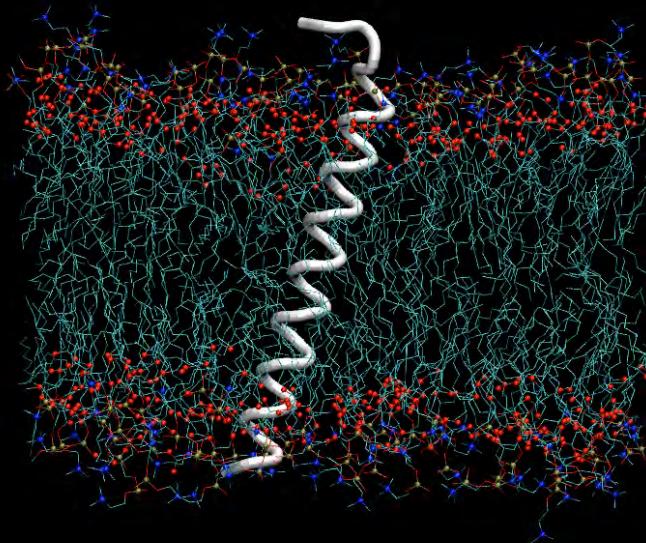


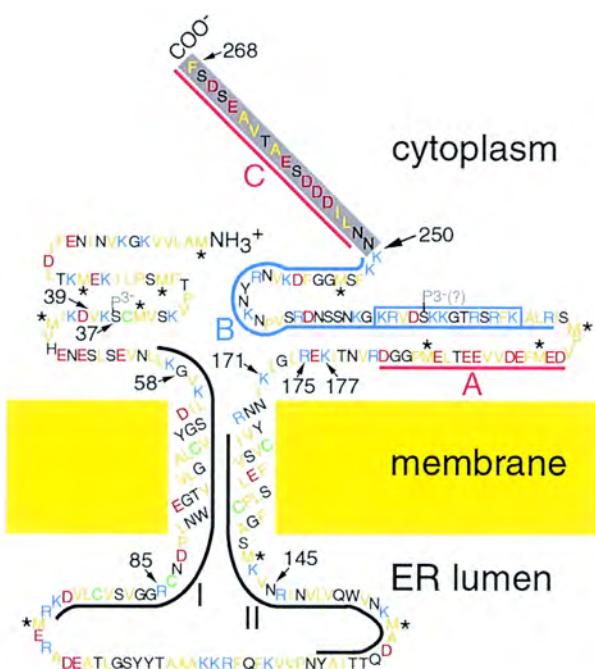
# Plant virus transport: what's the membrane's role?



