DNA helicase 3.6.4.12

1 Nomenclature

EC number
3.6.4.12

Systematic name
ATP phosphohydrolase (DNA helix unwinding)

Recommended name
DNA helicase

Synonyms
3' to 5' DNA helicase <28> [35]
3'-5' DNA helicase <11> [55]
3'-5' PfDH <11> [55]
5' to 3' DNA helicase <26,27> [19,42]
AvDH1 <47> [37]
BACH1 helicase <19> [34]
BLM <3> [28]
BLM protein <3> [28]
BRCA1-associated C-terminal helicase <19> [34]
BcMCM <8> [52]
CeWRN-1 <43> [9]
DDX25 <3,48> [36]
DNA helicase 120 <7> [15]
DNA helicase A <4> [8]
DNA helicase E <5> [44]
DNA helicase II <9> [7]
DNA helicase III <4> [27]
DNA helicase RECQL5β <44> [17]
DNA helicase VI <3> [45]
Dbp9p <46> (<46> a member of the DEAD box protein family [24]) [24]
DmRECQ5 <1> [50]
DnaB helicase <29> [23]
E1 helicase <17> [58]
GRTH/DDX25 <3,48> [36]
HCoV SF1 helicase <23> [3]
HCoV helicase <23> [3]
HDH IV <3> [45]
Hel E <5> [44]
Hmi1p <40> [60]
MCM helicase <6,35,38> [43,54]

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MCM protein <6,35> [43]
MER3 helicase <22> [30]
MER3 protein <22> [30]
MPH1 <28> [35]
NS3 <12,50> (<12,50> ambiguous [38,65,66]) [38,65,66]
NS3 NTPase/helicase <14> (<14> ambiguous [67]) [67]
NS3 protein <12> (<12> ambiguous [63]) [63]
NTPase/helicase <12,16> (<12> ambiguous [61]) [61,64]
PDH120 <7> [15]
PIF1 <33> [51]
PIF1 helicase <33> [51,53]
PcrA <37> [20]
PcrA helicase <37,41,49> [20,21,39]
PcrASpn <41> [21]
PfDH A <11> [55]
Pfh1p <27> [42]
RECQ5 <1> [49,50]
RECQ5 helicase <1> (<1> small isoform [49]) [49]
RECQL5β <44> [17]
REcQ <31> [13]
RSF1010 RepA <30> [5]
RecG <45> [6]
RecQ helicase <32> [56]
RecQsim <32> [56]
Rep52 <24> [40]
Rrm3p <26> [19]
Sgs1 <36> [29]
Sgs1 DNA helicase <36> [29]
TWINKLE <21> [33]
Tth UvrD <20> [16]
UvrD <20,42> [16,22]
UvrD helicase <39> [18]
WRN <18> [31]
WRN RecQ helicase <18> [12]
WRN helicase <18> [12]
WRN protein <18> [12]
WRN-1 RecQ helicase <43> [9]
Werner Syndrome helicase <18> [31]
Werner syndrome RecQ helicase <18> [12]
dheI I <1> [46]
dnaB <29> [23]
hPif1 <33> [53]
helicase DnaB <2> [10]
helicase II <25> [25]
helicase PcrA <49> [39]
helicase UvrD <20> [16]
helicase domain of bacteriophage T7 gene 4 protein <10> [47]

non structural protein 3 <12> (<12> ambiguous [61,62]) [61,62]
nonstructural protein 3 <12,14,50,51> (<12,14,50> ambiguous [38,63,65, 66,67]; <51> ambigous [4]) [4,38,63,65,66,67]
protein NS3 <12> (<12> ambiguous [62]) [62]
scHelI <4> [26]
urvD <25> [25]

2 Source Organism

<1> *Drosophila melanogaster* [46,49,50]
<2> *Escherichia coli* [10]
<3> *Homo sapiens* [28,36,45,48]
<4> *Saccharomyces cerevisiae* [8,26,27]
<5> *Bos taurus* [44]
<6> *Methanothermobacter thermautotrophicus* [43]
<7> *Pisum sativum* [14,15]
<8> *Bacillus cereus* [52]
<9> *Schizosaccharomyces pombe* [7]
<10> *Enterobacteria phage T7* [47]
<11> *Plasmodium falciparum* [55]
<12> *Hepatitis C virus* [1,11,38,61,62,63,65]
<13> *Human herpesvirus 1* [59]
<14> *West Nile virus* [2,67]
<15> *SARS coronavirus* (UNIPROT accession number: P0C6X7) [32]
<16> *SARS coronavirus* [64]
<17> *Human papillomavirus type 11* (UNIPROT accession number: P04014) [58]
<18> *Homo sapiens* (UNIPROT accession number: Q14191) [12,31]
<19> *Homo sapiens* (UNIPROT accession number: O14867) [34]
<20> *Thermus thermophilus HB8* (UNIPROT accession number: O24736) [16]
<21> *Homo sapiens* (UNIPROT accession number: Q96RR1) [33]
<22> *Saccharomyces cerevisiae* (UNIPROT accession number: P51979) [30]
<23> *Human coronavirus 229E* (UNIPROT accession number: P0C6X1) [3]
<24> *Adeno-associated virus - 2* (UNIPROT accession number: Q89270) [40]
<25> *Escherichia coli* (UNIPROT accession number: P03018) [25]
<26> *Saccharomyces cerevisiae* (UNIPROT accession number: P07271) [19]
<27> *Schizosaccharomyces pombe* (UNIPROT accession number: Q9UUA2) [42]
<28> *Saccharomyces cerevisiae* (UNIPROT accession number: P40562) [35]
<29> *Bacillus anthracis* (UNIPROT accession number: Q81JI8) [23]
<30> *Escherichia coli* (UNIPROT accession number: P20356) [5]
<31> *Escherichia coli* (UNIPROT accession number: P15043) [13]
<32> *Arabidopsis thaliana* (UNIPROT accession number: Q6Y5A8) [56]
<33> *Homo sapiens* (UNIPROT accession number: Q9H611) [41,51,53]
<34> *Homo sapiens* (UNIPROT accession number: P46063) [57]
<35> *Sulfolobus solfataricus* (UNIPROT accession number: Q9UXG1) [43]
<36> *Saccharomyces cerevisiae* (UNIPROT accession number: P35187) [29]

<37> *Bacillus anthracis* (UNIPROT accession number: Q6I4A9) [20]
<38> *Halobacterium sp.* NRC-1 [54]
<39> *Plasmodium falciparum* (UNIPROT accession number: Q8I3W6) [18]
<40> *Saccharomyces cerevisiae* (UNIPROT accession number: Q12039) [60]
<41> *Streptococcus pneumoniae* (UNIPROT accession number: Q8DPU8) [21]
<42> *Mycobacterium tuberculosis* (UNIPROT accession number: P64320) [22]
<43> *Caenorhabditis elegans* (UNIPROT accession number: Q19546) [9]
<44> *Mus musculus* (UNIPROT accession number: Q8VID5) [17]
<45> *Thermotoga maritima* (UNIPROT accession number: Q9WY48) [6]
<46> *Saccharomyces cerevisiae* (UNIPROT accession number: Q06218) [24]
<47> *Apocynum venetum* (UNIPROT accession number: A8D930) [37]
<48> *Rattus norvegicus* (UNIPROT accession number: Q9QY16) [36]
<49> *Geobacillus stearothermophilus* (UNIPROT accession number: P56255) [39]
<50> *Hepatitis C virus* (UNIPROT accession number: Q9WPH5) [66]
<51> *Japanese encephalitis virus* (UNIPROT accession number: P27395) [4]

3 Reaction and Specificity

Catalyzed reaction

ATP + H_2O = ADP + phosphate

Natural substrates and products

S ATP + H_2O <3,6,7,12,14,15,16,18,20,21,22,26,27,32,35,39,40,41,43,46,47,48,49,51> (<18> 3-5 helicase activity. WRN helicase is involved in preserving DNA integrity during replication. It is proposed that WRN helicase can function in coordinating replication fork progression with replication stress-induced fork remodeling [12]; <27> 5 to 3 DNA helicase. ATPase/helicase activity of Pfh1p is essential. Maintenance of telomeric DNA is not the sole essential function of Pfh1p. Although mutant spores depleted for Pfh1p proceed through S phase, they arrest with a terminal cellular phenotype consistent with a postinitiation defect in DNA replication. Telomeric DNA is modestly shortened in the absence of Pfh1p [42]; <26> 5 to 3 DNA helicase. The ATPase/helicase activity of Rrm3p is required for its role in telomeric and subtelomeric DNA replication. Because Rrm3p is telomere-associated in vivo, it likely has a direct role in telomere replication [19]; <47> AvDH1 belonging to the DEAD-box helicase family is induced by salinity, functions as a typical helicase to unwind DNA and RNA, and may play an important role in salinity tolerance [37]; <40> DNA helicase Hmi1p is involved in the maintenance of mitochondrial DNA [60]; <6,35> during chromosomal DNA replication, the replicative helicase unwinds the duplex DNA to provide the single-stranded DNA substrate for the polymerase. In archaea, the replicative helicase is the minichromosome maintenance complex. The enzyme utilizes the energy of ATP hydrolysis to translocate along one strand of the duplex and unwind the complementary strand [43]; <3,48> gonadotropin-regulated testicular he-

licase (GRTH/DDX25), a target of gonadotropin and androgen action, is a post-transcriptional regulator of key spermatogenesis genes [36]; <20> helicase UvrD protein plays an important role in nucleotide excision repair, mismatch repair, rolling circular plasmid replication, and in DNA replication [16]; <39> helicases play an essential role in nearly all the nucleic acid metabolic processes, catalyzing the transient opening of the duplex nucleic acids in an ATP-dependent manner [18]; <32> involved in DNA recombination, repair and genome stability maintenance [56]; <22> meiosis-specific MER3 protein is required for crossing over, which ensures faithful segregation of homologous chromosomes at the first meiotic division [30]; <41> PcrA is a chromosomally encoded DNA helicase of gram-positive bacteria involved in replication of rolling circle replicating plasmids [21]; <43> the ability of CeWRN-1 to unwind DNA structures may improve the access for DNA repair and replication proteins that are important for preventing the accumulation of abnormal structures, contributing to genomic stability [9]; <12> the C-terminal portion of hepatitis C virus nonstructural protein 3 (NS3) forms a three domain polypeptide that possesses the ability to travel along RNA or single-stranded DNA (ssDNA) in a 3' to 5' direction. Driven by the energy of ATP hydrolysis, this movement allows the protein to displace complementary strands of DNA or RNA [11]; <18> the DNA-dependent ATPase utilizes the energy from ATP hydrolysis to unwind double-stranded DNA. The enzyme unwinds two important intermediates of replication/repair, a 5-ssDNA flap substrate and a synthetic replication fork. The enzyme is able to translocate on the lagging strand of the synthetic replication fork to unwind duplex ahead of the fork. For the 5-flap structure, the enzyme specifically displaces the 5-flap oligonucleotide, suggesting a role of the enzyme in Okazaki fragment processing. The ability of the enzyme to target DNA replication/repair intermediates may be relevant to its role in genome stability maintenance [31]; <21> TWINKLE is the helicase at the mitochondrial DNA replication fork [33]; <12> catalytic DNA helicase activity is coupled with NTPase and is stimulated by ATP [62]; <16> DNA-unwinding activity [64]; <12> multifunctional enzyme possessing serine protease, NTPase, DNA and RNA unwinding activities [65]) (Reversibility: ?) [4,9,11,12,15,16,18,19,21,24,30,31,32,33,36,37,39,42,43,56,60,61,62,64,65,67]

P ADP + phosphate

S NTP + H_2O <12> (<12> different NTP binding rate and processivity, DNA unwinding of nonstructural protein 3 [38]) (Reversibility: ?) [38]

P NDP + phosphate

S dNTP + H_2O <12> (<12> dNTPs support faster DNA unwinding mediated by nonstructural protein 3 [38]) (Reversibility: ?) [38]

P dNDP + phosphate

Substrates and products

S 2'(3')-O-(N-methylanthraniloyl)ATP + H_2O <49> (<49> the fluorescent ATP analogue is used throughout all experiments to provide a complete ATPase cycle for a single nucleotide species [39]) (Reversibility: ?) [39]

