Tunneling nanotubes enabling enterovirus cell-to-cell transmission – a novel way of spreading

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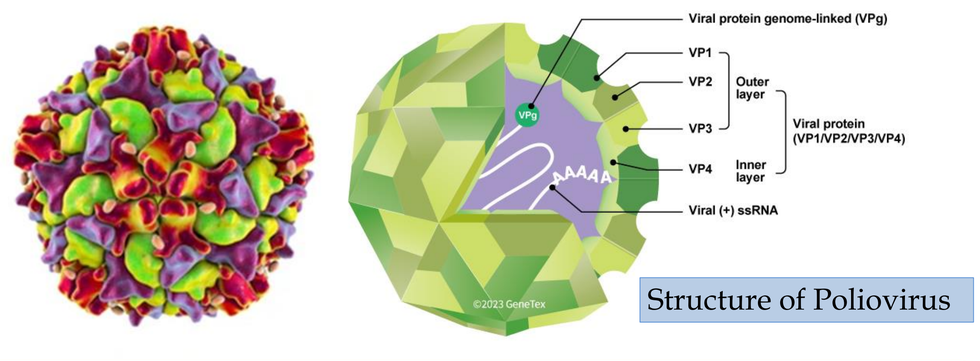
MOLECULAR SYSTEMS BIOLOGY

Introduction

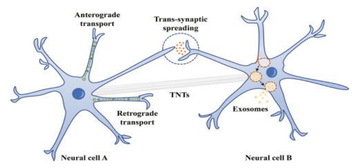
- Enteroviruses are small, non-enveloped, positive-sense single-stranded RNA viruses. Poliovirus, rhinovirus, enterovirus A71, coxsackieviruses, are members of enteroviruses. Non-enveloped viruses usually release virions lytically to infect neighbouring cells. However, cell-free transmission encounters immune barriers impacting viral spread efficiency.
- HIV and SARS-CoV-2 were found to use the cellular open membranous channels (Tunneling Nanotubes - TNTs), for direct cell-to-cell transmission and evade immune system recognition.