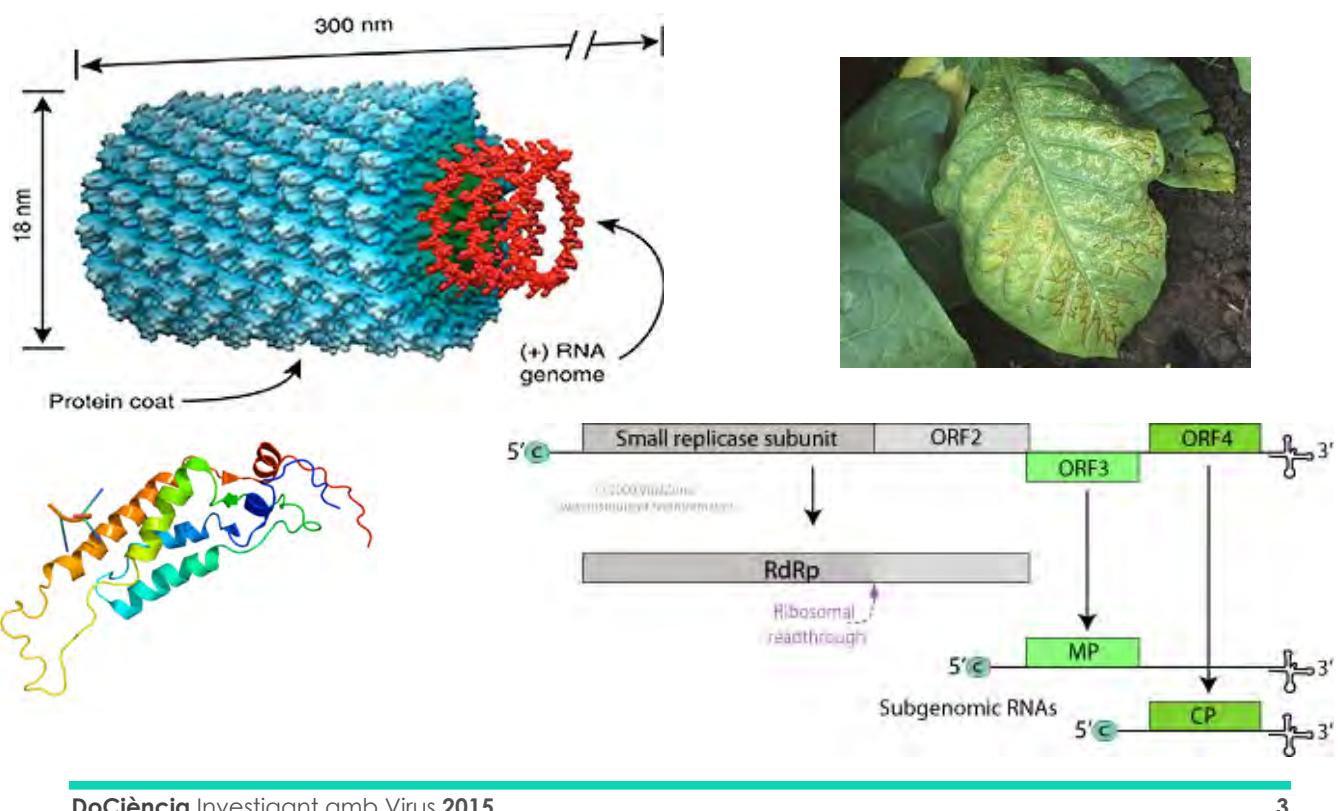
UNIVERSITAT  
DE VALÈNCIA

Ismael Mingarro  
**Membrane Proteins Lab**  
University of Valencia

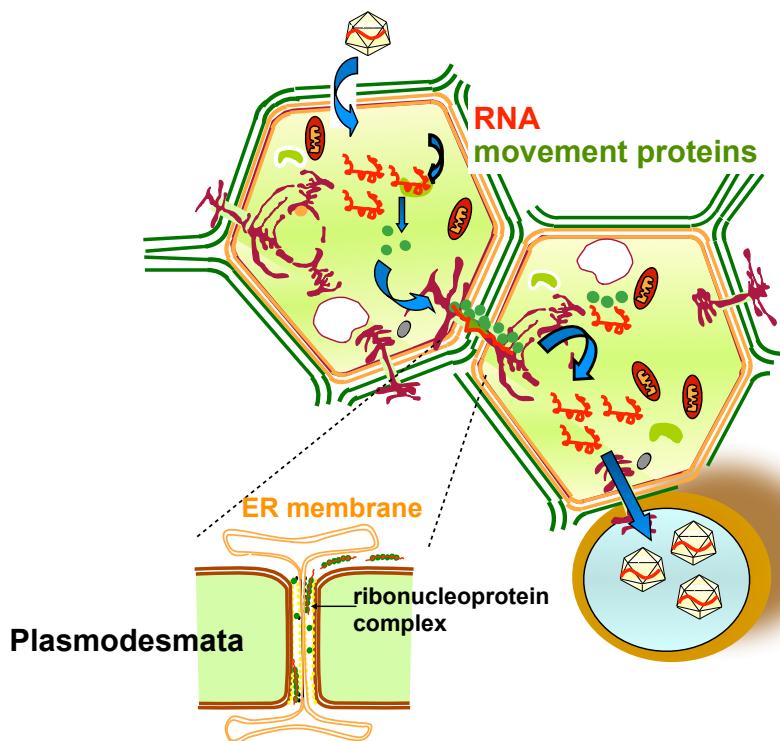
## Why did we get interested in Plant Virus?



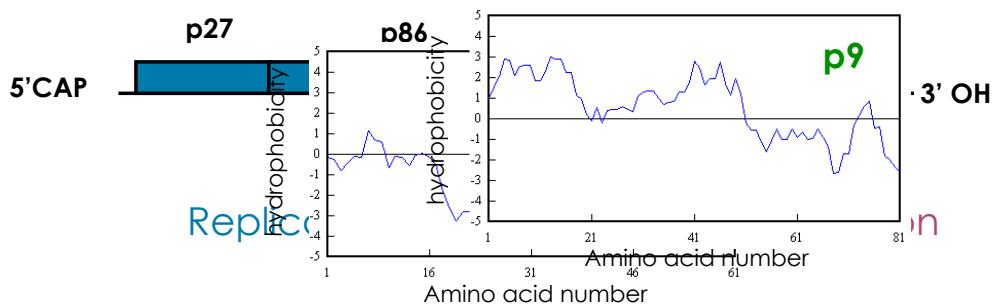
# Tobacco Mosaic Virus



## Plant virus infection



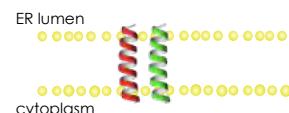
# Carnation Mottle Virus (CarMV) genome and movement proteins characterization



Vilar et al. (2002) J. Biol. Chem. **277**, 23447

Vilar et al. (2001) JBC **276**, 18122

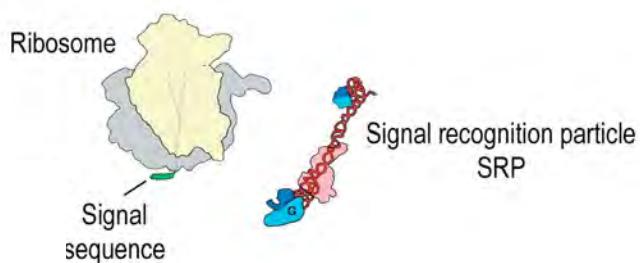
Vilar, Saurí et al. (2005) ChemBioChem **8**, 1396



DoCiència Investigant amb Virus 2015

5

## Targeting of membrane and secreted proteins



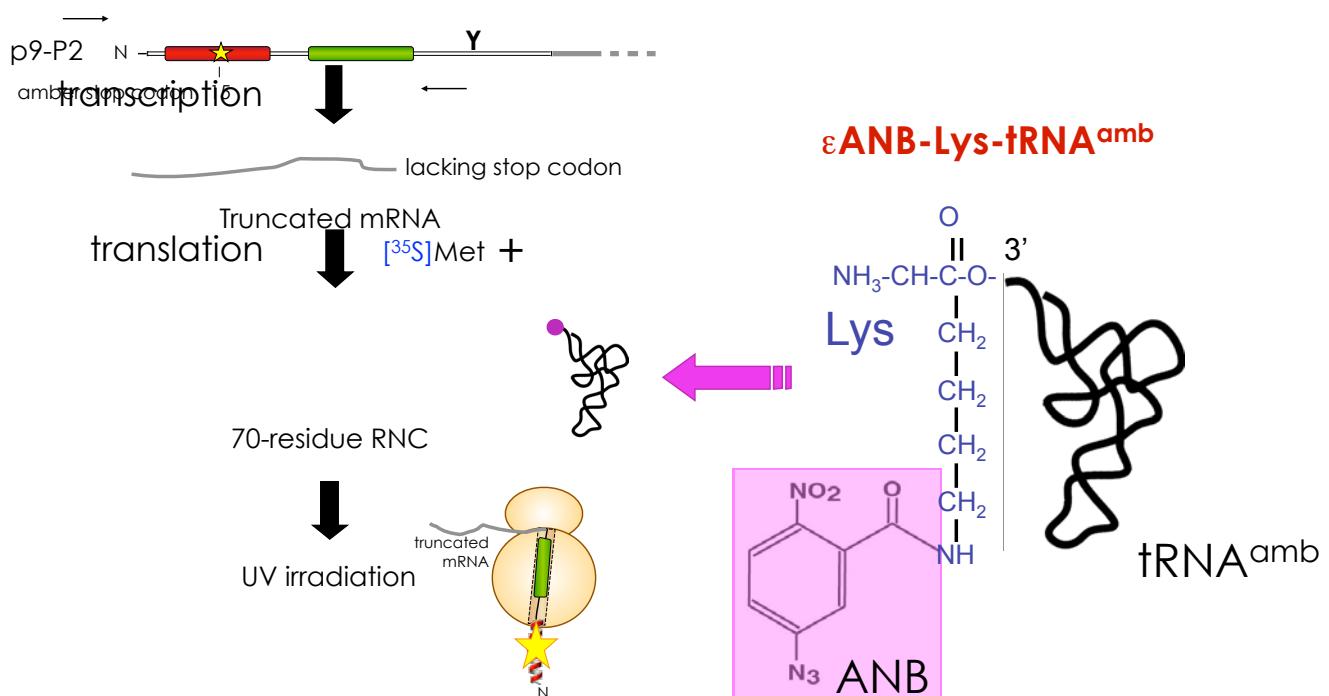
Modified from Halic & Beckmann (2005) Curr. Op. Struct. Biol., **15**, 116

