

Difficulties and barriers in developing RSV polymerase inhibitors

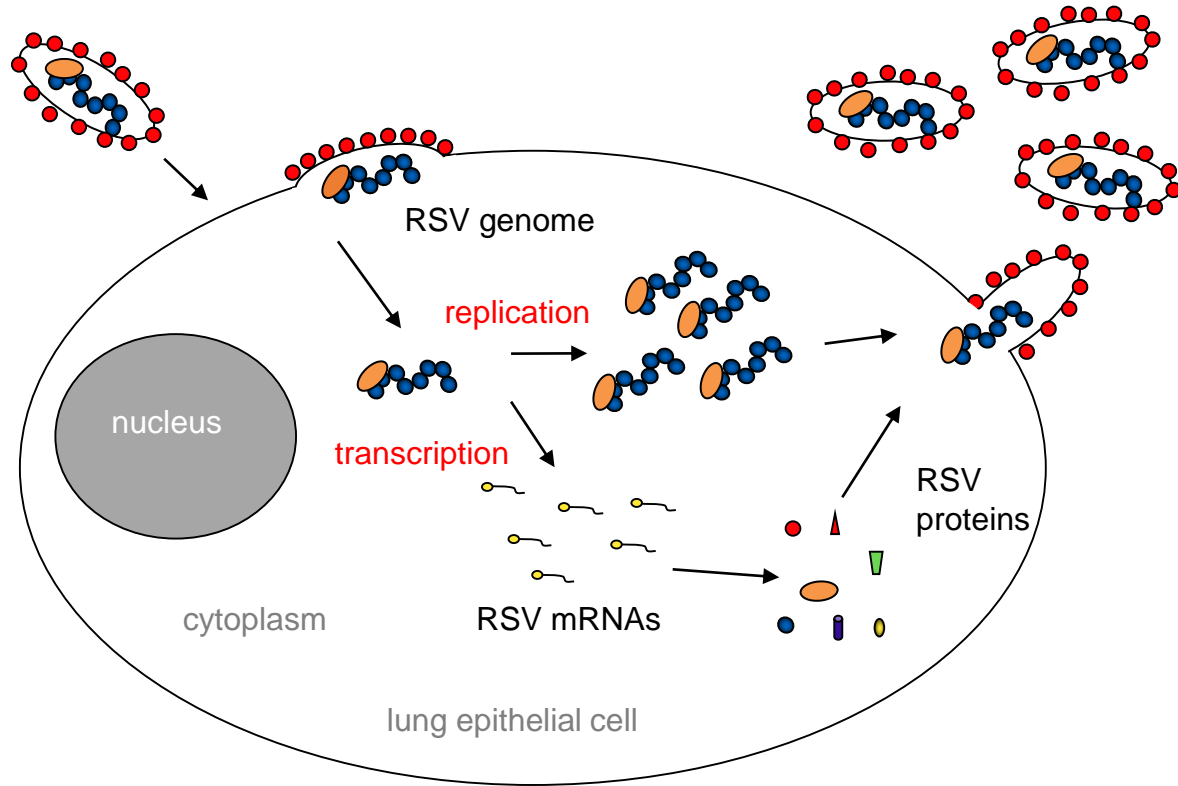
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National Emerging Infectious Diseases Laboratories

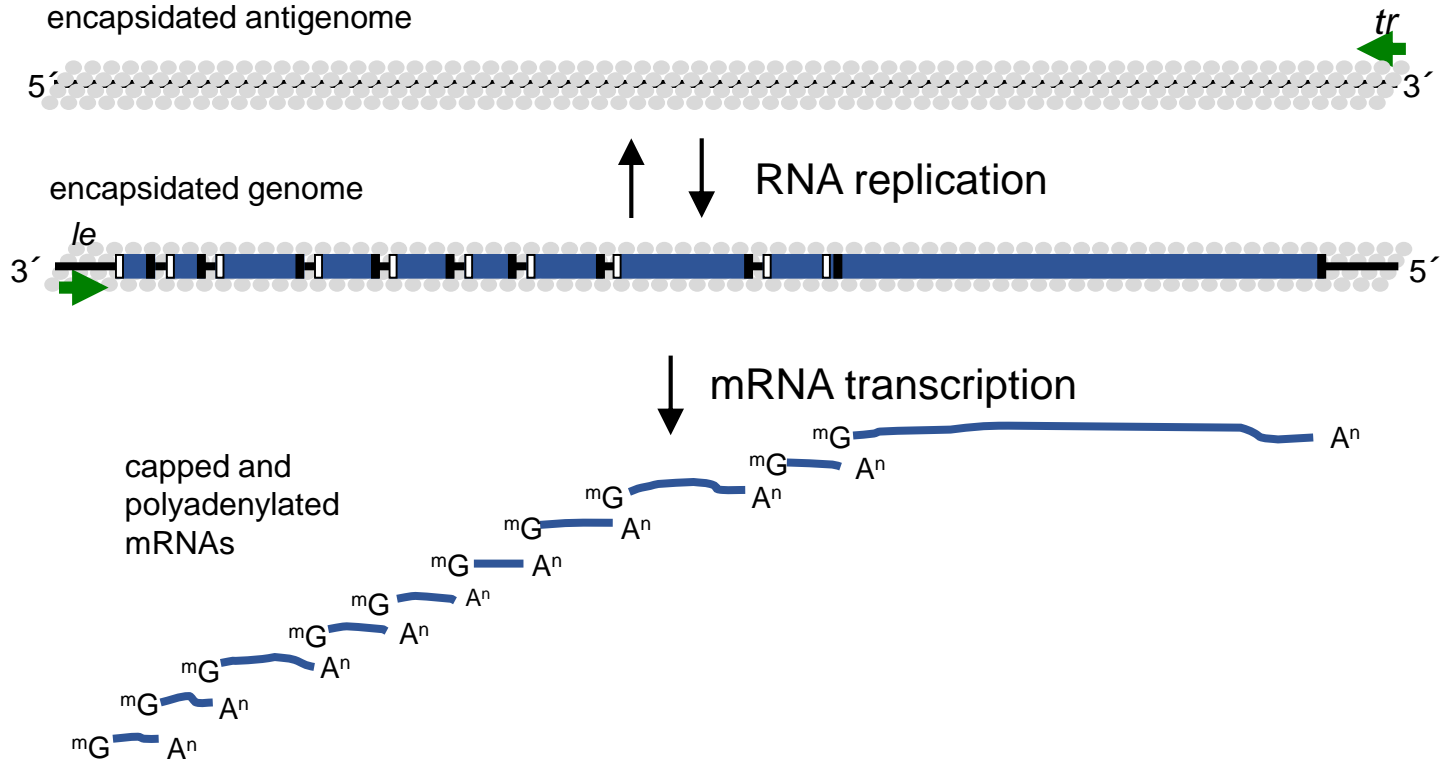
Boston University School of Medicine

Industry support: AstraZeneca, Gilead Biosciences, Merck, Alios Biopharma/ J&J, Enanta Pharmaceuticals

