The Abdus Salam
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International Atomic Energy Agency

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A Kinetic View of Viral RNA in Single Live Cells in Real Time

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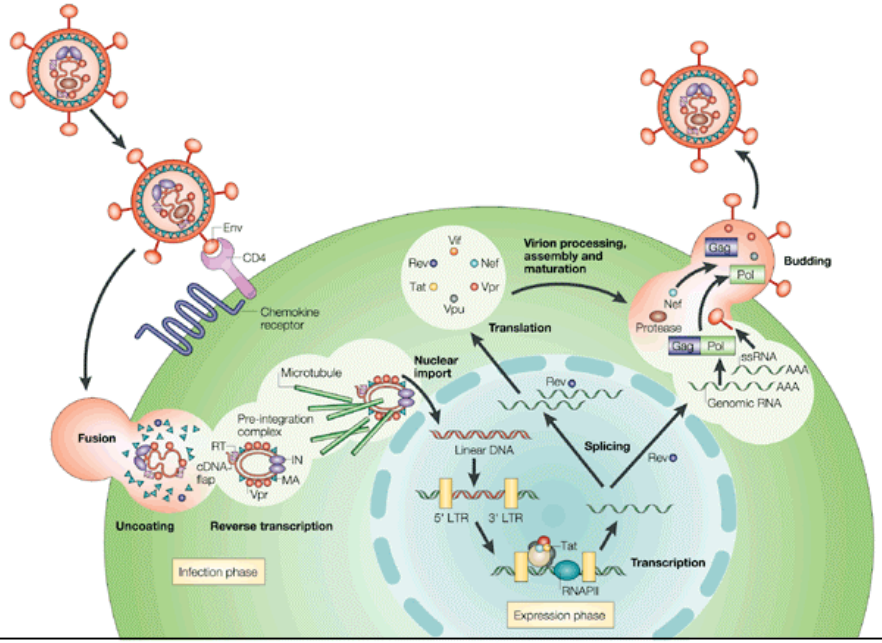
The International Centre for Genetic Engineering and Biotechnology (ICGEB)

PLEASE NOTE - EDITED PRESENTATION
- unpublished material has been removed -

An international organization dedicated to advanced research and training in molecular biology and biotechnology, with special regard to the needs of the developing world.



The HIV-1 life cycle

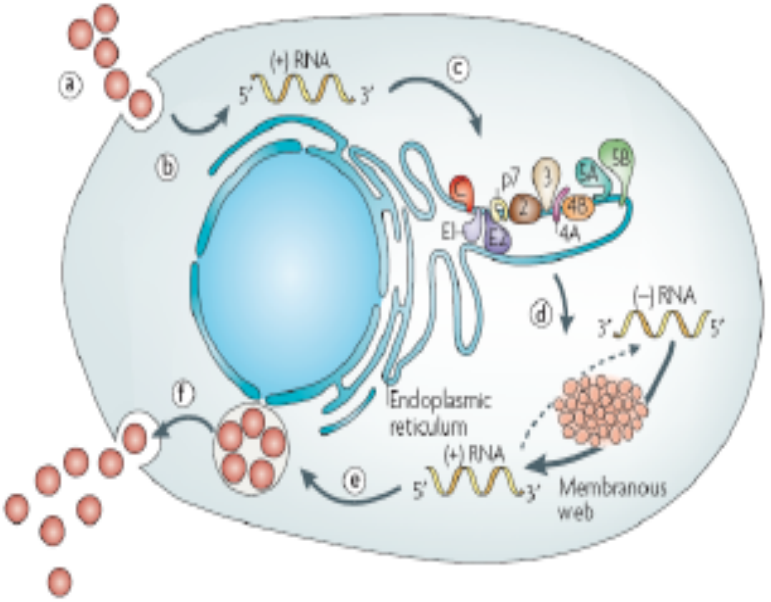
cytoplasm
- *Virus binding and internalization*
- *Uncoating*
- *PIC translocation*

