

Werner Syndrome as a Model of Human Aging

Raymond J. Monnat, Jr.

This chapter reviews clinical and basic science aspects of Werner syndrome (WS), a heritable human disease that displays features suggestive of premature aging. The resemblance of changes in WS to those observed in normal aging has long suggested WS may be a useful model in which to study the biology of aging and to identify mechanistic pathways responsible for age-associated diseases that are prevalent in WS patients and in normal aging such as atherosclerosis, neoplasia, diabetes mellitus, and osteoporosis. This chapter summarizes our understanding of the WS clinical phenotype. Current understanding of in vivo functions of the Werner syndrome protein is summarized, together with a discussion of how the loss of WRN function may promote disease pathogenesis in WS patients and in normal individuals. Subsections provide an historical overview of WS and WS research; a description of WS as a clinical disease entity, together with diagnostic criteria for WS; a discussion of the relationship of WS to normal aging; a summary of our current understanding of the WRN gene and the mutational basis for WS; a discussion of in vivo functions of the WS protein in human somatic cells, and how loss-of-function may be linked to disease pathogenesis; and an introduction to the more promising animal models of WS. A selection of the most useful additional resources on WS clinical medicine and biology are included to aid those interested in learning more about this fascinating and instructive human disease.

Introduction

Werner syndrome (WS) is an uncommon, autosomal recessive human disease that displays clinical features suggestive of premature aging. The initial description of WS was by Otto Werner, a German medical student, in 1904 (Werner, 1985). Werner saw a family in the north of Germany consisting of four siblings, ages 31 to 40, who shared common features including short stature, premature graying of the hair, bilateral cataracts, skin changes (hyperkeratosis, scleroderma-like changes and ulceration) that were most severe on the feet and ankles, atrophy of the extremities, and, in females, an early

cessation of menstruation. He noted that one of the siblings, a 36-year-old male, gave "the impression of extreme senility." Werner published these observations as part of his doctoral thesis, though he did not further study these or similar patients during the remainder of his career. He practiced general medicine in Eddelak, a small village on the North Sea near the Danish border, where he died in 1936 (Pehmoeller, 2001).

The eponym *Werner's syndrome* was first used in 1934 by Oppenheimer and Kugel in reporting findings in a patient (Oppenheimer and Kugel, 1934). Their paper, together with the more comprehensive study by Thannhauser (1945) of five additional cases, provided an accurate clinical description of WS that distinguished it from Rothmund (now Rothmund-Thomson) syndrome. The subsequent diagnosis of Werner syndrome in three affected, American-born sisters in a Japanese-American sibship seen in Seattle in the early 1960s led to further, detailed clinical and pathological characterization of Werner syndrome. Part of this characterization included a formal genetic analysis that firmly established an autosomal recessive mode of inheritance. These observations, together with a critical analysis of 122 additional cases, were published in 1966 (Epstein *et al.*, 1966). This landmark paper remains readily accessible and a key source of information for investigators interested in WS (see Recommended Resources).

The modern clinical and biological investigation of WS has been an international effort. This reflects both the worldwide occurrence of WS (Goto, 1997) and early collaborative efforts by investigators in Japan (where WS is prevalent), the United States, and Western Europe to better define and understand the WS clinical phenotype and its underlying biology. The first major gathering of investigators to discuss this work was in Kobe, Japan, in 1982. This United States-Japan Cooperative Seminar on Werner's Syndrome and Human Aging was sponsored by the U.S. National Science Foundation and the Japan Society for the Promotion of Science. The proceedings of this workshop were subsequently published and represent an important first summarization of modern work to better define and understand WS (Salk *et al.*, 1985). The published proceedings also include an important

selection of published primary sources of information on WS (e.g., Epstein *et al.*, 1966; Salk, 1982; Thannhauser, 1945) together with an edited translation of Otto Werner's thesis (Werner, 1985).

Several subsequent small workshops were sponsored by the U.S.–Japan Cooperative Cancer Research Program during the 1990s and led to further discussion of and interest in WS. These were part of a long-standing joint venture of the U.S. National Cancer Institute and the Japan Society for the Promotion of Science. Meetings held in 1994, 1996, and 1997 brought together investigators interested in WS as a cancer predisposition syndrome (1994), in clinical and biological aspects of WS (1996), and in the relationship of WS to other pediatric cancer syndromes (1997). The focus for these meetings broadened with a U.S.–Japan Workshop on Cancer in Human RecQ Helicase Gene Disorders (February 2002), the Keystone Symposia on DNA Helicases, Cancer and Aging (March 2002 and 2005), and a U.S. NIH-sponsored International Workshop on Werner Syndrome held in May 2003. Regrettably, the results of only one of these very productive workshops were captured in a meeting report (Bohr, 2003). The broader focus of these more recent meetings reflects the recognition that WS, Bloom syndrome, and Rothmund-Thomson syndrome are all human genetic instability/cancer predisposition disorders that result from mutations in different members of the five-member human RecQ helicase protein family (see later; Bachrati and Hickson, 2003; Opresko, Cheng *et al.*, 2004).

Attempts were made in the 1980s and early 1990s to isolate or map the affected gene in WS. These approaches took advantage of potentially useful cellular phenotypes for complementation such as a severe *in vitro* cell proliferation defect and chromosomal instability, and the fact that WS is an autosomal recessive disease that likely resulted from a single gene defect. Attempts at functional complementation to identify the *WRN* gene were not successful for at least two important reasons: the scarcity and poor growth properties of primary cells from WS patients that were used as complementation hosts, and the large size of the *WRN* gene and the *WRN* open reading frame (see later). In contrast, linkage mapping using the then-new technique of homozygosity mapping was successful, and led in 1992 to assignment of the *WRN* locus to the proximal short arm of chromosome 8 in a region defined by five anonymous DNA markers (Goto *et al.*, 1992). This initial linkage assignment, together with rapid maturation of methods for positional cloning in the early 1990s, led in 1996 to identification of the *WRN* locus and of unambiguous pathogenic mutations in the *WRN* gene of WS patients (Yu *et al.*, 1996).

Successful positional cloning of the *WRN* gene with delineation of WS-associated *WRN* mutations and predictions of potential activities encoded in the *WRN* protein provided a powerful stimulus for subsequent work on WS. There was an immediate effort in several

laboratories to confirm predicted biochemical activities, and then identify *in vivo* functions, of *WRN*. There was also renewed speculation on how the loss of *WRN* function could generate WS cellular and clinical phenotypes. Recent work has also begun to focus on genetic variation in the *WRN* gene, and the association of *WRN* mutations and polymorphisms with disease risk and disease pathogenesis in the general population. Each of these areas of investigation is discussed in greater detail below.

Werner Syndrome as a Clinical Disease Entity

The key clinical features of Werner syndrome were readily recognized by Otto Werner in the first patients he identified and described in 1904. These clinical signs or findings, outlined in Table 80.1, were subsequently confirmed and further elaborated by Oppenheimer and Kugel (1934), Thannhauser (1945), and Epstein *et al.*

TABLE 80.1
Diagnostic criteria for Werner syndrome

Consistent findings

- **clinical findings** (onset > age 10)
 - ◆ short stature
 - ◆ bilateral cataracts
 - ◆ premature graying and loss of scalp hair/eyebrows
 - ◆ scleroderma-like skin changes
- **history**
 - ◆ parental consanguinity (3rd cousin or closer)
 - ◆ affected sib
- **laboratory**
 - ◆ elevated 24 hr urinary hyaluronic acid secretion

Additional findings

- **clinical findings**
 - ◆ flat feet
 - ◆ voice changes
 - ◆ hypogonadism
- **history or laboratory findings**
 - ◆ diabetes mellitus
 - ◆ osteoporosis
 - ◆ soft-tissue/tendon calcification
 - ◆ premature atherosclerosis, myocardial infarction, stroke
 - ◆ neoplasia

Diagnostic likelihood of Werner syndrome

- **definite:** all of consistent clinical and history findings
- **probable:** short stature, bilateral cataracts and scleroderma-like skin changes, any two other clinical, history or laboratory findings
- **possible:** bilateral cataracts or scleroderma-like skin changes, any four other additional clinical, history or laboratory findings
- **exclusion:** onset of clinical or laboratory findings ≤ age 10

(1966) in their analyses of WS patients and pedigrees. Among the most consistent and earliest of the features of WS to be observed are short stature, bilateral cataracts, the early graying and loss of hair, and scleroderma-like skin changes. All four features have been observed in all or nearly all patients (Epstein *et al.*, 1966; Goto, 1997; Tollefsbol and Cohen, 1984). They appear *de novo*, and are not the secondary consequence of another systemic disease process, or the result of a primary endocrine deficiency or dysfunction syndrome. Each of these features or clinical signs of WS are discussed briefly, next, together with less consistently observed changes. This constellation of changes and the clinical appearance and progression of these changes have been used to develop criteria for the clinical diagnosis of WS.

SHORT STATURE

The short stature of WS patients results from a failure to undergo an adolescent or pubertal growth spurt. Short stature, together with progressive thinning and atrophy of the limbs and a stocky trunk, give patients a Cushingoid appearance that is readily apparent in full body clinical photographs (see, for example, patient photos in Figure 2 of Goto, 2001).

GRAYING AND LOSS OF HAIR

Early graying and loss of hair are, together with short stature, among the earliest and most consistent changes observed in WS patients. Hair graying and loss start late in the second decade of life, and first affect the scalp and eyebrows. The loss of hair pigmentation is progressive and may lead over the course of a decade or more to complete loss of pigmentation. The premature graying and loss of hair also extend to other areas of the body, although these changes usually start later and may not be as extensive as the changes observed in the scalp and eyebrows.

