RNA helicase 3.6.4.13

1 Nomenclature

EC number
3.6.4.13

Systematic name
ATP phosphohydrolase (RNA helix unwinding)

Recommended name
RNA helicase

Synonyms
1a NTPase/helicase <16> [5]
ATP/dATP-dependent RNA helicase <1,42> [32]
ATPase <10,12> [1,36]
ATPase/RNA helicase <1,42> [32]
ATPase/helicase <10> [36,41]
BMV 1a protein <16> [5]
BmL3-helicase <1,42> [32]
Brr2p <6> [50]
DBP2 <24> [30]
DDX17 <33> [12]
DDX19 <43> [56]
DDX25 <23,34,35> [12,21]
DDX3 <25> [8]
DDX3X <25> (<25> the gene is localized to the X chromosome [12]) [12]
DDX3Y <29> (<29> the gene is localized to the Y chromosome [12]) [12]
DDX4 <30> [12]
DDX5 <32> [12]
DEAD box RNA helicase <1,2,3> [32,45,52]
DEAD box helicase <2> [45]
DEAD-box RNA helicase <4,5,7,38,47,48> [9,14,16,25,53,55]
DEAD-box protein DED1 <38> [11]
DEAD-box rRNA helicase <5> [26]
DEAH-box RNA helicase <24> [30]
DEAH-box protein 2 <24> [30]
DED1 <38> [11,14]
DENV NS^3H <10> [41]
DEXD/H-box RNA helicase <43> [56]
DEx(H/D)RNA helicase <12> [23]
DHX9 <44> [58]
DbpA <5> [10,25,26]

D. Schomburg and I. Schomburg (eds.), *Class 3.4–6 Hydrolases, Lyases, Isomerases, Ligases: EC 3.4–6*, Springer Handbook of Enzymes S10, DOI 10.1007/978-3-642-36260-6_25,

Dhx9/RNA helicase A <13> [61]
EhDEAD1 <7> [16]
EhDEAD1 RNA helicase <7> [16]
FRH <9> [54]
FRQ-interacting RNA helicase <9> [54]
GRTH <3> [57]
GRTH/DDX25 <3,35> [21,51]
HCV NS3 helicase <12> [48]
KOKV helicase <27> [7]
Mtr4p <31> [22]
NPH-II <8> [18,28]
NS3 <10,12,17,20,39,41> (<12,39> ambiguous [27,42,44]) [1,2,4,27,35,36,39, 42,44,46]
NS3 ATPase/helicase <10> [41]
NS3 NTPase/helicase <17> (<17> ambiguous [46]) [46]
NS3 helicase <10,12,17> [15,44,46]
NS3 protein <10,12,17,18> (<12> ambiguous [39]) [15,39,40,41,62]
NTPase/helicase <12> (<12> ambiguous [37]) [37,39]
RHA <6> [31,49]
RNA helicase <2> [45]
RNA helicase A <6,44> [31,49,58]
RNA helicase CrhR <14> [59]
RNA helicase DDX3 <25> [8]
RNA helicase Ddx39 <47> [53]
RNA helicase Hera <4> [9]
RNA-dependent ATPase <37> [34]
RNA-dependent NTPase/helicase <12> [1]
RTPase <10> [36]
RhlB <5> [43]
SpolvlgA <48> [55]
Supv3L1 <46> [64]
TGBp1 NTPase/helicase domain <22,28> [24]
Tk-DeaD <15> [47]
VRH1 <26> [33]
YxiN <2> [45]
eIF4A <36> [20]
eIF4A helicase <36> [20]
eIF4AIII <37> [34]
eukaryotic initiation factor eIF 4A <36> [20]
gonadotropin-regulated testicular RNA helicase <3> [51,57]
helicase <10> [41]
helicase B <5> [43]
helicase/nucleoside triphosphatase <10> [4]
non structural protein 3 <12> (<12> ambiguous [37,38]) [37,38]
non-structural 3 <10> [36]
non-structural protein 3 <17> [46]
non-structural protein 3 protein <18> [40]

nonstructural protein 3 <12,17,20,39,40,41> (<12,17,39,40> ambiguous [6,27,39,42,44,46]) [1,2,6,27,35,39,42,44,46]
nucleoside 5'-triphosphatase <10> [4]
nucleoside triphosphatase/RNA helicase and 5'-RNA triphosphatase <20> [2]
nucleoside triphosphatase/helicase <16> [5]
p54 RNA helicase <45> [60]
p68 RNA helicase <3,6> [52,63]
protein NS3 <12> (<12> ambiguous [38]) [38]

2 Source Organism

<1> *Brugia malayi* [32]
<2> *Bacillus subtilis* [45]
<3> *Mus musculus* [51,52,57]
<4> *Thermus thermophilus* [9]
<5> *Escherichia coli* [10,25,26,43]
<6> *Homo sapiens* [17,19,31,49,50,63]
<7> *Entamoeba histolytica* [16]
<8> *Vaccinia virus* [18,28]
<9> *Neurospora sp.* [54]
<10> *Dengue virus* [4,15,36,41]
<11> *Yellow fever virus* [29]
<12> *Hepatitis C virus* [1,13,23,27,37,38,39,42,44,48]
<13> *Human immunodeficiency virus 1* [61]
<14> *Synechocystis sp. PCC 6803* [59]
<15> *Thermococcus kodakarensis* [47]
<16> *Brome mosaic virus* [5]
<17> *West Nile virus* [46,62]
<18> *Rice hoja blanca virus* [40]
<19> *Classical swine fever virus* [35]
<20> *Dengue virus 2* (NPP6 [2]) [2]
<21> *unidentified human coronavirus* (UNIPROT accession number: P0C6X1) [3]
<22> *Potato virus X* [24]
<23> *Rattus norvegicus* (UNIPROT accession number: Q9QY16) [12]
<24> *Homo sapiens* (UNIPROT accession number: O60231) [30]
<25> *Homo sapiens* (UNIPROT accession number: O00571) [8,12]
<26> *Vigna radiata var. radiata* (UNIPROT accession number: Q9M6R6) [33]
<27> *Kokobera virus* [7]
<28> *Poa semilatent virus* [24]
<29> *Homo sapiens* (UNIPROT accession number: O15523) [12]
<30> *Homo sapiens* (UNIPROT accession number: Q9NQI0) [12]
<31> *Saccharomyces cerevisiae* (UNIPROT accession number: P47047) [22]
<32> *Mus musculus* (UNIPROT accession number: Q61656) [12]
<33> *Mus musculus* (UNIPROT accession number: Q501J6) [12]
<34> *Mus musculus* (UNIPROT accession number: Q9QY15) [12]

<35> *Homo sapiens* (UNIPROT accession number: Q9UHL0) [12,21]
<36> *Oryctolagus cuniculus* (UNIPROT accession number: P29562) [20]
<37> *Homo sapiens* (UNIPROT accession number: P38919) [34]
<38> *Saccharomyces cerevisiae* (UNIPROT accession number: P06634) [11,14]
<39> *Hepatitis C virus* (UNIPROT accession number: Q9WPH5) [44]
<40> *Japanese encephalitis virus* (UNIPROT accession number: P27395) [6]
<41> *Classical swine fever virus* (UNIPROT accession number: Q9YS30) [35]
<42> *Brugia malayi (GenBank accession number: EF409381)* [32]
<43> *Homo sapiens* (UNIPROT accession number: Q9UMR2) [56]
<44> *Homo sapiens* (UNIPROT accession number: Q08211) [58]
<45> *Homo sapiens* (UNIPROT accession number: P26196) [60]
<46> *Mus musculus* (UNIPROT accession number: Q80YD1) [64]
<47> *Xenopus laevis* (UNIPROT accession number: Q7ZX48) [53]
<48> *Schmidtea polychroa* (UNIPROT accession number: B9VSG1) [55]

3 Reaction and Specificity

Catalyzed reaction

ATP + H_2O = ADP + phosphate (<5> models: DbpA functions as an active monomer that possesses two distinct RNA binding sites, one in the helicase core domain and the other in the carboxyl-terminal domain that recognizes 23 S rRNA and interacts specifically with hairpin 92 of the peptidyl transferase center [25]; <5> quantitative kinetic and equilibrium characterization of the rRNA-activated ATPase cycle mechanism of DbpA [26])

NTP + H_2O = NDP + phosphate (<10> catalytic mechanism involving a bound sulfate ion, NTPase active site structure, nucleic acid binding site [4])

Natural substrates and products

S ATP + H_2O <2,5,6,8,10,12,13,16,17,20,23,24,25,29,30,31,32,33,34,35,38,39,40,42> (<16> NTPase activity [5]; <35> gonadotropin-regulated testicular helicase (GRTH/DDX25), a target of gonadotropin and androgen action, is a post-transcriptional regulator of key spermatogenesis genes. GRTH has a negative role on its mRNA stability [21]; <31> Mtr4p can unwind duplex RNA in the presence of ATP and a single-stranded RNA tail in the 3 to 5 direction [22]; <8> phosphohydrolase and helicase activities of NPH-II are essential for virus replication [28]; <6> RHA is a coactivator in STAT6-mediated transcription, and this function is dependent on its helicase activity [31]; <25,29,30,32> the ability of RNA helicases to modulate the structure and thus availability of critical RNA molecules for processing leading to protein expression is the likely mechanism by which RNA helicases contribute to differentiation [12]; <23,33,34,35> the ability of RNA helicases to modulate the structure and thus availability of critical RNA molecules for processing leading to protein expression is the likely mechanism by which RNA helicases contribute to differentiation. DDX17 is involved in mRNA splicing [12]; <12> the C-terminal portion of hepatitis C virus nonstructural protein 3 (NS3) forms a three domain polypep-

tide 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 [13]; <38> the DEAD-box protein DED1 has the ability to balance RNA unwinding with a profound strand annealing activity in a highly dynamic fashion [11]; <10,20> RNA helicase activity [2,4]; <12> multifunctional enzyme possessing serine protease, NTPase, and RNA unwinding activities [42]; <12> NTPase activity analyzed, ambiguous helicase activity, enzyme capable for unwinding RNA and DNA [38]; <39> RNA-stimulated ATPase activities determined, interaction between the replicative component nonstructural protein 3 (NS3) with the nonstructural protein 4A (NS4A) [44]; <12> the Arg-rich amino acid motif HCV1487-1500, a fragment of domain 2 NS3 of Hepatitis C virus, as well as the complete domain 2, and domain 2 lacking the flexible loop localized between Val1458 and Thr1476, mediate competitive inhibition of diverse protein kinase C functions, inhibition of rat brain PKC, overview [39]; <17> the West Nile virus RNA helicase uses the energy derived from the hydrolysis of nucleotides to separate complementary strands of RNA [62]; <13> translation of HIV-1 gag mRNA is reliant on the ATP-dependent helicase activity of RNA helicase A [61]) (Reversibility: ?) [2,4,5,6,11,12, 13,21,22,28,30,31,32,37,38,39,41,42,43,44,45,46,61,62]

P ADP + phosphate

S RNA + H_2O <2,5,10,42> (<5> helicase/unwinding activity [43]; <42> helicase/unwinding activity, either ATP or dATP is required for the unwinding activity [32]; <2> RNA unwinding activity, the enzyme contains two RecA-like domains, opening and closing of the interdomain cleft during RNA unwinding [45]) (Reversibility: ?) [32,41,43,45]

P ?

