Stalking a Killer: Respiratory Syncytial Virus

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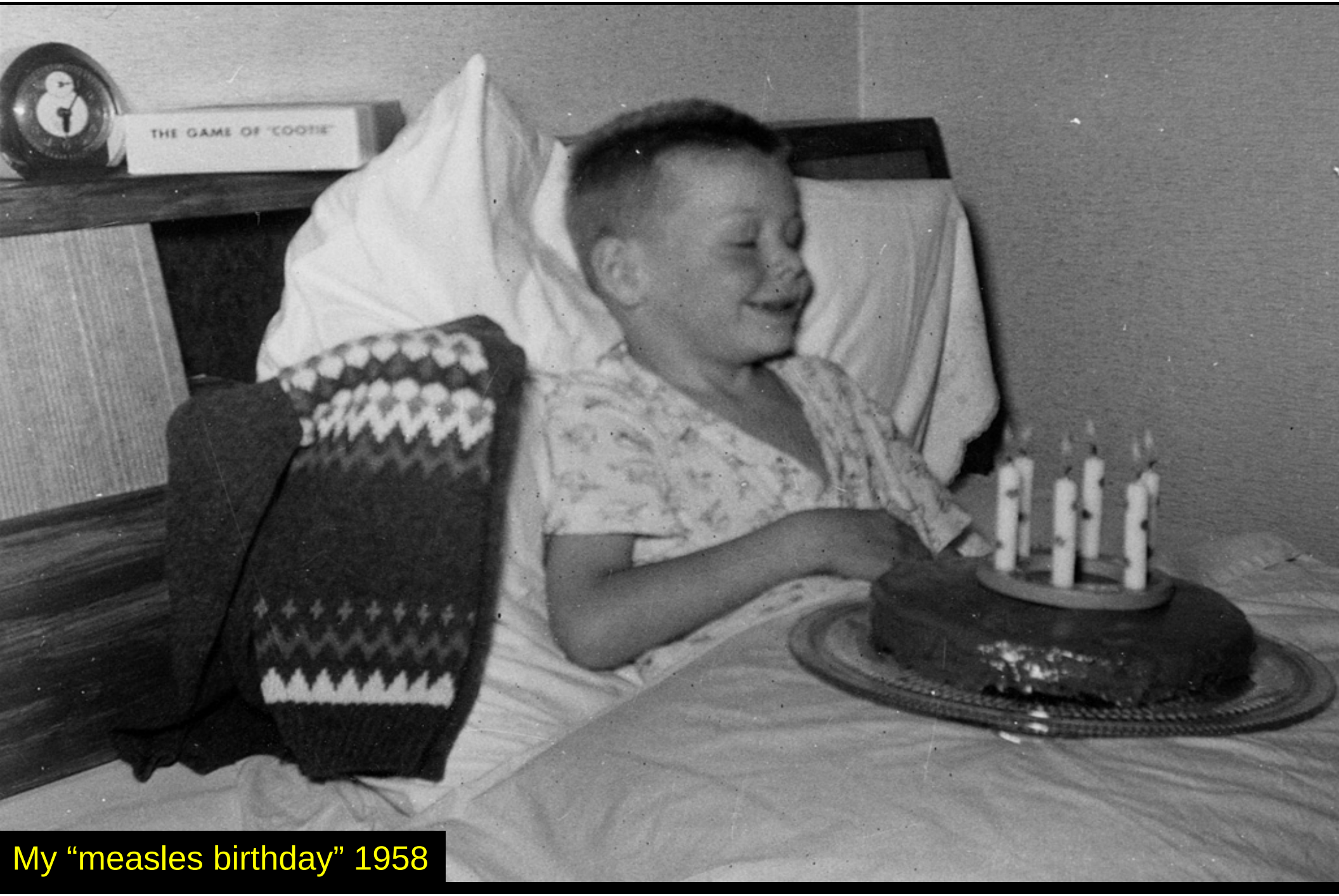
Goals for Today

- Describe the ‘life’ of a virus
- Discuss RSV and the disease it causes
- Explore
  - Attempts at treatments and prophylaxis
  - Why we don’t have a vaccine to protect us
  - What we are doing to
    - Understand RSV better
    - Solve the RSV problem
- What the heck does ‘syncytial’ mean?

What is a virus?

- Very small
- Has no life of its own
  - A freeloader, a parasite
- Gets inside one of your cells and takes over
- Produces 100's of identical viruses
  - These viruses infect more of your cells, and so on
    - Until your immune system stops it
  - Or they are spewed onto your friends (or whomever)



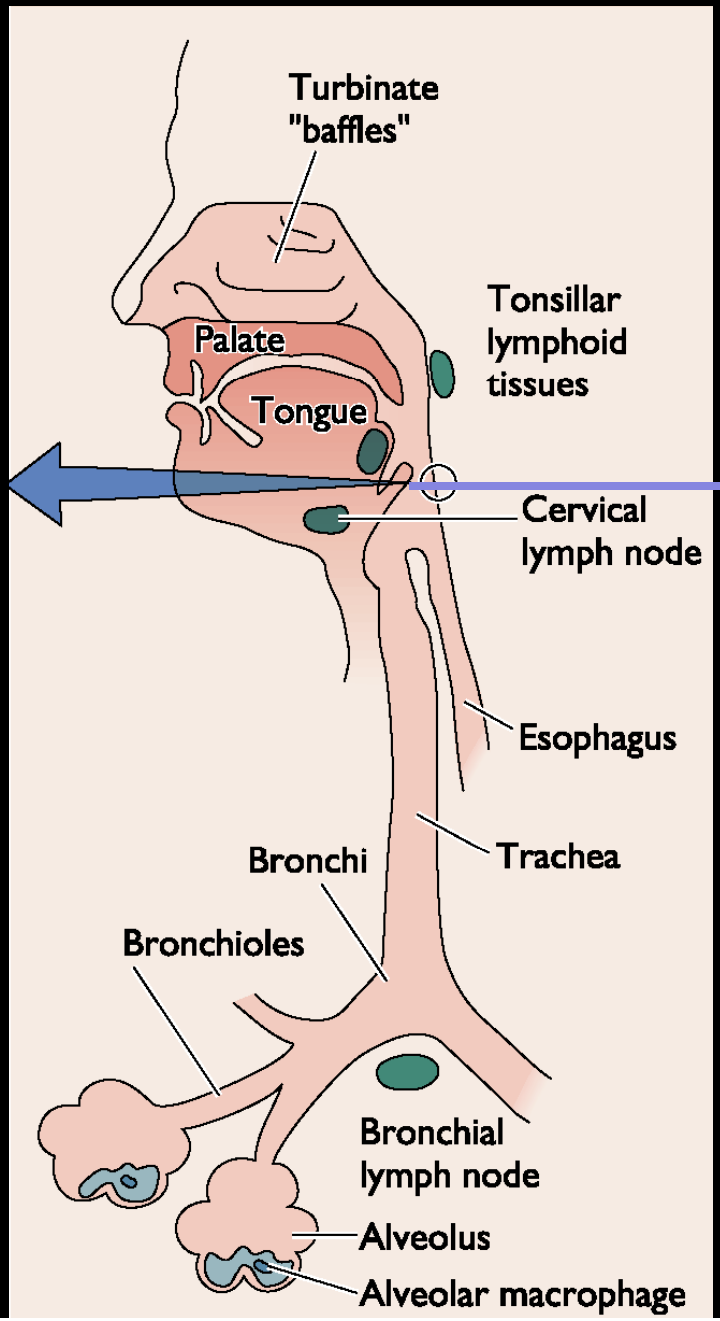
My “measles birthday” 1958

How is RSV spread?

RSV infects the upper respiratory tract 1st

- If the immune system stops infection: common cold (most of us)

- If it moves to the lower respiratory tract, RSV can cause disease
  - Infants (no immunity)
  - Elderly (losing immunity)