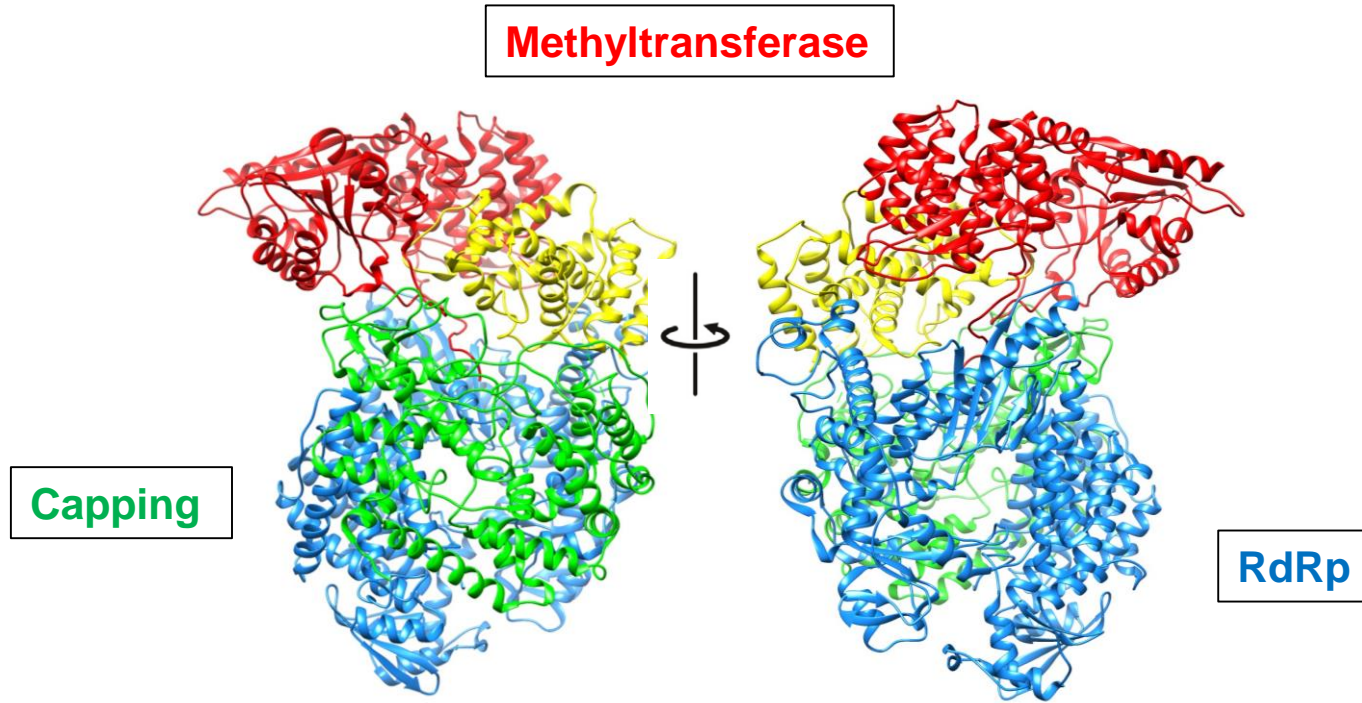
RSV replication cycle



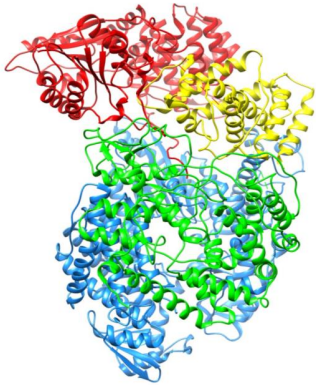
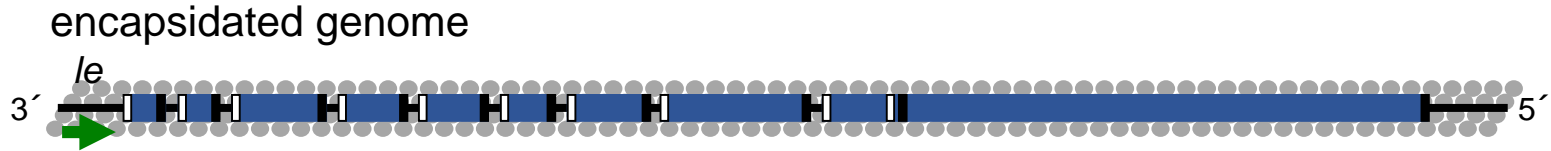
RSV transcription and replication