CATARACTS

Bilateral ocular cataracts are a consistent feature of WS and first appear or are reported in many patients by the second or third decades of life. The cataracts consist of

posterior cortical and subcapsular opacification. Vacuoles and small punctate opacifications in other parts of the lens have also been noted, though are not a consistent part of the lens changes. This type of cataract, often referred to as juvenile, can be readily distinguished from the more common opacification of the lens nucleus observed in the senile cataracts of normal aged individuals. Vision is otherwise unimpaired, and thus can be restored following cataract removal.

SCLEDERMA-LIKE SKIN CHANGES

Skin changes were first clearly described in the patient series reported by Thannhauser, who distinguished changes in WS patients from those commonly seen in scleroderma patients or in patients with Rothmund-Thomson syndrome (Thannhauser, 1945). The histologic appearance of skin biopsies from WS patients reveals an interesting mix of atrophic and proliferative changes. There is epidermal atrophy that extends to include skin appendages (e.g., hair follicles, sweat and sebaceous glands), in conjunction with focal hyperkeratosis and basal hypermelanosis. Dermal subcutaneous connective tissue atrophy is common, and often is found in conjunction with dermal fibrosis. Muscle, adipose, and connective tissue underlying the skin is often atrophic. This constellation of changes gives the skin a tight, white, and shiny or contracted appearance, with a loss of normal elasticity.

These cutaneous changes often are first seen and may be most prominent in the face and extremities. Skin changes in the face lead to a progressive sharpening of facial features to give patients what is often described as a pinched, beaked, or bird-like appearance (see the patient photo panel in Figure 3 of Goto, 2001). The lower extremities, especially the feet, are often markedly affected by these changes, leading to foot deformation, ulceration of nonpressure-bearing portions of the foot and ankles, and calcification of soft tissue and tendons (Hatamochi, 2001; Figure 80.1). Laryngeal changes consisting of a mix of proliferative and atrophic changes are likely responsible for the thin, high-pitched voice noted of many

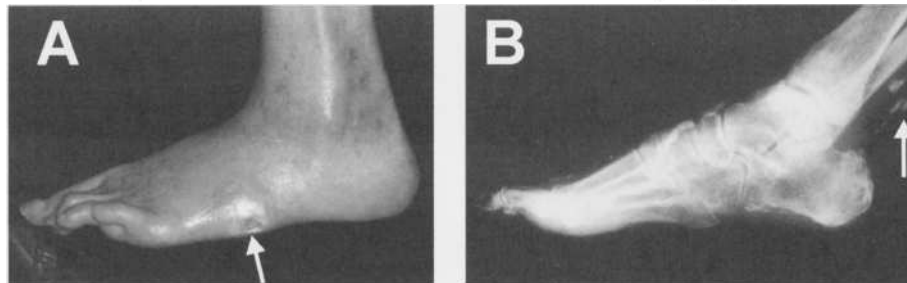


Figure 80.1 Skin and soft tissue changes in Werner syndrome patients. **A.** Scleroderma-like skin changes, hyper-pigmentation and ulceration (arrow) of the lateral border of the left foot in a male Japanese WS patient age 37. **B.** Localized calcification of the Achilles tendon (arrow) and plantar tendon insertion site calcification in a Japanese female WS patient age 54. Both photos were previously published (Hatamochi, 2001) and are used here with kind permission of Dr. Atsushi Hatamochi, Professor of Dermatology, Dokkyo University School of Medicine, and the Japanese Scientific Societies Press.

patients, although the associated laryngeal pathology has not been well studied.

OTHER PROMINENT CLINICAL FEATURES OF WERNER SYNDROME

Several less consistent clinical findings have been noted in a portion of WS patients. These additional findings (see Table 80.1) have been mentioned in many independent reports of WS, and thus are likely to be part—albeit a more variable part—of the WS phenotype. Most notable among these changes are clinically important disease states that we commonly associate with aging: atherosclerosis and its cardiovascular and cerebral sequella; a mix of nonepithelial and epithelial neoplasms (see later); osteoporosis that is characteristically most severe in the distal phalanges; diabetes mellitus; and hypogonadism affecting both males and females. Each of these disease states in part can be explained by proliferative and atrophic changes at the tissue level (see later). A few pertinent negatives also need to be mentioned. Among these, the most notable are that development, structure, and function of the central and peripheral nervous systems are normal, and WS patients are invariably of normal or higher-than-normal intelligence. Also, few or no indications of primary endocrine defects or abnormalities have been reported in WS patients beyond secondary changes that accompany the development of diabetes mellitus or with gonadal atrophy and the loss of reproductive function.

CANCER IN WERNER SYNDROME

WS patients are at increased risk of developing cancer (Goto *et al.*, 1996; Monnat, 2001; Monnat, 2002). The elevated risk of neoplasia in WS patients is of particular biological interest. As discussed later, neoplasia may be an expression of important mechanistic links between WRN function *in vivo*, genome stability assurance, and the limitation of cell proliferation defects. The elevated risk of neoplasia in WS is selective in that only a small subset of neoplasms are clearly elevated in incidence as compared with general population controls (see Table 80.2). The following neoplasms, in order of decreasing frequency, have been observed most often in WS patients and occur at higher or much higher frequency than in normal population controls: soft tissue sarcomas, thyroid carcinoma, meningioma, malignant melanoma, malignant or preneoplastic hematologic disease, and osteosarcoma. Many other neoplasms, including common adult epithelial malignancies, have been observed in WS patients. However, it is not clear whether the risk of developing these neoplasms is significantly elevated. Of note, the histopathologic spectrum of neoplasms observed in WS overlaps with, though is distinct from, that observed in patients with Bloom syndrome and Rothmund-Thomson syndrome,

TABLE 80.2
Neoplasia in Werner syndrome

Frequent (2/3 of neoplasms)	Less common (1/3 of neoplasms)
Soft tissue sarcomas	Non-melanoma skin cancer
malignant fibrous histiocytoma	Hepatobiliary carcinomas
leiomyosarcoma	hepatocellular
fibrosarcoma	cholangiocarcinoma
malignant schwannoma	gallbladder
synovial sarcoma	Genito-urinary
rhabdomyosarcoma	bladder carcinoma
Thyroid carcinoma	uterine/ovarian carcinoma
follicular	renal cell carcinoma
papillary	prostate carcinoma
anaplastic	seminoma
Malignant melanoma	Gastro-intestinal carcinoma
acral lentiginous melanoma	gastric
mucosal malignant melanoma	esophagus
Meningioma	pancreas
benign	colon
multiple/malignant	Breast carcinoma
Hematological	Oro-pharyngeal carcinoma
acute myelogenous leukemias (M1-5)	
erythroleukemia (M6)	
megakaryocytic leukemia (M7)	
myelofibrosis/myelodysplasia	
aplastic anemia	
Osteosarcoma	

two other RecQ helicase deficiency syndromes, (Monnat, 2001).

Several pathologic and clinical aspects of neoplasia in patients indicate that WS is a classical cancer predisposition syndrome. WS patients develop neoplasms at a comparatively early age and often display unusual sites of presentation (e.g., osteosarcoma of the patella) or less common histopathologic subtypes (e.g., follicular, as opposed to the more common papillary, type of thyroid carcinoma) than do population controls. The elevated risk of melanoma in WS provides a striking example of unusual histology. The melanoma seen in WS patients is exclusively acral lentiginous melanoma (ALM), a comparatively rare melanoma subtype that arises on the palms and soles, or in mucosa of the nasal cavity or esophagus. The risk of ALM is most clearly elevated in Japanese WS patients. This suggests the existence of patient- and population-specific modifiers for ALM in the absence of WRN function. A final expression of elevated cancer risk and predisposition in WS is the occurrence of

80. Werner Syndrome as a Model of Human Aging

multiple tumors: there are numerous reports of multiple, concurrent, or sequential neoplasms of different histology; for example, thyroid carcinoma and osteosarcoma, with up to five neoplasms having been reported in individual patients. Estimates of the increased risk of neoplasia in WS patients range from ~30-fold elevated overall lifetime risk across all tumor types to ~1000-fold elevated risk for acral lentiginous melanoma (Goto *et al.*, 1996; Monnat, 2001; Monnat, 2002).

CLINICAL PROGRESSION OF WS

One aspect of WS that is not well conveyed by Table 80.1 is the progressive nature of the WS clinical

phenotype: a complex constellation of changes that may develop over two or three decades, after having been first noticed beginning in the second decade of life. A sense of the progressive nature of the WS clinical phenotype and visible changes can be gleaned from pairs of patient photos taken in early adulthood, and later in mid-life when the clinical phenotype of WS is often well-developed (see Figure 80.2).

