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DNA helicases involved in DNA repair and their roles in cancer

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Abstract

Helicases have major roles in genome maintenance by unwinding structured nucleic acids. Their prominence is marked by various cancers and genetic disorders that are linked to helicase defects. Although considerable effort has been made to understand the functions of DNA helicases that are important for genomic stability and cellular homeostasis, the complexity of the DNA damage response leaves us with unanswered questions regarding how helicase-dependent DNA repair pathways are regulated and coordinated with cell cycle checkpoints. Further studies may open the door to targeting helicases in order to improve cancer treatments based on DNA-damaging chemotherapy or radiation.

Helicases are classically defined as molecular motors that are able to couple nucleoside triphosphate (NTP) hydrolysis (typically of ATP) to the unwinding of polynucleic acids¹ (FIG. 1). In doing so, the helicase translocates in a directionally specific manner (3' to 5' or 5' to 3') along the strand it predominantly interacts with. DNA helicases are classified according to their amino acid sequence homology in the ATPase/helicase core domain into two larger superfamilies, superfamily 1 (SF1) and SF2, and four smaller superfamilies¹. Although DNA helicases are conventionally known to unwind B-form duplex DNA, some can unwind alternative DNA structures or have specialized functions (FIG. 1). There are an estimated 95 helicases or putative helicases (31 DNA helicases and 64 RNA helicases) encoded by the human genome². Helicases are ubiquitous in nature and their functions depend on various factors including cell lineage, environmental stress, cell cycle stage and genetic background. Since the discovery of the first DNA helicase in 1976 (REF. 3), researchers have characterized ATP-dependent DNA-unwinding enzymes from all kingdoms of life, and from bacteriophages and eukaryotic viruses, thus providing an immense wealth of information about their mechanistic roles.

Competing interests statement

The author declares no competing financial interests.

FURTHER INFORMATION

Author's homepage: <http://www.irp.nia.nih.gov/branches/lmg/rbrosh.htm>

Fanconi Anemia Mutation Database: <http://www.rockefeller.edu/fanconi/mutate>

The International Registry of Werner Syndrome: <http://www.wernersyndrome.org>

The Bloom's Syndrome Registry: <http://www.med.cornell.edu/bsr>

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Chemical damage to DNA can perturb cellular replication and transcription, and is implicated in mutagenesis, cell lethality, carcinogenesis, ageing and neurological disorders. Helicase-dependent DNA repair systems and DNA damage tolerance mechanisms exist to preserve the informational content and integrity of the genome and to permit timely and efficient replication. Advances in understanding mechanistic and structural aspects of helicase function (BOX 1) suggest new avenues of research for helicase-targeted drugs to combat cancer and other diseases. The importance of DNA helicases in virtually all aspects of nucleic acid metabolism cannot be overestimated and places them at the forefront of biomedical research into genetic disorders, ageing and cancer biology. An important concept that governs all of helicase biology is the crucial role of protein–protein interactions in helicase function to preserve genomic stability, and understanding these interactions is a central area for future research.

Box 1

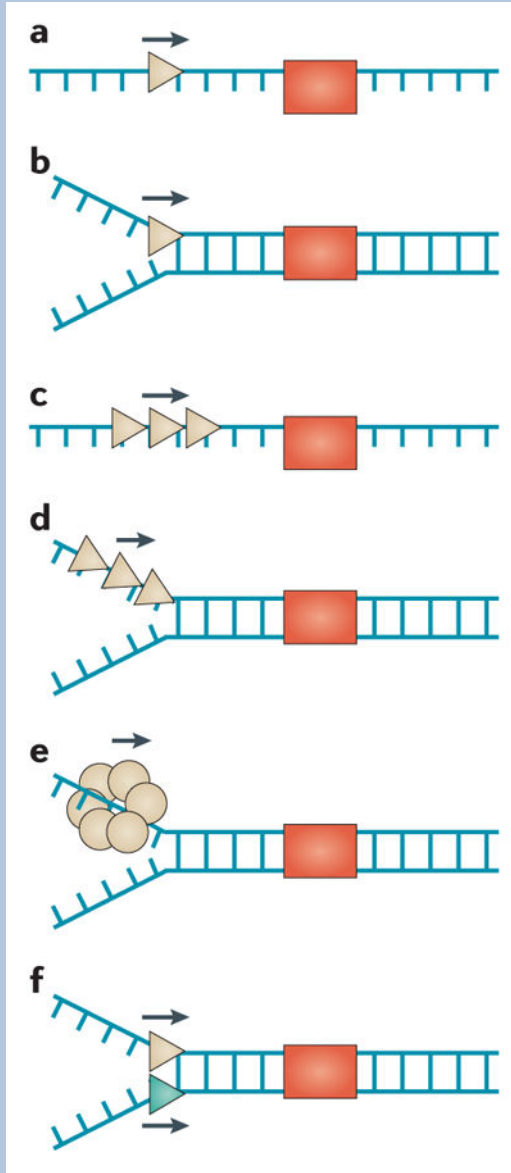
Advances in understanding helicase mechanisms

In certain cases, helicase monomers efficiently translocate along single-stranded DNA (ssDNA) and/or unwind double-stranded DNA (dsDNA) (see the figure, parts **a** and **b**), whereas multimerization or functional cooperation between monomers promotes dsDNA unwinding or the displacement of proteins bound to DNA, represented by red boxes¹⁹⁶ (see the figure, parts **c** and **d**). The prototypical *Escherichia coli* RecQ helicase operates by an inchworm mechanism involving ATP-driven movements of two DNA-binding domains remaining in the same relative orientation along the DNA lattice^{197,198}. By contrast, the replicative bacterial (DnaB) or eukaryotic (minichromosome maintenance (MCM)) helicase forms a hexameric ring-like structure that unwinds dsDNA by steric exclusion¹⁹⁹ (see the figure, part **e**). Coordinate action of helicases with opposite polarities provides a unique dual-motor mechanism^{89,200} (see the figure, part **f**). A rolling model for unwinding by a helicase homodimer has also been proposed²⁰¹. The efficiency of DNA translocation versus unwinding may be affected by mutations that uncouple catalytic activities^{202,203} or perturb oligomerization²⁰⁴. Certain helicases use their motor ATPase to disrupt protein DNA interactions to enable smooth replication progression or to regulate homologous recombination (HR) by stripping RAD51 from DNA²⁰⁵.

DNA helicases may act by repetitive movements on DNA^{206,207}. The Bloom syndrome helicase (BLM) was shown to unwind a single DNA duplex molecule, re-anneal it by strand switching, and re-initiate unwinding in successive cycles in a replication protein A (RPA)-dependent manner²⁰⁸. Helicase repetitive action may be important for remodelling stalled replication forks, displacing DNA-bound proteins, or preventing illegitimate recombination. This mechanism of action is quite distinct from a hexameric ring-shaped helicase that potentially regulates DNA unwinding during replication by ATP-induced slippage²⁰⁹. It remains to be shown if strand switching, repetitive unwinding, or slippage by a helicase occur *in vivo*.

Structural analyses of DNA helicases and their accessory domains (for example, human RECQ1 (REF. 210) and *Thermoplasma acidophilum* XPD²¹¹) have provided molecular insights to their unwinding mechanisms and disease pathology. Analysis of disease-

causing missense mutations in the context of domain structure has helped to explain the molecular basis for helicase dysfunction in DNA repair.



DNA helicases in genomic stability and cancer

Helicase-dependent mechanisms help cells to cope with endogenous or exogenous stress to prevent chromosomal instability and to maintain cellular homeostasis. Numerous genetic diseases that confer a predisposition to cancer are linked to mutations in genes encoding DNA-helicase-like proteins⁴ (TABLE 1). The expression of many DNA helicases is upregulated in transformed or neoplastic cells and tissues⁵⁻⁸ and is required for cancer cell proliferation or resistance to DNA damage imposed by chemotherapy (TABLE 2). Continuous upregulation of helicase gene expression in cancer reflects the need for an elevated DNA damage response to deal with replicative lesions that arise in highly

proliferative states. Conversely, chromosomal instability from loss of helicase functions in hereditary helicase disorders promotes carcinogenesis, thus implicating DNA helicases as a prominent class of genome caretakers.

The RecQ family

The five RecQ family DNA helicases (*RECQL1*, *BLM*, *WRN*, *RECQL4* and *RECQL5*) are highly conserved and are required for genomic stability^{9,10}. Three are implicated in genetic diseases that predispose to cancer: *BLM* in Bloom syndrome¹¹, *WRN* in Werner syndrome¹² and *RECQL4* in Rothmund–Thomson syndrome (RTS). In addition, *RECQL4* mutations are genetically linked to RAPADILINO syndrome and Baller–Gerold syndrome (BGS)^{13–15} (TABLE 1). Individuals affected by Bloom syndrome are diagnosed with a wide variety of cancer types earlier in life than the normal population. Patients with Werner syndrome display many features of premature ageing and frequently develop a broad range of neoplasias including thyroid epithelial tumours, malignant melanomas, meningiomas, soft-tissue sarcomas, haematological and lymphoid neoplasms, and osteosarcoma¹⁶. The three genetic disorders involving *RECQL4* mutations show overlapping clinical features and a broad range of severity, which is probably influenced by genetic or environmental factors. Individuals with RTS display growth retardation and photosensitivity with poikiloderma, hair thinning and loss, juvenile cataracts and a predisposition to osteogenic sarcomas. The range of tumour types in patients with *RECQL4* mutations includes lymphomas, predominantly in patients with RAPADILINO syndrome¹⁷. *RECQL4* has a crucial role in prostate carcinogenesis⁷. *RECQL4* expression increases as a function of tumour grade; furthermore, *RECQL4* depletion was shown to dramatically reduce the growth, survival and invasiveness of metastatic prostate cancer cells *in vitro*, and to decrease tumorigenicity in a mouse model⁷.

Although no genetic disorders have been linked to *RECQL1* or *RECQL5* mutations, these helicases are also likely to have important roles in cancer. *RECQL1* polymorphisms are associated with the survival of patients with pancreatic cancer^{18,19}. Despite the absence of phenotypes in unstressed *Recql1*-knockout mice, primary embryonic fibroblasts from these mice display chromosomal instability²⁰. *RECQL1*-depleted human cells display increased chromosomal instability²¹ and sensitivity to ionizing radiation²¹ — which is consistent with involvement in double-strand break (DSB) repair²² — or agents that induce oxidative damage²³ or replication stress²⁴ (TABLE 2). *RECQL1* is highly expressed in various cancers, and its depletion reduces tumour cell proliferation^{5,25–28}. *RECQL5* is also required for cancer cell proliferation²⁹. Moreover, *Recql5*-deficient mice are highly cancer-prone; approximately half of these cancers are lymphomas and the rest are solid tumours of different tissue origins³⁰. *RECQL5* has roles in transcription through its interaction with RNA polymerase II and in DNA repair by regulating homologous recombination (HR)^{30,31} and base-excision repair (BER)³².

