DNA helicases in inherited human disorders

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Six known or predicted helicases that are mutated in human syndromes are now recognized. These syndromes include xeroderma pigmentosum, Cockayne's syndrome, trichothiodystrophy, Bloom's syndrome, Werner's syndrome, and α-thalassemia mental retardation on the X chromosome. The clinical abnormalities in these syndromes cover a broad spectrum, pointing to different cellular processes of DNA manipulation that are defective in these syndromes.

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Abbreviations

BS	Bloom's syndrome
CHO	Chinese hamster ovary
CPDs	cyclobutane pyrimidine dimers
CS	Cockayne's syndrome
NER	nucleotide excision repair
NTPs	nucleotide triphosphates
TTD	trichothiodystrophy
WS	Werner's syndrome
XP	xeroderma pigmentosum

Introduction

DNA and RNA helicases are a ubiquitous and diverse group of enzymes defined by their capacity to catalyze the unwinding of duplex nucleic acid molecules. They participate in multiple cellular processes, including DNA replication, DNA repair, recombination, transcription, transcriptional regulation, RNA processing, and translation. In fact, in every process involving the manipulation of nucleic acids, helicases play important roles.

The diversity of functions in which helicases operate is reflected in the number of different helicases present in prokaryotic and eukaryotic cells. *Escherichia coli* contains at least 12 biochemically distinct helicases [1,2]. On the basis of amino acid sequence analysis of the complete DNA sequence of the *Saccharomyces cerevisiae* genome, yeast contains as many as 41 helicase genes. A similar but necessarily less complete analysis of helicase genes in human cells reveals 31 genes (see Table 1). As these estimates are largely based on the presence of seven amino acid motifs identified in many known helicases (see below), the actual number of helicases in yeast and human is not known precisely.

The well-characterized helicases are known to be fine-tuned to perform specific tasks in particular cellular processes and these tasks are inevitably carried out in cooperation with other proteins. The primary goal of most research in helicases is to learn what job they do, how they do it, and with whom. Here, I review the six DNA helicase genes known to be mutated in certain rare human syndromes with a view towards their known and potential functions in the cell.

Table 1

Human helicase genes containing the seven helicase motifs*.

Gene (or protein)†	Accession number‡
DExH-box DNA helicases	
XPB; *ERCC3*	M31899
XPD; *ERCC2*	X52221; L47234
DDX11 (CHLR1)	U33833
DDX12 (CHLR2)	U33834
RECQL	L36140; D37984
BLM	U39817
WRN	L76937
CSB; *ERCC6*	L04791
ATRX	U09820; U72936–U72938
HRAD54	X97795
SNF2L1 (SMBP2)	P28370§; L24544
SNF2L2 (HBRM)	X72889
SNF2L3 (HIP116; HTLF)	Z46606
SNF2L4 (BRG-1)	U29175
DEAD-box RNA helicases	
DDX5 (p68)	X15729; X52104
DDX6 (RCK; p54)	Z11685; D17532
(p72)	U59321
(BAT1)	Z37166
(MRDB)	X98743
DDX10	U28042
(Gu; RNA helicase II)	U41387
DDX7 (NP52)	D26528
EIF4A (eIF4A-1)	D30655
(eIF4A-like)	P38919§
DDX1 (cl. 1042)	X70649
DExH-box RNA helicases	
DDX9 (RNA helicase A)	L13848; Y10658
DDX8 (HRH1)	D50487
SKIV2L (SKI2W; 170A)	Z48796
KIAA0134	D50924
(Mi-2)	X86691
Non DEAD/DExH box RNA helicase	
HUPF1	U59323

*Entries in the table were ascertained by text search queries of the GenBank, Swiss-Prot, and Protein Information Resource (PIR) database, by selected amino acid sequence queries of the non-redundant protein database of GenBank using BLASTp, by queries of the major helicase families using XREFdb, and by Medline literature searches. An analysis of ESTs (expressed sequence tags) was not included in these searches. †Gene names are, in most cases, those assigned by the Human Genome Organization and they can be found in the Genome Data Base. ‡All accession numbers are to GenBank entries unless otherwise noted. §Swiss-Prot accession number.

