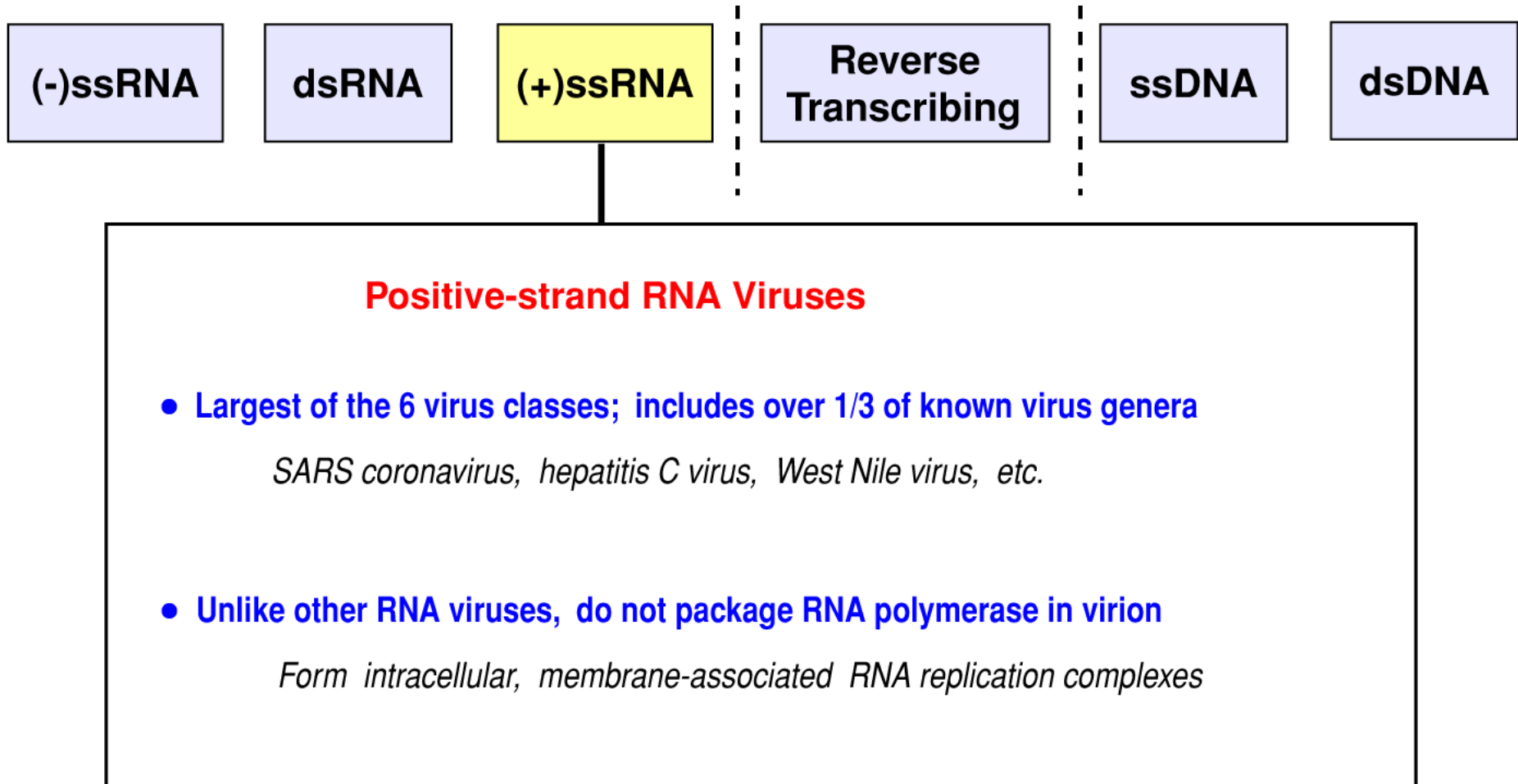


# Functional Genomics of Virus - Host Interactions

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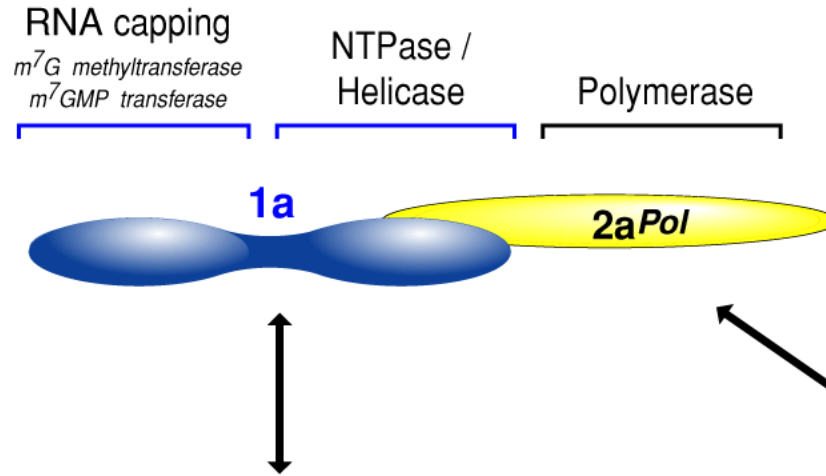
- *New parallels in replication by (+)RNA, retro & dsRNA viruses*
- *Genome-wide cataloging of host genes in (+)RNA virus replication*
- *Roles of Epstein-Barr virus in nasopharyngeal carcinoma*

