1996b; J. Oshima, unpublished observation]. The phenotypic effects of these WS-associated WRN mutations include direct protein truncation at newly generated stop codons and protein truncation as a consequence of frameshifts generated by insertions, deletions or point mutations. Mutations that lead to splicing abnormalities also have a high probability of generating truncated WRN proteins, as many of the 34 coding WRN exons include codons that are shared across exon–exon boundaries.

The WS-associated mutations listed in Table 1 have been found in both homozygous and heterozygous combinations in different WS patients and pedigrees (see references cited in Table 1 for detailed discussion). A higher proportion of WRN mutational heterozygotes have been reported thus far among Caucasian than among Japanese WS patients. A number of Japanese WS patients in initial mutation screens were reported to carry only a single mutant WRN allele (16/89, or 18%) or have no detectable WRN mutations (30/89, or 34%) [Goto et al., 1996a; Matsumoto et al., 1997b]. Potential explanations for this include uncertainties regarding the clinical ascertainment and diagnosis, the need to screen a large open reading frame to identify and characterize mutations, and the

TABLE 1. WS Associated Mutations in *WRN*

HGMD ^a	OMIM ^b	NWG ^c	Type of mutation	Mutation effect	Protein length (aa) ^e	Exon	Ref ^f	Comment(s)
CD972347		c.733-734delAA	2-bp deletion	FS/TER	176	5	5	AAAAA>AAA
CM961440	277700.0006	c.1336C>T	Transition	R369X	368	9	2	Nonsense mutation
CD962156	277700.0008	c.1396delA	1-bp deletion	FS/TER	391	9	2	AAA>AA
C1962549		c.1509-1510insATCT	4-bp insertion	FS/TER	429	10	2	ATCT>ATCTATCT
CM971492		c.1620T>A	Transversion	Y463X	462	11	5	Nonsense mutation
		c.2320-3056del	>15-kb deletion	Exon 19-23 skip, FS/TER	704	19	2	Large genomic deletion
		c.2319-2320ins105	Unknown	FS/TER	708	19	2	Unknown genomic mutation
CS971792		IVS20+1G>T	Transversion	Exon 20 skip, FS/TER	760	20	4	
CM961441		c.2896C>T	Transition	R889X	888	22	2	Nonsense mutation
CS961682	277700.0004	IVS25-1G>C	Transversion	Exon 26 skip, FS/TER	1,060	26	1	
		IVS26+1G>C	Transversion	Exon 26 skip, FS/TER	1,060	26	7	
		IVS28+1G>T	Transversion	Exon 28 skip, FS/TER	1,138	28	2	
CD962157	C591683	C.3677delA	1-bp deletion	FS/TER	1,160	30	5	Same stop codon as next
CS971793		IVS29-7A>T	Transversion	New splice site: 5 bp ins, FS/TER	1,162	30	5	Same stop codon as previous
CM961442	277700.0002	c.3724C>T	Transition	Q1165X	1,164	30	1	Nonsense mutation
CS961684	277700.0007	IVS30+2A>T	Transversion	Exon 30 skip, FS/TER	1,157	30	2	
CD962158	277700.0003	Complex ^d	4-bp deletion	New splice site: 4-bp del, FS/TER	1,245	30	1,2	tagACAGACC>tagACC
CM961443	277700.0001	c.4144C>T	Transition	R1305X	1,304	33	1	Nonsense mutation
CI972549	277700.0005	c.4146-4147insA	1-bp insertion	FS/TER	1,317	33	3	4146A>AA

^aHuman Gene Mutation Database <http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html>.

^bOnline Mendelian Inheritance in Man <http://www.ncbi.nlm.nih.gov/Omim/>.

^cNomenclature Working Group [Antonarakis et al., 1998].

^dBy RT-PCR, this mutation results in the deletion of the first 4 bp of exon 32. Genomic sequence shows one copy of a 4-bp tandem repeat spanning the splice junction is deleted resulting in the use of a new splice junction.