Biochemical properties of helicases

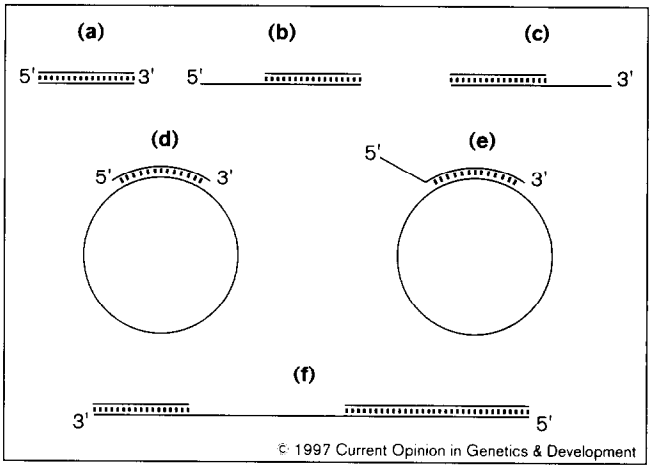
Helicase biochemistry has been reviewed recently [2,3], therefore only the most fundamental aspects will be treated here. Most of the firm biochemical knowledge of

DNA helicases comes from the analysis of bacterial and phage enzymes. DNA helicases can be distinguished by co-factor utilization, substrate preference, directionality of unwinding, processivity, and effects of other proteins on their activity; I will discuss these features briefly.

As DNA helicases unwind duplexes by hydrolysis of nucleotide triphosphates (NTPs) to catalyze the breakage of the hydrogen bonds holding the strands together, they are defined as DNA-dependent (or sometimes DNA-stimulated) NTPases. In general, helicases prefer to unwind a single type of substrate, namely, DNA–DNA, RNA–RNA, or DNA–RNA duplexes. The substrates typically used for defining specificity are composed of oligonucleotides annealed to each other or to a phage DNA molecule such as M13 (Fig. 1). Some helicases are active on blunt-ended duplexes (Fig. 1a); some are active on substrates with flanking 3′ or 5′ single-stranded DNA (Fig. 1 b–d); some require structures reminiscent of the replication fork (Fig. 1e). Helicases in general have a higher affinity for single-stranded than for double-stranded DNA. To show that unwinding occurs in a specific direction, a polarity substrate is used (Fig. 1f). Once actively unwinding a duplex, some helicases are highly processive, capable of unwinding thousands of base pairs without disengagement. On the other extreme, there are enzymes that unwind one or a few base pairs and then fall off. Helicase activity may either be inhibited or enhanced by the addition of other factors into the unwinding reaction, such as single-stranded binding proteins or topoisomerases.

All DNA helicases that have been characterized in detail are active as oligomeric structures, most often assemblages of two or six protomeric units. As each unit contains a DNA-binding site, oligomerization produces structures with multiple binding sites [3]. The multiple binding sites are thought to be required for processive unwinding or for translocating along a strand via intermediates in which two segments of DNA are simultaneously bound to two binding sites [3]. Models that involve 'rolling' or 'inching' have been proposed [3]. A recent crystal structure solution to a bacterial helicase has shed light on the mechanism by which NTP hydrolysis mediates helicase activity, as hydrolysis induces specific protein conformational changes [4•].