The Fe-S family

Conserved iron–sulphur (Fe–S) clusters are found in metalloproteins that are implicated in DNA replication and repair³³. Of the four human Fe-S DNA helicases (*XPD*, *FANCI* (also known as *BRIP1* and *BACH1*), *RTEL1* and *DDX11*), all are implicated in autosomal

recessive genetic diseases: *XPD* in xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy (TTD) and cerebro-oculo-facio-skeletal (COFS) syndrome³⁴; *FANCF* in Fanconi anaemia (FA)^{35–37}; *DDX11* in Warsaw breakage syndrome (WABS)^{38,39}; and regulator of telomere elongation helicase 1 (*RTEL1*) in dyskeratosis congenita^{40–42} (TABLE 1). *XPD*, which was originally discovered through its linkage to xeroderma pigmentosum^{43,44}, encodes a helicase subunit of the transcription factor II H (TFIIH) complex that is implicated in basal transcription and nucleotide-excision repair (NER); recessive mutations in *XPD* result in diseases with potentially related or overlapping clinical features³⁴. The hallmark of xeroderma pigmentosum is severe sun sensitivity and predisposition to skin cancer. Although less common, individuals with *XPD* mutations may display xeroderma pigmentosum combined with Cockayne syndrome, characterized by developmental and neurological abnormalities as well as ultraviolet light sensitivity, but not skin cancer.

FA, arising from autosomal recessive mutations in *FANCF* or in any of at least 14 other genes, is characterized by progressive bone marrow failure, acute myeloid leukaemia and other cancers⁴⁵. In some cases, individuals with FA display congenital defects or developmental abnormalities that affect organ systems. All FA-mutant cell lines are hypersensitive to DNA-crosslinking chemotherapies. *FANCF* mutations are also associated with breast cancer, which is consistent with the physical interaction between *FANCF* and *BRCA1* and with the role of *FANCF* as a tumour suppressor⁴⁶.

The Fe-S helicase *RTEL1* is essential and has a dominant role in setting telomere length in mice⁴⁷. Studies with *Rtel1*-deficient mouse embryonic stem cells demonstrated that the helicase is required for DNA damage resistance and acts as an antirecombinase in HR or counteracts telomeric G-quadruplex (G4) DNA structures⁴⁸. *RTEL1* knockdown in human cells renders them sensitive to the DNA-crosslinking agent mitomycin C (MMC)⁴⁹. Genome-wide association studies have identified *RTEL1* as a susceptibility locus for human glioma^{50–52}. Recently, germline mutations in *RTEL1* were linked to dyskeratosis congenita^{40–42}, which is characterized by inherited bone marrow failure and cancer predisposition.

Other human DNA helicases that are important for genomic stability

Other human DNA helicases have genome caretaker roles (TABLE 1). *XPB*, which was first discovered by its genetic linkage to combined xeroderma pigmentosum and Cockayne syndrome^{53,54}, is a TFIIH component (like *XPD*) and is also implicated in xeroderma pigmentosum with neurological abnormalities and in TTD³⁴. Another DNA helicase, *PIF1*, suppresses apoptosis in human tumour cells⁵⁵, and mutations are associated with breast cancer susceptibility⁵⁶. Mutations in *senataxin*, which is a putative RNA/DNA helicase that is involved in transcription termination at RNA polymerase pause sites, are genetically linked to the neurodegenerative disorder ataxia with oculomotor apraxia 2 (AOA-2)^{57,58}. *DNA2*, which is a helicase-nuclease that is involved in Okazaki fragment processing⁵⁹, has prominent roles in DNA end resection during HR-mediated repair and in mitochondrial DNA maintenance^{60–62}. Mutations in the mitochondrial DNA helicase *twinkle* (also known

as *PEOI*) co-segregate with several neuromuscular degenerative disorders⁶³. The number of DNA helicases implicated in cancer and other diseases is likely to grow.

Helicase functions in response to DNA damage

The DNA damage response mediated by the intra-S-phase checkpoint and DNA repair pathways help cells to cope with a variety of chromosomal lesions that are major contributing factors to various forms of cancer. Helicases have unique roles in these pathways through fork remodelling, DNA damage recognition, damaged strand removal, and recombination-based strategies to restore genomic integrity. The requirement for helicases to carry out roles in DNA repair through their catalytic functions or interactions with other proteins continues to be an important area of study.

Consequences of DNA lesions on helicase functions

Recognition of DNA damage among billions of normal base pairs in the human genome is formidable. Many helicases (for example, WRN⁶⁴) preferentially bind and unwind forked DNA structures; therefore, they may arrive early at blocked replication forks to facilitate DNA damage recognition. Generally speaking, bulky covalent adducts inhibit helicase-catalysed DNA unwinding in a strand-specific manner; however, the type of adduct and/or helicase under investigation and its assembly in a protein complex is relevant⁶⁵. Fe-S cluster DNA helicases (for example, XPD) may collaborate with other DNA repair proteins that are able to carry out DNA charge transport to search for DNA damage⁶⁶. Helicase partnerships with the heterotrimer replication protein A (RPA)⁶⁷ or other single-stranded DNA (ssDNA)-binding proteins (for example, POT1)⁶⁸ enable them to unwind past certain DNA lesions. In some cases, replicative helicases (and their associated replisomes) may bypass or hop over template lesions, thus providing the opportunity for ssDNA signalling mechanisms to mediate DNA-damage-induced intra-S-phase checkpoints⁶⁹. XPD bypasses DNA-bound proteins *in vitro*⁷⁰; however, the physiological importance remains unclear.

DNA damage recognition by TFIIH helicases implicated in NER

NER relies on faithful recognition of bulky helix-distorting lesions and subsequent incisions upstream and downstream in the lesion-containing strand⁷¹ (FIG. 2a). In order for this to occur, TFIIH must be recruited to the site of DNA damage. The multi-subunit TFIIH complex possesses two opposite-polarity DNA helicases, XPD (5' to 3') and XPB (3' to 5'), which orchestrate the opening of duplex DNA around the lesion and verify the distorting DNA damage⁷². Considerable efforts have been made to understand the precise roles of XPB, XPD and their protein partners in the various steps that are necessary for NER: TFIIH recruitment, duplex opening and damage verification. XPB ATPase (but not helicase) activity is required for TFIIH recruitment to DNA damage⁷². Damage recognition in NER involves a scanning mechanism that is dependent on XPD helicase activity⁷³. The ability of XPD to sense a DNA lesion during unidirectional ATP-dependent translocation is required for DNA damage verification, which is necessary for NER⁷⁴.

Lesion verification also involves XPB. One model depicts XPB melting a modest 5 nucleotide (nt) gap and XPD unwinding 22 base pairs (bp) by 5' to 3' translocation up to the

site of the lesion, thereby defining the characteristic size of the ssDNA bubble to be excised⁷⁵. Further studies are required to understand how DNA damage recognition by TFIIH-associated helicases is coordinated with subsequent steps in NER. Furthermore, the effects of mutations or polymorphisms in the NER helicases on their catalytic functions, protein interactions, disease pathology and cancer predisposition are of considerable interest^{4,76}.

DNA helicases implicated in crosslink resistance and DSB repair

A DNA interstrand crosslink (ICL) is highly toxic and poses a strong block to cellular replication and transcription⁷⁷. Reactive oxygen species from cellular biochemical processes may cause peroxidation of lipids, which in turn damage DNA to result in ICLs and other forms of oxidative DNA damage. Moreover, several DNA-damaging agents (for example, MMC and cisplatin) that are used for anticancer therapy induce DNA ICLs⁷⁷. Thus, a comprehensive understanding of the response to ICLs may provide diagnostic and therapeutic opportunities to combat cancer.

The FA pathway is a highly integrated protein network that provides cellular resistance to DNA ICLs and other forms of replication stress⁴⁵. Furthermore, it protects haematopoietic stem cells from endogenous aldehydes that produce DNA or protein crosslinks, thus ultimately suppressing bone marrow failure and leukaemia^{78,79}. The currently identified 15 FA gene products collaborate with other proteins that are involved in HR, NER and translesion synthesis to tolerate and repair DNA ICLs⁷⁷ (FIG. 2b). The FA pathway helps to channel DSBs through HR in order to prevent an inappropriate engagement of breaks by error-prone non-homologous end-joining (NHEJ)^{80,81}. FANCM and FANCI operate at distinct steps of ICL repair. FANCM shares sequence homology with DNA helicases and can translocate along, but not unwind, duplex DNA⁸². It is a component of the FA core complex that is important for early ICL recognition, for stabilizing and remodelling the blocked replication fork through interaction with the histone-fold-containing protein complex MHF1–MHF2 (REF. 83), and for signalling from the ICL damage by mono-ubiquitylation of FANCD2 and FANCI, which is a key activation step of the pathway. By contrast, FANCI operates downstream of FANCD2 and FANCI mono-ubiquitylation to facilitate recombinational repair of a DNA structure (presumably a DSB) that arises from the ‘unhooking’ process, which leaves crosslinked nucleotides covalently attached to the complementary strand. FANCI helicase activity and interaction with the mismatch repair (MMR) protein MLH1 is required for ICL resistance⁸⁴, raising the interesting possibility of targeting this protein interaction to sensitize cancer cells to chemotherapeutic agents that induce DNA ICLs.

