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# Molecular Basis of Progeroid Syndromes– the Werner and Hutchinson-Gilford Syndromes

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Segmental progeroid syndromes are members of a group of disorders in which affected individuals present various features suggestive of accelerated aging. The two best-known examples are the Werner syndrome (WS; "Progeria of the adult") and the Hutchinson-Gilford Progeria syndrome (HGPS; "Progeria of childhood"). The gene responsible for WS, *WRN*, was identified in 1996 and encodes a multifunctional nuclear protein with exonuclease and helicase domains. WS patients and cells isolated from the WS patients show various genomic instability phenotypes, including an increased incidence of cancer. The WRN protein is thought to play a crucial role in optimizing the regulation of DNA repair processes.

Recently, a novel recurrent mutation in the *LMNA* gene has been shown to be responsible for HGPS. *LMNA* encodes nuclear intermediate filaments, lamins A and C; mutant lamins are thought to result in nuclear fragility. There are at least six other disorders caused by *LMNA* mutations, most of which affect cells and tissues of mesenchymal origins, including atypical forms of WS. The pathophysiologies of these and certain other progeroid syndromes indicate an important role for DNA damage in the genesis of common age-related disorders.

**Key Words: Werner syndrome, WRN, RecQ, Hutchinson-Gilford Progeria syndrome, LMNA, Lamin, Genomic instability, Aging, Human**

## Introduction

Segmental progeroid syndromes encompass a group of disorders characterized by an apparent acceleration of senescence, in that multiple phenotypes associated with the last decades of life appear during early and mid-life.

The most striking example of an adult-onset segmental progeroid syndrome is the Werner syndrome (WS; OMIM277700) (McKusick 1998). WS was first reported by Dr. Otto Werner in his medical doctoral thesis at Kiel University in 1908, but the gene responsible for WS, *WRN*, was not discovered until 1996 (Yu et al. 1996). The *WRN* gene encodes a multifunctional nuclear protein with both exonuclease and helicase activities. As a result of the identification of *WRN*, the diagnosis of WS, previously based upon clinical criteria, can now be confirmed by molecular biological methods.

The most striking example of a segmental progeroid syndrome with an onset in childhood is the Hutchinson-Gilford Progeria syndrome (HGPS), also known as Progeria. Three independent groups have now shown that the syndrome is caused by a common mutation at the *LMNA* locus, which codes for nuclear intermediate filaments (De Sandre-Giovannoli et al. 2002, Cao et al. 2003, De Sandre-Giovannoli et al. 2003, Eriksson et al. 2003).

Approximately 80-90% of cases with a clinical diagnosis of WS have demonstrable mutations in the *WRN* gene. Our group has therefore been seeking mutations responsible for the remaining 10-20% of

**Abbreviations:** *BAF*, Barrier to autointegration factor; *BER*, base excision repair; *DSB*, double strand break; *IR*, Illegitimate recombination; *HGPS*, Hutchinson-Gilford Progeria Syndrome; *HR*, Homologous recombination; *Lamin A/C*, type A and C lamin; *NER*, Nucleotide excision repair; *NHEJ*, Non-homologous end joining; *WS*, Werner syndrome.

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cases. To our surprise, we recently discovered that mutant forms of *LMNA*, distinct from what has been observed in HGPS, are responsible for a subset of these "atypical" forms of WS.

It is not known how mutations in the *WRN* and *LMNA* genes cause progeroid features, much less why abnormalities of functionally different genes can sometimes lead to similar progeroid phenotypes or whether these genes are involved at all in human aging processes. In this review, the current understanding of the pathogenesis of disabilities due to mutations at these two loci will be explored.

## The Werner Synmdrome

### *Disease Course of Werner Syndrome (WS)*

WS patients develop normally until the end of their first decade. The first symptom, often recognized retrospectively, is short stature resulting from the lack of a growth spurt during the patient's early teens. Other clinical symptoms typically start toward the end of the second decade of life and include gray hair, alopecia (loss of hair), hoarseness or a high-pitched voice, and degenerative changes of skin. These symptoms are typically followed in the third decade by bilateral ocular cataracts, type 2 diabetes mellitus, hypogonadism, skin ulcers, and osteoporosis (Epstein 1966, Tollefsbol et al. 1984, Goto 1997).

WS patients exhibit several forms of arteriosclerosis (hardening of the arteries). Myocardial infarction, together with cancer, constitute the most common causes of death, typically around the age of forty-eight or about ten years after the typical age of diagnosis. The most recent review by Goto et al. (1996) shows that cancer is the most common cause of death among Japanese WS patients. The chronological onset of these symptoms is similar in all WS cases regardless of the type of underlying genetic mutations (Epstein 1966, Tollefsbol et al. 1984, Goto 1997).

The mean age of cancer detection in WS patients is 44 years of age, but there is a very wide range (25-64 years of age). A variety of benign and malignant neoplasms have been observed (Epstein 1966, Tollefsbol et al. 1984, Goto 1997). The spectrum of cancers is unusual, however, in that there are large numbers of sarcomas and some very rare types of cancers (Goto et al. 1996). The overall cancer risk is increased 30- to 50-fold over that of the general population and tumor specific risks are increased up to 1000-fold for the case of acral lentigenous melanoma. The ratio of carcinomas to sarcomas is approximately 1:1, as opposed to 10:1 in the general population. Thyroid carcinoma is the most commonly exhibited epithelial neoplasm. The most common sarcomas observed in WS patients are soft tissue sarcomas, osteosarcomas, acral lentigenous melanomas, and meningiomas. The occurrence of multiple tumors is also common. As many as five types of malignancies have been observed in a single patient (Tsuchiya et al. 1991).

In addition to the unusual types of cancers, there are several other discrepancies between Werner syndrome and usual normal aging. While osteoporosis is typically observed, it is unusual in that it especially affects the distal long bones. There are also unusual and characteristic osteolytic lesions of the distal joints of fingers. Deep, chronic ulcers around the ankles (malleoli and Achilles tendon) are highly characteristic—almost pathogenomic. There is some controversy concern-ing the extent to which the brain is affected. Central nervous system complications associated with arteriosclerosis clearly can occur, but while beta amyloid deposits have been observed in patients bearing the APOE epsilon 4 susceptibility allele for dementia of the Alzheimer type (Leverenz et al. 1998), that disease is not part of the clinical picture (Martin et al. 1999).