The viral polymerase has three enzymatic domains



Challenges in studying polymerase activities

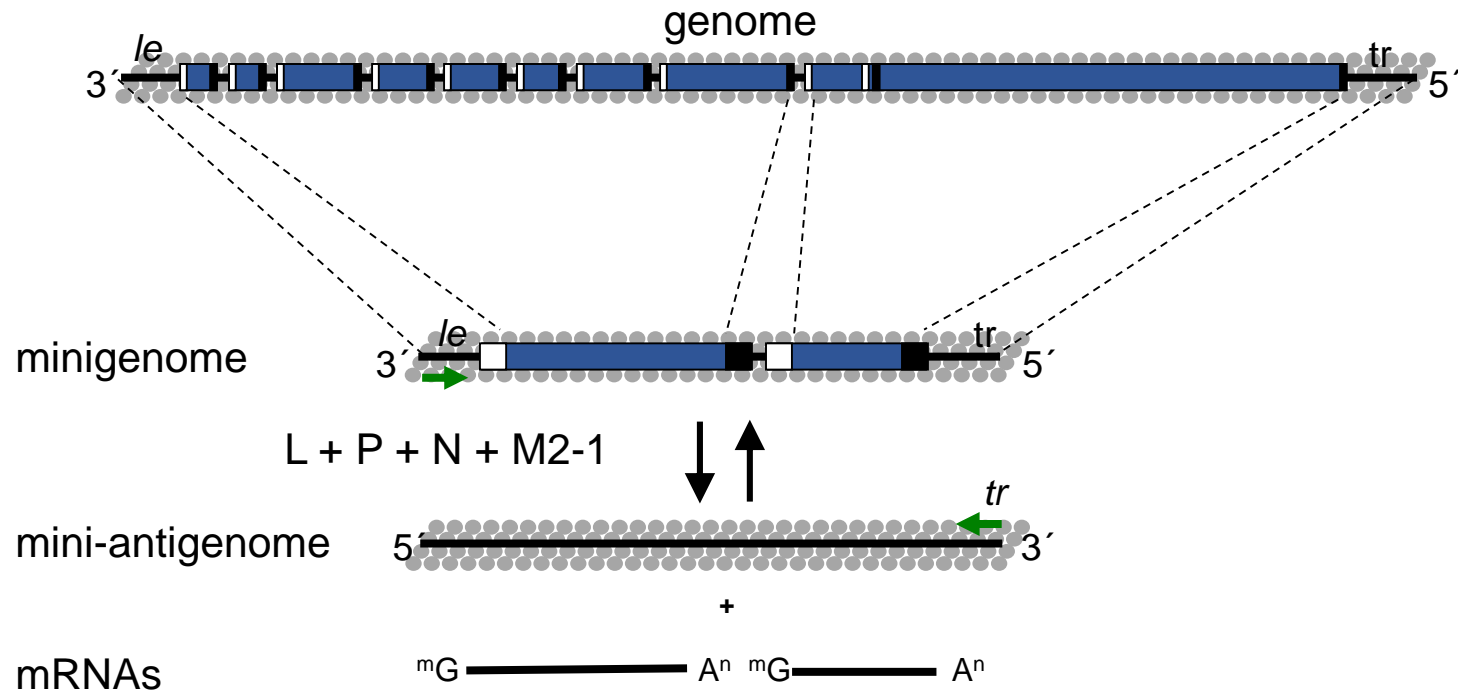


- Size and complexity of the polymerase
 - No high resolution structure
 - 2,165 aa, 250 kDa
 - requires a co-factor, P
- Encapsidated genome template
 - Reconstituting encapsidated RNA *in vitro* is challenging

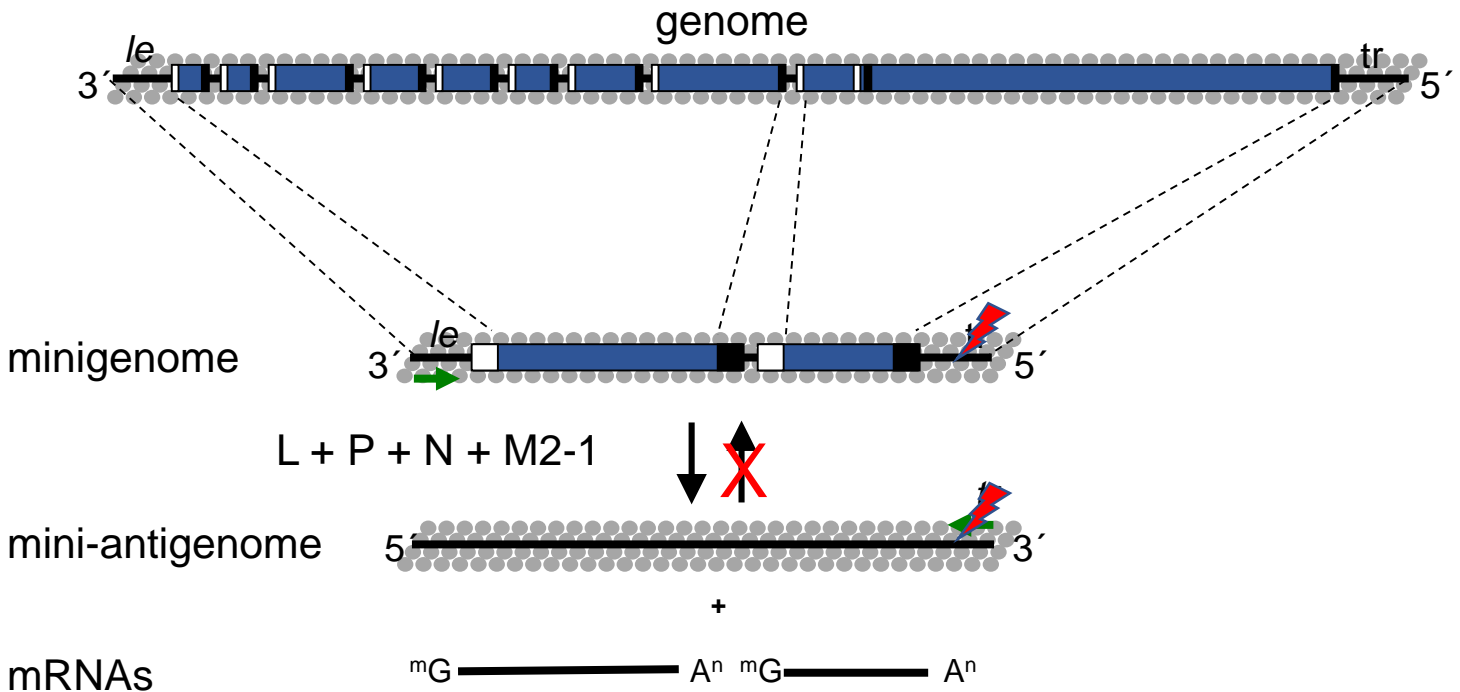
So what can we do?

- Mechanism of inhibition studies
 - Minigenome assays
 - Biochemical assays with purified enzyme