P 2'(3')-O-(N-methylanthraniloyl)ADP + phosphate
S 2',3'-ATP + H_2O <12> (<12> 274% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]) (Reversibility: ?) [38]
P 2',3'-ADP + phosphate
S 2',3'-dATP + H_2O <12> (<12> 274% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2',3'-dADP + phosphate
S 2'-O-methyl-ATP + H_2O <12> (<12> 34% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 34% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2'-O-methyl-ADP + phosphate
S 2'-amino-ATP + H_2O <12> (<12> 28% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 28% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2'-amino-ADP + phosphate
S 2'-ara-ATP + H_2O <12> (<12> 102% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]) (Reversibility: ?) [38]
P 2'-ara-ADP + phosphate
S 2'-azido-ATP + H_2O <12> (<12> 61% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 61% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2'-azido-ADP + phosphate
S 2'-dATP + H_2O <12> (<12> 157% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2'-dADP + phosphate
S 2'-fluoro-ATP + H_2O <12> (<12> 145% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 145% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2'-fluoro-ADP + phosphate
S 2-amino-ATP + H_2O <12> (<12> 78% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 78% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 2-amino-ADP + phosphate
S 3'-dATP + H_2O <12> (<12> 307% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]
P 3'-dADP + phosphate
S ATP + H_2O <1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,51> (<18> 3-5 helicase activity. WRN helicase is involved in preserving DNA

integrity during replication. It is proposed that WRN helicase can function in coordinating replication fork progression with replication stress-induced fork remodeling [12]; <27> 5 to 3 DNA helicase. ATPase/helicase activity of Pfh1p is essential. Maintenance of telomeric DNA is not the sole essential function of Pfh1p. Although mutant spores depleted for Pfh1p proceed through S phase, they arrest with a terminal cellular phenotype consistent with a postinitiation defect in DNA replication. Telomeric DNA is modestly shortened in the absence of Pfh1p [42]; <26> 5 to 3 DNA helicase. The ATPase/helicase activity of Rrm3p is required for its role in telomeric and subtelomeric DNA replication. Because Rrm3p is telomere-associated in vivo, it likely has a direct role in telomere replication [19]; <47> AvDH1 belonging to the DEAD-box helicase family is induced by salinity, functions as a typical helicase to unwind DNA and RNA, and may play an important role in salinity tolerance [37]; <40> DNA helicase Hmi1p is involved in the maintenance of mitochondrial DNA [60]; <6,35> during chromosomal DNA replication, the replicative helicase unwinds the duplex DNA to provide the single-stranded DNA substrate for the polymerase. In archaea, the replicative helicase is the minichromosome maintenance complex. The enzyme utilizes the energy of ATP hydrolysis to translocate along one strand of the duplex and unwind the complementary strand [43]; <3,48> gonadotropin-regulated testicular helicase (GRTH/DDX25), a target of gonadotropin and androgen action, is a post-transcriptional regulator of key spermatogenesis genes [36]; <20> helicase UvrD protein plays an important role in nucleotide excision repair, mismatch repair, rolling circular plasmid replication, and in DNA replication [16]; <39> helicases play an essential role in nearly all the nucleic acid metabolic processes, catalyzing the transient opening of the duplex nucleic acids in an ATP-dependent manner [18]; <32> involved in DNA recombination, repair and genome stability maintenance [56]; <22> meiosis-specific MER3 protein is required for crossing over, which ensures faithful segregation of homologous chromosomes at the first meiotic division [30]; <41> PcrA is a chromosomally encoded DNA helicase of gram-positive bacteria involved in replication of rolling circle replicating plasmids [21]; <43> the ability of CeWRN-1 to unwind DNA structures may improve the access for DNA repair and replication proteins that are important for preventing the accumulation of abnormal structures, contributing to genomic stability [9]; <12> the C-terminal portion of hepatitis C virus nonstructural protein 3 (NS3) forms a three domain polypeptide that possesses the ability to travel along RNA or single-stranded DNA (ssDNA) in a 3′ to 5′ direction. Driven by the energy of ATP hydrolysis, this movement allows the protein to displace complementary strands of DNA or RNA [11]; <18> the DNA-dependent ATPase utilizes the energy from ATP hydrolysis to unwind double-stranded DNA. The enzyme unwinds two important intermediates of replication/repair, a 5-ssDNA flap substrate and a synthetic replication fork. The enzyme is able to translocate on the lagging strand of the synthetic replication fork to unwind duplex ahead of the fork. For the 5-flap structure, the enzyme specifically displaces the 5-flap oligonucleotide, suggesting a role of the enzyme in Okazaki fragment processing. The ability of

the enzyme to target DNA replication/repair intermediates may be relevant to its role in genome stability maintenance [31]; <21> TWINKLE is the helicase at the mitochondrial DNA replication fork [33]; <18> 3-5 helicase activity [12]; <27> 5 to 3 DNA helicase [42]; <13> 5-3 unwinding activity, enzymatic functions of the two subunit helicase-primase complex (enzyme complex consisting of UL5 and UL52 gene functions): DNA-dependent ATPase, DNA primase, and DNA helicase activities [59]; <25> as a DNA-dependent ATPase, helicase II translocates processively along single-stranded DNA. The translocation of helicase II along single-stranded DNA is unidirectional and in th 3' to 5' direction with respect to the DNA strand on which the enzyme is bound [25]; <4> ATP hydrolysis is required for unwinding of DNA catalyzed by the DNA helicase, the enzyme moves in the 5' to 3' direction on a single-stranded DNA to catalyze unwinding of double-stranded regions of DNA in the 3 to 5 direction [27]; <7> ATP is the most active NTP. DNA helicase unwinds DNA unidirectionally from 3' to 5'. DNA helicase can unwind a 17-bp duplex whether it has unpaired single-stranded tails at both the 5' end and 3' end, at the 5' end or at the 3' end only, or at neither end. However, it fails to act on a blunt-ended 17-bp duplex DNA [14]; <43> ATP-dependent 3 to 5 helicase capable of unwinding a variety of DNA structures such as forked duplexes, Holliday junctions, bubble substrates, D-loops, and flap duplexes, and 3-tailed duplex substrates [9]; <6> ATP-dependent 3-5 helicase activity. During chromosomal DNA replication, the replicative helicase unwinds the duplex DNA to provide the single-stranded DNA substrate for the polymerase. In archaea, the replicative helicase is the minichromosome maintenance complex. The enzyme utilizes the energy of ATP hydrolysis to translocate along one strand of the duplex and unwind the complementary strand. ATP binding enhances DNA binding by the helicase. ATPase activity is substantially enhanced in presence of DNA. MCM protein binds DNA ends better than long circular substrates [43]; <35> ATP-dependent 3-5 helicase activity. During chromosomal DNA replication, the replicative helicase unwinds the duplex DNA to provide the single-stranded DNA substrate for the polymerase. In archaea, the replicative helicase is the minichromosome maintenance complex. The enzyme utilizes the energy of ATP hydrolysis to translocate along one strand of the duplex and unwind the complementary strand. Very limited stimulation of its ATPase activity by DNA [43]; <3> ATP-dependent DNA unwinding enzyme. HDH VI unwinds exclusively DNA duplexes with an annealed portion smaller than32 bp and prefers a replication fork-like structure of the substrate. It cannot unwind blunt-end duplexes and is inactive also on DNA-RNA or RNA-RNA hybrids. HDH VI unwinds DNA unidirectionally by moving in the 3 to 5 direction along the bound strand. ATP and dATP are equally good substrates [45]; <19> BACH1 preferentially binds and unwinds a forked duplex substrate compared with a duplex flanked by only one single-stranded DNA (ssDNA) tail. BACH1 helicase requires a minimal 5 ssDNA tail of 15 nucleotides for unwinding of conventional duplex DNA substrates. However, the enzyme is able to catalytically release the third strand of the homologous recombination intermediate D-loop structure irrespective of DNA tail

status. In contrast, BACH1 completely fails to unwind a synthetic Holliday junction structure. Moreover, BACH1 requires nucleic acid continuity in the 5 ssDNA tail of the forked duplex substrate within six nucleotides of the ssDNA-dsDNA junction to initiate efficiently DNA unwinding [34]; <8> BcMCM displays 3 to 5 helicase and ssDNA-stimulated ATPase activity. BcMCM is an active ATPase, and this activity is restricted to the MCM-AAA module [52]; <46> Dbp9p exhibits DNA-DNA and DNA-RNA helicase activity in the presence of ATP [24]; <1> Dhel I moves 5 to 3 on the DNA strand to which it is bound. Unwinding activity decreases with increasing length of the double-stranded region suggesting a distributive mode of action. ATP and dATP are the only nucleoside-5-triphosphates that support the strand displacement reaction. Both have an optimal concentration range between 1 and 2 mM [46]; <28> DNA helicase activity has a 3 to 5 polarity with respect to the DNA strand on which this protein translocates [35]; <24> DNA helicase with 3-to-5 polarity. No helicase activity in absence of NTP [40]; <3> DNA unwinding in 5 to 3 direction [48]; <36> exhibits an ATPase activity in the presence of single- or double-stranded DNA. Displacement of the DNA strand occurs in the 3 to 5 direction with respect to the single-stranded DNA flanking the duplex. The efficiency of unwinding is found to correlate inversely with the length of the duplex region. The recombinant Sgs1 fragment is found to bind more tightly to a forked DNA substrate than to either single or double-stranded DNA. Like the DNA-DNA helicase activity, unwinding of the DNA-RNA hybrid is driven by the hydrolysis of ATP or dATP [29]; <12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP. ATP is hydrolyzed far faster than DNA is unwound in the presence of both Mn^{2+} and Mg^{2+} [38]; <33> hPifHD (core helicase domain) only unwinds the substrate with a 5 single-stranded DNA (ssDNA) overhang and is a 5 to 3 helicase. Pif1 specifically recognizes and unwinds DNA structures resembling putative stalled replication forks. Notably, the enzyme requires both arms of the replication fork-like structure to initiate efficient unwinding of the putative leading replication strand of such substrates. This DNA structure-specific mode of initiation of unwinding is intrinsic to the conserved core helicase domain (hPifHD) that also possesses a strand annealing activity [53]; <4> hydrolyzes ATP and dATP with equal efficiency. ATPase activity of the enzyme is absolutely DNA-dependent. DNA sequences containing pyrimidine stretches are more effective activators than those containing purine stretches. poly(dC) appears to be the most effective activator of the ATPase activity. DNA helicase migrates on a DNA template in 5 to 3 direction [8]; <41> hydrolyzes both ATP and dATP at similar levels. The enzyme shows 5 to 3 and 3 to 5 DNA helicase activities and binds efficiently to partially duplex DNA containing a hairpin structure adjacent to a 6-nucleotide 5 or 3 single-stranded tail and one unpaired (flap) nucleotide in the complementary strand [21]; <42> only ATP and dATP support helicase activity. 80% of

the duplex is separated in the presence of 1 mM ATP in a 15 min reaction, 58% is unwound in the presence of 1 mM dATP. ATPase activity is dependent upon the presence of DNA. Oligonucleotides of 4 nucleotides are sufficient to promote the ATPase activity. UvrD preferentially unwinds 3-single-stranded tailed duplex substrates over 5-single-stranded ones, indicating that the protein has a duplex-unwinding activity with 3-to-5 polarity. A 3 single-stranded DNA tail of 18 nucleotides is required for effective unwinding. UvrD has an unwinding preference towards nicked DNA duplexes and stalled replication forks [22]; <37> PcrA shows 3 to 5 as well as 5 to 3 helicase activities, with substrates containing a duplex region and a 3 or 5 ss poly(dT) tail. PcrA also efficiently unwinds oligonucleotides containing a duplex region and a 5 or 3 ss tail with the potential to form a secondary structure [20]; <47> purified recombinant protein contains ATP-dependent DNA helicase activity, ATP-independent RNA helicase activity, and DNA- or RNA-dependent ATPase activity [37]; <1> RECQ5 unwinds duplex DNA with a 3-5 polarity. Unwinding of longer partial duplex DNA substrates requires a higher protein concentration than does unwinding of the 20bp partial duplex substrate. The unwinding reaction catalyzed by RECQ5 requires a nucleoside 5-phosphate. dATP is most effective. RECQ5 hydrolyzes dATP more rapidly than ATP regardless of the presence of ssDNA. Both ssDNA cofactors, M13mp18 ssDNA and poly(dT) strongly stimulate the dATPase activity of the protein [49]; <29> strong 5 to 3 DNA helicase activity. At both 0.1 and 0.5 mM, dATP produces comparable or slightly higher levels of unwinding than ATP [23]; <49> the chemical cleavage step is the rate-limiting step in the ATPase cycle and is essentially irreversible and results in the bound ATP complex being a major component at steady state. This cleavage step is greatly accelerated by bound DNA, producing the high activation of this protein compared to the protein alone. The data suggest the possibility that ADP is released in two steps, which results in bound ADP also being a major intermediate, with bound ADP*phosphate being a very small component. It therefore seems likely that the major transition in structure occurs during the cleavage step, rather than phosphate release [39]; <18> the DNA-dependent ATPase utilizes the energy from ATP hydrolysis to unwind double-stranded DNA. The enzyme unwinds two important intermediates of replication/repair, a 5-ssDNA flap substrate and a synthetic replication fork. The enzyme is able to translocate on the lagging strand of the synthetic replication fork to unwind duplex ahead of the fork. For the 5-flap structure, the enzyme specifically displaces the 5-flap oligonucleotide [31]; <7> the enzyme can unwind 17-bp partial duplex substrates with equal efficiency whether or not they contain a fork. It translocates unidirectionally along the bound strand in the 3 to 5 direction. NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP. The optimum concentration of ATP for DNA helicase activity is 1.0 mM. At 8 mM ATP the DNA unwinding activity of PDH120 is inhibited. No significant difference in the DNA unwinding activity of PDH120 with forked or nonforked substrates. The enzyme fails to unwind synthetic blunt-ended duplex DNA suggesting that PDH120 re-

quires ssDNA adjacent to the duplex as a loading zone [15]; <15> the enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates [32]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <11> the enzyme moves unidirectionally in the 3 to 5 direction along the bound strand and prefers a fork-like substrate structure and could not unwind blunt-ended duplex DNA [55]; <9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited extents GTP, CTP, dGTP and dCTP. ATP and dATP support unwinding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]; <3> the enzyme unwinds DNA in the 3-5 direction with respect to the strand to which the enzyme is bound [28]; <5> the helicase is capable of displacing DNA fragments up to 140 nucleotides in length, but is unable to displace a DNA fragment 322 nucleotides in length. Preference for displacing primers whose 5 terminus is fully annealed as opposed to primers with a 12 nucleotide 5 unannealed tail. The presence of a 12 nucleotide 3 tail has no effect on the rate of displacement. DNA helicase E is capable of displacing a primer downstream of either a four nucleotide gap, a one nucleotide gap or a nick in the DNA substrate. Helicase E is inactive on a fully duplex DNA 30 base pairs in length [44]; <2> the NTP hydrolysis step is significantly faster for the purine NTPs than for the pyrimidine NTPs, both in the absence and in the presence of the DNA. The nature of intermediates of the purine nucleotide, ATP, is different from the nature of the analogous intermediates of the pyrimidine nucleotide CTP [10]; <14> the number of ATP hydrolysis events per unwinding cycle is not a constant value. At optimum Mg^{2+} and saturating ATP concentrations 1 pmol of the enzyme unwinds 5.5 fmol (given as nucleotide bases) of the DNA duplex per s [2]; <12> the protein binds RNA and DNA in a sequence specific manner. ATP hydrolysis is stimulated by some nucleic acid polymers much better than it is stimulated by others. The range is quite dramatic. Poly(G) RNA does not stimulate at any measurable level, and poly(U) RNA (or DNA) stimulates best (up to 50 fold). HCV helicase unwinds a DNA duplex more efficiently than an RNA duplex. ATP binds HCV helicase between two RecA-like domains, causing a conformational change that leads to a decrease in the affinity of the protein for

