

Unwinding of duplex nucleic acids by cooperative translocation of SARS coronavirus helicase nsP13 is modulated with ATP concentration

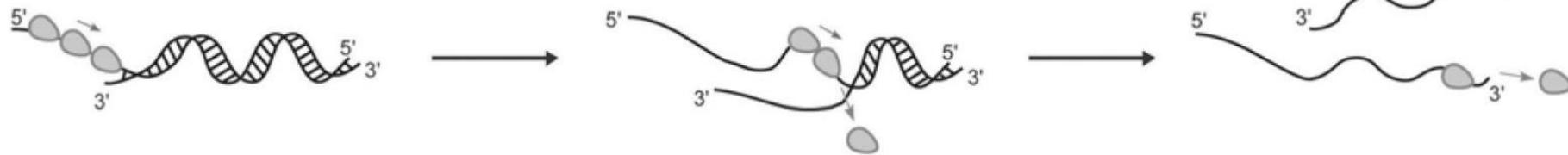


Dong-Eun Kim*

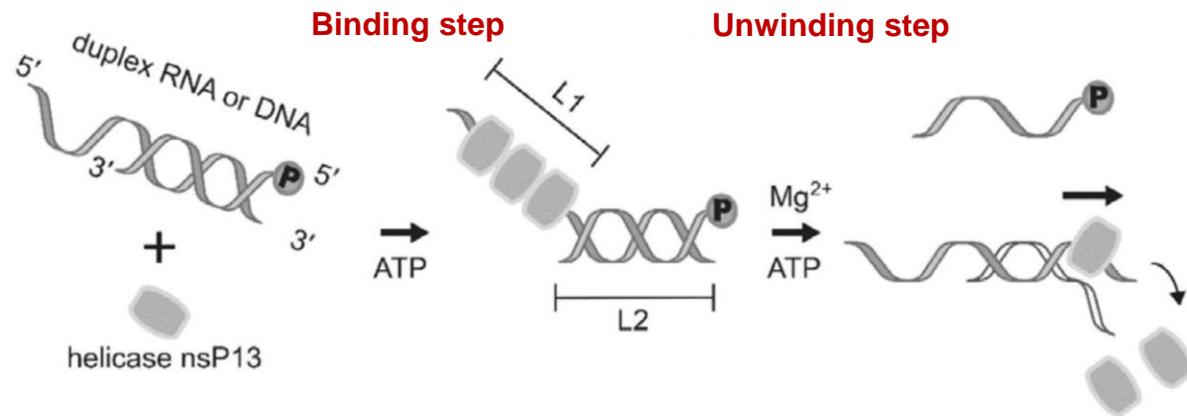
Department of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Republic of Korea