# Major Classes of Viruses



# BMV RNA Replication

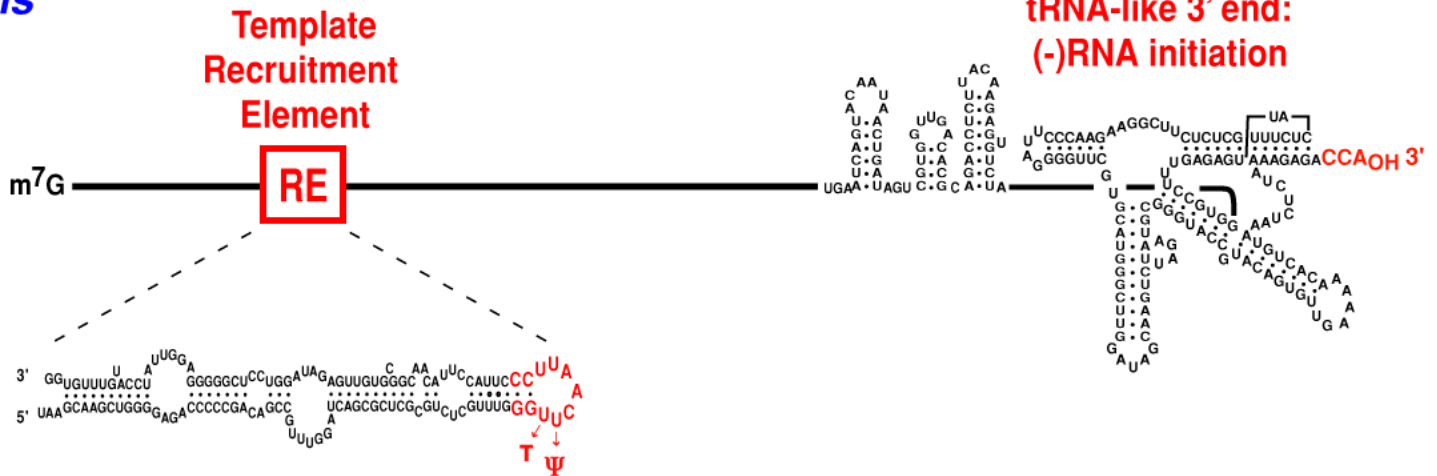
## BMV replication factors 1a and 2aPol



## tRNA-like cis signals

## tRNA-like 3' end: (-)RNA initiation

BMV Genomic RNA



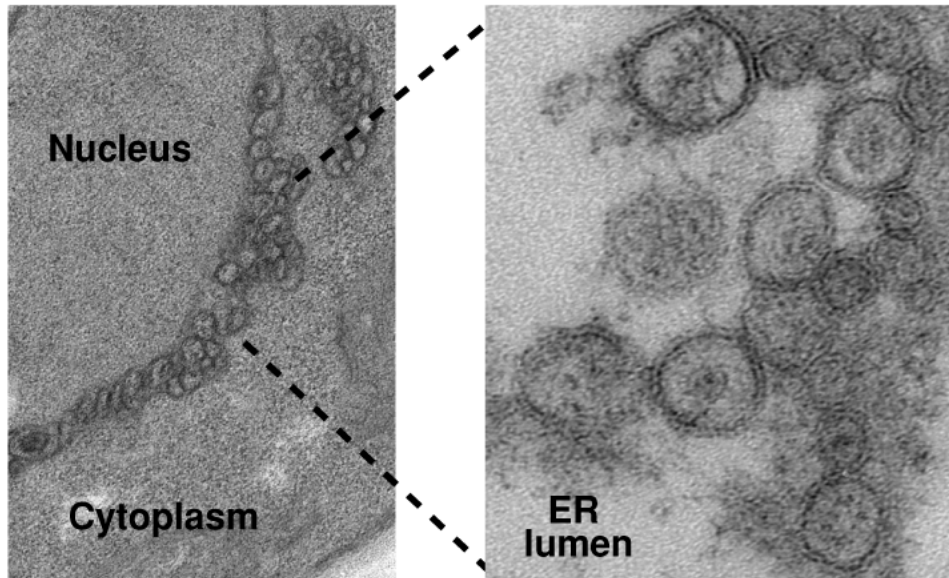
**RNA replication occurs in virus-induced,  
~60 nm membrane invaginations**

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**BMV**

(alphavirus superfamily)

ER membranes



**FHV**

(Nodavirus family)