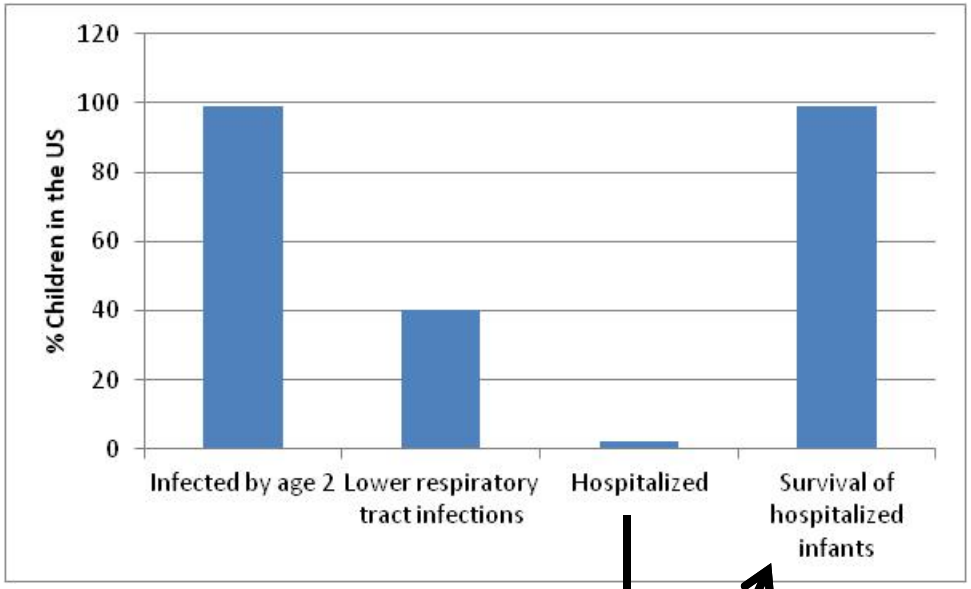


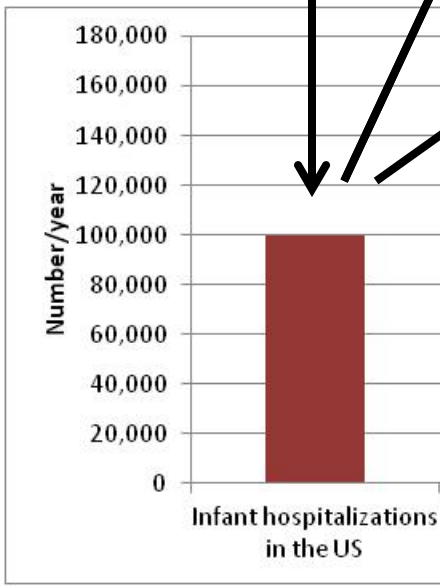
Respiratory Tr

Upper

Lower

RSV as a Public Health Problem





RSV is second only to influenza virus as cause of "excess deaths" of the elderly

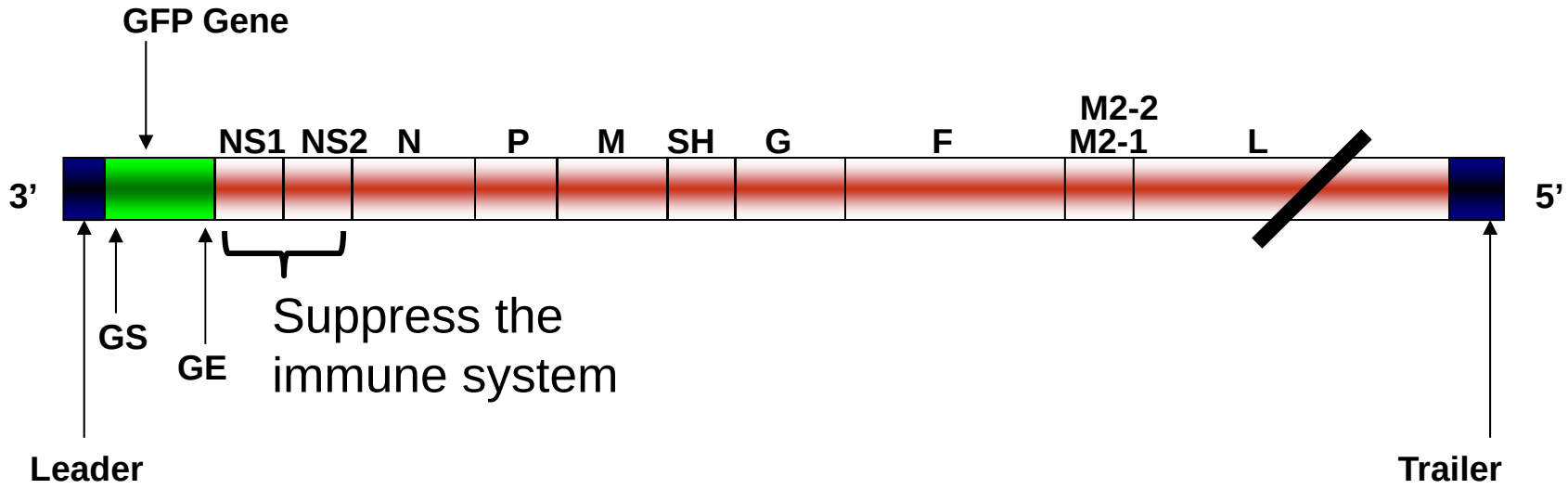
- RSV is the #1 cause for hospitalization at children's hospitals
- Only present November-March
- Worse for premies

RSV Prevention/Treatment

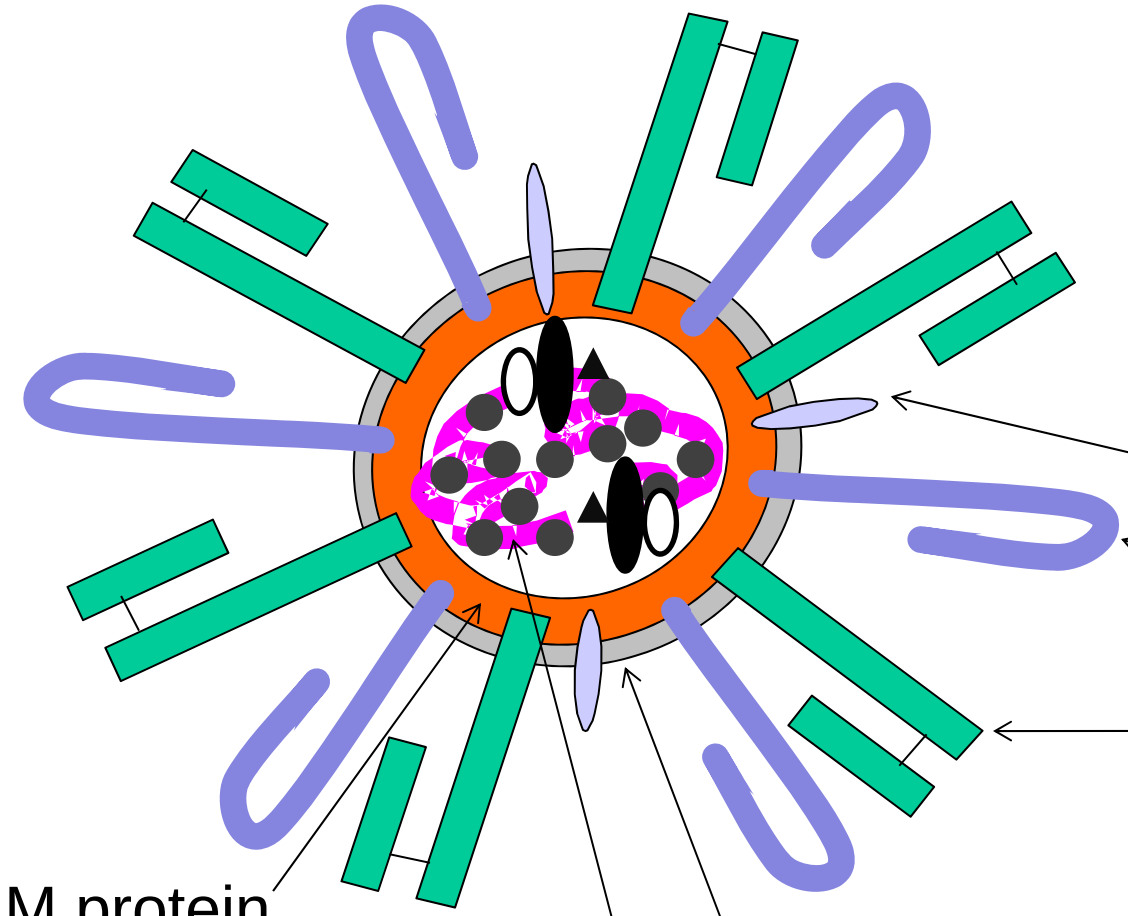
- No vaccine
  - Attempt in the 1960's was disastrous
    - Killed vaccine
  - Since then, focus on live, attenuated virus vaccine
    - Goal: prevent lower respiratory tract infection
- No effective antiviral agents
- Prophylaxis for premature infants
  - *Synagis* (MedImmune/AZ): monoclonal antibody
    - Expensive
      - But less than treatment in the PICU

Respiratory Syncytial Virus

- Single strand RNA virus
  - Negative sense
- Paramyxoviridae family
  - Pneumovirinae subfamily
- 10 genes encoding 11 proteins



RSV virion



N protein

P protein

L protein

M2-1 protein

Glycoproteins:

SH

G (attachment)

F (fusion)

M protein

Genome (RNA)