DoCiència Investigant amb Virus 2015

6

# Site-directed photocrosslinking approach

Johnson & van Waes (1999) Annu. Rev. Cell Dev. Biol. 15, 799



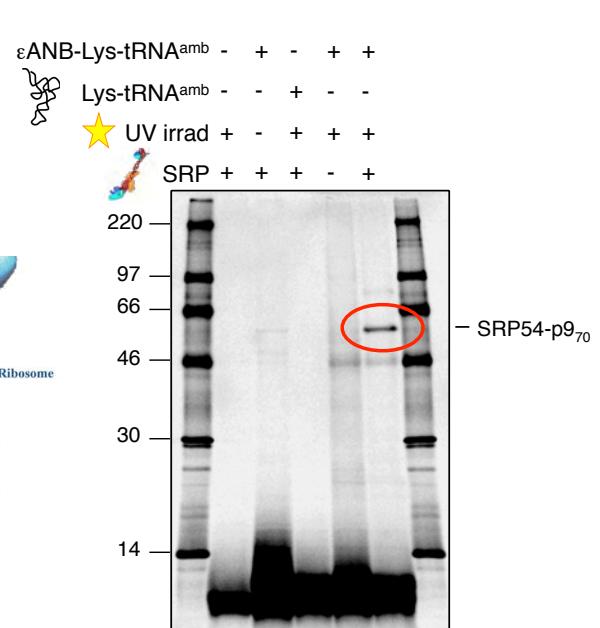
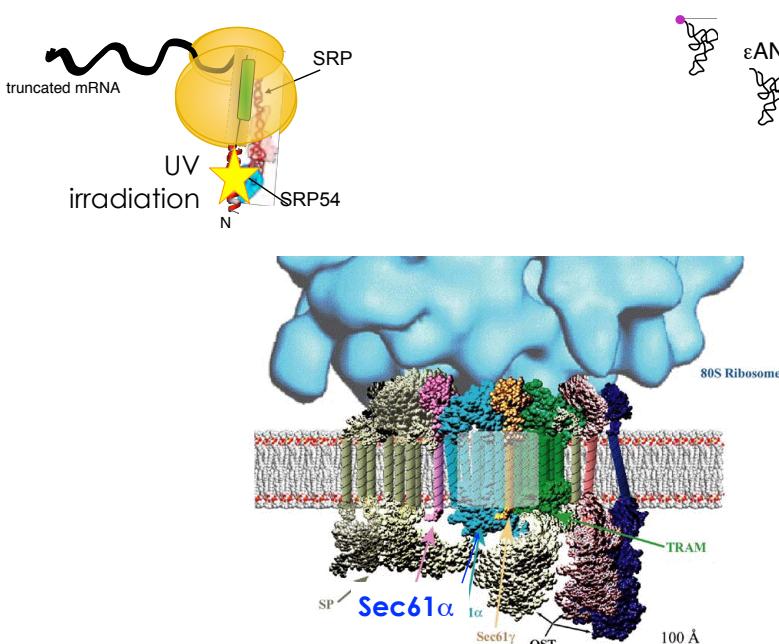
DoCiència Investigant amb Virus 2015

7

## p9 integrates in an SRP-dependent manner



Nt -MA<sup>70</sup>YGPSVNLHHLIVLKGVIGLMILLRLRCTFTSFLPPLVTLNQIIALSFCGLLLNSISRAERACYYDYS...



Saurí et al. (2005) J. Biol. Chem. 280, 25907

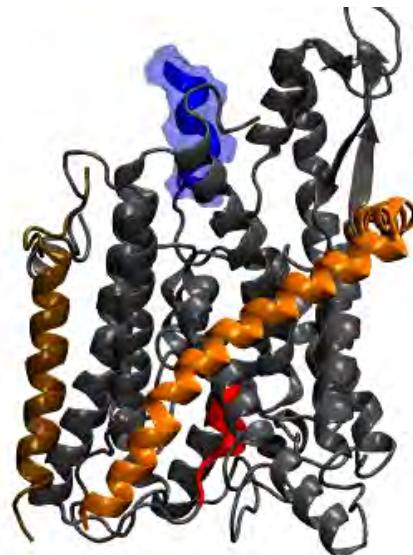
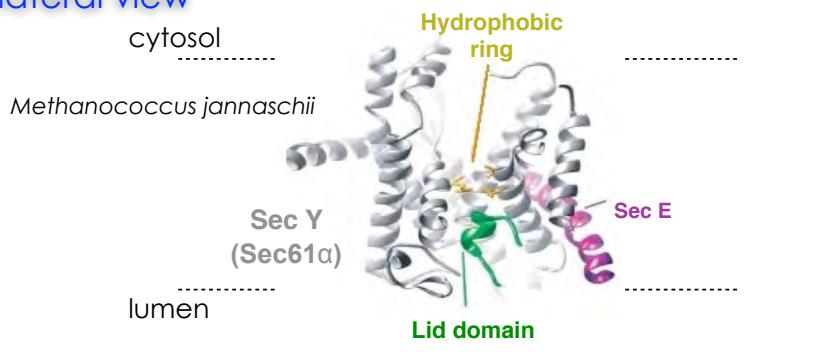
DoCiència Investigant amb Virus 2015

8

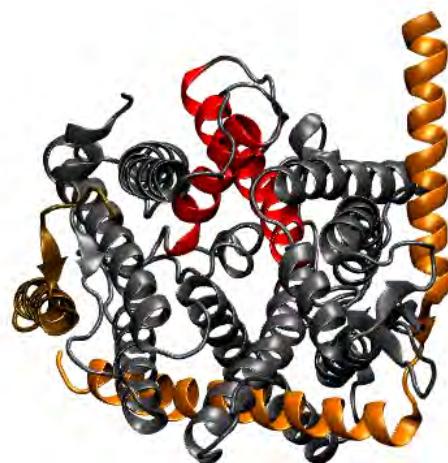
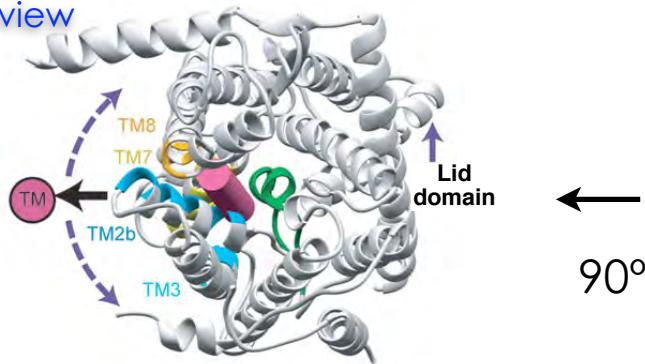
# Translocon structure

van den Berg et al. (2004) *Nature* **427**, 36

**lateral view**



**bottom view**



Gumbart & Schulten (2006) *Biophys. J.* **90**, 2356

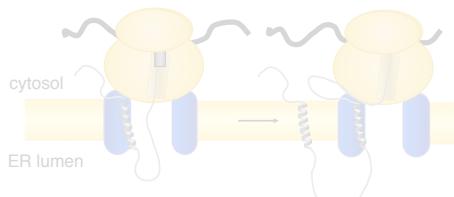
DoCiència Investigant amb Virus 2015



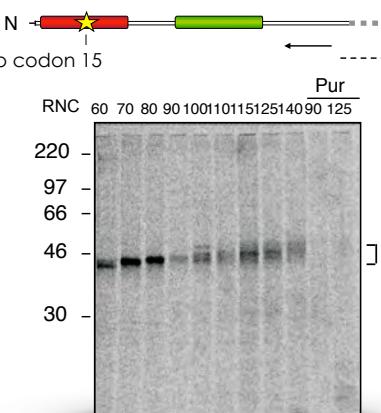
## The two TM segments partition into the membrane in a concerted manner