Other helicases are involved in resistance to ICLs or other forms of replication stress. BLM resides in a multi-protein nuclear complex with FA core complex proteins to recognize the DNA lesion and stabilize the stalled replication fork⁸⁵. Together with the RecQ-mediated genome instability (RMI) sub-complex (RMI1 and RMI2) and topoisomerase 3 α , BLM interacts with FANCM to help manage the repair of stalled replication forks through its ability to dissolve a double Holliday Junction (HJ) and related DNA structures⁸⁶; such repair is necessary to avoid loss of heterozygosity and to suppress the cancer that is observed in

patients with Bloom syndrome^{87,88}. BLM also interacts with FANCD1 to maintain chromosomal stability⁸⁹. Therefore, BLM probably has upstream and downstream roles in ICL repair, including recognition of the ICL and strand resection of a DSB that arises from an unhooked ICL. FANCD1 may also have a role in strand resection through its interaction with BLM and the MRN protein complex, which consists of MRE11–RAD50–Nijmegen breakage syndrome protein 1 (NBS1) and acts as a sensor of DSBs⁹⁰. Cellular studies suggest that the WRN helicase, in conjunction with BRCA1, has a role in processing DNA ICLs⁹¹. WRN is required for ataxia telangiectasia mutated (ATM) activation and the intra-S-phase checkpoint in response to ICL-induced DSBs⁹² and other forms of fork arrest⁹³. WABS-causing *DDX11* mutations render cells sensitive to the chemotherapeutics MMC or camptothecin³⁹, suggesting that they disrupt ICL repair. Depletion of HELQ (also known as HEL308) in human endocervical cancer cells rendered them MMC sensitive and defective in HR⁹⁴. HELQ was observed to disrupt RAD51–double-stranded DNA filaments. However, this filament-disrupting activity was not dependent on ATP hydrolysis by HELQ⁹⁵, leaving doubt as to the importance of HELQ catalytic activity in downstream events of HR repair. Human HELQ was shown to localize to damaged replication forks and to preferentially unwind the lagging strand of synthetic replication fork structures⁹⁶, suggesting a role in fork remodelling or replication restart.

It is difficult to comprehend how so many helicases coordinate their roles in ICL repair and possibly render certain tumours resistant to crosslinking drugs. A key nexus is BLM, which interacts with FA proteins and DNA repair factors to repair the damage that is associated with ICLs and other forms of replication stress. For example, BLM collaborates with the FA pathway during S phase to prevent, and during mitosis to resolve, sister-chromatid bridging at fragile sites, ultimately to avoid chromosomal breakage and aneuploidy^{97,98}. BLM and other helicases are involved in checkpoint signalling, strand resection and resolution of recombination intermediates during DSB repair of processed ICL DNA intermediates⁹⁹. RECQL5 efficiently displaces RAD51 bound to DNA¹⁰⁰, whereas BLM does so poorly¹⁰¹ and requires RAD51 inactivation to disrupt joint D-loop molecules¹⁰², thus suggesting that RECQL5 has a more prominent role than BLM in regulating HR by RAD51 filament disassembly. Further studies should delineate specialized functions of DNA helicases in response to drugs that induce ICLs and other forms of replication stress and that are widely used to treat leukaemia and other cancers⁷⁷.

Processing of ICLs can lead to DSBs, which also arise at broken replication forks as a consequence of topoisomerase inhibitors that trap topoisomerase–DNA complexes¹⁰³. Ionizing radiation or DNA-damaging agents can also lead to DNA breaks. The RecQ helicases, like numerous tumour suppressors, are implicated in HR-mediated repair of DSBs during the S or G2 phases of the cell cycle^{9,99} (FIG. 3a). HR is a high-fidelity repair mechanism, whereas NHEJ, which is the other major pathway of DSB repair and which occurs throughout the cell cycle, is error-prone. WRN interacts with the Ku protein complex¹⁰⁴ and DNA ligase IV¹⁰⁵, which are both implicated in NHEJ, but its precise role as a DNA helicase or exonuclease to trim DNA ends is still poorly understood. It may be that WRN facilitates an alternative form of NHEJ that is mediated by short (5–25 bp) microhomologous DNA sequences to align broken DNA ends, which contributes to the

repair of DSBs in chronic myeloid leukaemia cells¹⁰⁶. Studies in cell lines suggest that both helicase and exonuclease functions of WRN are necessary for optimal recombinational repair^{107–109}.

ICL-inducing DNA-damaging agents are among the most widely used classes of chemotherapies⁷⁷. Understanding the cellular response to ICL-induced DNA damage is a high priority in order to improve chemotherapeutic efficacy. Certain sporadic head and neck, lung, ovarian, cervical and haematological cancers are characterized by epigenetic silencing of wild-type FA gene expression¹¹⁰. It is estimated that 15% of all cancers harbour defects in the FA pathway, and loss of heterozygosity in FA carriers may increase cancer risk later in life⁴⁵. Therefore, a synthetic lethality approach to target DNA damage response proteins in tumours that are characterized by a defective FA pathway should be evaluated. If FA-deficient tumours become reliant on WRN or other helicases to cope with ICL-induced strand breaks, it will be worthwhile to explore helicase inhibitors as a new class of anticancer therapy (discussed further below).

Helicases help cells to cope with endogenous DNA damage

WRN¹¹¹, RECQL4 (REF. 112) and RECQL1 (REF. 23) interact with poly(ADP-ribose) polymerase 1 (PARP1), which is a sensor of DNA breaks and is implicated in BER. In mice, WRN and PARP1 collaborate to preserve chromosomal stability and to suppress the early development of neoplasms¹¹³. In addition to PARP1, WRN interacts with other BER proteins, notably DNA polymerase- β , apurinic/apyrimidinic endonuclease 1 (APE1), and flap endonuclease 1 (FEN1) to promote long-patch BER¹¹⁴ (FIG. 3b). In addition, RECQL5-depleted cells are sensitive to oxidative agents and accumulate endogenous damage, including strand breaks and 8-oxoguanine³², which is consistent with elevated cancer incidence in *Recql5*-deficient mice³⁰.

Mutations in MMR genes are linked to colorectal, endometrial, gastric and urothelial cancers¹¹⁵; however, no DNA helicase has been implicated in eukaryotic MMR, unlike the established role of the UvrD helicase in *Escherichia coli* MMR¹¹⁶. MMR proteins interact with WRN, BLM, RECQL1 and FANCI, which may regulate the roles of these helicases in recombinational repair¹¹⁷. Interestingly, exonuclease 1 (EXO1), the nuclease that is implicated in eukaryotic MMR, interacts with WRN¹¹⁸, RECQL1 (REF. 119) and BLM⁶¹, and the BLM–EXO1 interaction is important for DSB resection during HR-mediated repair.

DNA helicases at replication forks

DNA helicase proteins, which were previously thought to operate strictly in DNA repair or replication, are now known to have interwoven roles in both processes. Maintaining replication fork integrity *in vivo* is a challenging task that necessitates versatile strategies to cope with fork obstacles (FIG. 4). The importance of helicase-dependent pathways to deal with replication stress is becoming evident from cellular and biochemical studies.

Fork remodelling and checkpoint signalling by helicase proteins

Replication lesions are prominent in rapidly dividing cancer cells¹²⁰, and persistent replication fork stalling leads to genomic instability. Therefore, understanding how cancer

cells deal with such lesions will be informative for developing antitumor strategies. An intra-S-phase checkpoint helps to stabilize proteins at stalled replication forks, to curtail cell cycle progression and to invoke DNA repair pathways. The *Saccharomyces cerevisiae* RecQ helicase Sgs1, through its interaction with the Rpa70 (also known as Rpa1) subunit of the Rpa heterotrimer¹²¹, has a crucial role in checkpoint signalling by recruiting the kinase Rad53 to regions of ssDNA at stalled replication forks and facilitating subsequent Rad53 activation^{122,123}. Conservation of checkpoint activation mechanisms through RecQ protein interactions remains an area of active investigation that will yield new insights into helicase functions at stalled replication forks, the molecular pathology underlying RecQ helicase disorders and targeting DNA damage checkpoints in cancer. For example, the human WRN and BLM RecQ helicases also interact with RPA70 through an amino-terminal acidic domain, which is required for stimulation of helicase activity¹²⁴. WRN is required for normal replication fork progression after exposure to the DNA-damaging agent methylmethanesulphonate or the replication inhibitor hydroxyurea¹²⁵ (TABLE 2). Both WRN and BLM may help cells to cope with stalled replication forks by remodelling DNA structures, as demonstrated *in vitro*^{126–128} (FIG. 4a,b); however, their precise roles require further investigation. RECQL1 was shown to have a role in the restart of reversed replication forks induced by the topoisomerase 1 inhibitor camptothecin, which is used as an anticancer drug¹²⁹.

Unlike RecQ helicases, human F-box DNA helicase 1 (FBH1), in cooperation with the MUS81 nuclease¹³⁰, promotes DNA breakage and apoptosis in response to replication stress, thus suggesting a role for FBH1 during chronic replication stress to suppress oncogenic transformation by eliminating genetically unstable transformed cells that have been overwhelmed by broken replication forks¹³¹. Alternatively, FBH1 may promote DNA breakage following replication stress to promote a constitutively high number of DNA ends that can enhance chromosomal rearrangements and promote tumour progression. This would be consistent with the characteristic replicative trauma that transpires during oncogene activation. The role of FBH1 in carcinogenesis requires further study.

FANCI helicase activity is required for timely progression through S phase¹³². FANCI acts with DNA topoisomerase-2-binding protein TOPBP1 in early DNA replication checkpoint control after hydroxyurea-induced replication stress, suggesting that their interaction enables RPA to load onto chromatin, which is a prerequisite for the activation of ataxia telangiectasia and Rad3-related (ATR) and for the replication checkpoint mediated by checkpoint kinase 1 (CHK1)¹³³. The FANCD1 DNA translocase is recruited to ICL-blocked replication forks, where it suppresses sister-chromatid exchange (SCE) by remodelling DNA^{134,135}. SMARCA1 (also known as HARP) — which is defective in the autosomal T-cell immunodeficiency disorder Schimke immune-osseous dysplasia — surveys replication forks and catalyses fork regression and HJ branch migration to preserve genome stability¹³⁶. The ability of SMARCA1 to anneal complementary strands coated by RPA¹³⁷ distinguishes it from classic DNA helicases (for example, human RecQ proteins) in which RPA inhibits strand annealing. Helicase-like proteins known as chromatin remodelling enzymes (which are distinct from helicases because they do not separate complementary strands) reconfigure nucleosome interactions by displacing or repositioning histone octamers

bound to duplex DNA and by exchanging histone variants¹³⁸. Chromatin remodelling complexes — for example, the nucleosome remodelling and deacetylase (NuRD) complex, which contains a protein with an ATPase/helicase-like domain — regulate transcriptional events that are important for oncogenesis and cancer progression¹³⁹.

Collaboration between replicative and accessory helicases

Aside from drug-induced replication stalling, cells must cope with natural barriers to replication, such as stable protein–DNA complexes or a replication fork converging with the transcriptional apparatus. Helicase-dependent mechanisms exist to minimize these collisions¹⁴⁰ (FIG. 4c). If the fork collides with transcription complexes, accessory motors help to displace RNA polymerase from DNA to restore fork progression. Genetic evidence from *E. coli* suggests that replication fork reversal sets the stage for accessory helicases to enable replication restart¹⁴¹. It is unknown whether a similar arrangement exists in eukaryotes.