These two patients and many other WS patients appear remarkably normal until the time of puberty, after which the most prominent of the features outlined in Table 80.1 become apparent over the subsequent 10 to 20 years. A simple animation from one of these patient

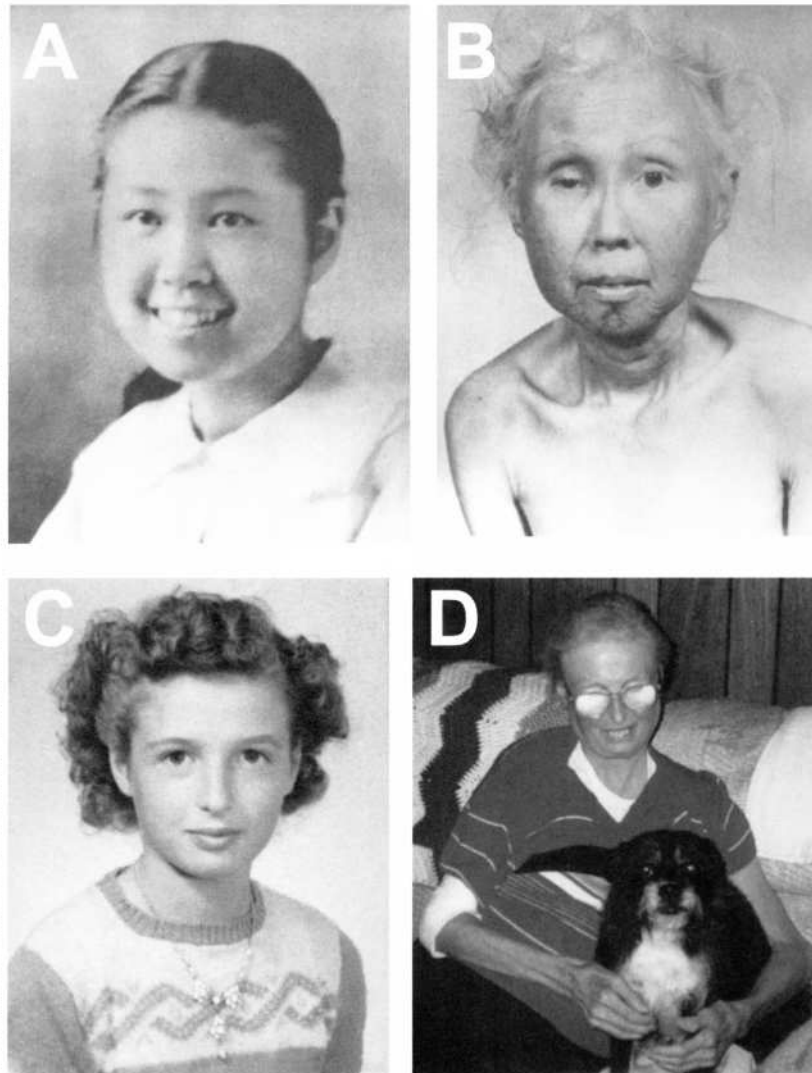


Figure 80.2 Clinical progression and features of Werner syndrome. **A, B.** Photographs of a Werner syndrome patient reported by Epstein *et al.* (1966) as Case 1, at ages 15 (**A**) and 48 (**B**). **C, D.** Photographs of a second patient at ages ~13 (**C**) and 56 (**D**). Note in both instances the rounded face, sharp features, graying, thinning, and loss of scalp and eyebrow hair and, in **D**, the thin, atrophic forearms and elbow ulceration. Panels **A** and **B** are used with kind permission of Drs. George Martin and Nancy Hanson of the International Registry of Werner Syndrome, and Lippincott Williams & Wilkins (**B**). The patient photographs in panels **C** and **D** were previously published in Martin (2005), and are used here courtesy of the patient's spouse with informed consent of the patient and of Drs. George Martin and Nancy Hanson, and Elsevier Press.

photo pairs can be viewed on the Web to get a sense of clinical progression of the changes in external appearance (see Recommended Resources).

The progression of clinical changes in WS can usefully be thought of as having three distinct phases. The first of these comprises the absence of an adolescent growth spurt followed over the subsequent decade by the appearance of graying and loss of hair, the development of skin changes, and of cataracts. A second wave of changes, often first seen late in the third or in the fourth decades of life, include skin ulceration, hypogonadism, and reproductive failure, together with a progressive worsening of the primary changes. A third phase may follow with the development of clinically important disease processes such as atherosclerosis, osteoporosis, diabetes mellitus, and cancer. These diseases occur proportionately earlier in WS patients than in otherwise normal individuals of comparable age, and are an important cause of premature morbidity and mortality. The three leading causes of death in WS patients are atherosclerotic cardiovascular disease, neoplasia, and, in a minority of cases, infection. The mean age at death in a Japanese clinical series was ~47 years, though well-documented patients have lived into the seventh decade of life (Goto, 1997).

DIAGNOSTIC CRITERIA AND DIFFERENTIAL DIAGNOSIS

The complex, progressive, and variable nature of the WS clinical phenotype makes the clinical diagnosis of WS challenging. This is especially true in young adults, where there are often few convincing signs or changes and where there may be no family history of WS to raise clinical suspicion. There are no universally accepted criteria for the diagnosis of WS. However, a useful set of diagnostic criteria and a scoring system have been developed by investigators at the International Registry for Werner Syndrome (Table 80.1, bottom panel; see also Recommended Resources). These criteria, together with molecular approaches to identify common *WRN* mutations or the loss of *WRN* protein expression (see later), can be used in most instances to confirm or exclude a diagnosis of WS in suspected affected individuals. The ability to couple clinical and molecular diagnostic approaches, as noted later, now allows the unambiguous identification of affected or at-risk homozygous mutant individuals, and of heterozygous carriers in families regardless of age or clinical findings.

The availability of a consistent set of clinical diagnostic criteria and of molecular diagnostic criteria for WS have provided sharper definition of WS as a disease entity, and provide a way to distinguish WS from other human diseases and syndromes that may mimic WS. Two groups of patients who may be confused with, or not easily distinguished from, typical or classic WS include patients who fulfill the clinical diagnostic criteria for WS as outlined in Table 80.1, though lack mutations in the *WRN*

gene; and patients who resemble WS patients though fail to fulfill the diagnostic criteria outlined in Table 80.1. This second group is often ascertained as potential variant or atypical WS. Both of these groups of patients are rare, and from the experience of the International Registry of Werner Syndrome together represent no more than a small fraction of suspected WS patients.

Despite their rarity, these WS look-alikes are of considerable clinical and biological interest as they are likely to be highly enriched for rare mutations in proteins that act with—or act on—*WRN* to modify or target its function *in vivo*. There are strong theoretical grounds for proposing the existence of these individuals, in light of the growing number of proteins that may act with *WRN* (see later). There are experimental hints, for example, of at least one additional complementation group for WS in addition to the group represented by inactivating mutations in the *WRN* gene (Prince *et al.*, 1999). These WS phenocopies or partial phenocopies represent an important area for additional clinical and molecular diagnostic work.

A portion of the second group of patients just mentioned, with what has been termed atypical Werner syndrome, share some clinical features of WS (see Table 80.1), though lack mutations in the *WRN* gene. A small number of these patients were recently found to carry germline mutations that alter splicing of the *LMNA* or lamin A/C gene (Chen *et al.*, 2003). A subset of different *LMNA* mutations that alter the splicing of *LMNA* mRNA have been found in patients with Hutchinson-Gilford progeria (HGPS), a rare, heritable, severe, and rapidly progressive disease that is often referred to simply as progeria or childhood progeria. Of note, other mutations that alter splicing or the properties of *LMNA* or lamin A/C have been associated with at least eight other clinically distinguishable diseases that have been collectively termed the laminopathies. This fascinating group of diseases affect predominantly nerve, muscle, connective tissue, or adipocytes (Broers *et al.*, 2005). HGPS and WS show little clinical overlap, and are unlikely to share any deep mechanistic similarities. Thus the existence of atypical WS as a distinct clinical entity has been controversial. It would appear to make the best sense at present to consider atypical WS patients with *LMNA* mutations that affect RNA splicing as having an atypical form of HGPS, rather than a variant form of WS (Hegele, 2003).

ADDITIONAL WORK NEEDED TO BETTER CHARACTERIZE WS

Our present understanding of clinical and pathologic features of WS is far from complete. This reflects both the comparative rarity of WS (see later) and the way in which patient data have been accumulated during the diagnosis or treatment of individual patients or their relatives rather than as part of a research protocol.

As a result, there is important work needed on all aspects of WS clinical medicine, pathology, and molecular and cellular function. A few of the more important areas that should receive emphasis to improve our understanding of WS are outlined here.

Systematic phenotyping

One way to improve our understanding of WS as a disease entity is to use our current understanding of clinical and pathologic features of WS to identify areas where systematic—or more quantitative—data need to be collected. For example, the clinical diagnosis of WS can often be made with confidence if the patient history and physical exam are systematic, and draw on aspects of history and clinical signs and symptoms already known to be associated with WS (see Table 80.1). Medical information forms available from the International Registry of Werner Syndrome Web site (see Recommended Resources) have been constructed with this end in mind, and can be used to collect key pieces of data to aid diagnosis during the history and physical examination of suspected WS patients. Table 80.1 also highlights those areas where we need to identify appropriate normal population control data for comparison with data collected from WS patients and their family members.

Longitudinal and cohort analyses

A second area where much productive work remains to be done is in the longitudinal analysis of WS in individual patients and their families. Few longitudinal data now exist, though will be essential to better understand the clinical expression and clinical progression of WS and of associated diseases (see Table 80.1). Careful family or pedigree studies linked to molecular data could also serve as the starting point for identification of important genetic or environmental factors that may modify either the clinical expression of WS or the development of associated diseases. Coupled clinical and molecular analyses that are longitudinal, within pedigrees, would also help resolve the question of whether there is a weakly penetrant haploinsufficiency or heterozygote phenotype in carriers of single mutant *WRN* alleles (Moser, Bigbee *et al.*, 2000). This is a point of considerable importance for, as noted later, heterozygote or haploinsufficiency phenotypes that may have clinical importance have been identified in cells and cell lines from *WRN* heterozygotes.

Patient-centered support to facilitate care and research

The ability to identify, follow, and support individual patients and their families would be immeasurably improved by a Werner patient-centered support group that provided consistent, accurate, general information on WS, developed guidelines for the clinical care of WS patients, and served to identify referral physicians knowledgeable about and skilled in the care of WS patients. An excellent example of this type of support

group is the Fanconi Anemia Research Fund (FARF). The FARF was founded to support patients and families with Fanconi anemia, a rare, heritable form of bone marrow failure that is also a predisposition to leukemia and squamous cell carcinoma. The FARF provides an example of how to create a support system that serves patients and their families by providing information and advice on patient care, while facilitating further study of biological and clinical aspects of a rare, heritable disease by linking patients and their families to investigators and clinicians (see Recommended Resources).

