Translocation Behavior of Hepatitis C NS3 Helicase

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Hepatitis C Virus (HCV)

- RNA virus (positive sense)
- RNA viruses are very mutable
- Mutable viruses are difficult to fight
- Forms capsules surrounded by host's own cell membrane
- Two major functions: making proteins, replication

NS3

- Boring name - "nonstructural protein #3"
- Protease: cleaves proteins into smaller pieces
- ATPase: hydrolyzes adenosine triphosphate (ATP)
- Helicase: translocates along the backbone of a nucleic acid, separating it from a complementary strand

Typical RNA Structure

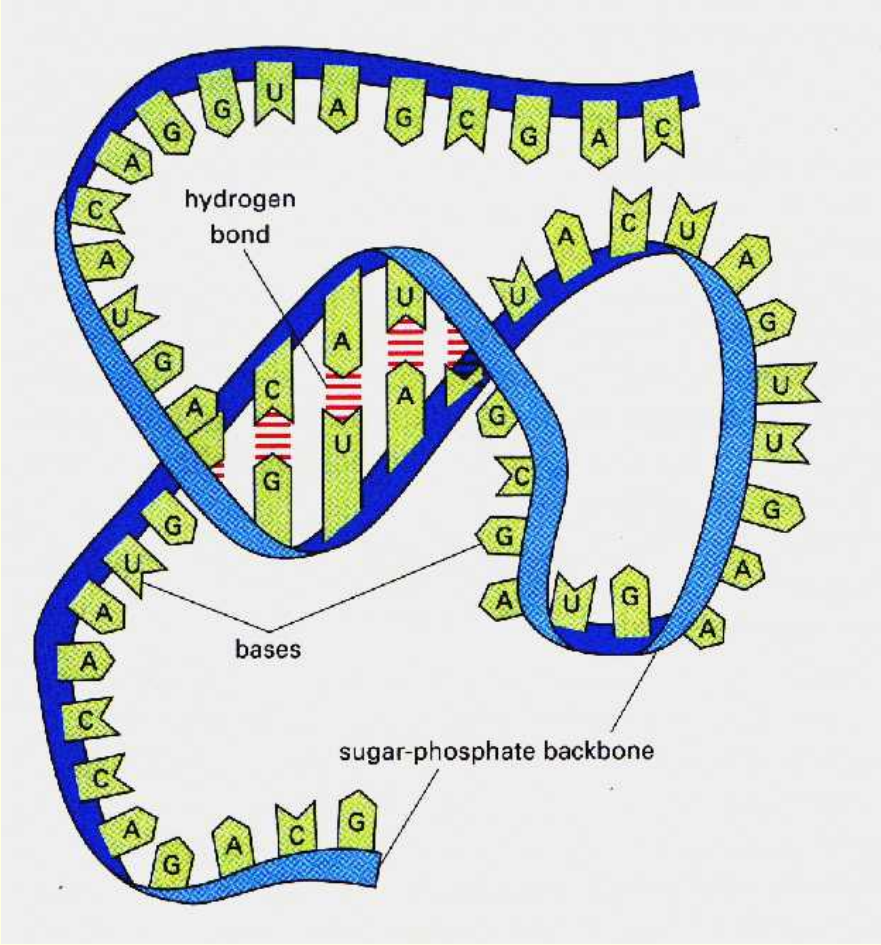


Image from www.uic.edu/classes/phys/phys461/phys450/ANJUM04/

NS3h

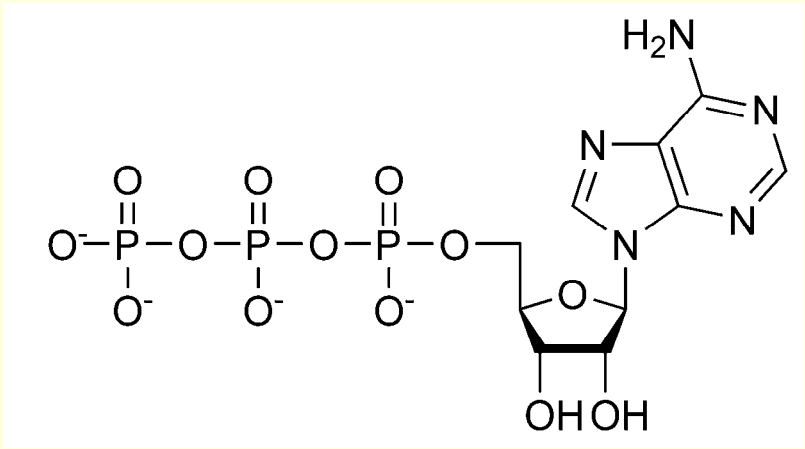
- Recombinant form of NS3
- Protease domain is eliminated, leaving helicase, ATPase domains
- Question: how is ATP binding and hydrolysis linked to NS3h translocation?
- Possibilities: 1) ATP binding and hydrolysis are necessary for translocation, 2) ATP binding is necessary but hydrolysis is not