nucleic acids. One strand of RNA binds in a second cleft formed perpendicular to the ATP-binding cleft and its binding leads to stimulation of ATP hydrolysis. RNA and/or ATP binding likely causes rotation of domain 2 of the enzyme relative to domains 1 and 3, and somehow this conformational change allows the protein to move like a motor [11]; <23> the recombinant protein has both RNA and DNA duplex-unwinding activities with 5-to-3 polarity. The DNA helicase activity of the enzyme preferentially unwinds 5-oligopyrimidine-tailed, partial-duplex substrates and requires a tail length of at least 10 nucleotides for effective unwinding [3]; <21> TWINKLE is a DNA helicase with 5 to 3 directionality. The enzyme needs a stretch of 10 nucleotides of single-stranded DNA on the 5-side of the duplex to unwind duplex DNA. In addition, helicase activity is not observed unless a short single-stranded 3-tail is present. UTP efficiently supports DNA unwinding. ATP, GTP, and dTTP are less effective [33]; <22> unwinds DNA in the 3 to 5 direction relative to single-stranded regions in the DNA substrates [30]; <4> unwinds partial duplex DNA substrates, as long as 343 base pairs in length, in a reaction that is dependent on either ATP or dATP hydrolysis. The direction of the unwinding reaction is 5 to 3 with respect to the strand of DNA on which the enzyme is bound [26]; <12> catalytic DNA helicase activity is coupled with NTPase and is stimulated by ATP [62]; <16> DNA-unwinding activity [64]; <12> multifunctional enzyme possessing serine protease, NTPase, DNA and RNA unwinding activities [65]; <51> genome structure, crystals and three-dimensional structure determined, structure of NTP-binding region, conserved residues within the NTP-binding pocket [4]; <12> modified malachite green assay, DNA unwinding by nonstructural protein 3, pH 6.5, 5 mM $MgCl_2$, 2 mM ATP, and 0.1mg/ml polyU, initiated by adding 5-100 nM enzyme [38]; <12> peptide inhibitors derived from amino acid sequence of motif VI analyzed, binding of the inhibitory peptides does not interfere with the NTPase activity, 4.7 pM DNA substrate used for determination of helicase activity [61]; <14> recombinant protein of C-terminal portion of NS3 protein, ATPase catalytic properties but no DNA helicase activities [67]) (Reversibility: ?) [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,51,52,53,54,55,56,57,58,59,60,61,62,64,65,67]

P ADP + phosphate

S CTP + H_2O <1,2,7,9,12,15,20,24> (<24> DNA helicase with 3-to-5 polarity. No helicase activity in absence of NTP [40]; <12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <1> RECQ5 unwinds duplex DNA with a 3-5 polarity. The unwinding reaction catalyzed RECQ5 requires a nucleoside 5-phosphate. dATP is most effective. ATP supports helicase reaction with 10% of the efficiency obtained with dATP [49]; <20> the enzyme hydrolyzes nucleoside tripho-

sphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited extents GTP, CTP, dGTP and dCTP. ATP and dATP support unwinding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]; <2> the NTP hydrolysis step is significantly faster for the purine NTPs than for the pyrimidine NTPs, both in the absence and in the presence of the DNA. The nature of intermediates of the purine nucleotide, ATP, is different from the nature of the analogous intermediates of the pyrimidine nucleotide, CTP [10]; <12> ability of various NTPs to support HCV helicase-catalyzed DNA unwinding by nonstructural protein 3 using a molecular-beacon-based helicase assay [38]) (Reversibility: ?) [7,10,15,16,32,38,40,49]

P CDP + phosphate

S GTP + H_2O <1,2,7,9,12,15,20,21,24> (<24> DNA helicase with 3-to-5 polarity. No helicase activity in absence of NTP [40]; <12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP. 21% of the ability to support NS3hb(con1)-catalyzed DNA unwinding compared to ATP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <1> RECQ5 unwinds duplex DNA with a 3-5 polarity. The unwinding reaction catalyzed by RECQ5 requires a nucleoside 5-phosphate. dATP is most effective. ATP supports helicase reaction with 35% of the efficiency obtained with dATP [49]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited

extents GTP, CTP, dGTP and dCTP. ATP and dATP support unwinding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]; <2> the NTP hydrolysis step is significantly faster for the purine NTPs than for the pyrimidine NTPs, both in the absence and in the presence of the DNA. The nature of intermediates of the purine nucleotide, ATP, is different from the nature of the analogous intermediates of the pyrimidine nucleotide, CTP [10]; <21> TWINKLE is a DNA helicase with 5 to 3 directionality. The enzyme needs a stretch of 10 nucleotides of single-stranded DNA on the 5-side of the duplex to unwind duplex DNA. In addition, helicase activity is not observed unless a short single-stranded 3-tail is present. UTP efficiently supports DNA unwinding. ATP, GTP, and dTTP are less effective [33]; <12> ability of various NTPs to support HCV helicase-catalyzed DNA unwinding by nonstructural protein 3 using a molecular-beacon-based helicase assay, 21% relative ability to support DNA unwinding, reported as percentage relative to ATP [38]) (Reversibility: ?) [7,10,15,16,32,33,38,40,49]

P GDP + phosphate

S N^1-methyl-ATP + H_2O <12> (<12> 47% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 47% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]

P N^1-methyl-ADP + phosphate

S N^6-methyl-ATP + H_2O <12> (<12> 122% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]; <12> 122% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]

P N^6-methyl-ADP + phosphate

S NTP + H_2O <12> (<12> different NTP binding rate and processivity, DNA unwinding of nonstructural protein 3 [38]; <12> ability of various dNTPs to support HCV helicase-catalyzed DNA unwinding by nonstructural protein 3 using a molecular-beacon-based helicase assay [38]) (Reversibility: ?) [38]

P NDP + phosphate

S TTP + H_2O <12> (<12> ability of various NTPs to support HCV helicase-catalyzed DNA unwinding by nonstructural protein 3 using a molecular-beacon-based helicase assay [38]) (Reversibility: ?) [38]

P TDP + phosphate

S UTP + H_2O <7,12,15,20,21> (<12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution.

The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <21> TWINKLE is a DNA helicase with 5 to 3 directionality. The enzyme needs a stretch of 10 nucleotides of single-stranded DNA on the 5-side of the duplex to unwind duplex DNA. In addition, helicase activity is not observed unless a short single-stranded 3-tail is present. UTP efficiently supports DNA unwinding. ATP, GTP, and dTTP are less effective [33]) (Reversibility: ?) [15,16,32,33,38]

P UDP + phosphate

S XTP + H_2O <12> (<12> 7% of the ability to support helicase catalyzed DNA unwinding compared to ATP [38]) (Reversibility: ?) [38]

P XDP + phosphate

S dATP + H_2O <1> (<1> RECQ5 unwinds duplex DNA with a 3-5 polarity. Unwinding of longer partial duplex DNA substrates requires a higher protein concentration than does unwinding of the 20bp partial duplex substrate. The unwinding reaction catalyzed by RECQ5 requires a nucleoside 5-phosphate. RECQ5 hydrolyzes dATP more rapidly than ATP regardless of the presence of ssDNA. dATP is most effective. ATP supports helicase reaction with 45% of the efficiency obtained with dATP. Both ssDNA cofactors, M13mp18 ssDNA and poly(dT) strongly stimulate the ATPase activity of the protein [49]) (Reversibility: ?) [49]

P ADP + phosphate

S dATP + H_2O <1,3,4,7,9,11,12,15,20,24,29,36,41,42> (<4> ATP hydrolysis is required for unwinding of DNA catalyzed by the DNA helicase, the enzyme moves in the 5´ to 3‘ direction on a single-stranded DNA to catalyze unwinding of double-stranded regions of DNA in the 3 to 5 direction. dATP shows 95% of the activity with ATP [27]; <3> ATP-dependent DNA unwinding enzyme. HDH VI unwinds exclusively DNA duplexes with an annealed portion smaller than32 bp and prefers a replication fork-like structure of the substrate. It cannot unwind blunt-end duplexes and is inactive also on DNA-RNA or RNA-RNA hybrids. HDH VI unwinds DNA unidirectionally by moving in the 3 to 5 direction along the bound strand. ATP and dATP are equally good substrates [45]; <7> dATP shows 25% of the activity compared to ATP. DNA helicase unwinds DNA unidirectionally from 3‘ to 5‘ [14]; <1> Dhel I moves 5 to 3 on the DNA strand to which it is bound. Unwinding activity decreases with increasing length of the double-stranded region suggesting a distributive mode of action. ATP and dATP are the only nucleoside-5-triphosphates that support the strand displacement reaction. Both have an optimal concentration range

between 1 and 2 mM [46]; <24> DNA helicase with 3-to-5 polarity. No helicase activity in absence of NTP. dATP is as efficient as ATP [40]; <36> exhibits an ATPase activity in the presence of single- or double-stranded DNA. Displacement of the DNA strand occurs in the 3 to 5 direction with respect to the single-stranded DNA flanking the duplex. The efficiency of unwinding is found to correlate inversely with the length of the duplex region. The recombinant Sgs1 fragment is found to bind more tightly to a forked DNA substrate than to either single or double-stranded DNA. Like the DNA-DNA helicase activity, unwinding of the DNA-RNA hybrid is driven by the hydrolysis of ATP or dATP [29]; <12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <4> hydrolyzes ATP and dATP with equal efficiency. ATPase activity of the enzyme is absolutely DNA-dependent [8]; <41> hydrolyzes both ATP and dATP at similar levels. The enzyme shows 5 to 3 and 3 to 5 helicase activities and binds efficiently to partially duplex DNA containing a hairpin structure adjacent to a 6-nucleotide 5 or 3 single-stranded tail and one unpaired (flap) nucleotide in the complementary strand [21]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <42> only ATP and dATP support helicase activity. 80% of the duplex is separated in the presence of 1 mM ATP in a 15 min reaction, 58% is unwound in the presence of 1 mM dATP. ATPase activity is dependent upon the presence of DNA. Oligonucleotides of 4 nucleotides are sufficient to promote the ATPase activity. UvrD preferentially unwinds 3-single-stranded tailed duplex substrates over 5-single-stranded ones, indicating that the protein has a duplex-unwinding activity with 3-to-5 polarity. A 3 single-stranded DNA tail of 18 nucleotides is required for effective unwinding. UvrD has an unwinding preference towards nicked DNA duplexes and stalled replication forks [22]; <29> strong 5 to 3 DNA helicase activity. At both 0.1 and 0.5 mM, dATP produces comparable or slightly higher levels of unwinding than ATP [23]; <1> structure-specific DNA helicase. DmRECQ5 preferentially unwinds specific DNA structures including a 3flap, a three-strand junction and a three-way junction. Unwinding of a Holliday junction, 5flap and 12 nt bubble structures, which can be unwound by other RecQ proteins (WRN, BLM and/or Escherichia coli RecQ), can not be detected or requires significantly higher protein concentrations [50]; <15> the enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates [32]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <11> the enzyme moves unidirectionally in the 3 to 5 direction

along the bound strand and prefers a fork-like substrate structure and could not unwind blunt-ended duplex DNA. dATP supports unwinding at 42% of the efficiency of ATP [55]; <9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited extents GTP, CTP, dGTP and dCTP. ATP and dATP support unwinding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]; <4> unwinds partial duplex DNA substrates, as long as 343 base pairs in length, in a reaction that is dependent on either ATP or dATP hydrolysis. The direction of the unwinding reaction is 5 to 3 with respect to the strand of DNA on which the enzyme is bound [26]; <12> helicase-catalyzed DNA unwinding by nonstructural protein 3 analyzed by molecular beacon-based helicase assay (MBHA), NTP binding occurs with similar affinities, dNTPs support faster DNA unwinding [38]) (Reversibility: ?) [7,8,14,15,16,21,22,23,26,27,29,32,38,40,45,46,50,55]

P dADP + phosphate

S dCTP + H_2O <7,12,15,20> (<12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <15> the enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates [32]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <12> helicase-catalyzed DNA unwinding by nonstructural protein 3 analyzed by molecular beacon-based helicase assay (MBHA), NTP binding occurs with similar affinities, dNTPs support faster DNA unwinding [38]) (Reversibility: ?) [15,16,32,38]

P dCDP + phosphate

S dCTP + H_2O <9> (<9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited extents GTP, CTP, dGTP and dCTP. ATP and dATP support un-

winding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]) (Reversibility: ?) [7]

P dCTP + phosphate

S dGTP + H_2O <1,7,9,12,15,20> (<12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <1> RECQ5 unwinds duplex DNA with a 3-5 polarity. The unwinding reaction catalyzed by RECQ5 requires a nucleoside 5-phosphate. dATP is most effective. ATP supports helicase reaction with 30% of the efficiency obtained with dATP [49]; <20> the enzyme hydrolyzes nucleoside triphosphates in order of decreasing efficiency: ATP, dATP, dGTP, GTP, CTP, dCTP, UTP. The enzyme is highly active on a double-stranded DNA with 5 recessed ends in comparison with substrates with 3 recessed or blunt ends, and supports enzyme translocation in a 3-5 direction relative to the strand bound by the enzyme [16]; <9> the enzyme translocates in a 5-to-3 direction with respect to the substrate strand to which it is bound. The enzyme favours adenosine nucleotides (ATP and dATP) as its energy source, but utilizes to limited extents GTP, CTP, dGTP and dCTP. ATP and dATP support unwinding activity with equal efficiency. GTP, dGTP, CTP, dCTP support unwinding activity to limited extents (5-12% of that with ATP at 1.5 mM). The ATPase activity of DNA helicase II increases proportionally with increasing lengths of single-stranded DNA cofactor. In the presence of circular DNA, ATP hydrolysis continues to increase up to the longest time tested (3 h), whereas it ceases to increase after 5-10 min in the presence of shorter oligonucleotides. The initial rate of ATP hydrolysis during the first 5 min of incubation time is not affected by DNA species used. The enzyme does not dissociate from the single-stranded DNA once it is bound and is therefore highly processive [7]; <12> helicase-catalyzed DNA unwinding by nonstructural protein 3 analyzed by molecular beacon-based helicase assay (MBHA), NTP binding occurs with similar affinities, dNTPs support faster DNA unwinding [38]) (Reversibility: ?) [7,15,16,32,38,49]