Models of polytopic membrane protein integration

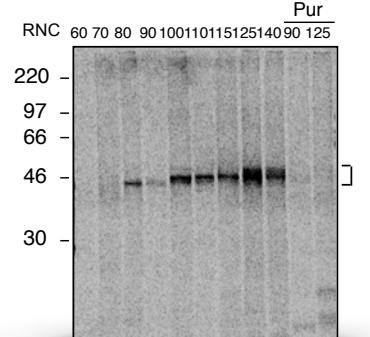
**Sequential integration**



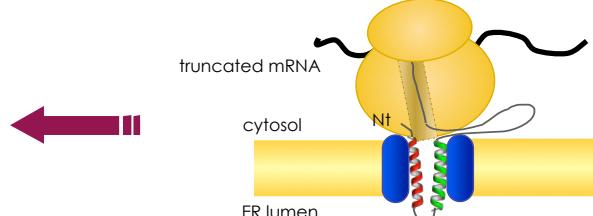
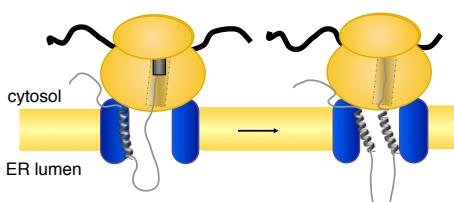
**Sec61 $\alpha$  IP**



N → red box → green box → stop codon 49 → Purine (Pur)



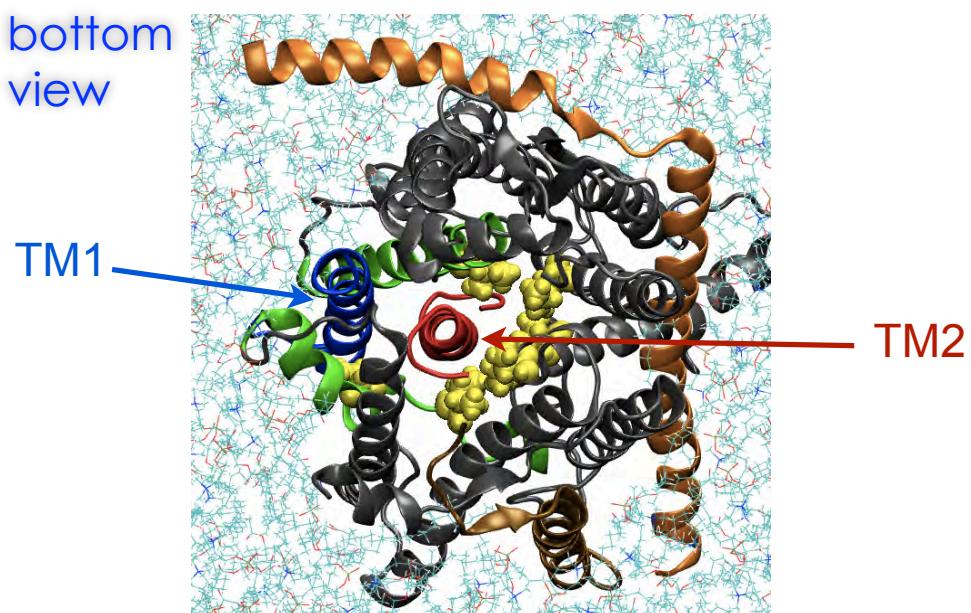
**Concerted integration**



Saurí et al. (2005) *J. Biol. Chem.* **280**, 25907

DoCiència Investigant amb Virus 2015

# but, can two helices fit?

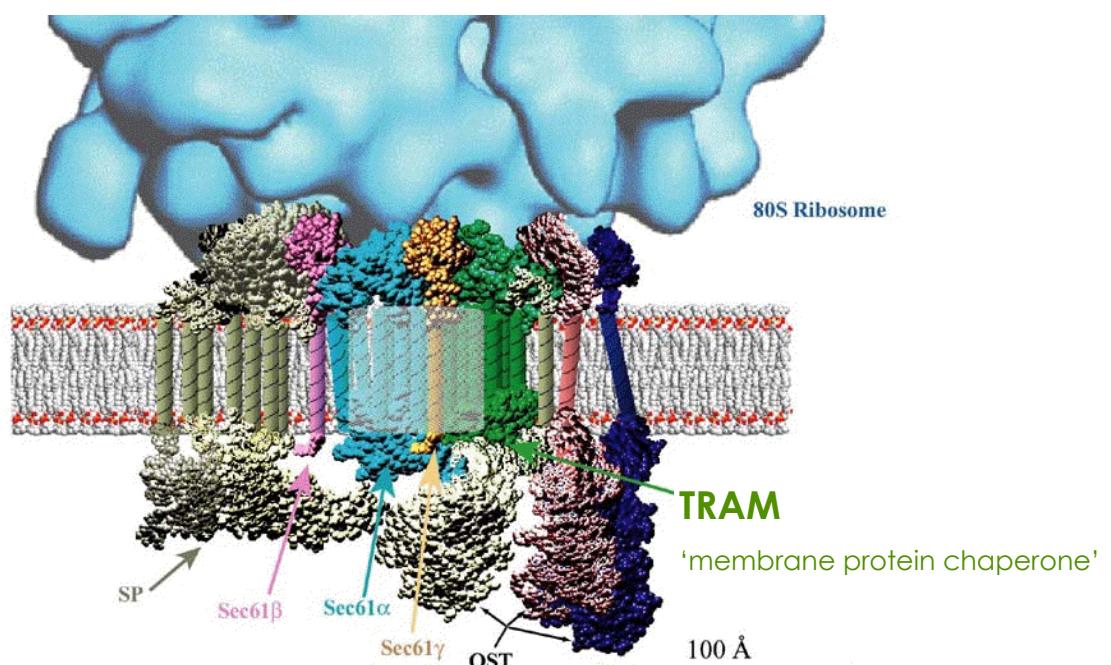


James Gumbart, Univ Chicago (personal communication)