nucleus
- ***Transcription and replication***

cytoplasm
- *Packaging and assembly*
- *Maturation and release*

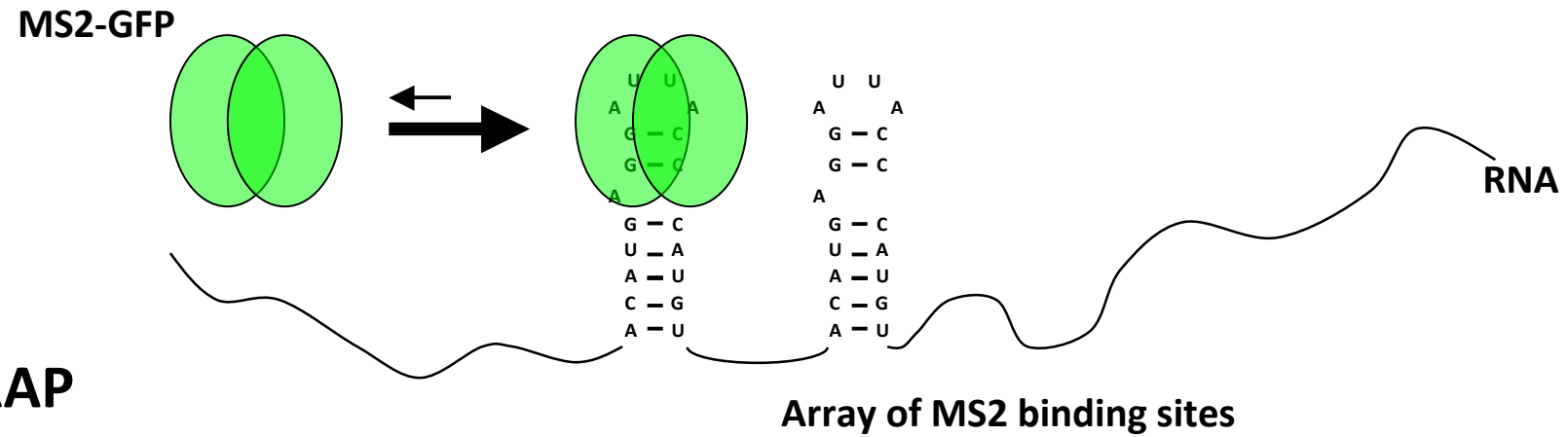
The TBEV life cycle

cytoplasm
- *Virus binding and internalization*
- *Uncoating*
- *Translation and polyprotein processing*
- ***Transcription and replication***
- *Packaging and assembly*
- *Maturation and release*