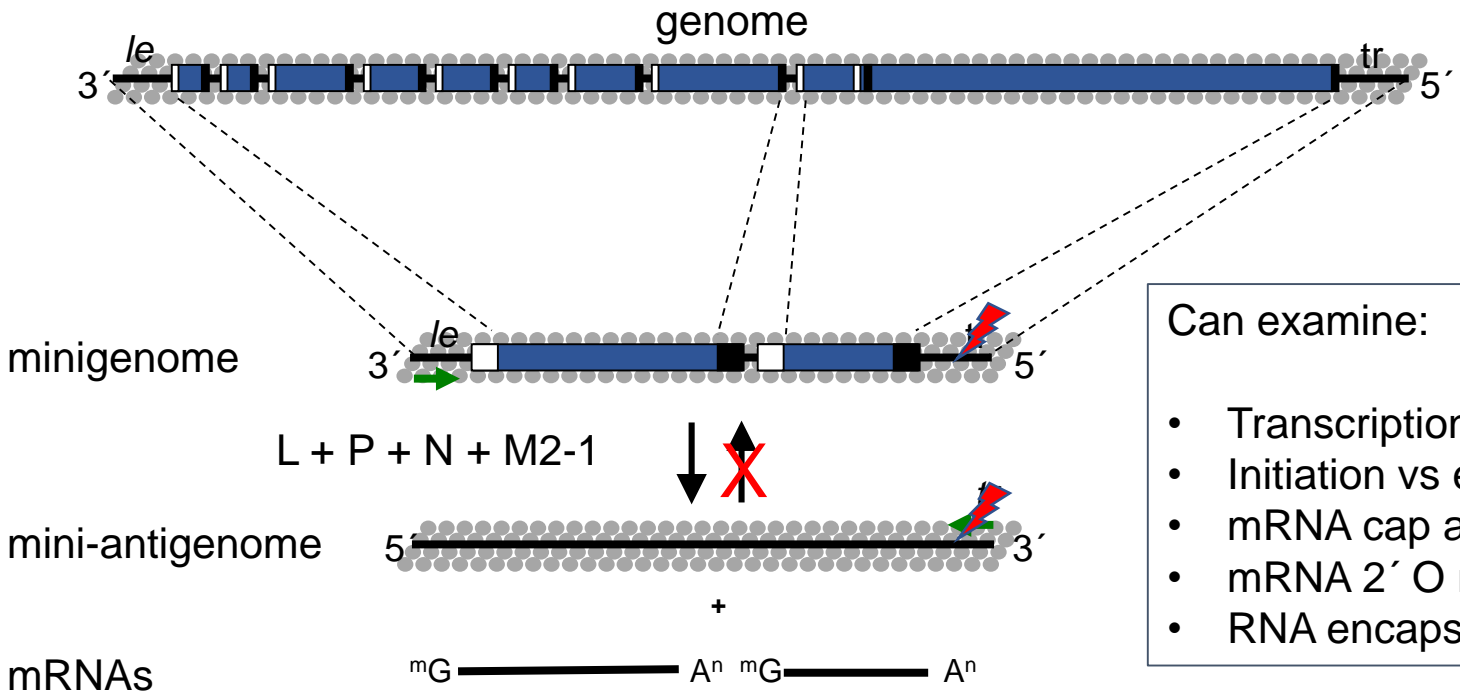
RSV minigenome assay



RSV minigenome assay – mechanism of inhibition studies

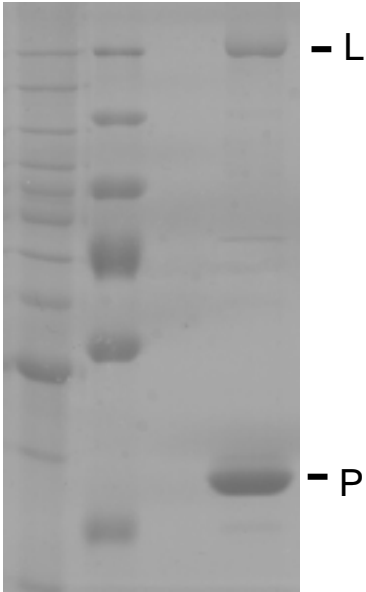


RSV minigenome assay – mechanism of inhibition studies

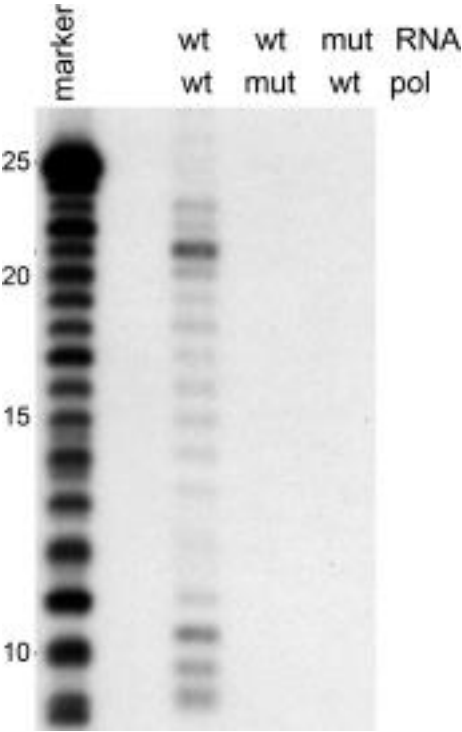
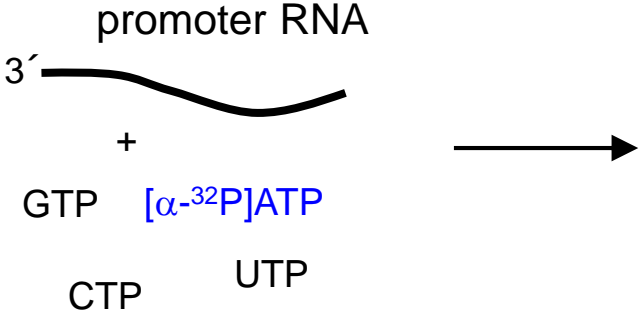


- Can examine:
- Transcription vs replication
 - Initiation vs elongation
 - mRNA cap addition
 - mRNA 2' O methylation
 - RNA encapsidation

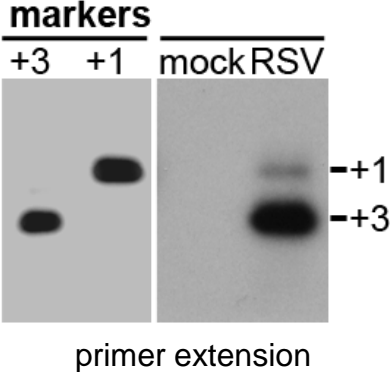
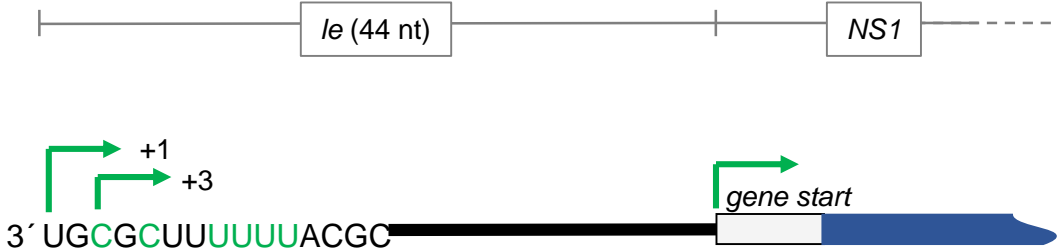
An *in vitro* assay to study RSV RNA synthesis activities



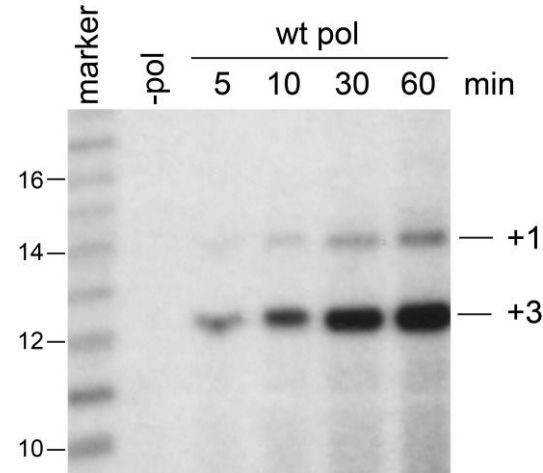
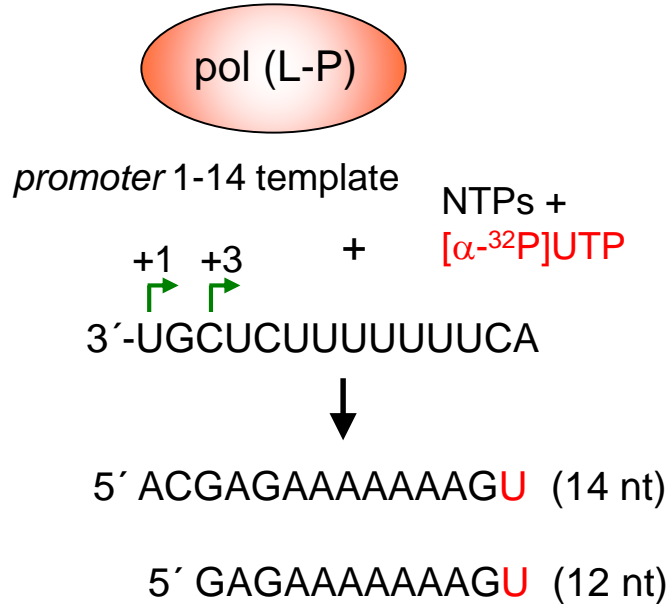
L and P proteins expressed from baculovirus vector