^eaa, amino acid residues

^fNumbers refer to the following references: 1, Yu et al., 1996; Oshima et al., 1996; 3, Goto et al., 1996; 4, Yu et al., 1997; 5, Matsumoto et al., 1997; 6, Meisslitzer et al., 1997; 7, J. Oshima (unpublished observations).

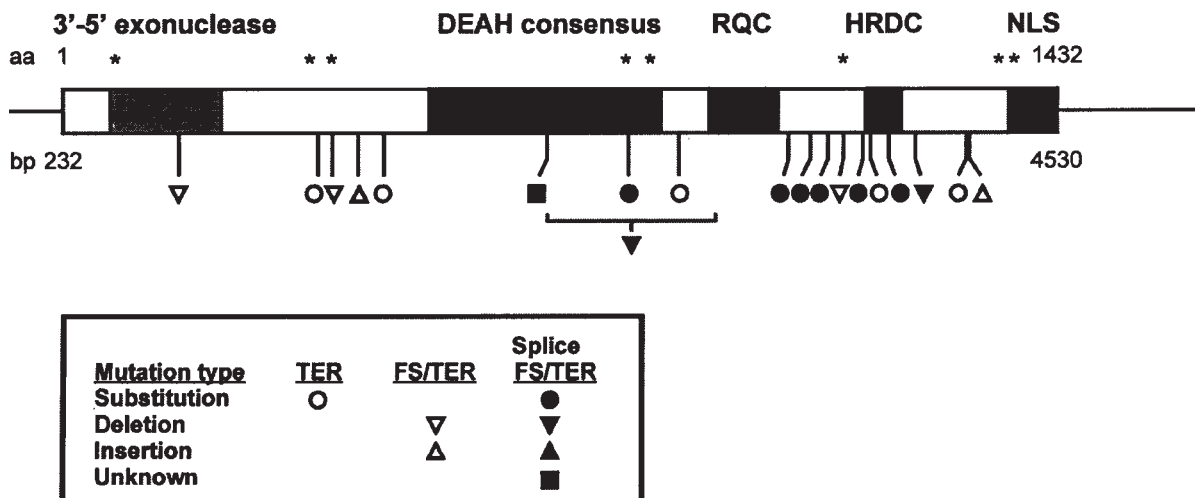


FIGURE 1. Spectrum of WS-associated *WRN* mutations. The thin horizontal line represents untranslated 5' and 3' portions of the *WRN* mRNA. Potential functional domains in the translated portion of the mRNA (central boxed region) identified by sequence (sa) or biochemical analyses (ba) are indicated by shaded or filled segments. These domains include the 3' -5' exonuclease domain, also referred to as the RNaseD/PMSC domain (sa,ba); the DEAH consensus helicase domain, consisting of seven conserved regions found in both RNA and DNA helicases (sa,ba); RQC, the RecQ helicase conserved region of unknown function found in many RecQ family helicases (sa); HRDC, the Helicase, RNaseD, C-terminal conserved region of unknown function found in several helicase and RNase proteins (sa); and NLS, a nuclear localization signal (sa,ba). The positions of polymorphisms within the coding region are indicated by asterisks (*). An amino acid scale is given above the box and a nucleotide scale is given below. The positions of mutations in the *WRN* open reading frame and their consequences are indicated below the protein by symbols. Horizontal bracket, position of the large deletion that eliminates exons 19–23. The key at the lower left (box) indicates the type of mutation and whether it leads to direct termination, a frameshift with premature termination, or a splicing error with the generation of a frameshift and termination.

corresponding difficulty in confidently excluding the presence of subtle disease-associated mutations in addition to characterized mutations in this large open reading frame. These patients raise the possibility of genetic heterogeneity in WS, which is discussed further below.

POLYMORPHIC VARIANTS OF *WRN*

Eight polymorphic variants of *WRN* have been identified in the course of screening for *WRN* mutations. Six are polymorphic at the DNA and protein level, while two are silent single nucleotide polymorphisms [Castro et al., 1999; Table 2]. One polymorphism, c.4330T>C leading to a C1367R substitution, has been associated with an elevated risk of myocardial infarction in a Japanese cohort study [Ye et al., 1997]. In light of the large size of the *WRN* locus, it is likely that additional polymorphisms will be identified in both the coding and intron regions of *WRN*.

