Minireview

Eukaryotic DNA helicases

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DNA is very stable in its double-stranded form. For many processes of DNA metabolism, such as replication, repair, recombination and transcription, the DNA has to be brought transiently into a single-stranded form. DNA helicases are enzymes capable of melting the hydrogen bonds of base pairs by using the energy of nucleoside-5'-triphosphate hydrolysis. This minireview focusses on the current knowledge of DNA helicases from eukaryotic cells.

DNA replication; DNA helicase; Eukaryotic; ATPase; Nucleoside triphosphate hydrolysis

1. INTRODUCTION

Many processes such as DNA replication, DNA repair mechanisms, DNA recombination and also DNA transcription require the DNA double helix to be melted to provide DNA polymerases and RNA polymerases with a single stranded DNA substrate. Since the double helix is energetically favoured the melting or unwinding of DNA is an extremely energy consuming process. The enzymes that can perform this unwinding of the DNA are called DNA helicases [1]. These enzymes contain an intrinsic ATPase activity which hydrolyses nucleoside-5'-triphosphates in the presence of DNA and divalent cations (mostly Mg^{2+}) as essential cofactors. This hydrolysis provides the energy for the DNA helicase to translocate along one strand of the duplex DNA, thus unwinding the DNA. This now single stranded DNA is then ready, e.g. for the primosome to initiate DNA synthesis and for the replisome (e.g. DNA polymerase holoenzyme) to replicate the DNA [2]. During the last decade advanced in vitro DNA replication systems have been developed in *Escherichia coli* [2] and, among many other enzymological details, have facilitated the discovery of at least 7 different DNA helicases [3]. These multiple DNA helicases underline the importance of DNA unwinding events in many cellular processes. In contrast to the situation in *Escherichia coli*, where a connection between gene product and its function can be found easily, the situation in eukaryotes is much more complex. Here we summarize our knowledge on DNA

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helicases from eukaryotic cells: In the first part these enzymes from some viral sources (simian virus 40, polyoma virus and herpes simplex 1 virus) will be reviewed, the second part will deal with DNA helicases of cellular origin.

2. VIRAL DNA HELICASES

Viral genomes provided the most promising in vitro replication systems so far and serve as models to study DNA replication events occurring in the cell [2]. The simian virus 40 (SV40) and the polyoma virus rely with one single exception on host cell enzymes and proteins for the replication of their approximately 5 kb DNA. The large tumor (T) antigen of both viruses is the only protein which is essential for viral DNA replication. Among many different functions, e.g. for the regulation of viral gene expression, transformation and regulation of several cellular properties the large T antigen was found to have an intrinsic DNA helicase activity [4]. The SV40 T antigen helicase (reviewed in [5]) is a 92 kDa protein that translocates and therefore unwinds processively in the $3' \rightarrow 5'$ direction [5]. The preferred substrates for nucleoside-5'-triphosphate hydrolysis are ATP and dATP. T antigen interacts with DNA polymerase α and appears to function during the unwinding at the origin of DNA replication as well as during fork movement [5]. The analogous T antigen from polyoma virus also has DNA helicase activity. This 100 kDa enzyme has similar DNA helicase properties as its SV40 counterpart [6]. The polyoma T antigen DNA helicase unwinds in the $3' \rightarrow 5'$ direction and also prefers ATP and dATP as the nucleoside-5'-triphosphate to be hydrolyzed for unwinding.

The genome of herpes simplex virus 1 has a size of approximately 150 kilobases and contains cis- and trans-acting functions involved in DNA replication. It appears that all enzymes involved in herpes simplex virus 1 replication are coded for by the virus [2]. The DNA helicase from herpes simplex 1 resides in a 3 subunit complex with molecular weights of 120,000, 97,000 and 70,000, respectively [7]. The complex contains two enzymatic activities which are DNA helicase and DNA primase. DNA primase is an enzyme required for the synthesis of the initiator RNA at the lagging strand of the replication fork and is coded for by the UL8 gene. The helicase-primase complex moves and unwinds in the $5' \rightarrow 3'$ direction. The helicase activity probably can be assigned to the combined action of the UL5 and UL52 gene products. Both polypeptides are required for DNA dependent ATPase and GTPase as well as for helicase activities and appear to be inactive as single polypeptides [8].