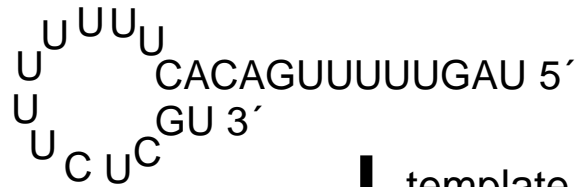
RSV RdRp initiates at positions 1 and 3 in infected cells



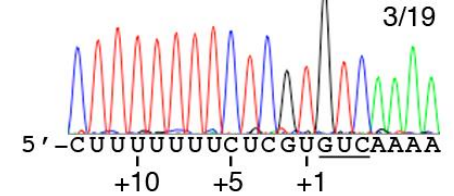
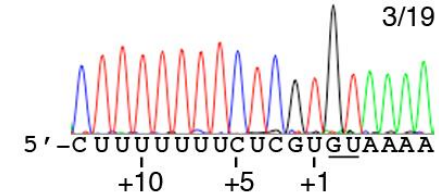
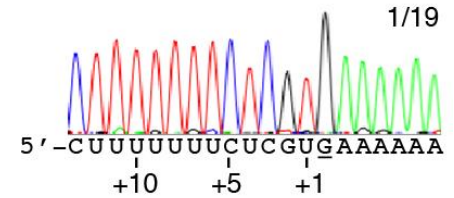
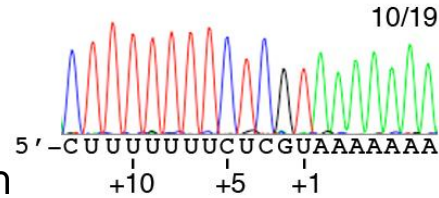
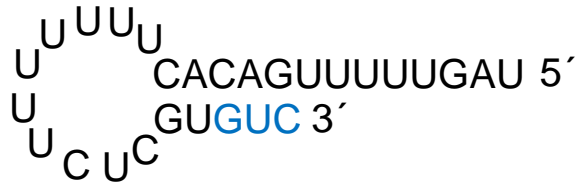
Purified RdRp initiates at +1 and +3 *in vitro*



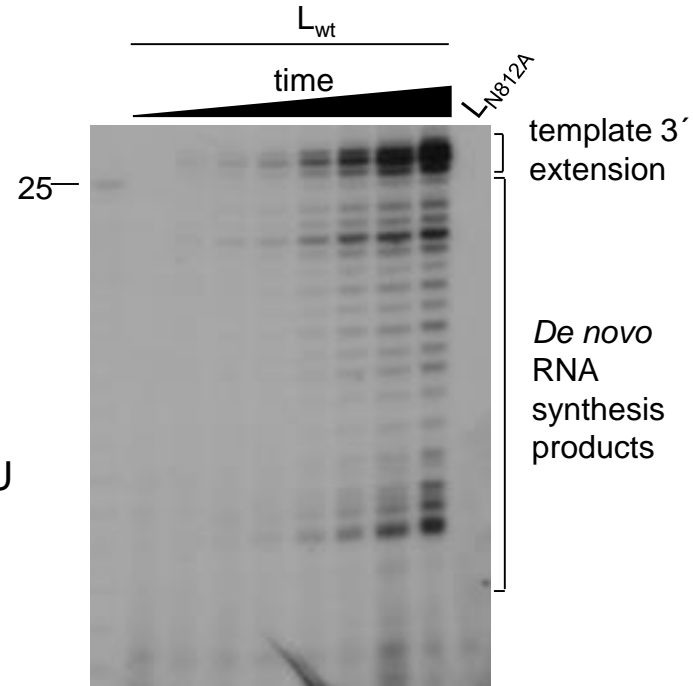
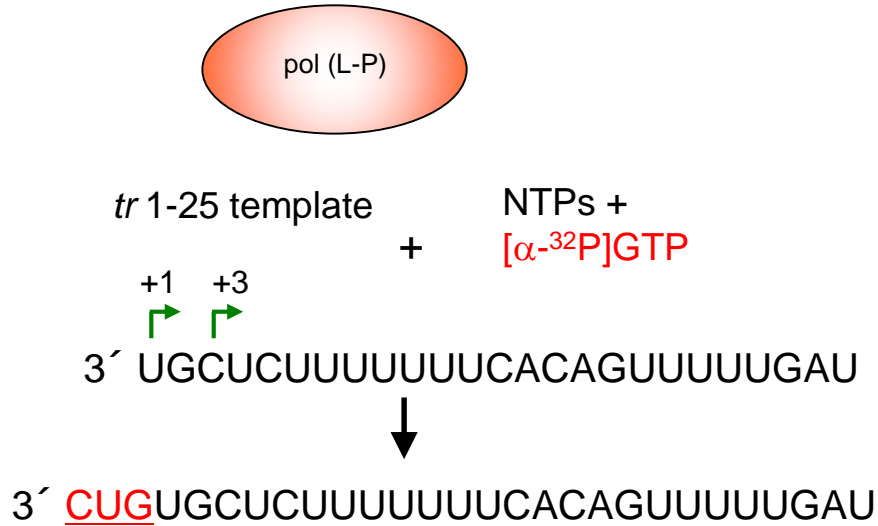
RSV RdRp can perform back-priming and 3' extension in infected cells