Figure 1



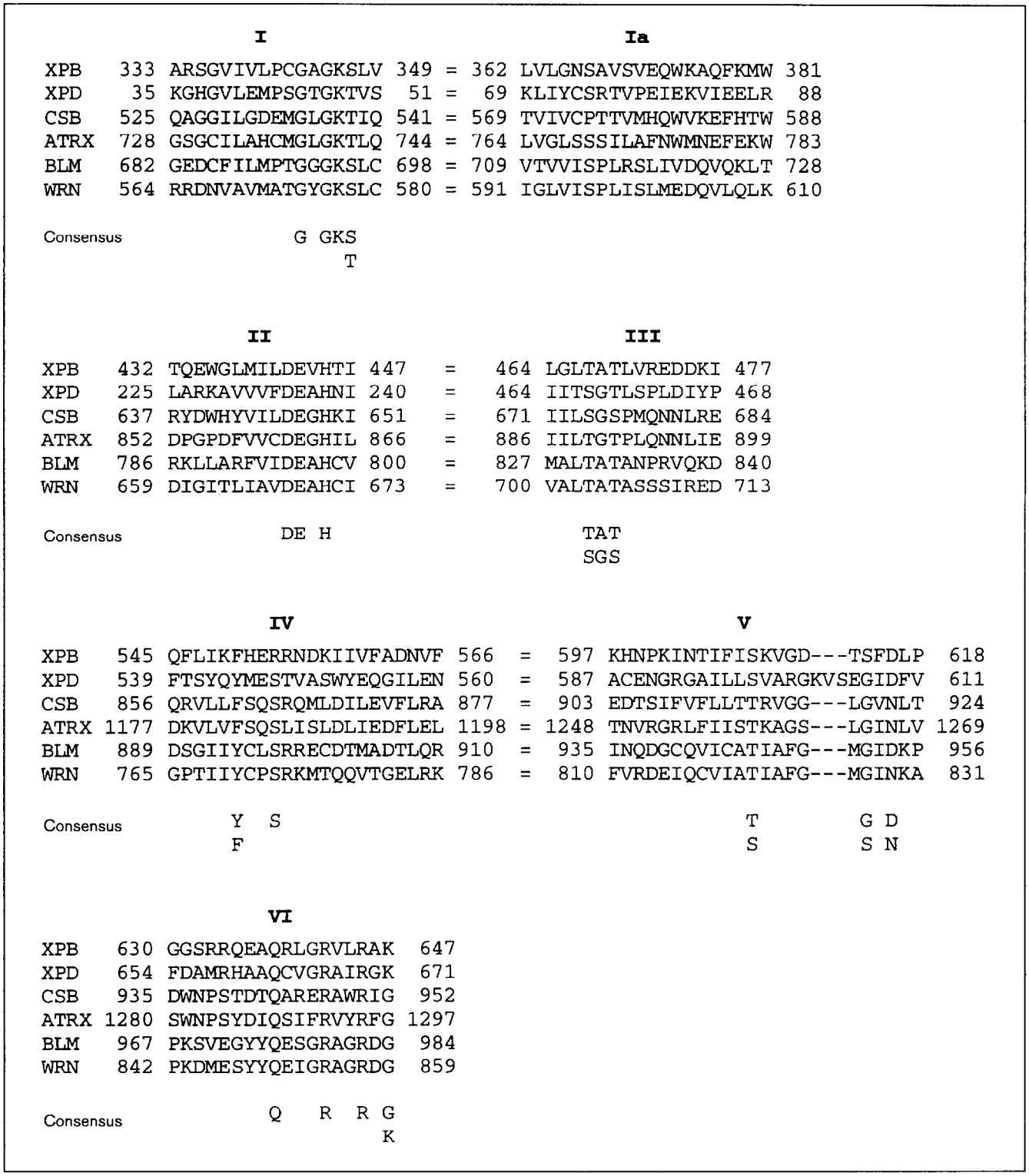
Substrates that are commonly used to assay helicase strand-displacement activity. A labeled oligonucleotide (typically 17 to 40 nucleotides long) is annealed either to an unlabeled complementary oligonucleotide or to single-stranded phage DNA, such as M13. Purified substrates are incubated with enzyme and NTP co-factor, and the products of the unwinding reaction are detected by nondenaturing polyacrylamide gel electrophoresis or by the sensitivity of the unwound product to single-strand-specific nucleases. In polyacrylamide gel electrophoresis, the singled-stranded, unwound product migrates faster. Many sorts of substrates can be generated for the assay; some representative substrates include **(a)** blunt ended duplex, **(b)** duplex with a single-stranded extension on the 5′ side, **(c)** duplex with a single-stranded extension on the 3′ side, **(d)** duplex with single-stranded extensions on both the 5′ and 3′ sides, **(e)** duplex with single-stranded extensions on the both the 5′ and 3′ sides as well as a 5′ non-homologous extension, resembling a replication fork, and **(f)** a polarity substrate. In (f), the oligonucleotide on the left side of the polarity substrate is smaller than the oligonucleotide on the right side, and the two oligonucleotides are distinguishable by polyacrylamide gel electrophorsis. If the oligonucleotide on the left side is unwound, then the polarity of unwinding is 5′ to 3′; if the oligonucleotide on the right side is unwound, then the polarity of unwinding is 3′ to 5′.