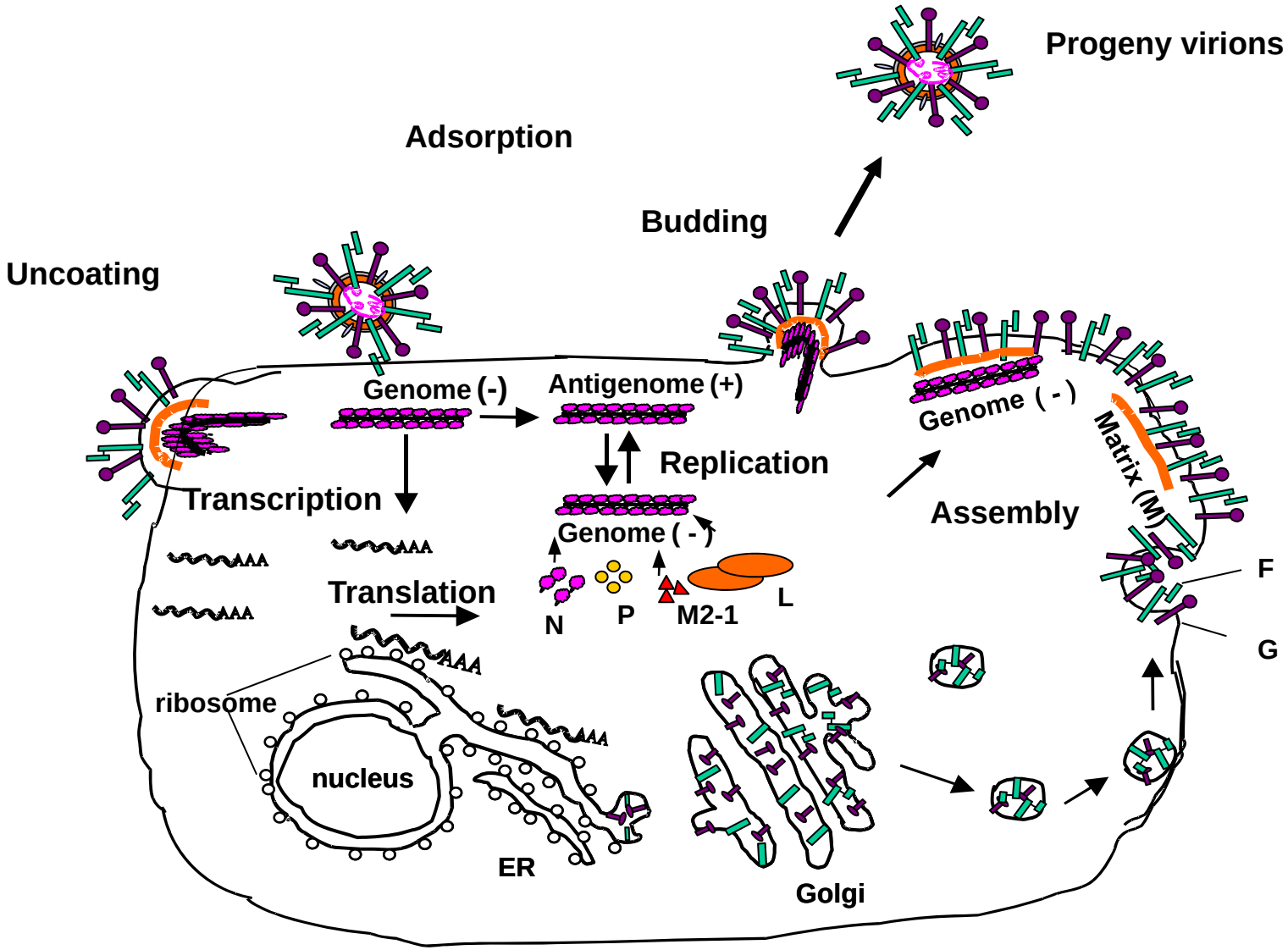
Lipid membrane

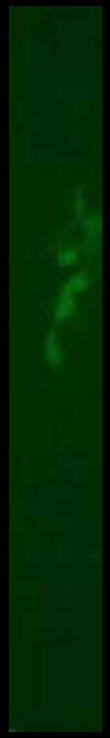
Virion's goal: Deliver the virus genome to the next cell

Initiation of RSV Infection

- Its G protein binds to a “receptor” on the cell surface
  - Only cells expressing the receptor are susceptible to RSV infection
- Its F protein causes the virion membrane to fuse with the target cell membrane
  - Result: the guts of the virus are spilled into the cytoplasm of the target cell
- The virus begins to replicate

Respiratory Syncytial Virus Life Cycle





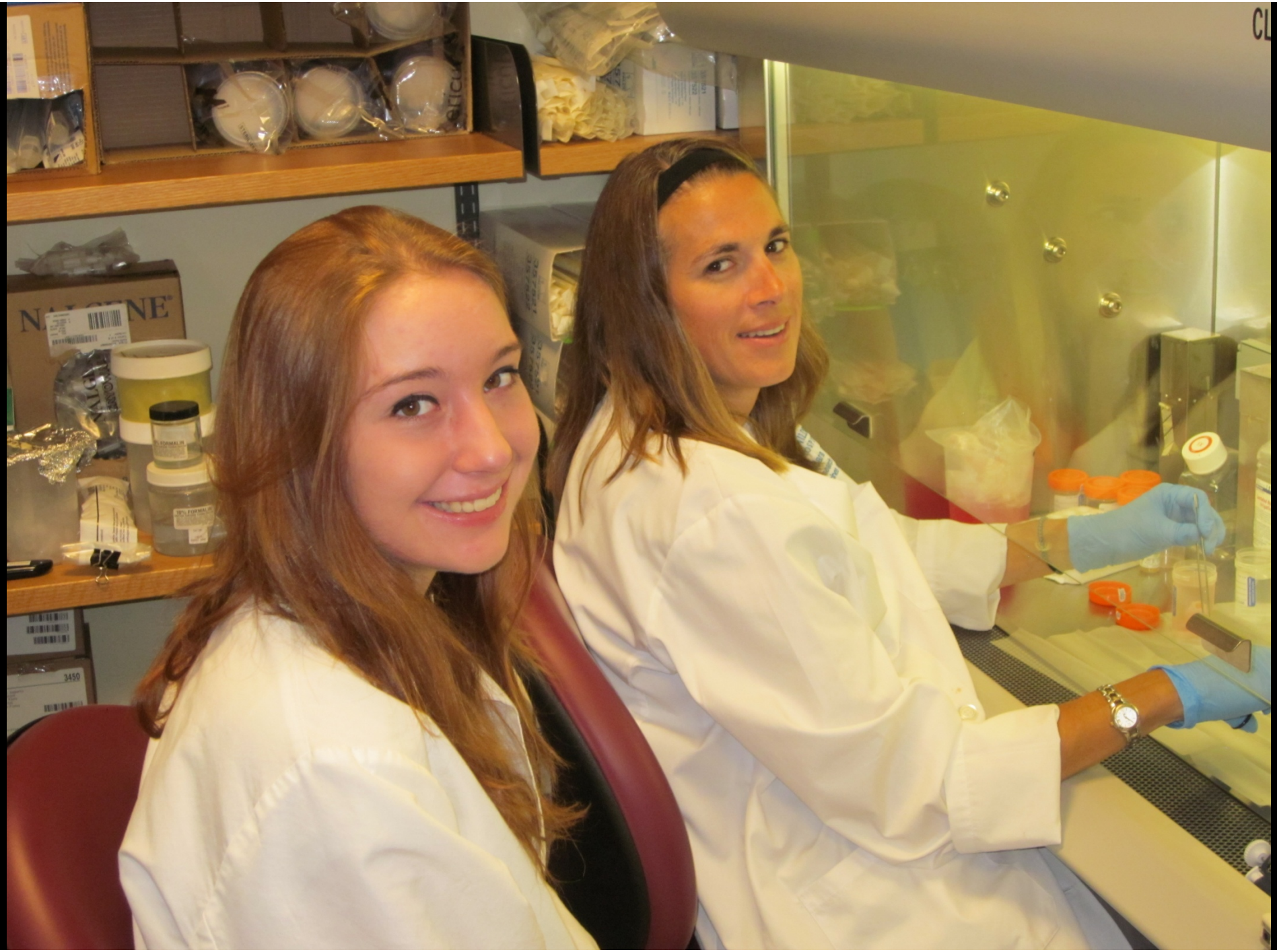
Syncytia

RSV infection of immortalized (HeLa) cells

(We have built the GFP gene into RSV)

What is the natural target cell for RSV?