Tumor specimens

Tumor studies of WS point to a fourth area where more work is needed. Careful pathologic analysis of even small series of tumors from WS patients already has been shown to be productive and revealing. For example, the analysis of a small number of osteosarcomas arising in Japanese WS patients revealed important clinical and pathologic differences between OS in WS patients as compared with the general population, and provided a first estimate of the relative risk of developing OS in WS (Goto *et al.*, 1996; Ishikawa *et al.*, 2000). Thus the collection of additional WS tumor specimens, together with information on the response to and the success of treatment and the presence of atypical or precursor lesions, has a very high priority. These materials, even if conventionally prepared for histopathology, often can be used for a growing range of immunocytochemical and molecular analyses including mutation typing of the *WRN* gene or of other loci of interest.

Tissue resources to facilitate research

The diagnosis and treatment of tumors or other lesions requiring surgical intervention, together with the rare opportunity to autopsy WS patients, represent our best opportunities to preserve tumor and normal tissue specimens from patients and to provide material appropriate for analyses that demand viable cells or fresh tissue for procedures such as RNA-based microarray analyses. Skin samples from autopsy can be readily cryopreserved as a source of primary dermal fibroblast cultures. Similar samples served as the source of primary fibroblast strains and their SV40 large T-antigen or telomerase catalytic subunit-immortalized cell line derivatives that have proven remarkably useful for analyses of WS cell proliferation, cytogenetic and DNA damage response abnormalities. Biopsy and autopsy specimens may also serve as a useful source for additional cell strains or cell lines.

The Relationship of WS to Normal Aging

Published accounts of WS from Otto Werner have commented on the similarities between WS and what might be expected in premature aging. This idea is supported by the striking clinical appearance and progression of

changes observed in WS patients (see Figure 80.2). The consistent appearance of features of advanced chronological age in patients in the fourth and fifth decades of life led, not surprisingly, to the idea that WS *is* a premature or accelerated aging syndrome and that there *must* be deep mechanistic links between WS and normal aging. Despite these apparent similarities, careful clinical and/or pathologic examination of WS patients has consistently pointed out differences in the nature or degree of change observed in WS as compared with normal aging.

Quantitative differences between WS and normal aging include the greater extent or severity of the loss of hair or hair color; of the extent of calcification of heart valve leaflets; of atherosclerosis and of osteoporosis; and of the loss of reproductive function. Qualitative differences between WS and normal aging include the unusual type of cataract observed in WS patients as opposed to normal aged individuals; the unusual changes observed in skin and subcutaneous connective tissue of the face and extremities, together with nontrophic ulceration; the unusual spectrum of neoplasms; the location and extent of soft tissue calcification; and the autosomal recessive nature of WS (Epstein *et al.*, 1966).

In light of these differences, there has been substantial discussion of the value of studying WS, related diseases, or related animal models as ways to gain insight into mechanistic aspects of aging or of age-associated disease pathogenesis. An oft-cited disadvantage of studying diseases such as WS is that they may represent at best a phenocopy of what we are *really* interested in understanding, and thus mechanistic or pathophysiologic blind alleys as regards the mechanisms underlying normal aging or disease pathogenesis (Miller, 2004). A more optimistic view is that a subset of the disorders that affect the apparent rate of aging or the appearance of age-associated diseases *will* have mechanistic overlap with normal aging. Thus these diseases may provide a useful way to bring data on many aspects of human biology, genetics, and medicine to a focus to formulate and test specific hypotheses about age-dependent disease pathogenesis. These diseases or syndromes may also provide useful instances in which to test ideas about more general mechanisms that contribute to human aging (Kipling *et al.*, 2004; Martin, 2005).

This issue was discussed in some detail by Epstein *et al.* (1966) in their clinical, pathologic, and genetic characterization of WS. Their conclusions on this point are worth revisiting. They stated that the many observed differences between normal aging and WS led to the conclusion that WS was neither a precocious or accelerated form of aging, but “may be better considered a ‘caricature’ of aging, exaggerating, although not necessarily by the same mechanisms, some of the clinical and pathologic changes which connote aging” (Epstein *et al.*, 1966). This conclusion is still largely sound after 40 years of additional work on WS, despite the large number of

references to WS in the popular and scientific presses as a premature aging syndrome.

The WRN Gene and Disease-Associated Mutations

The chromosome 8p12 *WRN* gene was identified in 1996 by positional cloning and predicted to encode a 162 kDa member of the human RecQ helicase family (Yu *et al.*, 1996). The human RecQ helicase family consists of five proteins that may possess 3' to 5' helicase and ATPase activities. WRN is unique among the human RecQ proteins in possessing an additional 3' to 5' exonuclease activity (Fry, 2002; Bachrati and Hickson, 2003; Opresko, Cheng *et al.*, 2004; Figure 80.3). Two stable RNAs are expressed from the *WRN* locus in human cells, and the shorter of these, of 5.8 kb, is expressed ubiquitously at varying levels in many cell types, tissues, and organs (Yu *et al.*, 1996). The 162 kDa WRN protein can be detected by Western blot analysis in cell lines and tissue samples from normal individuals, and from heterozygous carriers of single mutant copies of the *WRN* gene (Goto *et al.*, 1999; Kawabe *et al.*, 2000; Moser, Kamath-Loeb *et al.*, 2000). No systematic study of the level of expression of WRN protein as a function of human cell type or of development has as yet been published.

Thus far, a total of 25 different mutations in the *WRN* gene have been reported in WS patients. These published examples have been assembled in the form of a Web-accessible Locus-Specific Mutational Database that contains detailed information for each published mutation (Moser *et al.*, 1999; see Recommended Resources). All of these *WRN* mutations, regardless of molecular type, confer a common biochemical phenotype—they truncate the WRN open reading frame, and lead to the loss of WRN protein from patient cells (Goto *et al.*, 1999; Moser, Kamath-Loeb *et al.*, 2000). This biochemical phenotype of WS patient mutations is thus consistent with the autosomal recessive inheritance pattern of WS. The absence of missense mutations that selectively inactivate the WRN helicase or exonuclease activity from this series of WS patient mutations is notable, and suggests that both activities need to be lost to promote WS pathogenesis. This point has been further addressed experimentally (see later; Swanson *et al.*, 2004). At least 17 additional *WRN* mutations have been identified in WS patients that resemble those already described. These will be added to the *WRN* Mutational Database as they are published.

The availability of mutation typing has allowed reinvestigation of the prevalence of WS, and of the frequency of mutations and heterozygous carriers in defined populations. Previous methods to address these questions have included case counting in defined populations, and comparisons of the frequency of consanguinity in WS pedigrees with population estimates of consanguinity. Prevalence estimates by these methods have varied

80. Werner Syndrome as a Model of Human Aging

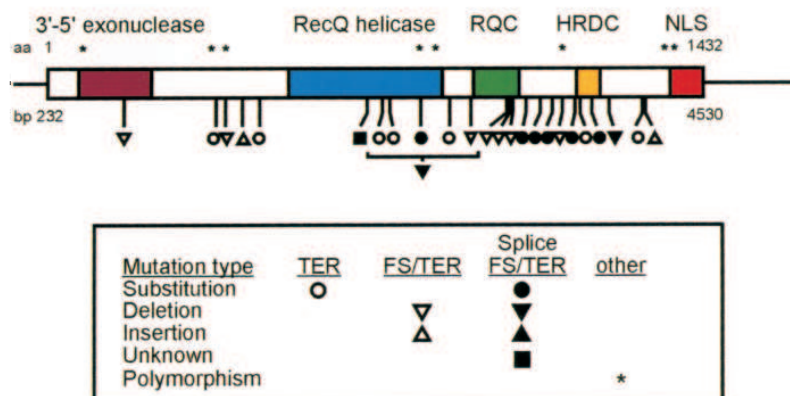


Figure 80.3 The human Werner syndrome protein WRN and a summary of mutations identified in the WRN gene of Werner syndrome patients. The central box indicates the WRN open reading frame with amino acid residue numbering indicated on top, and cDNA bp coordinates below, the left end of the open reading frame. The positions of five protein motifs are indicated by shaded boxes and labels: exonuclease and RecQ helicase domains, RecQ consensus (RQC) domain, the helicase and RNaseD-C-terminal (HRDC) domain, and the C-terminal nuclear localization signal (NLS). The location and molecular nature of mutations identified in Werner patients are indicated by symbols below the open reading frame to indicate the corresponding mutation location in the WRN cDNA sequence. All of the depicted mutations, stop codons (TER), frameshift mutations that alter the open reading frame (FS/TER), and mutations that alter splicing and the reading frame (Splice FS/TER) lead to truncation of the WRN open reading frame and loss of a C-terminal nuclear localization signal. The resulting proteins are unstable mislocalized and/or lost from patient cells. These data have been compiled and are available in the WRN Mutation and Polymorphism Database developed and maintained at the University of Washington (see Recommended Resources). See the color plate section.

over a 50-fold range ($1/22,000$ to $1/10^6$), with corresponding allele frequencies ranging from 0.0067 to 0.001. More recent estimates of allele frequency that make use of molecular diagnostic criteria are consistent with these allele frequency estimates. These wide-ranging estimates reflect uncertainties in several of the population estimates used to calculate each value, rather than inherent unreliability of the methods, and the comparatively small numbers of normal population controls who have been studied by molecular methods to determine allele frequencies (Schellenberg *et al.*, 2001).