Outer mitochondrial membranes

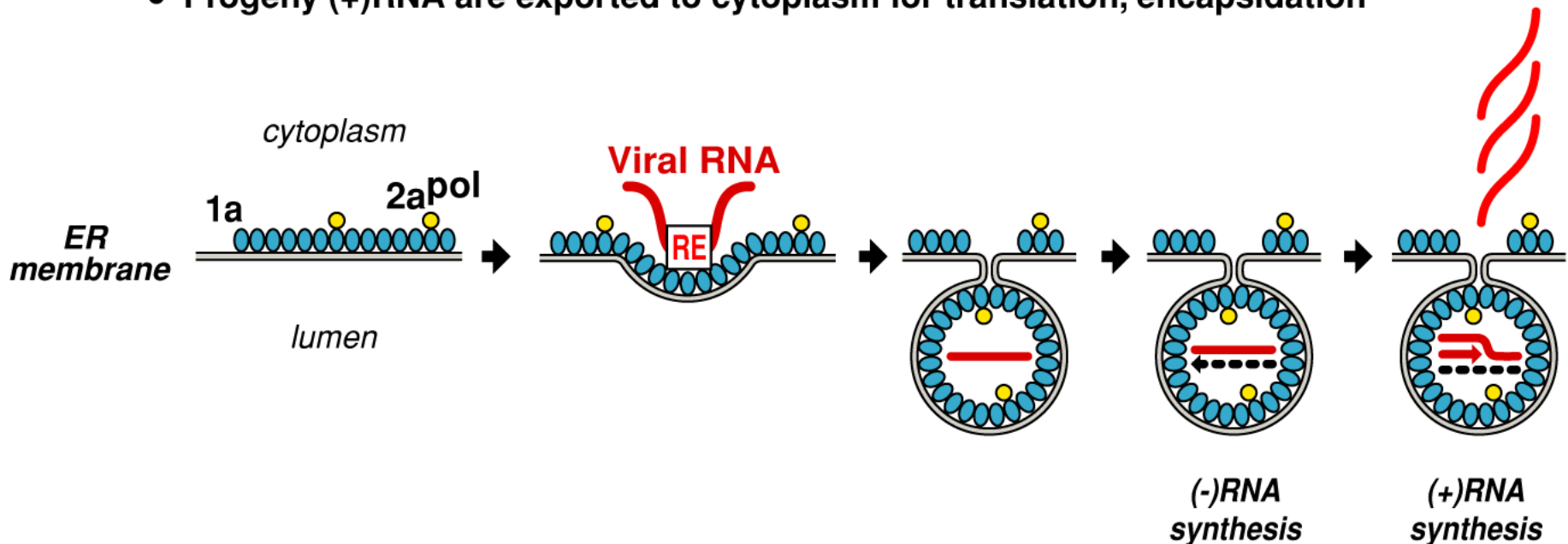


**Immunogold EM shows invaginations are the sites of:**

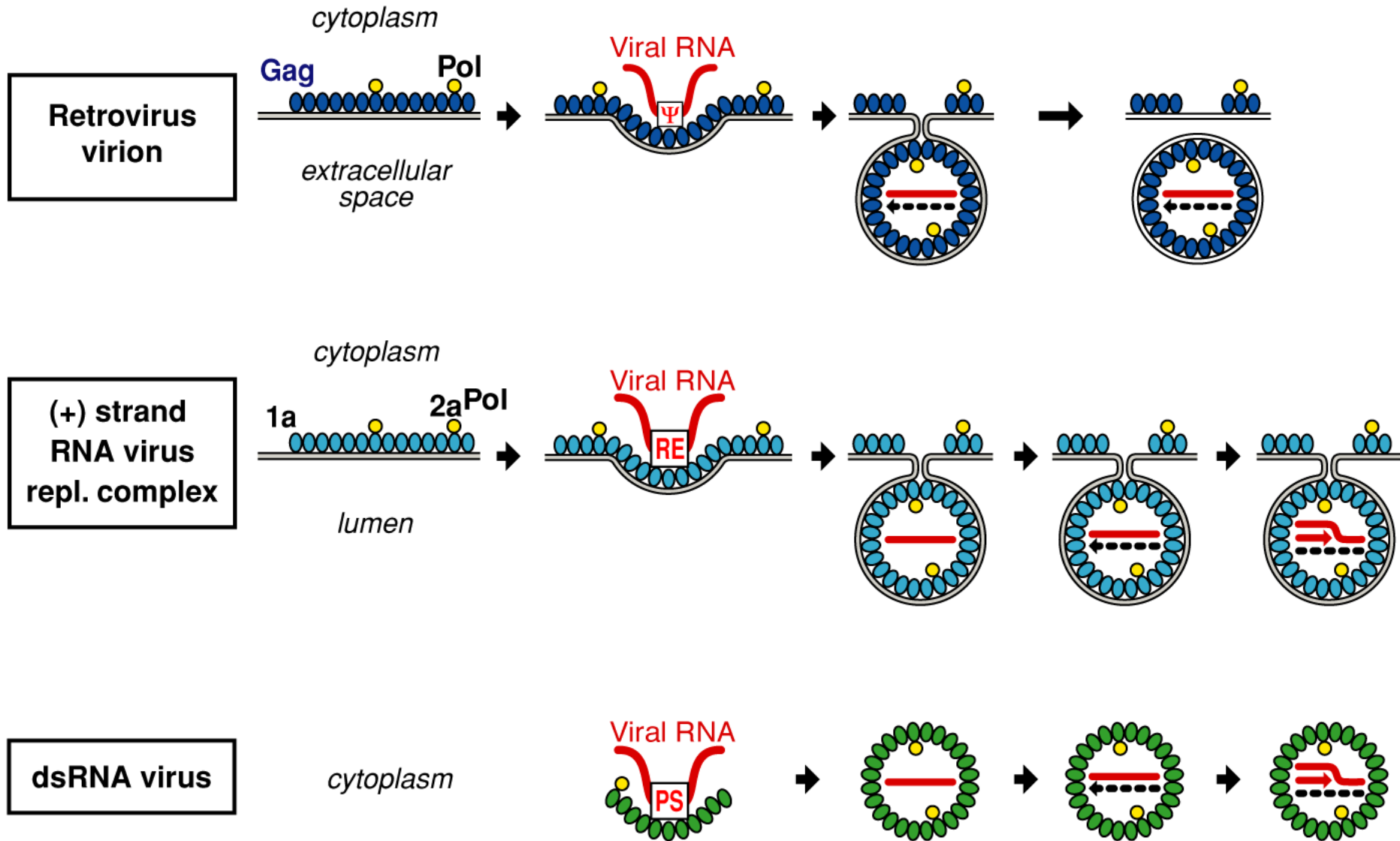
- *Viral RNA replication factors*
- *Virus-specific BrUTP incorporation into RNA*