Helicase motifs

Most helicases contain seven amino acid motifs set in a region of 300–500 residues (Fig. 2). The first and third of these motifs, called I and II (also known as Walker boxes A and B), are present together in proteins that bind nucleotide triphosphates and are not specific to helicases [5]. Motifs Ia and III–VI are unique to the helicases, presumably reflecting conserved protein structures that mediate DNA binding and the catalysis of unwinding. Table 1 lists the 30 human genes or gene products known to contain the seven helicase motifs. On the basis of similarity in these motifs, these genes fall into several large families. DNA helicases contain a DExH box in motif II; RNA helicases contain predominantly either a DExH box or a DEAD box (the letters refer to amino acids in the single letter code). On the basis of homology, the DNA helicase group can be divided into subfamilies: *XPB* is part of the *S. cerevisiae* RAD 25 subfamily; *XPD*, *DDX11*, and *DDX12* are part of the yeast RAD3 subfamily; *RECQL*, *BLM*, and *WRN* are part of the *E. coli* RecQ subfamily; and the remainder are part of a large group named after the yeast SWI2/SNF2 protein, a group which is itself divided into several subfamilies [6].

The identification of a protein that contains the seven motifs predicts it to be a helicase; however, as will be seen, some of these helicase candidates fail to possess the classic strand-displacement activities one might expect. Rejecting such failed candidates as helicases could be the solution to the problem but the conservation of amino acids in the seven motifs begs an evolutionary question: what aspect

Figure 2



The seven helicase motifs of the six genes in which mutations have been identified in human genetic disorders. The Roman numerals label each motif, and the segment of each motif shown is that segment that was analyzed statistically in Gorbalenya *et al.* [5]. The residue numbers flank each motif, and the intervals between motifs is represented by an equals sign. Below each motif is a consensus that applies to this group of six gene products. On the left, the name of each gene product is given: XPB, xeroderma pigmentosum gene product B; XPD, xeroderma pigmentosum gene product D; CSB, Cockayne's syndrome gene product B; ATRX, α-thalassemia mental retardation on the X chromosome gene product; BLM, Bloom's syndrome gene product; WRN, Werner's syndrome gene product. The amino acid residues are shown in the single-letter code.

of their function is being conserved? Ultimately, what is needed is an understanding of the function of these candidates, their mechanisms of action, and the role the motifs play in function and mechanism. For the purposes of this review, they will be called helicase genes, even though they may lack classic helicase activity.

DNA repair, transcription initiation, and human helicase genes

Nucleotide excision repair (NER) is the major pathway for repairing bulky DNA adducts and lesions caused by UV irradiation, which are predominantly cyclobutane pyrimidine dimers and pyrimidine-pyrimidone (6-4) photoproducts [7,8]. The genes of the NER pathway are conserved from yeasts to mammals and a closely related pathway exists in *E. coli* [7,8]. Genes acting in the NER pathway are mutated in persons with the rare human syndrome xeroderma pigmentosum (XP; see Table 2). As a consequence, individuals with XP are affected primarily by characteristic skin lesions and cancers because their cells are defective in repair of UV damage caused by sunlight.

In XP, there are seven genetic complementation groups; two genes, *XPB* and *XPD*, encode helicases that are defective in certain individuals with XP (Table 3). The

Table 2

Human genetic disorders in which known or predicted DNA helicases are mutated.

Syndrome*	Disease	Inheritance	Selected clinical features	Cancer predisposition	Selected cellular phenotypes
Xeroderma pigmentosum†	XP	Autosomal recessive	Skin abnormalities Photosensitivity Cataracts Neurological abnormalities‡	Skin cancer, all types	Increased UV sensitivity Increased mutation rate post-irradiation Decreased unscheduled DNA synthesis (UDS) Decreased repair of cyclobutane pyrimidine dimers (CPDs) and $6\text{-}4$ photoproducts
Cockayne's syndrome†	CS	Autosomal recessive	Growth deficiency Skeletal abnormalities Photosensitivity Mental degeneration Characteristic facies Cataracts Dental cavities	None§	Increased UV sensitivity Decreased repair of DNA lesions of the transcribed DNA strand Decreased recovery of DNA synthesis post-irradiation Decreased recovery of RNA synthesis post-irradiation
Trichothiodystrophy†#	TTD	Autosomal recessive	Sulfur-deficient brittle hair Growth deficiency Mental retardation Ichthyosis Characteristic facies Photosensitivity** Subfertility	None	Increased UV sensitivity Decreased repair of CPDs and/or $6\text{-}4$ photoproducts Decreased recovery of RNA synthesis post-irradiation Decreased UDS
Bloom's syndrome††	BS	Autosomal recessive	Proportional dwarfism Sun-sensitive facial erythema Characteristic facies Moderate immunodeficiency Infertility/subfertility Diabetes	All types, including (but not exclusively) non-Hodgkin's lymphoma, acute lymphocytic leukemia, acute non-lymphocytic leukemia, colon cancer, breast cancer, and skin cancer	Sensitivity to alkylating agents Increased spontaneous chromosome breakage Increased interchromatid exchange, including high sister-chromatid exchange Increased somatic-cell mutation frequency and rate Reduced replication fork elongation rate
Werner's syndrome††	WS	Autosomal recessive	Premature graying and/or alopecia Atrophy of extremities Atherosclerosis/arteriosclerosis Osteoporosis Diabetes Subfertility	Disproportionately many non-epithelial cancers, including soft tissue sarcoma, osteosarcoma, melanoma, meningioma, and hematological disorders	Reduced cell division potential of fibroblasts in culture Variegated chromosomal mosaicism Increased somatic cell mutation frequency and rate Increased telomere DNA shortening
α-Thalassemia mental retardation on the the X chromosome‡‡	ATR-X	X-linked recessive	α-Thalassemia Developmental delay Severe mental retardation Characteristic facies Genital abnormalities	None	HbH inclusions in red cells