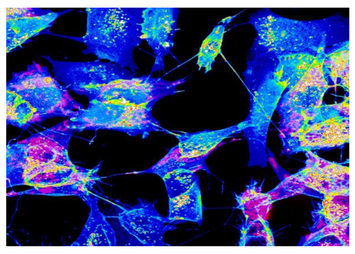


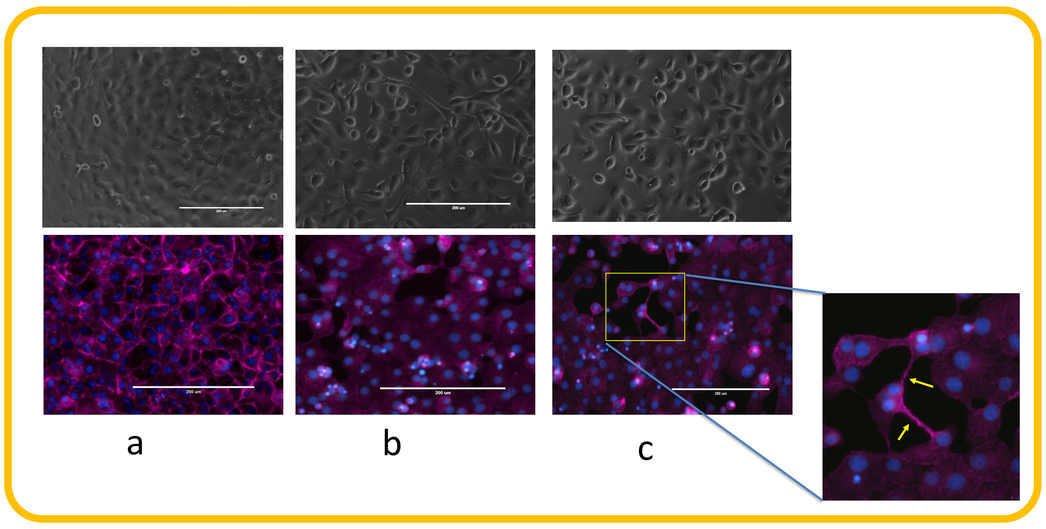
Structure of Poliovirus

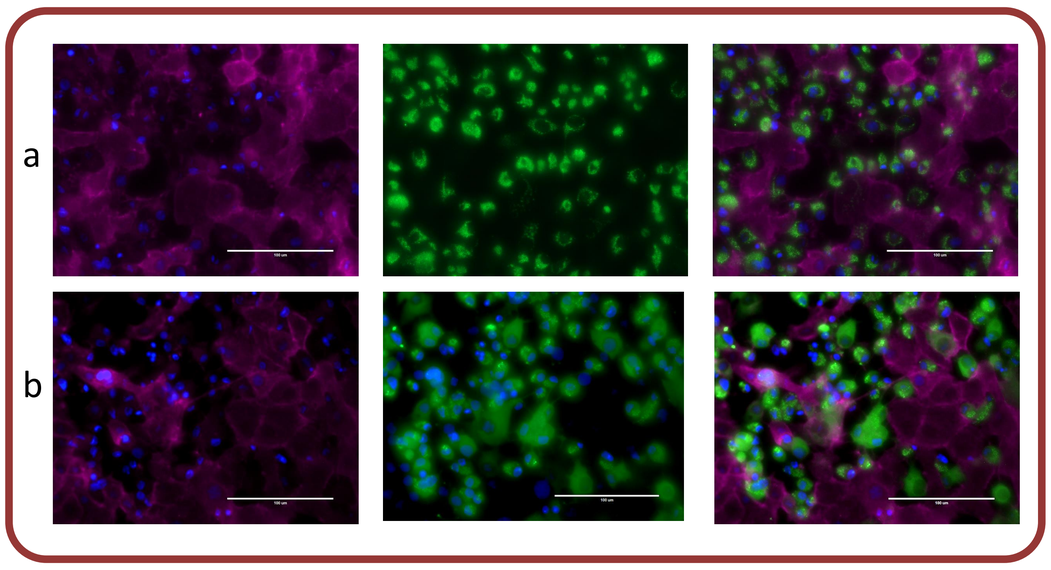
Tunneling Nanotubes (TNTs), discovered in 2004 by Rustom et al. are membranous channels between cells, typically 50 to 200 nm in diameter.

- They provide a direct communication pathway for various cellular materials, which viruses may exploit to spread infection.
- TNTs contain actin, tubulin (Wang et al., 2021)

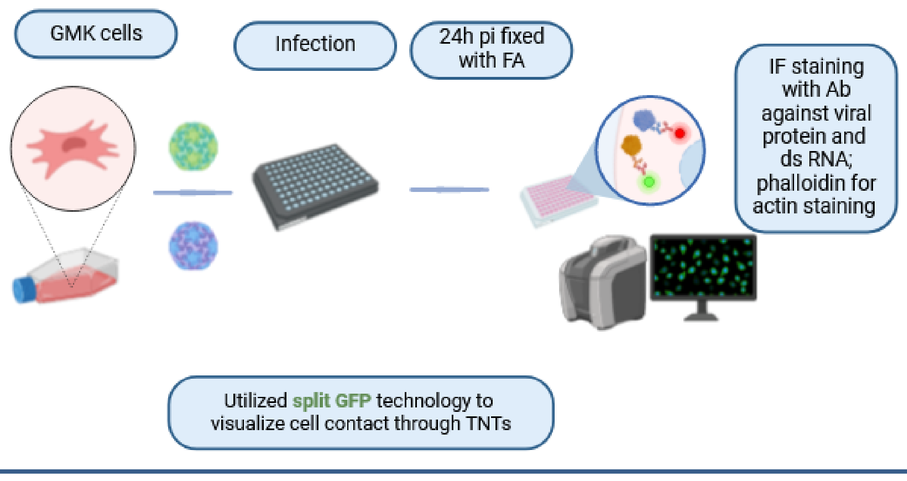








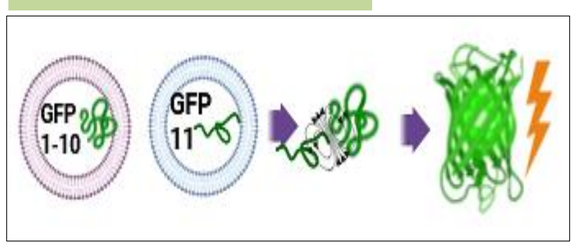
Experimental design



Aims

- To confirm Enterovirus infection induces TNTs.
- To investigate the cell-to-cell movement of Coxsackievirus B3 and CoxsackievirusA9 through TNTs.
- To utilize split GFP technology to visualize cell connection.