DoCiència Investigant amb Virus 2015

11

## The translocon is a multiprotein complex

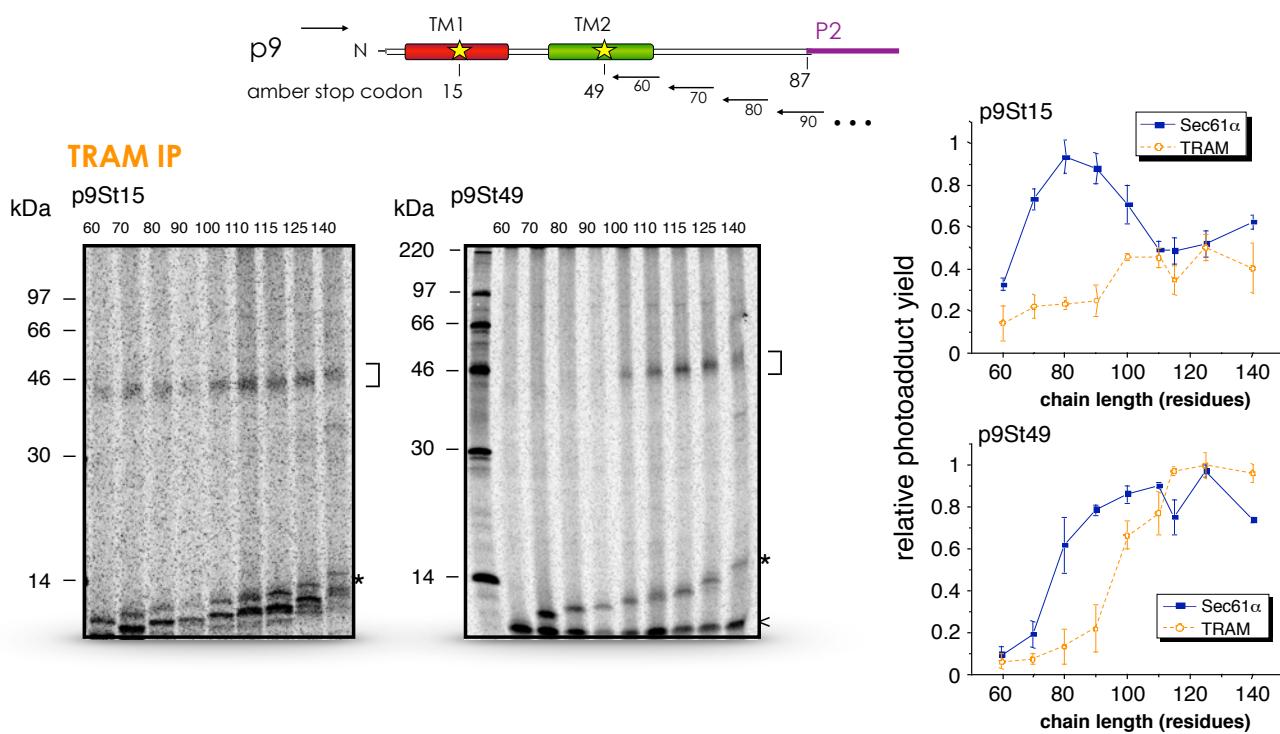


Taken from Johnson & van Waes (1999) Annu. Rev. Cell Dev. Biol. **15**, 799

DoCiència Investigant amb Virus 2015

12

# CarMV p9 is sequentially adjacent to Sec61 $\alpha$ and TRAM

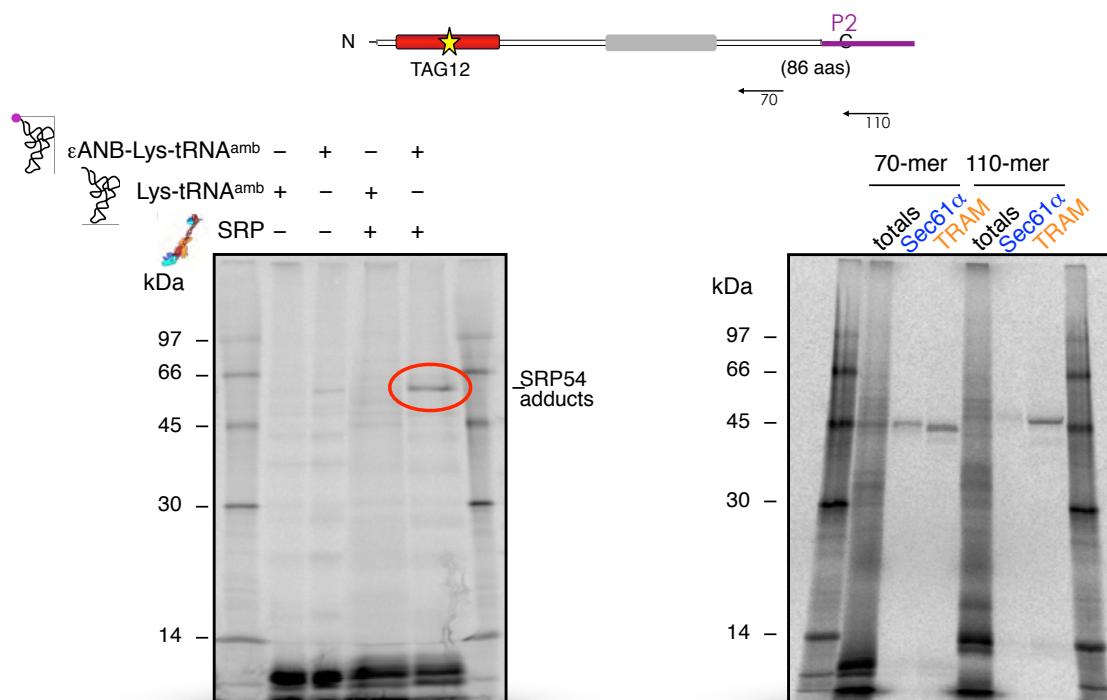


Saurí et al. (2007) J. Mol. Biol. **366**, 366

DoCiència Investigant amb Virus 2015

13

# Turnip Crinkle Virus movement protein insertion proceeds through the SRP/translocon complex

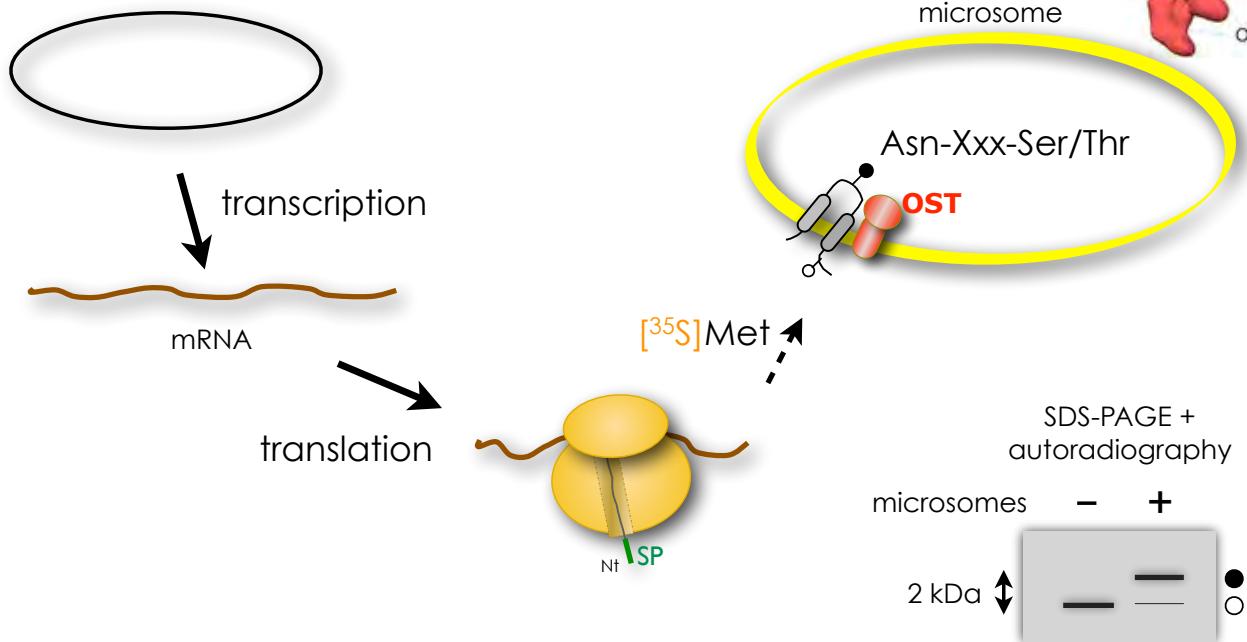


Martínez-Gil et al. (2010) J. Virol. **84**, 5520

DoCiència Investigant amb Virus 2015