The atypical helicase-related minichromosome maintenance 8 (MCM8) and MCM9 replication elongation proteins are required for efficient HR-mediated DNA DSB repair^{142,143}. Although MCM9 is dispensable for DNA replication, the characterization of mice lacking *Mcm9* demonstrated somatic chromosomal instability, embryonic germ cell depletion in both sexes and hepatocellular carcinoma in males¹⁴⁴. It will be important to determine how the recombination functions of MCM paralogues bear on pathways that affect germline stem cell maintenance and proliferation, fertility and cancer suppression.

Accessory DNA helicases facilitate replication fork progression when the forks encounter a protein–DNA complex such as an RNA polymerase, a DNA sequence that is prone to forming an alternative structure (for example, G-quadruplexes (see the next section)), or RNA–DNA hybrids known as R loops¹⁴⁵). In *Schizosaccharomyces pombe*, the Pfh1 helicase is required for replisome fork progression through difficult-to-replicate sequences¹⁴⁶, or past protein–DNA obstacles, to ensure chromosomal integrity¹⁴⁷. By sequence homology, only one Pfh1 family helicase (PIF1) exists in human cells, raising interest in its role in DNA synthesis at difficult-to-replicate sequences that may be sites of chromosomal fragility and markers or causative factors for cancer or disease.

Helicases at telomeres and G4 DNA

G-quadruplexes, which are composed of planar stacks of four guanines interacting by Hoogsteen hydrogen bonds, have attracted considerable attention, and there is evidence that they form *in vivo* and affect nucleic acid metabolism¹⁴⁸. Certain DNA helicases (for example, WRN, BLM, FANCD1 and PIF1) unwind G4 DNA substrates *in vitro*¹⁴⁹. Evidence has emerged that helicase resolution of G4 DNA has important biological outcomes for telomeres (FIG. 5) and other G-rich sequences.

Leading versus lagging strand G4 instability

S. cerevisiae Pif1 resolves G4 structures to preserve replication fork progression and suppress chromosomal breakage¹⁵⁰. Pif1 inactivation specifically destabilized a G-rich human sub-telomeric *CEBI* minisatellite inserted into a yeast chromosome¹⁵¹, and exposure

to a G4 ligand exacerbated *CEBI* instability¹⁵². The *CEBI* minisatellite was unstable only when the G-rich strand served as a template for leading-strand replication¹⁵³. This was not predicted because the lagging-strand template is believed to have more-extensive ssDNA. Genomic instability in *Caenorhabditis elegans* due to mutation of the Fe-S helicase DOG-1 is thought to be caused by structural blocks to lagging-strand synthesis¹⁵⁴. The 5' to 3' directionality of Pif1 (or FANCI¹⁵⁵) is well suited for unwinding G4 structures by loading onto downstream ssDNA of the leading-strand template; however, the helicase must be able to 'turn its shoulder' as it unwinds G4 to permit the replication machinery to move past the helicase and copy unwound G-rich sequence. Division of labour among G4 helicases may be handled in unique ways depending on the G4 topology or on the type of chromosomal G-rich region (for example, a telomere) that is undergoing replication or repair, or in the case of promoter elements, undergoing transcription.

Roles of vertebrate helicases in telomere maintenance and G4 DNA metabolism

Human cells that are deficient in FANCI, but not FANCD2 or FANCA, are sensitive to the G4 ligand telomestatin¹⁵⁵, which is known to cause telomere instability¹⁵⁶. Moreover, patient-derived FANCI-mutant cells accumulate deletions at genomic sequences that are predicted to form G-quadruplexes¹⁵⁷. These findings suggest a model in which FANCI suppresses genomic instability by resolving G-quadruplexes to enable smooth replication fork progression. A major advance would be obtaining direct evidence of G4-structure accumulation in FANCI-deficient cells.

The pronounced number of predicted G-quadruplexes in promoter regions suggests their involvement in transcriptional regulation¹⁵⁸. Fibroblasts from individuals with Werner syndrome or Bloom syndrome display upregulated expression of genes that are enriched for predicted G4-forming sequences¹⁵⁹. In the absence of WRN or BLM, G4 structures may accumulate to exclude nucleosomes or other DNA-binding proteins that repress transcription; however, this hypothesis remains untested.

Continuity of replicative DNA synthesis provides a mechanism for maintaining epigenetic memory by incorporating new histones and depositing parental histones with their characteristic post-translational modifications near their original locations¹⁶⁰. Perturbation of DNA synthesis by DNA lesions or structured DNA such as G4 can be overcome by translesion polymerases (for example, REV1)¹⁶¹. FANCI was shown to maintain epigenetic stability in chicken cells by collaborating independently with REV1 or the RecQ helicases WRN and BLM to replicate G4-forming DNA sequences¹⁶². Furthermore, FANCI counteracts chromatin compaction by enabling smooth fork progression¹⁶³. Although functional interactions between WRN and DNA polymerases are reported^{164,165}, there is currently no direct evidence that FANCI interacts with a DNA polymerase. Interaction of FANCI with BLM might be relevant to the replication or repair of G4 DNA⁸⁹.

Both FANCI and BLM are found at telomeres¹⁶⁶, where they potentially resolve G4 structures to repress telomere fragility¹⁶⁷ (FIG. 5). WRN is also found at telomeres and interacts with shelterin complex proteins that regulate WRN catalytic functions on telomeric D-loops (T-loops)¹⁶⁸. The connection of the role of WRN at telomeres to the pathophysiology of Werner syndrome was first made by the observation that late-generation

telomerase and WRN-deficient mice (*Terc*^{-/-} *Wrn*^{-/-}) have dysfunctional telomeres and chromosomal instability^{169–171}, and exhibit premature ageing and tumours (osteocarcinomas and soft tissue sarcomas) that are typically observed in patients with Werner syndrome¹⁶⁹. A role for human WRN in resolving telomeric G4 DNA was suggested by the observation that WRN-deficient cells are defective in replication of the telomeric G-rich lagging-strand template¹⁷²; however, the WRN–FEN1 interaction may also have a role^{173,174}. Although there is no evidence for a role for FANCD1 at telomeres, the FA pathway can influence telomere attrition and telomere recombination^{175,176}. A more-prominent player in telomere metabolism is probably RTEL1, which controls telomere length in mice and is required for telomere stability⁴⁷ and resistance to telomere fragility induced by the porphyrin G4 ligand TMPyP4 (REF. 48); however, direct evidence that RTEL1 catalytically unwinds G4 DNA substrates to preserve telomere integrity is lacking. Recently, germline mutations in *RTEL1* were linked to the telomere and cancer disorder dyskeratosis congenita^{40–42}. Cells from patients with dyskeratosis congenita harbouring biallelic *RTEL1* mutations display telomere shortening owing to defective T-loop resolution⁴².

Helicase-based biomarkers and therapeutics

The elevated expression of DNA helicases in rapidly proliferating cells and tumours suggests they have a role in resistance to DNA-damaging agents and may represent good biomarkers for response to chemotherapies. In general, RecQ and other helicase genes are not mutated or silenced in an appreciable number of tumours, and expression is most often upregulated. Thus, RecQ and perhaps other helicases may be important ‘survival’ factors in many tumours. One reported exception was the epigenetic inactivation of *WRN* in colorectal cancers¹⁷⁷. Studies that deplete helicases using RNA interference reinforce the idea that DNA helicases affect cancer cell proliferation and survival after chemotherapeutic DNA damage (TABLE 2). Recently, a screen to detect predictors of cancer cell response to DNA-damaging agents identified a putative DNA and RNA helicase schlafen 11 (SLFN11) as a potential biomarker¹⁷⁸. Inactivation of a single DNA-damage response gene (such as a helicase gene) may serve as a predictive biomarker for personalized cancer therapy¹⁷⁹.

Conventional anticancer therapies have relied on the administration of DNA-damaging chemotherapies or radiation; however, these approaches have drawbacks owing to cytotoxic effects on normal cells and the ability of tumours to become resistant¹⁸⁰. Personalized medicine to combat cancer is entering an age in which therapies make use of existing tumour deficiencies in the DNA damage response, cell cycle checkpoint signalling or DNA repair pathways¹⁷⁹. Synthetic lethality exploits pre-existing DNA repair deficiencies to enable an inhibitor of a given DNA repair pathway to be effective at eliminating those tumours. One potential caveat is therapy-related carcinogenesis that is driven by DNA-damage-induced genomic instability when DNA repair is compromised. Thus, further studies are required to elucidate the precise roles of DNA repair proteins to avoid secondary cancers.

The involvement of DNA helicases in the repair of endogenous DNA damage in rapidly dividing cells has prompted interest in their potential as therapeutic targets, following the path of inhibitors of the single-strand break repair protein PARP1. A proof-of-principle for

the synthetic lethal approach was made by the discovery that tumours that are deficient for BRCA1 or BRCA2, which are defective in HR, are highly sensitive to PARP inhibitors^{181,182}. Results from preclinical and clinical trials (Phase I and Phase II) with PARP inhibitors that potentiate the cytotoxicity and antitumor effects of chemotherapy drugs or radiation suggest that tumours that are defective in other DNA repair pathways (such as NER or BER) may also be susceptible¹⁷⁹. HR-defective tumours that are characterized by a deficiency of a key DNA repair helicase may also be amenable to DNA-damaging chemotherapies¹⁸³.

Small-molecule helicase inhibitors

DNA helicase inhibitors may improve the efficacy of anticancer therapies¹⁸³. Moreover, helicase inhibitors may have value as mono-therapies, as the inactivation of specific DNA helicases promotes synthetic lethality in contexts of specific genetic deficiencies and may impair the replication or division of actively dividing cancer cells. For example, recent work showed that conditional silencing of WRN expression in MYC-overexpressing non-small-cell lung cancer xenografts impaired tumour growth and establishment¹⁸⁴. Thus, targeting WRN may be effective to combat MYC-associated cancers.