P dGDP + phosphate

S dNTP + H_2O <12> (<12> dNTPs support faster DNA unwinding mediated by nonstructural protein 3 [38]; <12> ability of various NTPs

to support HCV helicase-catalyzed DNA unwinding by nonstructural protein 3 using a molecular-beacon-based helicase assay [38]) (Reversibility: ?) [38]

P dNDP + phosphate

S dTTP + H_2O <7,12,21> (<12> HCV helicase unwinds DNA at different rates depending on the nature and concentration of NTPs in solution. The fastest reactions are observed in the presence of CTP followed by ATP, UTP, and GTP. 3-deoxy-NTPs generally support faster DNA unwinding, with dTTP supporting faster rates than any other canonical (d)NTP [38]; <7> NTPs can support helicase activity in order of decreasing efficiency: ATP, GTP, dCTP, UTP, dTTP, CTP, dATP, dGTP [15]; <21> TWINKLE is a DNA helicase with 5 to 3 directionality. The enzyme needs a stretch of 10 nucleotides of single-stranded DNA on the 5-side of the duplex to unwind duplex DNA. In addition, helicase activity is not observed unless a short single-stranded 3-tail is present. UTP efficiently supports DNA unwinding. ATP, GTP, and dTTP are less effective [33]) (Reversibility: ?) [15,33,38]

P dTDP + phosphate

S dTTP + H_2O <12> (<12> helicase-catalyzed DNA unwinding by nonstructural protein 3 analyzed by molecular beacon-based helicase assay (MBHA), NTP binding occurs with similar affinities, dNTPs support faster DNA unwinding, dTTP supporting faster rates than any other canonical dNTP [38]) (Reversibility: ?) [38]

P TDP + phosphate

S dUTP + H_2O <15> (Reversibility: ?) [32]

P dUDP + phosphate

S xanthosine-5'-triphophate + H_2O <12> (<12> 7% relative ability to support DNA unwinding by nonstructural protein 3, reported as percentage relative to ATP [38]) (Reversibility: ?) [38]

P xanthosine-5'-diphophate + phosphate

S Additional information <6,9,12,24,41,46> (<46> exhibits RNA unwinding and binding activity in the absence of NTP, and this activity is abolished by a mutation in the RNA-binding domain [24]; <24> no helicase activity is observed with UTP, dCTP or dTTP, low levels of helicase activity is observed with dGTP [40]; <9> non-hydrolysable ATP analogues do not support helicase activity. DNA helicase II lacks any detectable RNA-unwinding activity [7]; <41> the enzyme is inefficient in in vitro replication of pT181, and perhaps as a consequence, this plasmid can not be established in Streptococcus pneumoniae [21]; <6> the helicase is capable of unwinding DNA substrates coated with various proteins, including histones, transcription inhibitors, and the transcription initiation complex. Thus, the helicase can displace at least some of the proteins associated with chromatin [43]; <12> the mature NS3 protein comprises 5 domains: the N-terminal 2 domains form the serine protease along with the NS4A cofactor, and the C-terminal 3 domains form the helicase. The helicase portion of NS3 can be separated form the protease portion by cleaving a linker. Since the protease portion is more hydrophobic, removing it allows

the NS3 helicase fragment to be expressed as a more soluble protein at higher levels in Escherichia coli. The fragment of NS3 possessing helicase activity is referred to as HCV helicase [11]) (Reversibility: ?) [7,11,21,24,40,43]

P ?

Inhibitors

(2Z)-4-[2-(benzyloxy)phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> low inhibitory activities [64]) [64]

(2Z)-4-[2-[(4-chlorobenzyl)oxy]phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> no or marginal inhibition activities towards ATPase activity or duplex DNA-unwinding activity [64]) [64]

(2Z)-4-[3-(benzylamino)phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> inhibition of duplex DNA-unwinding activity [64]) [64]

(2Z)-4-[3-(benzyloxy)phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> low inhibitory activities [64]) [64]

(2Z)-4-[3-[(4-chlorobenzyl)amino]phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> inhibition of duplex DNA-unwinding activity [64]) [64]

(2Z)-4-[3-[(4-chlorobenzyl)oxy]phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> inhibition of duplex DNA-unwinding activity [64]) [64]

(2Z)-4-[4-(benzyloxy)phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> low inhibitory activities, *para*-relationship between the diketoacid moiety and the OCH_2Ar group do not show antiviral activities [64]) [64]

(2Z)-4-[4-[(4-chlorobenzyl)oxy]phenyl]-2-hydroxy-4-oxobut-2-enoic acid <16> (<16> low inhibitory activities, *para*-relationship between the diketoacid moiety and the OCH_2Ar group do not show antiviral activities [64]) [64]

$(NH_4)_2SO_4$ <7> (<7> 45 mM [14]) [14]

2',3'-ddATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2',3'-ddGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2',3'-ddTTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2'-dATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2'-dGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2'-dTTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

2'-deoxythymidine 5'-phosphoryl-β,γ-hypophosphate <12> (<12> i.e. ppopT, dTTP analogue, most efficient inhibitor of NTPase activity among nucleotide derivaties, inhibits the ATP-dependent helicase reaction and also the ATP-independent duplex unwinding, structure of nucleic base and ribose fragment of NTP molecule have a slight effects on inhibitory properties [62]) [62]

3'-dATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

3'-dGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
3'-dUTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
5-fluoro-2-selenocytosine <14> (<14> reduces ATPase activity, no effect on helicase activity [2]) [2]
ADP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
AMP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
ATP <1,7,47> (<47> above 8 mM [37]; <7> 10 mM [14]; <1> substrate with optimal concentration range between 1 and 2 mM. At high concentrations inhibition of activity can be observed [46]; <7> the optimum concentration of ATP for DNA helicase activity is 1.0 mM. At 8 mM ATP the DNA unwinding activity of PDH120 is inhibited [15]) [14,15,37,46]
ATPγS <1,21> [33,49]
EDTA <3,4,7,11,15> (<7,11> 5 mM [14,55]; <3,7> 5 mM, complete inhibition [15,45]) [14,15,26,32,45,55]
GTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
K^+ <14> (<14> activation at 100-300 mM, inhibition above 500 mM [2]) [2]
KCl <3,7,11,12,28,47> (<11> 200 mM, inhibits [55]; <28> 30% inhibition occurs when KCl concentration is increased from 15 to 200 mM [35]; <7> 400 mM [14]; <47> helicase activity is inhibited at 200 mM [37]; <3> optimal concentration: 100 mM. Inhibition at 200 mM [45]; <7> optimum concentration: 250 mM. Completely inhibited at 400 mM [15]; <12> slight decrease of activity in presence of [62]) [14,15,35,37,45,55,62]
M13 dsDNA <7> (<7> 0.03 mM, complete inhibition [15]) [15]
M13 ssDNA <7> (<7> 0.03 mM, complete inhibition [15]) [15]
M13mp19 ssDNA <29> (<29> ATPase activity is slightly stimulated by ssDNA, and only M13mp19 ssDNA stimulates it significantly (increase in V_{max}) [23]) [23]
Mg^{2+} <3,7> (<7> absolute requirement for divalent cations. Mg^{2+} at 2.0 mM concentration optimally fulfills this requirement. At 8.0 mM $MgCl_2$ the activity is totally inhibited [15]; <3> required, optimal concentration: 0.8 mM. Inhibition at 4 mM [45]) [15,45]
N^1-hydroxyinosine 5'-triphosphate <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
N^1-oxoadenosine 5'-triphosphate <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
Na^+ <14> (<14> activation at 100-300 mM, inhibition above 500 mM [2]) [2]
NaCl <1,4,9,11,24> (<1> above 10 mM [46]; <11> 200 mM, inhibits [55]; <9> 57% inhibition at 0.2 M, 81% inhibition at 0.4 M [7]; <4> ATPase activity is inhibited by salt (NaCl) above 50 mM with a half-maximal inhibition at about 110 mM [8]; <24> optimal concentration is 50-100 mM, higher concentrations inhibit helicase activity [40]) [7,8,40,46,55]

O^6-benzyl-N^7-chloroethylguanine <14> (<14> weak inhibitor of the ATPase and helicase activity [2]) [2]
O^6-benzylguanine <14> (<14> weak inhibitor of the ATPase and helicase activity [2]) [2]
poly(C) <46> (<46> moderately inhibits ATPase activity [24]) [24]
poly(U) <46> (<46> moderately inhibits ATPase activity [24]) [24]
RNA <7> (<7> 0.01 mM [14]) [14]
UTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
aclarubicin <11> [55]
actinomycin C_1 <7> [15]
ammonium sulfate <7> (<7> 45 mM, complete inhibition [15]) [15]
β,γ-methylene-ATP <12> (<12> efficient inhibitor, like the N^1-oxides N^1-oxoadenosine 5-triphosphate and N^1-hydroxyinosine 5-triphosphate [62]) [62]
dATP <1,12> (<12> inhibits unwinding [11]; <1> substrate with optimal concentration range between 1 and 2 mM. At high concentrations inhibition of activity can be observed [46]) [11,46]
daunorubicin <7,11> (<7> 0.01 mM, completely inhibits DNA helicase reaction [14]) [14,15,55]
doxorubicin <11> [55]
dsDNA <7> (<7> 0.01 mM [14]) [14]
ethidium bromide <7> [15]
histone H1 <7> (<7> 0.001 mg/ml, inhibits of the DNA helicase activity [14]) [14]
imidodiphosphate <12> (<12> maximal inhibitory activity among diphosphate analogues, non-catalytic and catalytic conditions, inhibits the ATP-dependent helicase reaction but no effect on the ATP-independent duplex unwinding, structure of nucleic base and ribose fragment of NTP molecule have a slight effects on inhibitory properties [62]) [62]
nogalamycin <7,11> (<7> 0.01 mM, completely inhibits DNA helicase reaction [14]) [14,15,55]
poly(A) <46> (<46> moderately inhibits ATPase activity [24]) [24]
potassium phosphate <7> (<7> 100 mM, complete inhibition [15]) [14,15]
replication protein A <33> (<33> inhibits unwinding and annealing activities [51]) [51]
ribavirin 5'-triphosphate <14> (<14> competitive inhibitor with regard to ATP [2]) [2]
single-stranded DNA <20> [16]
single-stranded DNA-binding proteins <22> [30]
ssDNA <7,29> (<7> 0.01 mM [14]; <29> ATPase activity is slightly stimulated by ssDNA, and only M13mp19 ssDNA stimulats it significantly [23]) [14,23]
streptavidin <18> (<18> the enzyme is completely blocked by streptavidin bound to the 3-ssDNA tail 6 nucleotides upstream of the single-stranded/double-stranded DNA junction. The enzyme efficiently unwinds the forked duplex with streptavidin bound just upstream of the junction, suggesting that

the enzyme recognizes elements of the fork structure to initiate unwinding [31]) [31]
tetrabromobenzotriazole <12> (<12> inhibits unwinding, no inhibition of ATP hydrolysis [11]) [11]
trypsin <7> [15]
yeast total RNA <46> (<46> severly inhibits ATPase activity [24]) [24]
Additional information <5,12> (<5> Hel E is inhibited by replication fork structures [44]; <12> domain 2 of wild-type NS3 protein and domain 2 devoid of the loop structure used for inhibition studies on functions of protein kinase C (PKC), inhibitory potential towards the majority of protein kinase C isoforms shown [63]; <12> inhibitory potential of peptides deduced from amino acid sequence of motif VI tested, NTP-binding and hydrolyzing site not involved, 4.7 pM DNA substrate used for determination of helicase activity [61]) [44,61,63]

Activating compounds

ATP <12> (<12> catalytic DNA helicase activity coupled with NTPase stimulated by [62]) [62]
DNA <46> (<46> ATPase activity is stimulated by yeast genomic DNA and salmon sperm DNA [24]) [24]
N^7-chloroethylguanine <14> (<14> 2.2fold activation at 200-250 mM [2]) [2]
N^9-chloroethylguanine <14> (<14> 8.5fold activation at 200-250 mM [2]) [2]
O^6-benzyl-N^9-chloroethylguanine <14> (<14> stimulator of NTPase activity, with a maximum effect of 350% of control at 650 mM [2]) [2]
double-stranded DNA <3,22> (<22> MER3 ATPase activity is stimulated by either single- or double-stranded DNA [30]; <3> the enzyme is strongly stimulated by either single- or double-stranded DNA [28]) [28,30]
heterotrimeric single-stranded DNA binding protein <28> (<28> enhances DNA helicase activity of Mph1 [35]) [35]
homopolynucleotides <15> (<15> significantly stimulate the ATPase activity (15-25fold) with the exception of poly(G) and poly(dG), which are non-stimulatory. dT24 binds over 10 times more strongly than dA24 [32]) [32]
mitochondrial single-stranded DNA-binding protein <21> (<21> stimultes the enzyme [33]) [33]
poly(U) <23> (<23> strong stimulation [3]) [3]
poly(dA) <14,23> (<23> strong stimulation [3]; <14> 170-180% activation at 1.7-3.3 mM, no activation by other polynucleotides [2]) [2,3]
poly(dI*C) <9> (<9> weakly supports ATPase activity [7]) [7]
poly(dT) <9,23> (<23> strong stimulation [3]; <9> weakly supports ATPase activity [7]) [3,7]
polyadenylate <12> (<12> doubling of ATPase activity in the presence of [62]) [62]
polyuridylate <12> (<12> doubling of ATPase activity in the presence of, lowers K_m for the ATP substrate [62]) [62]
poly* <23> (<23> strong stimulation [3]) [3]

replication protein A <3,5,18,43> (<5> stimulates activity [44]; <43> from Caenorhabditis elegans, stimulates helicase activity [9]; <3> from yeast or human [48]; <18> stimulates helicase activity [12]) [9,12,44,48]
ribavirin <14> (<14> activates ATPase activity, no effect on helicase activity [2]) [2]
single-stranded DANN <3,4,7,8,22,26,28,33,37,47> (<26,37> stimulates [19,20]; <7> required [15]; <4,8> stimulates activity [8,52]; <22> MER3 ATPase activity is stimulated by either single- or double-stranded DNA [30]; <3> more than 5fold stimulation of ATPase activity [48]; <33> nonstructural single-stranded DNA greatly stimulates ATPase activity due to a high affinity for PIF1, even though PIF1 preferentially unwinds forked substrates. The N-terminal portion oF PIF1 helicase, named the PIF1 N-terminal (PINT) domain, contributes to enhancing the interaction with single-stranded DNA through intrinsic binding activity [51]; <47> the ATPase activity of AvDH1 is stimulated more by single-stranded DNA than by double-stranded DNA or RNA. Significantly stimulated by the presence of M13 ssDNA [37]; <3> the enzyme is strongly stimulated by either single- or double-stranded DNA [28]; <28> the enzyme requires single-stranded DNA for activation [35]) [8,15,19,20,28,30,35,37,48,51,52]
single-stranded DNA binding protein <4> (<4> of Escherichia coli (SSB), stimulates to a lower extent [8]) [8]
single-stranded DNA binding protein dRP-A <1> (<1> stimulates the activity on substrates with more than 300 nucleotides double-stranded region [46]) [46]
single-stranded DNA-binding protein <4,22,36> (<4> from Escherichia coli, strongly stimulates when long partial duplex substrates are used [26]; <36> of Escherichia coli, stimulates activity [29]; <22> single-stranded DNA-binding proteins stimulate [30]) [26,29,30]
ssDNA <2,41> (<41> stimulates ATPase activity [21]; <2> the effect of single-stranded DNA on the kinetics of NTP hydrolysis depends on the type of nucleotide cofactor and the base composition of the DNA and is centered at the hydrolysis step. Homoadenosine ssDNA oligomers are particularly effective in increasing the hydrolysis rate [10]) [10,21]
yeast replication protein A <4> (<4> stimulates significantly [8]) [8]
Additional information <3,9,23> (<3> no significant stimulation by Escherichia coli ssDNA-binding protein [48]; <23> no stimulation by poly(G) [3]; <9> synthetic RNA poly(U) does not support ATP hydrolysis at all. Unlike DNA helicase I, DNA helicase II is not stimulated by SpRPA or Escherichia coli SSB at low ATP concentrations [7]) [3,7,48]