The frequency of Werner syndrome can be expected to vary with the level of consanguinity in populations, as it is caused by an autosomal recessive mutation of *WRN*. In the Japanese population, the frequency may range from about 1/20,000 to 1/40,000 based upon the frequencies of detectible heterozygous mutations (Satoh et al. 1999). The prevalence in the US population is not known, but has been estimated to be on the order of 1/200,000 (Martin et al. 1999). *WRN* mutation heterozygotes do not appear to be at increased risk for any Werner syndrome-specific symptoms, but this question requires much more study. The male-to-female ratio of WS patients is believed to be 1:1. In our International Registry of Werner Syndrome, females are slightly over-represented, possibly due to a clinical ascertainment bias.

### *Clinical Diagnosis of Werner Syndrome*

The median age of WS diagnosis has been determined in two separate studies to be around thirty-eight years of age (Epstein 1966, Tollefsbol

et al. 1984, Goto 1997). By middle age, WS patients develop gray hair; baldness; wrinkled, tight skin; regional loss of subcutaneous fat; ocular cataracts; type 2 diabetes mellitus; osteoporosis; atherosclerosis; medical calcinosis; arteriolar sclerosis; atrophy of gonads; and certain types of cancers. It should be emphasized that, unlike HGPS, the onset of WS-related symptoms occurs after adolescence (with the exception of short stature).

The following diagnostic criteria have been proposed (Nakura et al. 1994). They were used prospectively for the successful mapping and positional cloning of the *WRN* locus (Yu et al. 1996).

*Cardinal signs and symptoms (onset over 10 years old):*

1. Cataracts (bilateral).
2. Characteristic dermatological pathology (tight skin, atrophic skin, pigmentary alterations, ulceration, hyperkeratosis, regional subcutaneous atrophy) and typical facies ('bird' facies).
3. Short stature.
4. Parental consanguinity (3rd cousin or greater) or affected sibling.
5. Premature graying and/or thinning of scalp hair.
6. (Elevated 24-hour urinary hyaluronic acid test, when available).

*Further signs and symptoms:*

1. Diabetes mellitus (Type 2).
2. Hypogonadism (secondary sexual underdevelopment, diminished fertility, testicular or ovarian atrophy).
3. Osteoporosis.
4. Osteosclerosis of distal phalanges of fingers and/or toes (x-ray diagnosis).
5. Soft tissue calcification.
6. Evidence of premature atherosclerosis (e.g. history of myocardial infarction).
7. Mesenchymal neoplasms, rare neoplasms or multiple neoplasms.
8. Voice changes (high pitched, squeaky or hoarse voice).
9. Flat feet.

*Diagnosis:*

Definite: All the cardinal signs and two others.
Probable: The first three cardinal signs and any two others.
Possible: Either cataracts or dermatological alterations and any four others.
Exclusion: Onset of signs and symptoms before adolescence (except stature, since current data on pre-adolescent growth patterns are inadequate.)

Goto proposes the clinical diagnosis of Werner syndrome if four of five of the following criteria are present (Goto 1997):

1. Consanguinity.
2. Characteristic "bird-like" appearance and body habitus.
3. Premature senescence.
4. Scleroderma-like skin changes.
5. Endocrine-metabolic disorders.

An increased production of urinary hyaluronic acid is observed in most cases of Werner syndrome (Tollefsbol et al. 1984, Goto 1997). Historically, elevated urinary hyaluronic acid production was used to support a clinical diagnosis. Due to the availability of modern molecular testing, however, such measures are rarely used today. Likewise, an increase in chromosomal aberrations (i.e., variegated translocation mosaicism) (Hoehn et al. 1975) has been observed in cells of WS, but is not currently considered among the standard diagnostic criteria. Other standard laboratory tests such as alteration in blood lipids, while informative, are not specific for WS.

## WRN Gene Product and WRN Mutations

### *The Wildtype WRN Protein*

The *WRN* gene consists of 35 exons that encode a multifunctional nuclear protein of 1,432 amino acids (Yu et al. 1996). The central region of the WRN protein contains the consensus domains of RecQ type helicases (Gray et al. 1997) and the N-terminal region contains exonuclease domains (Huang et al. 1998) (figure 1). There is a nuclear localization signal at the C-terminal end of the protein (Matsumoto et al. 1997). Between the exonuclease and helicase domains, there is a highly acidic transactivation sequence (Balajee et al. 1999). There are two consensus domains in the C-terminal region whose functions have not been completely elucidated: 1) a RecQ C-terminal conserved region speculated to be involved in protein-protein interactions, and 2) a helicase RNaseD C-terminal (HDRC) conserved region speculated to be involved in protein-DNA interactions.

There are at least four non-synonymous *WRN* polymorphisms (Castro et al. 1999, Passarino et al. 2001), two of which show evidence of a role in altering the risks of certain age-related human disorders. The first, Phe homozygosity at amino acid 1076, may be associated with reduced longevity and an age-dependent risk of atherosclerosis when compared to the common wildtype allele (Leu) (Castro et al. 2000). The second, Arg at amino acid 1367, has been associated with a decreased risk of myocardial infarction when compared to the common allele (Cys) (Ye et al. 1997).