* Email address: kimde@konkuk.ac.kr

● Cooperative translocation of SARS coronavirus helicase nsP13



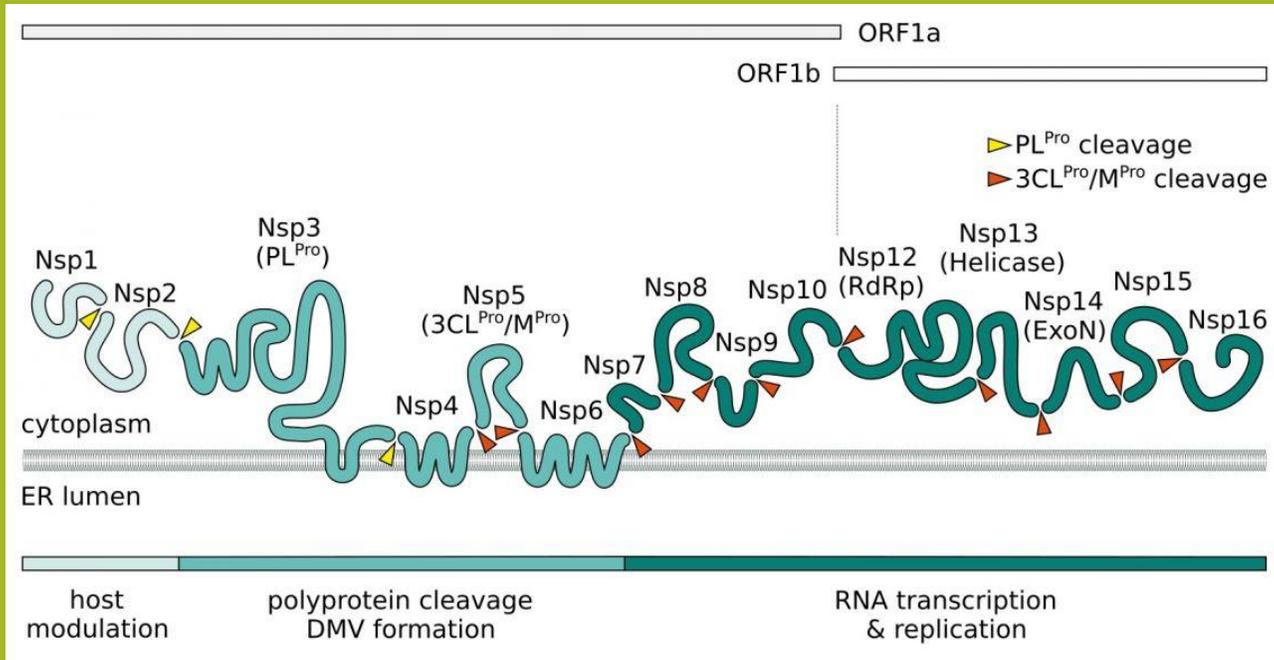
● High [ATP] enhances the cooperative translocation of nsP13 in duplex RNA unwinding



dsRNA unwinding	$L1 \propto \frac{1}{\text{Unwinding}}$	Unwound ss nucleotide	$\propto \frac{1}{L1}$	[ATP]	\propto Binding stability
	$L2 \propto \frac{1}{\text{Unwinding}}$		$\frac{1}{L2}$		Cooperative translocation

dsDNA unwinding	$L1 \propto \text{Unwinding}$	Unwound ss nucleotide	$\propto L1$	[ATP]	$\propto \frac{1}{\text{Unwinding}}$
	$L2 \propto \frac{1}{\text{Unwinding}}$		$\frac{1}{L2}$		$\frac{1}{\text{Cooperative translocation}}$

A. Generation of non-structural proteins, NSPs in SARS coronavirus



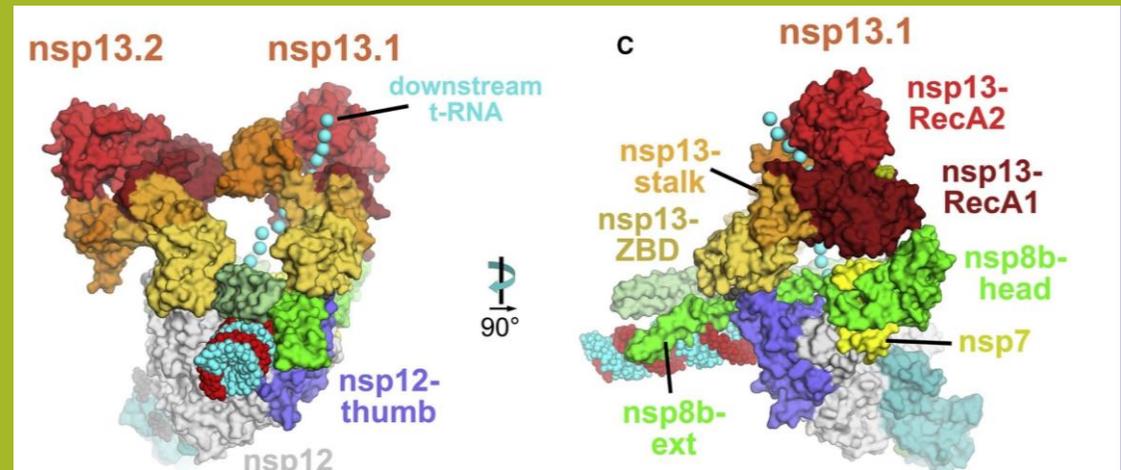
Two large polycistronic open reading frames ORF1a and ORF1b at the 5' end of the genome encode 16 non-structural proteins (NSPs) forming two replicase polyproteins pp1a and pp1b.