- NOT HeLa cells (from a cervical tumor)
  - (But they are easy to study)
- Human airway epithelial cells
  - Not so easy to study
  - Essential for understanding RSV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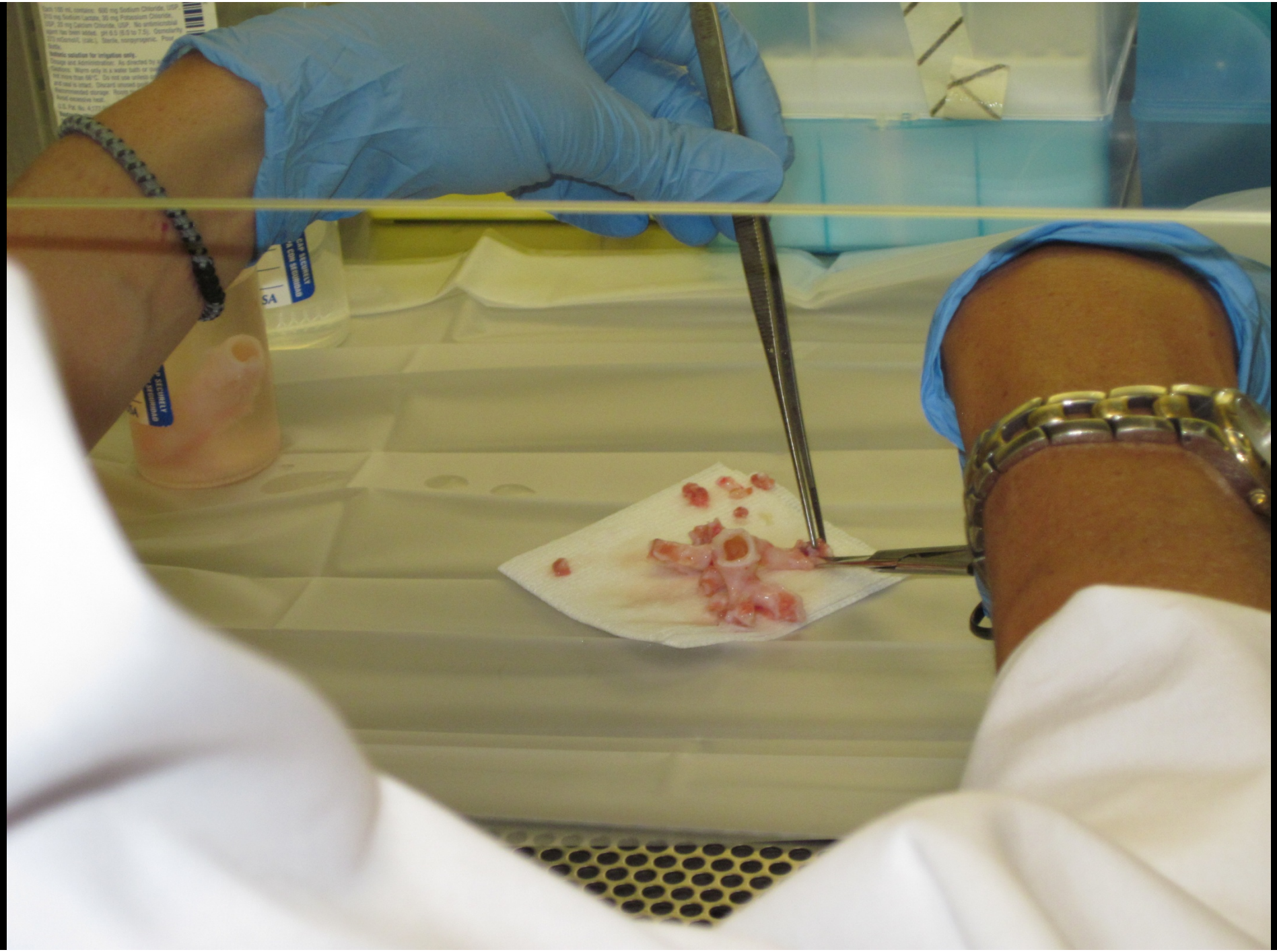
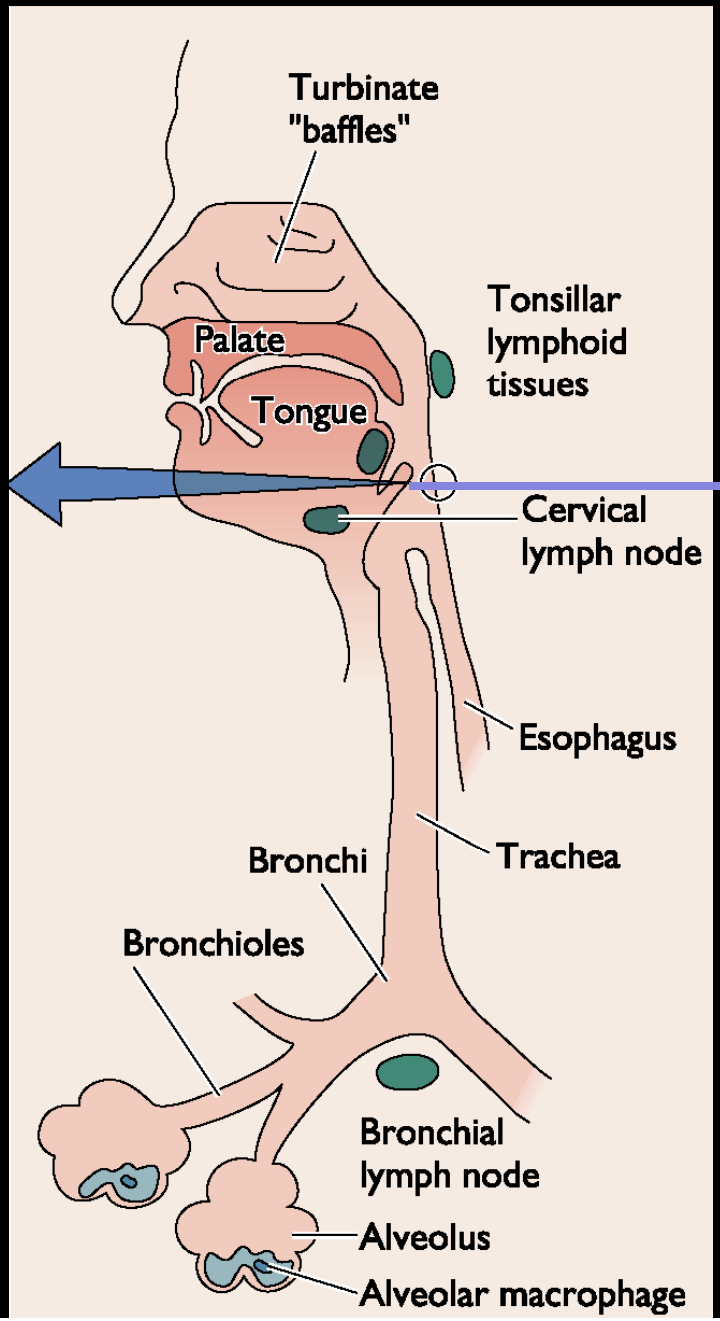