S Additional information <5,10,12,16,17,18,20,41,42> (<16> BMV 1a protein accumulates on endoplasmic reticulum membranes of the host cell, recruits the other RNA replication factor 2apol and induces 50- to 70-nm membrane invaginations serving as RNA replication compartments, BMV 1a protein also recruits viral replication templates such as genomic RNA3 depending on the BMV 1a protein helicase motif, in absence of 2apol, BMV 1a protein highly stabilizes RNA3 by transferring it to a membrane-associated, nuclease-resistant state, overview [5]; <20> nonstructural proteins NS3 and NS5 form complexes in infected mammalian cells [2]; <12> the enzyme is involved in viral replication [1]; <10> the enzyme plays an important role in viral replication [4]; <42> DEAD box proteins are putative RNA unwinding proteins, BmL3-helicase also is a DEAD box RNA helicase [32]; <5> helicase B, RhlB, is one of the five DEAD box RNA-dependent ATPases in Escherichia coli. ATPases found in Escherichia coli. RhlB requires an interaction with the partner protein RNase E for appreciable ATPase and RNA unwinding activities [43]; <17> NS3 possess both protease and helicase activities, the C-terminal portion of the NS3 contains the ATPase/helicase domain presumably involved in viral replication [46]; <41> NS3 possesses three enzyme activities that are

likely to be essential for virus replication: a serine protease located in the N-terminus and NTPase as well as helicase activities located in the C-terminus. Functions of NS3 and NS5B during positive-strand RNA virus replication, the NS3 protein is be involved in the unwinding of the viral RNA template while NS5B protein may be involved in catalyzing the synthesis of new RNA molecules [35]; <12> the C-terminal region of NS3 exhibits RNA-stimulated NTPase, e.g. ATPase, and helicase activity, while the N-terminal serine protease domain of NS3 enhances RNA binding and unwinding by the C-terminal region, NS4A mutants that are defective in ATP-coupled RNA binding are lethal in vivo [44]; <18> the NS3 protein of Rice hoja blanca virus is an RNA silencing suppressor, RSS, that exclusively binds to small dsRNA molecules. This plant viral RSS lacks interferon antagonistic activity, yet it is able to substitute the RSS function of the Tat protein of Human immunodeficiency virus type 1 based on the sequestration of small dsRNA. NS3 is able to inhibit endogenous miRNA action in mammalian cells [40]; <10> The NS3 protein physically associates with the NS5 polymerase, NS3 andNS5 carry out all the enzymatic activities needed for polyprotein processing and genome replication. NS3 possesses an ATPase/helicase and RNA triphosphatase at its C-terminal end that are essential for RNA replication. In addition to its known enzymatic functions, the NS3 protein appears to be involved in the assembly of an infectious flaviviral particle, through its interactions with NS2A and presumably host cell proteins [36]) (Reversibility: ?) [1,2,4,5,32,35,36,40, 4344,46]

P ?

Substrates and products

- **S** 2',3'-dideoxy-ATP + H_2O <17> (<17> 53% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2',3'-dideoxy-ADP + phosphate
- **S** 2',3'-dideoxy-GTP + H_2O <17> (<17> 28% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2',3'-dideoxy-GDP + phosphate
- **S** 2'-O-methyl-GTP + H_2O <17> (<17> 24% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2'-O-methyl-GDP + phosphate
- **S** 2'-deoxy-ATP + H_2O <17> (<17> 62% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2'-deoxy-ADP + phosphate
- **S** 2'-deoxy-GTP + H_2O <17> (<17> 39% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2'-deoxy-GDP + phosphate
- **S** 2'-deoxy-L-GTP + H_2O <17> (<17> 11% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
- **P** 2'-deoxy-L-GDP + phosphate
- **S** 2'-fluoro-2'-deoxy-GTP + H_2O <17> (<17> 22% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]

P 2'-fluoro-2'-deoxy-GDP + phosphate
S 2'-fluoro-2'-deoxy-ATP + H_2O <17> (<17> 63% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 2'-fluoro-2'-deoxy-ADP + phosphate
S 2-amino-ATP + H_2O <17> (<17> 103% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 2-amino-ADP + phosphate
S 2-hydroxy-ATP + H_2O <17> (<17> 40% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 2-hydroxy-ADP + phosphate
S 3'-O-methyl-GTP + H_2O <17> (<17> 35% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 3'-O-methyl-GDP + phosphate
S 3'-deoxy-ATP + H_2O <17> (<17> 60% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 3'-deoxy-ADP + phosphate
S 3'-deoxy-GTP + H_2O <17> (<17> 12% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 3'-deoxy-GDP + phosphate
S 6-methyl-thio-GTP + H_2O <17> (<17> 40% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 6-methyl-thio-GDP + phosphate
S 6-methyl-thio-ITP + H_2O <17> (<17> 16% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 6-methyl-thio-IDP + phosphate
S 6-thio-GTP + H_2O <17> (<17> 93% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 6-thio-GDP + phosphate
S 7-methyl-GTP + H_2O <17> (<17> 14% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 7-methyl-GDP + phosphate
S 8-bromo-ATP + H_2O <17> (<17> 124% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 8-bromo-ADP + phosphate
S 8-bromo-GTP + H_2O <17> (<17> 19% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 8-bromo-GDP + phosphate
S 8-iodo-GTP + H_2O <17> (<17> 54% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P 8-iodo-GDP + phosphate
S ATP + H_2O <1,2,4,5,6,7,8,10,12,13,15,16,17,19,20,21,22,23,24,25,26,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44> (<16,20> NTPase activity [2,5]; <12> preferred substrate for NTPase activity [1]; <35> gonadotropin-regulated testicular helicase (GRTH/DDX25), a target of gonadotropin and androgen action, is a post-transcriptional regulator of key spermatogenesis genes. GRTH has a negative role on its mRNA stability [21]; <31> Mtr4p can unwind duplex RNA in the presence of ATP and a single-

stranded RNA tail in the 3 to 5 direction [22]; <8> phosphohydrolase and helicase activities of NPH-II are essential for virus replication [28]; <6> RHA is a coactivator in STAT6-mediated transcription, and this function is dependent on its helicase activity [31]; <25,29,30,32> the ability of RNA helicases to modulate the structure and thus availability of critical RNA molecules for processing leading to protein expression is the likely mechanism by which RNA helicases contribute to differentiation [12]; <23,33,34,35> the ability of RNA helicases to modulate the structure and thus availability of critical RNA molecules for processing leading to protein expression is the likely mechanism by which RNA helicases contribute to differentiation. DDX17 is involved in mRNA splicing [12]; <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 [13]; <38> the DEAD-box protein DED1 has the ability to balance RNA unwinding with a profound strand annealing activity in a highly dynamic fashion [11]; <31> ATP and dATP are the preferred nucleotide substrates. In the presence of ATP or dATP Mtr4p unwinds the duplex region of a partial duplex RNA substrate in the 3 to 5 direction. Mtr4p displays a marked preference for binding to poly(A) RNA relative to an oligoribonucleotide of the same length and a random sequence [22]; <36> eIF4A may interact directly with double-stranded RNA, and recognition of helicase substrates occurs via chemical and/or structural features of the duplex. The initial rate and amplitude of duplex unwinding by eIF4A is dependent on the overall stability, rather than the length or sequence, of the duplex substrate. eIF4A helicase activity is minimally dependent on the length of the single-stranded region adjacent to the double-stranded region of the substrate. Interestingly, eIF4A is able to unwind blunt-ended duplexes. eIF4A helicase activity is also affected by substitution of 2-OH (RNA) groups with 2-H (DNA) or 2-methoxyethyl groups [20]; <1> either ATP or dATP is required for the unwinding activity [32]; <26> either ATP or dATP is required for the unwinding activity, VrRH1 catalyzes unwinding of a double-stranded RNA [33]; <19> helicase activity requires the substrates possessing a 3 un-base-paired region on the RNA template strand. The NS3h helicase activity is proportional to increasing lengths of the 3 un-base-paired regions up to 16 nucleotides of the RNA substrates. CSFV NS3 helicase activity requires a longer 3-end single-stranded overhang for efficient duplex unwinding and the directionality of NS3 helicase unwinding is 3 to 5 with respect to the template strand [35]; <38> promotes RNA unwinding. The enzyme also catalyzes strand annealing. The balance between unwinding and annealing activities of DED1 depends on the RNA substrate. ADP also modulates the balance between RNA unwinding and strand annealing [11]; <7> recombinant EhDEAD1 protein presents ATPase activity and is able to bind and unwind RNA in an ATPase-dependent manner [16]; <6> RNA helicase A utilizes all hydrolyzable NTPs without preference. RNA

helicase A unwinds dsRNA only in a 3 to 5 direction. The enzyme can only translocate on RNA possessing 3' single-stranded regions [17]; <37> RNA-dependent ATPase, helicase activity [34]; <5> the 3 to 5 helicase activity of DbpA can use a 3 single-stranded loading site on either strand of the substrate helix [10]; <6> the enzyme displaces partial duplex RNA exclusively in a 5 to 3 direction. This reaction is supported by ATP and dATP at relatively high concentrations. The enzyme displays only ATPase and dATPase activity. RNA helicase catalyzes the unwinding of duplex RNA and RNA*DNA hybrids provided that single-stranded RNA is available for the helicase to bind [19]; <21> the enzyme has both RNA and DNA duplex-unwinding activities with 5-to-3 polarity [3]; <22,28> the N-terminal part of the TGBp1 NTPase/helicase domain comprising conserved motifs I, Ia and II is sufficient for ATP hydrolysis, RNA binding and homologous protein-protein interactions [24]; <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 [13]; <38> the Q motif regulates ATP binding and hydrolysis, the affinity of the protein for RNA substrates and the helicase activity. At least three different protein conformations that are associated with free, ADP-bound and ATP-bound forms of the protein [14]; <8> unwinds duplex RNA exclusively in a 3 to 5 direction with respect to the strand to which the enzyme is bound and along which it is presumed to translocate. NTP hydrolysis by RNA bound NPH-II1 drives processive translocation of the protein in a 3' to 5' direction along the RNA strand [18]; <12> unwinds RNA in a discontinuous manner, pausing after long apparent steps of unwinding. It is proposed that the large kinetic step size of NS3 unwinding reflects a delayed, periodic release of the separated RNA product strand from a secondary binding site that is located in the NTPase domain (domain II) of NS3 [23]; <10,20> RNA helicase activity [2,4]; <12> multifunctional enzyme possessing serine protease, NTPase, and RNA unwinding activities [42]; <12> NTPase activity analyzed, ambiguous helicase activity, enzyme capable for unwinding RNA and DNA [38]; <39> RNA-stimulated ATPase activities determined, interaction between the replicative component nonstructural protein 3 (NS3) with the nonstructural protein 4A (NS4A) [44]; <12> the Arg-rich amino acid motif HCV1487-1500, a fragment of domain 2 NS3 of Hepatitis C virus, as well as the complete domain 2, and domain 2 lacking the flexible loop localized between