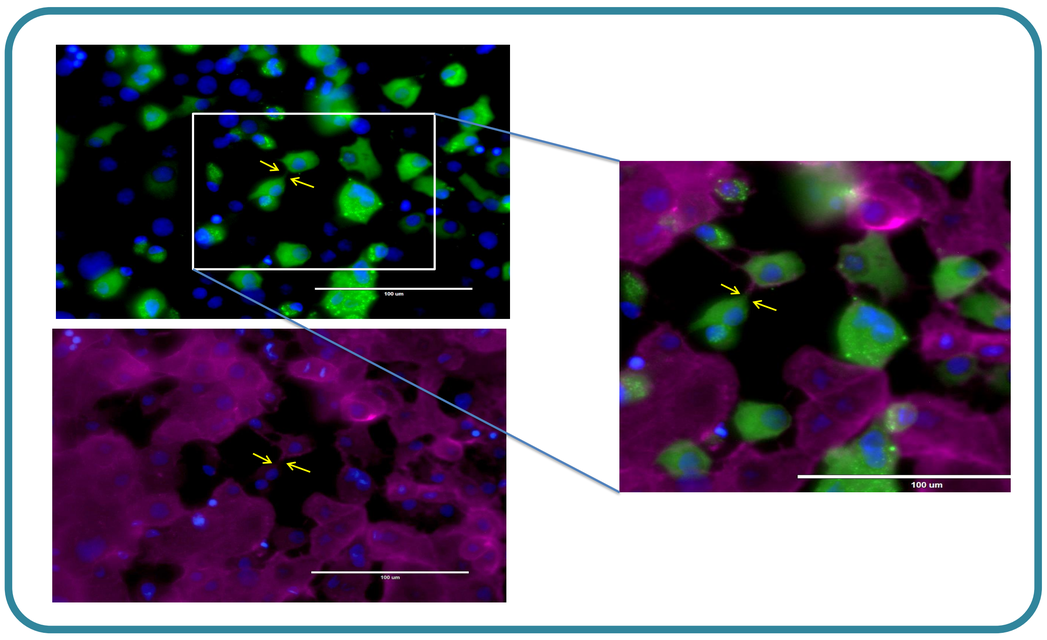
Split GFP Technology

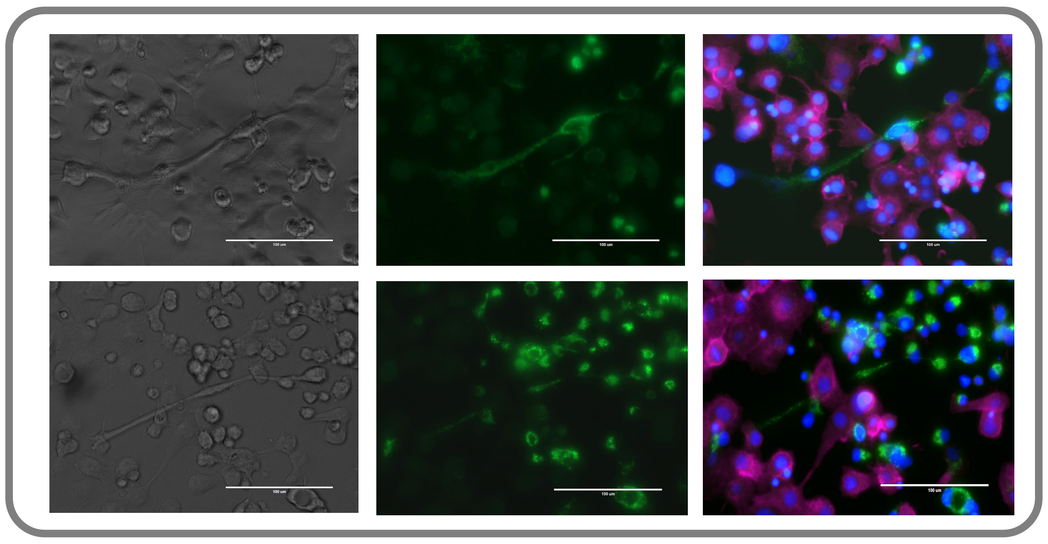


Result

All the staining was done 24h post infection. Phalloidin was used to stain **actin**, DAPI for **nucleus** staining to the fixed cells

- **Figure 1: Infection induces TNTs**. a. Non-infected GMK cells. Iinfection with, b. CAV9 & c. CBV3
- **Figure 2: Viral replication going on in infected cells.** Staining was done with Abs against a. **viral protein** b. **viral dsRNA** (CBV3)
- **Figure 3: Cellular connections/ TNTs.** Staining was done for **viral dsRNA**
- **Figure 4: Viral protein showing long continuous protrusion.**





Next Steps

- Plasma membrane staining to confirm the structures are open-ended.
- Iinfection in presence of neutralizing antibody
- To use split GFP technology to confirm intercellular connectivity via TNTs

Acknowledgement

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