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Helicases and aging

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Abstract. Studying monogenic hereditary disorders that manifest age-related phenotypes in cells, tissues, and the total organism would be helpful for clarifying the mechanisms of aging. In this context, seven human disorders that manifest age-related phenotypes have been found to be caused by aberrations of five proteins with seven helicase motifs conserved in most of the helicases. These disorders are xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy, Bloom syndrome, Werner syndrome, X-linked α-thalassemia/mental retardation syndrome, and Juberg-Marsidi syndrome. A decline of probably pleiotropic and fundamental function of helicases in these disorders is, therefore, implied to underlie not only the various age-related phenotypes of the disorders but also the pleiotropic and universal nature of ordinary aging. Consistent with this implication, studies of these seven disorders suggest that their various age-related phenotypes are caused by aberrations in multiple processes, especially transcription. Furthermore, a few studies imply some association between aberration of the helicases and phenotypes in ordinary aging.

Key words. Helicase disorder; aging; Werner syndrome; Cockayne syndrome; Bloom syndrome; transcription; segmental progeroid syndrome.

Introduction

Advances in molecular biology are making it easier to identify genes responsible for monogenic hereditary disorders. However, identifying genes for age-related common disorders including cancer, dementia, arteriosclerosis, hypertension, diabetes mellitus, and osteoporosis is still difficult, partly because most of these disorders are polygenic. A strategy to overcome such a difficulty is to identify genes responsible for age-related monogenic hereditary disorders, followed by clarifying the processes in which the gene products are involved. These causative genes and processes can then be studied in the corresponding non-hereditary age-related disorders, a strategy which has already succeeded with several such disorders, especially cancers.

Aging is a complex and multifactorial process. This makes aging studies difficult. In analogy to the strategy for studying common age-related disorders, studying monogenic hereditary disorders that manifest some age-related phenotypes in cells, tissues, and the total organism would be helpful for clarifying the mechanisms of aging. One example is cloning of the gene (WRN) responsible for Werner syndrome (WS), a well-known segmental progeroid syndrome with many age-related phenotypes [1]. This gene encodes a protein that has the seven helicase motifs conserved in most helicases, and the WRN protein has been shown to have helicase activity. This implies that a decline of probably pleiotropic and fundamental function in this and other helicases underlies the pleiotropic and universal nature of ordinary aging. Consistent with this implication, in addition to WS, at least six human disorders that manifest age-related phenotypes are caused by aberrations of five proteins that have the seven helicase motifs. These six disorders are xeroderma pigmentosum (XP), Cockayne syndrome (CS), trichothiodystrophy (TTD), Bloom syndrome (BS), X-linked α-thalassemia/mental retardation syndrome (ATR-X), and Juberg-Marsidi syndrome (JMS). Although not all proteins that have

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the seven helicase motifs have helicase activity, disorders that are caused by an aberration of such proteins have been tentatively called helicase disorders [2] in this review.

Is, then, an aberration of helicases involved in ordinary human aging? And if so, how does the aberration affect aging? A possible strategy to answer these questions is clarification of the pathways generating age-related phenotypes in helicase disorders, followed by study of these pathways in ordinary aging. Following this strategy, this paper reviews recent reports about helicase disorders and considers the following four subjects. First, how do age-related phenotypes occur in each helicase disorder? Second, what, if any, are the common causative pathways in helicase disorders? Third, are these pathways involved in ordinary aging? Fourth, if so, how are these pathways involved in ordinary aging?

Helicases

Helicases are a diverse group of enzymes defined by their ability to promote the unwinding of duplex nucleic acid molecules by breaking the hydrogen bonds holding the strands of the duplex together [3 and references therein]. They function in the fundamental processes of life, including DNA replication, DNA repair, recombination, transcription, RNA processing, and translation. To participate in the specific pathways in these processes, each helicase has unique amino acid sequences that bind with different molecules. In contrast, most helicases have conserved amino acid sequences known as the seven helicase motifs (I, Ia, II, III, IV, V, and VI), which reflect helicase activity in many cases. More than 30 human proteins are predicted to be putative helicases, mostly based on their having the seven helicase motifs.

Segmental progeroid syndromes

Aging is too complex and vague to define exactly and to study in a straightforward manner, and has, therefore, been studied and understood from various angles. One is the manifestation of many age-related phenotypes. In 1978, Martin [4] defined 21 criteria relating to different aspects of the phenotype of aging as seen in humans (table 1), and according to the criteria, he selected 83

Table 1. Helicase disorders and criteria for segmental progeroid syndromes.

Criteria*	Helicase disorders† and their scores						
	WS 14	CS 12	TTD 6	XP 5	BS 5	JMS 1	ATR-X 1
1) Potential relevance to the 'intrinsic mutagenesis' hypothesis of aging	○	○	○	○	○		
2) Increased frequencies of non-constitutional chromosomal aberrations	○		○	○	○		
3) Increased susceptibility to one or more types of neoplasm of relevance to aging	○			○	○		
4) Possibility of a defect in a stem cell population or in the kinetics of stem cell proliferation	○						
5) Premature graying or loss of hair or both	○						
6) Dementia or certain types of relevant degenerative neuropathology, or both		○	○	○		○	○
7) Possible susceptibility to 'slow virus'							
8) Various types of amyloid depositions							
9) Increased depositions of lipofuscin pigments		†					
10) Diabetes mellitus	○	○					
11) Disorder of lipid metabolism	○	○					
12) Hypogonadism	○	○	○		○		
13) Autoimmunity	○						
14) Hypertension‡		○					
15) Degenerative vascular disease	○	○					
16) Osteoporosis	○	○					
17) Cataracts whose morphologies may overlap, to some extent, with those of senile cataracts	○	○	○				
18) Abnormalities of mitochondria in one or more tissues							
19) Regional fibrosis	○	○		○	○		
20) Variations in amounts and/or distributions of adipose tissues	○	○	○				
21) Potential relevance to pathobiology of other aspects of aging							

*Taken from ref. 4.

† WS, Werner syndrome; TTD, trichothiodystrophy; CS, Cockayne syndrome; XP, xeroderma pigmentosum; BS, Bloom syndrome; JMS, Juberg-Marsidi syndrome; ATR-X, X-linked α-thalassemia/mental retardation syndrome.

‡ Hypertension, excluding the various genetic adrenal hyperplasias and several genetic renal diseases with secondary hypertension.