B. Structure of SARS-CoV-2 replication-transcription complex (RTC) with nsp13 helicases



Structure of stable nsp13:holo-RdRp:RNA complex was determined by cryo-EM to 3.5-Å resolution.

Chen et al., 2020, Cell 182, 1560–1573

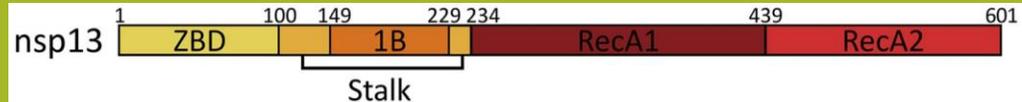


1. SARS coronavirus helicase nsP13



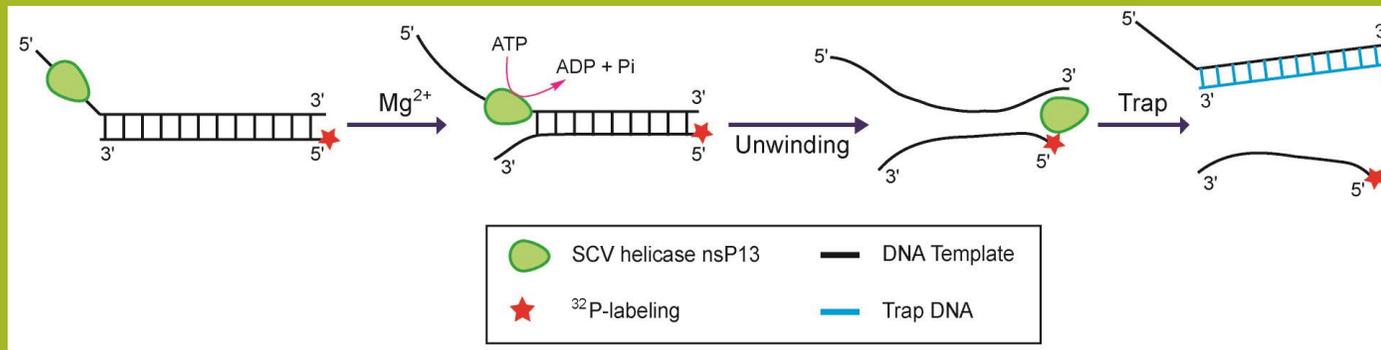
SARS-CoV hexahistidine (His₆)-nsp13

- ✓ nsp13, a superfamily 1B (SF1B) helicase, can unwind DNA or RNA in an NTP-dependent manner with a 5' → 3' polarity