The Plan

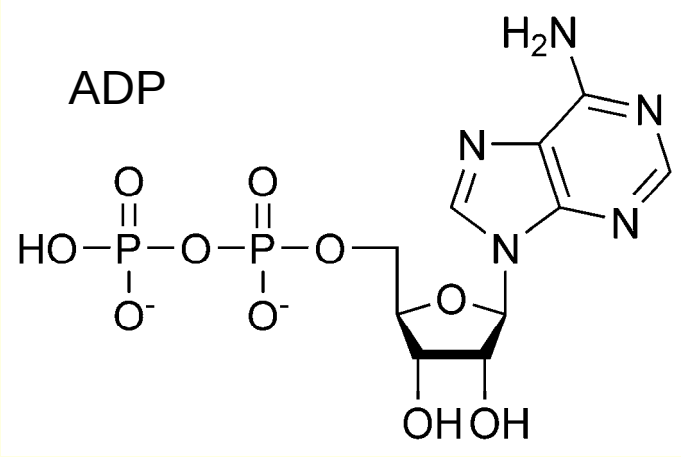
- Positive control: observe the translocation behavior of NS3h in the presence of ATP
- Negative Control: observe the translocation behavior with no ATP
- Observe the translocation behavior using two molecules similar to ATP, but more difficult to hydrolyze: adenosine diphosphate (ADP) and adenylyl-imidodiphosphate (AMP-PNP)

ATP, ADP, and AMP-PNP

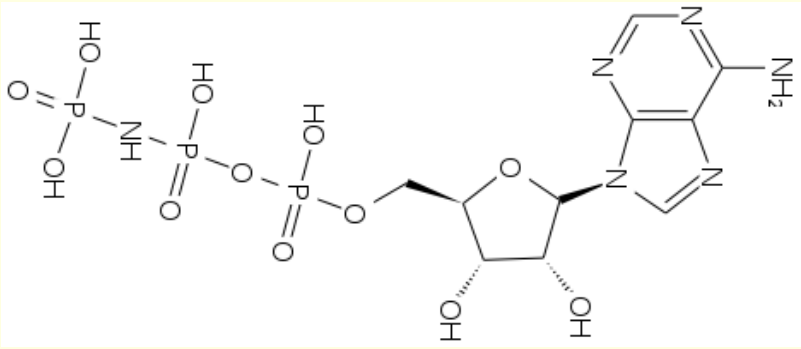
ATP



ADP



AMP-PNP



Images from https://eapbiofield.wikispaces.com/Chapter+Eight+reading+EBW?f=print, http://commons.wikimedia.org/wiki/File:ADP_chemical_structure.png, and http://www.h6.dion.ne.jp/~k-sugino/example/toki/2007/04051007_2.html

The Experiment, Part I: NS3h/DNA Preparation

- ssDNA labeled with fluorescein fluorophore at the phosphate end of the backbone (5’ end)
- ssDNA and NS3h mixed, allowed to bind before translocation experiment begins
- DNA is in excess (goal: no more than one NS3h molecule/DNA)