template 3' extension



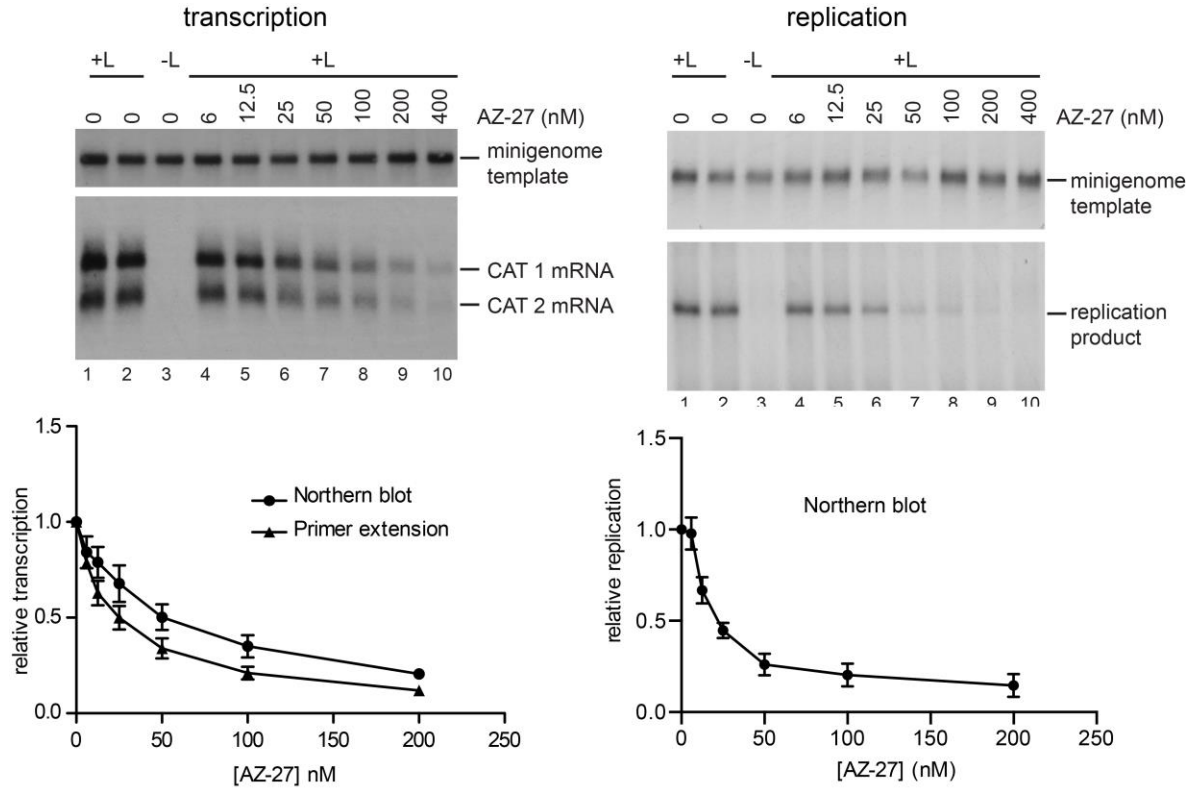
Purified RdRp can perform back-priming and 3' extension *in vitro*



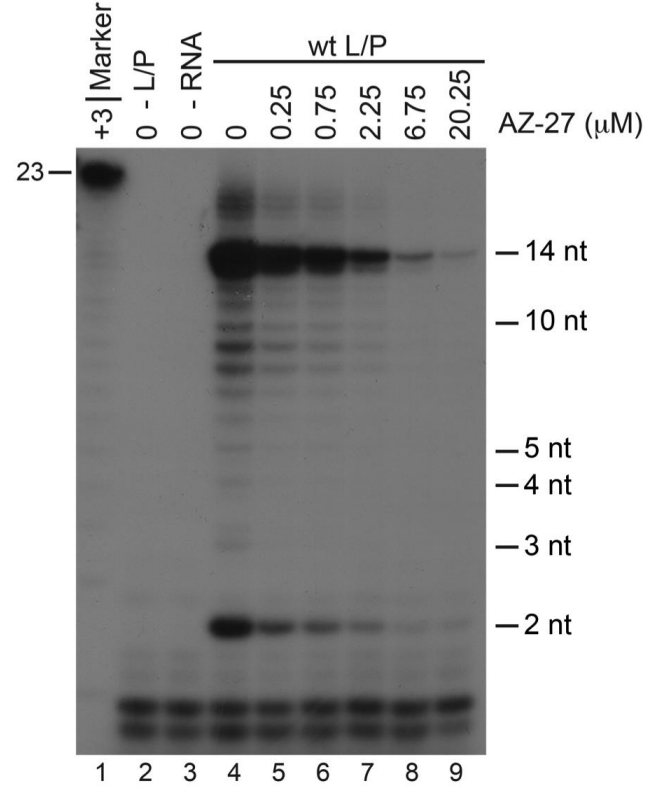
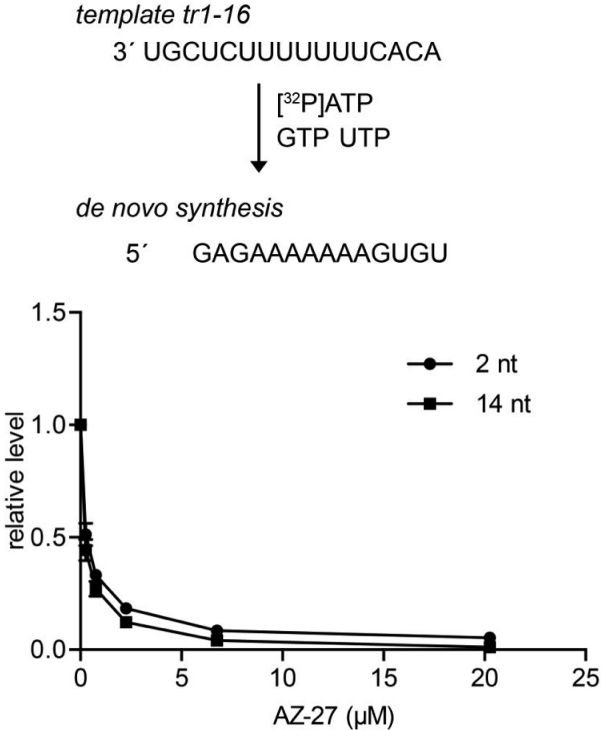
Inhibitor MOA studies (Fearn's lab)

compound	Mechanism of inhibition	reference
AZ-27	Inhibits RNA synthesis initiation, but not elongation, blocks both transcription and replication	Noton et al., J. Virol., 2015
BI-D	Increases processivity, causes abortive RNA synthesis, inhibits capping	Braun, Deflube et al., Plos Pathogens, 2017
GS-5734	Chain terminator (after multiple incorporations)	Warren et al., Nature, 2016