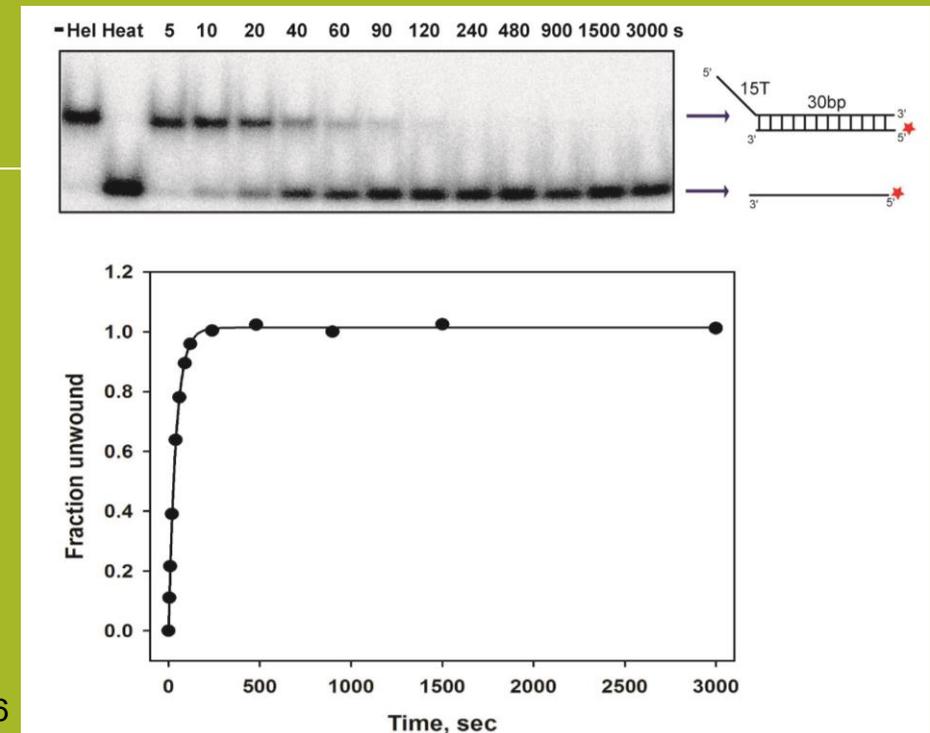


- ✓ nsp13 contains three domains unique to nidovirus helicases: an N-terminal zinc-binding domain (ZBD), a stalk, and a 1B domain in addition to two canonical RecA ATPase domains of SF1.

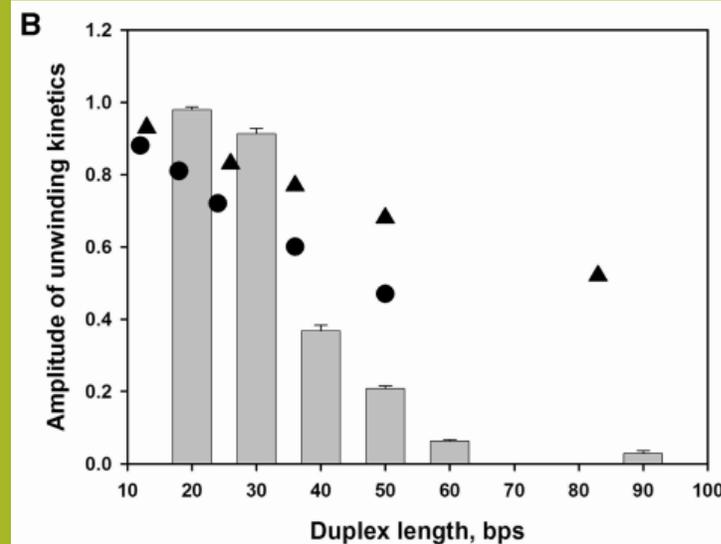
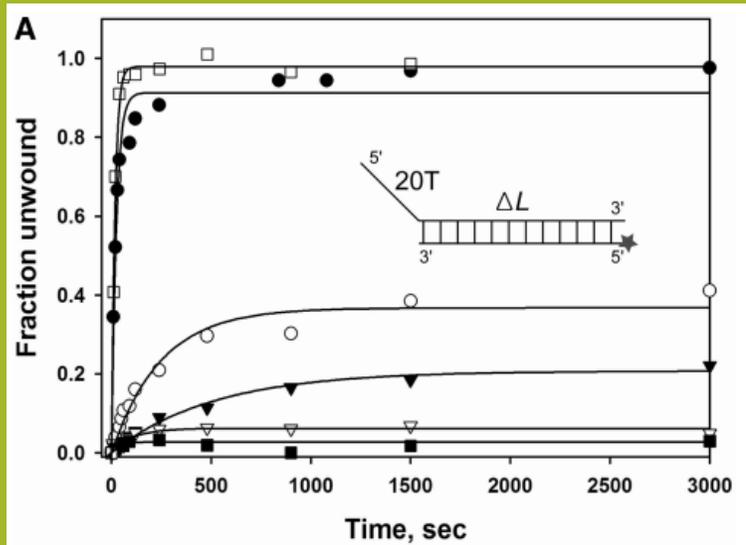
2. Single-turnover kinetics of duplex DNA unwinding by nsP13



