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Review

# Genetic alterations in accelerated ageing syndromes Do they play a role in natural ageing?

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**Abstract**

The molecular mechanisms leading to human senescence are still not known mostly because of the complexity of the process. Different research approaches are used to study ageing including studies of monogenic segmental progeroid syndromes. None of the known progerias represents true precocious ageing. Some of them, including Werner (WS), Bloom (BS), and Rothmund–Thomson syndromes (RTS) as well as combined xeroderma pigmentosa–Cockayne syndrome (XP–CS) are characterised by features resembling precocious ageing and the increased risk of malignant disease. Such phenotypes result from the mutations of the genes encoding proteins involved in the maintenance of genomic integrity, in most cases DNA helicases. Defective functioning of these proteins affects DNA repair, recombination, replication and transcription. Other segmental progeroid syndromes, such as Hutchinson–Gilford progeria (HGPS) and Cockayne syndrome are not associated with an increased risk of cancer. In this paper we present the clinical and molecular features of selected progeroid syndromes and describe the potential implications of these data for studies of ageing and cancer development.


*Keywords:* Ageing; Progeroid syndromes; Helicase; Lamin A; Mutation; Genomic integrity

**Contents**



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## 1. Introduction

Little is known about the pathophysiology of human senescence. It is thought to be a complex process involving genetic and environmental factors, affecting several physiological pathways. Identification of the genes for age-related disorders, such as circulatory system disorders, diabetes mellitus, cancer, dementia, osteoporosis, etc., is difficult because most of these disorders are polygenic and their occurrence is strongly related to modifying environmental factors. The other approach to the study of ageing is to identify the genes responsible for age-related monogenic hereditary disorders – so-called progeroid syndromes. In 1978, Martin (1978) defined 21 criteria of human ageing: 3 phenotypic alterations in cells and 18 phenotypic alterations in tissue or the total organism. He selected 83 autosomal dominant, 70 autosomal recessive, 9 sex-linked hereditary disorders, and 3 chromosomal syndromes as 'segmental progeroid syndromes'. To date, no progeroid syndrome is known that exactly resembles 'physiological' ageing, therefore the name 'segmental syndromes' is fully justified. Not all of them shorten patients' lifespan. Most features of ordinary ageing are present in Werner syndrome (WS) and Hutchinson–Gilford progeria (HGPS) patients. Most extensively studied are the progeroid syndromes caused by defective helicase proteins, including Werner (WS), Cockayne (CS), Rothmund–Thomson (RTS), and Bloom (BS) syndromes, and xeroderma pigmentosa (XP) and trichothiodystrophy (TTD). In contrast, the gene which mutation is responsible for Hutchinson–Gilford progeria, *LMNA*, has just recently been associated with this disease, and therefore little is known about the molecular mechanisms leading to this progeria phenotype. In other cases, e.g. Wiedemann–Rautenstrauch syndrome, the responsible gene is still not known. In this review we describe the clinical and molecular features of the best known and most well studied progeroid syndromes, with special emphasis on the function of the mutated syndrome-responsible proteins, and potential implications for the development of malignancy (Tables 1 and 2).

## 2. Hutchinson–Gilford progeria

It is estimated that this autosomal dominant disease occurs in 1:4–8 million births. Only approximately 100 cases have been described in medical journals. The first description of this disease was given by Hutchinson and independently by Gilford, in 1886. At birth, affected infants appear normal (only a few cases of intrauterine disease presentation are known (Rodriguez, Perez-Alonso, Funes, & Perez-Rodriguez, 1999)). Typical manifestations develop gradually beginning from sixth to twelfth month of life, and are evident by the first or second year of life. There is little phenotypic variation: all affected children are short, have a similar facial appearance with midface hypoplasia, micrognathia, prominent eyes, protruding ears with the absence of earlobes, delayed closure of fontanelles and sutures, alopecia, prominent scalp veins, atrophic skin, delayed dentition, and decreased subcutaneous fat, thin limbs with prominent stiff joints, coxa valga, generalised osteodysplasia with osteolysis and pathologic fractures, dystrophic nails and high-pitched voice. Metabolic, endocrine, serum lipid and immunologic examinations show no uniform abnormalities. There are no signs of precocious brain ageing. HGPS children's intelligence and emotional development are normal. The median age at death is 13.4 years, and death

Table 1
Selected progeroid syndromes – the summary of the genetic background

| Syndrome | Gene | Protein | Function |
|---|---|---|---|
| Hutchinson–Gilford | *LMNA* | Lamin A | Nucleus structure, mechanotransduction |
| Werner | *WRN* | WRN DNA helicase | DNA repair, recombination |
| Bloom | *BLM* | BLM DNA helicase | DNA repair, recombination |
| Rothmund–Thomson | *RECQL4* | RECQL4 DNA helicase | Unknown, possibly as other helicases |
| Cockayne | *CSA*, *CSB*, *XPB*, *XPD*, *XPG* | CSA WD-repeat protein, CSB DNA helicase, XPB DNA helicase, XPD DNA helicase, XPG exonuclease | Transcription-coupled DNA repair, transcription |

is usually the result of myocardial infarction or stroke. Remarkably, no increase in cancer frequency is associated with this syndrome.