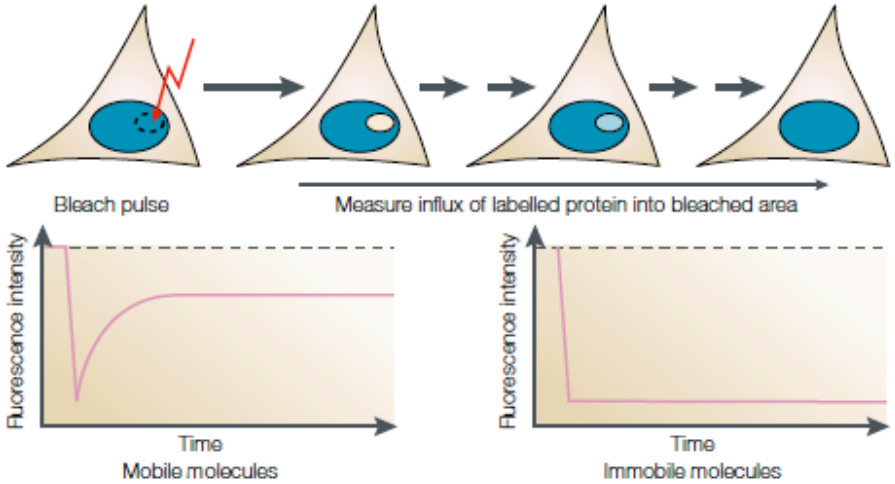


The MS2 RNA-tagging method

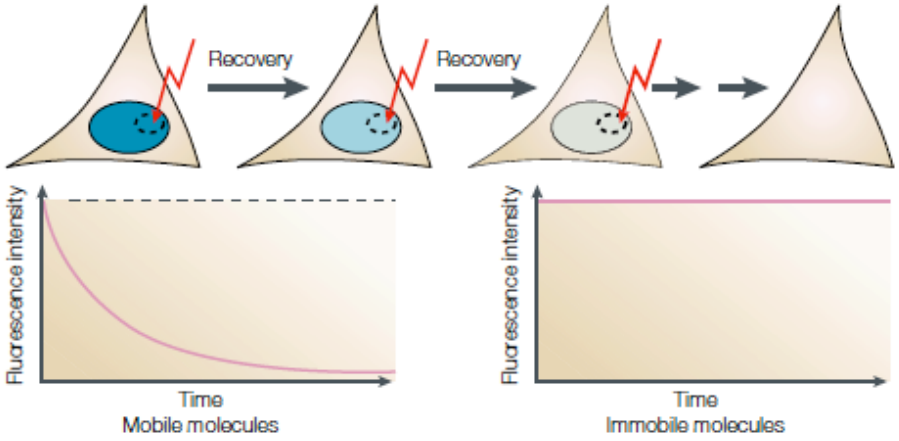
Bertrand/Singer

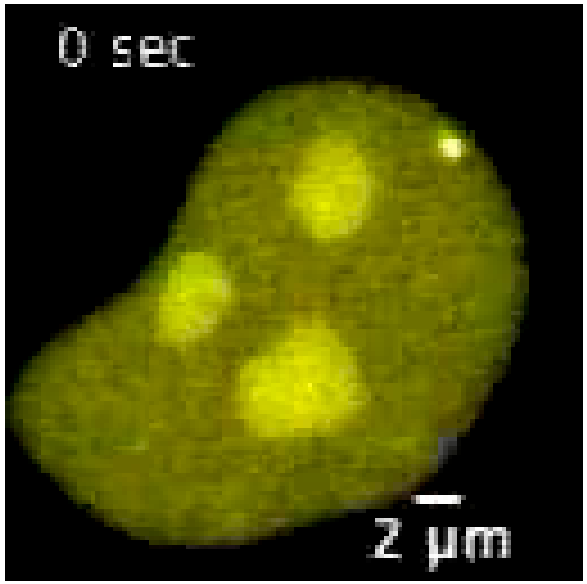


FRAP

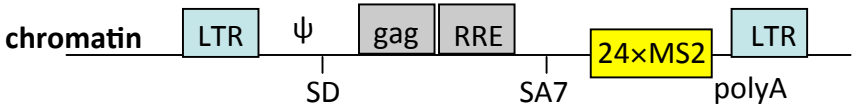


FLIP





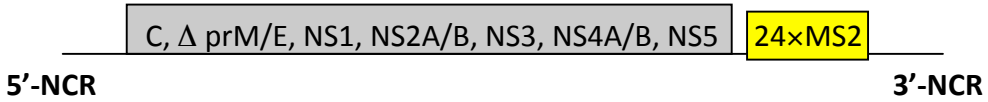
HIV-1 RNA dynamics



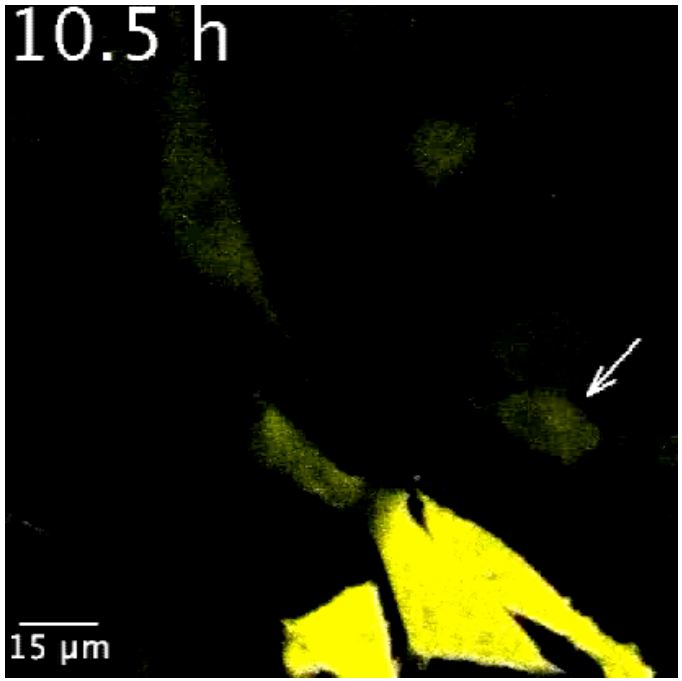
- kinetic analysis of RNA polymerase II transcription rates;
(Boireau et al. JCB 2007)