Lee et al., 2010, Nucleic Acids Research, 38, 7626



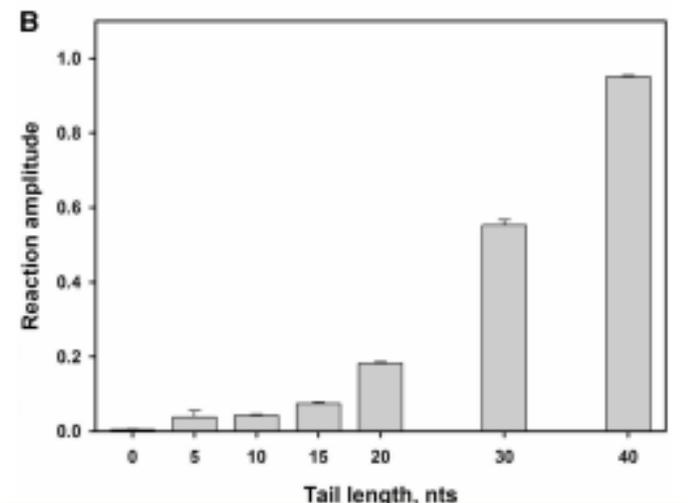
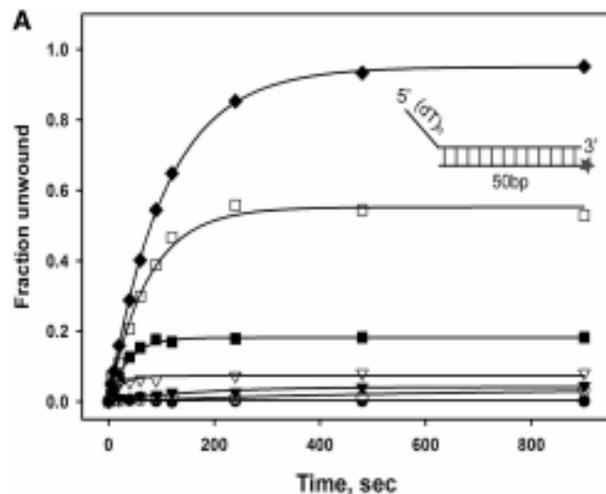
3. Processivity of duplex DNA unwinding is very low



(A) Single-turnover unwinding of duplex DNA substrates with different lengths by nsP13. dsDNA substrates contained 20 T tail and 20 bp (open square), 30 bp (filled circle), 40 bp (open circle), 50 bp (filled inverted triangle), 60 bp (open inverted triangle), 90 bp (filled square).

(B) Amplitudes of nP13 are shown in the bar graph against duplex length and amplitudes of NPH (filled triangle) and NS3 (filled circle) are marked on the same graph for comparison.