DNA

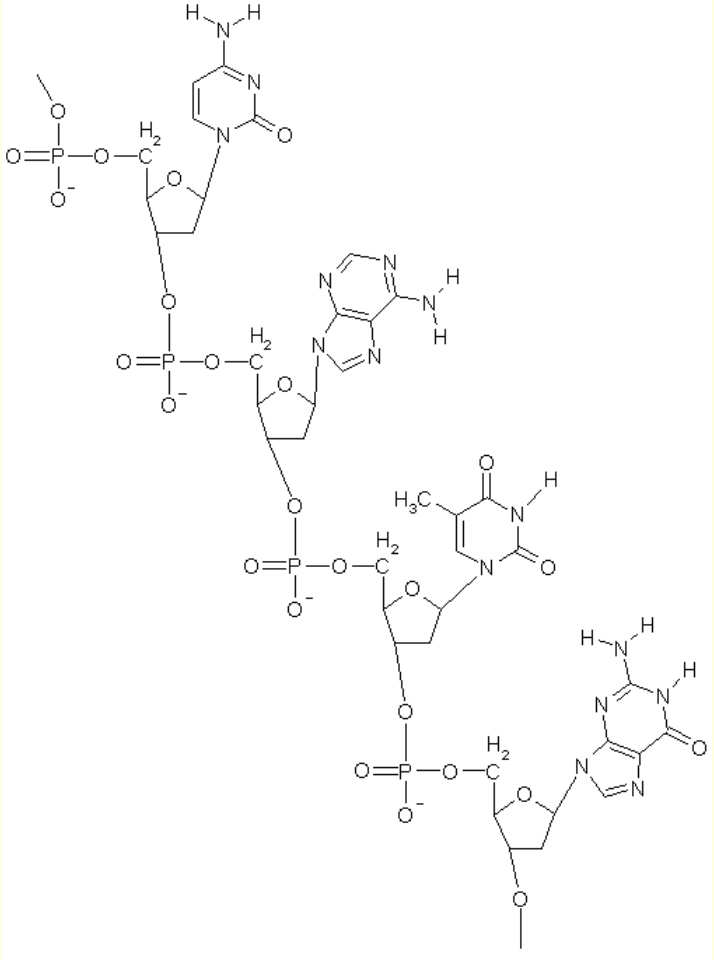


Image from www.steve.gb.com/science/molecules.html

The Experiment, Part II: ATP/Heparin Preparation

- ATP/ATP analog: fuels translocation (either successfully or unsuccessfully)
- Mg^{2+}: cofactor in ATP hydrolysis
- Heparin: binds protein as it dissociates from the DNA, only allows protein to attach to DNA once