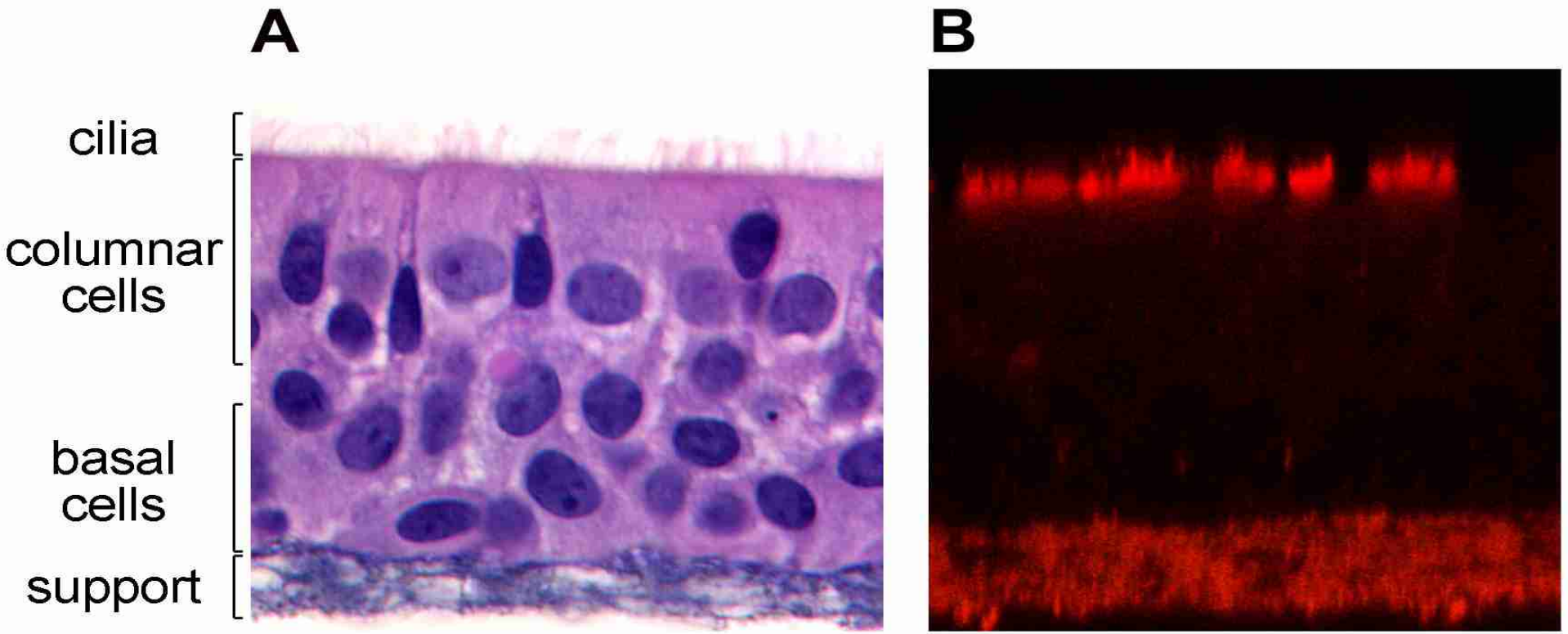
Respiratory Tract



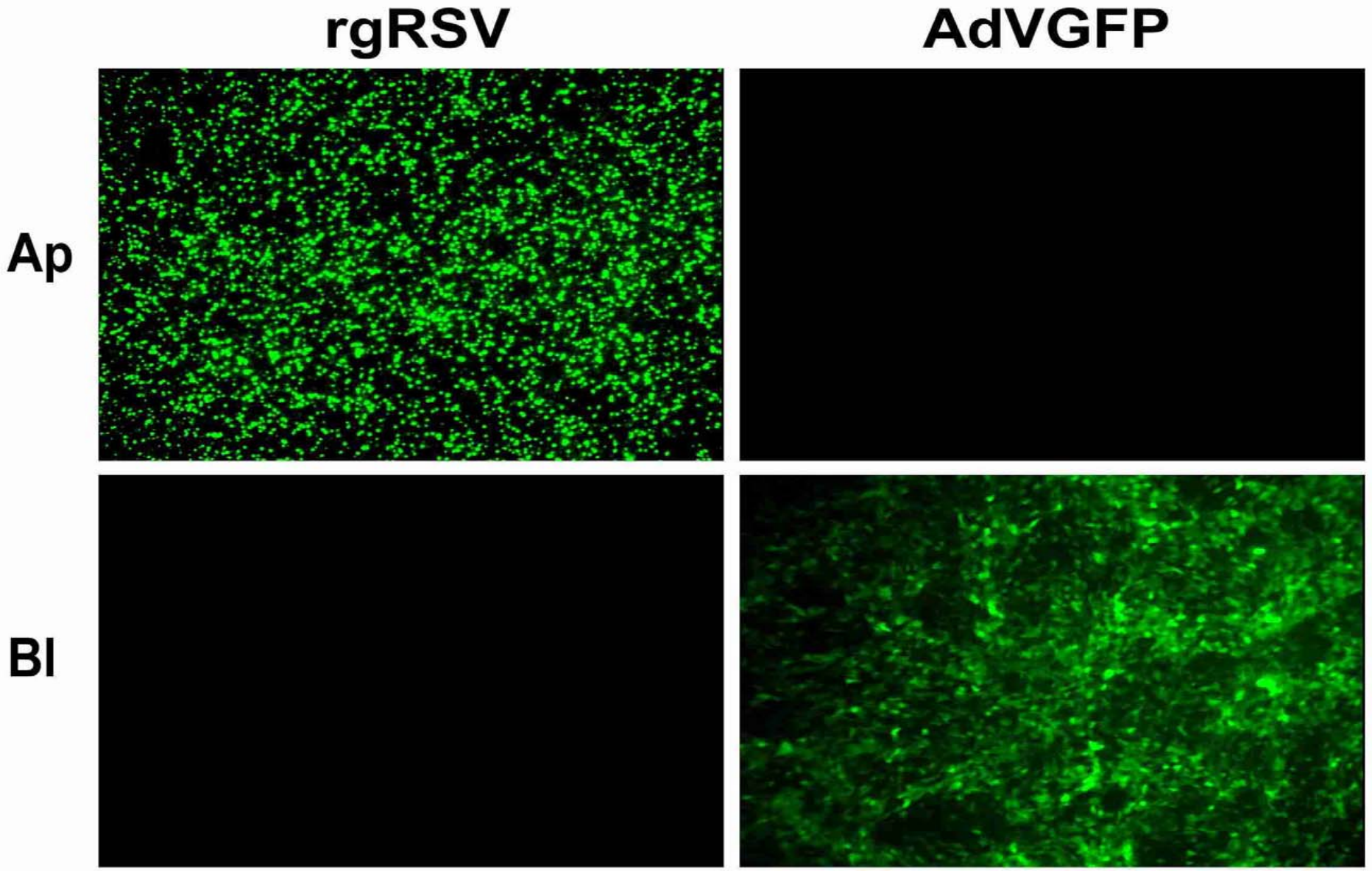
Upper

Lower

Primary Well Differentiated Human Airway Epithelial (HAE) Cultures



Zhang, et al, 2002



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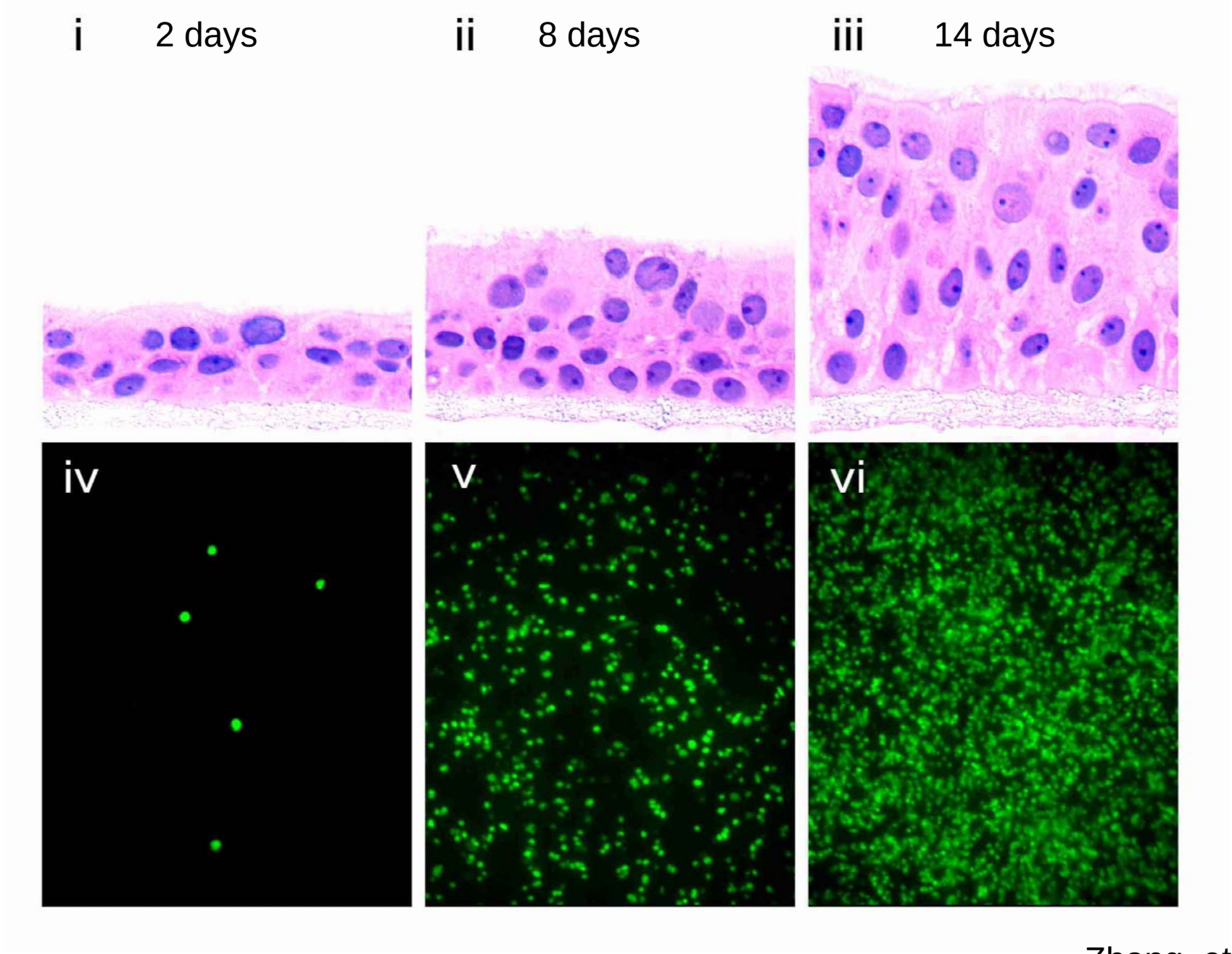

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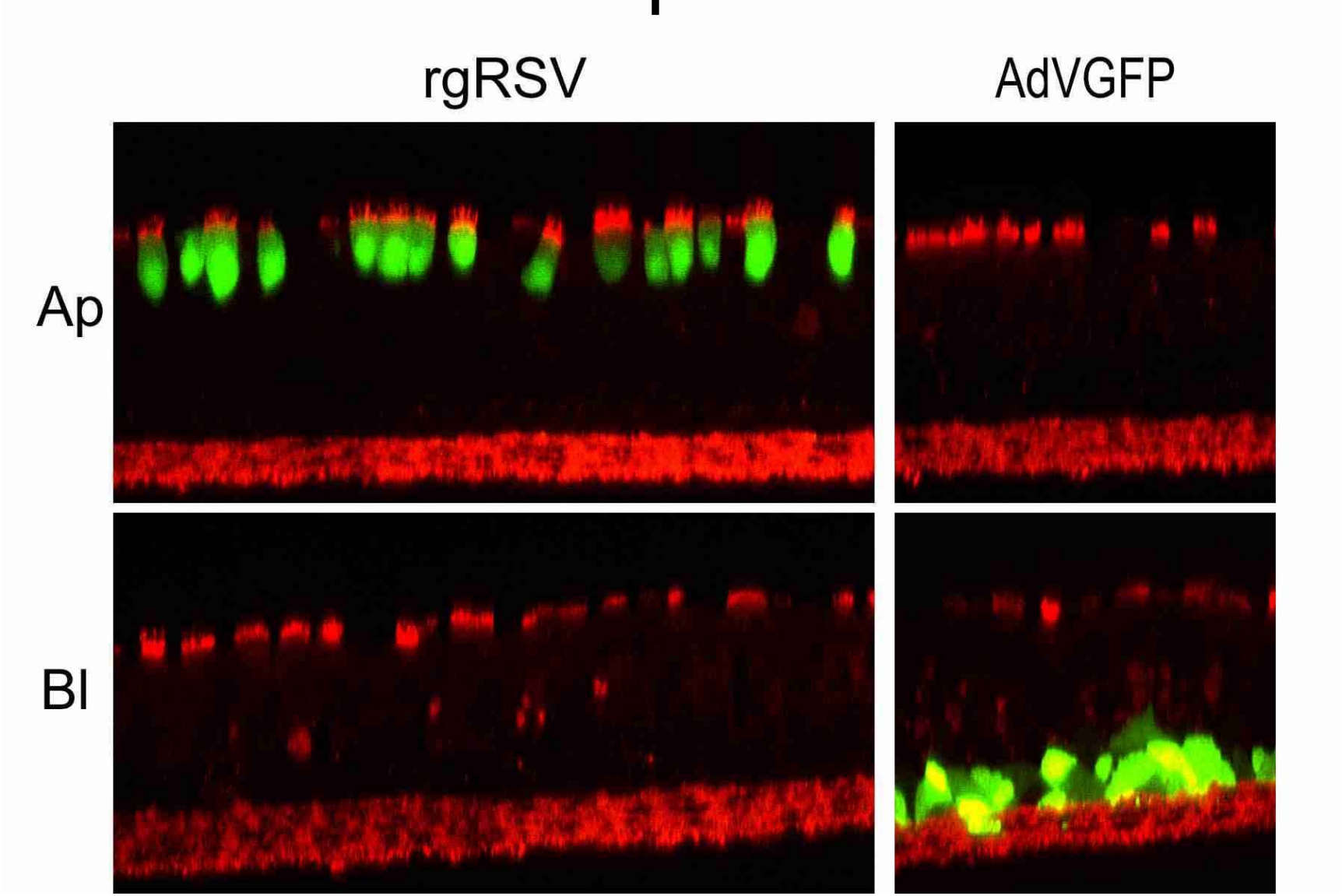
RSV Infects the Apical Ciliated Cells