Metals, ions

Ca^{2+} <22,40> (<40> ATP hydrolysis in the presence of $MgCl_2$ is 2fold higher than in the presence of either $MnCl_2$ or $CaCl_2$ [60]; <22> MER3 ATPase activity requires a divalent cation. Maximal activity can be observed in the presence of either Ca^{2+}, Mg^{2+}, or Mn^{2+}, whereas Zn^{2+} does not support the ATPase activity [30]) [30,60]

Co^{2+} <12,42> (<42> supports activity similar to that with Mg^{2+} [22]; <12> activity 3-5-fold lower when magnesium ions are replaced by [62]) [22,62]
K^+ <14> (<14> activation at 100-300 mM, inhibition above 500 mM [2]) [2]
KCl <3,7,47> (<47> optimal concentration for ATPase activity: 200 mM. Optimal concentration for helicase activity: 60 mM [37]; <3> optimal concentration: 100 mM. Inhibition at 200 mM [45]; <7> optimum concentration: 250 mM. Completely inhibited at 400 mM [15]) [15,37,45]
Mg^{2+} <1,3,4,7,9,11,12,14,15,20,22,24,26,28,40,43,47,51> (<3,4,26> required [19,26,28]; <15> activity is dependent on [32]; <7> absolute requirement for divalent cations. Mg^{2+} at 2.0 mM concentration optimally fulfills this requirement. At 8.0 mM $MgCl_2$ the activity is totally inhibited [15]; <40> ATP hydrolysis in the presence of $MgCl_2$ is 2fold higher than in the presence of either $MnCl_2$ or $CaCl_2$ [60]; <1> divalent cation required, optimum concentration: 0.5 mM [46]; <12> divalent metal cations are absolutely required for HCV helicase-catalyzed DNA unwinding. When compared with unwinding in the presence of Mg^{2+}, Mn^{2+} supports 10 times faster rates than Mg^{2+} regardless of the concentration of metal in solution. All NTPs support faster unwinding when 2 mM Mn^{2+} is used instead of 2 mM Mg^{2+}. The specificity profile remains mostly unchanged in the presence of Mn^{2+}, while the absolute magnitude of the rates increases [38]; <43> little or no unwinding is observed when Mg^{2+} was replaced with Zn^{2+} [9]; <28> maximal activity is obtained with Mg^{2+}, whereas Co^{2+} and Ca^{2+} are much less effective in this regard. No activity is observed with Mn^{2+} or Zn^{2+} [35]; <22> MER3 ATPase activity requires a divalent cation. Maximal activity can be observed in the presence of either Ca^{2+}, Mg^{2+}, or Mn^{2+}, whereas Zn^{2+} does not support the ATPase activity [30]; <24> Mg^{2+} or Mn^{2+} required, optimal activity at 4 mM $MgCl_2$ [40]; <7> Mg^{2+} or Mn^{2+} required, optimum concentration of $MgCl_2$ is 1 mM [14]; <47> required for ATPase and DNA-unwinding activity, optimal concentration for DNA unwinding reaction: 2.0 mM, optimal concentration for ATP-independent RNA unwinding reaction: 2 mM [37]; <3> required for maximal activity, optimal concentration: 0.8 mM. Inhibition at 4 mM [45]; <14> required, optimum concentration for ATPase reaction is 1-3 mM, optimum concentration for helicase reaction is 0.3-5 mM [2]; <20> requirement for divalent metal ions. Helicase activity is stimulated most by $MgCl_2$ at a concentration of 1.5 mM [16]; <4> requires divent cation, Mg^{2+} or Mn^{2+} [27]; <9> the enzyme requires $MgCl_2$ for its activity. It is not active in the presence of $MnCl_2$ or $CaCl_2$ (1 mM) [7]; <11> unwinding activity requires Mg^{2+} [55]; <12> influences DNA unwinding rates of recombinant nonstructural protein 3, metal ion specificity suggests that NTPs bind two different enzyme conformations [38]; <12> maximal NTPase activity achieved in the presence of 1.5-2 mM $MgCl_2$ [62]; <51> no ATPase activity of the wild-type in the absence of [4]) [2,4,7,9,14,15,16,19,26,27,28,30,32,35,37,38,40,45,46,55,60,62,65]
$MgCl_2$ <42> (<42> required, optimal concentration: 5 mM [22]) [22]
Mn^{2+} <4,7,12,15,20,22,24,40,42> (<7,24> Mg^{2+} or Mn^{2+} required [14,40]; <7> 2.0 mM, supports 80% of the activity compared to Mg^{2+} [15]; <40> ATP hydrolysis in the presence of $MgCl_2$ is 2fold higher than in the presence of either $MnCl_2$ or $CaCl_2$ [60]; <15> can substitute for Mg^{2+}, 40% of the efficiency

with Mg^{2+} at 2.5 mM [32]; <4> can substitute for Mg^{2+}, less effective [26]; <12> divalent metal cations are absolutely required for HCV helicase-catalyzed DNA unwinding. When compared with unwinding in the presence of Mg^{2+}, Mn^{2+} supports 10 times faster rates than Mg^{2+} regardless of the concentration of metal in solution. All NTPs support faster unwinding when 2 mM Mn^{2+} is used instead of 2 mM Mg^{2+}. The specificity profile remains mostly unchanged in the presence of Mn^{2+}, while the absolute magnitude of the rates increases [38]; <22> MER3 ATPase activity requires a divalent cation. Maximal activity can be observed in the presence of either Ca^{2+}, Mg^{2+}, or Mn^{2+}, whereas Zn^{2+} does not support the ATPase activity [30]; <20> $MnCl_2$ stimulates activity, though not as well as the $MgCl_2$ [16]; <4> requires divent cation, Mg^{2+} or Mn^{2+} [27]; <42> supports ATPase activity with 2fold lower efficiency compared to Mg^{2+} [22]; <12> activity 3-5-fold lower when magnesium ions are replaced by [62]; <12> influences DNA unwinding rates of recombinant nonstructural protein 3, supports about 10 times faster unwinding than Mg^{2+}, unlike Mg^{2+}, Mn^{2+} does not support helicase-catalyzed ATP hydrolysis in the absence of stimulating nucleic acids, metal ion specificity suggests that NTPs bind two different enzyme conformations [38]) [14,15,16,22,26,27,30,32,38,40,60,62]

Na^+ <14> (<14> activation at 100-300 mM, inhibition above 500 mM [2]) [2]

NaCl <24,42> (<42> optimal concentration: 50 mM [22]; <24> optimal concentration is 50-100 mM, higher concentrations inhibit helicase activity [40]) [22,40]

Ni^{2+} <12,42> (<42> supports ATPase activity with 3fold lower efficiency compared to Mg^{2+} [22]; <12> activity 3-5-fold lower when magnesium ions are replaced by [62]) [22,62]

Zn^{2+} <8,12> (<8> BcMCM amino-terminus can bind single-stranded DNA and harbors a zinc atom, BcMCM contains 0.11 zinc atoms per mole [52]; <12> activity 3-5-fold lower when magnesium ions are replaced by [62]) [52,62]

Additional information <7> (<7> Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Ag^{2+} and Co^{2+} are unable to support the activity [15]) [15]

Turnover number (s^{-1})

0.043 <1> (ATP, <1> pH 8.0, 30°C, without ssDNA [49]) [49]

0.1 <49> (2'(3')-O-(N-methylanthraniloyl)ATP, <49> pH 7.5, 20°C, without DNA [39]) [39]

0.25 <1> (dATP, <1> pH 8.0, 30°C, without ssDNA [49]) [49]

0.3 <49> (ATP, <49> pH 7.5, 20°C, without DNA [39]) [39]

1.2 <40> (ATP, <40> pH 7.5, 30°C [60]) [60]

1.5 <34> (ATP, <34> full-length RECQ1 helicase [57]) [57]

5.4 <15> (GTP, <15> pH 6.6, 25°C [32]) [32]

7.41 <34> (ATP, <34> mutant (RECQ1(T1))Y564A, a construct encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa [57]) [57]

7.5 <15> (dGTP, <15> pH 6.6, 25°C [32]) [32]

8.8 <22> (ATP, <22> pH 7.6, 30°C, in presence of M13mp18 single-stranded circular DNA [30]) [30]
9.2 <22> (ATP, <22> pH 7.6, 30°C, in presence of poly(dA) [30]) [30]
10.5 <15> (UTP, <15> pH 6.6, 25°C [32]) [32]
11.25 <34> (ATP, <34> (RECQ1(T1)), a construct encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa [57]) [57]
13.1 <42> (ATP, <42> pH 7.5, in presence of 1 mM Ni^{2+} [22]) [22]
13.9 <15> (CTP, <15> pH 6.6, 25°C [32]) [32]
14.3 <15> (dCTP, <15> pH 6.6, 25°C [32]) [32]
14.8 <15> (dATP, <15> pH 6.6, 25°C [32]) [32]
14.8 <15> (dUTP, <15> pH 6.6, 25°C [32]) [32]
15 <1> (ATP, <1> pH 8.0, 30°C, in presence of ssDNA [49]) [49]
16.9 <49> (ATP, <49> pH 7.5, 20°C, in presence of DNA [39]) [39]
17.2 <49> (2'(3')-O-(N-methylanthraniloyl)ATP, <49> pH 7.5, 20°C, in presence of DNA [39]) [39]
19.1 <15> (ATP, <15> pH 6.6, 25°C [32]) [32]
19.8 <42> (ATP, <42> pH 7.5, in presence of 1 mM Mn^{2+} [22]) [22]
22.2 <42> (ATP, <42> pH 7.5, in presence of 1 mM Co^{2+} [22]) [22]
23 <1> (dATP, <1> pH 8.0, 30°C, in presence of ssDNA [49]) [49]
29.8 <42> (ATP, <42> pH 7.5, in presence of 1 mM Mg^{2+} [22]) [22]
7980 <14> (ATP, <14> pH 7.5, 30°C, in presence of 2 mM Mg^{2+} [2]) [2]
Additional information <49> (<49> turnover numbers for cleavage of dTn DNA (dT10, dT20, dT30, dT40) [39]) [39]

Specific activity (U/mg)

160.8 <14> [2]
Additional information <7,12,14,16,50,51> (<7> highest specific activity among plant helicases [15]; <50> ambiguous helicase activity, also DNA unwinding [66]; <12> DNA helicase reaction can proceed in two modes depending on the ratio between enzyme and substrate concentration, non-catalytic in the case of enzyme excess and catalytic in the case of tenfold substrate excess, structure of nucleic base and ribose fragment of NTP molecule has a slight effect on inhibitory properties, duplex DNA oligonucleotides used for determination of DNA helicase activity [62]; <16> eight analogues of anti-HCV aryl diketoacide (ADK) investigated for inhibitory capacity, phosphate release assay and FRET-based assay [64]; <12> molecular beacon-based helicase assay (MBHA) developed, unwinding of DNA mediated by recombinant nonstructural protein 3 occurs at different rates depending on the nature and concentration of NTPs in solution, presence of an intact NS3 protease domain makes HCV helicase somewhat less specific than truncated NS3 bearing only its helicase region specificity determined by the nature of the Watson-Crick base-pairing region of the NTP base and the nature of the functional groups attached to the 2 and 3 carbons of the NTP sugar [38]; <12> overview of sequences of NTPase/helicase motifs VI derived peptides and their deleted derivatives, kinetic analyses reveals that binding of the peptides do not interfere with the NTPase activity of the enzymes, peptides do not interact with the ATP binding site [61]; <51> structural characterization of catalytic do-

main, mutation analysis of residue substitution in the Walker A motif (Gly199, Lys200 and Thr201), within the NTP-binding pocket (Gln457, Arg461 and Arg464) and of Arg458 in the outside of the pocket in the motif IV, residues crucial for ATPase and DNA helicase activities and virus replication, Lys200 cannot be substituted by other residues to establish sufficient activities, structure of the NTP-binding pocket well conserved among the viruses of the Flaviviridae [4]; <14> structural characterization of the C-terminal portion containing the ATPase/helicase domain, encompasses residues 181-619, monomer structure determined by analytical centrifugation and gel filtration, SDS-PAGE and immunoblotting, structure determined by circular dichroism and fluorescence spectroscopy, ATPase activity stimulated by RNA and ssDNA, DNA helicase activity assayed with different salt concentrations from 5 to 150 mM and ATP concentrations at 40, 80, 100, 250 and 500 microM, respectively, no DNA helicase activity at protein concentrations up to 500 nM, linker region between the protease and the helicase domains predicted as a prerequisite for protein-protein interactions leading to the formation of the active oligomer [67]; <12> surface of domain 2 of the NS3 NTPase/helicase in direct vicinity to a flexible loop that is localized between Val1458 and Thr1476, accessibility of the Arg-rich amino acid motif by this loop for protein kinase C inhibition analyzed, two variants of domain 2 generated, in vitro protein kinase C (PKC) phosphorylation studies, binding and competition assays, modelling of ribbon diagrams, presence of the intact loop abolishes the binding of domain 2 to a tailed duplex RNA, binding of dsDNA not affected, loop structure reduces the extent of inhibition of protein kinase C (PKC) by domain 2 and regulates the binding of dsRNA, various mechanisms by which the NS3 protein perturb signal transduction in infected cells [63]) [4,15,38,61,62,63,64,66,67]