14

# Experimental approach: N-Glycosylation *in vitro* assay

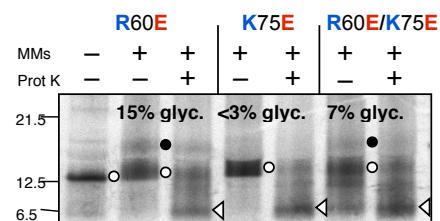
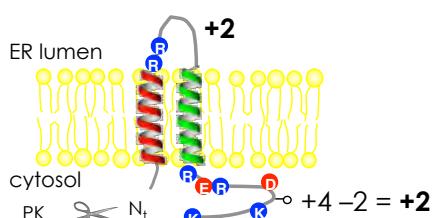
Nilsson & von Heijne (1990) *Cell* **62**:1135

DoCiència Investigant amb Virus 2015

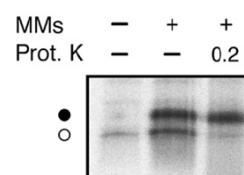
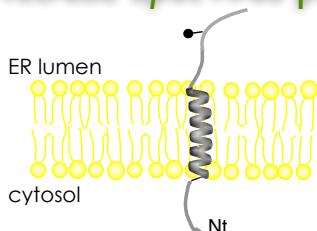
15

## Small viral movement proteins insert into biological membranes

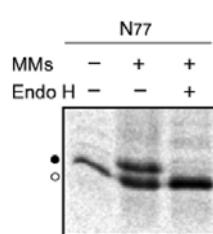
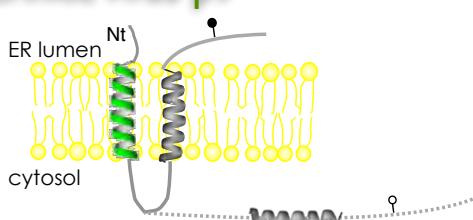
### Carnation Mottle Virus p9

Saurí et al. (2009) *J. Mol. Biol.* **387**, 113

### Melon Necrotic Spot Virus p7

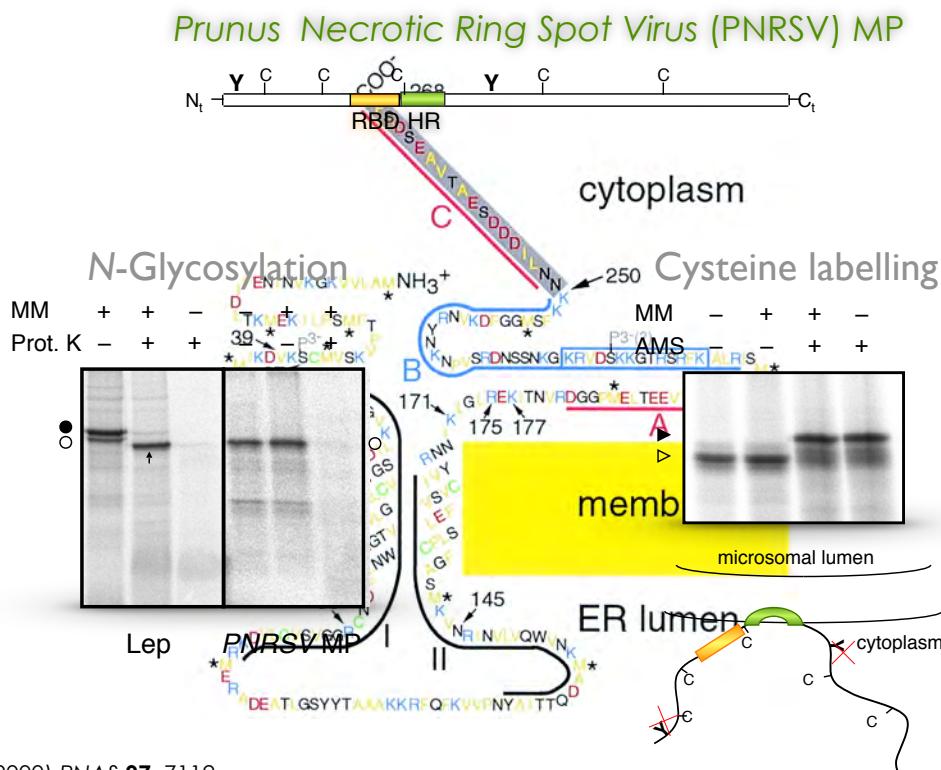
Martínez-Gil et al. (2007) *Virology* **367**, 348