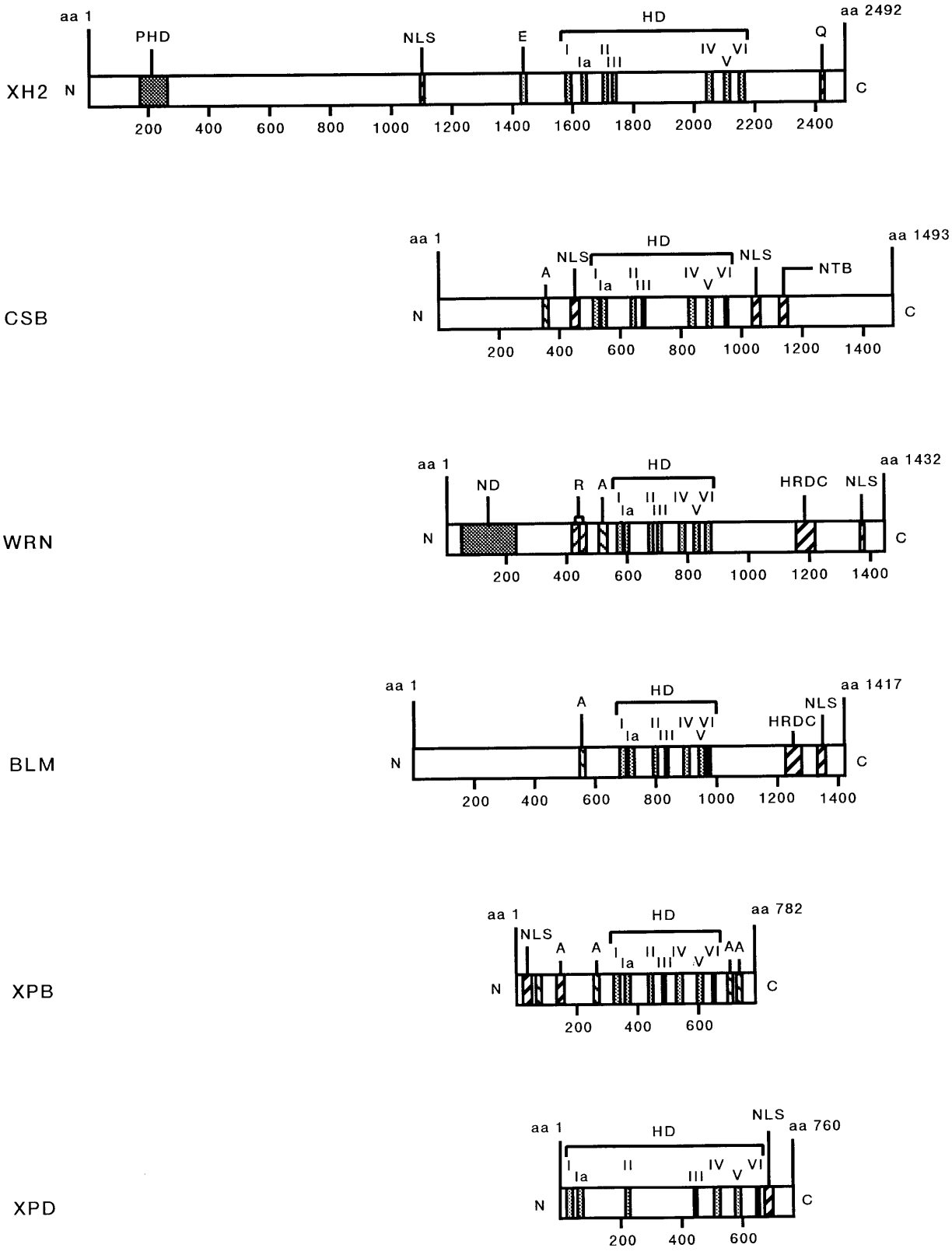

Figure 1. Schematic diagram of XH2, CSB, WRN, BLM, XPB, and XPD proteins. PHD, plant homeodomain-like motif; NLS, nuclear localization signal; E, stretch of 21 glutamic acid residues; HD, helicase domain (numbers I, I, and II–VI refer to corresponding helicase motifs); Q, glutamine-rich region, which may be involved in protein-protein interactions; A, acidic amino acid stretch, which has been found in a number of nuclear proteins that associate with chromatin or histones; NTB, nucleotide-binding fold; R, 27-amino acid repeat which is perfectly duplicated at the nucleotide level; HRDC, helicase and RNase D C-terminal domain, which is a putative nucleic-acid-binding domain.

autosomal dominant, 70 autosomal recessive, 9 sex-linked hereditary disorders, and 3 chromosomal syndromes as 'segmental progeroid syndromes.' The 21 criteria consist of 3 phenotypic alterations in cells and 18 phenotypic alterations in tissue or the total organism. Among the 70 autosomal recessive segmental progeroid syndromes, at least 4 have been found to be caused by defective helicases. These 4 syndromes are XP, CS, BS, and WS. In addition, other helicase disorders, TTD, ATR-X, and JMS, could also be regarded as segmental progeroid syndromes according to the 21 criteria, although ATR-X and JMS are relatively poor in age-related phenotypes (table 1). Thus, segmental progeroid syndromes are indispensable for studying the mechanisms of aging, especially in terms of the involvement of helicases.

Xeroderma pigmentosum

XP is an autosomal recessive segmental progeroid syndrome. The clinical phenotypes of XP include extreme photosensitivity, a dry scaly skin (xeroderma), abnormal pigmentation of sun-exposed areas of the skin (pigmentosum), an estimated 2000-fold increase in the risk of developing skin cancer (basal cell carcinoma, squamous cell carcinoma, and melanoma), and a 10- to 20-fold increase in the frequency of internal neoplasms including primary brain tumors, leukemia, lung tumors, and gastric carcinomas. Some XP patients show neurologic abnormalities, microcephaly, dwarfism, and gonadal hypoplasia (De Sanctis-Cacchione syndrome, table 1). Autopsy cases of XP show fetal maldevelopment and developmental retardation of various organs including the kidneys and lungs as well as brain atrophy resulting from progressive neurologic degeneration due to increased neuronal death.

Normal skin fibroblasts can repair ultraviolet light (UV)-induced DNA damage by inserting new bases into DNA by nucleotide excision repair (NER), whereas XP fibroblasts have a reduced capacity for NER. Exposure of XP fibroblasts to UV and chemical carcinogens therefore results in low cloning efficiency, more chromosomal aberrations, and a higher frequency of sister chromatid exchanges (SCEs). In practice, XP fibroblasts are usually characterized by low unscheduled DNA synthesis after UV irradiation. Recovery of RNA synthesis after UV irradiation is also depressed in XP fibroblasts. Cell fusion studies have identified at least eight XP complementation groups to date (A through G and variant). XP variant fibroblasts, in contrast to fibroblasts from the other complementation groups, are proficient in NER but defective in a poorly understood post-replication repair process.