Screening of chemical libraries for helicase-docking small molecules that perturb unwinding may lead to the development of anticancer drugs. Alternatively, chemical rescue may restore the function of a helicase protein that is misfolded owing to a disease-causing or cancer-promoting missense mutation. Further studies are required to determine the efficacy of these strategies. Recently, a small molecule (NSC 19630) was identified that specifically inhibited WRN helicase activity *in vitro*¹⁸⁵. Exposure of human cancer cells to NSC 19630 dramatically impaired growth and proliferation, and induced apoptosis and DNA damage in a WRN-dependent manner. The dominant-negative effect of the WRN helicase inhibitor is reminiscent of certain clinical PARP inhibitors that exert their poisonous effect by trapping PARP on DNA¹⁸⁶. Exposure of cancer cells to a sublethal dose of NSC 19630 sensitized them to telomestatin, a PARP inhibitor or topotecan chemotherapy¹⁸⁵. Further investigation, such as a recent study that identified a BLM inhibitor¹⁸⁷, is necessary to characterize helicase-specific small molecules, their mechanisms of action and their potential as therapeutic drugs. To achieve synthetic lethality, a helicase inhibitor should be successful in a genetic background that is defective in a key protein that is involved in HR or another repair pathway, checkpoint signalling, DNA replication or the cell cycle.

Targeting G-quadruplexes to combat cancer

In approximately 90% of cancers, progressive shortening of chromosomes by loss of telomeric DNA is counteracted by upregulated telomerase activity¹⁸⁸. Inhibiting telomerase may be useful to combat cancer, and a number of telomerase inhibitors are in clinical trials¹⁸⁹. There is growing interest in anticancer drugs that stabilize telomeric G-quadruplexes¹⁹⁰. Understanding the roles of G4-resolving DNA helicases may lead to new insights for the design of G4 ligands that block G4 helicases or related proteins, thereby interfering with telomere semiconservative replication or impeding telomerase-catalysed elongation in cancer cells and causing them to senesce (FIG. 5).

The G4 ligand telomestatin induces telomere destabilization by perturbing the interaction of the shelterin protein POT1 with telomeres, thus resulting in apoptosis of cancer cells¹⁹¹. Similarly, the synthetic G4-binding ligand pyridostatin inhibits the proliferation of numerous cancer cell lines and uncaps POT1 from telomeric ssDNA in fibrosarcoma cells to trigger a DNA damage response¹⁹². Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) analysis of DNA sequences associated with the DNA damage marker γ H2AX was used to search for pyridostatin-stabilized G-quadruplexes in human cells that displayed replication- and transcription-dependent DNA lesions¹⁹³. Fluorescently labelled pyridostatin, detected mainly at non-telomeric G4 foci, colocalized with human PIF1. Pyridostatin modulated the expression of genes with high G4-forming potential, including the proto-oncogene SRC. The prospect of targeting G-quadruplexes in gene promoters for anticancer therapy prompts a greater understanding of helicases that are involved in gene regulation. G4 ligands that target promoter G-quadruplexes are in Phase I and Phase II clinical trials for cancer¹⁵⁸. Pyridostatin analogues also cause telomere dysfunction, resulting in long-term growth arrest and senescence of cancer cells, suggesting a small-molecule approach to target G-quadruplexes for anticancer therapy¹⁹⁴ (FIG. 4).

Targeting helicases that resolve unusual DNA structures (such as G-quadruplexes and T-loops) found at telomeres is a possibility (FIG. 5). In addition, RecQ helicases have a role in the alternative lengthening of telomeres (ALT) pathway, which affects proliferation in 5–10% of tumours¹⁹⁵. Characterization of small molecules that modulate helicase function in the ALT pathway may be valuable for devising strategies to treat ALT-dependent tumours (for example, osteosarcomas) that are resistant to conventional anticancer therapies.

Concluding remarks

Helicases have crucial roles in DNA metabolism, and their clinical importance is evident from the discovery of links between helicase gene mutations and cancer-predisposing diseases. The prominent role of helicases in the DNA damage response and cell proliferation has prompted researchers to investigate their molecular and cellular functions. However, there is still much to learn about the underlying mechanisms that are responsible for genomic instability when human DNA helicases are defective. This challenge is particularly daunting owing to the complex network of interconnected roles of DNA helicases and their protein interactions, which profoundly influence DNA repair and replication.

Continued interest in DNA repair as a target for anticancer strategies is propelling the field forwards to develop clinical applications for the diagnosis or treatment of individuals that are predisposed to or experiencing cancer. As chemotherapy or radiation frequently introduces DNA damage, helicases that are involved in DNA repair are moving to the forefront of cancer research. To target helicase-dependent processes, a greater understanding of the molecular and cellular functions of helicases will be required. Modulation of helicase-mediated DNA damage response pathways to enhance anticancer efforts will require the development of tumour-targeted gene-silencing strategies and small molecules that bind to helicase proteins or their protein partners to alter catalytic functions and/or protein interactions. Whole-genome RNA interference screens should help to identify synthetic lethal relationships of target helicases that may potentially be exploited to sensitize tumours

by personalized medicine. The knowledge gained from mechanistic and structural studies of helicases (for example, BOX 1) and high-throughput screens to identify small molecules that directly bind to and modulate helicase function will hopefully result in translational advances. As we learn from the successes and limitations of the first wave of treatment strategies to target DNA damage response proteins, DNA helicases are now an important focus for understanding both the origins and treatment of human cancer.

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Glossary

Poikiloderma	A skin condition that consists of areas of increased and decreased pigmentation, prominent blood vessels and thinning of the skin
Homologous recombination (HR)	A type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. HR is most widely used by cells to accurately repair damage that involves both strands, such as double-strand breaks or interstrand DNA crosslinks
Base-excision repair (BER)	A DNA repair pathway that operates on small DNA lesions such as oxidized or reduced bases, fragmented or non-bulky adducts, or those produced by methylating agents. The resulting single-strand break can be processed by either short-patch BER (through which a single nucleotide is replaced) or long-patch BER (through which 2–10 new nucleotides are synthesized)
Transcription Factor IIIH (TFIIH)	A general transcription factor complex composed of multiple protein subunits that enables formation of the RNA polymerase II pre-initiation complex. TFIIH is also implicated in nucleotide-excision repair (NER)
Nucleotide-excision repair (NER)	This pathway recognizes bulky distortions in the DNA that occur after ultraviolet radiation or chemotherapy. Recognition of these distortions leads to the removal of a short single-stranded DNA segment that includes the lesion, creating a single-strand gap in the DNA, which is subsequently filled by a DNA polymerase
G-quadruplex (G4)	A four-stranded nucleic acid structure stabilized by non-Watson–Crick Hoogsteen base-pairing within stacks of four planar-orientated guanosine nucleotides. G-quadruplex structures can form within or between G-rich strands of telomeric DNA or other G-rich sequences

Intra-S-phase checkpoint	Single-stranded DNA created at the stalled replication fork generates a signal mediated by phosphorylation of target proteins (for example, mammalian ATR) that prevents the cell cycle from progressing to the G2 phase until replication is complete
DNA charge transport	The process of transporting electrons along the axis of a double helical DNA molecule through the overlapping π -orbitals of stacked DNA base pairs
Slippage	When a helicase loses its firm grasp on single-stranded DNA, causing it to slide backwards owing to re-annealing of complementary strands at the DNA fork
Translesion synthesis	A DNA damage tolerance process that allows replication past DNA lesions. If the normal replicative polymerase cannot insert a base owing to damage in the template strand, it is often replaced by a lower-fidelity translesion polymerase
Non-homologous end-joining (NHEJ)	Unlike homologous recombination-mediated repair, NHEJ rejoins broken ends of DNA following double-strand breaks without using a homologous DNA template and can therefore be accompanied by loss of nucleotides and errors
Mismatch repair (MMR)	A process that acts during DNA replication to correct base pairing errors made by the DNA polymerases
RAD51–double-stranded DNA filaments	During an early stage of homologous recombination, the major eukaryotic recombinase RAD51 forms nucleoprotein filaments on double-stranded DNA to initiate the homology search and exchange of DNA strands. Filaments can also occur on single-stranded DNA
Synthetic lethality	Cell death resulting from the combined inactivation or inhibition of two genes or gene products that are non-lethal when inactivated individually. Synthetic lethality can occur between genes and small molecules, which may lead to anticancer therapies
Sister-chromatid exchange (SCE)	A crossing-over event between sister chromatids, leading to the exchange of homologous stretches of DNA sequence
G4 ligand	A typically planar aromatic small molecule that preferentially binds G-quadruplex (G4) DNA with high affinity. There are also proteins that preferentially bind G4 DNA
Shelterin complex	A six-protein complex that localizes to the terminal TTAGGG repeats of mammalian chromosome ends, enabling cells to distinguish their natural chromosome ends from DNA breaks. The shelterin complex represses DNA repair reactions and regulates telomerase-based telomere maintenance

T-loops	The 3' single-stranded telomere DNA overhang circles around and hybridizes to the complementary strand of the adjacent double-stranded DNA to create a displacement loop (D-loop) by displacing one of the strands. T-loops are stabilized by telomere-binding proteins
Alternative lengthening of telomeres (ALT)	A recombination-based mechanism that allows telomere length maintenance in the absence of telomerase activity

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Key points

- Helicase-dependent DNA damage response and repair mechanisms help cells to cope with endogenous or exogenous stress to prevent chromosomal instability and maintain cellular homeostasis.
- Inactivating mutations in DNA helicase genes are linked to genetic disorders that are frequently associated with various cancers. However, the expression of many DNA helicases is upregulated in transformed or neoplastic cells and tissues and is required for cancer cell proliferation or resistance to DNA damage imposed by chemotherapies.
- The RecQ and iron-sulphur (Fe-S) families of DNA helicases have prominent roles in the maintenance of genomic stability through their catalytic functions and protein interactions in telomere maintenance and DNA repair pathways including nucleotide-excision repair (NER), homologous recombination (HR)-mediated repair of double-strand breaks (DSBs), interstrand crosslink (ICL) repair, and base-excision repair (BER).
- Specialized DNA helicases efficiently unwind G-quadruplex (G4) DNA and other forms of alternative DNA structures such as telomeric displacement loops (T-loops). Emerging evidence suggests that DNA helicases that unwind non-conventional DNA structures have important roles in the replication or repair of telomeres.
- Replication forks in rapidly dividing cancer cells are likely to encounter DNA lesions that perturb fork progression. Evidence suggests that certain DNA helicases help cells to cope with replicative lesions by remodelling the fork, restoring the integrity of broken replication forks, or having a role in the signalling mechanism for the intra-S-phase checkpoint.
- Elevated expression of DNA helicases in rapidly proliferating cells and tumours suggests that they have a role in resistance to DNA-damaging agents and may represent good biomarkers for response to chemotherapies.
- High-throughput screening of chemical libraries may prove to be beneficial for the discovery of small molecules that modulate helicase function *in vivo*. Such compounds may be useful in synthetic lethal approaches used in anticancer strategies that target tumours with existing DNA repair deficiencies.