By the organization of a helicase-primase complex the herpes simplex virus 1 obviously combines the action of two important replication enzymes in an economical way. This combination of two enzymes has also been evolved by the two bacteriophages T7 and T4. The T7 viral gene 4 protein possesses both primase and helicase in one polypeptide which can be seen as a minimal strategy for a small virus [9]. The genes 41 and 61 from bacteriophage T4 form a complex with potent primase and helicase activities [10].

Finally, herpes simplex 1 virus codes for another essential gene, the UL9, a 83 kDa protein that binds to the viral origin sequence and has an intrinsic DNA helicase activity. The directionality of unwinding is $3' \rightarrow 5'$ [11].

3. CELLULAR DNA HELICASES

None of these 3 viral replication systems requires a cellular DNA helicase. Accordingly during the fractionation of activities essentially for viral replication no cellular helicases could be identified. To find a counterpart for the viral helicase the hard way of protein purification had to be taken. The first candidate for an eukaryotic DNA helicase was described from meiotic cells of Lilium [12]. This enzyme unwinds DNA dependent on hydrolysis of ATP and appears to bind to nicks in the DNA strands from where it starts to unwind. The direction of unwinding is not known.

In the yeast, Saccharomyces cerevisiae 3 DNA helicases are known up to date. The best characterized one is the rad3 gene product [13], a 89 kDa polypeptide. The enzyme prefers ATP and dATP for helicase activity and can processively unwind DNA in the $5' \rightarrow 3'$ direction [14]. It needs single stranded gaps between 5-21 nucleotides to unwind double-stranded DNA. The rad3 DNA helicase is unique among all

eukaryotic DNA helicases for its acidic pH requirement, with an optimum around 5.5 and an optimal $MgCl_2$ concentration between 5 and 10 mM [13]. Its in vivo function is most likely during excision-repair. ATPase III from yeast also has DNA unwinding activity [15] and stimulates the DNA polymerase α/primase from yeast specifically (in a revised nomenclature proposal for eukaryotic DNA polymerases, the greek nomenclature has been adopted for all eukaryotic DNA polymerases including yeast, [16]). Finally, rad H is a gene of Saccharomyces cerevisiae that codes for a protein containing DNA helicase consensus sequence [17]. This protein has not been characterized at the biochemical level. Genetical evidence suggests that this DNA helicase is involved in error-prone repair events.

In vertebrates, a DNA helicase has been described from Xenopus laevis ovaries [18]. Even though the enzyme was not purified to homogeneity a native molecular weight of 140 000 was proposed with two proteins of 75 and 65 kDa copurifying with DNA helicase activity. The Xenopus laevis DNA helicase prefers ATP and dATP for hydrolysis. Neither the direction of movement nor its biological function are known. Dependency on monovalent cations, cofactor requirements and elution from single-stranded DNA-cellulose affinity columns suggested that this Xenopus laevis enzyme is different from the other eukaryotic DNA helicases.

The progress to isolate DNA helicases from mammalian cells had been hampered mainly by the following two facts: (i) purification of DNA dependent ATPases often led to pure enzymes that lacked DNA helicase activity (P. Thömmes and U. Hübscher, unpublished data) and (ii) contamination with DNase did not allow the determination of DNA helicase in early purification stages. The first mammalian DNA helicase was found in calf thymus [19] and had a tendency to copurify with DNA polymerase α/primase. Successful purification of a calf thymus DNA helicase to apparent homogeneity was recently possible since the combination of an ATPase and a DNA helicase assay allowed the fate of ATPase activities that separated from the DNA helicase to be followed [20]. Separation from the DNA polymerase α/primase complex as well as from the bulk of another DNA-dependent ATPase could be achieved on a heparin-Sepharose column [20]. The enzyme is a monomer of 47 000 Da and is able to use ATP, dATP, CTP and dCTP for helicase action. The calf thymus DNA helicase appears to bind processively to single-stranded DNA, to move in the $3' \rightarrow 5'$ direction to a single strand/double stranded transition and to unwind DNA fragments of at least 36 nucleotides [20]. It has a comparatively low K_m for the hydrolysis of ATP, which is around 200 μM, while e.g. the K_m for T antigen helicase for ATP is 3 to 5 times higher. No assignment of in vivo function can be made for this calf thymus DNA helicase so far.