Since 1990, seven genes (XPA–XPG) responsible for XP have been cloned and all of the gene products are found to be involved in the initial steps of NER [5]. Both XPA and XPE, which are DNA-binding proteins, are thought to function as DNA damage recognition proteins. Both XPB and XPD, which are helicases and subunits of the transcription/DNA repair factor IIH (TFIIH), unwind double-stranded DNA at the damaged region (fig. 1). Nine genes encoding the subunits forming TFIIH have been cloned. These genes are CDK7, cyclin H, MAT1 (cdk-activating kinase, CAK), XPD, XPB, p62, p52, p44, and p34. XPC, which is a single-stranded-DNA-binding protein, appears to stabilize the unwound structure. In addition, a recent finding suggests a two-step mechanism of damage recognition involving damage detection by the XPC/HR23B heterodimer followed by damage verification by the XPA protein [6]. The HR23B protein is one of two homologues of yeast RAD23. Both the XPF/ERCC1 complex and XPG, which are endonucleases, function to cut out the damaged region. In NER, two subpathways are recognized: rapid preferential repair of the transcribed strand of active genes (transcription-coupled repair; TCR) and the less efficient global genome repair (GGR) process. Among the seven XP genes, the XPC protein is unique to GGR, whereas the others are involved in both subpathways of NER. Mutation studies in XP patients show that mutation correlates well with the expression level of the corresponding causative gene, with the extent of the cellular DNA repair defect, and with the clinical severity of the disorder [7–11].

Based on molecular cytogenetic understanding of NER and XP, the skin lesions and the high risk of skin cancer are thought to result directly from a GGR defect in XP cells. Consistent with this concept, the clinical photosensitivity in XP patients is usually associated with cellular UV hypersensitivity. The progressive neurologic degeneration due to increased neuronal death might also be caused by a GGR defect, because neurons are usually non-dividing and vulnerable to various forms of damage. However, examination of many XPC patients has failed to detect the typical neurologic abnormalities of XP, which are therefore difficult to explain on the basis of a GGR defect only. Instead, the neurologic abnormalities might rather result from apoptotic neuronal cell death [12, 13] due to a defect in another pathway independent of the XPC protein. A candidate for such a pathway is TCR partly because, among the seven XP genes, the XPC protein is unique to GGR, whereas the others are involved in both GGR and TCR. However, the neurologic abnormalities of XP appear to be different from those of CS which has a TCR defect as described below. Another candidate pathway is proposed to be involved in repairing damage such as oxidative DNA lesions, on the basis that two major oxidative DNA lesions, 8-oxoguanine and thymine glycol, are excised from DNA in vitro by the same enzyme system responsible for removing pyrimidine dimers caused by UV irradiation [14].

Other proposed candidate pathways include an apoptotic pathway [13]. This pathway is probably p53 dependent, because XPB and XPD proteins are components of the p53-mediated apoptotic pathway [15]. Finally, a transcription defect in XP would explain some XP phenotypes [16].

Cockayne syndrome

CS is a high-ranked autosomal recessive segmental progeroid syndrome. The clinical phenotypes of CS include severe growth failure (cachectic dwarfism) with senile-like appearance, neurodevelopmental abnormalities, microcephaly, progressive ocular abnormalities including retinal atrophy and cataracts, hearing loss, and acute sun sensitivity (table 1). In contrast to XP, most CS patients do not have an increased frequency of skin cancers, although a few have the XP phenotypes including skin cancer predisposition (XP/CS complex). CS, unlike XP, causes primary neuronal dysmyelination leading to severe atrophy of the brain.

After UV irradiation, CS fibroblasts, like XP fibro-blasts, show low cloning efficiency and a failure of recovery of RNA synthesis. This failure is regarded as indicating a TCR defect. Some CS cells also show increased SCEs after UV irradiation. On the other hand, CS fibroblasts, unlike XP fibroblasts, show normal unscheduled DNA synthesis after UV irradiation. Cell fusion studies have identified five CS complementation groups to date (CSA, CSB, XPB, XPD, and XPG). Most patients show only CS symptoms; these patients belong to group CSA or CSB. In contrast, a few rare patients show clinical features of the XP/CS complex; most of these patients are assigned to XP group B, D, or G.

The two genes responsible for CS (CSA and CSB) have been cloned [17, 18]. The CSA gene encodes a WD (Trp-Asp) protein. A WD repeat is a motif implicated in protein-protein interactions. In fact, the CSA protein interacts with several proteins, including p44 and CSB protein [17]. The p44 protein is a subunit of TFIIH. The CSB protein belongs to the yeast SNF2/SWI2 protein family, members of which are involved in transcriptional activation of many genes and contain the seven helicase motifs. Although the CSB protein also contains the seven helicase motifs (fig. 1), it does not have helicase activity [19]. The CSB protein is found to interact with XPG protein [20] as well as CSA protein. p44, XPB, XPD, and XPG proteins are also found to interact with each other. Taken together, these findings connect the five causative proteins (CSA, CSB, XPB, XPD, and XPG) for CS.

In XP, some clinical features appear to result from a GGR defect in XP cells. In CS, do some clinical phenotypes, then, result from the TCR defect of CS cells? TCR is an NER subpathway and requires several proteins including XPB, XPD, XPG, CSA, and CSB. In addition, the XPA, XPE, XPF, and XPG proteins are also required for TCR. Mutations in the XPA, XPE, XPF, and XPG genes therefore confer a total defect in repair of both the transcribed and non-transcribed strand. Nevertheless, XPA patients do not show the various CS phenotypes. Even the hypersensitivity of CS cells to DNA-damaging agents fails to be explained by the TCR defect [21]. Thus, the CS phenotypes are difficult to explain on the basis of the TCR defect alone. In this context, TFIIH is not only a DNA repair factor but also a transcription factor [22], and in fact, RNA-polymerase-II-dependent transcription is decreased in extracts of CS cells [23]. At least part of the CS phenotype could therefore be explained by a primary transcription defect rather than by the TCR defect [24–26]. In addition, the XPB protein is found to interact with SUG1, an integral component of the 26S proteasome [27]. This finding implies that CS has a secondary transcription defect, because SUG1 is thought to act as a mediator of transcription [28]. More interestingly, SUG1 also has helicase activity [29]. Thus, although few specific genes are reported to express an aberrant transcriptional pattern and their involvement in specific phenotypes is unknown, most of the various CS symptoms would be a consequence of aberrant expression of genes due to primary and secondary transcription defects.