WRN PROTEIN STRUCTURE AND FUNCTION

The *WRN* locus contains a single open reading frame consisting of exons 2–35 that encodes a 1432 residue protein with a predicted molecular weight

of 162.5 kDa. Analyses of the sequence of the predicted *WRN* protein, in conjunction with biochemical and cellular analyses, have identified several potential functional domains within the *WRN* protein [Mushegian et al., 1997; Morozov et al., 1997; Moser et al., 1997]. Figure 1 summarizes these functional domains and indicates the positions of polymorphisms and the positions and effects of the mutations detailed in Table 1. Evidence for the functional role(s) of each of these protein regions is described below.

DEAH Helicase Consensus Domain

A DEAH helicase consensus domain occupies the central third of *WRN* (Fig. 1). This helicase consen-

TABLE 2. Polymorphisms in *WRN*

NWG	Amino acid change	Reference
c.744T>C	C171C	Castro et al., 1999
c.1201A>G	T324A	Castro et al., 1999
c.1392G>A	M387I	Meisslitzer et al., 1997; Vidal et al., 1997
c.2592T>G	L787L	Castro et al., 1999
c.2691T>G	A820A	Castro et al., 1999
c.3453T>G	F1054L	Meisslitzer et al., 1997
c.4314C>T	S1361S	Castro et al., 1999
c.4330T>C	C1367R	Ye et al., 1997

sus domain and a second C-terminal consensus domain, the RecQ consensus domain, are found in the Bloom syndrome helicase BLM and the human RecQL helicases, in addition to WRN [Puranam and Blackshear, 1994; Seki et al., 1994; Yu et al., 1996; Ellis, 1997]. RecQ homologues have been identified in many bacterial genomes, in budding and fission yeast, in *Caenorhabditis elegans*, in *Xenopus laevis*, and in *Mus musculus*. Purified WRN protein possesses 3'-5' helicase activity on DNA substrates and helicase activity on DNA/RNA templates, as do other several other members of the RecQ family [Gray et al., 1997; Suzuki et al., 1997; Lu et al., 1996; Umezu et al., 1990; Lu et al., 1996; Karow et al., 1997; Bennett et al., 1998; Shen et al., 1998a].

The physiologic substrate(s) for WRN and for the other RecQ helicases of human cells remain unknown; as a result, we do not have good clues to the in vivo function(s) of WRN. RecQ helicases in bacteria and yeast play roles in DNA recombination, repair, and genetic stability assurance. Mutants in *Escherichia coli* RecQ, for example, were initially found to be ultraviolet (UV) sensitive and recombination-deficient in *recBC sbcB(C)* mutant backgrounds [Nakayama et al., 1984]. More recently, *recQ* mutants have been found to have increased frequencies of illegitimate recombination [Hanada et al., 1997], and purified *E. coli* RecQ protein has been shown under different reaction conditions to stimulate or disrupt the formation of DNA recombination intermediates in vitro [Harmon and Kowalczykowski, 1998]. Budding yeast with mutations in the *SGS1* gene have increased rates of mitotic recombination, and a combination of genetic and two-hybrid data have identified interactions between the Sgs1 helicase and different topoisomerases [Gangloff et al., 1994; Watt et al., 1995, 1996; Lu et al., 1996]. Heterologous expression of the human WRN or BLM helicases in *sgs1* mutant yeast suggests that WRN and BLM can both suppress recombination, but that BLM alone can suppress the growth of *sgs1top3* double mutants and restore the hydroxyurea sensitivity of *sgs1* mutants [Yamagata et al., 1998]. It is not clear, however, which of these results represent partial cross-complementation, as opposed to non-specific suppressive effects. Absence of the Sgs1 helicase has also been implicated in nucleolar disruption and cellular senescence in yeast [Sinclair et al., 1997]. The fission yeast RecQ homologue *rqh1*⁺ was isolated by virtue of the sensitivity to hydroxyurea and UV irradiation and appears to play roles in DNA damage responses including recombination and checkpoint activation [Stewart et al., 1997; Murray et al., 1997; Davey et al., 1998].