K_m-Value (mM)

0.0026 <49> (2'(3')-O-(N-methylanthraniloyl)ATP, <49> pH 7.5, 20°C, in presence of DNA [39]) [39]
0.0035 <49> (ATP, <49> pH 7.5, 20°C, in presence of DNA [39]) [39]
0.0038 <33> (ATP, <33> pH 8.0, 30°C, with saturating concentration of M13 mp18 ssDNA [51]) [51]
0.005 <49> (ATP, <49> pH 7.5, 20°C, without DNA [39]) [39]
0.006 <49> (2'(3')-O-(N-methylanthraniloyl)ATP, <49> pH 7.5, 20°C, without DNA [39]) [39]
0.0095 <14> (ATP, <14> pH 7.5, 30°C, in presence of 2 mM Mg^{2+} [2]) [2]
0.013 <14> (ATP, <14> recombinant protein including C-terminal portion the ATPase/helicase domain encompassing residues 181-619, ATP concentration 1 mM ATP, ATPase but not DNA helicase activity [67]) [67]
0.0553 <42> (ATP, <42> pH 7.5, in presence of 1 mM Co^{2+} [22]) [22]
0.061 <3> (ATP) [48]
0.07 <12> (ATP, <12> helicase-catalyzed DNA unwinding activity mediated by recombinant nonstructural protein 3, reactions with 1 mM ATP contain 1.25 mM total $MgCl_2$, data are globally fit to a model for substrate inhibition [38]) [38]

0.08 <42> (ATP, <42> pH 7.5, in presence of 1 mM Mg^{2+} [22]) [22]
0.086 <42> (ATP, <42> pH 7.5, in presence of 1 mM Mn^{2+} [22]) [22]
0.09 <4> (ATP, <4> pH 7.5, 30°C [8]) [8]
0.1 <34> (ATP, <34> mutant (RECQ1(T1))Y564A, a construct encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa [57]) [57]
0.115 <34> (ATP, <34> full-length RECQ1 helicase [57]) [57]
0.128 <42> (ATP, <42> pH 7.5, in presence of 1 mM Ni^{2+} [22]) [22]
0.135 <34> (ATP, <34> (RECQ1(T1)), a construct encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa [57]) [57]
0.15 <1,40> (ATP, <40> pH 7.5, 30°C [60]; <1> pH 8.0, 30°C, in presence of ssDNA [49]) [49,60]
0.152 <29> (ATP, <29> pH 7.5, 37°C, addition of 60-mer oligonucleotide [23]) [23]
0.163 <12> (ATP, <12> addition of polyuridylate lowers K_m for the ATP substrate [62]) [62]
0.2 <4> (ATP, <4> pH 7.8, 30°C [27]) [27]
0.2 <15> (GTP, <15> pH 6.6, 25°C [32]) [32]
0.2 <15> (dATP, <15> pH 6.6, 25°C [32]) [32]
0.22 <29> (ATP, <29> pH 7.5, 37°C, addition of M13mp19 ssDNA [23]) [23]
0.22 <15> (dCTP, <15> pH 6.6, 25°C [32]) [32]
0.25 <1,4> (dATP, <4> pH 7.8, 30°C [27]; <1> pH 8.0, 30°C, in presence of ssDNA [49]) [27,49]
0.256 <12> (ATP, <12> wild-type [62]) [62]
0.3 <12> (dTTP, <12> helicase-catalyzed DNA unwinding activity mediated by recombinant nonstructural protein 3, reactions with 0.2 mM ATP contain 0.45 mM total $MgCl_2$, data are globally fit to a model for substrate inhibition [38]) [38]
0.33 <15> (ATP, <15> pH 6.6, 25°C [32]) [32]
0.35 <15> (dGTP, <15> pH 6.6, 25°C [32]) [32]
0.41 <12> (GTP, <12> helicase-catalyzed DNA unwinding activity mediated by recombinant nonstructural protein 3, data for reactions performed at or below 1 mM NTP, data are globally fit to a model for substrate inhibition [38]) [38]
0.47 <22> (ATP, <22> pH 7.6, 30°C, in presence of poly(dA) [30]) [30]
0.47 <15> (CTP, <15> pH 6.6, 25°C [32]) [32]
0.55 <15> (UTP, <15> pH 6.6, 25°C [32]) [32]
0.58 <22> (ATP, <22> pH 7.6, 30°C, in presence of M13mp18 single-stranded circular DNA [30]) [30]
0.65 <9> (ATP, <9> pH 7.8, 37°C [7]) [7]
0.93 <15> (dUTP, <15> pH 6.6, 25°C [32]) [32]
Additional information <49> (<49> K_M-values for cleavage of dTn DNA (dT10, dT20, dT30, dT40) [39]) [39]

K_i-Value (mM)

0.00071 <7> (nogalamycin, <7> pH 8.0, 37°C [15]) [15]
0.004 <7> (daunorubicin, <7> pH 8.0, 37°C [15]) [15]

0.0052 <7> (ethidium bromide, <7> pH 8.0, 37°C [15]) [15]
0.0056 <7> (actinomycin C1, <7> pH 8.0, 37°C [15]) [15]
0.09 <12> (GTP, <12> recombinant nonstructural protein 3 analyzed for DNA unwinding rates, acts as a noncompetitive inhibitor, 22°C, 2 mM $MgCl_2$, 25 nM enzyme, and 5 nM substrate, pH 6.5, initiation by adding each NTP to 0.5 mM [38]) [38]
0.09 <12> (NTP, <12> recombinant nonstructural protein 3 analyzed for DNA unwinding rates, data globally fit to a model for substrate inhibition, 22°C, 2 mM $MgCl_2$, 25 nM enzyme, and 5 nM substrate, pH 6.5, initiation by adding each NTP to 0.5 mM [38]) [38]
0.097 <12> (2'-deoxythymidine 5'-phosphoryl-β,γ-hypophosphate, <12> i.e. ppopT, dTTP analogue, inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.109 <12> (N^1-OH^-ITP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.116 <12> (2',3'-ddATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.116 <12> (2'-dTTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.141 <12> (3'-dATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.145 <12> (β,γ-methylene-ATP, <12> efficient inhibitor, like the N^1-oxides N^1-oxoadenosine 5-triphosphate and N^1-hydroxyinosine 5-triphosphate [62]) [62]
0.205 <12> (N^1-O-ATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.26 <12> (3'-dUTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.277 <12> (2'-dGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.291 <12> (2'-dATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.298 <12> (2',3'-ddTTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.443 <12> (3'-dGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.576 <12> (GTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
0.69 <12> (ATP, <12> recombinant nonstructural protein 3 analyzed for DNA unwinding rates, 22°C, 2 mM Mg^{2+}, 25 nM enzyme, and 5 nM substrate, pH 6.5, initiation by adding each NTP to 0.5 mM [38]) [38]
0.721 <12> (2',3'-ddGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
1.3 <12> (ADP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]
1.46 <12> (UTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

5 <12> (AMP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [62]) [62]

pH-Optimum

6.5 <12> (<12> assay at [38]; <12> helicase-catalyzed DNA unwinding activity at [38]) [38]
6.6 <15,16> (<15,16> assay at [32,64]) [32,64]
6.6-8.4 <28> [35]
7 <5> (<5> assay at [44]) [44]
7.4 <12> (<12> activity assay at [62]) [62]
7.5 <3,4,12,13,14,36,40,49> (<3,12,13,14,36,40,49> assay at [2,28,29,39,59,60,61,67]; <4> unwinding reaction [26]) [2,26,27,28,29,39,59,60,61,67]
7.6 <18,19,22,26,27> (<18,19,22,26,27> assay at [19,30,31,34,42]) [19,30,31,34,42]
7.8 <9,21> (<9,21> assay at [7,33]) [7,33]
8 <1,7,43,47> (<1,43> assay at [9,49,50]; <47> ATPase activity, DNA-unwinding activity [37]) [9,15,37,49,50]
8.9 <20> (<20> assay at [16]) [16]

pH-Range

6-9 <4> (<4> pH 6.0: about 35% of maximal activity, pH 9.0: about 65% of maximal activity [26]) [26]
6.5-8.5 <47> (<47> pH 6.5: about 60% of maximal activity, pH 8.5: about 60% of maximal activity, ATPase activity [37]) [37]
6.5-8.9 <9> (<9> the enzyme functions efficiently over wide ranges of pH from 6.5 to 8.9 [7]) [7]
7.5-9 <7> (<7> significant unwinding activity is observed in the broad pH range (pH 7.5-9.0) [15]) [15]

pi-Value

7.6 <47> (<47> calculated from sequence [37]) [37]
8 <39> (<39> calculated from sequence [18]) [18]

Temperature optimum (°C)

20 <49> (<49> assay at [39]) [39]
22 <12> (<12> helicase-catalyzed DNA unwinding activity at [38]) [38]
25 <15> (<15> assay at [32]) [32]
29 <47> (<47> ATPase activity [37]) [37]
30 <1,12,14,19,22,40> (<1,12,14,19,22,40> assay at [2,30,34,49,50,60,61]) [2,30,34,49,50,60,61]
37 <3,5,9,12,13,14,16,18,21,26,27,36,42,43,51> (<3,5,9,12,13,14,16,18,21,26,27,36,42,43,51> assay at [4,7,9,19,22,28,29,31,33,38,42,44,59,64,67]; <12> activity assay at [62]) [4,7,9,19,22,28,29,31,33,38,42,44,59,62,64,67]
50 <20> [16]

Temperature range (°C)

20-37 <43> (<43> similar active at 20°C, 30°C and 37°C [9]) [9]
25-34 <47> (<47> 25°C: about 55% of maximal activity, 34°C: about 40% of maximal activity, ATPase activity [37]) [37]

30-60 <20> (<20> 30°C: about 65% of maximal activity, 60°C: about 75% of maximal activity [16]) [16]

4 Enzyme Structure

Molecular weight

50480 <47> (<47> calculated from sequence [37]) [37]
54000 <51> (<51> molecular mass of the helicase/NTPase domain, SDS-PAGE [4]) [4]
65000 <9> (<9> gel filtration, glycerol gradient analysis [7]) [7]
66000 <14> (<14> recombinant protein of C-terminal portion the ATPase/helicase domain, residues 181-619, SDS-PAGE, gel filtration [67]) [67]
85000 <42> (<42> sedimentation equilibrium ultracentrifugation [22]) [22]
120000 <7> (<7> gel filtration, glycerol gradient centrifugation [15]) [15]
128000 <3> (<3> gel filtration [45]) [45]
136000 <7> (<7> gel filtration [14]) [14]
170000 <39> (<39> calculated from sequence [18]) [18]
200000 <1> (<1> gel filtration, glycerol gradient centrifugation [46]) [46]
500000 <4> (<4> gel filtration [8]) [8]
600000 <3> (<3> gel filtration [48]) [48]

Subunits

? <1,3,4,11,12,14,15,21,29,47> (<47> x * 54000, SDS-PAGE [37]; <11> x * 90000, SDS-PAGE [55]; <14> x * 60000, SDS-PAGE [2]; <12,15,21> x * 70000, SDS-PAGE [1,32,33]; <29> x * 50000, SDS-PAGE [23]; <3> x * 180000, SDS-PAGE [28]; <4> x * 12000, SDS-PAGE [27]; <47> x * 50478, calculated from sequence [37]; <1> x * 54000, small isoform of RECQ5 helicase, SDS-PAGE [49]) [1,2,23,27,28,32,33,37,49,55]
dimer <7> (<7> 2 * 68000, SDS-PAGE [14]) [14]
heptamer <6> (<6> structural polymorphism: in addition to helical filaments and heptameric rings the protein also forms double heptamers, hexamers and double hexamers, octamers and open rings [43]) [43]
heterodimer <7> (<7> 1 * 54000 + 1 * 66000, SDS-PAGE [15]) [15]
hexamer <3,4,6,35> (<4> 6 * 90000, SDS-PAGE [8]; <3> 6 * 116000, SDS-PAGE [48]; <6> structural polymorphism: in addition to helical filaments and heptameric rings the protein also forms double heptamers, hexamers and double hexamers, octamers and open rings [43]) [8,43,48]
homohexamer <30> (<30> 6 * 30000 [5]) [5]
monomer <3,4,8,9,14,33,42> (<3> 1 * 128000, SDS-PAGE [45]; <9> 1 * 63000, SDS-PAGE [7]; <33> 1 * 71000, SDS-PAGE [51]; <4> 1 * 135000, SDS-PAGE [26]; <42> 1 * 85000, the lack of cooperativity observed for both the ATPase and helicase activities lends support to the view that UvrD monomers are the functional unit [22]; <8> BcMCM is a monomer in solution but likely forms the functional oligomer in vivo [52]; <14> $\alpha\beta$, 29% α-helix, 15% β-sheet, and 56% non-regular structures, globular monomer accounts for 90%, a small

percentage (7%) of dimers or trimers, higher oligomers almost absent (3%), analytical centrifugation and gel filtration [67]) [7,22,26,45,51,52,67]
octamer <6> (<6> structural polymorphism: in addition to helical filaments and heptameric rings the protein also forms double heptamers, hexamers and double hexamers, octamers and open rings [43]) [43]
Additional information <24> (<24> posttranslational modifications lacking in in vitro- or bacterially synthesized Rep52 may be required for efficient Rep52 multimerization [40]) [40]