Val1458 and Thr1476, mediate competitive inhibition of diverse protein kinase C functions, inhibition of rat brain PKC, overview [39]; <10> ATPase activity, ATP binding mode, the ATP binding site is housed between these two subdomains. In the ATP binding pocket, a Mg ion is coordinated in a octahedral manner by the β- and γ-phosphate oxygen atoms from ATP, two equatorial water molecules and oxygen atoms from residues Glu285 in motif II, and Thr200 in motif I, overview [36]; <2> cooperative binding of ATP and RNA leads to a compact helicase structure [45]; <40> genome structure, crystals and three-dimensional structure determined, structure of NTP-binding region, conserved residues within the NTP-binding pocket, ATPase and RNA helicase activities determined [6]; <10> NS3 C-terminal domain catalyzes ATP hydrolysis in the presence of $MgCl_2$ or $MnCl_2$. $MgCl_2$ is more effective than $MnCl_2$ at inducing ATPase activity at concentrations ranging from 0.1 mM to 5 mM. ATP hydrolysis is required for the unwinding activity of DENV NS3H [41]; <12> peptide inhibitors derived from amino acid sequence of motif VI analyzed, binding of the inhibitory peptides does not interfere with the NTPase activity [37]; <17> recombinant protein of C-terminal portion of NS3 protein, ATPase catalytic properties but no RNA helicase activities [46]; <10> wild-type and mutant, NTPase activity analyzed, functional binding of RNA analyzed [41]; <17> the West Nile virus RNA helicase uses the energy derived from the hydrolysis of nucleotides to separate complementary strands of RNA [62]; <13> translation of HIV-1 gag mRNA is reliant on the ATP-dependent helicase activity of RNA helicase A [61]; <17> the amino acids Arg185, Arg202 and Asn417 are critical for phosphohydrolysis [62]; <15> unwinding activity specific for single-strand paired RNA, does not unwind dsRNAs [47]; <12> unwinding of dsRNA [48]) (Reversibility: ?) [1,2,3,4,5,6,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,28,30,31,32,33,34,35,36,37,38,39,41,42,43,44,45,46,47,48,56,58,61,62]

P ADP + phosphate

S Ara-ATP + H_2O <17> (<17> 18% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]

P Ara-ADP + phosphate

S CTP + H_2O <12,19,44> (<12> NTPase activity [1]; <19> helicase activity is about 85% of the activity with ATP [35]) (Reversibility: ?) [1,35,58]

P CDP + phosphate

S GTP + H_2O <12,17,19,44> (<12> NTPase activity [1]; <19> helicase activity is about 55% of the activity with ATP [35]; <17> 49% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [1,35,58,62]

P GDP + phosphate

S ITP + H_2O <17> (<17> 49% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]

P IDP + phosphate

S N^1-methyl-ATP + H_2O <17> (<17> 66% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]

P N^1-methyl-ADP + phosphate

S N^1-methyl-GTP + H_2O <17> (<17> 49% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P N^1-methyl-GDP + phosphate
S N^6-methyl-ATP + H_2O <17> (<17> 43% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P N^6-methyl-ADP + phosphate
S O6-methyl-GTP + H_2O <17> (<17> 17% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P O6-methyl-GDP + phosphate
S RNA + H_2O <2,5,10,12,41,42> (<5> helicase/unwinding activity [43]; <42> helicase/unwinding activity, either ATP or dATP is required for the unwinding activity [32]; <2> RNA unwinding activity, the enzyme contains two RecA-like domains, opening and closing of the interdomain cleft during RNA unwinding [45]; <10> helicase/unwinding activity, ATP hydrolysis is required for the unwinding activity of DENV NS3H [41]; <41> NS3 helicase domain helicase activity is dependent on the presence of NTP and divalent cations, with a preference for ATP and Mn^{2+}, and requires a substrates possessing a 3 un-base-paired region on the RNA template strand. The helicase activity is proportional to increasing lengths of the 3 un-base-paired regions up to 16 nucleotides of theRNA substrates, overview [35]; <10> RNA helicase actiivty [36]; <2> RNA unwinding activity, substrate is a 154mer of 23S rRNA generated by T7 polymerase from in vitro transcription [45]; <12> unwinding helicase activity, NS3 is ahighly basic protein with multiple RNA binding sites [44]) (Reversibility: ?) [32,35,36,41,43,44,45]
P ?
S UTP + H_2O <12,19,44> (<12> NTPase activity [1]; <19> helicase activity is about 55% of the activity with ATP [35]) (Reversibility: ?) [1,35,58]
P UDP + phosphate
S XTP + H_2O <17> (<17> 40% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P XDP + phosphate
S dATP + H_2O <1,6,19,26,31> (<31> ATP and dATP are the preferred nucleotide substrates. In the presence of ATP or dATP Mtr4p unwinds the duplex region of a partial duplex RNA substrate in the 3 to 5 direction. Mtr4p displays a marked preference for binding to poly(A) RNA relative to an oligoribonucleotide of the same length and a random sequence [22]; <1> either ATP or dATP is required for the unwinding activity [32]; <26> either ATP or dATP is required for the unwinding activity, VrRH1 catalyzes unwinding of a double-stranded RNA [33]; <19> helicase activity is about 10% of the activity with ATP [35]; <6> the enzyme displaces partial duplex RNA exclusively in a 5 to 3 direction. This reaction is supported by ATP and dATP at relatively high concentrations. The enzyme displays only ATPase and dATPase activity. RNA helicase catalyzes the unwinding of duplex RNA and RNA*DNA hybrids provided that single-stranded RNA is available for the helicase to bind [19]) (Reversibility: ?) [19,22,32,33,35]
P dADP + phosphate

S dCTP + H_2O <19> (<19> helicase activity is about 25% of the activity with ATP [35]) (Reversibility: ?) [35]
P dCDP + phosphate
S dGTP + H_2O <19> (<19> helicase activity is about 10% of the activity with ATP [35]) (Reversibility: ?) [35]
P dGDP + phosphate
S dTTP + H_2O <19> (<19> helicase activity is about 55% of the activity with ATP [35]) (Reversibility: ?) [35]
P dTDP + phosphate
S ribavirin triphosphate + H_2O <17> (<17> 36% of the phosphohydrolase activity with ATP [62]) (Reversibility: ?) [62]
P ribavirin diphosphate + phosphate
S Additional information <2,5,8,10,11,12,16,17,18,19,20,41,42> (<16> BMV 1a protein accumulates on endoplasmic reticulum membranes of the host cell, recruits the other RNA replication factor 2apol and induces 50- to 70-nm membrane invaginations serving as RNA replication compartments, BMV 1a protein also recruits viral replication templates such as genomic RNA3 depending on the BMV 1a protein helicase motif, in absence of 2apol, BMV 1a protein highly stabilizes RNA3 by transferring it to a membrane-associated, nuclease-resistant state, overview [5]; <20> nonstructural proteins NS3 and NS5 form complexes in infected mammalian cells [2]; <12> the enzyme is involved in viral replication [1]; <10> the enzyme plays an important role in viral replication [4]; <10> multifunctional enzyme showing protease, helicase, and NTPase activities [4]; <16> multifunctional enzyme showing protease, helicase, and NTPase activities, the enzyme has a function in RNA replication complex assembly besides its function in RNA synthesis/capping, the enzyme activity is located in the C-terminal nucleoside triphosphatase/helicase domain of the BMV 1a protein RNA replication factor [5]; <20> substrate specificity, bifunctional enzyme, NS3 is an RNA-stimulated nucleoside triphosphatase NTPase/RNA helicase and a 5-RNA triphosphatase RTPase, overview, the full-length NS3 with or without NS2B cofactor domain exhibits a catalytically more efficient RNA helicase activity than the N-terminally-truncated NS3 helicase domain, suggesting that the protease domain enhances RNA helicase activity [2]; <12> the multifunctional enzyme shows RNA-dependent NTPase and helicase activities, no activity with ADP and AMP [1]; <19> nonstructural protein 3 (NS3) possesses three enzyme activities that are likely to be essential for virus replication: a serine protease located in the N-terminus and NTPase as well as helicase activities located in the C-terminus [35]; <11> NS3 includes a protease and a helicase that are essential to virus replication and to RNA capping [29]; <8> the enzyme is unable to unwind duplex DNA [18]; <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 [13]; <42> DEAD box proteins are putative RNA unwinding proteins, BmL3-helicase also is a DEAD box RNA helicase [32]; <5> helicase B, RhlB, is one of the five DEAD box RNA-dependent ATPases in Escherichia coli. ATPases found in Escherichia coli. RhlB requires an interaction with the partner protein RNase E for appreciable ATPase and RNA unwinding activities [43]; <17> NS3 possess both protease and helicase activities, the C-terminal portion of the NS3 contains the ATPase/helicase domain presumably involved in viral replication [46]; <41> NS3 possesses three enzyme activities that are likely to be essential for virus replication: a serine protease located in the N-terminus and NTPase as well as helicase activities located in the C-terminus. Functions of NS3 and NS5B during positive-strand RNA virus replication, the NS3 protein is be involved in the unwinding of the viral RNA template while NS5B protein may be involved in catalyzing the synthesis of new RNA molecules [35]; <12> the C-terminal region of NS3 exhibits RNA-stimulated NTPase, e.g. ATPase, and helicase activity, while the N-terminal serine protease domain of NS3 enhances RNA binding and unwinding by the C-terminal region, NS4A mutants that are defective in ATP-coupled RNA binding are lethal in vivo [44]; <18> the NS3 protein of Rice hoja blanca virus is an RNA silencing suppressor, RSS, that exclusively binds to small dsRNA molecules. This plant viral RSS lacks interferon antagonistic activity, yet it is able to substitute the RSS function of the Tat protein of Human immunodeficiency virus type 1 based on the sequestration of small dsRNA. NS3 is able to inhibit endogenous miRNA action in mammalian cells [40]; <10> The NS3 protein physically associates with the NS5 polymerase, NS3 andNS5 carry out all the enzymatic activities needed for polyprotein processing and genome replication. NS3 possesses an ATPase/helicase and RNA triphosphatase at its C-terminal end that are essential for RNA replication. In addition to its known enzymatic functions, the NS3 protein appears to be involved in the assembly of an infectious flaviviral particle, through its interactions with NS2A and presumably host cell proteins [36]; <10> conformational changes during ATP hydrolysis and RNA unwinding: on ssRNA binding, the NS3 enzyme switches to a catalytic competent state imparted by an inward movement of the P-loop, interdomain closure and a change in the divalent metal coordination shell, providing a structural basis for RNA-stimulated ATP hydrolysis. Determination of enzyme structure-function relationship of enzyme bound to single-stranded RNA, to an ATP analogue, to a transition-state analogue and to ATP hydrolysis products. RNA recognition appears largely sequence independent, reaction mechanism and RNA recognition, overview. RNA-unwinding mechanism, overview [15]; <17> NS3 possess both protease and helicase activities, the C-terminal portion of the NS3 contains the ATPase/helicase domain [46]; <2> open helicase conformation in the absence of nucleotides, or in the presence of ATP, or ADP, or RNA. In the presence of ADP and RNA, the open conformation is retained. By contrast, cooperative binding of ATP and