Most recently, the *Xenopus* oocyte WRN homologue has been identified as FFA-1 (focus forming activity-1), a protein that plays a critical role in inducing DNA replication foci in *Xenopus* egg extracts [Yan et al., 1998]. All these reports indicate that WRN and other RecQ helicases act on DNA substrates in vivo, and may play a role in the replication of specific DNA sequences or in the generation or resolution of DNA recombination or repair events that require at least a limited degree of DNA synthesis [Fry and Loeb, 1998].

Nuclease and HRDC Domains

WRN contains an N-terminal domain with conserved residues that has been found in several other proteins with 3'-5' exonuclease activity [Mushegian et al., 1997; Morozov et al., 1997; Moser et al., 1997]. These proteins include the proofreading domain of *E. coli* DNA polymerase I, *E. coli* RNaseD and the 100-kDa human scleroderma autoantigen, PMSC. Sequence analyses also indicate that this putative nuclease domain is found only in WRN among the human RecQ helicases. Recently, a 3'-5' exonuclease activity has been identified in biochemical assays using WRN protein synthesized in insect cells [Huang et al., 1998; Shen et al., 1998b; Kamath-Loeb et al., 1998]. A second, short region of homology that may be related to this nuclease domain is the HRDC (Helicase, RNaseD C-terminal) domain. This domain, of unknown functional importance, is found near the C-terminus of both RecQ helicase and nuclease proteins from several species [Mushegian et al., 1997; Morozov et al., 1997].

Nuclear Localization Signal

The last 69 residues of WRN encode a nuclear localization signal that can direct the localization of WRN-GFP fusion proteins to the nucleus of human cells [Matsumoto et al., 1997a, 1998]. More recently, nucleolar localization for the Werner syndrome protein in human cells has been reported by two groups using different antisera [Marciniak et al., 1998; Gray et al., 1998]. A similar pattern of nucleolar localization has been previously reported for the budding yeast Sgs1 helicase [Sinclair et al., 1997]. Surprisingly, the murine WRN protein does not appear to be nucleolar localized [Marciniak et al., 1998].

Post-translational Processing and Modification

There have been no reports documenting post-translational processing or modification of the WRN protein, or the effects of in vitro modi-

fication on biochemical functions of purified WRN protein. There is, however, one intriguing report in which nucleolar staining for WRN protein was lost in human cells following serum starvation and refeeding, or in response to 4NQO treatment [Gray et al., 1998]. This type of conditional localization may be functionally important, and could be mediated by post-translational modifications of WRN.

MOUSE MODELS OF WS

Identification of a murine homologue of WRN [Imamura et al., 1997] served as the starting point for the development of murine models of WS. Several groups are pursuing these models using different inactivating WRN mutations. One murine model of WS has been reported thus far [Lebel and Leder, 1998]. ES cells and mice lacking murine WRN helicase consensus domains III and IV were generated and characterized. The resulting ES cells displayed sensitivity to camptothecin; mice displayed reduced embryonic survival but were otherwise normal during the first year of life. It is not clear how well this model recapitulates WS: the mutation used has not been documented in any WS patient, and the resulting mutant murine protein that is stably expressed in both ES cells and mice has not been characterized for helicase activity [Lebel and Leder, 1998].

CLINICAL IMPORT OF WRN MUTATIONS

The most conspicuous feature of the spectrum of WS-associated WRN mutations summarized in Table 1 is the absence of missense mutations: all of the WS-associated mutations described to date directly (chain termination) or indirectly (frameshift mutations) truncate the WRN protein. This is in contrast to Bloom syndrome [Ellis et al., 1995] and suggests that WS results from the loss of all functions encoded within WRN. This uniformity of effect of many different WS-associated WRN mutations may also explain the consistency of the WS clinical phenotype in different geographic regions and ethnic groups [Epstein et al., 1966; Goto, 1997].