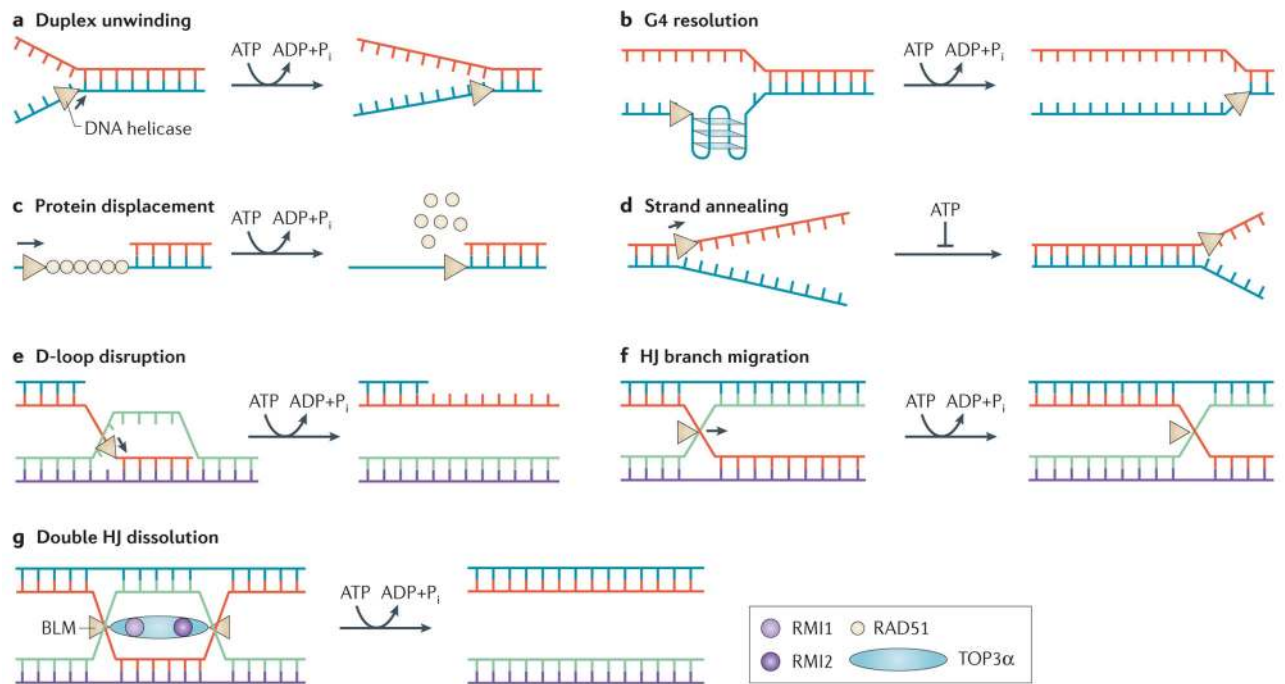


Figure 1. Molecular functions of DNA helicases

DNA helicases (beige triangles) catalytically disrupt base pairs between complementary strands in an ATP-dependent manner (**a**), and may have specialized functions. For example, Fanconi anaemia group J protein (FANCI), the Werner syndrome helicase (WRN), the Bloom syndrome helicase (BLM), and PIF1 disrupt G-quadruplex (G4) DNA structures (**b**). RECQL5 and FANCI strip off proteins (for example, RAD51) that are bound to DNA (**c**). Some helicases (for example, RECQL1, RECQL4, RECQL5, WRN and BLM) carry out strand annealing by promoting base pairing²¹² (**d**). Strand annealing directionality by a DNA helicase has not been demonstrated. ATP inhibits strand annealing and promotes duplex unwinding by inducing a conformational change in the helicase protein (for example, RECQL1 (REF. 213)). Some helicases (for example, BLM and regulator of telomere elongation helicase 1 (RTEL1)) suppress homologous recombination (HR)-mediated repair by unwinding displacement loop (D-loop) intermediates (**e**). Branch-migration of three- or four-stranded joint DNA molecules by a DNA helicase (for example, BLM, WRN or RECQL1) (**f**) can suppress or promote the formation of Holliday Junction (HJ) structures that can be resolved by specialized endonucleases to create crossover products that are responsible for loss of heterozygosity and cancer predisposition²¹⁴. The BLM helicase, together with topoisomerase 3α (TOP3α) and RecQ-mediated genome instability 1 (RMI1) and RMI2, dissolves double HJ structures (**g**) during HR or at converging replication forks to generate non-crossover DNA molecules²¹⁵. See the main text for details.

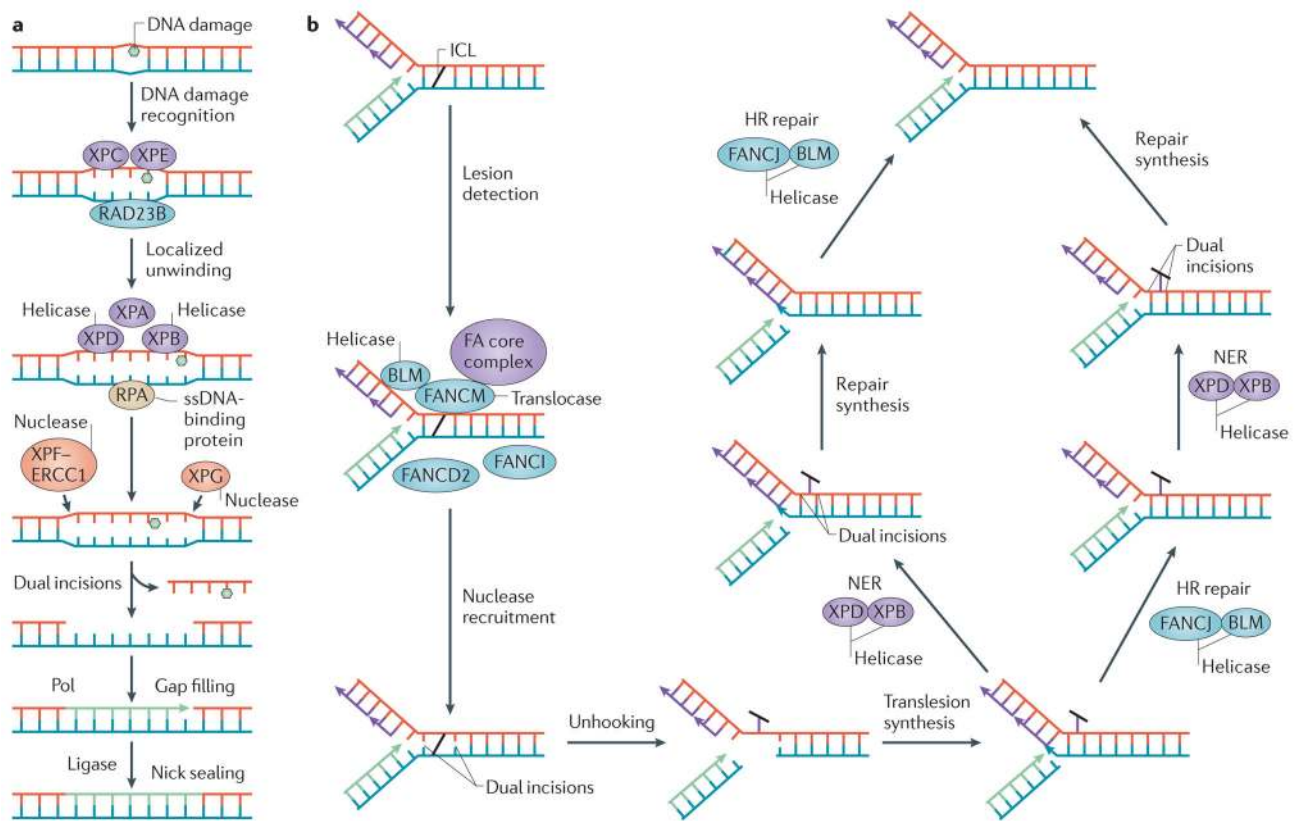


Figure 2. Involvement of helicases in nucleotide excision and interstrand crosslink DNA repair mechanisms

Basic steps of nucleotide-excision repair (NER) (**a**) and interstrand crosslink (ICL) DNA repair (**b**) are shown, highlighting the roles of helicases. **a** | After DNA damage recognition by proteins including xeroderma pigmentosum complementation group C (XPC), XPE and RAD23B, the XPB and XPD helicases act with XPA to create a single-stranded bubble coated by replication protein A (RPA) that is processed by nucleases (the XPF-ERCC1 complex and XPG) to remove the damaged strand in NER^{72,75}. **b** | Multiple helicases are implicated in crosslink resistance pathways that intersect with the Fanconi anaemia (FA) pathway⁷⁷. After lesion detection, nuclease-catalysed incisions on each side of the crosslink remove (unhook) it from one strand, leaving a small gap that converts the fork to a double-strand break (DSB). Parallel pathways resolve the lesion. For simplicity, the double replication fork model²¹⁶ is not shown. HR, homologous recombination; Pol, DNA polymerase; ssDNA, single-stranded DNA.