The first clues regarding the location of the gene responsible for HGPS on chromosome 1q arose from the observation of a case with 1q23 6 Mb deletion and two other cases of 1q20–24 inverted insertion in twins with progeria. In 2003 two reports describing mutations within the lamin A/C gene (*LMNA*) in HGPS patients were published simultaneously. Eriksson et

Table 2
Selected progeroid syndromes – the summary of the clinical signs

| Syndrome | Age at presentation | Clinical signs | Length of life, typical cause of death |
|---|---|---|---|
| Hutchinson–Gilford | 6–12 months | Short stature, midface hypoplasia, micrognathia, prominent eyes, alopecia, prominent scalp veins, atrophic skin, decreased subcutaneous fat, osteoporosis with pathologic fractures, cardiovascular disease | 10–20 years, myocardial infarction, stroke, no malignant disease |
| Werner | Puberty | Short stature, alopecia, atrophic skin, fat deposits on the trunk, trophic ulcerations of the legs, diabetes mellitus type II, osteoporosis, juvenile bilateral cataract, hypogonadism, atherosclerosis, neoplastic disease | 40–50 years, myocardial infarction, cancer |
| Bloom | At birth | Short stature, narrow face, small mandible, prominent nose and big ears, sensitivity to sunlight, teleangiectatic skin lesions, hyper- and hypopigmentation, variable degree of immunodeficiency, diabetes mellitus, defective fertility, neoplastic disease | Almost normal lifespan, cancer |
| Rothmund–Thomson | 3–6 months | Short stature, photosensitivity, polikiloderma, hyperkeratosis, alopecia, cataract, reduced fertility, neoplastic disease (usually sarcoma) | Normal lifespan, cancer |
| Cockayne | CSA: 1–3 years, CSB: at birth, XP–CS: first year | Severe growth retardation with lack of subcutaneous fat (so-called cachectic dwarfism), atrophic skin, sparse hair, progressive neurodevelopmental abnormalities with microcephaly, diffuse dysmyelination and calcium deposits in the cortex and basal ganglia, progressive retinal atrophy and cataracts, sensorineural hearing loss, acute sun sensitivity. XP–CS – as above, and neoplastic disease | CSA: 20–40 years, CSB: 6–7 years, central nervous system deterioration, infections, no malignant disease, XP–CS: as CS, central nervous system deterioration, cancer |

al. (2003) revealed that 18 out of 20 classic HGPS cases harboured an identical mutation, a GGC > GGT single-base substitution at position 1824 within exon 11. Such substitution located to codon 608 did not alter the amino acid sequence of the encoded protein. Identical mutation has been found by DSG et al. (2003) in two patients who had a heterozygous C to T transition at nucleotide 1824. This change was not found in 300 control samples from healthy subjects or in the parents of the affected children. Further analysis revealed that an additional cryptic donor splicing site has been introduced by this mutation in exon 11 at position 1819–1820. As a result, lamin A encoded by the mutated gene lacks 50 amino acids near the carboxy terminus, while lamin C also encoded by this gene is normal. de Sandre-Giovannoli et al. also showed that both normal and truncated transcripts were produced by the same allele (T at position 1824). The mutation would then inhibit transcriptional processing of the normal allele, acting as a dominant negative mutation. It is important to note that other *LMNA* mutations in HGPS patients have been described, namely R471C, R527C, G608S and 2036C>T (Cao & Hegele, 2003).