The *WRN* locus resembles many other human genes in displaying a large number of genetic variants. A small subset of these are mutant alleles segregating in the human population. However, most of the genetic variation identified thus far in the human *WRN* gene consists of single nucleotide polymorphisms or sequence variants of uncertain functional importance. The number of these variants is surprisingly large: 375 *WRN* sequence variants were detected during the recent resequencing of *WRN* exons, promoter region and downstream untranslated region at the University of Washington as part of the NIEHS-funded Environmental Genome Project. Of note, these variants were detected in a small number of different DNA samples, the 90 contained in the EGP's Polymorphism Discovery Resource (see Recommended Resources for additional information). A few of the more common polymorphic sites in the *WRN* open reading frame are shown in Figure 80.3 by asterisks. At least one of these common polymorphic variants has been shown to have a substantial impact on catalytic function of the *WRN* protein (Kamath-Loeb *et al.*, 2004). Several additional common sequence variants are also being

examined for association with clinically important diseases prevalent in WS patients (e.g. atherosclerotic cardiovascular disease; see, e.g., Castro *et al.*, 2000; Ye *et al.*, 1997), or for possible associations with enhanced longevity (Castro *et al.*, 1999).

Another line of investigation of the potential of *WRN* sequence variants to modify human disease risk has come from the study of *WRN* heterozygotes. Heterozygous carriers of WS-associated mutant *WRN* alleles are likely to be present worldwide at frequencies ranging up to 1:100 in selected populations (Schellenberg *et al.*, 2001). Estimates of the prevalence or frequency of carriers of single, clearly pathogenic mutant *WRN* alleles in the United States are $\sim 1:250$, which places the number of carriers of mutant alleles in the U.S. population at $>10^6$. These frequency estimates suggest it will be important to determine whether the WS clinical phenotype is even weakly penetrant in heterozygous carriers.

There are some suggestions that *WRN* heterozygosity may be manifest at the cellular—and perhaps organismal—level. These data include the identification of genetic instability *in vivo* in mutation-typed heterozygous carriers of several *WRN* mutations (Moser, Bigbee *et al.*, 2000). Moreover, lymphoblastoid cell lines from otherwise healthy *WRN* heterozygotes display an intermediate sensitivity to killing by DNA damaging agents that selectively kill *WRN*-deficient cells (Ogburn *et al.*, 1997; Okada *et al.*, 1998). These two results indicate that *WRN* heterozygote effects may play an important role in cancer risk or the outcome of cancer therapy.

The clonal growth of many tumors may provide a way to identify inherited or somatically acquired *WRN*

mutations or *WRN* gene silencing events that play a role in cancer pathogenesis or progression. The first place to look for this type of mutational enrichment will be in sporadic tumors of the histopathologic types most commonly observed in WS patients (see Table 80.2). Tumors or normal tissue in a tumor patient that displayed an aberrant or exaggerated response to chemotherapy might also be further investigated to identify heritable or acquired compromise of the *WRN* functional pathway. The most productive place to look for this type of response would be in patients receiving camptothecin, mitomycin-C or cis-Pt, as these agents selectively kill cells that are *WRN*-deficient or haploinsufficient (Ogburn *et al.*, 1997; Okada *et al.*, 1998; Poot *et al.*, 2001).

WRN Protein Function in Human Cells

The identification of *WRN* as a RecQ helicase, and later as an exonuclease, were made initially on the basis of protein sequence analyses (Mian, 1997; Mushegian *et al.*, 1997; Yu *et al.*, 1996). These suggestions were subsequently verified by work from several laboratories (reviewed in Fry, 2002; Bachrati and Hickson, 2003; Opresko, Cheng *et al.*, 2004). Helicases are motor proteins that use the chemical energy obtained from nucleotide triphosphate hydrolysis to break hydrogen bonds in DNA or RNA. The disruption of hydrogen bonding and base pairing is essential for the expression, repair, and replication of DNA, and helicases together with additional proteins provide a way to do this in a controlled fashion. Exonucleases, in contrast to helicases, degrade DNA (or RNA) by hydrolyzing the phosphodiester bonds between adjacent nucleotide bases. Exonucleases facilitate many DNA metabolic processes such as repair or recombination by making single-stranded DNA ends of defined polarity available as substrates. They also play key roles in insuring the fidelity of DNA replication by acting as proofreading exonucleases, and in cellular nucleotide metabolism by insuring the turnover and salvage of DNA precursors.

The predicted helicase and exonuclease activities of *WRN* have been confirmed and further characterized by several groups using recombinant *WRN* protein and short oligonucleotide substrates of defined sequence and structure. These analyses indicate that *WRN* can bind, unwind, or degrade several types of 3- and 4-way DNA junctions and gapped, branched, or unpaired DNA regions, and that exonuclease and helicase activities often are coordinated in acting on these DNA substrates. Subsequent protein interaction studies of *WRN* indicated physical and/or functional cross-talk between *WRN* and general nucleic acid metabolic proteins such as RPA, together with more specialized proteins involved in DNA synthesis, on similar nucleic acid substrates (reviewed in Fry, 2002; Bachrati and Hickson, 2003; Opresko, Cheng *et al.*, 2004). Knowledge of these properties of *WRN* and results from prior work on *in vivo* functions of RecQ homologs

in *E. coli* and in budding and fission yeast suggested potential roles for *WRN* in recombination, replication, or repair.

Many of the types of DNA substrates that *WRN* can act upon *in vitro* resemble intermediates that are common to and generated during DNA replication, recombination, or repair *in vivo*, or may be present at telomeres. DNA replication and recombination are often thought of as discrete and rather distinct aspects of nucleic acid metabolism. Both processes are now recognized to be tightly linked and interdependent upon one another in many or all organisms. In human and other mammalian cells, the close functional interrelationship between DNA replication and homology-dependent recombinational repair (HR repair) is indicated by the importance of HR repair of DNA breaks, the critical role played by HR repair in mammalian development, and the critical role for HR proteins and HR function in the rescue of stalled replication forks (Cox *et al.*, 2000; Thompson and Schild, 2001). Consistent with these observations, abnormalities in DNA replication have been noted in WS cells for many years, and there are more recent data indicating an important role for *WRN* protein in insuring the completion of late stages of homologous recombination (Prince *et al.*, 2001; Saintigny *et al.*, 2002).

Several lines of evidence argue that telomeres are physiologic substrates for *WRN* function *in vivo*. *WRN* protein can be found at telomeres, and associates with key telomeric proteins such as TRF1 and TRF2. Moreover, TRF1 and TRF2 can modulate *WRN* biochemical activities on telomeric substrates *in vitro* (Opresko, Otterlei *et al.*, 2004). The T-loop structure of telomeres resembles the D-loop intermediate involved in recombination and replication, which is one of the branched DNA structures that may be a preferred *in vivo* substrate for *WRN*. Moreover, upon replication, telomeres are transiently converted into another postulated substrate for *WRN*, a single-ended DNA double-strand break. A final line of evidence for *WRN* function at telomeres comes from the observation that telomerase expression can extend the lifespan and modify the DNA damage responses of primary WS fibroblasts (Hisama *et al.*, 2000; Wyllie *et al.*, 2000), and that WS-like changes have been observed in mouse models in which telomerase function and *Wrn* function have been simultaneously ablated (see the section, "Animal Models").

If *WRN* plays a role in DNA replication and recombination, in telomere maintenance, or in HR repair in human cells, how does the loss of *WRN* function lead to characteristic molecular and cellular abnormalities in WS patients? A model for *WRN* function that reflects the close connection between replication and recombination is shown in Figure 80.4.

In this simplest version of *WRN* function *in vivo*, *WRN* acts on DNA molecules that are generated during the HR repair of DNA damage, from potential recombination substrates that are generated during stalled or

80. Werner Syndrome as a Model of Human Aging

disrupted DNA replication, or from intermediates or products of telomere maintenance. Successful resolution of these substrates suppresses genomic instability and insures high cell viability. In the absence of WRN function, these processes either stall or fail and leave cells with potentially toxic DNA intermediates that can trigger both DNA damage and apoptotic response pathways. Thus a loss of WRN function may lead in many cell lineages to apoptosis or mitotic death, while leading to gene rearrangement, mutation, or loss in surviving cells.

Experimental evidence supporting this model has been recently reviewed (Monnat and Saintigny, 2004).

For example, the predictions of genomic instability and reduced cell viability following the loss of WRN function are reflected in the characteristic chromosomal rearrangements in primary lymphocyte and fibroblast cultures from WS patients (Hoehn *et al.*, 1975; Melcher *et al.*, 2000; Figure 80.5), and in the reduced proliferation of primary WS fibroblasts in culture (Martin *et al.*, 1970).

The most surprising aspect of the model depicted in Figure 80.4 is that WS disease pathogenesis may be driven by a recombination *defect*. This conclusion is the opposite of what has been widely assumed—that WS is

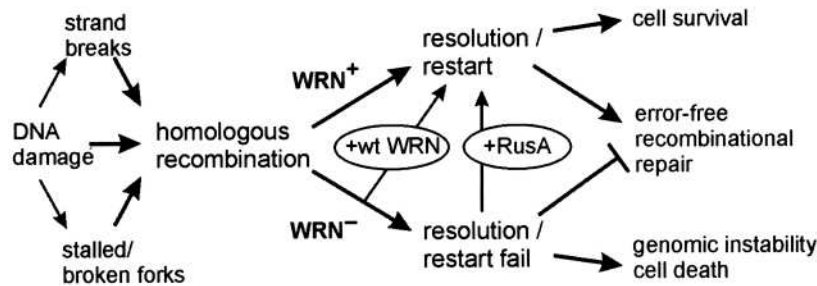


Figure 80.4 Model of Werner protein function in human cells. A requirement for WRN function can be initiated by DNA damage that leads to DNA strand breaks or that stalls or disrupts DNA replication. Damage to chromosomal DNA, replication forks, or telomeres can initiate homologous recombination repair (HR repair) that in the presence of WRN is successfully resolved to insure high cell viability and genetic stability (WRN^+ arrow). In the absence of WRN (WRN^- arrow), HR resolution and/or replication restart fail, leading to mitotic arrest, cell death, and genetic instability. Two of the experimental tests of this model are shown by the ovals: re-expressing WRN protein ($+wt\ WRN$) improves both cell survival and the recovery of viable mitotic recombinants, as does expression of the bacterial resolvase protein RusA ($+RusA$).