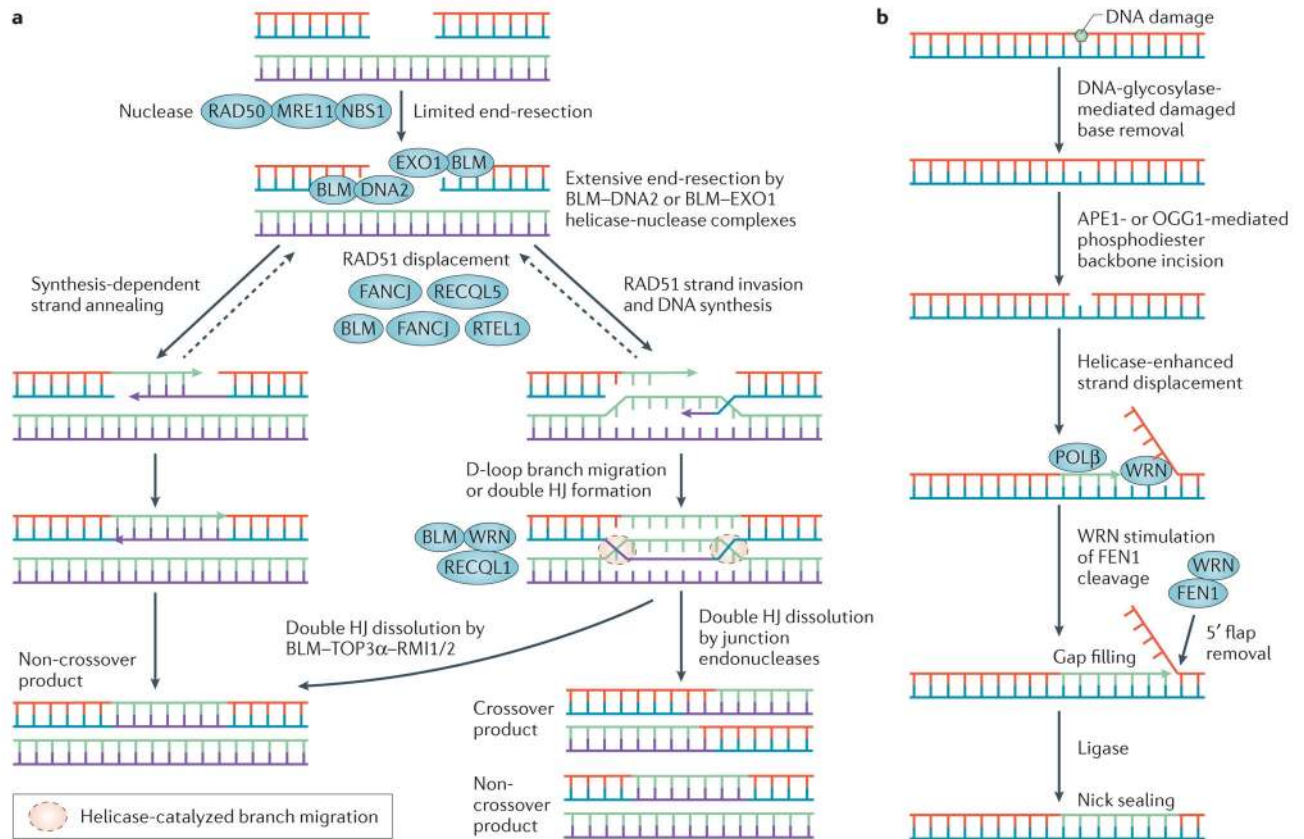


Figure 3. Involvement of helicases in homologous recombination and base excision DNA repair mechanisms

Basic steps of homologous recombination (HR)-mediated DNA repair (**a**) and base-excision repair (BER) (**b**) are shown, highlighting the roles of helicases. **a** | Helicases participate in various steps of conservative HR-mediated repair in somatic cells⁹⁹. Suppression of crossover and rearrangement events can occur pre- and post-synaptically. Specific roles for helicases are being identified. Helicase disruption of displacement loops (D-loops) promotes synthesis-dependent strand annealing by decreasing double Holliday Junction (HJ) formation. **b** | The Werner syndrome helicase (WRN) participates in long-patch BER by unwinding 5' flaps and interacting with BER proteins¹¹⁴, including flap endonuclease 1 (FEN1) (REF. 217). APE1, apurinic/apyrimidinic endonuclease 1; DNA2, DNA replication helicase/nuclease 2; EXO1, exonuclease 1; FANCI, Fanconi anaemia complementation group J protein; NBS1, Nijmegen breakage syndrome protein 1; OGG1, 8-oxoguanine DNA glycosylase; POL β , DNA polymerase- β ; RTEL1, regulator of telomere elongation helicase 1.

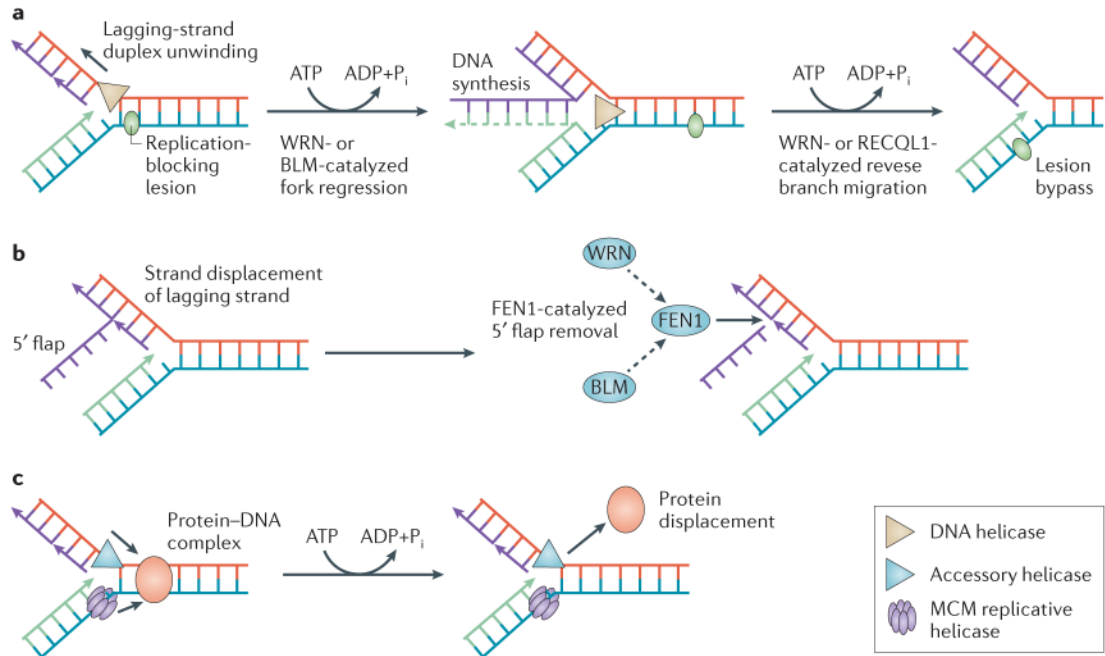


Figure 4. Roles of DNA helicases during replication stress

a | When the replisome (not shown) encounters a replication-blocking lesion (green oval) or other form of replication stress (for example, nucleotide starvation), certain helicases (for example, the Werner syndrome helicase (WRN) or Bloom syndrome helicase (BLM)^{126,128}), carry out fork regression to create a Holliday Junction (HJ)-like ‘chicken-foot’ DNA structure. Reverse branch-migration of the regressed fork by a helicase (for example, WRN¹²⁷ or RECQL1 (REF. 129)) potentially allows replication bypass of the lesion in a non-recombinogenic mode. **b** | The WRN²¹⁷ or BLM²¹⁸ helicases can stimulate flap endonuclease 1 (FEN1)-catalyzed cleavage of 5′ flap structures that might arise during lagging-strand synthesis. BLM stimulates FEN1-mediated cleavage on flaps with secondary 5′ flap structure²¹⁹, and WRN is involved with DNA polymerase-δ in a hairpin repair pathway²²⁰. DNA replication helicase/nuclease 2 (DNA2) also facilitates FEN1-mediated cleavage of 5′ flaps²²¹. **c** | Based on evidence from studies of *Escherichia coli* replication²²², accessory DNA helicases (blue triangle) may coordinate with a eukaryotic minichromosome maintenance (MCM) helicase complex (purple) and replication machinery to displace a protein (orange oval) bound to duplex DNA that impedes fork progression. Replication fork reversal has a role in bacterial replication restart¹⁴¹. Further studies are required to substantiate this model for eukaryotes.

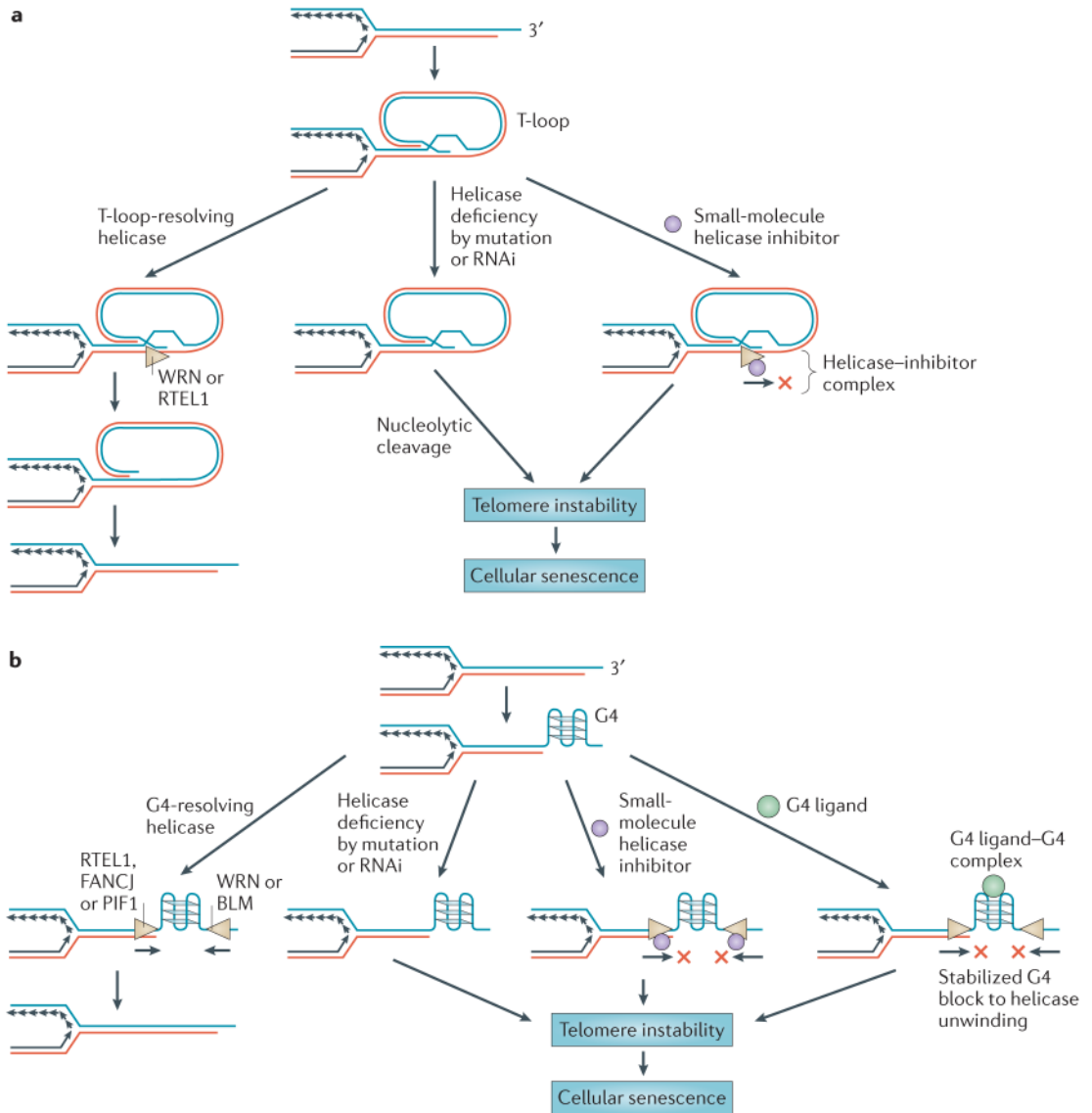


Figure 5. Helicase functions at telomeres and potential for anticancer therapy

Helicases that regulate telomere capping may be targeted for depletion by RNA interference (RNAi) or small-molecule inhibition to selectively kill cancer cells. **a** | 3' single-stranded DNA (ssDNA) tail invasion into adjacent telomeric double-stranded DNA (dsDNA) results in displacement of the complementary strand to create a displacement loop (D-loop), the so-called T-loop structure. Human DNA helicases (regulator of telomere elongation helicase 1 (RTEL1)⁴⁸ and the Werner syndrome helicase (WRN)¹⁶⁸; indicated by the beige triangles) resolve T-loops to enable telomere replication or repair. Helicase deficiency by mutation or RNAi-mediated depletion results in the persistence of T-loops which can be nucleolytically cleaved, leading to telomere instability. A helicase inhibitor (purple circle) may prevent T-loop unwinding, thus resulting in telomere instability. **b** | ssDNA that arises at telomeres or during lagging-strand synthesis can form a G-quadruplex (G4). Certain human helicases with 5' to 3' polarity (RTEL, Fanconi anaemia complementation group J protein (FANCI))

