Visualizing cellular entry of single viruses & folding of single enzymes
Single Molecule Experiments ⇒ Complex Dynamics

Non-accumulative intermediates
Single Molecule Experiments ⇒ Complex Dynamics

Multiple Pathways

Spontaneous transition
Outline

- Cellular entry of influenza viruses
- Folding and function of RNA enzymes
Influenza Virus

- Lipid bilayer
- Hemagglutinin
- Matrix protein M1
- Viral RNP
Influenza infection

Single particle experiments

- Dissect individual infection steps
Single Virus Investigation

Single Virus Investigation

Viruses are transported in three stages.

Passive transport in cells

Diffusion

\[ \langle \Delta r^2 \rangle = D \Delta t \]

Active transport in cells

\[ \langle \Delta r^2 \rangle = a \Delta t + b \Delta t^2 \]

Active transport in cells

\[ \langle \Delta r^2 \rangle = a \Delta t + b \Delta t^2 \]

Stage I

Stage I

Virus transport is actin-dependent.

Stage II

Viruses are transported by dyneins on microtubules.

Viruses are transported by both plus- and minus-end-directed motors on microtubules.

How are viruses transported?

How are viruses acidified?

Endocytic Acidification

Initial acidification occurs after stage II movement.

Both acidification processes occur after stage II movement!

Endocytic Traffic of Influenza Viruses

Acidification to pH 6

Early endosome or endocytic vesicle

Acidification to pH 5

Late endosome

Virus

Actin

Microtubule

Nucleus

Endocytosis of influenza viruses

Single particle experiments

Endocytosis mechanism?
Endocytosis of influenza viruses

- Phagocytosis (particle-dependent)
- Macropinocytosis (>1 µm)
- Pinocytosis
  - Clathrin-mediated endocytosis (~120 nm)
  - Caveolin-mediated endocytosis (~60 nm)
  - Clathrin- and caveolin-independent endocytosis (~90 nm)
Endocytosis of influenza viruses

Viruses stimulate CCP formation.

Viruses move to existing CCPs.

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Endocytosis Pathways

Pinocytosis

- Clathrin-mediated endocytosis (~120 nm)
- Caveolin-mediated endocytosis (~60 nm)
- Clathrin- and caveolin-independent endocytosis (~90 nm)

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CCPs and Viruses

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CCP-dependent Endocytosis

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CCP-dependent Endocytosis

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CCP dynamics

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Virus binding induces the formation of CCP.
Virus can be endocytosed via a clathrin-independent pathways.
Endocytosis of Influenza

Stimulate CCP formation

61% Via pre-existing CCP

35% Via clathrin- and caveolin-independent pathway

4% Via clathrin- and caveolin-independent pathway

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Influenza infection

Single particle experiments

- Viral Endocytosis mechanisms.
- Trafficking on the endocytic pathway.
- Nuclear import of viral RNPs.
Outline

- Cellular entry of influenza viruses
- Folding and function of RNA enzymes
Why are RNA enzymatic reactions slow?

How do protein cofactors help RNA enzymes?
Hairpin Ribozyme
Proposed Catalysis Scheme
Rate-limiting Mechanism

Conformation transitions are rate-limiting.

Fluorescence Resonance Energy Transfer (FRET)
Hairpin Ribozyme --- FRET System

Complex Structural Dynamics

Complex Structural Dynamics

Very complex conformational dynamics!

Why RNA Enzymes?

What are the capability and limitation of RNA enzymes?

How do protein cofactors help RNA enzymes?
An RNA-protein system

- 7mM Mg$^{2+}$: No activity
- 40mM Mg$^{2+}$: moderate activity
- 40mM Mg$^{2+}$ + CBP2: full activity

BI5 group I intron

Bokinsky, Nivon, Zhuang
An RNA-protein system

- 7mM Mg$^{2+}$
- 7mM Mg$^{2+}$ + CBP2
- 40mM Mg$^{2+}$ + CBP2
- 40mM Mg$^{2+}$
An RNA-protein system

7mM Mg^{2+} + CBP2
Conclusions

- RNA has complex and rugged energy landscape.

- Structural dynamics can be a significant rate limiting mechanism for the RNA’s enzymatic reaction.

- The protein cofactors significantly alter the structural dynamics of RNA.
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