### Turnip Crinkle Virus p9

Martínez-Gil et al. (2010) *J. Virol.* **84**, 5520

16

# What about 30K family?

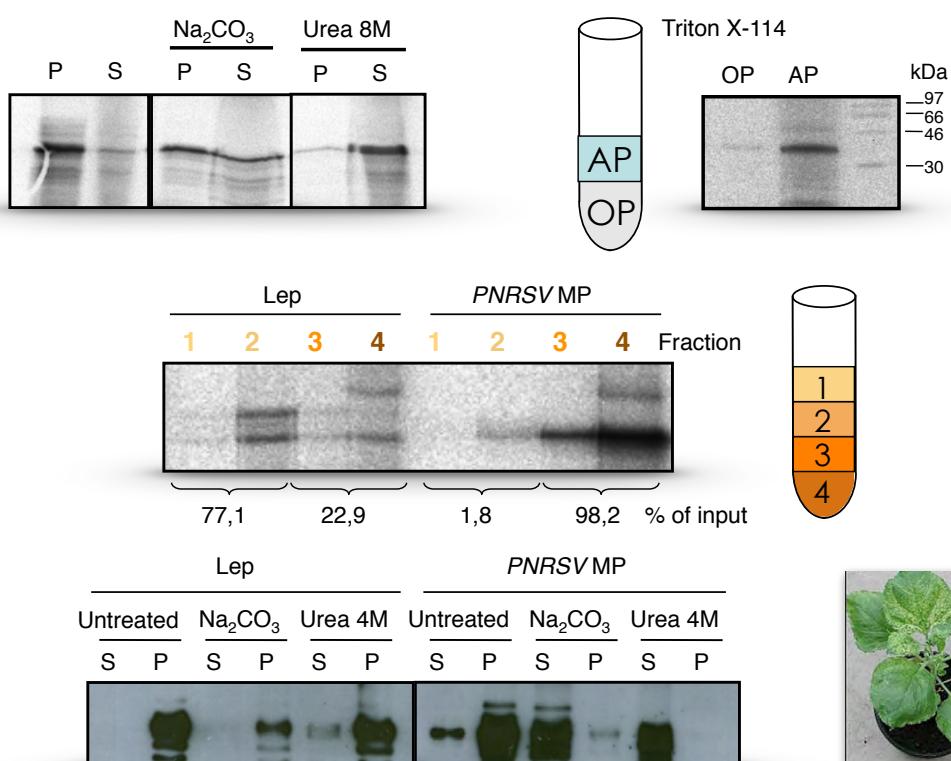


Brill et al. (2000) PNAS 97, 7112

DoCiència Investigant amb Virus 2015

17

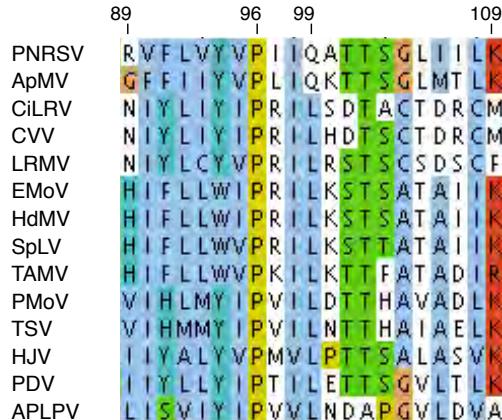
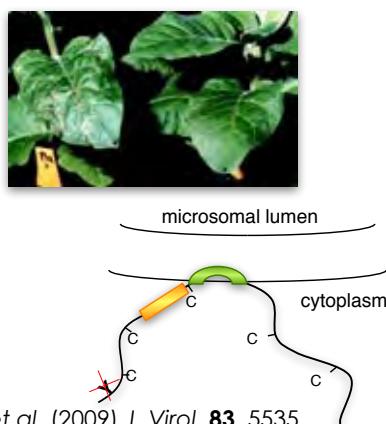
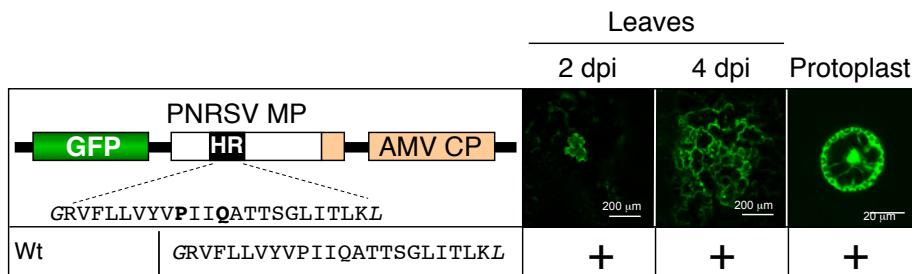
## Membrane association characterisation of PNRSV MP



DoCiència Investigant amb Virus 2015

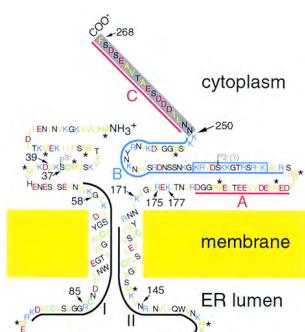
18

# Cell to cell movement in plants

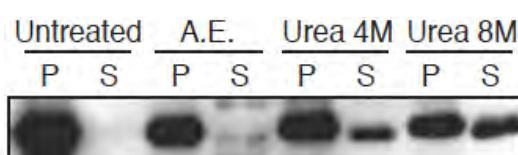
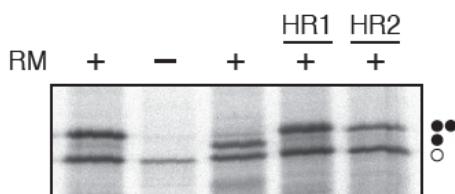
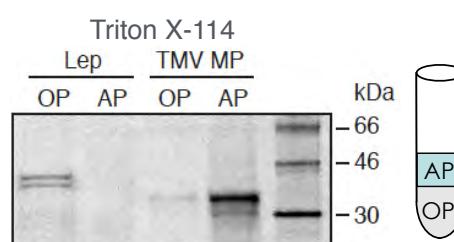
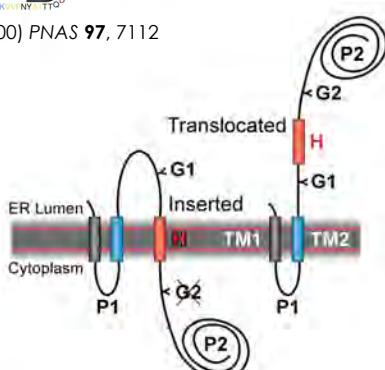