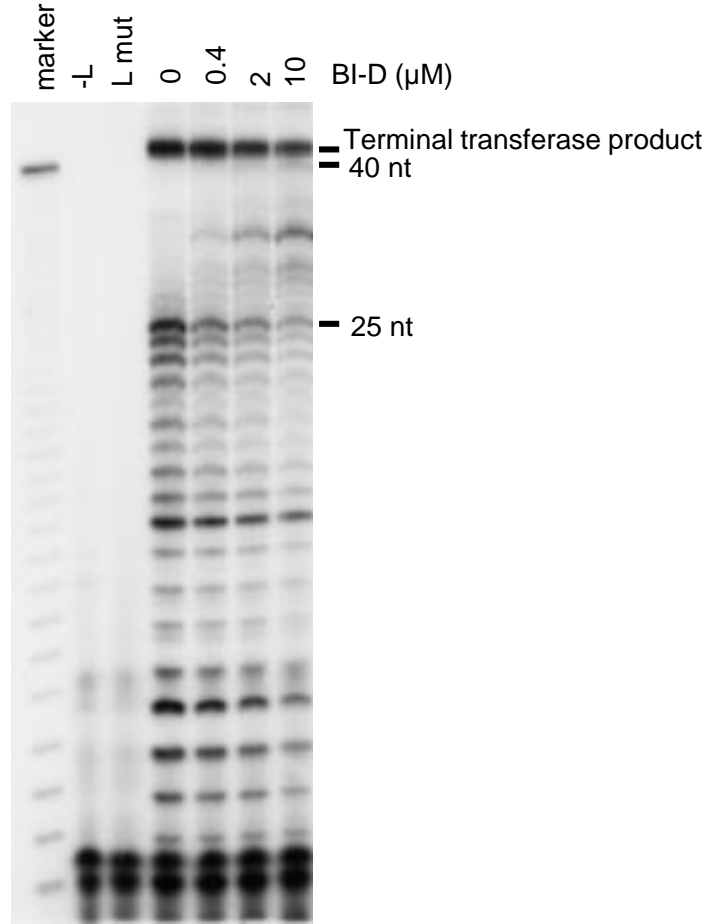
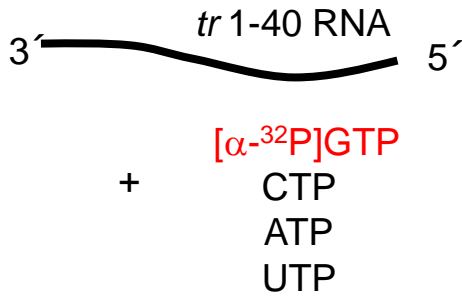
AZ-27 inhibits transcription and replication



AZ-27 inhibits RNA synthesis initiation

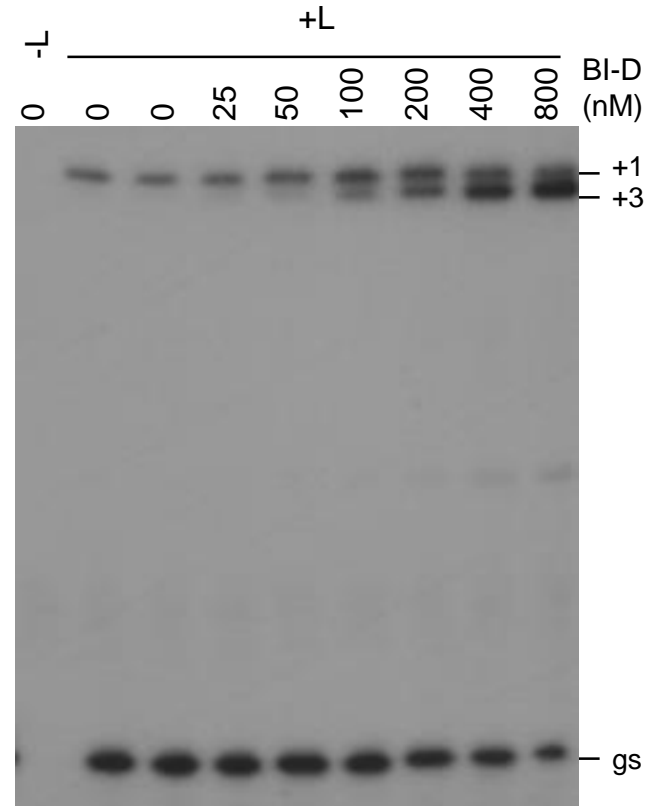
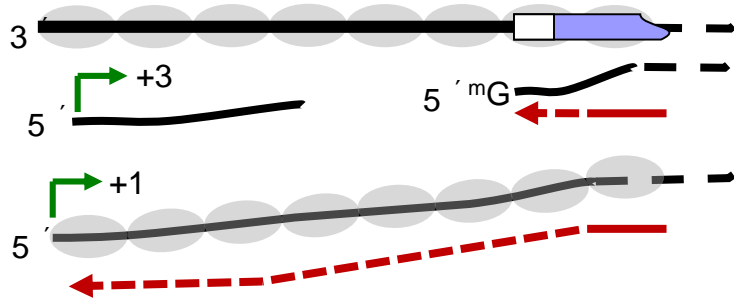


BI-D causes abnormal elongation *in vitro*



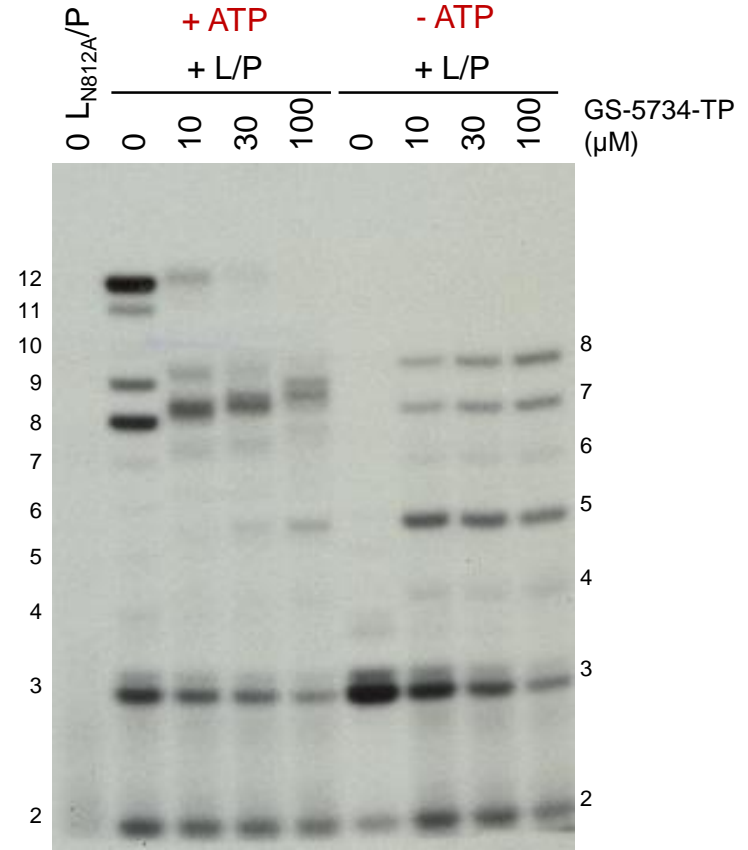
BI-D prevents the polymerase from releasing the RNA