Lamin A/C proteins encoded by the *LMNA* gene are ubiquitous components of the nuclear lamina, a structure near the inner nuclear membrane and the peripheral chromatin. The nuclear lamina is involved in many processes that occur inside the nucleus, including chromatin organisation, cell cycle and apoptosis regulation, DNA and RNA processing, etc. Microscopic analysis of HGPS fibroblasts probed with antibodies directed against lamin A revealed abnormalities of the nuclear membrane. Similarly, HGPS lymphocytes exhibit nuclear size and shape alterations with envelope interruptions and chromatin extrusions. It is important to note that a wide spectrum of human disorders – Emery–Dreifuss and limb girdle muscular dystrophies, dilated cardiomyopathy with conduction disease, autosomal recessive axonal neuropathy, mandibuloacral dysplasia, familial partial lipodystrophy, Greenberg skeletal dysplasia, Pelger–Huet anomaly, and finally Hutchinson–Gilford progeria – are all ascribed to *LMNA* mutations. It is not known why the abnormalities within this gene result in diseases with such diverse phenotypes. This is partly because the function of lamins is still not completely determined. Muscle manifestation of some *LMNA*-mutated phenotypes suggests that the mechanical sensitivity of the cell could be altered. Indeed, the experiments with lamin A/C-deficient mouse embryo fibroblasts subjected to mechanical strain showed defective nuclear mechanics and impaired mechanically activated gene transcription (Lammerding et al., 2004; Worman & Courvalin, 2004). Other experiments show that cellular ageing of Hutchinson–Gilford progeria syndrome fibroblasts is characterised by a period of hyperproliferation and terminates with a large increase in the rate of apoptosis (Bridger & Kill, 2004), and that fibroblast clones derived from HGPS donors frequently fail to immortalise with telomerase despite the restoration of telomerase activity and the stabilisation of telomere length (Wallis et al., 2004). Clearly, the cellular and molecular mechanisms that play a role in the pathophysiology of Hutchinson–Gilford progeria still await their explanation.

## 3. Werner syndrome

The first written description of the clinical symptoms of the syndrome was delivered by Werner in 1904. It is estimated that, worldwide, there are currently approximately 1300 people suffering from this disease, which makes Werner syndrome the most common known progeroid syndrome. The majority of patients are Japanese. The reason for this concentration within one ethnic group is not known. Most probably it is a result of inbreeding, although other reasons are also possible. WS is an autosomal recessive progeroid syndrome most closely resembling 'physiological' ageing. Still, the typical WS phenotype consists not only of typical ageing signs but also of other features not commonly present in normal ageing (such as skin ulcers around the ankles and elbows, atherosclerotic changes mostly in arterioles, osteoporosis affecting mostly long bones of the lower extremities, and calcification of cardiac valves). WS patients develop normally until they reach puberty. The first sign of the disease is absence of the pubertal growth spurt, which results in short stature in the affected adult. By the third decade of life, premature greying, loss of hair and skin atrophy become apparent. Subcutaneous fat is deposited mostly on the trunk. In contrast, extremities look thin. Trophic ulceration of the legs, diabetes mellitus type II, osteoporosis, juvenile bilateral cataract, hypogonadism and atherosclerosis develop. Werner syn-

drome is a cancer-prone disease. Compared with the general population, an excess of soft tissue sarcomas, osteosarcomas, myeloid disorders and benign meningiomas have been observed in WS patients. In Japanese subjects an excess of thyroid cancers and melanomas was also observed. The ratio between cancers of epithelial origin and sarcomas of mesenchymal origin in WS patients is 1:1, while in the general population it is 10:1. In addition, the primary sites of the cancers are also not typical in WS patients. For example, melanomas frequently develop in regions not exposed to the sun, and osteosarcomas frequently localise to the lower extremities, whereas in the general population they localise to upper extremities (Goto, Miller, Ishikawa, & Sugano, 1996; Ishikawa, Miller, Machinami, Sugano, & Goto, 2000). There is a suggestion that the site of mutation in the *WRN* gene might be linked to the type of cancer: papillary thyroid carcinomas seem to be more common in patients with mutation in the N-terminal end of the WRN protein, while follicular carcinomas tend to develop in WS patients bearing mutations within the C-terminal end of this protein (Ishikawa et al., 1999). WS patients typically die in the fifth decade of life (at an average age of 47 years), owing to cardiovascular disease or cancer.

The genetic hallmark of WS is genomic instability. The defect responsible for this syndrome was tracked down in 1996, when *WRN* (*RECQL2*) gene was cloned and its mutations in WS patients found (Yu et al., 1996). The gene is localised on 8p11–12 locus and encodes a 1432 amino acid protein (WRN) homologous to RecQ subfamily helicases (for a review of RecQ helicases in cancer and ageing see Bachrati & Hickson, 2003; Furuichi, 2001; Karow, Wu, & Hickson, 2000; Mohaghegh & Hickson, 2002). Indeed, WRN functions as a $3' \rightarrow 5'$ DNA helicase (Gray et al., 1997), but additionally acts as a $3' \rightarrow 5'$ exonuclease (Huang et al., 1998). The presence of exonuclease domain makes WRN protein different from other RecQ helicases. Helicase and exonuclease domains do not overlap. Approximately 40 different mutations have been described so far in the *WRN* gene. Mutations are either homozygous or compound. They are frameshift mutations, nonsense mutations or large genomic deletions, and result in the production of truncated WRN protein lacking a nuclear localisation signal. Therefore, truncated WRN fails to translocate into the nucleus, and cannot perform its physiological function (Matsumoto, Shimamoto, Goto, & Furuichi, 1997). This uniformity of molecular events possibly accounts for the low phenotypic variation observed in WS patients. It is important to note that missense changes in the *WRN* gene are also known, and are typically considered polymorphic changes. It is possible, though, that mutations of this type might also result in WS phenotype.