5 Isolation/Preparation/Mutation/Application

Source/tissue

B-cell <3> [28]
HeLa cell <3> (<3> maximal level of expression is observed in late G1/early S phase [48]) [45,48]
culture medium <14> [2]
embryo <1> [46]
leaf <7,32> [14,15,56]
testis <3,48> (<3,48> GRTH is a negative regulator of apoptosis in spermatocytes and promotes the progress of spermatogenesis [36]) [36]
thymus <5> (<5> calf [44]) [44]

Localization

chloroplast <7> [14]
membrane <12> [61]
mitochondrion <40> (<40> mitochondrial localization of Hmi1p is essential for its role in mtDNA metabolism [60]) [60]
nucleus <7> [15]

Purification

<1> [46]
<1> (enzyme recombinantly expressed in Escherichia coli) [49]
<3> [45,48]
<3> (recombinant) [28]
<4> [8,26,27]
<7> [14,15]
<9> [7]
<10> [47]
<11> [55]
<12> [1,11]
<12> (gel filtration, SDS-PAGE) [61,63]
<12> (gel filtration, recombinant nonstructural protein 3) [38]
<12> (gel filtration, recombinant protein) [62]
<12> (truncated and full-length complexes between nonstructural protein 3 (NS3) and nonstructural protein 4A (NS4), NS3-4A complex purifies as two separable proteins, gel filtration, SDS-PAGE) [65]

<13> (one-step column purification of helicase-primase subcomplex (helicase-primase enzyme complex consisting of UL5 and UL52 gene functions) using C-terminally His-tagged UL5 subunit) [59]
<14> [2]
<14> (gel filtration, recombinant protein, soluble form) [67]
<15> [32]
<20> [16]
<21> (generation of recombinant baculovirus encoding the human TWINKLE gene, expression in insect cells) [33]
<22> [30]
<23> [3]
<26> [19]
<27> [42]
<28> [35]
<29> [23]
<30> [5]
<31> [13]
<33> [53]
<33> (full-length PIF1 with a 6* histidine tag at the N-terminus, a C-terminal truncated form (PIF1N) and a N-terminal truncated form (PIF1C)) [51]
<33> (streamlined purification for the production of near-homogeneous and high yield recombinant forms of the human mitochondrial DNA helicase, minimizing the number of steps and the time elapsed for purification) [41]
<34> (a construct (RECQ1(T1)) encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa is produced in Escherichia coli and purified to more than 95% homogeneity) [57]
<36> (recombinant Sgs1 fragment (amino acids 400-1268 of the 1447-amino acid full-length protein)) [29]
<37> [20]
<38> [54]
<40> (recombinant) [60]
<42> (recombinant histidine-tagged form of the protein) [22]
<43> [9]
<46> (recombinant enzyme) [24]
<47> [37]
<50> (gel filtration) [66]
<51> (gel filtration, recombinant protein) [4]

Crystallization

<10> (crystallization of the helicase domain of bacteriophage T7 gene 4 protein) [47]
<12> [11]
<30> (hanging-drop vapour-diffusion method with polyethyleneglycol monomethyl ether as precipitating agent) [5]
<31> (1.8 A resolution crystal structure of the catalytic core of Escherichia coli RecQ in its unbound form and a 2.5 A resolution structure of the core bound to ATPγS) [13]

<34> (purified RECQ1T1 protein is crystallized in the presence of ATP-γS and oligonucleotides by vapor diffusion from sitting drops equilibrated against 0.2 M sodium bromide, 20% PEG 3350, 10% ethylene glycol, 0.1 M bis-Tris propane (pH 7.5). Crystal structure of a truncated form (RECQ1(T1)) of the human RECQ1 protein with $MgADP^{2-}$) [57]
<45> (hanging-drop vapour diffusion at room temperature) [6]
<51> (enzymatically active fragment of the JEV NTPase/helicase catalytic domain, recombinant protein, crystal structure determined at 1.8 A resolution, data collection and refinement statistics) [4]

Cloning

<3> [36]
<3> (expression in Escherichia coli) [48]
<3> (overexpression of an oligohistidine-tagged version of the BLM gene product in Saccharomyces cerevisiae) [28]
<4> (expression in Escherichia coli) [8]
<10> [47]
<12> [1]
<12> (NS3-plus and NS3/4a-plus genes expressed in Escherichia coli, generation of NS3-4A expression product, pET15b and pet-SUMO vector) [65]
<12> (expressed in Escherichia coli) [61]
<12> (expressed in Escherichia coli BL21(DE3), recombinant protein, NS3d2wt variant corresponding to wild-type domain 2, NS3d2D construct comprises the complete domain, HCV(1361-1503) without loop, pET21b andpET16b vectors) [63]
<12> (expressed in Escherichia coli, strain Rosetta (DE3), recombinant nonstructural protein 3) [38]
<12> (expressed in Escherichia coli, strains XL-1 Blue, Rosetta (DE3), M15 (pREP4), vector pET-21-2c, kinetics of NS3 protein accumulation upon its expression in Escherichia coli at 25°C for 1-5 h shown) [62]
<13> (His6-tagged DNA helicase expressed via recombinant baculovirus) [59]
<14> (expressed in Escherichia coli, C-terminal portion with the ATPase/helicase domain, plasmid pET-30a) [67]
<15> [32]
<20> [16]
<23> (baculovirus expression system) [3]
<26> (expression of a truncated version of Rrm3p as a GST fusion protein in Saccharomyces cerevisiae. This polypeptide (Rrm3pΔN), contains amino acids 194 to 723 of the 723-amino-acid protein, including all seven helicase motifs as well as 56 amino acids amino-terminal of the first helicase motif. Rrm3pΔN is expressed under the control of a galactose-inducible promoter) [19]
<28> (the carboxyl-terminal His6 epitope is attached to the MPH1-coding sequence and the tagged gene is placed under the galactose inducible GAL1 promoter in the vector pYES) [35]
<29> [23]

<32> [56]
<33> (human hPif1 (nuclear form amino acids 1-641) and the hPif helicase domain (hPifHD, amino acid residues 206-620) are cloned as a fusion protein with glutathione S-transferase in pET11c. GST-hPifHD is expressed in Escherichia coli BL21(DE3) cells) [53]
<34> (a construct (RECQ1(T1)) encompassing amino acids 49-616 (of 649) of RECQ1, followed by a C-terminal tag of 22 aa is produced in Escherichia coli) [57]
<36> (a recombinant Sgs1 fragment (amino acids 400-1268 of the 1447-amino acid full-length protein) is overexpressed in yeast) [29]
<37> (PcrA protein is overexpressed with a His6 fusion at its amino-terminal end) [20]
<38> [54]
<40> [60]
<42> (histidine-tagged form of the protein is expressed) [22]
<43> [9]
<44> [17]
<46> (expression of a recombinant Dbp9p in Escherichia coli) [24]
<47> (expression in Escherichia coli) [37]
<48> [36]
<50> (NS3-plus and NS3/4a-plus genes expressed in Escherichia coli, composition of NS3-4A expression product using the pet-SUMO vector) [66]
<51> (expressed in Escherichia coli BL21 (DE3), recombinant protein, pET21b vector) [4]

Engineering

C261A <8> (<8> mutant with a disrupted zinc-binding site. One mol of the C261A mutant contains 0.03 atoms [52]) [52]
D523N <17> (<17> DNA binding and ATPase activity is comparable to wild-type enzyme, no in vitro replication activity [58]) [58]
D542N <17> (<17> DNA binding and ATPase activity is comparable to wild-type enzyme, no in vitro replication activity [58]) [58]
H293A <12> (<12> mutation results in a protein with a significantly higher level of ATPase in the absence of RNA. The mutant protein still unwinds RNA. In the presence of RNA, the H293A mutant hydrolyzes ATP slower than wild-type [11]) [11]
K116H <24> (<24> an MBP-Rep52 chimera bearing K116H mutation within a consensus helicase- and ATPase-associated motif (motif I or Walker A site) is deficient for both DNA helicase and ATPase activities [40]) [40]
K337A <27> (<27> K337A and the K337R alleles are unable to supply the essential function of Pfh1p [42]) [42]
K337R <27> (<27> K337A and the K337R alleles are unable to supply the essential function of Pfh1p [42]) [42]
K340H <24> (<24> in a Rep78 A-site mutant protein bearing mutation K340H, the MBP-Rep52 A-site mutant protein fails to exhibit a trans-dominant negative effect when it is mixed with wild-type MBP-Rep52 or MBP-Rep78 in vitro [40]) [40]

K484E <17> (<17> mutant enzyme binds the immunoaffinity column poorly, the heparin purified E1/K484E is tested for the above activities. The protein that is recovered shows no activity [58]) [58]
K653A <8> (<8> mutation of the ATP-binding site reduces activity to about 30% of wild-type. This drop in ATPase activity corresponds to an abrogation of helicase activity observed in the same mutant [52]) [52]
P479S <17> (<17> DNA binding and ATPase activity is comparable to wild-type enzyme, 50% of in vitro replication activity compared to wild-type enzyme [58]) [58]
Additional information <6,35> (<35> a mutation of the MCM N-terminal β-hairpin reduces but does not abolish DNA binding and helicase activity [43]; <6> a mutation of the zinc finger motif of the MCM protein reduces single-stranded and double-stranded DNA binding and abolishes helicase activity. Removal of the HTH domain from the MCM protein results in an enzyme with increased ATPase and helicase activity. A mutation of the MCM N-terminal β-hairpin completely abolishes DNA binding and helicase activity [43]) [43]

Application

medicine <39> (<39> UvrD helicase is a potential drug targets for chemotherapy of malaria. As Plasmodium falciparum contains only one homologue of UvrD helicase and human lacks this helicase, detailed studies including cloning and characterization of UvrD helicase of malaria parasite may be helpful in identifying a compound that has no effect on the cellular machinery of the host and consequently could be used as the potential drug for the treatment of malaria [18]) [18]
pharmacology <12,51> (<51> conservation of the NTP-binding pocket among viruses of the family Flaviviridae as potential for development of therapeutics [4]; <12> peptide inhibitors reproducing the structure of the autoregulatory motif as possibility to develop effective antivirals [61]) [4,61]

6 Stability

Temperature stability

56 <7> (<7> 1 min, inactivated [14]; <7> 1 min, loss of activity [15]) [14,15]
60 <47> (<47> enzyme is heat labile and loses its activity upon heating at 60°C for 1 min [37]) [37]

General stability information

<3>, enzyme activity is destroyed if trypsin is included in the reaction [45]
<7>, trypsin destroys activity [14]

Storage stability

<3>, 4°C, DNA helicase VI loses 90% of its activity in 24 h [45]
<7>, 4°C, inactivation after prolonged storage [14]
<11>, -70°C, loses 25% of its activity following storage for 6 months [55]

References

[1] Poliakov, A.; Hubatsch, I.; Shuman, C.F.; Stenberg, G.; Danielson, U.H.: Expression and purification of recombinant full-length NS3 protease-helicase from a new variant of hepatitis C virus. Protein Expr. Purif., **25**, 363-371 (2002)
[2] Borowski, P.; Niebuhr, A.; Mueller, O.; Bretner, M.; Felczak, K.; Kulikowski, T.; Schmitz, H.: Purification and characterization of West Nile virus nucleoside triphosphatase (NTPase)/helicase: evidence for dissociation of the NTPase and helicase activities of the enzyme. J. Virol., **75**, 3220-3229 (2001)
[3] Ivanov, K.A.; Ziebuhr, J.: Human coronavirus 229E nonstructural protein 13: characterization of duplex-unwinding, nucleoside triphosphatase, and RNA 5-triphosphatase activities. J. Virol., **78**, 7833-7838 (2004)
[4] Yamashita, T.; Unno, H.; Mori, Y.; Tani, H.; Moriishi, K.; Takamizawa, A.; Agoh, M.; Tsukihara, T.; Matsuura, Y.: Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A. Virology, **373**, 426-436 (2008)
[5] Röleke, D.; Hoier, H.; Bartsch, C.; Umbach, P.; Scherzinger, E.; Lurz, R.; Saenger, W.: Crystallization and preliminary X-ray crystallographic and electron microscopic study of a bacterial DNA helicase (RSF1010 RepA). Acta Crystallogr. Sect. D, **53**, 213-216 (1997)
[6] Singleton, M.R.; Scaife, S.; Raven, N.D.; Wigley, D.B.: Crystallization and preliminary X-ray analysis of RecG, a replication-fork reversal helicase from Thermotoga maritima complexed with a three-way DNA junction. Acta Crystallogr. Sect. D, **57**, 1695-1696 (2001)
[7] Lee, C.; Seo, Y.S.: Isolation and characterization of a processive DNA helicase from the fission yeast Schizosaccharomyces pombe that translocates in a 5'-to-3' direction. Biochem. J., **334**, 377-386 (1998)
[8] Biswas, E.E.; Fricke W.M.; Chen, P.H.; Biswas, S.B.: Yeast DNA helicase A: cloning, expression, purification, and enzymatic characterization. Biochemistry, **36**, 13277-13284 (1997)
[9] Hyun, M.; Bohr, V.A.; Ahn B.: Biochemical characterization of the WRN-1 RecQ helicase of Caenorhabditis elegans. Biochemistry, **47**, 7583-7593 (2008)
[10] Roychowdhury, A.; Szymanski, M.R.; Jezewska, M.J.; Bujalowski, W.: Mechanism of NTP hydrolysis by the Escherichia coli primary replicative helicase DnaB protein. 2. Nucleotide and nucleic acid specificities. Biochemistry, **48**, 6730-6746 (2009)
[11] Frick, D.N.: The hepatitis C virus NS3 protein: a model RNA helicase and potential drug target. Curr. Issues Mol. Biol., **9**, 1-20 (2007)
[12] Sidorova, J.M.: Roles of the Werner syndrome RecQ helicase in DNA replication. DNA Repair, **7**, 1776-1786 (2008)
[13] Bernstein, D.A.; Zittel, M.C.; Keck, J.L.: High-resolution structure of the E.coli RecQ helicase catalytic core. EMBO J., **22**, 4910-4921 (2003)