XPB, XPD, and XPG proteins appear to have additional functions, defects in which could also contribute to the phenotypic expression of CS. Recent mutation studies of the XPG gene found that three XPG/CS patients had mutations that produced severely truncated XPG proteins, whereas two sibling XPG patients without CS symptoms made full-length XPG, but with a missense mutation that inactivated its function in NER [30]. This finding suggests that XPG/CS mutations abolish the interactions required for a second important XPG function and that loss of this second function leads to the clinical CS phenotypes. A candidate for this second function is TCR of oxidative damage, which is independent of the XPG incision function in NER [31], on the basis of the finding that ionizing-radiation-induced damage was repaired by TCR which requires CSB but not XPA [32]. Thus, TCR is now thought to be a universal phenomenon that is involved not only in NER but also in other repair processes including DNA mismatch repair [33]. A probable function of XPB and XPD proteins is related to apoptosis, because these two proteins are components of the p53-mediated apoptotic pathway [34]. Furthermore, XPA-, XPC-, or CSB-deficient mice are viable whereas XPB- or XPG-deficient mice undergo embryonic death, indicating that the XPB and XPG proteins are also involved in some pathway other than NER during prenatal development. Thus, the various CS symptoms are likely to be caused by a combination of

defects in pleiotropic and multiple functions rather than by a defect in a single function.

An unexplained finding in CS is that CS patients have no skin cancer predisposition despite the TCR defect in CS cells. Moreover, in contrast to humans, CSB-deficient mice are found to have a skin cancer predisposition [35]. The lack of skin cancer predisposition in CS patients might be explained by a more effective GGR in humans.

Trichothiodystrophy

TTD is a rare autosomal recessive disorder. The clinical phenotypes of TTD include brittle hair and nails with reduced sulfur content, ichthyosis, peculiar facial features, and mental and physical retardation. TTD can therefore be regarded as a segmental progeroid syndrome (table 1). Although some TTD patients show photosensitivity, in contrast to XP patients and like CS patients, they do not have cutaneous lesions and a skin cancer predisposition. Hair mounts show the characteristic banding pattern with polarizing microscopy and hair amino acid analysis shows decreased high-sulfur matrix proteins.

Fibroblasts from TTD patients with photosensitivity have a defect in GGR, indistinguishable from that in XP patients. That is, after UV irradiation, fibroblasts from TTD patients with photosensitivity, like XP fibroblasts, show low cloning efficiency, low unscheduled DNA synthesis, and a failure of recovery of RNA synthesis. On the other hand, after UV irradiation, fibroblasts from most TTD patients without photosensitivity show a normal level of cloning efficiency and unscheduled DNA synthesis [36–38]. Cell fusion studies have identified three TTD complementation groups to date: XPB, XPD, and TTDA. Although the TTDA gene from a photosensitive TTD patient has not yet been cloned, it is predicted to be a new gene involved in GGR. In addition, besides the XPB and XPD proteins, the TTDA protein is also predicted to be part of TFIIH [39, 40].

At least several symptoms appear to be shared between CS and TTD. These include photosensitivity, short stature, mental retardation, aged appearance, microcephaly, cataracts, hypogonadism, and joint contractures. The TTD symptoms, like the CS symptoms, would therefore be caused by primary and secondary transcription defects and some other defects. Moreover, a reduced transcriptional level of the SPRP2 gene is associated with the development of the hair and skin abnormalities in a mouse model of TTD [41], also indicating a transcription defect in TTD.

Why, then, are three distinct disorders (XP, CS, and TTD) caused by mutations in the same genes, XPB and XPD? Mutations in the XPG gene also cause at least XP and CS. The most likely explanation is that effects dependent on the sites of mutations in these genes determine the disorder. To date, the same mutation pattern of both alleles of the XPB, XPD, and XPG genes has not been found in the three distinct disorders. On the other hand, several mutation studies show that the three distinct disorders are caused by the same XPD mutation in one allele and different XPD mutations in the other alleles, resulting in several disorder-specific mutations of the XPD gene [42–45]. In addition, a mutation study of the XPD gene suggests that the severity of the clinical TTD symptoms does not correlate with the degree of cellular UV hypersensitivity, but it is influenced by the dosage of the mutated allele [43].

How, then, do disorder-specific mutations affect the functions of the defective proteins, resulting in the clinical symptoms of XP, CS, or TTD? One possible mechanism is that disorder-specific mutations impede disorder-specific interactions of the causative proteins with some molecules. A candidate for the disorder-specific interactions is that between the XPD protein and the p44 protein, another subunit of TFIIH [46]. Impediment of such an interaction would result from a change in the amino acid sequence contained in the indispensable region for the interaction. Alternatively, such an impediment might result from a causative gene mutation that does not change the amino acid sequence containing the indispensable region but alters the causative protein conformation [41].

Bloom syndrome

BS is a rare autosomal recessively transmitted chromosome instability syndrome and is also regarded as a segmental progeroid syndrome. The clinical phenotypes of BS include pre- and postnatal growth retardation, sun-sensitive, erythematous, hyper- and hypopigmented skin, severe immune deficiency, and infertility. The three major complications are chronic lung disease, diabetes mellitus, and cancer (table 1). Cancers of the types and sites seen in the general population arise frequently and unusually early.

Cells from individuals with BS have retarded rates of progression of DNA replication forks, delayed conversion of replication intermediates to high-molecular-weight DNA, and a slow growth phenotype in culture, suggesting a possible defect in DNA replication. Furthermore, BS cells have highly reduced linear-plasmid-DNA-joining ability with an elevated spontaneous rate of mutations consisting predominantly of deletions, insertions, or complexes at the joining sites [47], and an elevated spontaneous mutation rate to 6-thioguanine resistance, a high frequency of multiple, spontaneous chromosomal aberrations, and frequent SCEs, suggesting a possible defect in recombination. Although the

evidence that BS cells have a DNA defect repair is slight, a recombination defect could affect some repair pathways including recombinational DNA repair which is the most complex and least understood of the DNA repair pathways. In contrast to XP cells, BS cells in general are not hypersensitive to UV irradiation by standard assays, although after UV irradiation, some BS cells show a significantly higher frequency of SCE and complete absence of p53 accumulation. Another finding was that both topoisomerase IIα mRNA and protein levels were decreased in high-SCE cells derived from a BS patient, whereas they were normal in low-SCE cells derived from the same BS patient, suggesting a possible defect in transcription [48]. In addition, there are several reports indicating that BS cells have various abnormalities of several enzymes, such as DNA ligase I [49].