# RNA Replication Complex Assembly and Function

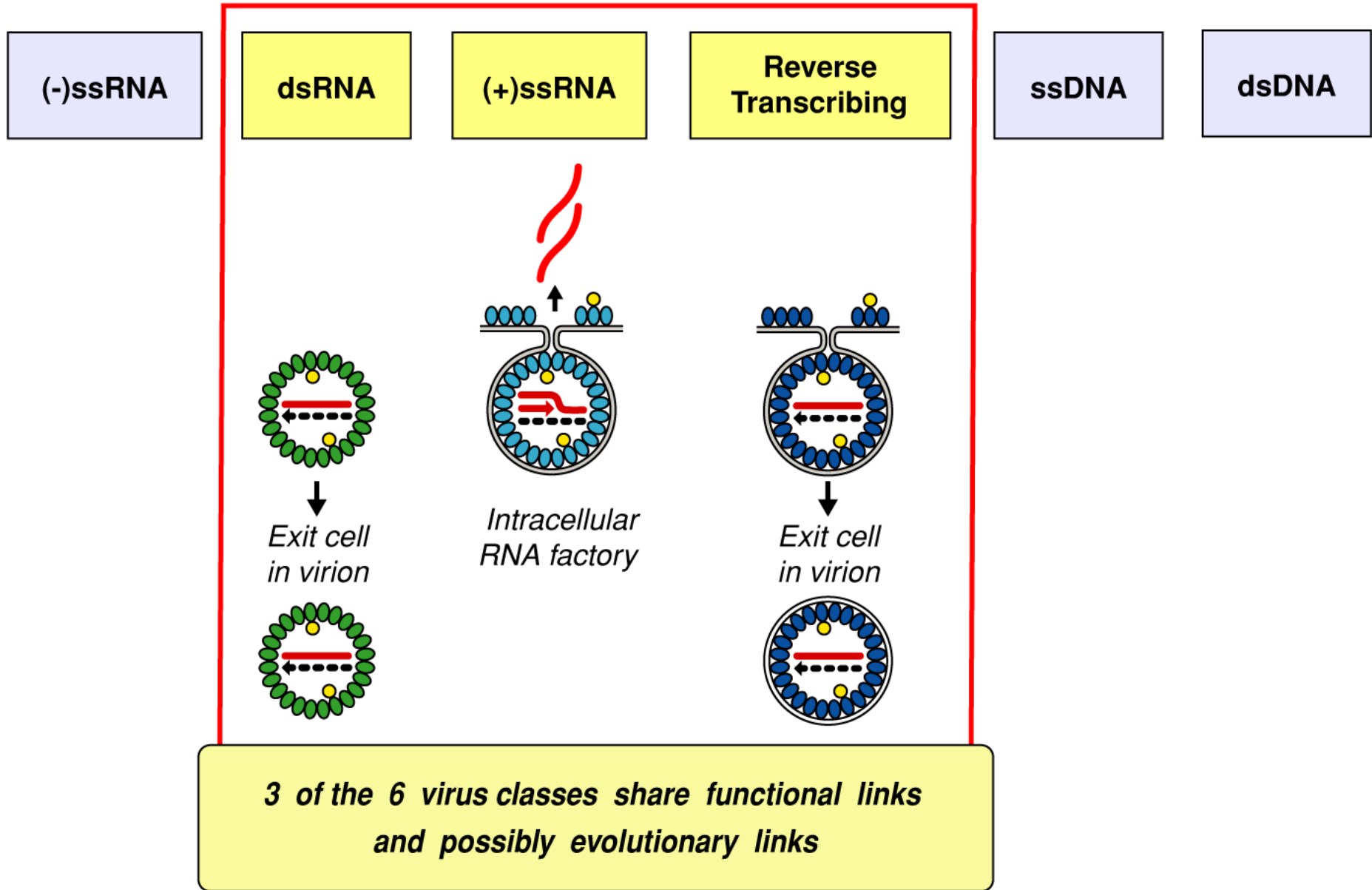
- 1a localizes to ER membranes & invaginates spherules containing 200-400 1a's
- 1a uses cis-acting RE as packaging signal to sequester viral RNA templates
- 2a<sup>Pol</sup> interacts with 1a and co-localizes to spherules, which become the sites of (-) and (+) RNA synthesis
- Progeny (+)RNA are exported to cytoplasm for translation, encapsidation