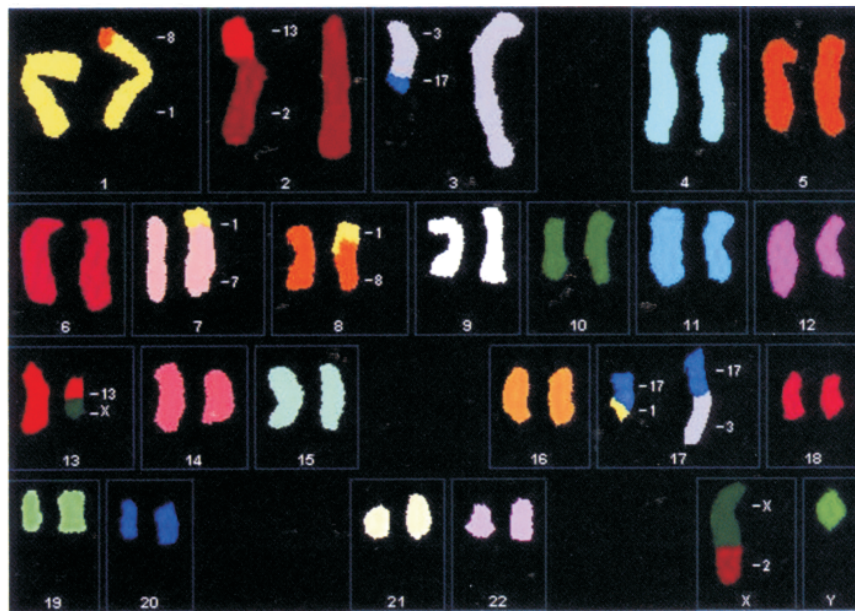


Figure 80.5 Variegated translocation mosaicism in primary fibroblasts from a Werner syndrome patient. The figure is a spectral (or SKY) karyotype of the chromosomal complement of a skin fibroblast from a male WS patient (reported in Melcher *et al.*, 2000). Note the many stable chromosomal changes including reciprocal translocations (e.g., involving chromosomes 1 and 8), together with translocations that are not obviously reciprocal in nature and may be accompanied by deletions (e.g., the translocation of material from chromosome 1 to chromosomes 7 and 17). This original spectral karyotype was kindly provided by Dr. Holger Hoehn, University of Würzburg, Würzburg, Germany. See the color plate section.

a hyper-recombination syndrome—and thus of particular conceptual value.

The model depicted in Figure 80.4 provides a useful way to integrate molecular, biochemical, and cytologic data on WS, and begins to explain mechanistic links among recombination, cell viability, and mutagenesis in WS cells. It also provides a useful way to further explore mechanistic aspects of WRN function. One example of this is illustrated by recent work to determine whether one or both of the WRN catalytic activities must be lost in order to generate the HR repair and cell survival defects characteristically seen in cells from WS patients. These experiments used single amino acid substitutions to disrupt the WRN exonuclease or helicase activities, together with the reexpression of mutant protein in WS cell lines. We found that both the WRN exonuclease and helicase activities needed to be lost to reveal the WRN recombination defect. However, and in contrast to WRN-deficient cells, the expression of either single missense mutant supported high cell viability after DNA crosslink damage (Swanson *et al.*, 2004).

These results indicate that the spectrum of *WRN* mutations identified in WS patients reflects the need to lose *both* WRN catalytic activities from cells in order to generate the cellular and clinical defects characteristic of WS. Our results also raise the intriguing possibility that *WRN* missense mutations that selectively affect helicase or exonuclease activity may be segregating in the human population, and could be associated with disease phenotypes in addition to WS that resulted from a selective loss of WRN-mediated HR repair.

WRN Function and Disease Pathogenesis in Cell Lineages

The elucidation of molecular aspects of WRN function, and of how the loss of WRN function generates WS

cellular phenotypes, together begin to suggest how the WS clinical phenotype may originate and progress. The postulated role for WRN in insuring successful, high-fidelity DNA replication, HR repair, and telomere maintenance, as outlined earlier (see Figure 80.4), indicates that a loss of function will be accompanied by genomic instability and reduced cell viability in many cell lineages during and after development. These two cellular consequences are likely to be intermediate phenotypes that lead to mutation accumulation and cell loss, that together drive the development of cell type-, cell lineage-, or tissue-specific defects in WS patients.

This idea of pathogenesis is shown in Figure 80.6. Further rounds of mutation accumulation, cell dysfunction, and cell loss can occur in continuously or conditionally replicating cell lineages, with the eventual compromise of tissue or organ structure and function and, in some tissues, the emergence of mutation-dependent neoplastic proliferation. This model of clinical progression indicates that the appearance of the first features of WS during adolescence is a reflection of the progressive accumulation of cellular defects during development and over the first decade of life, rather than something that is driven by the endocrine and physiologic changes that accompany puberty. Thus puberty reveals, rather than generates, the WS phenotype in affected individuals.

This view is consistent with the clinical and mechanistic view of WS as outlined earlier, and emphasizes the importance of genomic instability and replicative senescence in the generation of the WS phenotype. If this picture is accurate, however, why are dividing cell lineages not *selectively* affected during adult life by the loss of WRN function? And why are some organs such as the CNS spared the consequences of loss of WRN function? One likely explanation is the following. All cell lineages are generated by mitotic division during development and thus have the potential to be affected by mutation

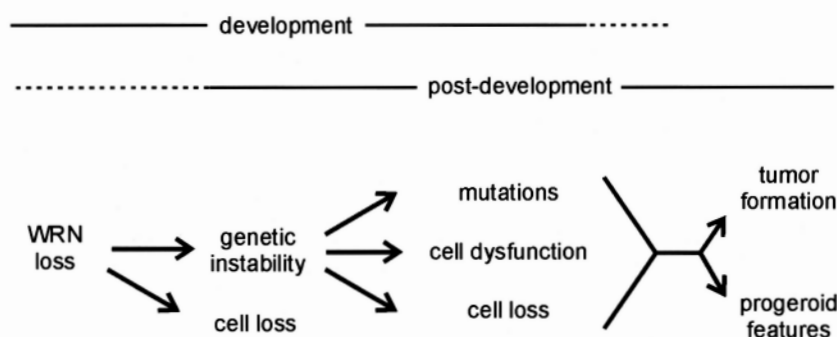


Figure 80.6 Model for pathogenesis of disease in the absence of WRN function. WRN loss as a result of inherited germline mutations (see Figure 80.3) leads from the beginning of development to genetic instability and cell loss in many or all cell lineages. These changes, following the completion of development, can be perpetuated or amplified in specific cell lineages or tissues where division potential is retained. How the intermediate consequences or phenotypes of the absence of WRN function, i.e., mutation accumulation, cell dysfunction and cell loss, affect specific lineages or tissues to lead to the emergence of either neoplastic, atrophic, or progeroid outcomes is heavily conditioned by normal lineage biology (see text for additional discussion). Two different time lines above the figure indicate the progressive nature of cell and cell lineage defects, and their origins during development.

accumulation or cell loss in the absence of WRN function. Continuously dividing lineages during adult life such as skin, gut, and bone marrow may be tolerant of the loss of WRN function by virtue of mutation expansion-limiting lineage architecture, normally stringent cell editing by a combination of apoptosis and terminal differentiation, and large reserves of stem cells or lineage repopulating cells.

Conversely, in the absence of WRN function, those cell lineages or tissues that are largely postmitotic following the completion of development (e.g., many CNS cell lineages) may take advantage of normal developmental regulatory mechanisms such as compensatory cell proliferation and cell editing by programmed cell death to compensate for increased cell loss or dysfunction to insure the completion of development with normal structure and function. Key variables that determine the eventual outcome are the number of cell divisions required to generate a mature lineage; how much cell editing via apoptotic cell death occurs during and after development; and what functional redundancy is present to compensate for cell loss or dysfunction.

The fibroblast lineage and other mesenchymal or mesodermally derived cell lineages may be selectively affected by the loss of WRN function for several of these reasons, including the persistence of conditional cell division throughout life; the ability to accumulate genetic variation, and perhaps genetic damage, in conjunction with a comparative resistance to damage-induced apoptosis; and the absence of a compartmentalized tissue architecture that could effectively suppress the proliferative defects that result in neoplasia. In the absence of WRN function this combination of features may predispose mesenchymal lineages to the progressive accumulation of mutant and dysfunctional or senescent cells, together with progressive disruption of trophic or regulatory interactions with adjacent epithelial or stromal cells (reviewed in Campisi, 2005).

This line of pathogenetic reasoning leads to two important conclusions. First, we need to know more about the normal biology of specific human cell lineages before we will be able to understand and predict *in vivo* consequences of a loss or absence of WRN function. A second important conclusion is that we clearly need experimentally tractable animal models in which to study cell lineage-specific functions of WRN.

Animal Models of WS

Animal models are clearly important if we are to study WRN function and WS pathogenesis at the levels of cell lineage and the whole organism. The only mammalian models of WS that have been developed thus far have been in the mouse. Three different types of mouse model have been published: a complete knockout or null of murine *Wrn* leading to a loss of Wrn protein expression in all tissues (Lombard *et al.*, 2000); an in-frame deletion of

the helicase domain of murine *Wrn*, leading to a truncated protein that retains exonuclease activity though lacks helicase activity (Lebel and Leder, 1998); and transgenic expression of a human K577M WRN variant protein that lacks helicase activity against a background of normal murine *Wrn* expression (Wang *et al.*, 2000).