Wild type WRN protein binds to the single-stranded part of the longer strand of DNA duplex and moves in a $3' \rightarrow 5'$ direction. It unwinds DNA-DNA and DNA-RNA duplexes, DNA triple helix, G2 tetraplexes, and G4 tetraplexes made by two hairpin loops of d(CGG)n (interestingly, the sequences that can form G4 structures are present in telomeres, among others), and promotes branch migration of a Holliday junction. With regard to its exonuclease activity, double-stranded DNA with multiple base-pair mismatches and structures similar to Holliday junctions are more susceptible to digestion by WRN than DNA without mismatches (for a review of WRN see Chen & Oshima, 2002).

WRN interacts with several protein components of the DNA replication complex, such as proliferating cell nuclear antigen (PCNA) and topoisomerase I (Lebel, Spillare, Harris, & Leder, 1999). It also interacts with DNA polymerase δ, so one of its functions might be the recruitment of this polymerase to the complex secondary structures of DNA and alleviation of stalled DNA synthesis (Kamath-Loeb, Loeb, Johansson, Burgers, & Fry, 2001). A direct interaction between WRN and Bloom syndrome helicase (BLM) has been shown. Both proteins colocalised to nuclear foci, and BLM inhibited the exonuclease activity of WRN (von Kobbe et al., 2002). The absence of both WRN and BLM proteins synergistically increased hypersensitivity of the cell to genotoxic agents and UV light, compared with cells with deletions of either WRN or BLM proteins alone. This suggests that the two proteins may both be involved in DNA repair in a complementary fashion (Inamura et al., 2002). In WS cells decreased telomeric DNA repair efficiency was observed (Kruk, Rampino, & Bohr, 1995). WRN protein has been found on telomeres (Shiratori et al., 1999), where it is recruited by TRF2, a telomeric repeat binding factor essential for correct telomeric structure (Machwe, Xiao, & Orren, 2004). WRN was shown to unwind up to 23 kb of a PCR-generated telomere repeat sequence (Ohsugi et al., 2000). Telomeres found in lymphoblastoid cell lines originating from WS patients are unstable and

of varying length. In WS fibroblasts telomeres shorten quickly, and cells stop dividing while telomeres are still quite long. Overexpression of telomerase in these cells decreases the rate of their senescence (Wyllie et al., 2000). These findings suggest that WRN protein plays an important role in telomere maintenance. WRN has been shown to suppress increased homologous and illegitimate recombination (Yamagata et al., 1998).

Although global repair ability of WS cells is normal (Fujiwara, Higashikawa, & Tatsumi, 1977), the absence of the above described functions of WRN results in defective replication, inefficient transcription-coupled DNA repair, deficient mismatch repair, and chromosome rearrangements such as deletions and multiple translocations, as well as increased spontaneous mutation and deletions generated by non-homologous hyper-recombination (Fukuchi, Martin, & Monnat, 1989; Fukuchi et al., 1985). Altogether, these alterations result in major genomic instability and cancer predisposition (Bachrati & Hickson, 2003; Furuichi, 2001; Mohaghegh & Hickson, 2002; Moser et al., 2000). Interestingly, a direct interaction between WRN and p53 has been shown. In the absence of WRN, p53-directed apoptosis was attenuated (Spillare et al., 1999), while overexpression of WRN potentiated p53-dependent apoptosis (Blander et al., 1999). *p53*-null, *WRN*-defective double mutant transgenic mice developed a variety of tumours not detected in either type of single mutant (Lebel, Cardiff, & Leder, 2001). The overexpression of MYC oncoprotein in WS fibroblasts or after WRN depletion led to cellular senescence. So direct up-regulation of wild type *WRN* by MYC in normal cells may promote MYC-driven tumourigenesis by preventing cellular senescence (Grandori et al., 2003). Physiologically, such interactions may be of great importance for the prevention of the accumulation of genetic aberrations that, if not removed (such as in Werner syndrome), can lead to premature senescence or neoplastic transformation.