# Parallels in (+)RNA, Retro and dsRNA Virus Replication



# New Parallels in (+)RNA, Retro and dsRNA Virus Replication



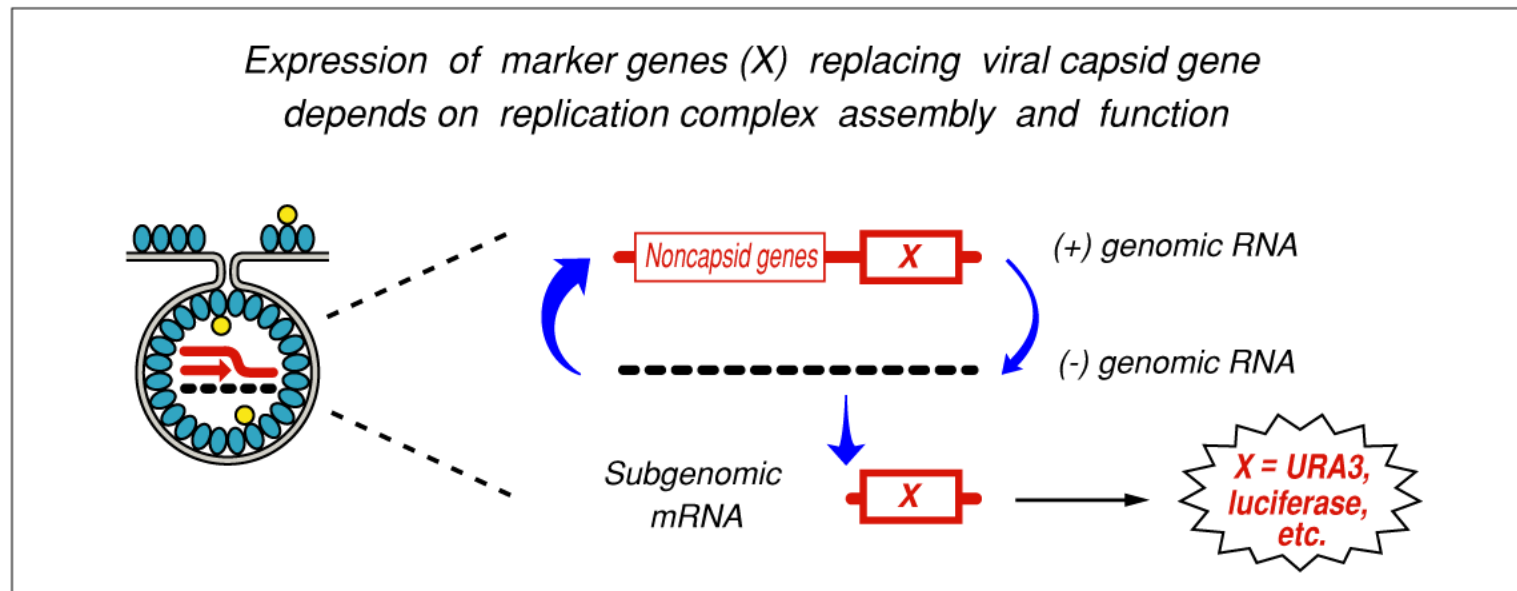
## Host Genes Required for Virus Replication

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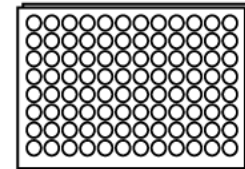
- **Most steps in virus replication depend on interaction of virus factors with largely undetermined host factors**
- **Virus - host interactions are crucial determinants of virus replication, host range and pathology**
- **Virus dependence on host functions offers important avenues for virus control and new insights to cell functions**

## Yeast Genetic Analysis of Host Factors in Virus Replication

- *Yeast support natural pathways of RNA replication, gene expression and virion assembly for two model (+)RNA viruses, BMV and FHV*
- *Selectable and counter-selectable phenotypes can be tightly linked to BMV and FHV RNA replication*



# Systematic Analysis of Host Gene Effects on Viral RNA Replication



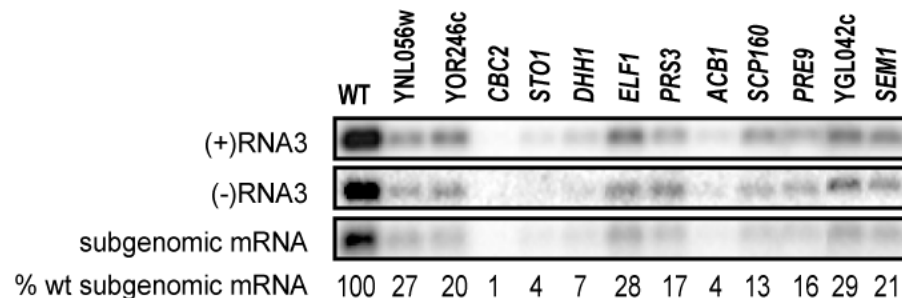
- **Ordered Yeast Deletion Strain Array**

- ~4800 viable deletion strains ordered on 96- or 384-well plates
- Each strain has one pre-selected ORF replaced by Kan<sup>R</sup> cassette
- Allows testing 80% of all ~6000 yeast genes