and PIF1) or 3' to 5' polarity (WRN and the Bloom syndrome helicase (BLM)) may resolve G4 structures to enable replication or repair¹⁴⁹. Helicase deficiency by mutation or RNAi-mediated depletion results in telomere fragility. A helicase inhibitor or G4-specific ligand (green circle) may prevent G4 resolution at telomeres or other G-rich genomic sequences. Telomere instability can lead to cellular senescence of rapidly dividing cancer cells. Figure is modified, with permission, from REF. 48 © (2012) Elsevier Science.

Table 1

DNA helicase gene defects linked to cancer

Gene	Disease	Cancer type	Genome metabolic pathway	Biochemical function
<i>WRN</i>	Werner syndrome	Thyroid neoplasms, melanomas, meningiomas, sarcomas, haematological and lymphoid neoplasms, and osteosarcomas	DSB repair and replication stress response	3'-5' helicase, 3'-5' exonuclease, HJ branch migration, G4 resolution, replication fork regression and strand annealing
<i>BLM</i>	Bloom syndrome	Adult epithelial tumours, leukaemias, lymphomas and rare paediatric tumours	DSB repair and repair of replication-associated DNA damage	3'-5' helicase, HJ branch migration, G4 resolution, replication fork regression and strand annealing
<i>RECQLA</i>	RTS, BGS and RAPADILINO syndrome	Osteogenic sarcomas, and lymphomas	Replication, mitochondrial genome stability and repair of endogenous base damage	3'-5' helicase and strand annealing
<i>RECQL1</i>		Pancreatic cancer (polymorphisms)	Replication and oxidative DNA damage response	3'-5' helicase, HJ branch migration and strand annealing
<i>FANCF</i>	FA	Acute myeloid leukaemia and breast cancer (heterozygotes)	DSB repair and ICL repair	5'-3' helicase and G4 resolution
<i>XPD</i>	Xeroderma pigmentosum, xeroderma pigmentosum with Cockayne syndrome, TTD and COFS	Skin cancer	NER and transcription	5'-3' helicase
<i>XPB</i>	Xeroderma pigmentosum, xeroderma pigmentosum with Cockayne syndrome, and TTD	Skin cancer	NER and transcription	3'-5' helicase
<i>RTEL1</i>	Dyskeratosis congenita	Adult glioma (polymorphisms)	Telomere maintenance and homologous recombination	5'-3' helicase, and disassembly of D-loops and T-loops
<i>PIF1</i>		Breast cancer predisposition	Replication fork progression, telomere maintenance and mitochondrial DNA metabolism	5'-3' helicase and G4 resolution

BGS, Baller–Gerold syndrome; COFS, cerebro-oculo-facial-skeletal syndrome; D-loop, displacement loop; DSB, double-strand break; FA, Fanconi anaemia; G4, G-quadruplex; HJ, Holliday junction; ICL, interstrand crosslink; NER, nucleotide-excision repair; RAPADILINO, syndrome involving radial hypoplasia/aplasia, patellae hypoplasia/aplasia and cleft or highly arched palate, diarrhoea and dislocated joints, little size (height at least 2 standard deviations smaller than average height) and limb malformation, nose slender and normal intelligence; RTS, Rothmund–Thomson syndrome; T-loop, telomeric displacement loop; TTD, trichothiodystrophy.

Table 2

Effect of helicase depletion on cancer cell proliferation, tumour growth and sensitivity to chemotherapy or radiation

Helicase	Growth or proliferation	Radiation or chemotherapy sensitivity
RECQL1	RECQL1-targeted siRNA decreased proliferation, induced DNA damage and elevated SCE of endocervical carcinoma HeLa cells ²¹ , and induced mitotic cell death in lung, prostate, bladder, colon and liver cancer cells ²⁶ and HCC cells ⁵ <i>in vitro</i> . RECQL1-targeted siRNA also suppressed tumour growth in mouse xenograft lung, liver, pancreatic and colorectal cancer models ²⁷ , liver cancer with transplanted human HCC ⁵ , and nude mice carrying FaDu or D-562 hypopharyngeal carcinoma xenografts ²⁵ . RECQL1-targeted siRNA decreased proliferation of human hypopharyngeal carcinoma ²⁵ and glioblastoma cells <i>in vitro</i> ²⁸ .	RECQL1-targeted siRNA decreased proliferation of HeLa cells exposed to camptothecin or ionizing radiation ²¹ , and caused a camptothecin-induced replication restart defect in U2OS osteosarcoma cells <i>in vitro</i> ¹²⁹ . RECQL1-targeted siRNA enhanced an antitumour effect of cisplatin in nude mice carrying FaDu hypopharyngeal carcinoma xenografts, and co-treatment with cisplatin increased DNA damage, apoptosis and mitotic catastrophe ²⁵ . RECQL1-targeted siRNA and RECQL1-targeted shRNA sensitized glioblastoma ²⁸ and HeLa and U2OS cells ²⁴ , respectively, to hydroxyurea <i>in vitro</i> . RECQL1-targeted siRNA sensitized glioblastoma cells to TMZ <i>in vitro</i> ²⁸ . RECQL1-targeted shRNA sensitized HeLa and U2OS cells to 8-MOPS <i>in vitro</i> ²⁴ .
WRN	WRN-targeted siRNA decreased proliferation of human hypopharyngeal carcinoma cells <i>in vitro</i> and suppressed tumour growth in nude mice carrying FaDu or D-562 hypopharyngeal carcinoma xenografts ²⁵ . WRN-targeted shRNA decreased tumour establishment and tumour growth in MYC overexpressing non-small-cell lung cancer mouse xenografts ¹⁸⁴ .	WRN-targeted siRNA enhanced the antitumour effect of cisplatin in nude mice carrying FaDu hypopharyngeal carcinoma xenografts; this combination increased DNA damage and induced apoptosis and mitotic catastrophe ²⁵ .
BLM	BLM-targeted siRNA caused a 2.5-fold increase in SCE in HeLa cells ²²³ .	NR
WRN and BLM	WRN-targeted shRNA or BLM-targeted shRNA decreased proliferation of U2OS cells <i>in vitro</i> with BLM depletion having a greater effect ²²⁴ . Cells co-depleted for WRN and BLM showed reduced proliferation that was comparable to BLM-depleted cells.	BLM-targeted or WRN-targeted shRNA sensitized U2OS cells to camptothecin, cisplatin or 5-FU. BLM-targeted shRNA sensitized U2OS cells to hydroxyurea. Co-depletion did not further sensitize cells to any tested chemotherapies ²²⁴ .
RECQL4	RECQL4-targeted siRNA inhibited proliferation and increased DNA damage and apoptosis of prostate cancer cells <i>in vitro</i> . RECQL4-targeted shRNA caused metastatic cancer cells to display reduced cell invasiveness and suppressed tumour growth in nude mice ⁷ .	NR
RECQL5	RECQL5-targeted shRNA decreased proliferation of HeLa cells <i>in vitro</i> ²⁹ .	RECQL5-targeted shRNA increased 53BP1 foci in HeLa cells ²²⁵ .
FANCI	FANCI-targeted siRNA caused HeLa cells to display delayed G1/S progression and G1 accumulation ¹³² .	FANCI-targeted siRNA decreased hydroxyurea-induced CHK1 phosphorylation in U2OS cells ¹³³ and proliferation of HeLa cells ⁸⁹ <i>in vitro</i> . FANCI-targeted siRNA reduced the proliferation of MMC-exposed MCF7 breast cancer cells ³⁷ or HeLa cells ⁸⁹ . FANCI-targeted shRNA reduced the growth of MCF7 cells following exposure to ionizing radiation ²²⁶ .
DDX11	DDX11-targeted siRNA caused mitotic failure and sister-chromatid cohesion defects in HeLa cells ^{227,228} ; it also reduced proliferation and elevated chromosome segregation, telomere defects and apoptosis in melanoma cells ²²⁹ .	NR
PIF1	PIF1-targeted siRNA reduced colony formation, increased sub-G1 DNA, and prolonged S phase in colon cancer cells (HCT116) ⁵⁵ .	PIF1-targeted siRNA reduced survival and increased apoptosis of HCT116 cells exposed to thymidine, hydroxyurea or gemcitabine. Elevated apoptosis was observed in PIF1-depleted <i>TP53</i> ^{-/-} SW480 colon cancer and <i>TP53</i> ^{+/+} MCF7 breast cancer cells ⁵⁵ .
FBH1	NR	FBH1-depleted U2OS cells exposed to hydroxyurea show reduced DSBs and apoptosis ¹³¹ .
RTEL	NR	RTEL-targeted siRNA decreased MMC resistance in HeLa cells ⁴⁹ .

5-FU, 5-fluorouracil; 8-MOPS, 8-methoxypsoralen; 53BP1, p53-binding protein 1; CHK1, checkpoint kinase 1; DSB, double-strand break; HCC, hepatocellular carcinoma; MMC, mitomycin C; NR, not reported; SCE, sister-chromatid exchange; shRNA, short hairpin RNA; siRNA, small interfering RNA; TMZ, temozolomide.

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