## 4. Bloom syndrome

The first description of the syndrome, by Bloom, was published in 1954. BS is an autosomal recessive disorder that may occur in different ethnic groups but is most common in Ashkenazi Jews (descendants of Eastern European Jews), owing to intensive inbreeding. It is estimated that 1 in 100 Ashkenazi Jews is a carrier of the defective gene. Clinically, the syndrome is characterised by growth deficiency of prenatal onset and other features that develop later, such as sensitivity to sunlight, teleangiectatic skin lesions, hyper- and hypopigmentation, variable degree of immunodeficiency (decreased levels of immunoglobulin A and M with recurrent respiratory and gastrointestinal tract infections), diabetes mellitus and defective fertility (men are infertile, women have reduced fertility). Sufferers have a narrow, birdlike face, small mandible, prominent nose and big ears. Despite patients' short stature, the extremities (especially upper ones) are disproportionately long with large hands and feet. Compared with the general population BS patients are at 150–300-fold increased risk of a wide variety of malignancies, including blood cell-derived and epithelial cancers. The cancers arise unusually early, with leukaemia developing at an average age of 22 years and solid tumours in the fourth decade of life. Interestingly, a clinical difference between leukaemia patients with and without BS is that leukaemia in BS usually presents itself with leukopenia rather than leukocytosis.

The genetic hallmark of Bloom syndrome is an inability to suppress hyper-recombination. The frequency of homologous recombination events is increased 10-fold in BS cells in comparison to normal cells. The gene responsible for this condition was cloned in 1995 (Ellis et al., 1995). *BLM* is located on chromosome 15 (locus 15q26.1) and encodes 1417 amino acid BLM (RECQL3) protein homologous to RecQ helicases. BLM is a $3' \rightarrow 5'$ DNA helicase that, in addition to unwinding duplex DNA, is able to unwind G4 tetraplexes, triple helix, Holliday junctions, bubbles and forked DNA. BLM is necessary for normal double stranded break repair (Langland et al., 2002).

The alterations affecting *BLM* gene are missense, nonsense or frameshift mutations, and large genomic deletions. All but missense mutations result in premature translation termination and production of the truncated BLM protein. Like truncated WRN, shortened BLM protein also fails to translocate to the nucleus since the nuclear localisation signal normally present in its C-terminus is deleted. Although BLM protein bearing a missense mutation is present in the nucleus, its function is abolished due to the lack of ATPase and/or DNA helicase activity. The absence of physiological function of BLM results in a markedly elevated rate of

mutations, and a high frequency of chromosomal aberrations and sister chromatid exchange (SCE), owing to excessive recombination. Expression of wild type BLM in BS cells can decrease their high SCEs to normal levels.

BLM physically interacts with topoisomerase IIIα (Johnson et al., 2000). Expression of a BLM deletion mutant defective in topoisomerase IIIα binding results in intermediate SCE levels. This suggests the involvement of both proteins in the regulation of recombination in somatic cells (Hu et al., 2001). It is proposed that interaction of RecQ helicase with topoisomerase IIIα might be a well conserved mechanism that disrupts recombination intermediates between erroneously paired nucleic acid molecules (Harmon, DiGate, & Kowalczykowski, 1999). Indeed, it has been shown that BLM and topoisomerase IIIα catalyse the resolution of a recombination intermediate containing a double Holliday junction. This mechanism prevents exchange of flanking sequences (Wu & Hickson, 2003).

BLM interacts with single-stranded DNA-binding protein – replication protein A. Such binding stimulates BLM protein helicase activity (Brosh et al., 2000). BLM also interacts with RAD51, the protein accumulating in nuclear foci that are thought to correspond to sites of recombinational repair (Wu, Davies, Levitt, & Hickson, 2001). This and the ability of BLM to unwind G4 structures suggest the involvement of this protein in DNA replication and repair. BLM also interacts with 5′-flap endonuclease/5′–3′ exonuclease (FEN-1), a genome stability factor involved in Okazaki fragments processing and DNA repair. BLM protein stimulates both the endonucleolytic and exonucleolytic cleavage activity of FEN-1 (Sharma et al., 2004).

BLM interacts with so-called BRCA1-associated genome surveillance complexes (BASCs) which contain BRCA1, among others. Many BASC-forming proteins play a role in the recognition of damaged DNA or unusual DNA structures (Wang et al., 2000). This and the ability of BLM to unwind difficult DNA structures further support the involvement of this protein in DNA repair. Thus, the absence of normal function of BLM in BS cells leads to increased recombination and defective DNA replication and repair. An important alteration observed in BS cells is decreased DNA ligase I activity (Runger & Kraemer, 1989), which can further contribute to the defective processing of DNA in these cells.

p53-mediated apoptosis is defective in BS fibroblasts and can be rescued by expression of the normal *BLM* gene (Wang et al., 2001). Further inactivation of p53 prevented the death of damaged BS cells and delayed recruitment of BRCA1 to nuclear foci (Davalos & Campisi, 2003). Expression of BLM in p53 wild type cells causes an anti-proliferative effect that is not present in p53-deficient cells. p53-mediated transactivation is attenuated in BS fibroblasts (Garkavtsev, Kley, Grigorian, & Gudkov, 2001). After hydroxyurea treatment, p53 and BLM co-localised with each other and with RAD51 at sites of stalled DNA replication forks. The absence of p53 enhanced the rate of spontaneous sister chromatid exchange in BS cells. These results indicate that p53 and BLM functionally interact during resolution of stalled DNA replication forks (Sengupta et al., 2003). Interactions between p53 and BLM protein suggest that Bloom syndrome cancer-prone phenotype may in part be a result of the deregulation of the p53 tumour suppressor pathway.