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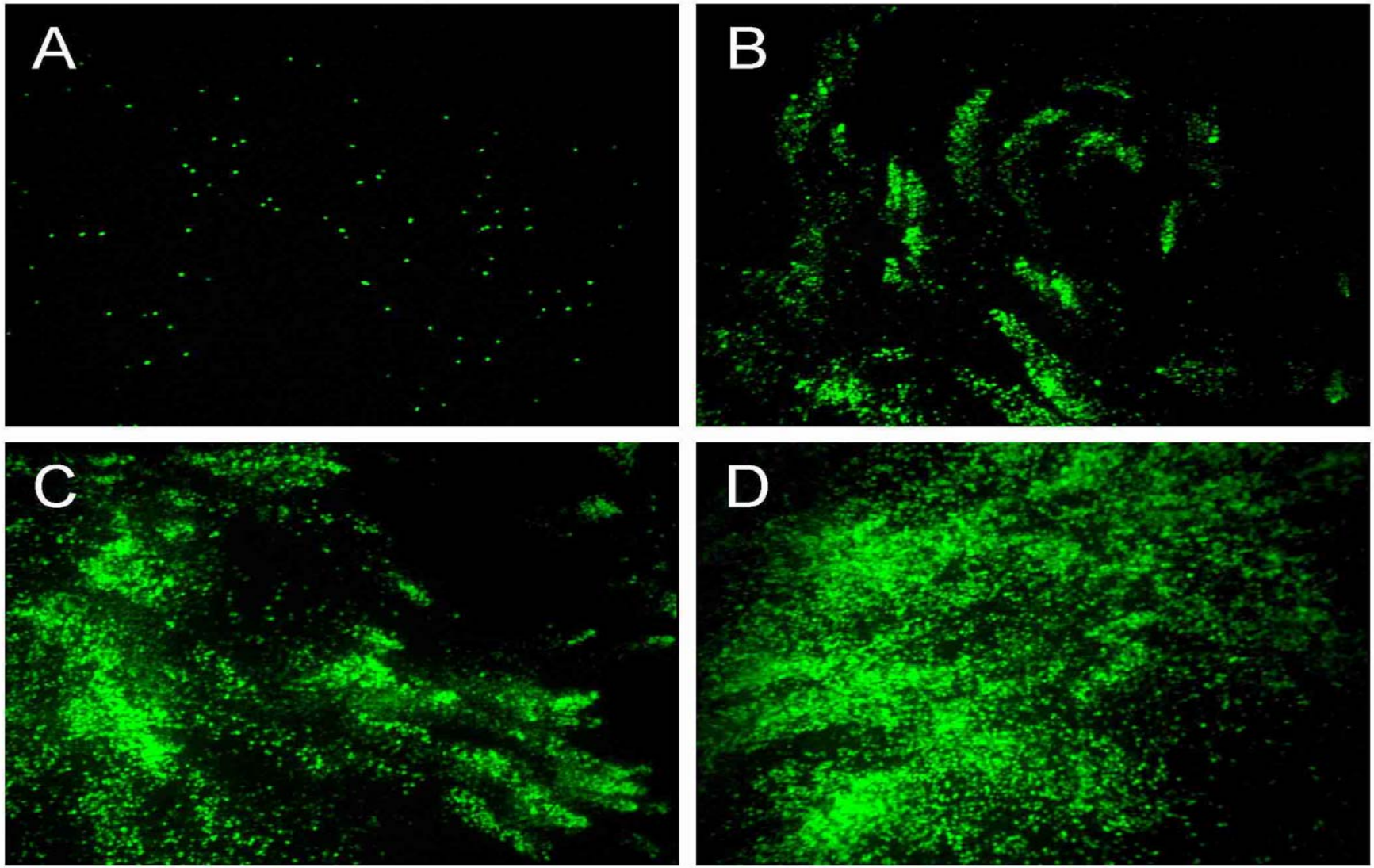


Zhang, et al, 2002

Does the Cell Source of RSV Matter?

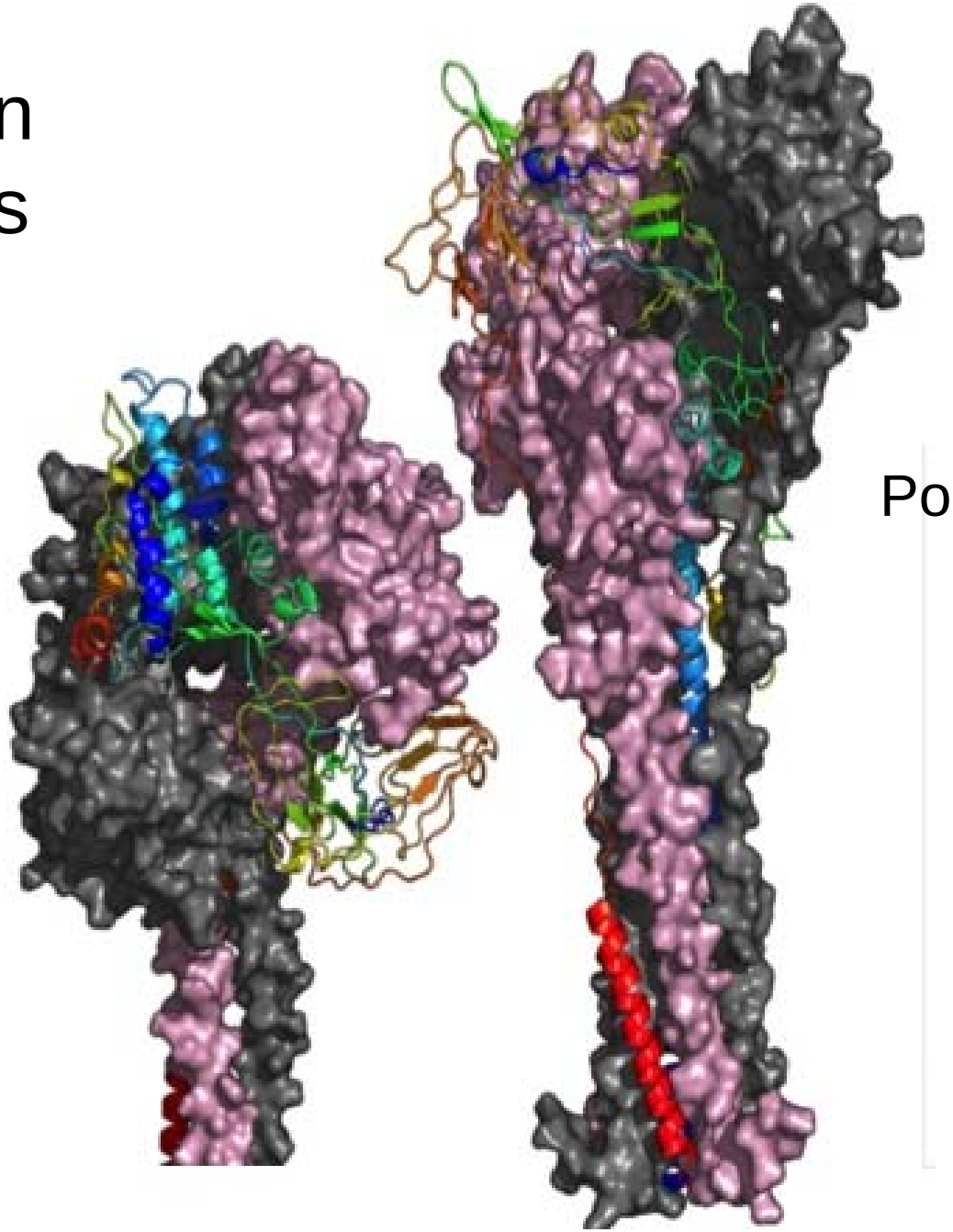
Things We've Learned that May Improve the Attenuated Vaccine

- Problem: can't grow enough attenuated RSV vaccine to make it economically feasible
  - Must grow in a WHO-approved cell line (Vero)
- We have found that:
  - This vaccine is 1,000 times less infectious for HAE cells than the same virus produced in HAE cells
    - The RSV G protein is destroyed in Vero cells
    - The RSV G protein is not modified properly in any cell line
- If we can fix both of these problems, we should be able to boost vaccine production 1,000 times, easily making it economically feasible

Adult Vaccine: RSV F Protein

- A live attenuated virus vaccine might cause disease if
  - A weakened immune system
- A protein vaccine would not have that problem
  - Would ‘remind’ the adult immune system
- It probably would not be used in infants
  - Fear: infants might react as they did to the killed virus vaccine
- Previous attempts to immunize adults with F protein
  - Not toxic
  - But, not very potent
- Since that time we have learned…