*For all the syndromes listed except WS, a person is usually diagnosed with the syndrome in their first decade of life. In WS, diagnosis is post-pubertal, usually in the third decade of life. †In XP, CS, and TTD, multiple disease genes are known that can be mutated in these syndromes. Gene mutations can be categorized on the basis of a complementation test in which cell strains derived from different individuals are fused and resulting hybrids are exposed to UV light and tested for unscheduled DNA synthesis (UDS) or recovery of RNA or of DNA synthesis. Complementation occurs when the mutations are in different genes and non-complementation when they are in the same gene. By this means, seven complementation groups have been defined in XP (XP groups A–G), five groups in CS (CS groups A and B and XP groups B, D and G), and three groups in TTD (TTD group A and XP groups B and D). XP and CS are sometimes present together in the same person and some TTD cells fail to complement XPB and XPD cells. There is also a form of XP known as XP variant in which the classical clinical features of XP are present but in which the typical cellular phenotypes are absent. ‡Neurological abnormalities are expressed in XP complementation groups A, B, D, and G, but not in groups C, E, and F. §Cancer predisposition in CS has not been reported; however, mean life expectancy in CS is 12 years. As development of cancer is an age-dependent phenomenon and may depend on an environmental stimulus, such as sun exposure, the opportunity to record cancer in CS may not have arisen. #TTD is defined clinically by hair anomalies and characteristic facies. The other clinical features associated with TTD are variably expressed. **Photosensitivity is known to be expressed in TTD in which mutations have been defined in *XPB* and *XPD* by complementation and/or mutational analysis. ††All current evidence in BS and WS supports the interpretation that these disorders are monogenic. ‡‡The ATR-X syndrome was defined initially by those cases of α-thalassemia and mental retardation in which the α-globin genes on human chromosome 16 were not deleted and in which X-linkage was detectable by pedigree analysis. Subsequently, it was shown that α-thalassemia is variably expressed and that at least one X-linked mental retardation syndrome, Coffin–Lowry syndrome in which HbH inclusions and genital abnormalities are not clinical features, results from mutation in the gene mutated in ATR-X families (see text).

XPB and *XPD* genes were cloned by complementation of the UV sensitivity of CHO cell strains mutated in the orthologous Chinese hamster genes [9,10]. There are but a few individuals known with mutations in the *XPB* gene [10–12], and some of these individuals exhibit a second striking phenotype in addition to XP, namely Cockayne's syndrome (CS; see Table 2). The remainder exhibit the syndrome trichothiodystrophy (TTD; see Table 2) an entity that is distinct from XP but which shares certain features with CS [13]. The variation of phenotype resulting from *XPB* mutations is, in a way not completely understood, dependent on the kind of mutation in the *XPB* gene. Many of the same considerations apply to mutations in the *XPD* gene (Table 3), except that most of them result in XP in the absence of CS and mutations in *XPD* are more frequent than mutations in *XPB* [14–16]. No person has been identified who is homozygous for a null mutation in either *XPB* or *XPD*.