The gene responsible for BS (BLM) has been cloned [50]. It encodes a 1417-amino-acid peptide with homology to RecQ subfamily helicases (fig. 1). The BLM protein functions as a 3′-5′ DNA helicase [51]. Mutation studies in BS patients reveal more than 20 mutations including missense, nonsense, and frameshift mutations, and a large genomic deletion mutation. The mutations, except the missense mutations, cause premature translation termination of the BLM protein. The truncated BLM protein fails to translocate into the nucleus due to the absence of a nuclear localization signal in the C terminus of the protein, resulting in loss of protein function [52]. The missense mutations abolish ATPase and DNA helicase activities of the protein, being found in or near the conserved helicase domain of the BLM protein [53].

Genes homologous to the BLM gene are found in several organisms including *Saccharomyces cerevisiae* SGS1, *Schizosaccharomyces pombe* rad12+, also known as hus2+ or rqh1+, and *Escherichia coli* recQ. In mutant *sgs1 S. cerevisiae* cells, the BLM protein is found to suppress the increased homologous and illegitimate recombinations, to suppress cell growth in the *top3 sgs1* mutation background, to restore the increased sensitivity of the sgs1 mutant to hydroxyurea [54], and to restore the shortened life span of the *sgs1* mutant [55]. The Sgs1 protein interacts with topoisomerase II [56] and topoisomerase III [57]. Topoisomerase II catalyzes the decatenation of interlinked DNA molecules and is essential for chromosome segregation. Topoisomerase III is a poorly characterized type I topoisomerase. In *S. pombe*, rad12+ is required to prevent recombination [58] and regulates cell cycle checkpoint control [59]. Rad12 null cells are sensitive to DNA damage induced by UV light and γ radiation [59]. In *E. coli*, RecQ DNA helicase, part of a multicomponent recombination pathway involved in processing DNA damage at replication forks, is a suppressor of illegitimate recombination, and the recombination is mediated by none of the known recombination pathways [60]. RecQ DNA helicase is also involved in the initial step of recombinational DNA repair [61]. RecQ helicase activity is stimulated by single-strand binding (SSB) protein [62]. These studies of BLM homologues are helpful to deduce the functions of the BLM gene, although some of the functions may be gene or organism specific. Despite many molecular cytogenetic studies of BS, the pathways generating age-related phenotypes in BS remain largely to be elucidated. Several speculations, however, are possible. For example, the short stature could be caused by increased apoptosis in the BS epiblast [63]. The high incidence of malignancy could be caused by the elevated spontaneous mutation rate due to a possible defect in recombination [64]. Another speculation is that many BS phenotypes are caused by aberrant expression of genes due to a possible defect in transcription, based on the finding that the murine BLM protein interacts with ATFa which belongs to the CREB/ATF family of transcription factors [53].

Werner syndrome

WS is a high-ranked autosomal recessive segmental progeroid syndrome. The clinical phenotypes of WS include short stature, stocky trunk with thin extremities, premature graying and loss of hair, juvenile cataracts, scleroderma-like skin changes, trophic ulceration of the legs, diabetes mellitus, osteoporosis, hypogonadism, and

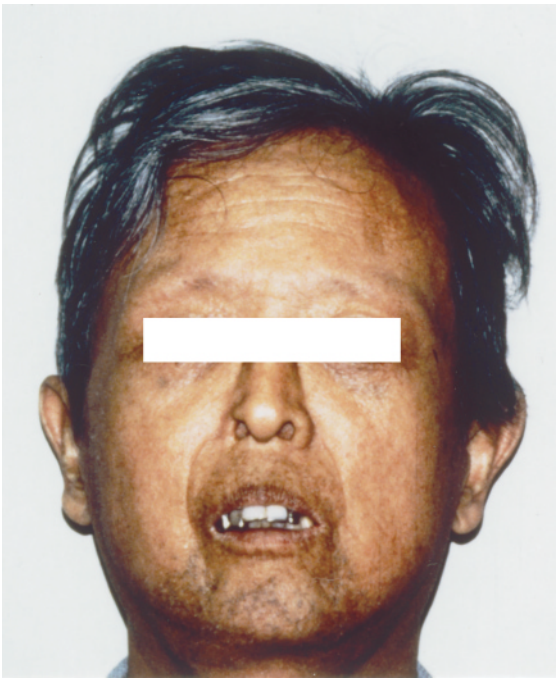

Figure 2. Patient with Werner syndrome aged 51 years. The appearance of senility is illustrated, and includes loss and graying of hair, increase in irregular pigmentation, beaked nose, small mouth, cataracts, and atrophic scleroderma-like skin.

an increased incidence of neoplasia (table 1, fig. 2) [65]. Autopsies of WS patients disclose calcifications in various organs, various forms of arteriosclerosis including atherosclerosis, and atrophy of the epidermis, subcutaneous fat, testis, and cerebral cortex. In WS patients, death occurs at an average age of 47 years, and the major causes of death are malignant tumors and myocardial infarction.

Various defects in DNA replication, repair, recombination, and transcription are found in WS fibroblasts and lymphoblasts. With respect to DNA replication, WS fibroblasts have a reduced life span in vitro [66], lengthened mean population doubling time [67], prolonged S phase [68], altered frequency of initiation sites of DNA replication [69], and decreased average size of replicons [70]. WS lymphoblasts also show poor growth [71]. Global repair ability is apparently normal in WS fibroblasts [72, 73]. However, slightly lower telomeric DNA repair efficiency in WS fibroblasts [74], deficient gene and strand-specific DNA repair in WS lymphoblasts, but not in primary WS fibroblasts [75], and deficient mismatch repair in WS fibroblasts, but not in WS lymphoblasts [76], have been reported recently. As far as recombination is concerned, fibroblasts and lymphocytes derived from WS patients have chromosome rearrangements ranging from partial chromosome deletions to multiple translocations [71]. An increased spontaneous mutation rate characterized by extensive deletions generated by non-homologous recombination in WS fibroblasts and lymphocytes has also been detected [77–79]. Concerning transcription, sets of multiple gene sequences are overexpressed in WS fibroblasts [80, 81]. Similar sets of sequences are also overexpressed in late-passage normal human fibroblasts, leading to the hypothesis that senescent and WS fibroblasts enter a final common pathway where similar sets of overexpressed genes generate diverse antiproliferative mechanisms and pathogenic sequelae. Another study demonstrated that WS fibroblasts have a reduced response to platelet-derived growth factor and fibroblast growth factor [82]. This leads to the hypothesis that failure of a step in these growth-factor-mediated pathways in WS cells may contribute to the phenotypic expression of the disorder.