RNA leads to a compact helicase structure, direct transitions between open and closed conformations, overview [45]; <5> RhlB is the only Escherichia coli DEAD box protein that requires a protein partner to stimulate its ATPase activity [43]; <10> the C-terminal region of NS3 forms the RNA helicase domain, an ATP-driven molecular motor [36]; <10> the helicase domain of Dengue virus NS3 protein, i.e. DENV NS3H, contains RNA-stimulated nucleoside triphosphatase, NTPase, ATPase/helicase, and RNA 5-triphosphatase, RTPase, activities that are essential for viral RNA replication and capping. A 5-tailed RNA is a better RTPase substrate than an RNA containing no 5-dangling nucleotide [41]) (Reversibility: ?) [1,2,4,5,13,15,18,29,32,35,36,40,41,43,44,45,46]

P ?

Inhibitors

2',3'-ddATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2',3'-ddGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2',3'-ddTTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2',3'-dideoxy-ATP <17> [62]
2',3'-dideoxy-GTP <17> [62]
2'-deoxy-ATP <17> [62]
2'-dATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2'-dGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2'-dTTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
2'-deoxy-GTP <17> [62]
2'-deoxy-L-GTP <17> [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 [38]) [38]
2'-fluoro-2'-deoxy-ATP <17> [62]
2-amino-ATP <17> [62]
2-hydroxy-ATP <17> [62]
3'-dATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
3'-dGTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
3'-dUTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
3'-deoxy-ATP <17> [62]
6-methyl-thio-ITP <17> [62]

6-thio-GTP <17> [62]
7-methyl-GTP <17> [62]
8-bromo-ATP <17> [62]
ADP <12,38> (<38> competitive [14]; <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [14,38]
AMP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
ATP <17> [62]
ATP-γ-S <12> (<12> 5.4 mM, about 50% of the original helicase activity is inhibited, competitive inhibitor [48]) [48]
Ara-ATP <17> [62]
Cu^{2+} <12> (<12> inhibits ATPase activity, IC50: 0.13 mM [1]) [1]
EDTA <6> [17]
Fe^{2+} <12> (<12> inhibits ATPase activity, IC50: 0.75 mM [1]) [1]
GTP <12,17> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38,62]
Hg^{2+} <12> (<12> inhibits ATPase activity, IC50: 49 nM, targets the cysteine residue in the DECH box, competitive, cysteine or DTT protect at large concentrations [1]) [1]
ITP <17> [62]
KCl <6,10,12> (<6> RNA-dependent ATPase activity is sensitive to high salt concentrations. Maximal activity is obtained in the absence of KCl, and it is inhibited 50% at 0.1 M KC1, completely inhibited at 0.3 M KCl [19]; <6> slight stimulation at 0.05-0.1 M, inhibition at 0.2 M [17]; <12> slight decrease of activity in presence of [38]) [17,19,38,41]
N^1-O-ATP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
N^1-OH-ITP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
N^1-methyl-ATP <17> [62]
N^1-methyl-GTP <17> [62]
N^6-methyl-ATP <17> [62]
NEM <6> (<6> 5 mM, ATPase activity is blocked [19]) [17,19]
PCMB <12> (<12> inhibits ATPase activity, IC50: 88 nM [1]) [1]
UTP <12> (<12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
benzoyl-Nle-Lys-Arg-Arg <10> (<10> competitive inhibition, structure-activity relationship [36]) [36]
β,γ-methylene-ATP <12> (<12> efficient inhibitor, like the N^1-oxides N^1-O-ATP and N^1-OH-ITP [38]) [38]
dATP <12> (<12> inhibits unwinding [13]) [13]
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 [38]) [38]
ribavirin triphosphate <17> [62]

tetrabromobenzotriazole <12> (<12> inhibits unwinding, no inhibition of ATP hydrolysis [13]) [13]
Additional information <12> (<12> no or poor inhibition by Mn^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Ni^{2+} [1]; <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 [39]; <12> inhibitory potential of sequences of NTPase/helicase motifs VI derived peptides and their deleted derivatives analyzed, NTP-binding and hydrolyzing site not involved [37]; <12> several extracts of marine organisms exhibit different inhibitory effects on the RNA and DNA helicase activities of HCV NS3 [48]) [1,37,39,48]

Activating compounds

MLN51 <37> (<37> stimulates the RNA-helicase activity of eIF4AIII [34]) [34]
RNA <17,20> (<20> RNA-stimulated enzyme [2]; <17> the ATPase activity is stimulated by the presence of RNA and single-stranded DNA molecules [46]) [2,46]
RNase E <5> (<5> is required for ATPase and RNA unwinding activities of the enzyme, forms a complex with the enzyme, interaction analysis, overview. Avid, enthalpy-favored interaction between the helicase and RNase E 696-762 with an equilibrium binding constant Kaof at least 1 x 108 M-1 determined by isothermal titration calorimetry. Protein-protein and RNA-binding surfaces both communicate allosterically with the ATPase catalytic center [43]) [43]
deoxyribonucleotides <42> (<42> ATPase activity of the bacterially expressed BmL3-helicase is triggered by both the ssRNA and the dsRNA and, to a much lesser extent, by the ssDNA and dsDNA [32]) [32]
double-stranded DNA <1> (<1> weak stimulation [32]) [32]
double-stranded RNA <1> (<1> ATPase activity of the recombinant BmL3-helicase is strongly stimulated by dsRNA [32]) [32]
nonstructural protein 4A <12,39> (<39> i.e. NS4A, enhances the coupling between RNA binding and ATPase activity of nonstructural protein 3 (NS3), does not influence the kinetic parameters for RNA unwinding by NS3 [44]; <12> i.e. NS4A, stimulates serine protease activity of NS3 protein, helicase domain enhances serine protease activity and vice versa [42]; <12> NS4A binds to the NS3 protease domain and serves as an obligate cofactor for NS3 serine protease activity, thus NS4A enhances the ability of the C-terminal helicase to bind RNA in presence of ATP acting as a cofactor for helicase activity, 100fold lower K_m of NS3 with RNA in presence of NS4A. NS4A mutants that are defective in ATP-coupled RNA binding sre lethal in vivo [44]) [42,44]
nonstructural protein 5 <20> (<20> interaction with NS5, the viral RNA-dependent RNA polymerase, stimulates NS3 NTPAse and RTPase activities as well as thr RNA helicase activity [2]) [2]
poly(U) <21> (<21> strong stimulation of ATPase activity [3]) [3]
poly(dA) <21> (<21> strong stimulation of ATPase activity [3]) [3]
poly(dT) <21> (<21> strong stimulation of ATPase activity [3]) [3]

poly(rU) <10> (<10> stimulates the ATPase activity of NS3 [41]) [41]
polyU <10> (<10> stimulatory effect of polyU on ATP hydrolysis is significantly attenuated when NaCl concentration is 50 mM or higher, functional binding of polyU mainly through electrostatic interaction, binding triggers a conformational rearrangement that activates the catalytic core of the enzyme for ATP hydrolysis [41]) [41]
poly* <6,21> (<6> stimulates ATPase and dATPase activity [19]; <21> strong stimulation of ATPase activity [3]) [3,19]
rRNA <5> (<5> activates the ATPase activity of DbpA by promoting a conformational change after ATP binding that is associated with hydrolysis [26]) [26]
ribonucleotides <42> (<42> ATPase activity of the bacterially expressed BmL3-helicase is triggered by both the ssRNA and the dsRNA and, to a much lesser extent, by the ssDNA and dsDNA, recombinant BmL3-helicase is strongly stimulated by dsRNA [32]) [32]
single-stranded DANN <1,10,17> (<1> weak stimulation [32]; <10> on single-stranded RNA binding, the NS3 enzyme switches to a catalytic competent state imparted by an inward movement of the P-loop, interdomain closure and a change in the divalent metal coordination shell, providing a structural basis for RNA-stimulated ATP hydrolysis [15]; <17> the ATPase activity is stimulated by the presence of RNA and single-stranded DNA molecules [46]) [15,32,46]
single-stranded RNA <1,6,31> (<1,31> stimulates [22,32]; <6> RNA helicase catalyzes the unwinding of duplex RNA and RNA*DNA hybrids provided that single-stranded RNA is available for the helicase to bind [19]) [19,22,32]
single-strandede DNA <8> (<8> stimulates [18]) [18]
tRNA <31> (<31> stimulates [22]) [22]
Additional information <12,20,21,31,41> (<20> NS3 requires the hydrophilic domain of NS2B for activation [2]; <31> a 20-bp duplex RNA is ineffective in stimulating the (d)ATPase activity of Mtr4p [22]; <12> low salt conditions enhance unwinding by monomeric NS3 [23]; <21> no stimulation by poly(G) of ATPase activity [3]; <41> the NS3 protease domain enhances the helicase activity of NS3 but has no effect on its NTPase activity. For the truncated NS3 helicase domain both NTPase and helicase activities are up-regulated by NS5B, for the full-length NS3, the NTPase activity, but not the helicase activity, is stimulated by NS5B, specific interaction between NS3 and NS5B [35]) [2,3,22,23,35]