DoCiència Investigant amb Virus 2015

19



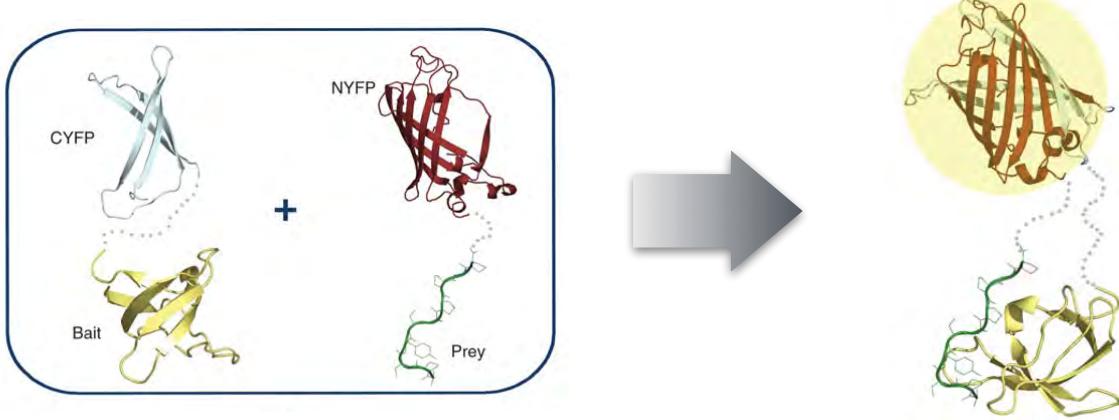
## Tobacco Mosaic Virus MP



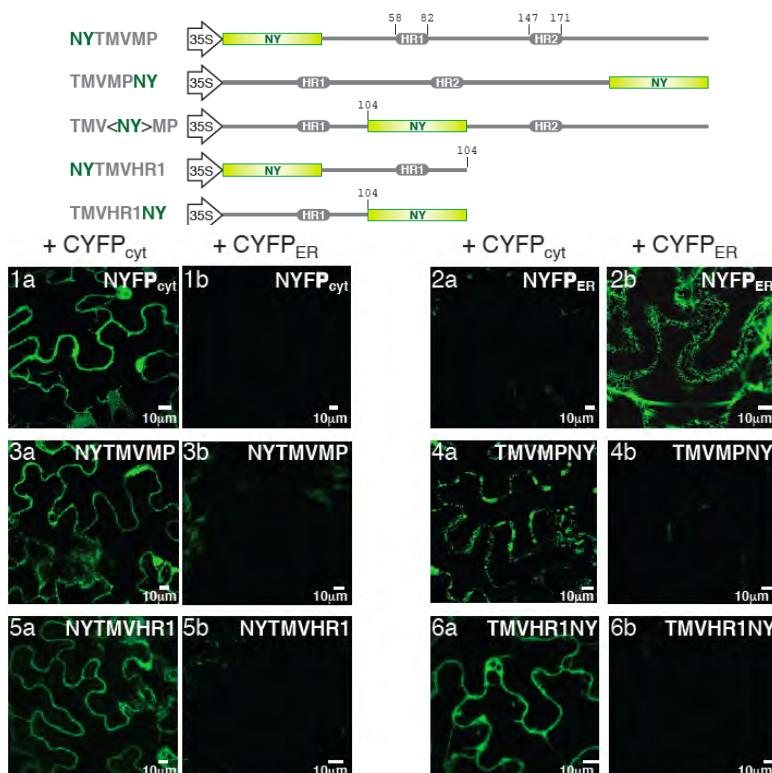
DoCiència Investigant amb Virus 2015

20

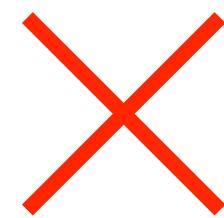
# Bimolecular Fluorescence Complementation in plant cells



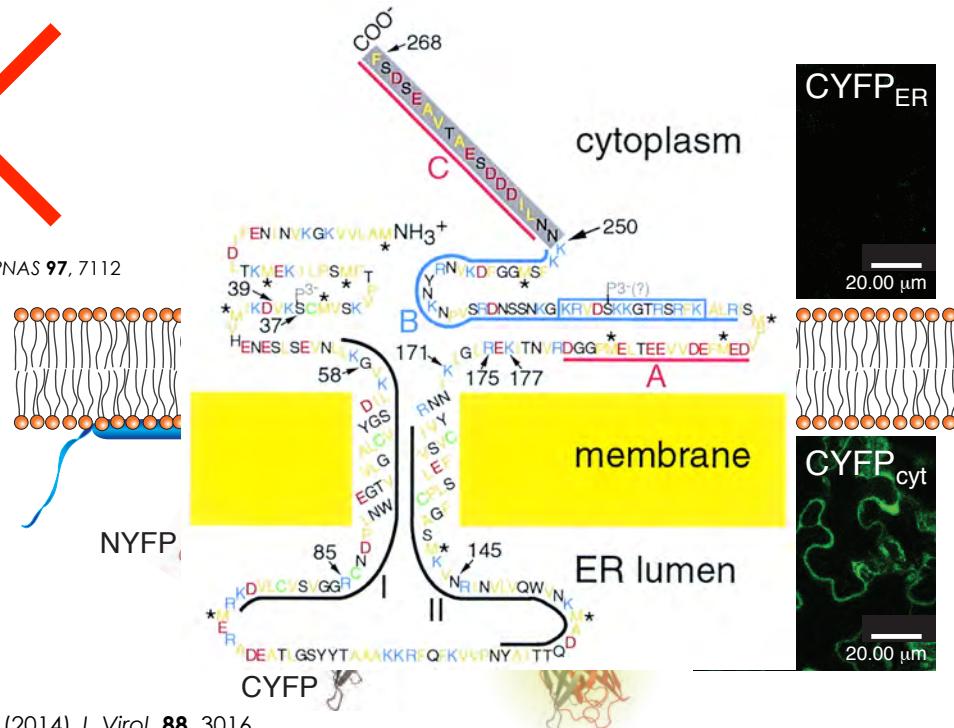
# Bimolecular Fluorescence Complementation in plant cells



# Tobacco Mosaic Virus MP associates but do not span cell membranes



Brill et al. (2000) PNAS **97**, 7112



Peiró et al. (2014) J. Virol. **88**, 3016

DoCiència Investigant amb Virus 2015

23

## Conclusions

- ▶ Small plant virus movement proteins target/insert into the ER membrane through the SRP/translocon machinery
- ▶ 30K family members associate but do not span cell membranes

# Acknowledgements

<http://research.uv.es/membrana/>



C. Abad



M. Sánchez



L. Martínez-Gil



C. Baeza



C. Martínez



V. Andreu



M.J. García



Arthur E. Johnson  
TAMU, Texas



G. von Heijne  
CBR/Stockholm University

Past members:



M. Orzáez  
CIPF



A. García-Sáez  
Tübingen Univ.



A. Saurí  
OCTOPLUS



D. Lukovic  
CABIMER



M. Vilar  
CARLOS III



S. Tamborero  
VLPbio



M. Bañó-Polo  
Stanford Univ.