Interestingly, it has been shown that human BLM can prevent premature ageing in yeast (Heo et al., 1999). However, the role of this protein in ordinary human ageing remains to be elucidated.

## 5. Rothmund–Thomson syndrome

Rothmund first described this syndrome in 1868. Only approximately 300 cases have been described since then in English language scientific journals. Some sufferers are born with skeletal abnormalities such as underdeveloped or absent thumbs and/or forearm bones. Prominent forehead, prognathism and saddle nose make patients' faces characteristic. Erythematous plaques accompanied by oedema appear on the cheeks at around age 3–6 months. Subsequently, poikilodermatous changes with telangiectasia, skin atrophy and regions of hypo- and hyperpigmentation develop. Growth retardation results in dwarfism. Greying and loss of hair, juvenile cataract, and hypogonadism with delayed puberty are further clinical signs of the disease. RTS sufferers are at increased risk of cancer. In the majority of cases they suffer from osteosarcoma or skin cancer. The cancers develop at a younger age than would normally be expected. Even though some clinical symptoms suggest precocious ageing, the patients' lifespan is not altered (providing that the neoplastic

disease is diagnosed early and is adequately treated). Inheritance is autosomal recessive.

The disease is caused by abnormalities within the *RECQL4* gene localised on the long arm of chromosome 8 (position 8q24.3) (Kitao et al., 1999). As in the above-described WS and BS, this gene encodes another protein homologous to RecQ helicases. Its function has not been extensively studied, but based on its structural similarity to WRN and BLM helicases it is predicted to be an ATP-dependent enzyme unwinding DNA structures.

Mutations of *RECQL4* gene found in RTS patients affect either the coding sequence or the splice junctions and frequently result in the production of truncated protein. The correlation of mutation type with cancer development has been studied. Truncating *RECQL4* mutations were found in two-thirds of 33 RTS patients examined. However, all RTS patients suffering from osteosarcoma had such mutations. In other words, there was a significant correlation between truncating mutations and the risk of osteosarcoma (Wang et al., 2003). As in other syndromes associated with helicase dysfunction, RTS cells are characterised by marked genomic instability associated with chromosomal rearrangements resulting in acquired somatic mosaicism. *RECQL4* transcripts are down-regulated in cells from RTS patients (Kitao, Lindor, Shiratori, Furuichi, & Shimamoto, 1999). Embryonic fibroblasts from transgenic mice with deletions within the RecQ helicase domain of *RECQL4* genes show a defect in cell proliferation (Hoki et al., 2003).

The interactions of RECQL4 with other proteins are still unknown. Interestingly, *RECQL4* mutations have also been found in another inborn genetic defect – RAPADILINO syndrome characterised by growth retardation, forearm, thumb and patella defects, infantile diarrhoea and hypogonadism, but not by a significant cancer risk (Siitonen et al., 2003).

## 6. Cockayne syndrome

CS is another autosomal recessive segmental progeroid syndrome, first described by Cockayne in 1936. It is estimated that a child suffering from CS is born once in 250,000 live births. Cell fusion studies have identified five CS complementation groups. Most sufferers belong to the ‘pure’ CS complementation groups: milder CSA and severe CSB. Some patients with xeroderma pigmentosa phenotypes caused by mutations in the *XPB*, *XPD*, and *XPG* genes also present clinical features of CS (XPB-CS, XPD-CS, XPG-CS). Only approximately 25% of ‘pure’ CS patients are diagnosed with CSA, the rest being diagnosed with CSB. There is marked variability in the clinical manifestation of this disease. In CSA the child’s development is normal during the first years of life, although so-called ‘early CSA’ has also been described. CSB manifests itself intrauterinally. The cardinal features of CS are: senile-like appearance (atrophic skin, sparse hair), severe growth retardation with a lack of subcutaneous fat (cachectic dwarfism), progressive neurodevelopmental abnormalities with microcephaly, diffuse dysmyelination and calcium deposits in the cortex and basal ganglia, progressive ocular abnormalities including retinal atrophy and cataract, sensorineural hearing loss, and acute sun sensitivity. The appearance of cataract during the first 3 years of life indicates the severe phenotype and predicts early death. Despite the sensitivity to ultraviolet radiation, only xeroderma pigmentosa–Cockayne syndrome (XP–CS) sufferers have an increased frequency of skin cancers. CSA patients usually die in the second or third decade of life (the mean age of death is 12.5 years), while death in CSB patients occurs much earlier, usually in the sixth to seventh year of life. The most common cause of death is deterioration of the central nervous system or a variety of respiratory tract infections. However, XP–CS patients (as all XP patients) are at 1000-fold increased risk of developing skin cancer (basal cell carcinomas, squamous cell carcinomas and melanomas), mostly in sun exposed areas. Tumours occur by the fourth to eighth year of life and most patients die in the second or third decade of life, owing to malignancy.