RecQ type helicases are ATP-dependent 3'?5' helicases that belong to the DEAH (Asp-Glu-Ala-His) subfamily of DNA and RNA helicases. Originally identified as the *RecQ* of *E. coli*., RecQ helicases consist of seven canonical helicase motifs: I (ATPase), Ia II, III, IV, V and VI. *E. coli* and yeast have only one member of the RecQ helicase whereas humans have at least five. Other human RecQ helicases include RecQL1, BLM (mutations at which are responsible for the Bloom Syndrome), RecQ4 (mutations at which are responsible for the Rothmund-Thomson Syndrome; RTS) and RecQ5 (figure 1)(reviewed by Oshima (Martin et al. 2000, Oshima 2000, Oshima 2000)). The unique feature of the WRN helicase is that it has the exonuclease domain on the same protein. *E. coli* RecQ helicase activity is associated with 3'→5' exonuclease activity (Phillips et al. 1988, Myers et al. 1995) raising the possibility that *WRN* evolved to efficiently coordinate exonuclease and helicase activities during DNA metabolism. Neither RecQL nor RecQ5 have yet been associated with any human disease.

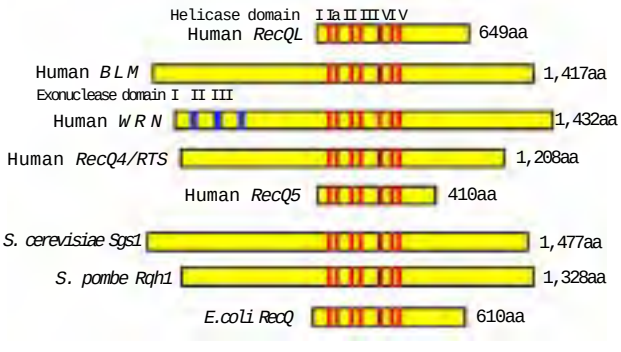



**Figure 1.** Human RecQ helicases. The yellow rectangles represent the five human RecQ helicases. Helicase domains are shown in red. Exonuclease domains, shown in blue, are present only in the WRN protein.

### *WRN* Mutations in WS Patients

The mechanism by which *WRN* mutations cause the WS phenotype is not clear. All of the mutations identified to date result in truncation of the WRN protein and the loss of the nuclear localization signal at the C-terminal region of the WRN protein (Moser et al. 1999). The known mutations correspond either to stop codons, insertions, or deletions that result in frame shifts or splicing donor/acceptor site mutations, ultimately causing one or more exons to be skipped (figure 2). Dominant negative forms of *WRN* have not been identified in WS patients. Mutant mRNAs and the resulting mutant proteins exhibit shorter half-lives than do the wild-type mRNA and WRN protein (Yamabe et al. 1997). Unlike the case for HGPS, Werner syndrome is the only disease known to be associated with mutations at the *WRN* locus.

The specific cell type in which WS-associated cancers develop may differ depending on the type of mutation in the *WRN* gene. Whereas papillary carcinoma has been associated with an N-terminal mutation, follicular carcinoma is more frequently

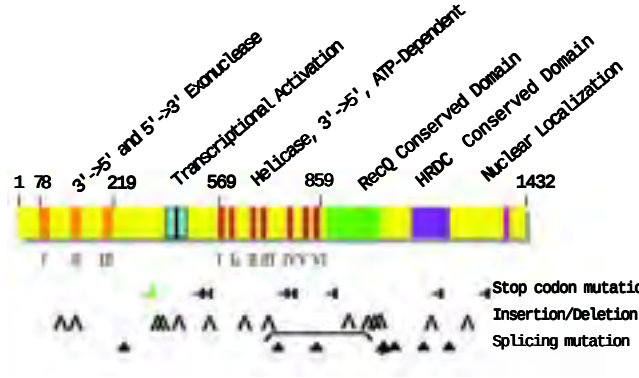



**Figure 2.** Functional domains and disease mutations of the *WRN* gene product. Arabic numerals above the rectangle indicate the segments of amino acid numbers of the WRN protein. Roman numerals indicate exonuclease or helicase domains. The functions of the RecQ conserved domain (green) and the helicase RNaseD C-terminal (HRDC) conserved domain (purple) have not yet been determined. WS mutations are grouped by type below the main figure. The green symbol indicates the most frequent WRN mutation among Caucasians. Note that all the identified WS mutations eliminate the nuclear localization signal at the C-terminal region.

observed in association with C-terminal mutations (Ishikawa et al. 1999). This finding is inconsistent with the assumption that all identified mutations within *WRN* result in loss of the nuclear localization signal of WRN protein, and thereby act as null mutations. Further studies may reveal additional correlations between specific genotypes and phenotypes.

### *Enzymatic Activities of WRN Protein*

WRN helicase unwinds double-stranded DNA and DNA-RNA hybrids, but its catalytic activities may not be limited to those substrates. Several unusual DNA substrates have been tested as potential physiological targets for WRN helicase activity (figure 3). WRN protein is able to efficiently unwind double-stranded DNA with mismatch "bubbles." It also unwinds G4 quartets made by two hairpin loops (G'2 biomolecular tetraplex) of d(CGG)n (Fry et al. 1999). Though its presence has not been demonstrated *in vivo*, G4 quartets can potentially be formed from two GC-rich regions of unwound single-stranded DNA (or RNA) during replication, repair, recombination or transcription.

Another interesting structure is the recombination intermediate, or a-structure. WRN protein is able to promote branch migration of Holliday junctions (Constantinou et al. 2000). In fact, the WRN helicase appears to more effectively dissociate the a-structure than the simple DNA duplex, as assessed by the length of the migration. Double-stranded DNA with mismatched tails is also a preferred substrate for WRN helicase (Suzuki et al. 1997). These structures can be formed both during recombination and replication, such as break-induced DNA replication or repair of a stalled replication. Recombinant WRN protein does not unwind a G'2 tetraplex containing a telomere repeat sequence (Fry et al. 1999). However, in a process which is stabilized by human replication protein A, WRN protein does appear to unwind up to 23 kb of a PCR-generated telomere repeat sequence to single-stranded DNA(Ohsugi et al. 2000). WRN exonuclease also preferentially digests single strands in complex DNA structures, such as double-stranded DNA with mismatched ends or bubbles (Oshima 2000, Shen et al. 2000).

Biochemical and cell biological studies suggest that WRN protein is involved in DNA repair, recombination, replication, and transcription as well as complex functions such as DNA repair during replication (Oshima 2000).