4. Enhancement of duplex DNA unwinding by increasing the length of 5'-overhang

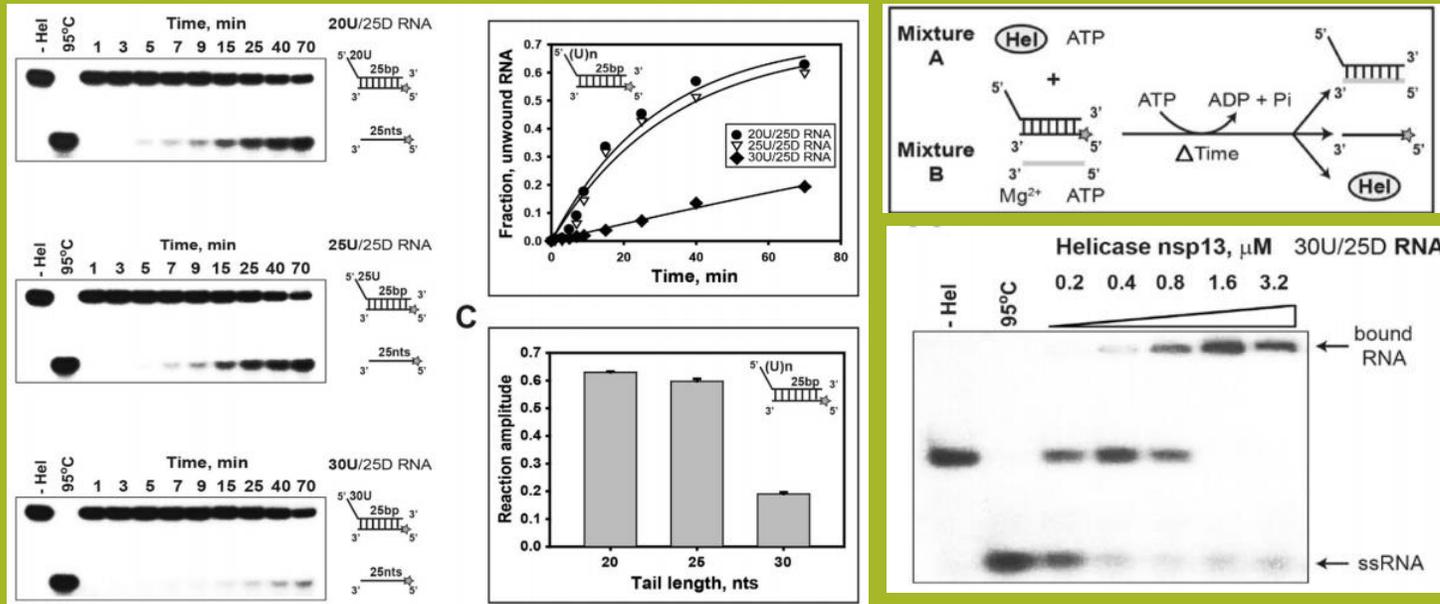


Unwinding of DNA substrates with varying length of 5'-overhang by nsP13.

(A) Single-turnover unwinding of 50 bp duplex DNA substrates with 5'-overhangs of varying lengths by 0 T (filled circle), 5 T (open circle), 10 T (filled inverted triangle), 15 T (open inverted triangle), 20 T (filled square), 30 T (open square) and 40 T (filled diamond).

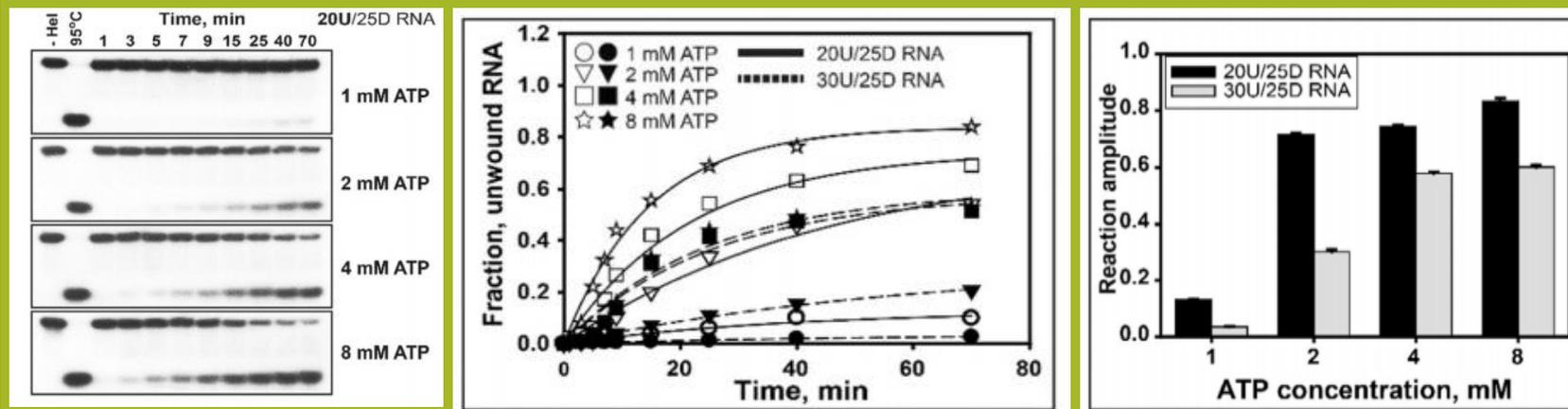
(B) The bar graph shows the reaction amplitudes of nsP13-catalyzed dsDNA unwinding as the length of the 5'-overhang increased from 0 to 40 nt.