The WS gene (WRN) has been cloned [1]. It encodes a 1432-amino-acid peptide with homology to RecQ subfamily helicases (fig. 1). The WRN protein functions as a 3′-5′ DNA helicase [83]. To date, at least 19 mutations have been found in WS patients. The mutations are nonsense mutations, frameshift mutations, and a large genomic deletion mutation, all of which truncate the WRN protein. The truncated WRN protein fails to translocate into the nucleus due to the absence of a nuclear localization signal in the C terminus of the protein, resulting in loss of protein function [84]. This is consistent with the fact that WS patients have only slight phenotypic variance, which would be caused by environmental and other genetic factors. In contrast to the truncation mutations, to date, missense changes in the WRN gene have been found as polymorphisms, but not as mutations.

Because the WRN gene is homologous to the BLM gene, findings about BLM gene homologues are important not only to understand the functions of the WRN gene but also to explain shared phenotypes between BS and WS. For example, in mutant *sgs1 S. cerevisiae* cells, the WRN protein, like the BLM protein, suppresses increased homologous and illegitimate recombinations [54]. In contrast, the WRN protein, unlike the BLM protein, fails to restore the shortened life span of the *sgs1* mutant [55]. SSB protein stimulates the helicase activity not only of RecQ DNA helicase but also of the WRN protein [83]. Nucleolar localization of the SGS1 protein and the WRN protein in replicating cells implies that the two proteins have a function related to ribosomal DNA [85, 86], although the nucleoplasmic WRN protein in quiescent cells could also have important functions. The homology between the WRN and BLM proteins is restricted mostly to their helicase domains. Shared phenotypes between the two disorders would therefore reflect the fact that the two proteins interact with the same proteins through their helicase domains. Nevertheless there are many clinical and cytological differences between WS and BS, which could result from the non-helicase domains. In fact, the WRN protein has not only helicase activity but also 3′-5′ exonuclease activity [87], and the exonuclease domain is outside the helicase domain [87–89]. The exonuclease activity distinguishes the WRN protein from other RecQ subfamily helicases including the BLM protein. The *E. coli* enzyme RecBCD and *Bacillus subtilis* AddAB also have both helicase and exonuclease activity, leading to the idea that the enzymes overlap functionally with the WRN protein. These enzymes are required for at least DNA repair and recombination. In addition, the unique sequences in the non-helicase domains in each of the BLM and WRN proteins are probably indispensable for interactions of the two proteins with different proteins. Since the WRN gene was isolated, WRN-defective mice have been created and studied [90]. The defective WRN protein is thought to lack helicase activity, because the defective WRN gene in mice expresses a 156-kDa protein with deletion of 121 amino acids containing the helicase motifs III and IV. Like WS fibroblasts, mouse fibroblasts derived from WRN-defective embryos show premature loss of proliferative capacity, and WRN-defective embryonic stem (ES) cells show a high mutation rate. In addition, WRN-defective ES cells are sensitive to topoisomerase inhibitors, suggesting an interaction between the WRN protein and topoisomerases. Mortal B lymphoblastoid cell lines from WS patients are also found to be sensitive to a topoisomerase inhibitor [91]. In

contrast to WRN-defective ES cells, WRN-defective mice, unlike WS patients, appear to grow normally although some develop extensive myocardial fibrosis or T cell lymphoma; most mice, however, have been observed for less than 13 months. This discrepancy might arise from species or genetic background specificity. Alternatively, some function that is involved in the phenotypic expression of WS might be other than helicase activity and independent of the helicase motifs III and IV. The WRN protein, but not the defective WRN protein with deletion of 121 amino acids containing the helicase motifs III and IV, in mouse ES cells is copurified through a series of centrifugation and chromatography steps with a multiprotein DNA replication complex [90]. This suggests that the WRN protein interacts with the multiprotein DNA replication complex through the 121 amino acids containing the helicase motifs III and IV. In addition, the findings that *Xenopus laevis* FFA-1 (focus-forming activity 1), which is required for the formation of replication foci, has DNA helicase activity and is a homologue of the WRN protein [92] support primary involvement of the WRN protein in DNA replication.

On the basis of observations from molecular cytogenetic studies of WS, several speculations about the pathways generating abnormalities in WS are possible. For example, the high incidence of malignancy could be caused by the elevated spontaneous mutation rate due to a possible defect in recombination. Speculations are also possible that most of the WS phenotypes, such as short stature, diabetes mellitus, arteriosclerosis, and atrophy of the epidermis, subcutaneous fat and cerebral cortex, are caused by the slow growth phenotype of WS cells due to a possible defect in DNA replication, and that diabetes mellitus and atherosclerosis are caused by the high mutation rate of WS cells due to a possible defect in DNA recombination and/or repair [77]. These speculations are not, however, supported by the observation that WRN-defective mice appear to grow normally despite the slow-growth phenotype and the high mutation rate of fibroblasts derived from the mice [90]. How, then, do the variety of phenotypes occur in WS? A most likely mechanism involves the aberrant expression of several genes, including osteonectin, thrombospondin, plasminogen activator inhibitor-1 (PAI-1), αB-crystallin, α1(I) and α2(I) procollagen, fibronectin, and insulin-like growth factor binding protein-3 (IGFBP-3), in WS cells [80] due to a possible defect in DNA transcription. For example, overexpression of osteonectin and thrombospondin might relate to osteoporosis and calcifications in various organs. Overexpression of PAI-1 and thrombospondin may relate to atherosclerosis. Overexpression of αB-crystallin might relate to juvenile cataracts. Overexpression of α1(I) and α2(I) procollagen may relate to scleroderma-like skin changes and arteriosclerosis. Overexpression of fibronectin may relate to arteriosclerosis. Overexpression of IGFBP-3 might relate to short stature. Thus, several WS phenotypes appear to be secondary consequences of aberrant expression of several genes, due to a possible defect in transcription. In addition, such phenotypes would be associated with other WS phenotypes: muscle atrophy would be associated with diabetes mellitus, hyperlipidemia with fatty liver and atherosclerosis, atherosclerosis and diabetes mellitus with trophic ulceration of the legs, and resistance to several hormones including insulin and growth hormone with short stature and stocky trunk with thin extremities. Several WS cellular phenotypes also appear to occur secondarily. For example, the cytoplasmic as well as nuclear environment is responsible for the retarded DNA synthesis in WS fibroblasts, suggesting that the retardation is a secondary consequence [93]. The WRN protein fails to restore the hypersensitivity to 4-nitroquinoline 1-oxide and the extensive deletion mutations in simian-virus-40-transformed WS cells, and this failure is difficult to explain from the standpoint of primary effects [94]. Another circumstantial piece of evidence of secondary effects is that the expression pattern of the WRN gene is not compatible with the WS phenotypes. WS appears to affect connective tissue cells predominantly, whereas the WRN gene is expressed ubiquitously and at lower levels in fibroblasts. This apparent discrepancy might reflect the cell-dependent expression of proteins that are affected by the WRN protein.