### *Roles of WRN Protein in Regulation of Recombination*

The repair of double-strand breaks in eukaryotic cells occurs through one of two different mechanisms: 1) homologous recombination (HR), or 2) illegitimate recombination (IR), also called nonhomologous end-joining (NHEJ). The latter process is error-prone as it requires little or no DNA homology. The *E. coli* RecQ helicase has been shown to initiate or to disrupt recombinational intermediates (Harmon et al. 1998). Combined with the variegated translocation mosaicism seen in WS cells, a number of findings suggest a model in which WRN protein participates both in homologous recombination and in illegitimate recombination by modulating the structures of recombination intermediates (Saintigny et al. 2002). Several investigators have observed a prolonged S-phase during the active cell cycle. This may be due to the mechanistic similarity of DNA strand-processing in DNA replication, recombination/double-strand break repair, and transcription (Kodadek 1998).

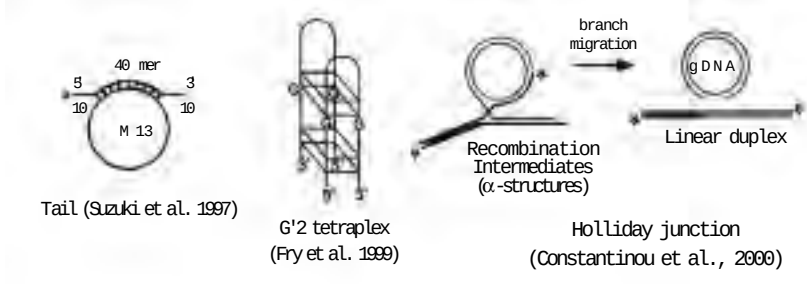



**Figure 3.** Preferred substrates of WRN helicase. Double-stranded DNA with tail (left), G4 structure (center) and alpha structure during recombination (right) are shown.

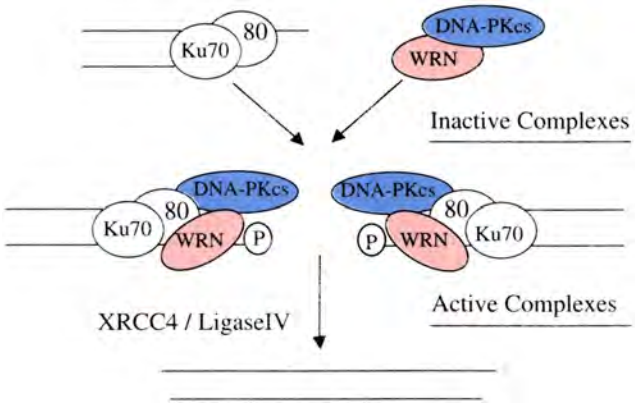



**Figure 4.** Potential role of WRN protein during non-homologous end joining. The NHEJ complex proposed by Yannone et al. (Yannone et al. 2001) is shown. See the text for details.

The illegitimate recombination process involves DNA-dependent protein kinase (DNA-PK) complexes that consist of Ku70/80 and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). Ku80 has been shown to directly interact with WRN protein and to modulate its exonuclease activity (Cooper et al. 2000, Li et al. 2000, Li et al. 2001). The association of WRN protein with Ku complex enhances its exonuclease activity, but does not alter WRN helicase activity. Upon binding with Ku, WRN protein is thus able to degrade DNA in the 3′→5′ direction from 3′ protruding and blunt ends as well as 3′ recessed ends (Li et al. 2001).

DNA-PKcs have also been shown to directly interact with WRN protein (Yannone et al. 2001). Association with DNA-PKcs inhibits both exonuclease and helicase activities of WRN protein. When WRN protein forms a stable complex on DNA with DNA-PKcs and Ku70/80, this assembly reverses WRN enzymatic inhibition (figure 4). WRN protein can potentially open up the double strand breaks (DSBs) for microhomology scanning and/or for trimming the DSB ends in preparation for the completion of repair by XRCC4 and ligase IV. The interaction of WRN protein might make the Ku-DNA end-joining complex more stable.

Our laboratory recently observed that when linear double-stranded DNA with non-compatible ends are introduced to primary WS fibroblasts, they undergo extensive deletion at the end-joining sites prior to re-ligation of the plasmids (Oshima et al. 2002). We hypothesize that WRN protein is involved in "fine-tuning" the processing of DNA recombination during NHEJ.

## The Hutchinson-Gilford Progeria Syndrome (HGPS)

### *Clinical Features of Hutchinson-Gilford Progeria Syndrome*

In contrast to WS, HGPS has its onset in early childhood—hence the use of the phrase "Progeria of childhood." HGPS patients typically exhibit growth retardation within the first three to six months of life along with accelerated degenerative changes of the cutaneous, musculoskeletal, and cardiovascular systems (DeBusk 1972, Brown 1979). Baldness and a characteristic "plucked-bird appearance" generally develop by age 2. The median age of death of HGPS patients is 13.5 years. Over 90% of the deaths are due to myocardial infarction or congestive cardiac failure. Ogihara et al. (1986) reported a clinically typical HGPS patient who died of myocardial infarction at age 45, an exceptionally long-lived HGPS patient. This raises the question of significant suppressor alleles and/or major environmental modulations.

The most biomedically significant feature of HGPS is the unusually early and rapidly progressive atherosclerosis (a specific type of arteriosclerosis). Serum cholesterol and triglyceride levels are reported to be normal (Szamosi et al. 1984). Unlike normal old individuals, Alzheimer-type dementia is not observed, nor are there such markers of CNS aging as large deposits of lipofuscins. Moreover, these children are intellectually intact.

There is little information on the prevalence of benign and malignant neoplasms in HGPS. The observation of a case of HGPS with death due to chondrosarcoma of the chest wall at age 13 years at least raises the question of underlying genomic instability (Oshima et al. 1996).

Prior to the identification of the responsible gene, the following genetic evidence supported an autosomal dominant mutation in HGPS:

1. Paternal age is relatively high in HGPS pedigrees (DeBusk 1972). In 7 U.S. cases, the mean paternal age was 37.1 (Jones et al. 1975). More recently, Brown (Brown 2003) reported that the average paternal age (31.3 years) was 4.5 years older that the average maternal age (26.7 years), which

was higher than the control value of 2.8 years. Approximately 20% of the fathers were about 20 years older than mothers. Advanced paternal age is often seen in autosomal dominant mutations.