Metals, ions

Co^{2+} <12> (<12> activity 3-5-fold lower when magnesium ions are replaced by [38]) [38]
KCl <6> (<6> slight stimulation at 0.05-0.1 M, inhibition at 0.2 M [17]) [17]
Mg^{2+} <2,5,6,10,12,16,17,19,20,40,41,42> (<2,5,17,41,42> activates [32,35,43,45,46]; <16> required for ATPase activity [5]; <10> metal-dependent NTPase activity, mechanism involves a bound sulfate ion [4]; <12> preferred metal ion, ATPase activity [1]; <6> divalent cation required, Ca^{2+} or Mn^{2+} do not substitute for Mg^{2+} [17]; <6> requirement for divalent ions for ATP hydroly-

sis is specific for Mg^{2+} and is not supported by Mn^{2+} and Ca^{2+}. Half-maximum activity is achieved with concentrations of Mg^{2+} of 0.120 mM [19]; <19> the helicase activity requires divalent ions. Mn^{2+} is preferred over Mg^{2+} [35]; <10> activates, binding complexes, overview [15]; <10> activates, preferred metal ion [41]; <10> activity depends on divalent cations, assay concentration 1.5 mM, rate of ATP hydrolysis 10 times enhanced with Mg^{2+} as divalent cation cofactor, rate of ATP hydrolysis increases slightly when the NaCl concentration is elevated in the range of 10 mM and 200 mM [41]; <10> can be substituted by a Mn^{2+} ion for the enzymatic reaction [36]; <12> maximal NTPase activity achieved in the presence of 1.5-2 mM $MgCl_2$ [38]; <40> no ATPase activity of the wild-type in the absence of [6]) [1,2,4,5,6,15,17,19,32,35,36,38,41,42,43,45,46]

Mn^{2+} <10,12,19> (<10> activates [41]; <12> can substitute partially for Mg^{2+}, ATPase activity [1]; <19> the helicase activity requires divalent ions. Mn^{2+} is preferred over Mg^{2+} [35]; <12> activity 3-5-fold lower when magnesium ions are replaced by [38]; <10> activity depends on divalent cations, assay concentration 1.5 mM, rate of ATP hydrolysis more than 100 times over basal levels with Mn^{2+} as divalent cation cofactor [41]; <10> can substitute for a Mg^{2+} ion in the enzymatic reaction [36]) [1,35,36,38,41]

NaCl <8,10> (<8> stimulates at 300-500 mM [18]; <10> slightly activating ATPase activity at 10-200 mM, and RTPase at 15-50 mM [41]) [18,41]

Ni^{2+} <12> (<12> activity 3-5-fold lower when magnesium ions are replaced by [38]) [38]

Zn^{2+} <12> (<12> activity 3-5-fold lower when magnesium ions are replaced by [38]) [38]

sulfate <10> (<10> bound, required for the metal-dependent NTPase reaction mechanism [4]) [4]

Additional information <10,12> (<12> no or poor effect by Mn^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Ni^{2+} [1]; <10> the ATPase activity requires a divalent cation cofactor to function but is not sensitive to high ionic strength [41]) [1,41]

Turnover number (s^{-1})

0.043 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme Q169E [14]) [14]

0.3 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme F162A [14]) [14]

0.58 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme D172A [62]) [62]

0.64 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutant S108C/S229C [45]) [45]

0.85 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutant S108C/E224C [45]) [45]

0.92 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E173A [62]) [62]

0.92 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme D172A [62]) [62]

0.98 <2> (RNA, <2> pH 7.5, 37°C, recombinant wild-type YxiN [45]) [45]

1 <17,38> (ATP, <38> pH 7.5, 30°C, mutant enzyme Q169A [14]; <17> pH 7.5, 37°C, mutant enzyme R170A [62]) [14,62]

1 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme K187A [62]) [62]

1.1 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E180A [62]) [62]

1.1 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E173A [62]; <17> pH 7.5, 37°C, mutant enzyme F179A [62]) [62]
1.2 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme F179A [62]) [62]
1.25 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E169A [62]) [62]
1.3 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E180A [62]) [62]
1.3 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/S229C [45]) [45]
1.4 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/E224C [45]) [45]
1.41 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/D262C [45]) [45]
1.48 <2> (RNA, <2> pH 7.5, 37°C, native wild-type enzyme [45]) [45]
1.7 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme K186A [62]; <17> pH 7.5, 37°C, mutant enzyme Q188A [62]; <17> pH 7.5, 37°C, mutant enzyme R170A [62]) [62]
1.8 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme T166S [14]) [14]
1.83 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E169A [62]) [62]
2 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme F162L [14]; <38> pH 7.5, 30°C, mutant enzyme T166A [14]) [14]
2.2 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme K187A [62]) [62]
2.7 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme K186A [62]) [62]
2.75 <17> (GTP, <17> pH 7.5, 37°C, wild-type enzyme [62]) [62]
3 <17> (ATP, <17> pH 7.5, 37°C, wild-type enzyme [62]) [62]
3.3 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E182A [62]; <17> pH 7.5, 37°C, mutant enzyme Q188A [62]) [62]
3.5 <38> (ATP, <38> pH 7.5, 30°C, wild-type enzyme [14]) [14]
3.6 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E182A [62]) [62]
22.2 <31> (ATP, <31> pH 7.5, 37°C [22]) [22]

Specific activity (U/mg)

0.00000001 <5> (<5> about, wild-type enzyme, RNA helicase activity [43]) [43]
Additional information <10,12,16,17,20,39,40> (<16> RNA replication, RNA protection, spherule formation size, relative ATPase activity, RNA accumulation and stabilization, of wild-type and mutant enzymes, overview [5]; <20> specificities and activities of wild-type and mutant enzymes [2]; <10> biochemical properties and enzymatic activity of the RNA-helicase domain, functional characterization to get information about the flavivirus replication mechanism, NTPase-deficient mutant generated, RNA binding features, electrostatic interaction with RNA, basal ATPase activity insensitive to high ionic strength [41]; <12> helicase capable of unwinding duplex RNA or DNA, ambiguous [27]; <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, peptides do not interact with the ATP binding site [37]; <40> structural characterization of catalytic domain, 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 RNA 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 [6]; <17> 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, no RNA 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 [46]; <12> structure of nucleic base and ribose fragment of NTP molecule has a slight effect on inhibitory properties [38]; <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 [39]; <39> the nonstructural protein 4A (NS4A) enhances the ability of the N-terminal domain of NS3 protein to bind RNA in the presence of ATP, stimulates helicase activity, interaction between nonstructural protein 3 (NS3) and nonstructural protein 4A (NS4) mediated by amino acids of the C-terminus of NS4, mutation of the C-terminus of NS4 reduces ATP-coupled RNA binding, RNA binding studies, RNA-stimulated ATPase activity of N3-4a variants [44]; <12> the nonstructural protein 4A (NS4A) stimulates NS3 serine protease activity, truncated and full-length complexes between nonstructural protein 3 (NS3) and nonstructural protein 4A (NS4) purified, serine protease activities analyzed, NS3 protease domain enhances the RNA binding, ATPase, and RNA unwinding activities of the C-terminal NS3 helicase domain, isolated protease domain is much less reactive than full-length NS3, NS3 protease activity is enhanced by the presence of the NS3 helicase domain, indicating that the two domains have evolved to become completely interdependent [42]; <12> development of continuous fluorescence assay based on fluorescence resonance energy transfer for the monitoring of RNA helicase activity in vitro. This assay will be useful for monitoring the detailed kinetics of RNA unwinding mechanisms and screening RNA helicase inhibitors at high throughput [48]) [2,5,6,27,37,38,39,41,42,44,46,48]

K_m-Value (mM)

0.000001 <12> (ATP, <12> pH 6.5, 37°C, RNA-stimulated ATPase activity of mutant NS3-4A [44]) [44]

0.000002 <12> (ATP, <12> pH 6.5, 37°C, RNA-stimulated ATPase activity of mutant S1369R/M1708A [44]) [44]
0.0001 <12> (ATP, <12> pH 6.5, 37°C, RNA-stimulated ATPase activity of wild-type NS3 [44]) [44]
0.000114 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutant S108C/S229C [45]) [45]
0.000156 <2> (RNA, <2> pH 7.5, 37°C, native wild-type enzyme [45]) [45]
0.00017 <2> (RNA, <2> pH 7.5, 37°C, recombinant wild-type YxiN [45]) [45]
0.000324 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/S229C [45]) [45]
0.000422 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/D262C [45]) [45]
0.000458 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutant S108C/E224C [45]) [45]
0.000496 <2> (RNA, <2> pH 7.5, 37°C, recombinant YxiN mutantA115C/E224C [45]) [45]
0.0005 <12> (ATP, <12> pH 6.5, 37°C, RNA-stimulated ATPase activity of mutant M1708A [44]; <12> pH 6.5, 37°C, RNA-stimulated ATPase activity of mutant Y1702A [44]) [44]
0.001 <39> (ATP, <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A construct [44]; <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A S1369R mutant [44]) [44]
0.002 <39> (ATP, <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A S1369R/M1708A mutant [44]) [44]
0.0095 <17> (ATP, <17> pH 7.5, 37°C, native WNV NS3 protein purified from infected cells [46]) [46]
0.013 <17> (ATP, <17> recombinant protein including C-terminal portion the ATPase/helicase domain encompassing residues 181-619, ATP concentration 1mM ATP, ATPase but not RNA helicase activity [46]) [46]
0.0157 <6> (ATP, <6> pH 7.6, 37°C [17]) [17]
0.03 <39> (ATP, <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A S1369R/Y1702A mutant [44]) [44]
0.05 <39> (ATP, <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A M1708A mutant [44]; <39> RNA-stimulated ATPase activity, recombinant protein, NS3-4A Y1702A mutant [44]) [44]
0.09 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme F179A [62]; <17> pH 7.5, 37°C, mutant enzyme K187A [62]) [62]
0.1 <39> (ATP, <39> RNA-stimulated ATPase activity, NS3, recombinant protein [44]) [44]
0.1 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme K186A [62]) [62]
0.11 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E173A [62]) [62]
0.13 <17> (ATP, <17> pH 7.5, 37°C, recombinant C-terminal portion of the NS3 [46]) [46]
0.14 <17> (ATP, <17> pH 7.5, 37°C, wild-type enzyme [62]) [62]
0.163 <12> (ATP, <12> addition of polyuridylate lowers K_m for the ATP substrate [38]) [38]

0.18 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme D172A [62]; <17> pH 7.5, 37°C, mutant enzyme F179A [62]) [62]
0.2 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E173A [62]; <17> pH 7.5, 37°C, mutant enzyme R170A [62]) [62]
0.22 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E169A [62]) [62]
0.23 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E182A [62]) [62]
0.24 <17,37> (ATP, <37> pH 7.5, 25°C, in absence of MLN51 [34]; <17> pH 7.5, 37°C, mutant enzyme E180A [62]) [34,62]
0.256 <12> (ATP, <12> wild-type [38]) [38]
0.27 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme E182A [62]) [62]
0.27 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E180A [62]; <17> pH 7.5, 37°C, mutant enzyme R170A [62]) [62]
0.29 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme Q188A [62]) [62]
0.33 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme D172A [62]) [62]
0.34 <38> (ATP, <38> pH 7.5, 30°C, wild-type enzyme [14]) [14]
0.34 <17> (GTP, <17> pH 7.5, 37°C, mutant enzyme E169A [62]) [62]
0.35 <17> (GTP, <17> pH 7.5, 37°C, wild-type enzyme [62]) [62]
0.39 <17,31> (ATP, <31> pH 7.5, 37°C [22]; <17> pH 7.5, 37°C, mutant enzyme K186A [62]) [22,62]
0.51 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme T166A [14]) [14]
0.64 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme Q188A [62]) [62]
0.66 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme T166S [14]) [14]
0.78 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme F162L [14]) [14]
0.94 <17> (ATP, <17> pH 7.5, 37°C, mutant enzyme K187A [62]) [62]
1.1 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme Q169E [14]) [14]
3.5 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme F162A [14]) [14]
33 <38> (ATP, <38> pH 7.5, 30°C, mutant enzyme Q169A [14]) [14]
Additional information <12,20,39> (<20> kinetic study [2]; <12> kinetics of wild-type and mutant enzymes, overview [44]; <12> protease activities of NS3-4A variants analyzed, uncleaved NS3/4A polyprotein lacks protease and helicase activities [42]; <39> RNA-stimulated ATPase activities of NS3-4A variants analyzed, functionally important ATP-bound state of NS3 binds RNA much more tightly in the presence of NS4A, effectively coupling RNA binding to ATPase activity [44]) [2,42,44]