The observation of clinically distinct syndromes in an individual points to a dual role played by the *XPB* and *XPD* products. The two products fulfill that dual role as components of a six-protein complex, TFIIH, which is an essential part of the transcription initiation machinery in human and yeast [17,18]. One key function of TFIIH is to unwind the DNA duplex in order that RNA polymerase II can gain access and enter the duplex. TFIIH also unwinds the duplex at a site of a DNA lesion prior to excision of the damaged strand and DNA repair synthesis [7,8]. The helicase activities of XPB and XPD have opposite polarities [19,20] and mutational analysis indicates that both activities are required in NER; therefore, unwinding in both the 5′ to 3′ and 3′ to 5′ directions occurs in the execution of normal THIIH function.

From the abnormalities in development that are characteristic of CS (Table 2) and the fact that TFIIH is an essential component of the transcription initiation machinery that is mutated in CS, it has been suggested that the developmental abnormalities arise from a defect in transcription [21]. This hypothesis has been challenged recently with evidence that the repair of oxidative damage — which unlike UV damage, is expected to occur spontaneously — is also defective in persons with both XP and CS [22•]. In addition to NER, the *XPB* and *XPD* products have been implicated in apoptosis. THIIH activity is modulated *in vitro* by p53 [23•] and XPB and XPD cells have a deficiency in apoptotic response [24].

In both prokaryotes and eukaryotes, the transcribed strand is repaired more efficiently than the non-transcribed strand or DNA that is untranscribed (transcription-coupled repair; reviewed in [21]). During transcription, RNA polymerase will stall at a site if it encounters a DNA lesion there. For repair to occur, specific factors must act to get the polymerase away from the site of the DNA lesion. In human, defects in transcription-coupled repair are observed in cells from individuals with CS. As mutations in *XPB* and *XPD* can result in CS and the XPB and XPD gene products are part of TFIIH, TFIIH plays a role in transcription-coupled repair. In addition to *XPB* and *XPD*, three other genes are known in which mutations result in CS; these are *XPG*, *CSA*, and *CSB*. Like the XPB and XPD gene products, CSB (Table 3) contains seven helicase motifs; these motifs are most homologous to those proteins of the yeast SWI2/SNF2 subfamily [25]. CSB interacts with various components of the NER machinery and it has DNA-stimulated ATPase activity; however, it does not possess DNA strand-displacement activity [26]. It has been suggested that CSB displaces RNA polymerase when it is stalled at the site of a DNA lesion in a manner analogous to bacterial Mfd (mutation frequency decline) activity [27]. However, *in vitro* evidence indicates that human RNA polymerase is not dissociated from the DNA by CSB [26], therefore the mechanism may be novel.

Transcriptional regulation and DNA helicase genes

Yeast SWI2/SNF2 contains the seven helicase motifs and is part of a large ten-protein complex (the SWI/SNF complex) that operates as part of the transcriptional regulation machinery [28]. When components of the SWI/SNF complex are mutated, the transcriptional activation of many genes fails to occur [28,29]. Transcriptional activation depends on the SWI/SNF complex's function in providing access of transcription factors to DNA in chromatin [30,31,32•]. DNA in the nucleus is packaged into nucleosomes in a way that, in general, leads to transcriptional silencing. The SWI/SNF complex generates a transcriptionally active form of chromatin perhaps through histone acetylation and by helping to displace a histone octamer from the promoter regions of genes [32•,33,34]. Consistent with this notion, the SWI/SNF complex interacts with specific transcriptional activators [30,35]. SWI2/SNF2 in the SWI/SNF complex has DNA-stimulated ATPase but not strand-displacement activity [30]. SWI2/SNF2, thus, apparently does not open the DNA duplex; instead, its function in the SWI/SNF complex may be as a kind of molecular motor. Perhaps SWI2/SNF2 is a protein in which the helicase domain has evolved a DNA–protein displacement activity.

The SWI/SNF complex is conserved in higher eukaryotes [6,36–38]. At least four human proteins are known with a helicase domain homologous to SWI2/SNF2 (see Table 1). By the way these genes were identified, they are implicated in transcriptional regulation [39–42]. Two of these gene products, HBRM and BRG-1, are present in specific SWI/SNF complexes [36].

In addition to these four genes, there is a recently defined *SWI2/SNF2*-like gene on the X chromosome [43••] that, when mutated, results in a severe mental retardation syndrome (see Table 2). This mental retardation syndrome (ATR-X) was defined initially on the basis of α-thalassemia [44] as the red cells in such persons contain HbH

Table 3

Features and functions of genes identified in disorders of human helicase genes.