Table I

Eukaryotic DNA helicases

Source	Molecular weight (kDa)	Direction of unwinding[1]	Preferred (d)NTP	Special features	Function	Authors
Viral origin						
SV40 T antigen	92	$3' \rightarrow 5'$	A>dA>dT=U	interacts with polα	ori unwinding and	5
Polyoma T antigen	100	$3' \rightarrow 5'$	A=dA>C=U	–	fork movement	6
Herpes simplex 1	120,97,70	$5' \rightarrow 3'$	A>G>C=U	helicase (UL5/UL52) - primase (UL8)	replication	7
	83	$3' \rightarrow 5'$	?	UL9	replication	11
Cellular origin						
Yeast						
Rad 3	89	$5' \rightarrow 3'$	A>dA	–	excision-repair	14
ATPase III	63	?	A	stimulates yeast polα[4]	?	15
Rad H	134[2]	?	?	only sequence known	error-prone-repair	17
Lilly	?	?	A	–	?	12
Xenopus laevis	140(75,62)[3]	?	A=dA	–	?	18
Mouse	58[3]	$5' \rightarrow 3'$	A>dA=dG=G	–	replication ?	23
Calf thymus						
copurifies with α	47	$3' \rightarrow 5'$	A>dA>C=dC	low km for ATP hydrolysis (<200 μM)	replication ?	20
copurifies with δ[4]	58[5]	$5' \rightarrow 3'$	A>dA>C=U	enables polδ to perform strand-displacement	replication ?	21
copurifies with ϵ[4]	90-100[5]	$3' \rightarrow 5'$	A=dA	dependent on ssb		22

[1] Movement on the single-stranded DNA, where the enzyme binds.
[2] Deduced from the DNA sequence.
[3] Not homogenous.
[4] For a revised nomenclature of eukaryotic DNA polymerases see [16].
[5] Calculated from S-value.

Another DNA helicase from calf thymus has been separated from DNA polymerase δ, an enzyme possibly involved in replication of the leading strand at the replication fork [21]. This helicase functions as a strand displacement factor and specifically affects the activity of DNA polymerase δ but not of DNA polymerase α on a poly(dA)-tailed pBR322 template [21]. It prefers ATP and dATP as nucleoside-5'-triphosphates but can also use CTP and UTP and has a $5' \rightarrow 3'$ directionality of unwinding.

A third DNA helicase from calf thymus was found to copurify with DNA polymerase ϵ, the DNA polymerase discovered most recently. In contrast to the two DNA helicases mentioned, this enzyme requires the presence of a single-stranded DNA binding protein (ssb) for its action. The directionality of unwinding is $3' \rightarrow 5'$ [22].

Studies with a DNA helicase characterized from mouse FM3A cells [23] indicated that this enzyme initially copurified with DNA polymerase α/primase. The enzyme has not been purified to homogeneity although the authors claim that a 58 kDa polypeptide copurifies with the enzyme. The mouse DNA helicase has a broad spectrum for nucleoside 5'-triphosphates since it can use ATP, dATP, dGTP, GTP, UTP and to some extent dCTP for DNA helicase action. The enzyme binds to single-stranded DNA and unwinds the DNA in the $5' \rightarrow 3'$ direction.

4. CONCLUSION AND PERSPECTIVE

Various DNA helicases from viral and cellular origins have been discovered in eukaryotes during the last few years. We predict that the multiplicity of 7 DNA helicases found in *Escherichia coli* might soon be reached in eukaryotes. Molecular genetic techniques (e.g. PCR), as well as the use of the yeast system which allows the isolation of conditional lethal mutants, will contribute to our knowledge. These approaches will eventually lead to a better understanding of the functional roles of eukaryotic DNA helicases in the unwinding of DNA.

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