[14] Tuteja, N.; Phan, T.N.; Tewari, K.K.: Purification and characterization of a DNA helicase from pea chloroplast that translocates in the 3'-to-5' direction. Eur. J. Biochem., **238**, 54-63 (1996)
[15] Phan, T.N.; Ehtesham, N.Z.; Tuteja, R.; Tuteja, N.: A novel nuclear DNA helicase with high specific activity from Pisum sativum catalytically translocates in the 3'→5' direction. Eur. J. Biochem., **270**, 1735-1745 (2003)
[16] Collins, R.; McCarthy, T.V.: Purification and characterization of Thermus thermophilus UvrD. Extremophiles, **7**, 35-41 (2003)
[17] Ohhata, T.; Araki, R.; Fukumura, R.; Kuroiwa, A.; Matsuda, Y.; Abe, M.: Cloning, genomic structure and chromosomal localization of the gene encoding mouse DNA helicase RECQL5β. Gene, **280**, 59-66 (2001)
[18] Shankar, J.; Tuteja, R.: UvrD helicase of Plasmodium falciparum. Gene, **410**, 223-233 (2007)
[19] Ivessa, A.S.; Zhou, J.Q.; Schulz, V.P.; Monson, E.K.; Zakian, V.A.: Saccharomyces Rrm3p, a 5' to 3' DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. Genes Dev., **16**, 1383-1396 (2002)
[20] Naqvi, A.; Tinsley, E.; Khan, S.A.: Purification and characterization of the PcrA helicase of Bacillus anthracis. J. Bacteriol., **185**, 6633-6639 (2003)
[21] Ruiz-Maso, J.A.; Anand, S.P.; Espinosa, M.; Khan, S.A.; del Solar, G.: Genetic and biochemical characterization of the Streptococcus pneumoniae PcrA helicase and its role in plasmid rolling circle replication. J. Bacteriol., **188**, 7416-7425 (2006)
[22] Curti, E.; Smerdon, S.J.; Davis, E.O.: Characterization of the helicase activity and substrate specificity of Mycobacterium tuberculosis UvrD. J. Bacteriol., **189**, 1542-1555 (2006)
[23] Biswas, E.E.; Barnes, M.H.; Moir, D.T.; Biswas, S.B.: An essential DnaB helicase of Bacillus anthracis: identification, characterization, and mechanism of action. J. Bacteriol., **191**, 249-260 (2009)
[24] Kikuma, T.; Ohtsu, M.; Utsugi, T.; Koga, S.; Okuhara, K.; Eki, T.; Fujimori, F.; Murakami, Y.: Dbp9p, a member of the DEAD box protein family, exhibits DNA helicase activity. J. Biol. Chem., **14; 279**, 20692-20698 (2004)
[25] Matson, S.W.: Escherichia coli helicase II (urvD gene product) translocates unidirectionally in a 3' to 5' direction. J. Biol. Chem., **261**, 10169-10175 (1986)
[26] Bean, D.W.; Kallam, W.E. Jr.; Matson, S.W.: Purification and characterization of a DNA helicase from Saccharomyces cerevisiae. J. Biol. Chem., **268**, 21783-21790 (1993)
[27] Shimizu, K.; Sugino, A.: Purification and characterization of DNA helicase III from the yeast Saccharomyces cerevisiae. J. Biol. Chem., **268**, 9578-9584 (1993)
[28] Karow, J.K.; Chakraverty, R.K.: Hickson, I.D.: The Bloom's syndrome gene product is a 3'-5' DNA helicase. J. Biol. Chem., **272**, 30611-30604 (1997)
[29] Bennett, R.J.; Sharp, J.A.; Wang, J.C.: Purification and characterization of the Sgs1 DNA helicase activity of Saccharomyces cerevisiae. J. Biol. Chem., **273**, 9644-9650 (1998)

[30] Nakagawa, T.; Flores-Rozas, H.; Kolodner, R.D.: The MER3 helicase involved in meiotic crossing over is stimulated by single-stranded DNA-binding proteins and unwinds DNA in the 3' to 5' direction.. J. Biol. Chem., **276**, 31487-31493 (2001)
[31] Brosh, R.M. Jr.; Waheed, J.; Sommers, J.A.: Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. J. Biol. Chem., **277**, 23236-23245 (2002)
[32] Tanner, J.A.; Watt, R.M.; Chai, Y.B.; Lu, L.Y.; Lin, M.C.; Peiris, J.S.; Poon. L.L.; Kung, H.F.; Huang, J.D.: The severe acute respiratory syndrome (SARS) coronavirus NTPase/helicase belongs to a distinct class of 5' to 3' viral helicases. J. Biol. Chem., **278**, 39578-3982 (2003)
[33] Korhonen, J.A.; Gaspari, M.; Falkenberg M.: TWINKLE has 5'→3' DNA helicase activity and is specifically stimulated by mitochondrial single-stranded DNA-binding protein. J. Biol. Chem., **278**, 48627-48632 (2003)
[34] Gupta, R.; Sharma, S.; Sommers, J.A.; Jin, Z.; Cantor, S.B.; Brosh, R.M.Jr.: Analysis of the DNA substrate specificity of the human BACH1 helicase associated with breast cancer. J. Biol. Chem., **280**, 25450-25460 (2005)
[35] Prakash, R.; Krejci, L.; van Komen, S.; Schürer, A.K.; Kramer, W.; Sung, P.: Saccharomyces cerevisiae MPH1 gene, required for homologous recombination-mediated mutation avoidance, encodes a 3' to 5' DNA helicase. J. Biol. Chem., **280**, 7854-7860 (2005)
[36] Gutti, R.K.; Tsai-Morris, C.H.; Dufau, M.L.: Gonadotropin-regulated testicular helicase (DDX25), an essential regulator of spermatogenesis, prevents testicular germ cell apoptosis. J. Biol. Chem., **283**, 17055-17064 (2008)
[37] Liu, H.H.; Liu, J.; Fan, S.L.; Song, M.Z.: Han, X.L.; Liu, F.; Shen, F.F.: Molecular cloning and characterization of a salinity stress-induced gene encoding DEAD-box helicase from the halophyte Apocynum venetum. J. Exp. Bot., **59**, 633-644 (2008)
[38] Belon, C.A.; Frick, D.N.: Fuel specificity of the hepatitis C virus NS3 helicase. J. Mol. Biol., **388**, 851-864 (2009)
[39] Toseland, C.P.; Martinez-Senac, M.M.; Slatter, A.F.; Webb, M.R.: The ATPase cycle of PcrA helicase and its coupling to translocation on DNA. J. Mol. Biol., **392**, 1020-1032 (2009)
[40] Smith, R.H.; Kotin, R.M.: The Rep52 gene product of adeno-associated virus is a DNA helicase with 3'-to-5' polarity. J. Virol., **72**, 4874-4881 (1998)
[41] Ziebarth, T.D.; Kaguni, L.S.: Purification strategy for recombinant forms of the human mitochondrial DNA helicase. Methods Mol. Biol., **554**, 121-126 (2009)
[42] Zhou, J.Q.; Qi, H.; Schulz, V.P.; Mateyak, M.K.; Monson, E.K.; Zakian, V.A.: Schizosaccharomyces pombe pfh1+ encodes an essential 5' to 3' DNA helicase that is a member of the PIF1 subfamily of DNA helicases. Mol. Biol. Cell, **13**, 2180-2191 (2002)
[43] Sakakibara, N.; Kelman, L.M.; Kelman, Z.: Unwinding the structure and function of the archaeal MCM helicase. Mol. Microbiol., **72**, 286-296 (2009)
[44] Turchi, J.J.; Murante, R.S.; Bambara, R.A.: DNA substrate specificity of DNA helicase E from calf thymus. Nucleic Acids Res., **20**, 6075-6080 (1992)

[45] Tuteja, N.; Ochem, A.; Taneja, P.; Tuteja, R.; Skopac, D.; Falaschi, A.: Purification and properties of human DNA helicase VI. Nucleic Acids Res., **23**, 2457-2463 (1995)
[46] Thömmes, P.; Marton, R.F.; Cotterill, S.: Purification and characterisation of a DNA helicase, dheI I, from Drosophila melanogaster embryos. Nucleic Acids Res., **23**, 4443-4450 (1995)
[47] Bird, L.E.; Hakansson, K.; Pan, H.; Wigley, D.B.: Characterization and crystallization of the helicase domain of bacteriophage T7 gene 4 protein. Nucleic Acids Res., **25**, 2620-2626 (1997)
[48] Biswas, E.E; Nagele, R.G.; Biswas, S.: A novel human hexameric DNA helicase: expression, purification and characterization.. Nucleic Acids Res., **29**, 1733-1740 (2001)
[49] Ozsoy, A.Z.; Sekelsky, J.J.; Matson, S.W.: Biochemical characterization of the small isoform of Drosophila melanogaster RECQ5 helicase. Nucleic Acids Res., **29**, 2986-2993 (2001)
[50] Ozsoy, A.Z.; Ragonese, H.M.; Matson. S.W.: Analysis of helicase activity and substrate specificity of Drosophila RECQ5. Nucleic Acids Res., **31**, 1554-1564 (2003)
[51] Gu, Y.; Masuda, Y.; Kamiya, K.: Biochemical analysis of human PIF1 helicase and functions of its N-terminal domain. Nucleic Acids Res., **36**, 6295-6308 (2008)
[52] Samuels, M.; Gulati, G.; Shin, J.H.; Opara, R.; McSweeney, E.; Sekedat, M.; Long, S.; Kelman, Z.; Jeruzalmi, D.: A biochemically active MCM-like helicase in Bacillus cereus. Nucleic Acids Res., **37**, 4441-4452 (2009)
[53] George, T.; Wen, Q.; Griffiths, R.; Ganesh, A.; Meuth, M.; Sanders, C.M.: Human Pif1 helicase unwinds synthetic DNA structures resembling stalled DNA replication forks. Nucleic Acids Res., **37**, 6491-6502 (2009)
[54] Sakakibara, N.; Han, M.; Rollor, C.R.; Gilson, R.C.; Busch, C.; Heo, G.; Kelman, Z.: Cloning, purification, and partial characterization of the Halobacterium sp. NRC-1 minichromosome maintenance (MCM) helicase. Open Microbiol. J., **2**, 13-17 (2008)
[55] Suntornthiticharoen, P.; Petmitr, S.; Chavalitshewinkoon-Petmitr, P.: Purification and characterization of a novel 3'-5' DNA helicase from Plasmodium falciparum and its sensitivity to anthracycline antibiotics. Parasitology, **133**, 389-398 (2006)
[56] Bagherieh-Najjar, M.B.; de Vries, O.M.; Kroon, J.T.; Wright, E.L.; Elborough, K.M.; Hille, J.; Dijkwel, P.P.: Arabidopsis RecQsim, a plant-specific member of the RecQ helicase family, can suppress the MMS hypersensitivity of the yeast sgs1 mutant. Plant Mol. Biol., **52**, 273-284 (2003)
[57] Pike, A.C.; Shrestha, B.; Popuri, V.; Burgess-Brown, N.; Muzzolini, L.M.; Costantini, S.; Vindigni, A.; Gileadi, O.: Structure of the human RECQ1 helicase reveals a putative strand-separation pin. Proc. Natl. Acad. Sci. USA, **106**, 1039-1044 (2009)
[58] Dixon, E.P.; Pahel, G.L.; Rocque, W.J.; Barnes, J.A.; Lobe, D.C.; Hanlon, M.H.; Alexander, K.A.; Chao, S.F.; Lindley, K.; Phelps, W.C.: The E1 helicase of human papillomavirus type 11 binds to the origin of replication with low sequence specificity. Virology, **270**, 345-357 (2000)

[59] Schreiner, U.; Theune, M.; Althof, F.; Kehm, E.; Knopf, C.W.: One-step column purification of herpes simplex virus 1 helicase-primase subcomplex using C-terminally his-tagged UL5 subunit. Virus Genes, **39**, 19-29 (2009)
[60] Monroe, D.S. Jr.; Leitzel, A.K.; Klein, H.L.; Matson, S.W.: Biochemical and genetic characterization of Hmi1p, a yeast DNA helicase involved in the maintenance of mitochondrial DNA. Yeast, **22**, 1269-1286 (2005)
[61] Borowski, P.; Heising, M.V.; Miranda, I.B.; Liao, C.L.; Choe, J.; Baier, A.: Viral NS3 helicase activity is inhibited by peptides reproducing the Arg-rich conserved motif of the enzyme (motif VI). Biochem. Pharmacol., **76**, 28-38 (2008)
[62] Mukovnya, A.V.; Tunitskaya, V.L.; Khandazhinskaya, A.L.; Golubeva, N.A.; Zakirova, N.F.; Ivanov, A.V.; Kukhanova, M.K.; Kochetkov, S.N.: Hepatitis C virus helicase/NTPase: an efficient expression system and new inhibitors. Biochemistry (Moscow), **73**, 660-668 (2008)
[63] Hartjen, P.; Medom, B.K.; Reinholz, M.; Borowski, P.; Baier, A.: Regulation of the biochemical function of motif VI of HCV NTPase/helicase by the conserved Phe-loop. Biochimie, **91**, 252-260 (2009)
[64] Lee, C.; Lee, J.M.; Lee, N.R.; Jin, B.S.; Jang, K.J.; Kim, D.E.; Jeong, Y.J.; Chong, Y.: Aryl diketoacids (ADK) selectively inhibit duplex DNA-unwinding activity of SARS coronavirus NTPase/helicase. Bioorg. Med. Chem. Lett., **19**, 1636-1638 (2009)
[65] Beran, R.K.; Pyle, A.M.: Hepatitis C viral NS3-4A protease activity is enhanced by the NS3 helicase. J. Biol. Chem., **283**, 29929-29937 (2008)
[66] Beran, R.K.; Lindenbach, B.D.; Pyle, A.M.: The NS4A protein of hepatitis C virus promotes RNA-coupled ATP hydrolysis by the NS3 helicase. J. Virol., **83**, 3268-3275 (2009)
[67] Feito, M.J.; Gomez-Gutierrez, J.; Ayora, S.; Alonso, J.C.; Peterson, D.; Gavilanes, F.: Insights into the oligomerization state-helicase activity relationship of West Nile virus NS3 NTPase/helicase. Virus Res., **135**, 166-174 (2008)