2. The lack of evidence of an increase in the frequency of parental consanguinity argues against an autosomal recessive inheritance. In an argument against autosomal recessive inheritance, DeBusk (1972) reported that the parents were related only in 3 out of 19 then-reported cases. Brown reported no consanguinity in 50 cases he examined (Brown 2003).
3. A report of monozygotic twins concordant for HGPS with 14 normal sibs also is consistent with more recent data in autosomal dominant inheritance (Brown 1979).

Based on these findings, sporadic dominant mutations of the fertilizing sperm or ovum were suggested as causes of HGPS (Badame 1989). Rare instances of HGPS sibling cases (Maciel 1988, Khalifa 1989) have been considered to be due to germline mosaicism in the parents.

### *Cell Biological Studies of HGPS Cells*

Primary fibroblasts isolated from HGPS have diminished replicative capacities (Colige et al. 1991, Saito 1991), but the limitation may not be as consistently severe as is observed in the WS (Martin et al. 1970). The cumulative population doublings (CPD) of HGPS fibroblasts is limited to approximately fifteen, while CPDs of control fibroblasts are approximately forty. The replicative senescence of HGPS cells appears to be associated with an accelerated rate of loss of telomeres (Allsopp et al. 1992).

Several studies have shown altered growth-related gene expression in HGPS. Reduced insulin receptor gene expression has been shown in HGPS lymphoblastoid cell lines (LCLs) (Briata et al. 1991). Elevated c-myc, but not c-erbB or csrc, expression has been reported (Nakamura et al. 1988) although the significance is not known. Somatomedin-C binding to its receptor, or the cellular response to somatomedin-C in HGPS cells, was similar to control cells (Conover et al. 1985). Although there is resistance to growth hormone in HGPS patients, aberrations in the growth hormone cascade do not appear to be primarily responsible for HGPS. Genetic regulation of general metabolism in HGPS cells is similar to control cells. This includes the rate of decline of thyroid hormone induction of (Na + K) ATPase in cell membranes during replicative senescence (Guernsey et al. 1986) and replicative senescence-dependent declines of ornithine decarboxylase activity (Chen et al. 1986).

Markedly increased urinary hyaluronic acid (HA) (Tokunaga et al. 1978, Kieras et al. 1986) has been claimed to be characteristic to HGPS. A 17-fold mean increase of HA was reported in HGPS patients (Zebrower et al. 1986). These findings have recently been challenged, however (Gordon et al. 2003). Various components of the extracellular matrix are expressed differently in HGPS fibroblasts compared to controls. Elastin and type IV collagen were markedly increased in early passage HGPS fibroblasts; this is observed in senescent normal fibroblasts (Sephel et al. 1988, Giro et al. 1993); there is variation for these phenotypes among HGPS fibroblasts, however (Colige et al. 1991). The secretions of elastin and fibronectin were said to be increased (Maquart et al. 1988). Unlike control cell lines, stimulation of elastin production by serum or transforming growth factor (TGF)-beta in HGPS cells was only modestly increased, suggesting that elastin is near-maximally expressed. An increase in the expression levels of metalloproteinases, collagens and stromelysin, and a decrease in tissue inhibitor of metalloproteinase-1 (TIMP-1) gene expression while undergoing replicative senescence in HGPS fibroblasts was similar to that of senescent normal fibroblasts (Millis et al. 1992). A marked increase of glycoprotein gp200 has been noted (Clark et al. 1993). This may reflect the cutaneous and vascular alterations in HGPS patients. Another glyco-protein, decorin, was remarkably decreased at the transcriptional level in HGPS fibroblasts, but only in one of two patients (Beavan et al. 1993). We can probably conclude that the overall expression pattern of extracellular matrix components in HGPS fibroblasts is similar to senescent normal fibroblasts and is more likely to be a consequence rather than a cause of the HGPS phenotype.

### *Genomic Instability in HGPS Cells*

There is some evidence to suggest genomic instability in cells derived from HGPS patients

(Goldstein et al. 1985). Unscheduled DNA synthesis following UV irradiation in HGPS cells has been independently reported by at least three groups as being reduced (Stefanini et al. 1986, Lipman et al. 1989, Wang et al. 1990). The reduction of UV-induced, unscheduled DNA synthesis was 30-50% in HGPS cells as compared to control cells. This suggests defective nucleotide excision repair (NER) in HGPS. Lack of induction of NER prior to unscheduled DNA synthesis has been reported (Lipman et al. 1989).

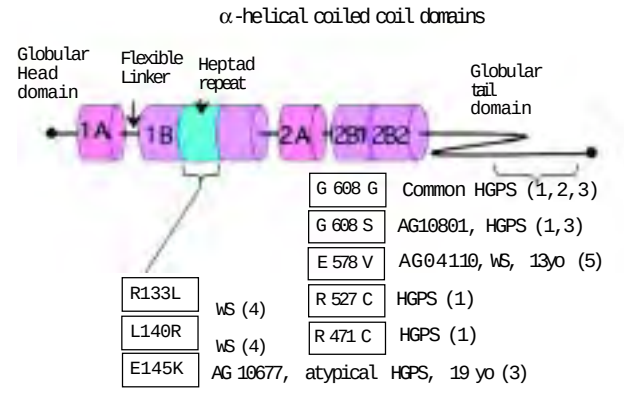
In contrast, the cell cycle-mediated regulation of two-base excision repair (BER) enzymes, uracil DNA glycosylase and hypoxanthine DNA glycosylase, appear to be normal (Cool et al. 1990) indicating that regulation of BER, at least, seems to be intact. The results of assays for the sensitivity to gamma rays and rejoining of double-strand DNA breaks of HGPS cells following gamma irradiation have been controversial (Little et al. 1975, Rainbow et al. 1977, Arlett et al. 1980). The specific activities of DNA polymerase alpha, beta, and gamma appear to be normal in HGPS as well (Bertazzoni et al. 1978). Cytogenetic studies of HGPS have not shown consistent abnormalities (Gahan et al. 1984). Sister chromatid exchange frequencies did not differ between HGPS and controls (Darlington et al. 1981). Transcriptional rates and transcriptional fidelity also appear to be normal (O'Brien et al. 1995).