The disease is caused by *CSA* or *CSB* mutation. *CSA* (*ERCC8*) defective in Cockayne syndrome group A was cloned in 1995. It is located on the long arm of chromosome 5 (5q12.3) and encodes CSA protein belonging to the family of WD-repeat proteins (Henning et al., 1995). *CSB* (*ERCC6*), mutations in which result in Cockayne syndrome group B, was cloned in 1992 (Troelstra et al., 1992). It is localised to locus 10q11, and encodes another member of the helicase family. Mutations found in *CS* genes are homozygous or compound, missense, nonsense mutations or deletions (Cao, Williams, Carter, & Hegele, 2004;

Colella, Nardo, Botta, Lehman, & Stefanini, 2000; Ren et al., 2003). Both proteins encoded by *CS* genes are components of the transcription-coupled DNA repair (TCR) subpathway of the nucleotide excision repair (NER) system. Briefly, TCR involves damage recognition by the stalled RNA polymerase II complex (Mu & San-car, 1997), local DNA unwinding, incision on both sides and removal of damaged oligonucleotide, gap-filling DNA synthesis with the intact complementary strand as a template, ligation of the newly synthesised oligonucleotide and the resumption of RNA synthesis. More than 20 proteins are involved in this process, including all XP proteins, mutations in which result in xeroderma pigmentosa types A through G, or a combined syndromes XPB-CS, XPD-CS and XPG-CS, and both CSA and CSB proteins, damage of which results in Cockayne syndrome A or B, respectively (for review see de Boer & Hoeijmakers, 2000). The unwinding step of TCR requires the presence of TFIIH complex. TFIIH was originally identified as a factor crucial for transcription initiation. It consists of nine subunits including XPB and XPD, the two helicases that unwind double stranded DNA in $3' \rightarrow 5'$ and $5' \rightarrow 3'$ direction, respectively. Subsequently, the damaged strand is excised by the two exonucleases. The one restricting at the $3'$ side of the damage site is XPG.

After UV irradiation or oxidative DNA damage, CSA protein rapidly translocates to the nuclear matrix in a CSB-dependent manner. CSA interacts with CSB and p44 subunit of TFIIH (Henning et al., 1995). In UV-irradiated cells CSA co-localises with the phosphorylated form of RNA polymerase II engaged in RNA transcript elongation (Kamiuchi et al., 2002). It is proposed that CSA is required for efficient DNA repair only during the elongation stages of RNA polymerase II transcription (Tu, Bates, & Pfeifer, 1998). CSB interacts with the stalled transcription complex (van Gool et al., 1997) and plays a role in the recruitment of TFIIH to the damaged DNA. CSB also induces chromatin remodelling (Citterio et al., 2000). The conformational changes in the stalled complex or its translocation from the lesion site as well as chromatin remodeling may allow CSA to attach and the repair complex to access the damaged DNA strand. Thus, both CSA and CSB are components of RNA polymerase II-associated complexes, and their possible role is to assist this polymerase in dealing with transcription blocks and the resumption of transcription.

Based on the fact that xeroderma pigmentosa complementation group A patients who are completely deficient in nucleotide excision repair do not develop CS symptoms, it is suggested that, in addition to NER defects, transcription deficiency contributes to the development of CS clinical symptoms. This is supported by the fact that TFIIH with which CS proteins interact during transcription-coupled repair, is not only a factor involved in DNA repair, but is also a transcription factor, and that RNA polymerase II-dependent transcription is decreased in CS cell extracts (Balajee, May, Dianov, Friedberg, & Bohr, 1997). Abnormal transcription-coupled repair of oxidative DNA damage was detected in CS cells, but not, as mentioned before, in XPA cells that are completely NER deficient (Leadon & Cooper, 1993). Defective TCR of oxidative damage was also found in XPG–CS cells but not in XPG cells (Cooper, Nouspikel, Clarkson, & Leadon, 1997), and in XPB-CS and XPD-CS cells but not in XPB or XPD cells. In addition, it has been shown that unrepaired 8-oxoguanine blocked transcription by RNA polymerase II (Le Page et al., 2000). These data further support the notion that CS phenotype also results from the defective TCR of oxidative lesions.