RSV F protein has two forms



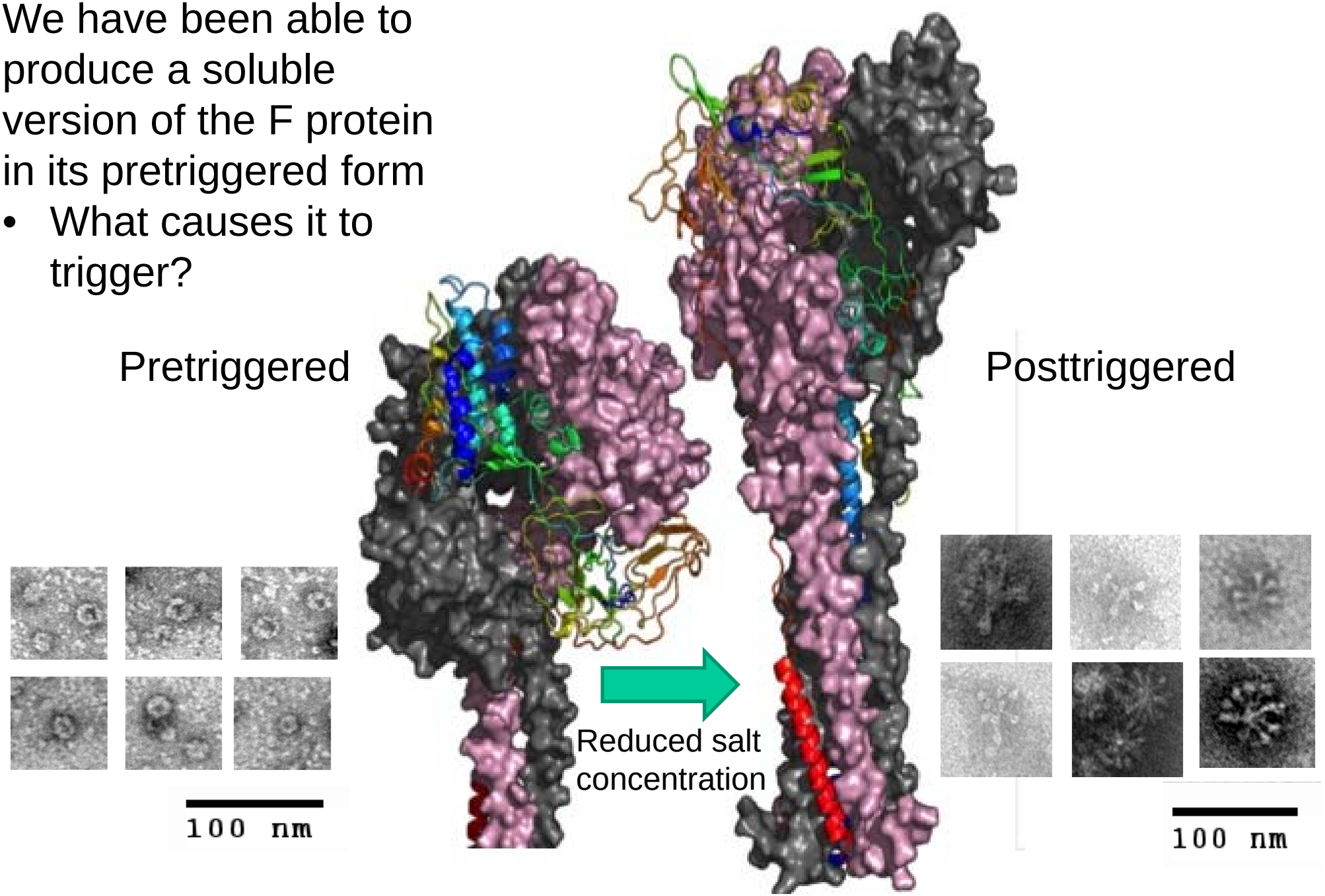
Pretriggered

Posttriggered

Movie Time!

We have been able to produce a soluble version of the F protein in its pretriggered form

- What causes it to trigger?



Pretriggered Soluble F Protein

- Better vaccine for adults?
  - Induce better antibodies than previous F vaccines?
  - We are working to stabilize the pretriggered form
    - Supported by a pharmaceutical company
- I was recently appointed to a Board
  - Funded by the Bill and Melinda Gates Foundation
  - Produce a vaccine for financially restricted countries
  - Vaccinate pregnant women
    - Transfer of protective antibodies across the placenta

Pretriggered Soluble F Protein

- Use to test antiviral drugs
  - Do the drugs cause triggering?
    - Or do they prevent triggering?
- Use the F protein model to design better drugs
  - Crystallize the F protein, determine its exact structure
  - We are working with a different pharmaceutical company

Summary of a Killer Virus: RSV

- Virus ‘life’ begins at infection
  - After fusion of the viral membrane with the cellular membrane
- RSV causes disease in the youngest and oldest
  - Can’t stop movement of the virus to the lower respiratory tract
    - Major burdens on the health care system in US
    - Major cause of death in the developing world
  - Common cold in the rest of us

Summary of a Killer Virus: RSV

- Treatments/Prevention
  - Antibody treatment protects premies (expensive)
  - No vaccine yet
    - Killed vaccine trial was a disaster (1960's)
    - Attenuated virus is being explored for infants
      - Can't produce enough vaccine to be economically viable
      - Our lab has discovered 2 problems in the G protein
        - » Solutions could increase vaccine production by 1,000-fold
    - F protein vaccine is being explored for the elderly
      - Our lab has produced the F protein in the pretriggered form
      - Does it induce better immunity than the posttriggered F?
  - No small molecule antiviral drug yet
    - We are using our pretriggered F protein and triggering conditions: Do drugs prevent or cause triggering?
- 'Syncytia' are giant cells, fused by the RSV F protein

Acknowledgements

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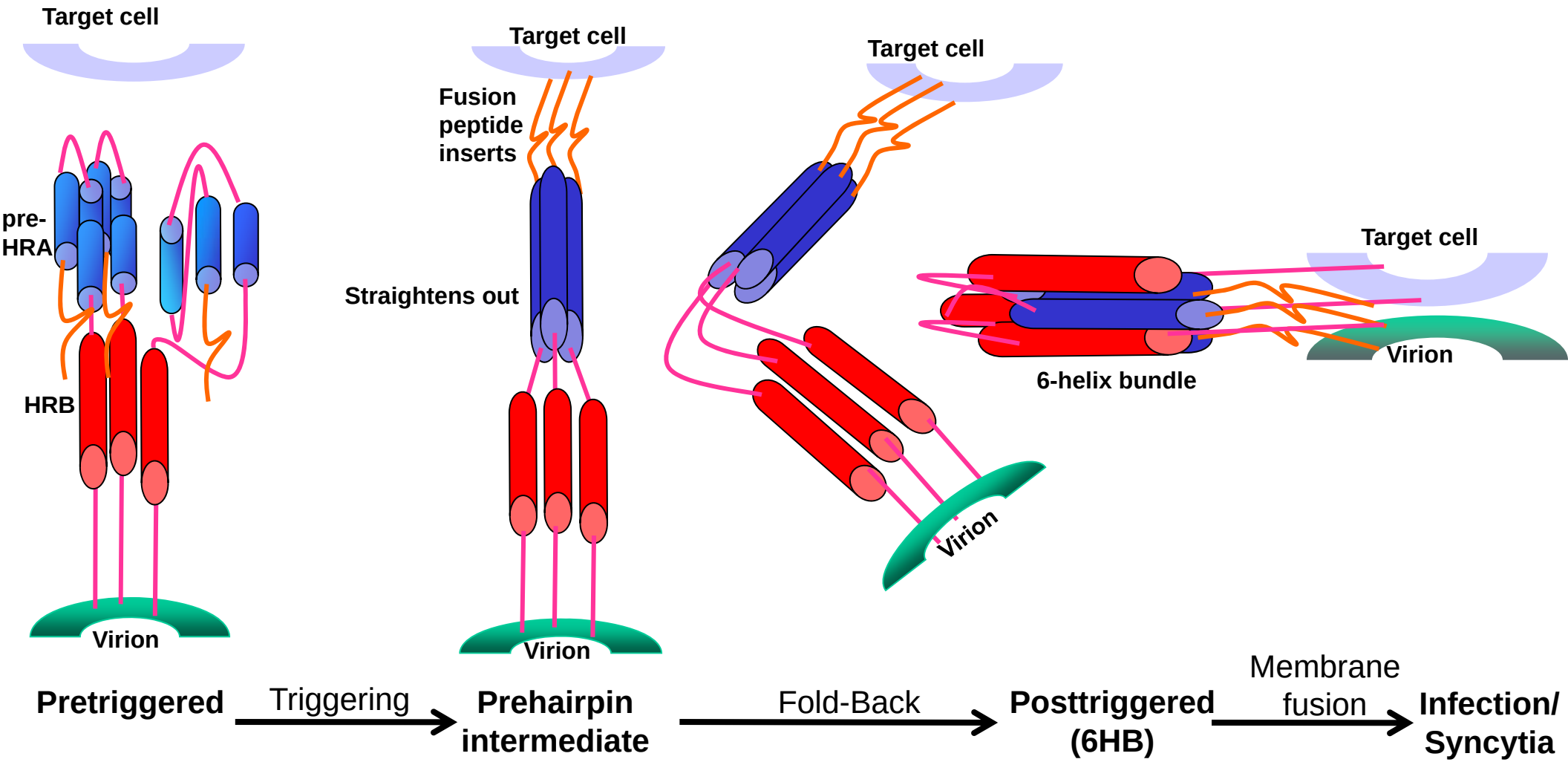
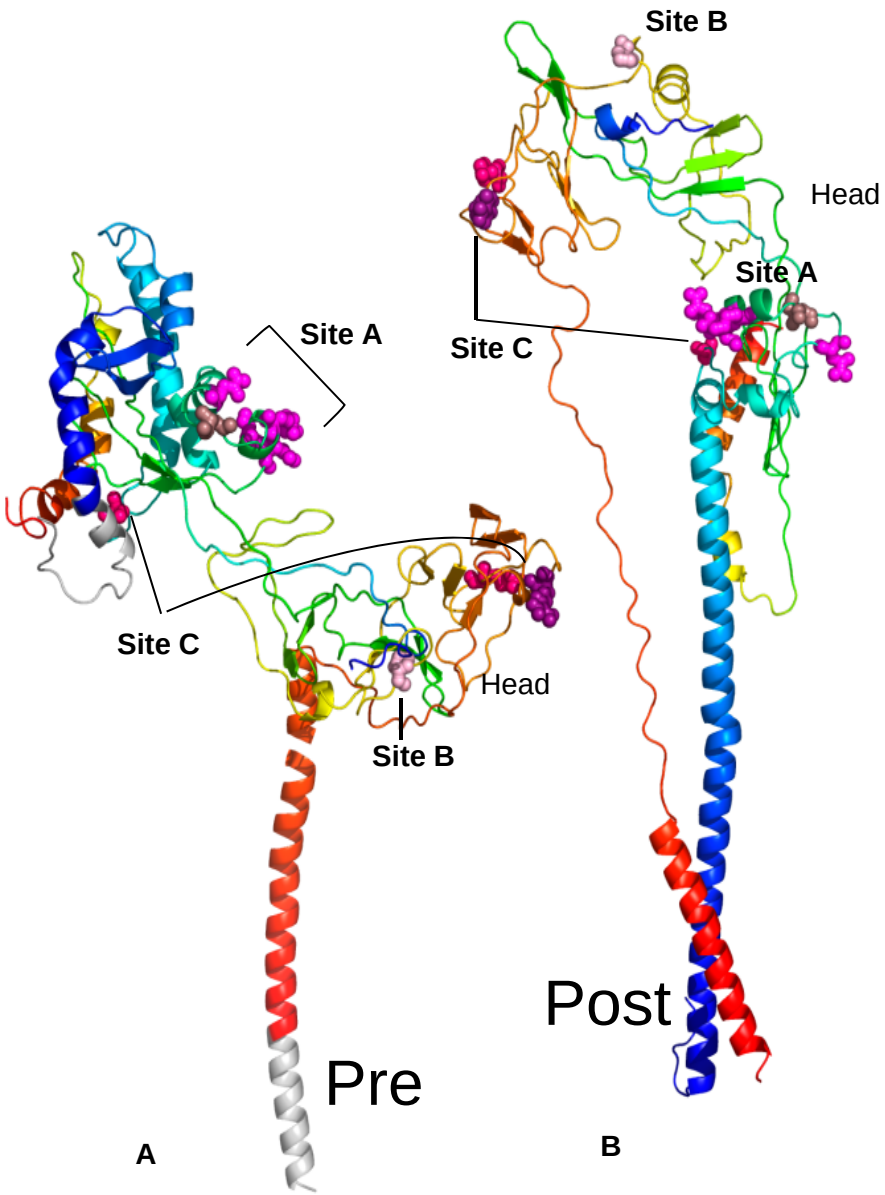
F protein mediated fusion