Gene	Chromosomal localization	Mutated in	Gene product	Factor	Function
XPB (*ERCC3**)	2q21	XP/CS† and TTD	782 amino acids *S. cerevisiae* Rad25p (Ssl2p)	TFIIH	Transcription and nucleotide excision repair DNA helicase activity with 3′→5′ polarity
XPD (*ERCC2**)	19q13	XP, XP/CS†, and TTD	760 amino acids *S. cerevisiae* Rad 3p *S. pombe* Rad15	TFIIH	Transcription and nucleotide excision repair DNA helicase activity with 5′→3′ polarity
CSB (*ERCC6**)	10q11	CS	1493 amino acids *S. cerevisiae* Rad 26p SWI2/SNF2 helicase subfamily	Binds to XPA, TFIIH, CSA, TFIIE, and XPG	Transcription-coupled nucleotide excision repair DNA-stimulated ATPase
BLM	15q26.1	BS	1417 amino acids *S. cerevisiae* Sgs1p *S. pombe* Rqh1 RecQ DNA helicase subfamily	Unknown Unknown	Unknown Unknown
WRN	8p11.1-21.1	WS	1432 amino acids *S. cerevisiae* Sgs1p *S. pombe* Rqh1 RecQ DNA helicase subfamily		
ATRX‡	Xq13.3	ATR-X	2375 amino acids SWI2/SNF2 helicase subfamily	SWI/SNF complex (proposed)	Chromatin accessibility (proposed)

**ERCC, excision repair cross-complementing.* These are human genes that can complement the UV sensitivity of CHO cell strains that were isolated on the basis of UV hypersensitivity. Some of these cell strains contain mutations in Chinese hamster genes homologous to certain human XP and CS genes. †This is a clinical phenotype in which a person manifests the features of both XP and CS. ‡Formally referred to as XNP, XH2, and RAD54L.

inclusions (that is, hemoglobin B tetramers) that stain with brilliant cresyl blue. The α-thalassemia and genital abnormalities, however, are variably expressed [45,46,47•]. The gene, which has been given several names including *XNP*, *XH2*, *RAD54L*, and now *ATRX*, was cloned as part of an effort to characterize a region of the X chromosome that contains interesting genes [48,49]. The mutations that have been characterized in *ATRX* lead to the reduction or complete loss of function of the gene product and some of the variability in expressivity in the syndrome is caused by the mutation types [50•]. The depression of α-globin synthesis, the absence of any defects relating to the synthesis or repair of DNA, and the genetic relationship of the mutated gene to the *SWI2/SNF2* family implicates *ATRX* in transcriptional regulation, possibly as a component of a particular SWI/SNF complex. If this is true, one might predict that ATRX possesses DNA-stimulated ATPase but not strand-displacement activities. Like CSB, ATRX contains domains other than its helicase domain, which presumably contribute to the specificity of interaction with as yet unidentified gene products [43••,50•].

Chromosome instability and helicases

In XP and CS, lesions that are repaired by the NER pathway stimulate chromosome abnormalities seen at metaphase. In two human syndromes in which chromosome instability is detected spontaneously — that is, in the absence of any agents added to the culture medium — two helicase genes have been identified recently [51••,52••]. In Bloom's syndrome (BS; see Table 2), the chromosome instability is exhibited by increased chromosome breakage and increased chromatid exchanges both between homologous chromosomes and between sister chromatids [53]. The high sister-chromatid exchange is a diagnostic feature of BS and one that is unique in human genetics. In Werner's syndrome (WS; see Table 2), chromosome instability is exhibited by variegated translocation mosaicism, that is, WS cells carrying different karyotypic abnormalities — including inversions, translocations, and chromosomes losses — are detected in different clonal populations [54]. In both syndromes, the rate of spontaneous mutations is increased [55,56] but DNA repair pathways appear to be functioning normally. Altogether, the evidence suggests that BS and WS cells are defective in some aspect of DNA synthesis.