- **Even under highly permissive conditions for BMV replication,**

**58 deletions inhibited** virus replication 3-fold to >50-fold

**39 deletions stimulated** virus replication 3-fold to >10-fold



# Host Functions Contribute to Numerous Steps in Virus Replication

Infesting virion RNA

## 1. Regulated viral translation

*LSM1-LSM7, PAT1, DHH1, DED1, RPL19b, RPA1, RPA34, RRN3*

## 2. Protein targeting, regulated stability

*SCS2, PRE9*

## 3. RNA template recruitment from translation to replication

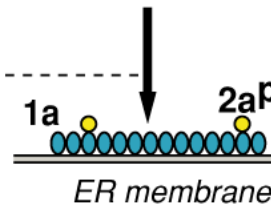
*LSM1-LSM7, PAT1, DHH1, SCP160*

## 5. Chaperone activation of replication complex

*YDJ1 (HSP70,90)*

## 6. Survival, fate of progeny (+)RNA

*SKI2,3,7,8*

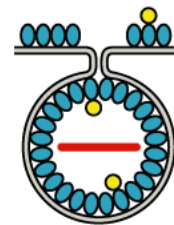


Viral RNA

RE

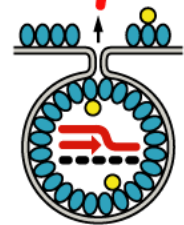
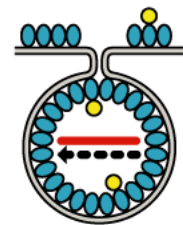
## 4. Membrane synthesis, trafficking, lipid composition

