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DNA Unwinding Mechanism by T7 Bacteriophage Helicase-Primase

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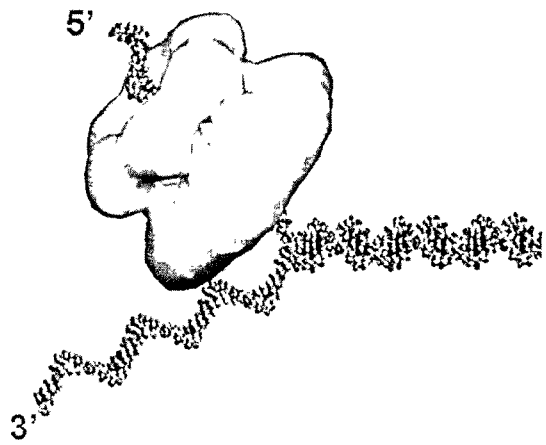
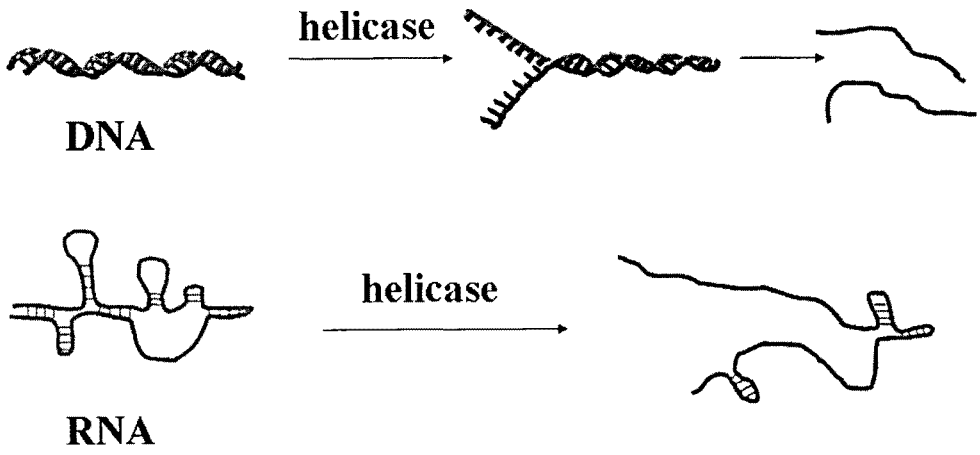
Helicases are motor proteins that use the chemical energy of NTP hydrolysis to drive mechanical processes such as translocation and nucleic acid strand separation. Bacteriophage T7 helicase functions as a hexameric ring to drive the replication complex by separating the DNA strands during genome replication. Measurements of the single-turnover kinetics of DNA strand separation and comparison to the translocation properties of T7 helicase on ssDNA provide insights into the mechanism of strand separation that might be general to ring helicases. T7 helicase unwinds DNA with a kinetic step-size of 9 bp that decreases to 6 bp at a lower dTTP. T7 helicase has a low processivity of unwinding even though DNA is bound in the central channel of the ring; this is because the helicase dissociates from the DNA upon ring opening. The global fitting of the unwinding kinetics to a modified stepping model show that T7 helicase unwinds DNA with a rate of 15 bp/s, which is 9-fold slower than the translocation speed along ssDNA. This implies that the duplex DNA poses a barrier to the movement of T7 helicase. Distinct from the unwinding mechanism of monomeric or dimeric helicases that bind and destabilize duplex DNA, T7 helicase achieves DNA unwinding by its ability to translocate unidirectionally along ssDNA and by its ability to exclude the complementary strand from the active site. Our results also imply that the free energy of dTTP hydrolysis is not utilized directly for bp separation but used for unidirectional movement along ssDNA.

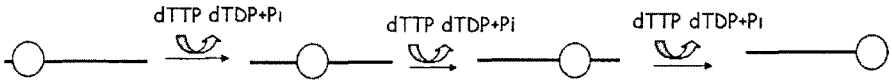
ssDNA Passes through the Central Channel of the Hexameric Ring



Nucleic Acid Strand Separation by Helicase



Translocation of T7 helicase along ssDNA

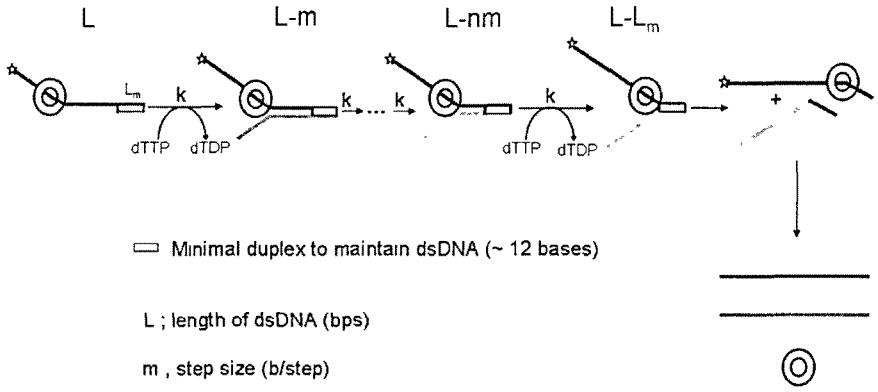


Translocation rate: 130 bases/s (18°C)

dTTPase rate: 50 s^{-1} (18°C)

Coupling Ratio: ~ 3 bases translocated /dTTP

Model of dsDNA unwinding



dsDNA unwinding vs ssDNA translocation

	rate(b/s)	processivity	dissociation ate (/s)	falling off after
dsDNA nwinding	15	0.9835	0.25	60 bases
ssDNA ranslocation	130	0.99996	0.002	66 Kbases