5. Duplex RNA unwinding decreased as the length of the 5'-ss tail increased

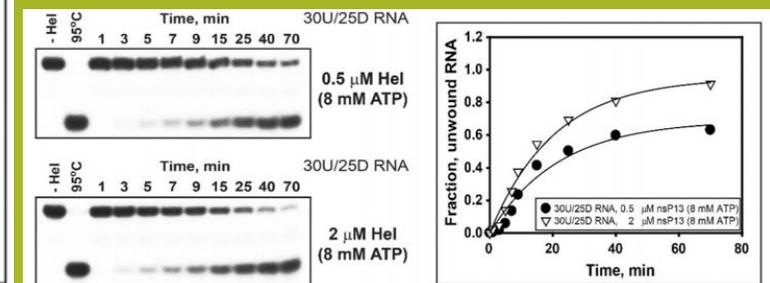


- In the experiment that blocked the preloading of nsP13 onto the 5'-ss loading strand, 30U/25D RNA was unwound almost to incompleteness (~5%), implying that preloading of nsP13 is strongly needed to unwind duplex RNA. Unlike the case of duplex RNA, 30T/25D DNA was unwound almost to completion regardless of preloading onto the 5'-ss tail by nsP13.
- The quantity of ssRNA unwound from dsRNA was decreased as the length of the 5'-ss tail increased. The amplitudes were decreased as the length of the 5'-ss tail increased on duplex RNA unwinding.
- Strong binding affinity of nsP13 onto RNA can be explained as a decrease in unwinding and translocation with an increasing 5'-ss tail.

6. Enhanced cooperative translocation in duplex RNA unwinding by nsP13 with increasing [ATP]



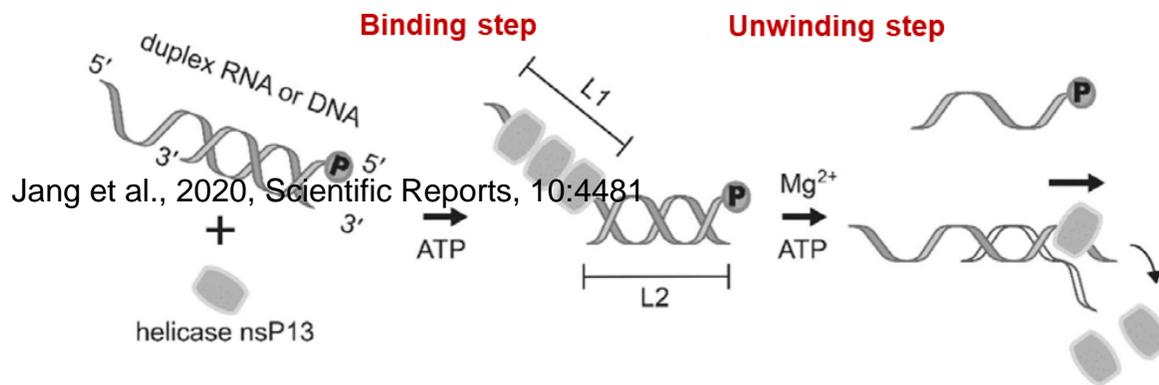
Additional ATP requirement to promote ATP-dependent translocation of helicase nsP13.