The genes mutated in BS and WS, *BLM* and *WRN* (see Table 3), were identified by positional cloning using detailed physical and genetic maps [57,58]. Both *BLM* and *WRN* were localized by homozygosity mapping [59,60], and linkage disequilibrium in Ashkenazi Jews with BS and in Japanese with WS was detected, having arisen in these groups by genetic drift/founder effect [61–63]. In

addition, *BLM* was localized by a novel technique using cell lines in which intragenic recombination had corrected to normal the high sister chromatid exchange phenotype of the BS cells [64]. The kinds of mutations that have been identified in *BLM* [51••] and *WRN* [52••,65–67] result in loss-of-function of the gene products, that is, in the generation of premature translation termination. Both the BLM and WRN products are members of the RecQ helicase subfamily.

Two groups have purified RECQL, a major ATPase in HeLa cells that is 44% homologous to bacterial RecQ in its helicase domain [68,69]. Mutations in bacterial *recQ* confer a hypo-recombinogenic phenotype to the cell because the gene product is required in the RecF pathway of recombination [70]; but no mutations in human *RECQL* are known. RECQL and RecQ [71] are DNA helicases, having amino acid lengths that are similar to those of XPB and XPD. BLM and WRN contain twice as many amino acids. Thus, like CSB and ATRX, additional domains are present that most likely play important roles in BLM and WRN function.

Clinically, BS and WS are very different entities. Individuals with BS exhibit growth retardation that is detectable *in utero* and is present throughout life [72]. BS individuals exhibit a defect in immunity and in fertility, and suffer from photosensitivity. Individuals with WS have normal stature until puberty, when they fail to undergo the adolescent growth spurt [73]. WS individuals have many features of premature aging (for example, hair loss, diabetes, osteoporosis) and WS cells in culture have a more limited life span than normal cells. Both syndromes feature cancer predisposition but of different types: in BS, the cancer spectrum resembles that seen in the general population [72]; in WS, non-epithelial cancers predominate [74]. The differences in the clinical features and cellular phenotypes thus point to differences in both the function of BLM and WRN as well as the cell types in which they are functioning.

In *S. cerevisiae* and *Schizosaccharomyces pombe*, mutations have been identified in genes homologous in their helicase domains to RecQ, BLM, and WRN. In *S. cerevisiae*, one RecQ helicase exists, known as SGS1, and *sgs1* mutants exhibit an increase in chromosome loss and a hyper-recombination phenotype [75,76]. *Sgs1* mutations were first identified as suppressers of the slow growth phenotype of topoisomerase 3 [75] and biochemical studies indicate that a direct physical interaction occurs between both topoisomerases 2 and 3 and Sgs1p [75,77•]. Mutation in *sgs1* need not affect topoisomerase activities directly, however, because *sgs1* genes encoding amino acid substitutions that inactivate the helicase activity can complement the slow-growth defect of *sgs1/top3* cells [78].

In *S. pombe*, a RecQ helicase gene, *rqh1*, was identified in certain mutants that are sensitive to hydroxyurea [79], a drug that blocks DNA replication. *Rqh1* mutants are allelic to *S. pombe rad12* mutants, which are hypersensitive to both UV and γ-ray irradiation. It has been suggested that BLM, WRN, Sgs1p, and Rqh1 all function to control the levels of genetic exchange in mitotic cells, as mutations in these genes result in hyper-recombination [75,79]. It is not clear, however, that hyper-recombination is a direct effect of these mutations as opposed to a secondary effect, such as a response to failure during DNA synthesis to resolve or maturate certain replication intermediates. For example, some of these products may function in assissting DNA polymerase when it is blocked at the site of a DNA lesion. The increased genetic exchange in these mutants is a vital clue to understanding the role their products play during DNA synthesis. Yet it is possible that BLM, WRN, Sgs1p and Rqh1 have different functions in the cell and their absence leads to hyper-recombination by different means.

Conclusions

As many new predicted human helicases are coming to light—the complete characterization of which is inseparable from an understanding of the processes and functions in which they engage—they are in a sense signposts of a hidden machinery in the cell. The state of genetic and biochemical knowledge is developed to a different degree for each of the human helicase genes reviewed here. It ranges from a detailed description of function of XPB and XPD, which operate as the DNA-unwinding enzymes for TFIIH in transcription initiation and NER; to the less well defined functions of CSB in transcription-coupled repair, which operates on RNA polymerases stuck at DNA lesions, and of ATRX in transcriptional regulation, which may be a member of a specific SWI/SNF complex; to an undefined role of BLM and WRN in DNA synthesis and genomic stability, the defect resulting in increased mutation and hyper-recombination. In the future, there is no doubt that additional human helicase genes will be discovered that are mutated in interesting clinical entities.

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