K_i-Value (mM)

0.007 <10> (benzoyl-Nle-Lys-Arg-Arg, <10> full-length enzyme in presence of cofactor CF40-Gly4-Ser-Gly4-NS3FL [36]) [36]
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 [38]) [38]
0.109 <12> (N^1-OH-ITP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.116 <12> (2',3'-ddATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.116 <12> (2'-dTTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]

0.141 <12> (3'-dATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.145 <12> (β,γ-methylene-ATP, <12> efficient inhibitor, like the N^1-oxides N^1-O-ATP and N^1-OH-ITP [38]) [38]
0.2 <17> (2-amino-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.205 <12> (N^1-O-ATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.26 <12> (3'-dUTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.277 <12> (2'-dGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.291 <12> (2'-dATP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.298 <12> (2',3'-ddTTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.3 <17> (2',3'-dideoxy-GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.3 <17> (ATP, <17> pH 7.5, 37°C, wild-type enzyme [62]) [62]
0.34 <38> (ADP, <38> pH 7.5, 30°C, mutant enzyme T166S [14]) [14]
0.36 <38> (ADP, <38> pH 7.5, 30°C, wild-type enzyme [14]; <38> pH 7.5, 30°C, mutant enzyme T166A [14]) [14]
0.4 <17> (2'-fluoro-2'-deoxy-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.443 <12> (3'-dGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.5 <17> (3'-deoxy-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.5 <17> (Ara-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.5 <17> (N^6-methyl-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.576 <12> (GTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.6 <17> (2',3'-dideoxy-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.6 <17> (2'-deoxy-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.6 <17> (6-methyl-thio-ITP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.7 <17> (8-bromo-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
0.721 <12> (2',3'-ddGTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
0.9 <17> (ITP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]

1 <17> (7-methyl-GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
1.3 <17> (2'-deoxy-L-GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
1.3 <12> (ADP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
1.46 <12> (UTP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
1.5 <17> (N^1-methyl-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
2.3 <17> (ribavirin triphosphate, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
2.4 <17> (2-hydroxy-ATP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
2.6 <17> (N^1-methyl-GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
3 <38> (ADP, <38> pH 7.5, 30°C, mutant enzyme F162L [14]) [14]
3.4 <17> (2'-deoxy-GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]
5 <12> (AMP, <12> inhibition of NTPase activity of NS3 protein by NTP derivatives [38]) [38]
8.1 <17> (GTP, <17> pH 7.5, 37°C, wild-type enzyme, inhibition of the ATPase reaction [62]) [62]

pH-Optimum

6.5 <12,39,41> (<41> ATPase assay at [35]; <12> helicase assay at [44]; <39> ATPase and RNA binding assay at [44]) [35,44]
7 <12> (<12> ATPase activity [1]) [1]
7.2 <36> (<36> assay at [20]) [20]
7.4 <16> (<16> ATPase assay at [5]) [5]
7.5 <2,5,10,17,19,20,26,31,37,38,41,42> (<2,5,10,17,20,26,31,37,38,42> assay at [2,4,14,22,32,33,34,41,43,45,46,62]; <41> RNA helicase assay at [35]) [2,4,14,22,32,33,34,35,41,43,45,46,62]

pH-Range

6-9 <12> (<12> activity range, ATPase activity [1]) [1]
6.5 <19> (<19> pH 6.5: about 50% of maximal activity, pH 8: about 80% of maximal activity [35]) [35]

Temperature optimum (°C)

25 <37> (<37> assay at [34]) [34]
30 <38> (<38> assay at [14]) [14]
37 <2,10,12,16,17,20,26,31,36,39,40,41,42> (<2,10,17,20,26,31,36,40,41,42> assay at [2,4,6,20,22,32,33,35,41,45,46,62]; <16> ATPase assay at [5]; <12> helicase assay at [44]; <39> ATPase and RNA binding assay at [44]) [2,4,5,6,20,22,32,33,35,41,44,45,46,62]
50 <15> (<15> maximal ATPase activity and unwinding activity specific for single-strand paired RNA [47]) [47]

Temperature range (°C)

40-50 <15> (<15> 40°C: about 40% of maximal activity, 50°C: optimum, 60°C: less than 10% of maximal activity [47]) [47]

4 Enzyme Structure

Molecular weight

54000 <40> (<40> molecular mass of the helicase/NTPase domain, SDS-PAGE [6]) [6]

66000 <17> (<17> recombinant protein of C-terminal portion the ATPase/helicase domain, residues 181-619, SDS-PAGE, gel filtration [46]) [46]

130000 <6> (<6> glycerol gradient centrifugation [17]) [17]

140000 <6> (<6> MALDI-TOF mass spectrometry [31]) [31]

246000 <6> (<6> calculated from sequence. Apart from an N-terminal domain of unknown function, Brr2p consists of two putative helicase domains, each connected at its C-terminus to a Sec63-like domain [50]) [50]

Subunits

? <6,8,18,24,27> (<6> x * 100000, gel filtration [19]; <24> x * 119037, calculated from sequence [30]; <27> x * 49800, recombinant C-terminal helicase domain (amino-acid sequence corresponding to that between residues 189 and 620 of the predicted NS3 polypeptide), SDS-PAGE [7]; <8> x * 70000, His-tagged enzyme, SDS-PAGE [28]; <18> x * 66000, recombinant NS3, SDS-PAGE, x * 109000, recombinant MBP-fusion NS3 protein, SDS-PAGE [40]) [7,19,28,30,40]

dimer <10> (<10> crystal structure, three-domain structure with asymmetric distribution of charges on the surface and a tunnel structure for RNA substrate access, overview [4]) [4]

monomer <5,6,17> (<6> 1 * 130000, SDS-PAGE [17]; <5> DbpA is monomeric in solution up to a concentration of 25 mM and over the temperature range of 4°C to 22°C [25]; <17> $\alpha\beta$, 29% α-helix, 15% β-sheet, and 56% nonregular structures, globular monomer accounts for 90%, a small percentage (7%) of dimers or trimers, higher oligomers almost absent (3%), analytical centrifugation and gel filtration [46]; <17> in solution, x * 66000, about, recombinant soluble His6-tagged C-terminal portion of NS3, SDS-PAGE [46]) [17,25,46]

Additional information <5,10,16,17,20> (<20> DEN2 nonstructural protein 3, NS3, has a serine protease domain and requires the hydrophilic domain of NS2B for activation [2]; <16> the enzyme activity is located in the C-terminal nucleoside triphosphatase/helicase domain of the BMV 1a protein RNA replication factor, BMV 1a protein contains an N-terminal capping domain with m^7G-methyltransferase and m^7GMP binding activities, and a C-terminal NTPase/helicase-like domain, comprising residues 562-961, containing 7 conserved helicase motifs, the two domains are separated by a proline-rich region, overview [5]; <5> location of the nine conserved sequence motifs in the DEAD box helicase RhlB, structure modelling, overview [43]; <10> the C-

terminal domain of the enzyme contains the Walker A and Walker B motifs, i.e. motif I, GK(S/T) and motif II, DExD/H [41]; <10> the C-terminal region of NS3 forms the RNA helicase domain. The ATP binding site is housed between these two subdomains, structure modelling, overview [36]; <17> the linker region plays a critical role in determining the protein-protein interactions that leads to the formation of the active oligomer [46]) [2,5,36,41,43,46]

Posttranslational modification

phosphoprotein <6,23,32,33,34,35> (<23,32,33,34,35> helicase activity of DDX5 is regulated by phosphorylation and calmodulin binding [12]; <6> phosphorylation of p68 RNA helicase at Y593 upregulates transcription of the Snail1 gene [63]) [12,63]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HEK-293T cell <6> [49]
HeLa cell <6> [17,19,49]
Leydig cell <3,23,34,35> (<3> the 1 kb fragment (5 to the ATG codon) of GRTH gene contains sequences for androgen regulation of its expression in Leydig cells [57]) [12,57]
NIH-3T3 cell <3> [52]
SW-480 cell <6> [63]
SW-620 cell <6> [63]
blastomere <48> [55]
brain <33,46> (<33> DDX17 transcripts are abundant in rat brains in early embryonic stages and are downregulated in late post-natal and adults, suggesting involvement during neuronal differentiation during development of the central nervous system [12]) [12,64]
branchial arch <47> [53]
central nervous system <47> [53]
embryo <47,48> (<48> expressed in blastomeres and embryonic cells in planarian embryonic development [55]) [53,55]
eye <47> (<47> in the ciliary marginal zone adjacent to the neural retina and within the lens epithelium, present in the anterior eye during fibroblast growth factor 2 (FGF2)-mediated retinal regeneration. Ddx39 message is restricted to a subpopulation of proliferating cells in the developing and regenerating optic cup [53]) [53]
germ cell <32> (<32> high level of expression in male germ cells [12]) [12]
larva <42> [32]
limb <47> (<47> developing limb buds at stages 48-55 [53]) [53]
liver <46> (<46> low expression level [64]) [64]
mesenchyme <47> (<47> facial mesenchyme [53]) [53]
muscle <46> [64]
neural tube <47> (<47> Ddx39 is present in the ventricular region of the developing neural tube up to and including stage 48 [53]) [53]

otic vesicle <47> (<47> Ddx39 message is restricted to a subpopulation of proliferating cells in the developing and regenerating optic cup [53]) [53]
reticulocyte <36> [20]
seedling <26> (<26> VrRH1 may play a role in the viability of mung bean seeds [33]) [33]
spermatid <3,23,34,35,48> (<3> GRTH resides in the nucleus, cytoplasm and chromatoid body of round spermatids [51]) [12,51,55]
spermatocyte <23,34,35,48> [12,55]
spermatogonium <48> [55]
spleen <46> [64]
testis <3,23,29,34,35,46> (<23,34,35> highly expressed in [12]; <46> high expression level [64]; <29> expression is restricted to the male germ cell line [12]; <35> GRTH is a negative regulator of apoptosis in spermatocytes and promotes the progress of spermatogenesis [21]; <3> the expression of GRTH in testicular cells is differentially regulated by its 5 flanking sequence [57]) [12,21,57,64]
thymus <46> [64]
trophozoite <7> [16]
Additional information <16,25,30,42> (<16> the virus is propagated in yeast cells [5]; <25> the protein is expressed in all tissues [12]; <30> vasa (DDX4) mRNA and protein are abundantly and specifically expressed in germ cells in both sexes throughout development [12]; <42> presence of BmL3-helicase in various life stages of Brugia malayi [32]) [5,12,32]