*OLE1, ACB1, DRS2, RCY1, NEM1, SPO7*



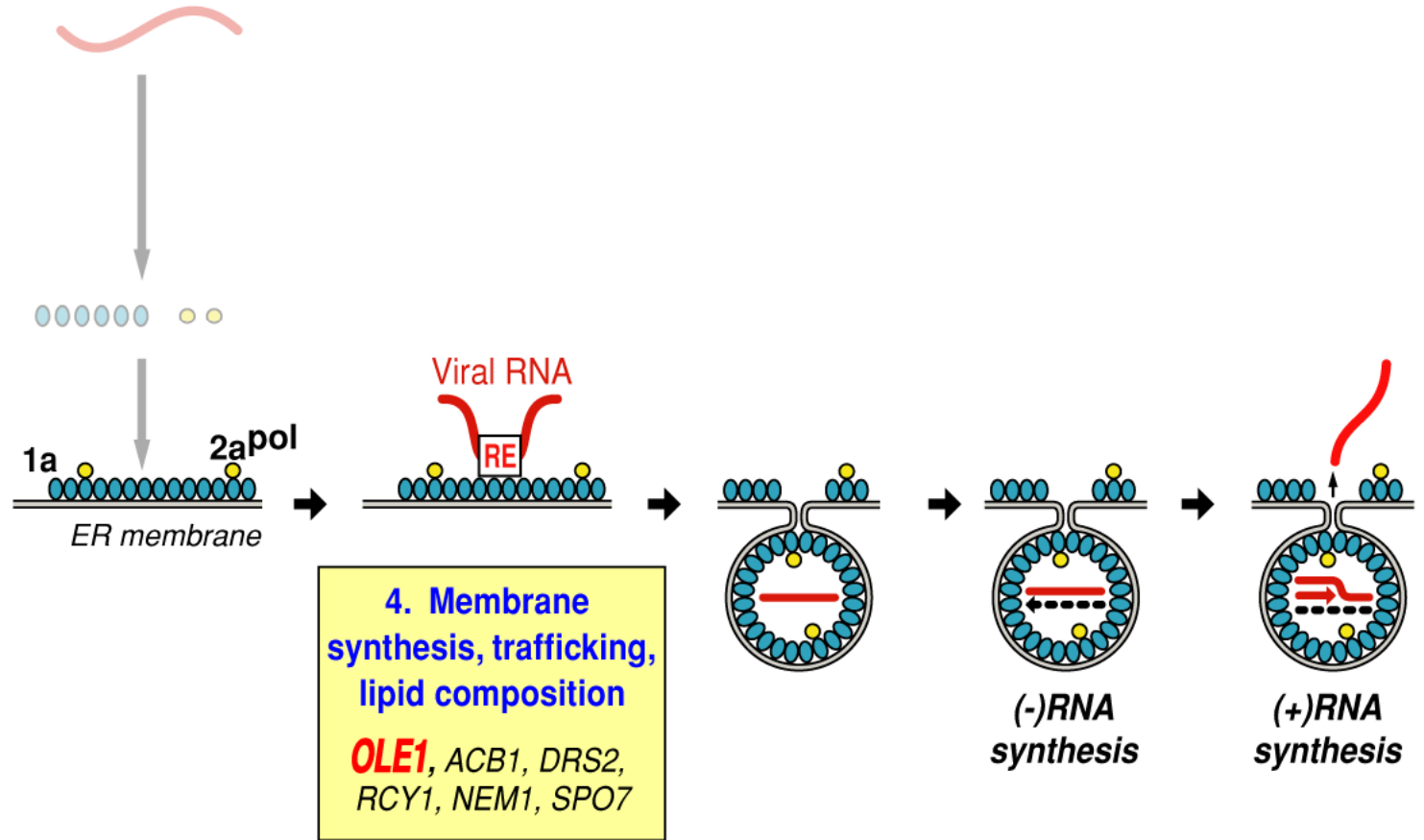
(-)RNA synthesis

(+)RNA synthesis



# Membrane Lipid Requirements for RNA Replication

Infecting virion RNA

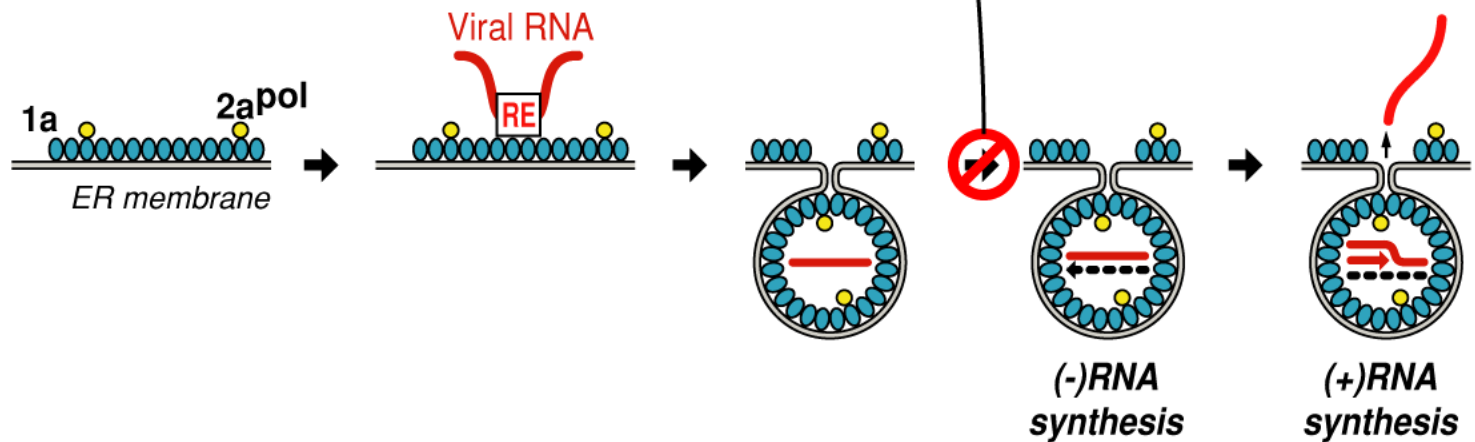


# Membrane Lipid Requirements for RNA Replication

*RNA replication is blocked by mutating **OLE1***

*$\Delta 9$  fatty acid desaturase*

*Key regulator of unsat'd: sat'd fatty acids*

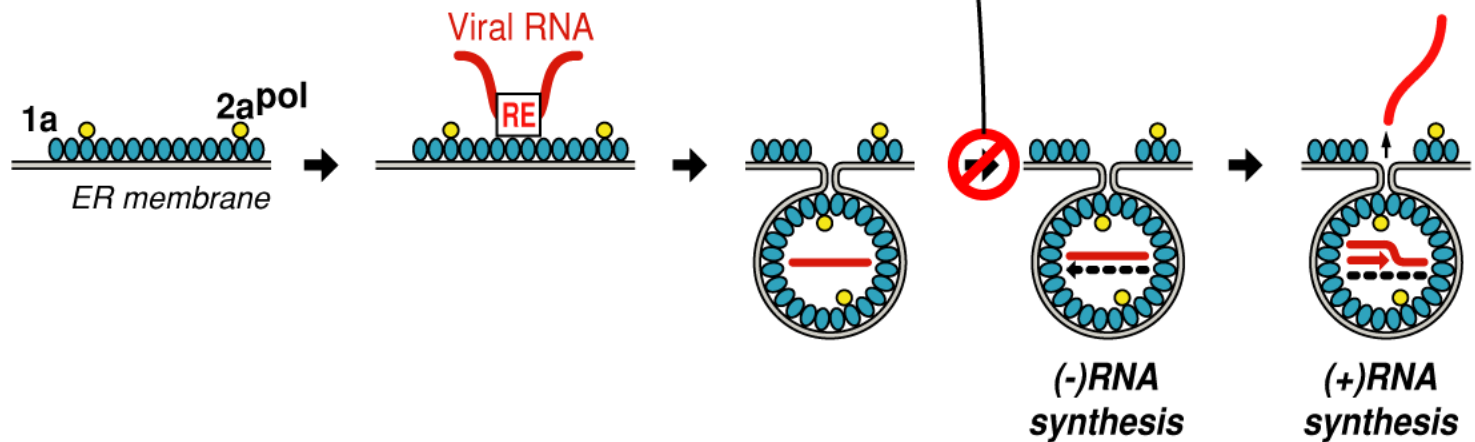


*RNA replication is highly sensitive  
to membrane lipid composition*

# Membrane Lipid Requirements for RNA Replication

## High lipid sensitivity = new class of antiviral targets

- (+)RNA, retro & other viruses all depend on membranes
- OLE1 (SCD1) inhibitors are in pharma development for heart disease, diabetes



# Summary

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- **Systematically testing 80% of yeast genes identified >100 genes that support or inhibit BMV RNA replication**
- **Diverse host genes and pathways are crucial for**
  - *Expression and activation of viral proteins*
  - *Assembly and function of RNA replication complexes*
  - *Survival and fate of progeny RNA*
- **All virus - host interactions are potential targets for viral control**