Heparin

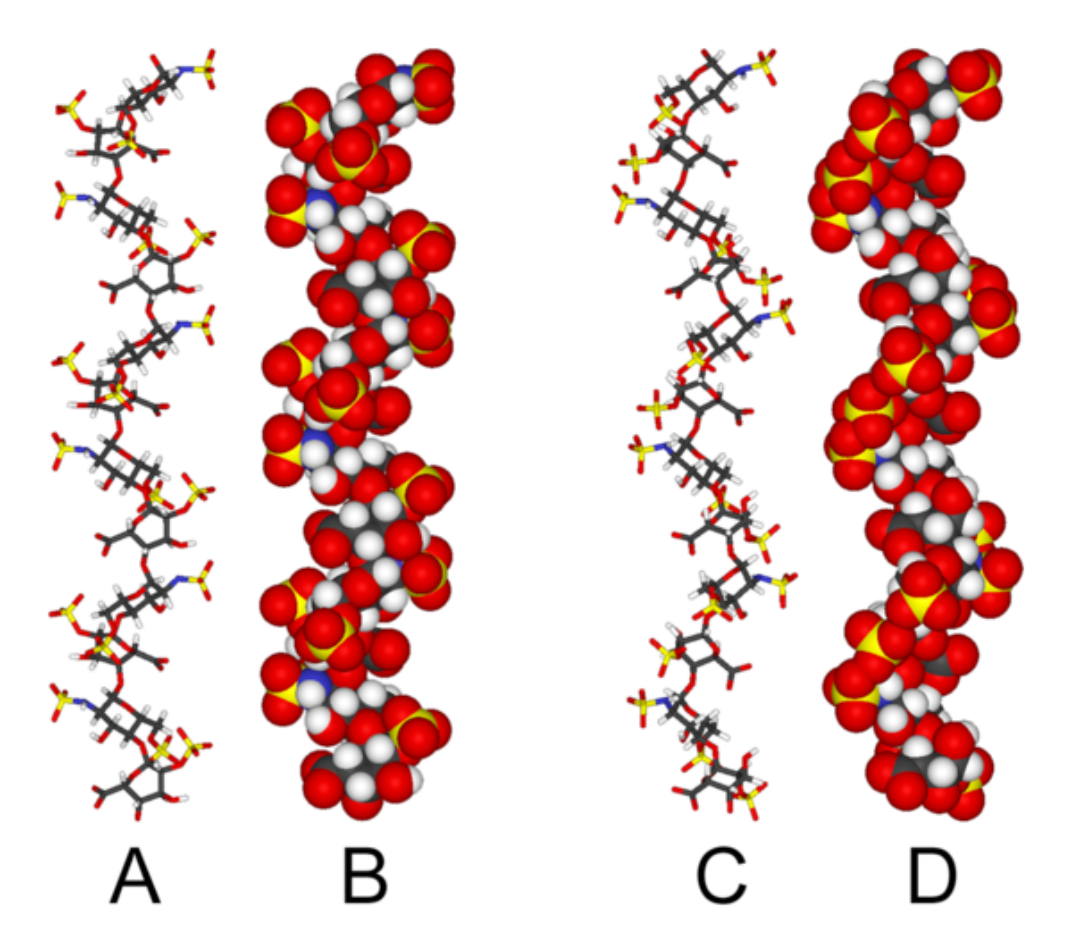


Image from http://www.absoluteastronomy.com/topics/Heparin

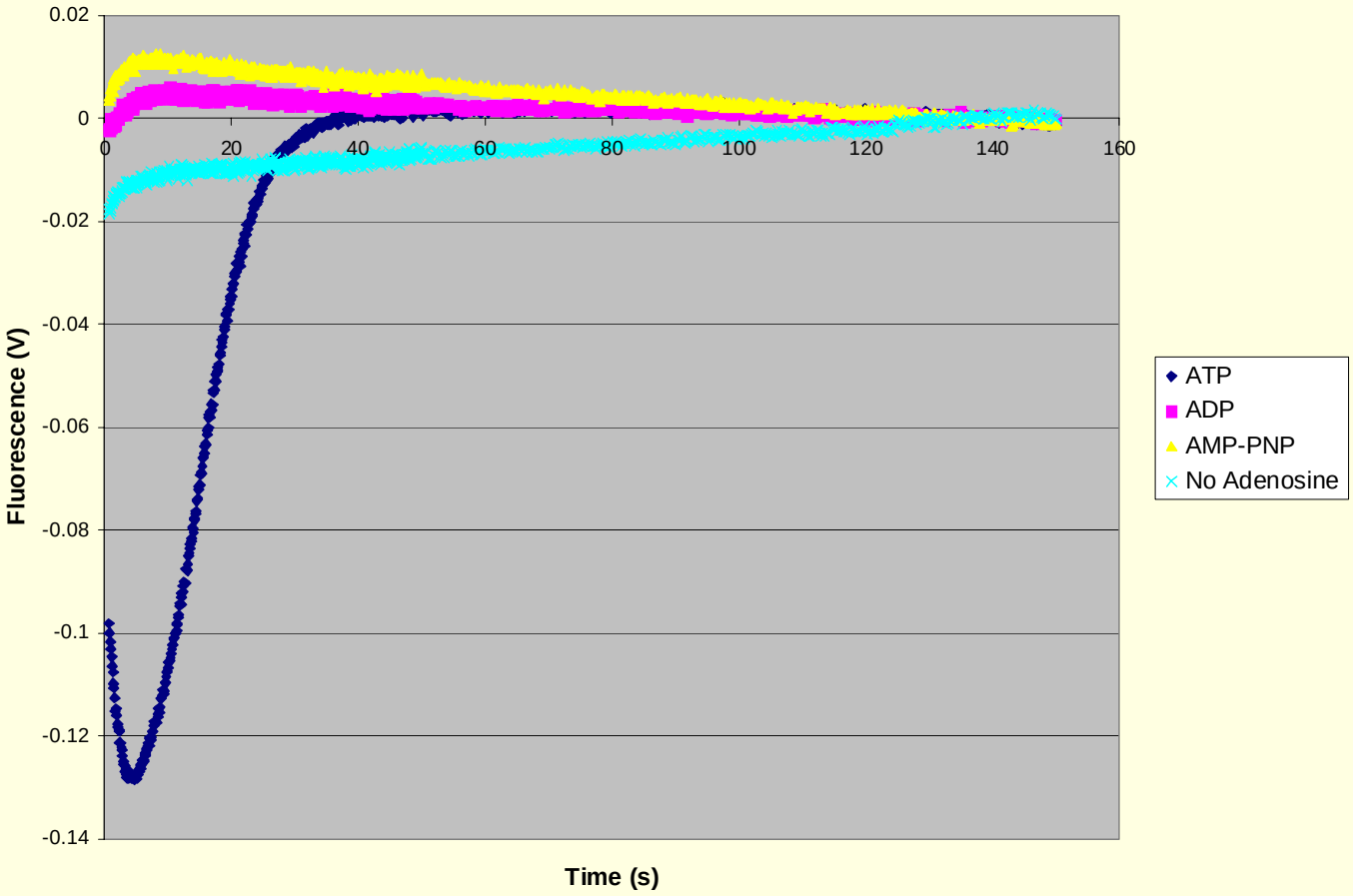
The Experiment, Part III: Putting It Together

- NS3h/DNA solution must be mixed with the ATP (or analog)/Mg^{2+}/Heparin solution for translocation to start
- Stopped-flow: solutions are rapidly mixed by introducing them into a common chamber under pressure; when pressure is transferred to a sensor plate, reaction monitoring begins

The Experiment, Part IV: Monitoring Fluorescence

- Fluorophore is excited with 492 nm light
- Photons from fluorescence produce electrical signal in a photoelectric sensor, magnified by photomultiplier tube
- Proximity of NS3h to the fluorophore changes the electromagnetic environment, changes fluorescence

Results


0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
0
20
40
60
80
100
120
140
160
Fluorescence (V)
Time (s)
ATP
ADP
AMP-PNP
No Adenosine

Conclusion

- ATP hydrolysis is necessary for the translocation of NS3h; adenosine binding alone is insufficient
- ATP hydrolysis is probably necessary for HCV replication