Localization

chromatoid body <3> [51]
cytoplasm <3,7> (<3> p68 shuttles between the nucleus and the cytoplasm. The nucleocytoplasmic shuttling of p68 is mediated by two nuclear localization signal and two nuclear exporting signal sequence elements. p68 shuttles via a classical RanGTPase dependent pathway [52]) [16,51,52]
membrane <12> [37]
mitochondrion <46> [64]
nucleus <3,6,7,24> (<24> the GFP-DBP2 gene product, transiently expressed in HeLa cells, is localized in the nucleus [30]; <3> p68 predominately localizes in the cell nucleus [52]) [16,19,30,51,52]
Additional information <16> (<16> BMV 1a protein accumulates on endoplasmic reticulum membranes of the host cell [5]) [5]

Purification

<4> [9]
<5> [25]
<5> (recombinant His-tagged wild-type and mutant RhlB and RNaseE from Escherichia coli strain BL21(DE3) by nickel affinity chromatography and gel filtration) [43]
<6> [17,19,49]
<7> [16]
<8> [28]
<10> [15]

<10> (recombinant His-tag C-terminal domain of NS3 protein from Escherichia coli by nickel affinity chromatography) [41]
<10> (recombinant His-tagged catalytic domain of the NS3 helicase domain from dengue virus serotype 4 from Escherichia coli strain BL21 by nickel affinity chromatography and gel filtration) [15]
<10> (recombinant protein, gel filtration, SDS-PAGE) [41]
<11> [29]
<12> [13]
<12> (gel filtration, SDS-PAGE) [37,39]
<12> (gel filtration, recombinant nonstructural protein 3) [27]
<12> (gel filtration, recombinant protein) [38]
<12> (recombinant C-terminally His-tagged truncated NS3 NTPase/helicase domain from Escherichia coli by nickel affinity chromatography) [1]
<12> (recombinant His10-tagged Arg-rich amino acid motif HCV1487-1500, complete domain 2, and domain 2 lacking the flexible loop from Escherichia coli strain BL21(DE3)) [39]
<12> (recombinant enzyme) [48]
<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) [42]
<15> (recombinant enzyme) [47]
<16> (recombinant GST-fusion wild-type and mutant enzymes from Escherichia coli strain C41(DE3) by glutathione affinity chromatography) [5]
<17> [62]
<17> (gel filtration, recombinant protein, soluble form) [46]
<17> (recombinant soluble His6-tagged C-terminal portion of NS3 in Escherichia coli by nickel affinity chromatography and gel filtration) [46]
<20> (recombinant wild-type and N-terminally truncated enzyme from Escherichia coli) [2]
<21> [3]
<26> [33]
<27> [7]
<37> [34]
<38> [14]
<39> (gel filtration) [44]
<40> (gel filtration, recombinant protein) [6]
<42> (recombinant N-terminally His-tagged enzyme from Escherichia coli strain BL21(DE3) by nickel affinity chromatography) [32]
<44> [58]

Crystallization

<4> (RNA helicase Hera C-terminal domain, vapour diffusion, microbatch under oil) [9]
<10> (hanging drop vapour diffusion method, crystallization of native enzyme, enzyme in complex with adenylyl imidodiphosphate, enzyme in complex with ADP, enzyme in complex with single-stranded RNA and enzyme in complex with single-stranded RNA and ADP) [15]

<10> (purified catalytic domain fragment, hanging drop vapour diffusion method, 0.002 ml of 10 mg/ml protein in 0.1 M MES, pH 6.5, 0.2 M ammonium sulfate, 14% PEG 8000, mixed with an equal volume of precipitation solution, 18°C, macroseeding, cryoprotection by 25% glycerol, X-ray diffraction structure determination and analysis at 2.4 A resolution, modeling) [4]
<10> (purified recombinant His-tagged catalytic domain of the NS3, hanging drop vapour diffusion method, at 13°C over a well solution containing 0.1M MES, pH 6.5, and 20% PEG 3350, X-ray diffraction structure determination and analysis. Crystals for the AMPPNP complex are obtained by cocrystallization of NS3h at 5 mg/ml with 5 mM $MnCl_2$ and 5 mM AMPPNP using a precipitating solution containing 0.1M MES, pH 6.5, and 10% PEG 3350, at 13°C. Crystals with ADP are obtained by cocrystallization at a concentration of 2.5 mg/ml with 5 mM $MnCl_2$ and 5 mM ADP in 0.1 M Tris-HCl, pH 7.0, and 7.5% PEG 3350 at 23°C, further preparation of ternary complexes,overview) [15]
<11> (hanging-drop vapor diffusion method, the 1.8 A crystal structure of the helicase region of the YFV NS3 protein (includes residues 187 to 623) and the 2.5 A structure of its complex with ADP) [29]
<12> [13]
<25> (hanging-drop method, crystallization of recombinant DDX3 RNA helicase domain) [8]
<27> (crystals of the recombinant C-terminal helicase domain are obtained by the hanging-drop vapour-diffusion method) [7]
<40> (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) [6]
<43> (crystals of DDX1954-475 in complex with RNA and Mg/adenosine 5'-(β,γ-imido)triphosphate are obtained by vapor diffusion in sitting drops incubated at 4°C by mixing 0.0001 ml of protein solution (20 mg/ml) including 10-molar excess of decauracil ssRNA, adenosine 5'-(β,γ-imido)triphosphate, and $MgCl_2$ and 0.0002 ml of reservoir solution containing 14% polyethylene glycol monomethyl ether 2000, 0.25 M trimethylamine n-oxide, 0.1 M Tris, pH 8. The crystal structures of DDX19, in its RNA-bound prehydrolysis and free posthydrolysis state, reveal an α-helix that inserts between the conserved domains of the free protein to negatively regulate ATPase activity) [56]
<44> (sitting-drop vapor diffusion method at 4 °C. Crystal structures of the conserved domain 1 of the DEIH-motif-containing helicase DHX9 and of the DEAD-box helicase DDX20. Both contain a RecA-like core, but DHX9 differs from DEAD-box proteins in the arrangement of secondary structural elements and is more similar to viral helicases such as NS3. The N-terminus of the DHX9 core contains two long α-helices that reside on the surface of the core without contributing to nucleotide binding) [58]

Cloning

<1> (overexpressed as His-tag fusion protein in Escherichia coli) [32]
<4> (C-terminal domain of Hera is overproduced in Escherichia coli) [9]

<5> (construction of a di-cistronic vector that overexpresses a complex comprising RhlB and its recognition site within RNase E, corresponding to residues 696-762, the expression construct is termed pRneRhlBΔ1-397. Expression of His-tagged wild-type and mutant RhlB and RNaseE in Escherichia coli strain BL21(DE3)) [43]
<6> (expression in Escherichia coli) [49]
<7> [16]
<8> [28]
<10> (expressed in Escherichia coli, recombinant protein) [41]
<10> (expression in Escherichia coli) [15]
<10> (expression of NS3 ATPase/helicase in Escherichia coli, expression of the His-tag C-terminal domain) [41]
<10> (expression of the His-tagged catalytic domain of the NS3 helicase domain from dengue virus serotype 4 in Escherichia coli strain BL21) [15]
<11> [29]
<12> (NS3-plus and NS3/4a-plus genes expressed in Escherichia coli, generation of NS3-4A expression product, pET15b and pet-SUMO vector) [42]
<12> (expressed in Escherichia coli) [37]
<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) [39]
<12> (expressed in Escherichia coli, strain Rosetta (DE3), recombinant nonstructural protein 3) [27]
<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) [38]
<12> (expression of C-terminally His-tagged truncated NS3 NTPase/helicase domain in Escherichia coli) [1]
<12> (expression of the Arg-rich amino acid motif HCV1487-1500, of the complete domain 2, and of domain 2 lacking the flexible loop localized between Val1458 and Thr1476 as His10-tagged proteins in Escherichia coli strain BL21(DE3)) [39]
<12> (expression of wild-type and mutant NS3, cloning of a His6-tag to the N-terminus of NS3 greatly increases its affinity for RNA) [44]
<15> (expression in Escherichia coli) [47]
<16> (expression of GST-fusion wild-type and mutant enzymes in Escherichia coli strain C41(DE3)) [5]
<17> (expressed in Escherichia coli, C-terminal portion with the ATPase/helicase domain, plasmid pET-30a) [46]
<17> (expression of the soluble His6-tagged C-terminal portion of NS3 in Escherichia coli C41 cells) [46]
<18> (expression of NS3 as maltose-binding protein furion protein using the constitutive elongation factor-1α promoter in HEK-293T cells. Plant viral RSS protein NS3 complements HIV-1 Tat based on the sequestration of small dsRNA) [40]

<20> (expression of wild-type and N-terminally truncated enzyme in Escherichia coli) [2]
<21> (baculovirus expression system) [3]
<24> [30]
<25> (overexpression in Escherichia coli) [8]
<26> (overexpression in Escherichia coli) [33]
<27> (expression of the C-terminal helicase domain (amino-acid sequence corresponding to that between residues 189 and 620 of the predicted NS3 polypeptide) in Escherichia coli) [7]
<35> [21]
<37> [34]
<38> [14]
<39> (NS3-plus and NS3/4a-plus genes expressed in Escherichia coli, composition of NS3-4A expression product using the pet-SUMO vector) [44]
<40> (expressed in Escherichia coli BL21 (DE3), recombinant protein, pET21b vector) [6]
<41> (expression of wild-type and mutant His-tagged NS3 helicase domain in Escherichia coli) [35]
<42> (expression of the N-terminally His-tagged enzyme as soluble protein in Escherichia coli strain BL21(DE3)) [32]
<44> (expression in Escherichia coli) [58]