X-linked α-thalassemia/mental retardation syndrome and Juberg-Marsidi syndrome

The clinical phenotypes of ATR-X include severe psychomotor retardation, characteristic facial features, genital abnormalities, and α-thalassemia (table 1). The gene responsible for ATR-X is found to be the XH2 gene [95], also known as the ATRX or XNP gene. The XH2 protein, like the CSB protein, contains the seven helicase motifs (fig. 1) and belongs to the yeast SNF2/SWI2 protein family. The XH2 protein might, however, lack helicase activity in analogy to the CSB protein [19] and the yeast SNF2/SWI2 protein [96]. The XH2 protein also has three multicysteine zinc finger motifs, two of which are included in a PHD-like domain that may be involved in chromatin-mediated transcriptional control, a nuclear localization signal, a stretch of 21 glutamic acid residues and a glutamine-rich region, both of which may be involved in protein-protein interactions, and a region with up to 50% identity to other SNF2 proteins.

Mutation analyses of the XH2 gene reveal various mutations including missense, nonsense, and frameshift mutations, and a deletion mutation [97]. Most of the mutations are found in ATR-X, whereas a single missense mutation in the helicase motif V is found in JMS. JMS is a rare X-linked recessive condition characterized by severe

mental retardation, growth failure, sensorineural deafness, microgenitalism, and early death (table 1). The difference in JMS and ATR-X phenotypes and the variation in ATR-X would result from different mutations in the XH2 gene by mechanisms including an alteration in the XH2 protein structure and destruction of the domains indispensable for interactions with specific proteins. Furthermore, the phenotypes would vary depending on environmental and other genetic factors. In support of this concept, the dosage of XH2 mRNA appears to be associated with variable degrees of urogenital abnormalities even within a single affected family [97]. The XH2 protein, in analogy to proteins belonging to the yeast SNF2/SWI2 protein family, is thought to be involved in the regulation of gene expression by influencing chromatin structure. This concept is supported by the facts that the XH2 protein interacts with the chromatin-associated EZH2 protein [98], and that the level of α-globin mRNA is reduced in ATR-X. α-Globin expression, but not β-globin expression, is affected by the XH2 protein, reflecting their different chromosomal environments [99]. Thus, α-thalassemia in ATR-X is thought to result from reduced α-globin expression due to XH2 gene defects, and other ATR-X and JMS phenotypes are also thought to result from perturbation in the expression of many other as yet unidentified genes induced by XH2 gene defects [100]. Among the phenotypes, mental retardation is a common feature to ATR-X and JMS. It is suggestive that the XH2 transcript is particularly abundant in human and mouse brain, and that, in the developing mouse brain, the gene is highly expressed in areas of neural proliferation [98].

Helicase disorders and ordinary aging

Aging appears to affect several pathways in multiple processes in humans, resulting in the occurrence of various senescent phenotypes. At least some of the pathways appear to be unique to humans. In this context, segmental progeroid syndromes are potentially relevant to aging in humans. These syndromes should therefore be useful to investigate pathways generating age-related phenotypes specific to humans. Among these syndromes, the seven disorders, including several high-ranked segmental progeroid syndromes in particular, have been found to be caused by mutations in helicase genes. This implies some contribution of aberrant helicases to phenotypes in ordinary aging. Indeed, there are a few findings consistent with this implication. An example is that frameshift mutation in a polyadenine repeat is found in the coding region of the BLM gene in genetically unstable sporadic gastrointestinal tumors [101]. Another example is that a case control study suggested that homozygotes, but not heterozygotes, for a polymorphic missense variant at amino acid 1367 in the WRN protein have a higher risk for myocardial infarction [102], although this study did not use family-based controls. This result suggests that the missense change itself may affect some function of the WRN protein, because the protein with certain missense change may be inhibited from interacting with specific molecules, possibly causing some of the WS phenotypes. Alternatively, this result could reflect that variation in some locus other than the WRN gene or of some other gene in linkage disequilibrium with the missense change is associated with myocardial infarction. The finding that heterozygotes for the missense variant fail to show an association with myocardial infarction is also reasonable because a relatively small amount of the WRN protein functions adequately [103].

Do the helicase disorders share any causative pathways for their age-related phenotypes, and if so, are these pathways also involved in ordinary aging? Each of the helicase disorders appears to have, with a few exceptions, unique pathways generating phenotypes. Nevertheless, overall, these disorders have pathway defects involved in the same specific processes corresponding to the phenotypes (table 2). Cancer predisposition appears to be caused by either a spontaneous or UV-induced elevated mutation rate due mainly to defects in DNA repair and/or recombination; neurologic abnormalities appear to be caused by apoptotic neuronal cell death due to defects in DNA repair and/or aberrations in transcription; and various other phenotypes in the disorders are most likely caused by aberrations in transcription. Thus, the various phenotypes of helicase disorders appear to be caused by aberrations in multiple processes, especially in transcription. If such phenotypes include age-related alterations to some extent, helicase disorders may well have some phenotypic similarities to aging.