Of these three models, the only one that faithfully recapitulates the genetic and biochemical defect observed in WS patients is the knockout that does not express Wrn protein. However, despite having faithfully recapitulated the biochemical defect observed in WS patients, the *Wrn* knockout mouse model does not have an obvious aging, genetic instability, or cancer phenotype. Several reasons have been suggested to explain the apparent absence of an obvious organismal phenotype in *Wrn* knockout mice: that mice simply do not live long enough to develop the changes first observed in WS patients beginning in the second and third decades of life; that mice may have different, or more robust, ways to compensate for the loss of *Wrn* function; or that humans may represent for reasons of lack of redundancy, long life span or environmental exposures, the equivalent of a sensitized background for revealing WRN function at the cell and organismal levels. Three caveats in interpreting these results and arguments are that the phenotyping of the *Wrn* knockout mouse model has been very modest to date; there has not as yet been a careful aging cohort study taken to completion; and knockout mice have not been systematically challenged with DNA damaging agents such as cross-linkers that may reveal defects in the murine *Wrn* functional pathway that parallel those observed in human cells.

One obvious way to test these ideas and further develop the murine model of WS is to generate sensitized mouse genetic backgrounds by altering the genetic constitution of *Wrn* knockout mice to place additional stress on the replication, HR repair or telomere maintenance pathways where *Wrn* is likely to function. One example of this approach was taken by two collaborating groups that made use of a murine telomerase RNA template-deficient (or *Terc*-deficient) mouse in conjunction with the loss of *Wrn* (Chang *et al.*, 2004), or of *Wrn* and *Blm* (Du *et al.*, 2004), function to reveal the progressive appearance of changes observed in WS patients.

Changes observed in these double or triple mutants include the graying and loss of hair, osteoporosis, diabetes mellitus, and cataracts. These changes appear to depend on critically short telomeres as a primary driver of the phenotype in the absence of *Wrn* and *Blm*. This provides an explanation as to why changes are observed in only a portion of mice, and in those affected only in late-generation *Terc*-deficiency where telomere erosion is substantial. These results are encouraging and have begun to provide mouse models in which to investigate *Wrn* function while identifying telomeres as a potential substrate for *Wrn* function *in vivo*.

Conclusion

Werner syndrome is one of a growing number of human diseases that have revealed the importance of genetic instability, replicative senescence, and cell death as intermediate phenotypes that can shape the risk of human disease or modify disease pathogenesis. WS is further distinguished as one of the growing number of human cancer predispositions that appear to result from a defect in homologous recombination function. One prominent role for WRN function *in vivo* is as part of one or more resolution complexes that act on common nucleic acid substrates that are generated during DNA replication or recombination, or that are part of the structure or metabolism of telomeres. This resolution function of WRN insures the successful completion of replication, recombination, or telomere maintenance, and thus high cell viability and genetic stability in all human cell lineages during development. WRN may also play comparable roles in cell lineages that retain the ability to divide during adult life.

Much work remains to be done to improve our understanding of WS as a biological and clinical human disease state. WRN function clearly modulates normal biology and physiology, and thus disease risk or pathogenesis in many human tissues or cell lineages. There are also tantalizing suggestions that the importance of WRN function in human biology will extend well beyond the small number of individuals affected with classic WS, as a modulator of both neoplastic and non-neoplastic disease risk in the general population. These hints suggest that additional work on the biology and medicine of WS will be challenging though richly rewarding.

Recommended Resources

PRINT RESOURCES

- Epstein, C.J., Martin, G.M., Schultz, A.L., and Motulsky, A.G. (1966). Werner's syndrome: A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine* 45, 177–221. (A modern classic detailing the clinical, pathological, and formal genetic analysis of contemporary and 122 reported WS cases.)
- Salk, D., Fujiwara, Y., and Martin, G.M. (Eds.) (1985). *Werner's Syndrome and Human Aging, Advances in Experimental Medicine and Biology*, Vol. 190. New York: Plenum Press. (Proceedings of the Kobe, Japan meeting in 1982 that contains edited reprints of important primary references, a translation of Otto Werner's thesis of 1904, and many useful chapters detailing clinical, pathologic, and biological aspects of WS.)
- Tollefsbol, T.O. and Cohen, H.J. (1984). Werner's syndrome: An underdiagnosed disorder resembling premature aging. *Age* 7, 75–88. (Useful compilation of clinical and biological information on WS gleaned from literature reports.)
- Goto, M. and Miller, R.W. (2001). From Premature Gray Hair to Helicase–Werner Syndrome: Implications for Aging and Cancer. *Gann Monograph Cancer Res.* 49. (Relatively recent compilation of reviews covering historical, clinical, and biological features of WS.)
- Monnat, R. J. Jr. and Saintigny, Y. (2004). The Werner syndrome protein: Unwinding function to explain disease. *SAGE-KE*. <http://sageke.sciencemag.org/cgi/reprint/2004/13/re3>. (Recent summary of biological and clinical aspects of WS that provides a detailed discussion of information supporting the model of WRN function shown in Figure 80.4.)

WEB RESOURCES

- On-Line Mendelian Inheritance in Man*, listing for Werner Syndrome:
<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=277700>
- International Registry of Werner Syndrome:
<http://www.pathology.washington.edu/research/werner/registry/frame2.html>
- WRN Locus-Specific Mutational Database:
http://www.pathology.washington.edu/research/werner/ws_wrn.html
- Animation of Werner syndrome clinical progression and links to the International Registry and Locus-Specific Mutational Database:
<http://www.pathology.washington.edu/research/werner/>
- GeneClinics Review of Werner Syndrome (authoritative and up-to-date review written by members of the International Registry that includes information on counseling and molecular diagnostics):
<http://www.geneclinics.org/> (search for 'Werner Syndrome Review' link)
- NIEHS SNPs Project Homepage (source of information on WRN gene polymorphisms using data generated by the UW Genome Center):
<http://egp.gs.washington.edu/data/wrn/>
- Fanconi Anemia Research Fund (exemplary example of a patient-oriented support group that is facilitating clinical care and research on another rare, heritable human genetic instability and cancer predisposition syndrome).
<http://www.fanconi.org/>

ACKNOWLEDGMENTS

I thank past and present members of my laboratory and members of the University of Washington NCI-funded Werner Syndrome Program for contributing ideas, hard work,

and enthusiasm to our attempts to understand WS. I would like to extend a special thanks to my senior colleagues and WS pioneers Drs. George M. Martin and Arno Motulsky, and to WS colleagues worldwide for their help and encouragement. Alden Hackmann provided graphics support, and Dr. Ali Ozgenc provided a thoughtful reading of the finished manuscript. Work in my laboratory on WS has been supported by grants from the U.S. National Institutes of Health including the NIA and NCI, and by a grant from the Nippon Boehringer Ingelheim Virtual Research Institute on Aging. There are no conflicts of interest to disclose in this work.

REFERENCES

- Note:* For reasons of space only a small number of primary references have been included below. My apologies to those whose work I have not been able to cite in full.
- Bachrati, C.Z. and Hickson, I.D. (2003). RecQ helicases: Suppressors of tumorigenesis and premature aging. *Biochem. J.* 374, 577–606.
- Bohr, V.A. (2003). Werner syndrome and its protein: Clinical, cellular and molecular advances. *Mech. Ageing Dev.* 124, 1073–1082.
- Broers, J.L.V., Hutchison, C.J., and Ramaekers, F.C.S. (2005). Laminopathies. *J. Pathol.* 204, 478–488.
- Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell* 120, 513–522.
- Castro, E., Edland, S.D., Lee, L., Ogburn, C.E., Deeb, S.S., Brown, G. *et al.* (2000). Polymorphisms at the Werner locus: II. 1074Leu/Phe, 1367Cys/Arg, longevity, and athero-sclerosis. *Am. J. Med. Genet.* 95, 374–380.
- Castro, E., Ogburn, C.E., Hunt, K.E., Tilvis, R., Louhija, J., Penttinen, R. *et al.* (1999). Polymorphisms at the Werner locus: I. Newly identified polymorphisms, ethnic variability of 1367Cys/Arg, and its stability in a population of Finnish centenarians. *Am. J. Med. Genet.* 82, 399–403.
- Chang, S., Multani, A.S., Cabrera, N.G., Naylor, M.L., Laud, P., Lombard, D. *et al.* (2004). Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat. Genet.* 36, 877–882.
- Chen, L., Lee, L., Kudlow, B.A., Dos Santos, H.G., Sletvold, O., Shafeghati, Y. *et al.* (2003). LMNA mutations in atypical Werner's syndrome. *Lancet* 362, 440–445.
- Cox, M.M., Goodman, M.F., Kreuzer, K.N., Sherratt, D., Sandler, S.J., and Marians, K.J. (2000). The importance of repairing stalled replication forks. *Nature* 404, 37–41.
- Du, X., Shen, J., Kugan, N., Furth, E.E., Lombard, D.B., Cheung, C. *et al.* (2004). Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. *Mol. Cell Biol.* 24, 8437–8446.
- Epstein, C.J., Martin, G.M., Schultz, A.L., and Motulsky, A.G. (1966). Werner's syndrome: A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine* 45, 177–221.
- Fry, M. (2002). The Werner syndrome helicase-nuclease-one protein, many mysteries. *SAGE-KE*, <http://sageke.sciencemag.org/cgi/reprint/2002/13/re2>.
- Goto, M. (2001). Clinical characteristics of Werner syndrome and other premature aging syndromes: Pattern of aging in progeroid syndromes. *Gann Monograph. Cancer Res.* 49, 27–39.
- Goto, M. (1997). Hierarchical deterioration of body systems in Werner's syndrome: Implications for normal aging. *Mech. Ageing Dev.* 98, 239–254.
- Goto, M., Miller, R.W., Ishikawa, Y., and Sugano, H. (1996). Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiol. Biomarkers Prev.* 5, 239–246.
- Goto, M., Rubenstein, M., Weber, J., Woods, K., and Drayna, D. (1992). Genetic linkage of Werner's syndrome to five markers on chromosome 8. *Nature* 355, 735–738.
- Goto, M., Yamabe, Y., Shiratori, M., Okada, M., Kawabe, T., Matsumoto, T. *et al.* (1999). Immunological diagnosis of Werner syndrome by down-regulated and truncated gene products. *Hum. Genet.* 105, 301–307.
- Hatamochi, A. (2001). Dermatological features and collagen metabolism in Werner syndrome. *Gann Monograph. Cancer Res.* 49, 51–59.
- Hegele, R.A. (2003). Drawing the line in progeria syndromes. *Lancet* 362, 416–417.
- Hisama, F., Chen, Y.-H., Meyn, M.S., Oshima, J., and Weissman, S.M. (2000). WRN or telomerase constructs reverse 4-nitroquinoline 1-oxide sensitivity in transformed Werner syndrome fibroblasts. *Cancer Res.* 60, 2372–2376.
- Hoehn, H., Bryant, E.M., Au, K., Norwood, T.H., Boman, H., and Martin, G.M. (1975). Variegated translocation mosaicism in human skin fibroblast cultures. *Cytogenet. Cell Genet.* 15, 282–298.
- Ishikawa, Y., Miller, R.W., Machinami, R., Sugano, H., and Goto, M. (2000). Atypical osteosarcomas in Werner syndrome (adult progeria). *Jpn. J. Cancer Res.* 91, 1345–1349.
- Kawabe, T., Tsuyama, N., Kitao, S., Nishikawa, K., Shimamoto, A., Shiratori, M. *et al.* (2000). Differential regulation of the human RecQ family helicases in cell transformation and cell cycle. *Oncogene* 19, 4764–4772.
- Kipling, D., Davis, T., Ostler, E.L., and Faragher, R.G.A. (2004). What can progeroid syndromes tell us about human aging? *Science* 305, 1426–1431.
- Lebel, M. and Leder, P. (1998). A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc. Natl. Acad. Sci. USA* 95, 13097–13102.
- Lombard, D.B., Beard, C., Johnson, B., Marciniak, R.A., Dausman, J., Bronson, R. *et al.* (2000). Mutations in the WRN gene in mice accelerate mortality in a p53-null background. *Mol. Cell Biol.* 20, 3286–3291.
- Martin, G.M. (2005). Genetic modulation of senescent phenotypes in *Homo sapiens*. *Cell* 120, 523–532.
- Martin, G.M., Sprague, C.A., and Epstein, C.J. (1970). Replicative life-span of cultivated human cells. Effects of donor's age, tissue, and genotype. *Lab. Invest.* 23, 86–92.
- Melcher, R., von Golitschek, R., Steinlein, C., Schindler, D., Neitzel, H., Kainer, K. *et al.* (2000). Spectral karyotyping of Werner syndrome fibroblast cultures. *Cytogenet. Cell Genet.* 91, 180–185.
- Mian, I.S. (1997). Comparative sequence analysis of ribonucleases HII, III, PH, and D. *Nucleic Acids Res.* 25, 3187–3195.