An interesting feature of CS is the unchanged risk of cancer, despite UV hypersensitivity. In fact, it has been demonstrated that cancer-predisposed Ink4a/ARF−/− mice develop fewer neoplastic lesions if their *CSB* gene had been disrupted (Lu et al., 2001). Based on the data regarding Cockayne syndrome and xeroderma pigmentosa molecular defects, this can be explained by normal global genome nucleotide excision repair (GG-NER) in CS cells and abnormal GG-NER in XP cells. In fact, GG-NER defect seems to be of crucial importance for neogenesis, since XPC patients develop cancers while only their GG-NER but not their TCR function is affected. In other cases of XP both GG-NER and TCR are defective, so XP patients, including XPB-CS, XPD-CS and XPG-CS patients, suffer from neoplastic disease.

What kind of XPB, XPD or XPG mutations result either in xeroderma pigmentosa or XP–CS is a matter of interest. It has been reported that XPG-CS patients had truncating mutations, while XPG patients had missense mutations that resulted in the production of full-length XPG protein defective in its exonuclease function (Nouspikel, Lalle, Leadon, Cooper, & Clarkson, 1997). The role of the C-terminal end of XPG protein in the development of CS phenotype is supported by

the fact that homozygous transgenic mice with XPG lacking the last 360 amino acids exhibit growth retardation and short lifespan, although this phenotype was milder than in XPG-null mice (Shiomi et al., 2004). Individuals suffering from XPD-CS were shown to exhibit unique *XPD* mutations. Even though the extent of their NER defect was similar to XPD patients with other *XPD* mutations, their cells were extremely sensitive to UV light (van Hoffen et al., 1999). The reason for such hypersensitivity was the introduction of DNA breaks not in close proximity to the lesion site (as a necessary NER step) but in other locations. Therefore in addition to their inability to remove oxidative damage, XPD-CS cells break their own DNA after UV irradiation (Berneburg et al., 2000). Another mechanism responsible for the development of certain phenotypes has been proposed namely the relative expression of mutated XPB genes (Riou et al., 1999). Others have demonstrated that mutations found in two XPB-CS patients decreased TFIIH transcriptional activity by preventing promoter opening (Coin, Bergmann, Tremeau-Bravard,& Egly, 1999).

## 7. Summary

A number of genes, including mitochondrial, Fas, FasL, MHC class I, different lipid metabolism genes, etc., have been studied in ordinary ageing. Some studies delivered negative results while others described potential associations (Barzilai et al., 2003; Niemi et al., 2003). A genomewide linkage study performed on 308 centenarians and near-centenarians found a statistically significant linkage within chromosome 4. Further refining of the search identified a microsomal transfer protein gene encoding protein involved in lipoprotein synthesis as a factor potentially modulating lifespan (Geesaman et al., 2003).

Another approach to the study of ageing is the functional analysis of the genes involved in the development of progeroid syndromes. Unfortunately, none of the known progerias, including those most closely resembling ordinary ageing (Hutchinson–Gilford and Werner syndromes) represent true precocious ageing. Still, a disturbed function of such proteins in these disorders implies their role in 'physiological' ageing. This is further supported by the fact that many of these proteins are involved in the basic mechanisms that maintain genome integrity and function, while signs of genomic damage accumulate with age, and genomic instability characterises one of the age-related diseases – malignant disorder. Some clues to their potential involvement in normal ageing or in longevity promotion come from the analysis of polymorphisms of their genes in different age groups. The analysis of a polymorphism at amino acid 1367 of WRN protein (Cys (TTG)/Arg(CTG) in *WRN* gene) in Finnish newborns, centenarians and the general population were similar across age groups suggesting that there is no association of this WRN polymorphism with longevity. However, if there is no association with longevity, there might be link between the gene and common age-related diseases. 1367 Cys/Arg seems to be associated with a variation in the risk of myocardial infarction in the Japanese population, with 1367 Arg allele being less common but protective against this disease (Castro et al., 1999, 2000). Frameshift mutation in the coding region of the *BLM* gene has been found in genetically unstable sporadic gastrointestinal tumours (Calin, Herlea, Barbanti-Brodano, & Negrini, 1998). It might be of interest to check the sequence/function of the other proteins implicated in progeroid syndromes associated with malignant disease in different human tumours characterised by marked genomic instability.

Until now, there is many more data suggesting the involvement of different lipid metabolism genes than genome integrity genes in the control of human lifespan. Although progress in resolving the molecular function of helicases and other progeroid syndrome-associated proteins is rapid, our understanding of their role in ordinary ageing (if any) remains to be elucidated.

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