Engineering

A115C/D262C <2> (<2> site-directed mutagenesis, the mutant shows activity, structure and substrate specificity similar to the wild-type [45]) [45]
A115C/E224C <2> (<2> site-directed mutagenesis, the mutant shows activity, structure and substrate specificity similar to the wild-type [45]) [45]
A115C/S229C <2> (<2> site-directed mutagenesis, the mutant shows activity, structure and substrate specificity similar to the wild-type [45]) [45]
D172A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 41% of the ratio determined for the wild-type enzyme [62]) [62]
D310H <5> (<5> site-directed mutagenesis of the V motif, leads to altered enzyme activity, overview [43]) [43]
D31^{3}H <5> (<5> site-directed mutagenesis of the V motif, leads to altered enzyme activity, overview [43]) [43]
D755A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 90% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
E169A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 38% of the ratio determined for the wild-type enzyme [62]) [62]
E173A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 17% of the ratio determined for the wild-type enzyme [62]) [62]
E180A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 35% of the ratio determined for the wild-type enzyme [62]) [62]

E182A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 29% of the ratio determined for the wild-type enzyme [62]) [62]
E300A <8> (<8> mutation causes severe defect in RNA unwinding that correlates with reduced rate of ATP hydrolysis [28]) [28]
F162A <38> (<38> k_{cat}/K_M for ATP is 1% of wild-type value [14]) [14]
F162L <38> (<38> k_{cat}/K_M for ATP is 25% of wild-type value [14]) [14]
F179A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 19% of the ratio determined for the wild-type enzyme [62]) [62]
F788A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 30% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
G199A <40> (<40> mutation in WALKER A motif, PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
G460A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no effect on either ATPase or RNA-unwinding activities [6]) [6]
G463A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no effect on either ATPase or RNA-unwinding activities [6]) [6]
G781S <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 75% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
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 [13]) [13]
H299A <8> (<8> mutation elicits defects in RNA unwinding but spares the ATPase activity [28]) [28]
H320D <5> (<5> site-directed mutagenesis of the V motif, leads to altered enzyme activity, overview [43]) [43]
H51A <20> (<20> site-directed mutagenesis, the mutant has an inactivated protease domain showing enhanced RNA helicase compared to wild-type full-length enzyme [2]) [2]
H903A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 45% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
K177A <31> (<31> mutant enzyme shows no stimulation of ATPase activity by single-stranded RNA [22]) [22]
K186A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 15% of the ratio determined for the wild-type enzyme [62]) [62]

K187A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 8% of the ratio determined for the wild-type enzyme [62]) [62]
K191A <8> (<8> mutation causes severe defect in RNA unwinding that correlates with reduced rate of ATP hydrolysis [28]) [28]
K199A/T200A <10> (<10> site-directed mutagenesis, mutant avoid of basal and of RNA-stimulated NTPase activity [41]; <10> site-directed mutagenesis, the mutation in the C-terminal domain of NS3 eliminates both the basal and the RNA-stimulated NTPase activity [41]) [41]
K200A <40> (<40> mutation in WALKER A motif, PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200D <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200E <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200H <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200N <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200Q <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K200R <40> (<40> PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
K232A <41> (<41> site-directed mutagenesis in the helicase domain of NS3 [35]) [35]
K691A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 80% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
M1708A <12,39> (<39> NS3-4A construct, ability to bind and unwind RNA in vitro, mutation reduces functional NS3-4A binding affinity for RNA by 500-fold relative to the wild-type [44]; <12> site-directed mutagenesis, the NS3-4A mutant shows decreased ATPase activity and reduced RNA stimulation activity compared to wild-type NS3 [44]) [44]
Q169A <38> (<38> k_{cat}/K_M for ATP is 0.3% of wild-type value [14]) [14]
Q169E <38> (<38> k_{cat}/K_M for ATP is 0.4% of wild-type value [14]) [14]
Q188A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 33% of the ratio determined for the wild-type enzyme [62]) [62]
Q457A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, 80% reduction of ATPase activity, no RNA helicase activity [6]) [6]
Q785A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 65% reduced ATPase activity compared to the wild-type enzyme [5]) [5]

Q785E <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 75% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
R170A <17> (<17> the ration of (k_{cat}/K_m)ATP/(k_{cat}/K_m)GTP is 31% of the ratio determined for the wild-type enzyme [62]) [62]
R184Q/K185N/R186G/K187N <20> (<20> construction of the N-terminally truncated mutant NS3Δ180 containing a mutated RNA substrate biding motif, the mutant shows reduced RTPase activity [2]) [2]
R185A <17> (<17> inactive mutant enzyme [62]) [62]
R229A <8> (<8> mutation causes severe defect in RNA unwinding that correlates with reduced rate of ATP hydrolysis [28]) [28]
R458A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, 90% reduction of ATPase activity, no RNA helicase activity [6]) [6]
R459A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no effect on either ATPase or RNA-unwinding activities [6]) [6]
R461A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no ATPase activity, no RNA helicase activity [6]) [6]
R464A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no ATPase activity, no RNA helicase activity [6]) [6]
R791A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 10% increased ATPase activity compared to the wild-type enzyme [5]) [5]
R806H <9> (<9> interacts with the circadian oscillator component FREQUENCY (FRQ), but interaction between the FRQ-FRHR806H complex (FFC) and White Collar Complex is severely affected [54]) [54]
R815L <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 60% increased ATPase activity compared to the wild-type enzyme [5]) [5]
R938A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 45% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
S108C/E224C <2> (<2> site-directed mutagenesis, the mutant shows activity, structure and substrate specificity similar to the wild-type [45]) [45]
S108C/S229C <2> (<2> site-directed mutagenesis, the mutant shows activity, structure and substrate specificity similar to the wild-type [45]) [45]

S1369R <12,39> (<39> NS3-4A construct, suppressor mutant, ATP-coupled RNA affinity identical to that of wild-type NS3-4A [44]; <12> site-directed mutagenesis, the NS3-4A mutant shows increased ATPase activity and RNA stimulation activity compared to wild-type NS3 [44]) [44]
S1369R/M1708A <12,39> (<39> NS3-4A construct, reduced ATP-coupled RNA affinity of the single mutant suppressed by the addition of the S1369R mutation [44]; <12> site-directed mutagenesis, the NS3-4A mutant shows increased ATPase activity and reduced RNA stimulation activity compared to wild-type NS3 [44]) [44]
S1369R/Y1702A <12,39> (<39> NS3-4A construct, reduced ATP-coupled RNA affinity of the single mutant suppressed by the addition of the S1369R mutation [44]; <12> site-directed mutagenesis, the NS3-4A mutant shows decreased ATPase activity and reduced RNA stimulation activity compared to wild-type NS3 [44]) [44]
S790A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 60% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
S790W <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows 70% reduced ATPase activity compared to the wild-type enzyme [5]) [5]
T166A <38> (<38> k_{cat}/K_M for ATP is 37% of wild-type value [14]) [14]
T166S <38> (<38> k_{cat}/K_M for ATP is 26% of wild-type value [14]) [14]
T192A <8> (<8> mutation causes severe defect in RNA unwinding that correlates with reduced rate of ATP hydrolysis [28]) [28]
T201A <40> (<40> mutation in WALKER A motif, PCR-based mutagenesis, ATPase and RNA helicase activity lost [6]) [6]
T326A <8> (<8> mutation elicits defects in RNA unwinding but spares the ATPase activity [28]) [28]
T328A <8> (<8> mutation elicits defects in RNA unwinding but spares the ATPase activity [28]) [28]
T812A/Y813A <16> (<16> site-directed mutagenesis, mutation in the conserved BMV 1a protein helicase motif, the mutant shows abolished RNA recruitment and RNA stabilization, and thus RNA replication function, but normal accumulation, localization, and 2apol recruitment, the mutant shows unaltered ATPase activity compared to the wild-type enzyme [5]) [5]
V462A <40> (<40> mutation of residues of the arginine finger within the active sites of ATP hydrolysis, no effect on either ATPase or RNA-unwinding activities [6]) [6]
Y1702A <12,39> (<39> NS3-4A construct, ability to bind and unwind RNA in vitro, mutation reduces functional NS3-4A binding affinity for RNA by 500-fold relative to the wild-type [44]; <12> site-directed mutagenesis, the NS3-4A mutant shows decreased ATPase activity and reduced RNA stimulation activity compared to wild-type NS3 [44]) [44]

Y383A <5> (<5> site-directed mutagenesis, the mutation causes the formation of a higher order molecular weight species in binding of RNaseE by RhlB [43]) [43]
Y593F <6> (<6> expression of the mutant enzyme in SW620 cells leads to Snail repression, E-cadherin upregulation and vimentin repression [63]) [63]
Additional information <12,16,18,22,28,41,45> (<16> trans interference by BMV 1a protein helicase mutants with BMV 1a protein-stimulated RNA3 accumulation, overview [5]; <22,28> the N-terminal part of the TGBp1 NTPase/helicase domain comprising conserved motifs I, Ia and II is sufficient for ATP hydrolysis, RNA binding and homologous protein-protein interactions. Point mutations in a single conserved basic amino acid residue upstream of motif I have little effect on the activities of C-terminally truncated mutants of both TGBp1 proteins. When introduced into the full-length NTPase/helicase domains, these mutations cause a substantial decrease in the ATPase activity of the protein, suggesting that the conserved basic amino acid residue upstream of motif I is required to maintain a reaction-competent conformation of the TGBp1 ATPase active site [24]; <18> an NS3 mutant, that is deficient in RNA binding and its associated RSS activity, is inactive in complementing the RNA silencing suppressor function of the Tat protein of Human immunodeficiency virus type 1 [40]; <12> construction of the NS3-4A mutant affected in its acidic domain, the mutant shows altered RNA binding and increased ATPase activity, kinetics, overview [44]; <41> for the truncated NS3 helicase domain both NTPase and helicase activities are up-regulated by NS5B, for the full-length NS3, the NTPase activity, but not the helicase activity, is stimulated by NS5B, specific interaction between NS3 and NS5B [35]; <45> mutagenesis of conserved p54 helicase motifs activates translation in the tethered function assay, reduces accumulation of p54 in P-bodies in HeLa cells, and inhibits its capacity to assemble P-bodies in p54-depleted cells [60]) [5,24,35,40,44,60]

Application

drug development <10,12> (<12> the enzyme is a target for anti-HCV drug development [1]; <10> the enzyme is a target for development of specific antiviral inhibitors [4]; <10> the multifunctional NS3 protein from Dengue virus is a target for the design of antiviral inhibitors [36]) [1,4,36]
medicine <6,23,30,32,33,34,35> (<23,30,32,33,34,35> DDX4 can serve as a useful and highly specific biomarker for the diagnosis of germ cell tumors [12]; <6> mutation within hBrr2p can be linked to autosomal dominant retinitis pigmentosa [50]) [12,50]
pharmacology <12,40> (<40> conservation of the NTP-binding pocket among viruses of the family Flaviviridae as potential for development of therapeutics [6]; <12> peptide inhibitors reproducing the structure of the autoregulatory motif as possibility to develop effective antivirals [37]) [6,37]

6 Stability

Temperature stability

20-70 <15> (<15> the enzyme starts to unfold at 20°C and fully unfolds at 70°C [47]) [47]

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