It is clear that we require much more research in order to assess the significance of genomic instability in HGPS cells. A glaring deficiency is the lack of investigations of forward mutation rates in cultured somatic cells. Such studies have demonstrated 10- to 100-fold increases in mutation rates in WS cells (Fukuchi et al. 1989).

## The LMNA Gene Product and LMNA Mutations

### *Biology of Lamins*

The *LMNA* gene encodes type A and type C lamins (lamin A/C). These gene products result from alternative splicing (Fisher et al. 1986). Type A lamin (lamin A) is first translated as a 664 amino-acid prelamin A; the 18 amino acid C-terminal is then cleaved to generate mature lamin A. Lamin A and lamin B (encoded by *LMNB1* and *LMNB2*) are the major components of nuclear lamina, a 20~50nm mesh network which lines the inner nuclear membrane of the nuclear envelope (Stuurman et al. 1998). Like other intermediate filaments, nuclear lamins possess a central a-helical coiled-coil rod domain, flanked by globular N-terminal head and C-terminal tail domains (figure 5). Hydrophobic repeats within the central rod domain promote formation of the a-helical coiled-coil dimer and charged residues along the surface of the coiled-coil dimer promote interactions between rod dimers, thus producing a complex assembly of the filaments (Stuurman et al. 1998).




(1) Cao and Hegele 2003
(2) De Sandre-Giovannoli et al. 2003.
(3) Eriksson et al. 2003
(4) Chen et al. 2003. (5) Oshima unpublished.

**Figure 5.** Functional domains of lamin A and the sites of mutations identified in atypical WS and HGPS. The diagram shows the functional and structural domains of lamin A. Only mutations identified in atypical WS and HGPS are shown.

While lamin B is expressed in all cell types, lamin A/C is developmentally regulated. Mouse development can be divided into three phases: 1) germ layer formation, 2) organogenesis, and 3) tissue differentiation. Lamin A/C expression starts during the third phase in the trunk, head and appendages (Rober et al. 1989). Lamin A/C expression does not appear until well after birth, when it appears in the simple epithelia of lung, liver, kidney and intestine. In general, lamin A/C is expressed in differentiated cell types. In humans, lamin A is most prominent in well-differentiated epithelial cells. Relatively undifferentiated cells and proliferating epithelial cells show reduced expressions of lamin A (Broers et al. 1997).

### *Potential Role of Lamins in Gene Regulation*

In addition to its structural role, the nuclear envelope might be involved in the global pattern of gene expression. There is considerable circumstantial evidence to support this hypothesis. Some

of the lamina-associated proteins have a so-called "LEM domain" which interacts with BAF (barrier to autointegration factor), an abundant chromatin-associated protein (Shumaker et al. 2001). Both human and mouse studies indicate that the lamina exerts a profound influence on the organization of heterochromatin in the nucleus (Fidzianska et al. 1998, Sullivan et al. 1999). As heterochromatin is generally transcriptionally silent, changes in heterochromatin structure could lead to changes in the gene expression patterns with potentially deleterious effects. As a result, lamins may have a direct effect on gene expression.

Transcriptional regulators such as the retinoblastoma gene product, a tumour suppressor, have been shown to interact with lamin A *in vitro*. Lamin A also interacts with sterol response element binding protein, a transcriptional factor that is involved in adipocyte differentiation (Lloyd et al. 2002).

### *LMNA Mutations in a typical WS and HGPS*

The International Registry of Werner Syndrome has 127 WS index cases and 72 family members from many parts of the world. WS referrals are by primary care physicians or specialists who suspect a WS diagnosis. Among 127 index cases, 29 cases were found to not have mutations at the *WRN* locus. In collaboration with Dr. Abhimanyu Garg at the Southwestern Medical Center, University of Texas at Dallas, we sequenced all 12 exons of *LMNA* with previously published primers (Brown et al. 2001), and identified three new mutations in the *LMNA* gene (Chen et al. 2003).

A base substitution in exon 2, which alters amino acid 133, arginine (CGG) to leucine (CTG), was seen in two pedigrees (ATLAN and PORTU). In the PORTU pedigree, family samples were available and we did not find this mutation in any of the family members, indicating that R133L mutation is a *de novo* mutation. In the ATLAN pedigree, the father of the patient was clinically diagnosed with WS and died at age 49 as a result of insulin-resistant diabetes mellitus with progressive neuropathy, angiopathy and retinopathy, suggesting that the R133L mutation was inherited. An unaffected brother did not have this mutation. A similar mutation, R133P, has been reported in one case of Emery-Dreifuss muscular dystrophy, though a detailed clinical description of this case is not available (Brown et al. 2001). Another mutation, a leucine (CTG) to arginine (CGG) substitution at amino acid 140, was seen in the NORWAY pedigree. All three mutations were heterozygous mutations (figure 6).

Unlike classical WS, the symptomatic onset of atypical WS due to *LMNA* mutation is in the early teens. In the PORTU index case, poor growth was first noted at age 4. The cases of interest were initially referred to us because of a general "prematurely aged" appearance, short stature, and other features suggesting WS. Cardiac symptoms, osteoporosis, lipodystrophy and muscular atrophy appear to have been common features. Progression of these age-related disorders may be more accelerated than in classical WS cases.