At least two different mutation-dependent mechanisms have been identified that lead to WRN protein loss from human cells. First, all the WS-associated WRN mutations thus far identified truncate the WRN protein with loss of the C-terminal nuclear localization signal. Thus, most or all, of the predicted mutant WRN proteins (Table 1) would not be appropriately localized to the nucleus. Many of these truncated mutant proteins

may be incorrectly folded as well, thus promoting proteolytic destruction. A second mutation-dependent mechanism may operate at the level of WRN transcript stability. Initial quantitative analyses have shown that the level of WRN polyA⁺ mRNA in several WS patient-derived cell lines is ~30% that of control lines. This suggests that WRN mRNAs containing nonsense codons may be targeted by a nonsense-mediated or other decay pathway. Surprisingly, in this study the levels of WRN mRNA within cell lines derived from clinically unaffected WS heterozygotes in a single Japanese pedigree also appeared to be reduced to levels below the predicted 50% of normal [Yamabe et al., 1997]. This suggestion was further supported by WRN promoter activity assays, in which lower activity was found in WS as compared with control fibroblast cell lines [Wang et al., 1998; see also Yamabe et al., 1998]. Thus the presence of mutant WRN alleles or the proteins they encode may effect the production or stability of both mutant and wildtype WRN mRNA in human cells.

Loss of the 162-kDa WRN protein by one or more of the mutation-dependent mechanisms described above appears to be necessary and sufficient, in most patients, to promote development of the WS cellular and clinical phenotypes discussed above. The requirement for WRN protein loss also explains the autosomal recessive inheritance of WS. WRN appears to participate in one or more aspects of cellular nucleic acid metabolism that require at least limited DNA synthesis and the concerted or sequential activities of 3'-5' helicase and nuclease activities (see above). WRN, as is the case for virtually all other helicases, is likely to function as part of a multiprotein complex [Matson et al., 1994]. If WRN protein functions as part of such a complex, mutation or inappropriate modification of any component in addition to WRN could confer a common biochemical defect on human cells and promote the appearance of WS.

An instructive conceptual parallel here is provided by our understanding of the pathogenesis of xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD). These genetic instability/DNA repair deficiency syndromes are clinically and genetically heterogeneous. Several of the proteins encoded by the involved genes, most notably XP-B and XP-D and the CS-associated proteins CS-A and -B, are part of or associate with the TFIIH basal transcription complex that plays roles in transcription, repair and perhaps cell cycle regulation [Friedberg et al., 1995;

Friedberg, 1996; Hoeijmakers et al., 1996; Lehmann, 1998]. The XP-B and XP-D proteins, for example, are ATP-binding proteins with DNA helicase activity that unwind DNA duplexes with opposite polarity. The participation of these different proteins in a common functional complex begins to explain how the inactivation or loss of any member can lead to distinct though related biochemical defects and clinical disease patterns.

XPB and XPD disease-associated mutations are almost exclusively missense mutations, and XPB or -D null mutations, as predicted, are early embryonal lethal in mice [reviewed in Friedberg et al., 1998]. The WRN functions that are lost in WS patients, in contrast, are unlikely to be essential. Mutations in WRN are thus likely to lead to disease by promoting genetic instability and mutation accumulation in vivo with cell dysfunction, proliferation, or loss. The clinical phenotype of WS indicates that the consequences of WRN loss are to some degree cell lineage specific. The sequence of changes that occur in response to the loss of WRN function should be revealed as we learn more about biochemical functions and in vivo roles of WRN in nucleic acid metabolism in different human cell lineages.

ACKNOWLEDGMENTS

We thank Lawrence Loeb for helpful comments on a draft of this manuscript and Alden Hackmann for help with Figure 1. This work was supported by grants from the National Cancer Institute and National Institute on Aging (to R.J.M., Jr.). M.J.M. was supported by Training Grant T32 AG00057. Mutation characterization was supported by grant R24 CA78088 to support the International Registry of Werner Syndrome and by grant RO1 AG14446 (to J.O.).

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