Primer extension analysis

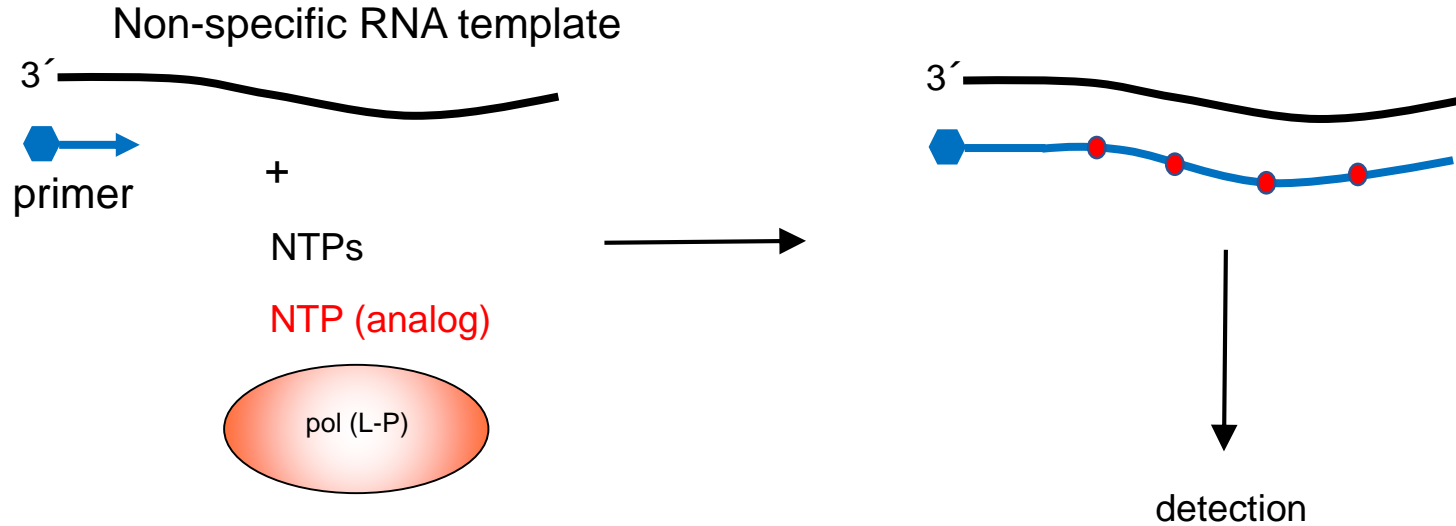


RSV polymerase can incorporate multiple copies of GS-5734-TP

Template: 3' UGCGCUUUUUUACG
Product: 5' GCGAAAAAAUGC

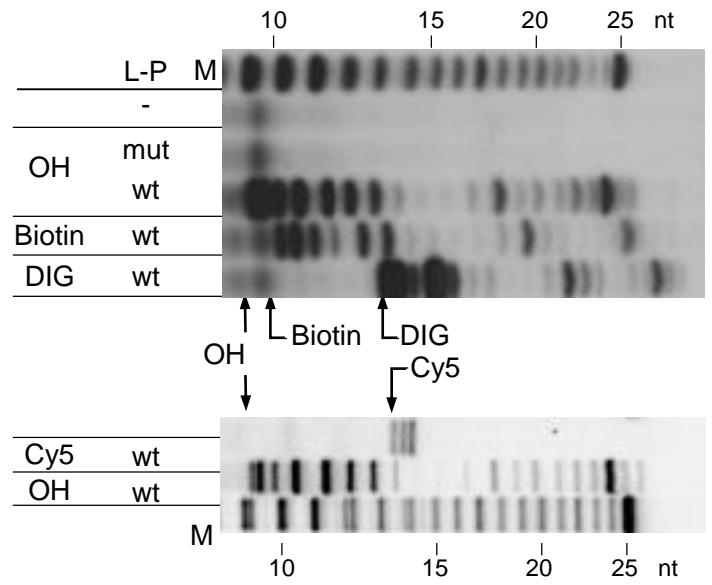
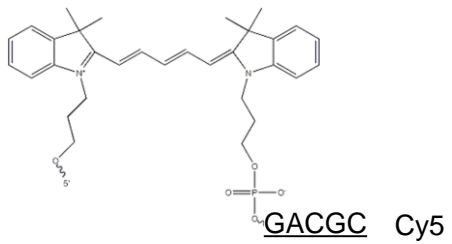
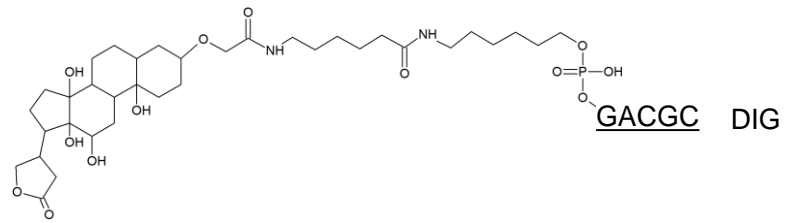
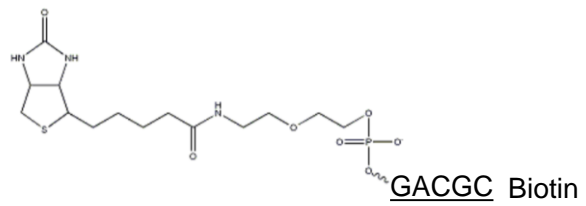


Development of a non-isotopic *in vitro* assay



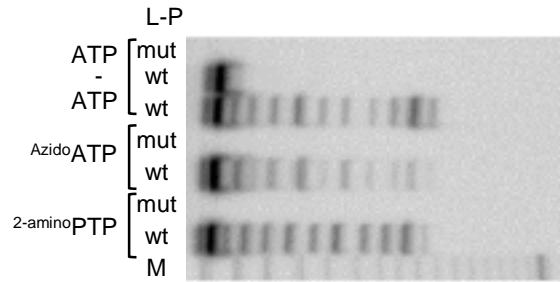
Analysis of primers with 5' modifications

primer

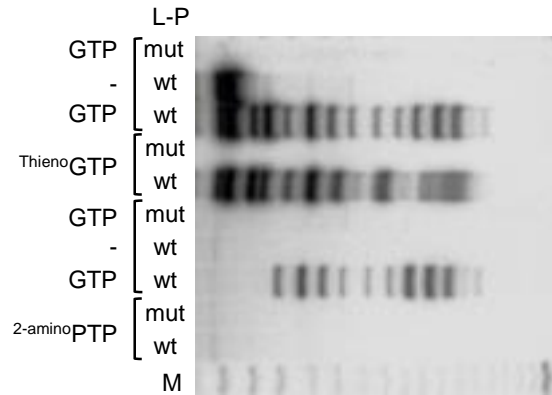


Analysis of NTP analogs

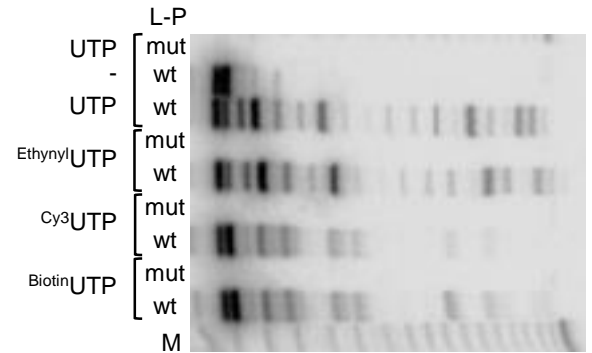
ATP analogs



GTP analogs



UTP analogs



Summary

- RSV polymerase is a promising target
- Challenges lie in the size and complexity of the polymerase, and nature of template
- Cell-based minigenome system and biochemical assay can be used to determine MOI
- Future work is focused on developing non-isotopic enzyme assays

Acknowledgements

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