We also found a third new mutation, which alters alanine (GCA) to proline (CCA) at amino acid 57 in Registry IRAN1010. This patient presented with features of the mandibuloacral dysplasia, including sloping shoulders, small jaw and relatively short fingers. The A57P mutation resides at the N-terminal region of an a-helical domain. Since the structure of the proline residue is incompatible with

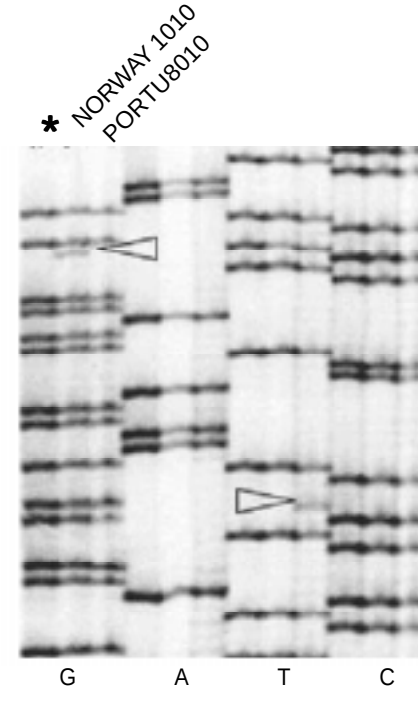



**Figure 6** LMNA mutations identified in atypical WS. Sequencing analysis shows a heterozygous Arg (CGG)→eu (CTG) substitution at amino acid 133 and a Leu (CTG)→Arg (CGG) substitution at amino acid 140.

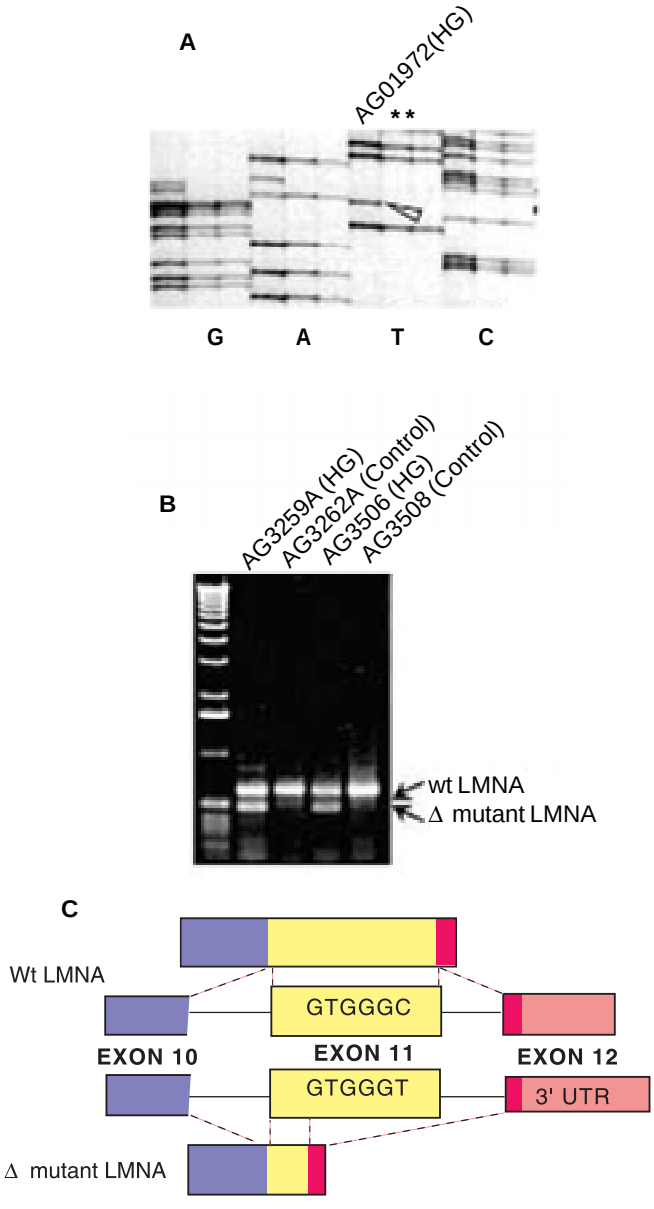



**Figure 7.** LMNA mutation identified in HGPS. (A). Sequencing analysis showing C→T mutation in exon 12. Due to the compression, C in the wildtype allele was seen only faintly. (B). RT-PCR products showing smaller products only in HGPS patients. (C). Diagram of cryptic splicing sites generated by the G→T mutation.

the a-helix, the Ala to Pro substitution is expected to cause misfolding of lamin A/C.

Sequencing of genomic PCR products from the HGPS pedigrees showed a heterozygous mutation at G608G (GGC→GGT) in *LMNA* exon 12 in the patients but not in the normal family members or unrelated controls (figure 7A). This is consistent with the three independent reports describing *LMNA* mutations in HGPS. All but one resides in the C-terminal region of the protein (figure 5) (Cao et al. 2003, De Sandre-Giovannoli et al. 2003, Eriksson et al. 2003).

RT-PCR spanning exons 10 though 12 showed the expected bands in all samples and an additional smaller band only in HGPS patients (figure 7B). Sequencing of the smaller RT-PCR products revealed that the G608G (GGC→GGT) mutation generated a cryptic splicing site which results in an in-frame deletion of amino acid 607 through 656 (D mutation)(figure 7C). This deletion eliminates the proteolytic site that converts prelamin A to mature lamin A. Sequencing showed that the sequence of the third position of amino acid 608 was C and T, indicating that the mutation created an incomplete cryptic splicing site.

***Other Laminopathies Caused by LMNA Mutations***

*LMNA* mutations are responsible for at least six disorders: Emery-Dreifuss muscular dystrophy (Bonne et al. 1999, Raffaele Di Barletta et al. 2000, Brown et al. 2001), dilated cardiomyopathy type 1A (Fatkin et al. 1999, Brodsky et al. 2000), limb-girdle muscular dystrophy type 1B (Muchir et al. 2000), familial partial lipodystrophy (Cao et al. 2000, Muchir et al. 2000, Speckman et al. 2000, Garg et al. 2002), Charcot-Marie-Tooth disease type 2 (Speckman et al. 2000), mandibuloacral dysplasia (Novelli et al. 2002) and HGPS (De Sandre-Giovannoli et al. 2002, Cao et al. 2003, De Sandre-Giovannoli et al. 2003, Eriksson et al. 2003, Genschel et al. 2000, Burke et al. 2002). As noted above, we have identified heterozygous *LMNA* mutations in a subset of "atypical" Werner syndrome cases. These cases had some clinical features of Werner syndrome but no detectable mutations in the *WRN* helicase gene (Oshima, in press).