7. Proposed models of duplex RNA and DNA unwinding by nsP13

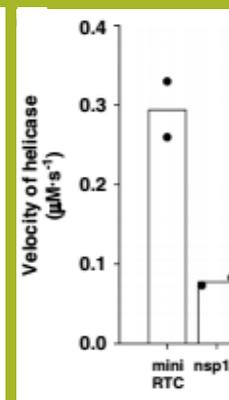
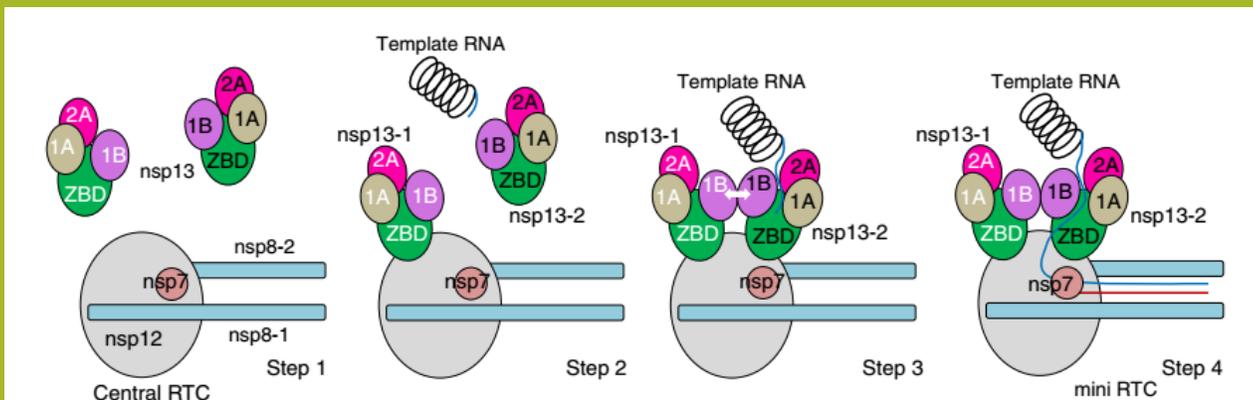
- nsP13 exhibits higher binding affinity and lower unwinding activity for RNA substrates than DNA substrates in the same ATP concentration, which may be explained by different ATP demand of nsP13 depending on the substrate.
- In addition, these results may imply that the open state of nsP13 binds with a higher affinity to RNA than to DNA and hence requires higher concentrations of ATP to drive it into the closed state.
- The unwinding of duplex RNA by nsP13 is a considerably energy-consuming reaction, which also implies the requirement of higher ATP concentration for its open and closed states or stable binding to ssRNA in translocation process.

- High [ATP] enhances the cooperative translocation of nsP13 in duplex RNA unwinding



dsRNA unwinding	L1	$\propto \frac{1}{\text{Unwinding}}$	Unwound ss nucleotide	$\propto \frac{1}{L1}$	[ATP]	\propto	Binding stability
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							Cooperative translocation

dsDNA unwinding	L1	\propto Unwinding	Unwound ss nucleotide	$\propto \frac{1}{L1}$	[ATP]	\propto	$\frac{1}{\text{Binding stability}}$
	L2	$\propto \frac{1}{\text{Unwinding}}$		$\frac{1}{L2}$		Unwinding	
							Cooperative translocation



- It is suggested that nsP13 helicase couples with nsP12 polymerase in the formation of SARS-CoV-2 RTC.
- Dimeric nsP13 helicases that are coordinated with nsP12 RdRp in RTC displayed enhanced dsRNA unwinding efficiency.
- Our result supports roles of nsP13 helicase in the RTC architecture complexed with nsP12 RdRp.

Yan et al., 2020, Nature Communications 11; 5874