- Miller, R.A. (2004). "Accelerated aging": A primrose path to insight? *Aging Cell* 3, 47–51.
- Monnat, R.J., Jr. (2002). Werner syndrome. In C. Fletcher, K. Unni, and F. Mertens (Eds.), *WHO/IARC Monograph on Pathology and Genetics of Tumours of Soft Tissue and Bone*, pp. 273–274. Lyon: IARC Press.
- Monnat, R.J., Jr. (2001). Cancer pathogenesis in the human RecQ helicase deficiency syndromes. *Gann Monograph Cancer Res.* 49, 83–94.
- Monnat, R. J., Jr. and Saintigny, Y. (2004). The Werner syndrome protein: Unwinding function to explain disease. *SAGE-KE*. <http://sageke.sciencemag.org/cgi/reprint/2004/13/re3>.
- Moser, M.J., Bigbee, W.L., Grant, S.G., Emond, M.J., Langlois, R.G., Jensen, R.H. *et al.* (2000). Genetic instability and hematologic disease risk in Werner syndrome patients and heterozygotes. *Cancer Res.* 60, 2492–2496.
- Moser, M.J., Kamath-Loeb, A.S., Jacob, J.E., Bennett, S.E., Oshima, J., and Monnat, R.J., Jr. (2000). WRN helicase expression in Werner syndrome cell lines. *Nucleic Acids Res.* 28, 648–654.
- Moser, M.J., Oshima, J., and Monnat, R.J., Jr. (1999). WRN mutations in Werner syndrome. *Hum. Mutation* 13, 271–279.
- Mushegian, A.R., Bassett, D.E., Jr., Boguski, M.S., Bork, P., and Koonin, E.V. (1997). Positionally cloned human disease genes: Patterns of evolutionarily conservation and functional motifs. *Proc. Natl. Acad. Sci. USA* 94, 5831–5836.
- Ogburn, C.E., Oshima, J., Poot, M., Chen, R., Hunt, K.E., Gollahon, K.A. *et al.* (1997). An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type and homozygous mutants. *Hum. Genet.* 101, 121–125.
- Okada, M., Goto, M., Furuichi, Y., and Sugimoto, M. (1998). Differential effects of cytotoxic drugs on mortal and immortalized B-lymphoblastoid cell lines from normal and Werner's syndrome patients. *Biol. Pharm. Bull.* 21, 235–239.
- Oppenheimer, B.S. and Kugel, V.H. (1934). Werner's syndrome—a heredo-familial disorder with scleroderma, bilateral juvenile cataract, precocious graying of hair and endocrine stigmatization. *Trans. Assoc. Amer. Physicians* 49, 358–370.
- Opresko, P.L., Cheng, W.-H., and Bohr, V.A. (2004). Junction of RecQ helicase biochemistry and human disease. *J. Biol. Chem.* 279, 18099–18102.
- Opresko, P.L., Otterlei, M., Graakjaer, J., Bruheim, P., Dawut, L., Kolvraa, S. *et al.* (2004). The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. *Mol. Cell* 14, 763–74.
- Pehmoeller, G. (2001). Memory of my father, Otto Werner. *Gann Monograph Cancer Res.* 49, vii–viii.
- Poot, M., Yom, J.S., Whang, S.H., Kato, J.T., Gollahon, K.A., and Rabinovitch, P.S. (2001). Werner syndrome cells are sensitive to DNA cross-linking drugs. *FASEB J.* 15, 1224–1226.
- Prince, P.R., Emond, M.J., and Monnat, R.J., Jr. (2001). Loss of Werner syndrome protein function promotes aberrant mitotic recombination. *Genes Dev.* 15, 933–938.
- Prince, P.R., Ogburn, C.E., Moser, M.J., Emond, M.J., Martin, G.M., and Monnat, R.J., Jr. (1999). Cell fusion corrects the 4-nitroquinoline 1-oxide sensitivity of Werner syndrome fibroblast cell lines. *Hum. Genet.* 105, 132–138.
- Saintigny, Y., Makienko, K., Swanson, C., Emond, M.J., and Monnat, R.J., Jr. (2002). Homologous recombination resolution defect in Werner syndrome. *Mol. Cell Biol.* 22, 6971–6978.
- Salk, D. (1982). Werner syndrome: A review of recent research with an analysis of connective tissue metabolism, growth control of cultured cells, and chromosomal aberrations. *Human Genetics* 62, 1–15.
- Salk, D., Fujiwara, Y. and Martin, G.M. (Eds.) (1985). Werner's Syndrome and Human Aging, Vol. 190, *Advances in Experimental Medicine and Biology*. New York: Plenum Press.
- Schellenberg, G.D., Miki, T., Yu, C.-E., and Nakura, J. (2001). Werner syndrome. In C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle (Eds.) *The Metabolic & Molecular Basis of Inherited Disease*, pp. 785–797. New York: McGraw-Hill.
- Swanson, C., Saintigny, Y., Emond, M.J., and Monnat, R.J., Jr. (2004). The Werner syndrome protein has separable recombination and viability functions. *DNA Repair* 3, 1–10.
- Thannhauser, S.J. (1945). Werner's syndrome (progeria of the adult) and Rothmund's syndrome: Two types of closely related hederofamilial atrophic dermatoses with juvenile cataracts and endocrine features; a critical study of five new cases. *Ann. Intern. Med.* 23, 559–626.
- Thompson, L.H. and Schild, D. (2001). Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat. Res.* 477, 131–153.
- Tollefsbol, T.O. and Cohen, H.J. (1984). Werner's syndrome: An underdiagnosed disorder resembling premature aging. *Age* 7, 75–88.
- Wang, L., Ogburn, C.E., Ware, C.B., Ladiges, W.C., Youssoufian, H., Martin, G.M. *et al.* (2000). Cellular Werner phenotypes in mice expressing a putative dominant-negative human WRN gene. *Genetics* 154, 357–362.
- Werner, O. (1985). On cataract in conjunction with scleroderma (translated by H. Hoehn). In D. Salk, Y. Fujiwara, and G.M. Martin (Eds.), *Werner's Syndrome and Human Aging*, Vol. 190, *Advances in Experimental Medicine and Biology*, pp. 1–14. New York: Plenum Press.
- Wyllie, F.S., Jones, C.J., Skinner, J.W., Houghton, M.F., Wallis, C., Wynford-Thomas, D. *et al.* (2000). Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts. *Nat. Genet.* 24, 16–17.
- Ye, L., Miki, T., Nakura, J., Oshima, J., Kamino, K., Rakugi, H. *et al.* (1997). Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. *Am. J. Hum. Genet.* 68, 494–498.
- Yu, C.-E., Oshima, J., Fu, Y.-H., Wijsman, E.M., Hisama, F., Ouais, S. *et al.* (1996). Positional cloning of the Werner's syndrome gene. *Science* 272, 258–262.