Most of the mutations found in autosomal Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy, and dilated cardiomyopathy are heterozygous missense mutations and have been extensively mapped throughout the *LMNA* exons (Burke et al. 2002). *LMNA* mutations in limb-girdle muscular dystrophy with atrio-ventricular conduction disturbance are also heterozygous missense mutations. Many of these are predicted to cause failure of lamina assembly. A homozygous mutation found in autosomal recessive Charcot-Marie-Tooth disease type 2 (Arg to Cys substitution at amino acid 298) resides at a rod domain, which likely perturbs

lateral interaction of lamin A (De Sandre-Giovannoli et al. 2002). A homozygous mutation had been previously been identified in Italian mandibuloacral dysplasia patients (Arg to His substitution at amino acid 527) (Novelli et al. 2002).

### *Postulated Disease Mechanism of LMNA Mutations*

Disease mutations of laminopathies are predicted to cause misfolding of lamin A/C or a failure to dimerize and assemble the nuclear lamina, resulting in nuclear structural abnormalities and nuclear fragility (Burke et al. 2002). The major fraction of lamin A is localized along the inner surface of the nuclear envelope and co-localizes with lamin B. Some of the lamin A mutants show a dramatically abnormal intranuclear localization of lamins. In one study, four out of fifteen *LMNA* mutants (N195K, E358K, M371K and R386K) overexpressed in human cells were observed to have decreased nuclear rim staining in conjunction with the formation of intranuclear foci (Ostlund et al. 2001). An independent study also demonstrated that three out of four *LMNA* mutant proteins (L85R, N195K and L530) overexpressed in Hela cells form nuclear aggregates and cause the relocation of endogenous wildtype lamin (Raharjo et al. 2001). Abnormal localization of mutant lamins was also shown to be associated with the loss of nuclear envelope-associated emerin. The authors of both studies noted that *LMNA* mutations associated with Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy cause abnormal lamin localization and loss of emerin. Primary fibroblasts isolated from an individual with mandibuloacral dysplasia showed a honeycomb pattern of lamin A distribution and lobulation of nuclei (Novelli et al. 2002). By contrast, lamin A/C carrying mutations associated with familial partial lipodystrophy behave in a manner indistinguishable from that of wildtype lamin (Ostlund et al. 2001, Raharjo et al. 2001).

We can tentatively conclude that there are at least three non-exclusive models for the pathogenesis of laminopathies:

1. Increased nuclear fragility: general alterations in nuclear structure resulting from *LMNA* mutation leading to cell instability and ultimately tissue atrophy (Burke et al. 2002).
2. Altered chromatin silencing: many lamina associated proteins bind to BAF, an abundant chromatin associated protein. Though the function of BAF is not well-understood, abnormal lamin protein could alter global transcriptional regulation (Fidzianska et al. 1998, Sullivan et al. 1999, Shumaker et al. 2001).
3. Mislocalization of nuclear proteins. Lamins A and C act to regulate a number of critical nuclear components whose activities may be important for the cell growth (Markiewicz et al. 2002).

## Future Directions of Research on Progeroid Syndromes

WS and HGPS share at least one probable common denominator—genomic instability. Genomic instability of WS is the result of the absence of nuclear enzymes that participate in DNA repair processes, while that of HGPS is secondary to defective nuclear structure. Other segmental progeroid syndromes are caused by mutations in genes involved in DNA repair . For example, the Cockayne syndrome, whose manifestation includes skeletal and neurological abnormalities, is caused by a mutation in *CSB* that encodes a DNA helicase involved in transcription-coupled DNA repair. Ataxia telangiectasia, a disorder causing skin atrophy and neurodegeneration, is caused by mutations in *ATM*, which encodes a protein kinase involved in DNA damage signaling. These observations collectively suggest that the inability to maintain genomic stability could lead to progeroid phenotypes in humans. The phenotypic variability of these syndromes may reflect the differences in the regulation and specific functions of the affected genes. The fact that *WRN* mutations and *LMNA* mutations can give rise to very similar phenotypes suggests that there are common downstream event. A task for the future is to carefully delineate these downstream events—for example, via microarray analysis of gene expression and proteomics. Ideally, this research will require robust animal models. While such models may exist for the case of HGPS (Mounkes et al. 2003), attempts to reproduce WS in transgenic mice have been disappointing (Lebel et al. 1998, Lombard et al. 2000, Wang et al. 2000).

The accumulation of somatic DNA damage is a potential unifying theory of aging (Martin et al. 1996). Currently, the leading causative process is oxidative damage. According to this theory, natural by-products of aerobic energy production, such as reactive oxygen species (ROS), cause damage to biological macromolecules, including both nuclear and mitochondrial DNA (Martin et al. 1996). DNA damage caused by ROS induces base modifications, single-strand breaks, or double-strand breaks. Base modifications can be repaired by base excision repair and nucleotide excision repair.Transcriptional-coupled repair may be particularly important. Double-strand DNA breaks can be repaired either by homologous recombination or non-homologous end joining. Despite the observation that many disease genes responsible for the progeroid syndromes participate in DNA repair systems, which in itself is surprising, a direct connection has not been established between ROS-induced DNA damage and each of the progeroid syndrome genes.

Both *WRN* and HGPS mutations may also impact upon gene expression. Direct evidence that this may be the case for WS cells has been published (Balajee et al. 1999). For the case of HGPS, there are strong theoretical rationales for how alterations in the regulation of gene expression may occur with lamin A mutations, as discussed earlier in the essay (e.g., altered chromatin silencing, mislocalization of nuclear proteins).

Which of these two broad aberrations in gene action is most significant for our understanding of the pathogenesis of WS and HGPS? Which is most significant for our understanding of normative aging? This is a core challenge for future research on the pathobiology of aging.

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