However, of the helicase disorders, WS has the most similarities to aging (table 2). For example, at least some of the genes overexpressed in WS fibroblasts are also overexpressed in senescent normal fibroblasts. This finding leads to the idea that WS and senescent fibroblasts enter a similar final pathway where multiple gene overexpression generates various age-related phenotypes [81]. Senescent phenotypes may then derive, at least in part, from altered gene expression in senescent fibroblasts [104]. According to this concept, altered gene expression in relatively few senescent fibroblasts would affect other cells and tissues through an altered, especially increased, amount of secreted functional molecules, resulting in some senescent phenotypes. Moreover, senescent fibroblasts are resistant to apoptotic death due to failure to suppress Bcl-2, and are therefore slow to clear [105]. Thus, if senescent fibroblasts lack the functions of the WRN protein, this lack could explain some senescent phenotypes in ordinary aging. Consistent with this concept, WRN gene expression is downregulated during

Table 2. Selected possible age-related phenotypes in helicase disorders and ordinary aging.

Phenotypes*	Helicase disorders† and ordinary aging							
	WS	CS	TTD	XP	BS	JMS	ATR-X	Aging
1) Helicase activity of causative proteins	○	○	○	○	○			
2) Aberration in DNA replication	○				○			○
3) Aberration in DNA repair	○	○	○	○	○			○
4) Aberration in recombination	○				○			○
5) Aberration in transcription	○	○	○	○	○	○	○	○
6) Aberration in apoptosis	○	○	○	○	○			○
7) Possible relation to chromatin structure	○							
8) Possible relation to telomere metabolism	○							
9) Partially dominant phenotype	○							
10) Evidence of direct involvement in age-related phenotype of ordinary aging	○				○			○

* Details in text.
† Abbreviations are as in Table 1.

fibroblast aging [106] and fibroblasts lacking functional WRN protein have decreased WRN promoter activity [107], although relatively low expression of the WRN gene is sufficient to prevent the onset of WS [103]. Furthermore, WS lymphoid cells [108], like senescent T cells [109], have increased susceptibility to Fas-induced apoptosis, with increased Fas and decreased Bcl-2 expression. This similarity suggests the presence of a common primary or secondary transcriptional pathway alteration in lymphocytes of WS and ordinary aging. This alteration might cause susceptibility to apoptosis, resulting in immunodeficiency in ordinary aging. However, the apparent absence of immunodeficiency in WS counters this simple explanation. Instead, the common alteration in the apoptotic pathway could have some association with increased autoantibody production in WS andordinary aging [110]. Other similarities between WS and ordinary aging include the partially dominant phenotype of WS and senescent cells with respect to DNA synthesis on the basis of cell hybridization studies. The cytoplasmic and nuclear environments are responsible for the retarded DNA synthesis in WS fibroblasts, suggesting that the retardation is a secondary consequence [93]. Similarly, the partial dominant phenotype of senescent cells could derive from a secondary effect on DNA synthesis. In fact, expression of several cyclin-dependent kinase inhibitors including p21 and p16 increases transcriptionally during aging, contributing to decreased DNA synthesis. Taken together, a possible decline in function of the WRN protein in senescent cells could explain some phenotypes in ordinary aging. In other words, aberrant WRN protein could play a role in pathways generating senescent phenotypes in ordinary aging. Alternatively, WS and ordinary aging may merely have similar pathways generating age-related phenotypes. Whatever the case, the many and various common features between WS and ordinary aging are unlikely to occur independently.

Helicase disorders other than WS are less easy to relate to ordinary aging. Nevertheless because helicase activity is important for various aspects of DNA metabolism including transcriptional regulation which could be involved in the expression of many genes, at least pleiotropy resulting from aberrations in multiple systems due to helicase defects appears to be related to the apparent relationship between helicase disorders and ordinary aging (table 2). Among the putative helicases, the XPB, XPD, BLM, and WRN proteins have helicase activity. The CSB protein lacks helicase activity but functions in pathways in which helicases are involved. Whether the XH2 protein has helicase activity and whether it functions in pathways in which helicases are involved remain to be established, but it appears to be involved in the regulation of gene expression. Moreover, in this context, the ATM protein, which is a kinase not a helicase, encoded by the gene responsible for ataxia telangiectasia, a high-ranked segmental progeroid syndrome, also has a pleiotropic fundamental function involved in cell cycle checkpoints, DNA repair, and apoptosis, although the involvement of the protein in transcription is unknown.

Why do some helicases including CSB protein and yeast SNF2/SWI2 protein lack helicase activity despite having the seven helicase motifs? A possible explanation is that the motifs have functions other than helicase activity. A candidate is some function requiring nucleic-acid-dependent NTPase activity, because the CSB protein [19] and the SNF2/SWI2 protein [111] have DNA-stimulated ATPase activity. The helicase-like region of the SNF2/SWI2 protein is found to be necessary for transcriptional activation [111]. Other candidate functions of the motifs include interaction with some molecules such as p53 [112].

Finally, alterations in telomere metabolism [113] and in chromatin structure are known to cause altered gene expression. The probable relationships of the WRN protein to telomere metabolism [74, 114] and to chro-

matin structure [92], and of the XH2 protein to chromatin structure [98] therefore raise the possibility that altered transcriptional regulation in the two disorders is a secondary consequence and that the two disorders and ordinary aging have similar mechanisms for this altered transcriptional regulation (table 2).

Concluding remarks and future work

Helicase disorders and ordinary aging appear to share some similar pathways generating age-related phenotypes. Strategies to confirm this tentative conclusion include identifying proteins interacting with the causative helicases in helicase disorders, because there are many unknown pathways to be elucidated, most of which would include protein-protein interactions. This strategy should identify many unknown pathways generating age-related phenotypes. Subsequent association studies using protein polymorphisms affecting the pathways could reveal the pathological significance of the pathways in humans. Furthermore, similar pathways in other organisms could be studied and altered to investigate their pathological significance. However, completing these studies in every helicase disorder is time consuming. In addition, at least in humans, different kinds of cells sometimes respond differently to the same stimulus. For example, in ordinary aging, fibroblasts and lymphocytes appear to have different alterations in apoptotic pathways, and in WS, fibroblasts and lymphocytes show defects in different DNA repair pathways [75, 76]. Thus, different kinds of cells appear to make unique contributions to age-related phenotypes. However, identifying many unknown pathways in every kind of cell is much more time-consuming. In this context, high-throughput methods used in proteomics and genomics including microchip technology are helpful to obtain preliminary information about protein-protein interactions. These methods are also helpful to study alterations in gene expression in the helicase disorders and in ordinary aging. Research based on such information will be effective for clarifying the pathways through which each helicase disorder generates age-related phenotypes, leading to an improved understanding of the relationship between the disorders and ordinary aging. This should be a basis for developing further strategies for humans to live a healthy life, with prevenion of many disorders.

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