**Functional Genomics of  
Nasopharyngeal Carcinoma**

## Nasopharyngeal Carcinoma (NPC)

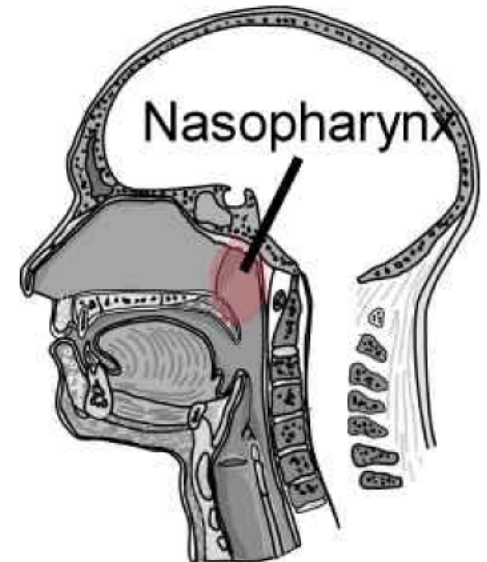
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- **Cancer of the epithelial lining of nasopharynx**

*~70,000 NPC deaths / year*

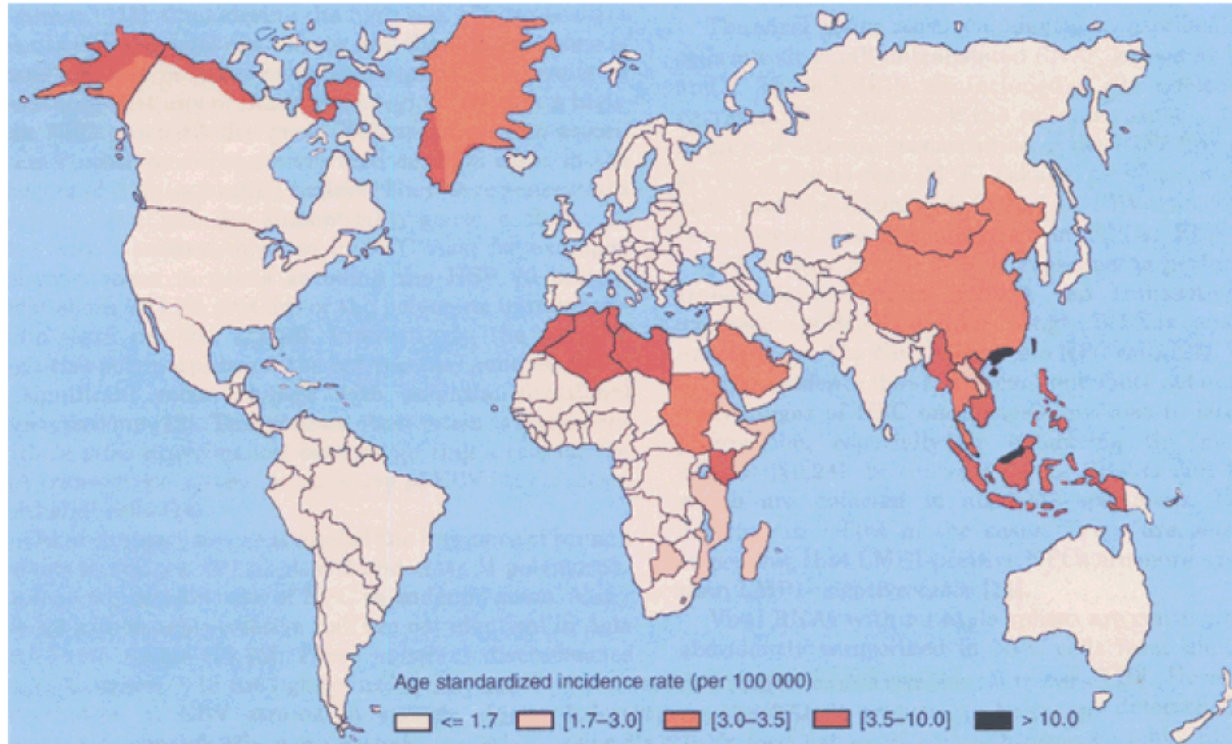
- **The major NPC type (non-keratinizing) is**  
**~always positive for Epstein-Barr virus (EBV)**

*EBV is a widespread herpesvirus  
causing lymphomas and carcinomas*



## Nasopharyngeal Carcinoma (NPC)

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In S China,  
NPC incidence  
>25-fold higher  
than world average

**In addition to EBV, NPC risk factors include**

- *HLA-A2 subtype, prevalent among Chinese*
- *Dietary nitrosamines in S Chinese diet*

# Summary

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- *Unexpected parallels link (+)RNA virus replication complexes with retrovirus and dsRNA virus virions*
- *Global knockout screening identifies >100 diverse host genes that modulate varied steps in (+)RNA virus replication*
- *Epstein-Barr Virus interacts with other risk factors to promote nasopharyngeal carcinoma*

**RNA Replication  
Complex Structure,  
Assembly, & Function**

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**Michael Schwartz**

**Chen Jianbo**

**Wang Xiaofeng**

**Michael Janda**

**Ben Kopek**

**RNA Virus  
Host Genes**

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**David Kushner**

**Amine Noueiry**

**Lee Waiming**

**Brandi Gancarz**

**Hao Linhui**

**EBV and  
Nasopharyngeal  
Carcinoma**

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**Srikumar Sengupta**

**Johan den Boon**

**Bill Sugden**

**Allan Hildesheim (NCI)**

**Chen Chienjen**