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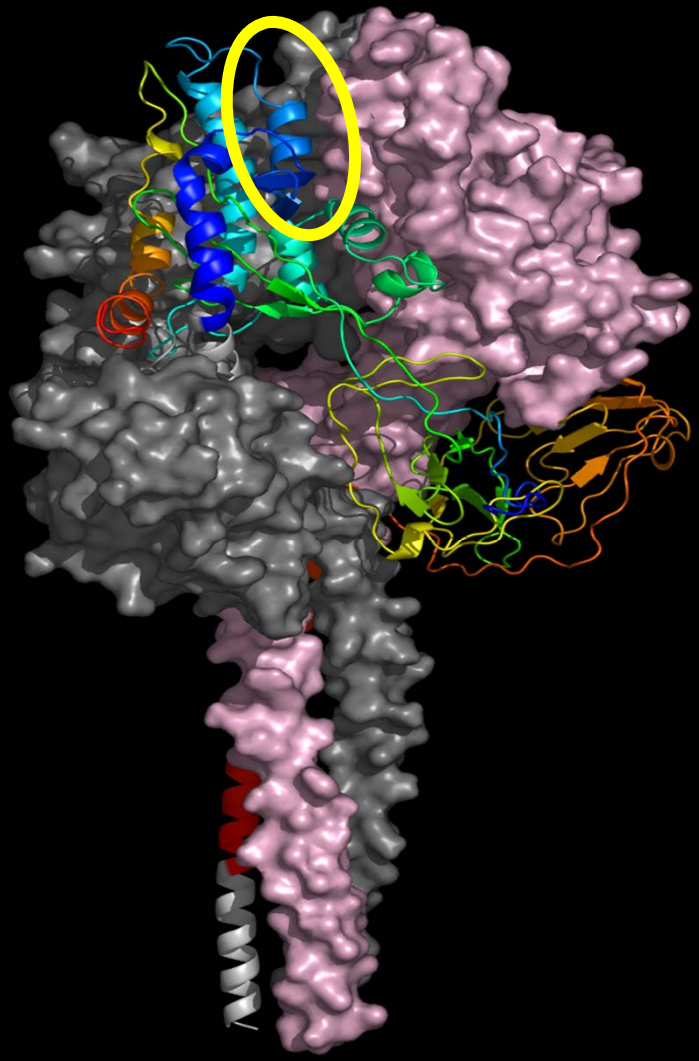
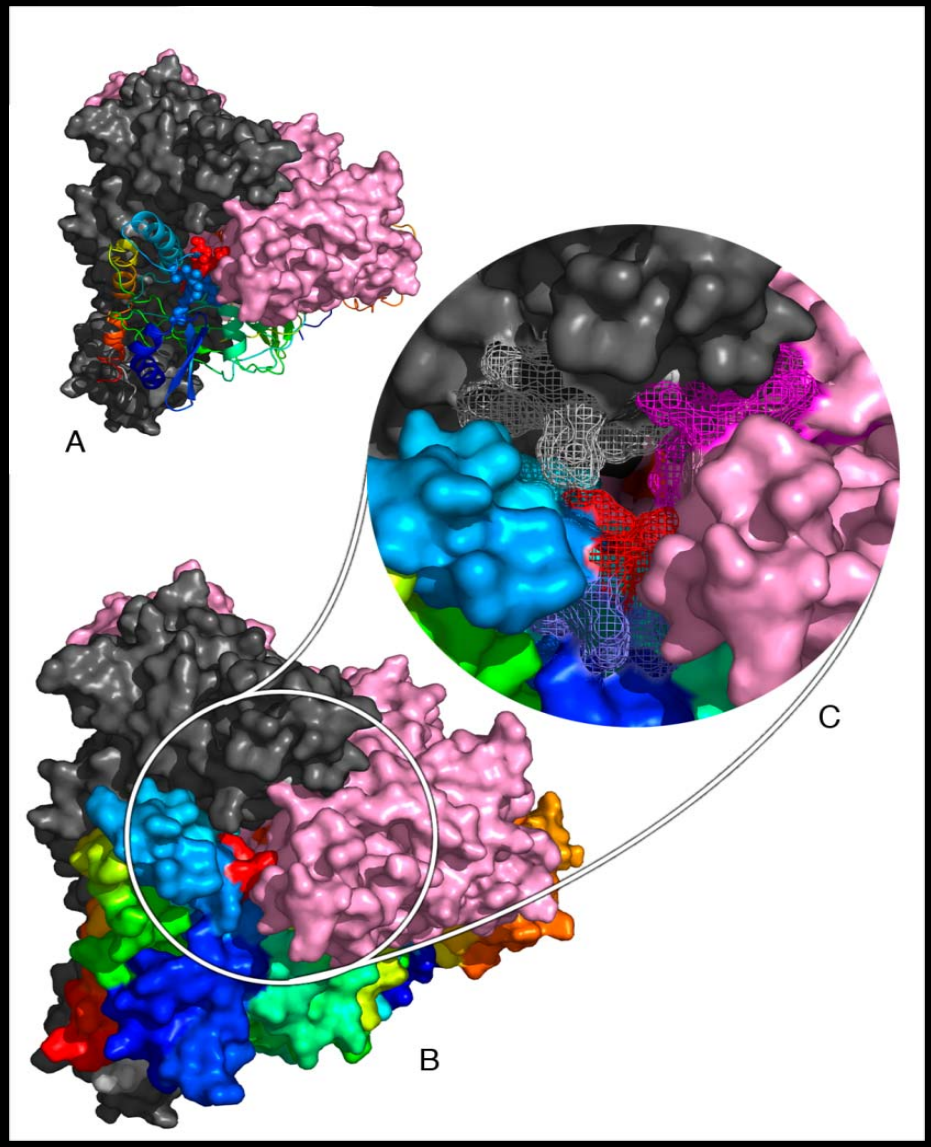
Site C
Head
Site B
Pre
A
Site B
Head
Site A
Site C
Post
B

Model of RSV F Protein Trimer

Will Ray



Pre-triggered form



Top view