- kinetic analysis of viral/host factors to the transcription site;
(Molle et al. Retrovirology 2007)

TBEV RNA dynamics



- kinetic analysis of TNE RNA replication;
(Miorin et al. Virology, 2008; Hoenninger et al. Virology, 2008)



Functional organization of the nucleus in 4D

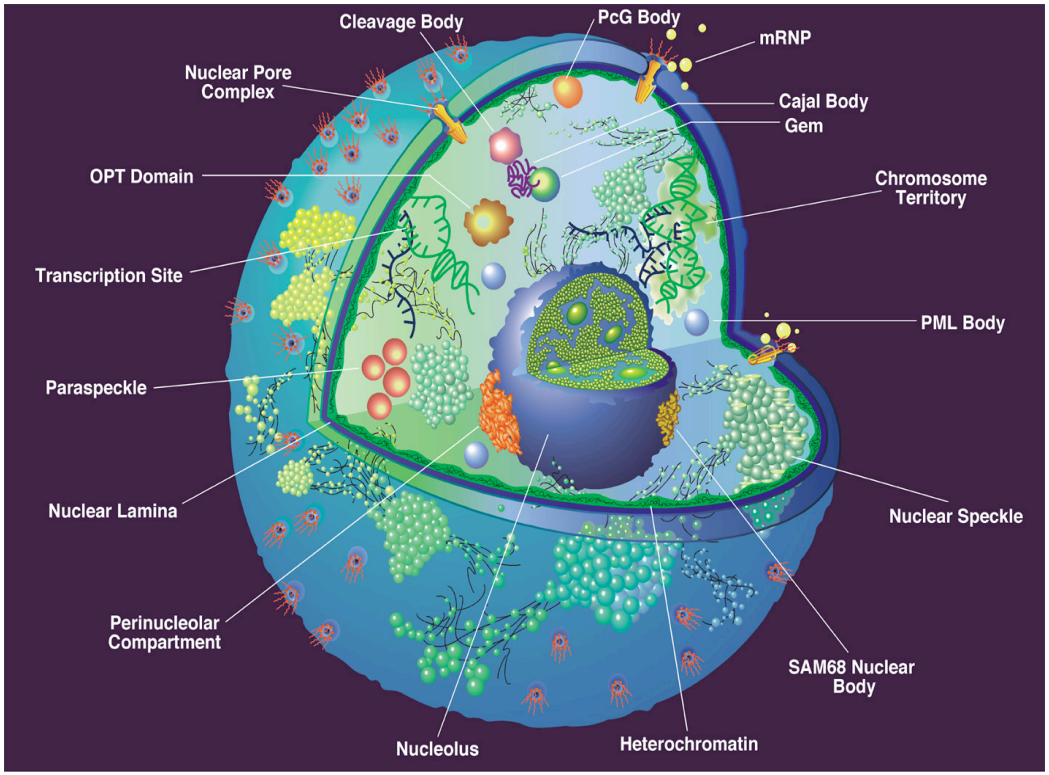
The 'bowl of spaghetti with meatballs' model

A dated view of the nucleus:
chromatin (spaghetti) is disorganized in the nucleus (bowl) with interspersed organelles not delimited by membranes like the nucleolus, speckles, PML bodies, Cajal bodies etc (meatballs)…

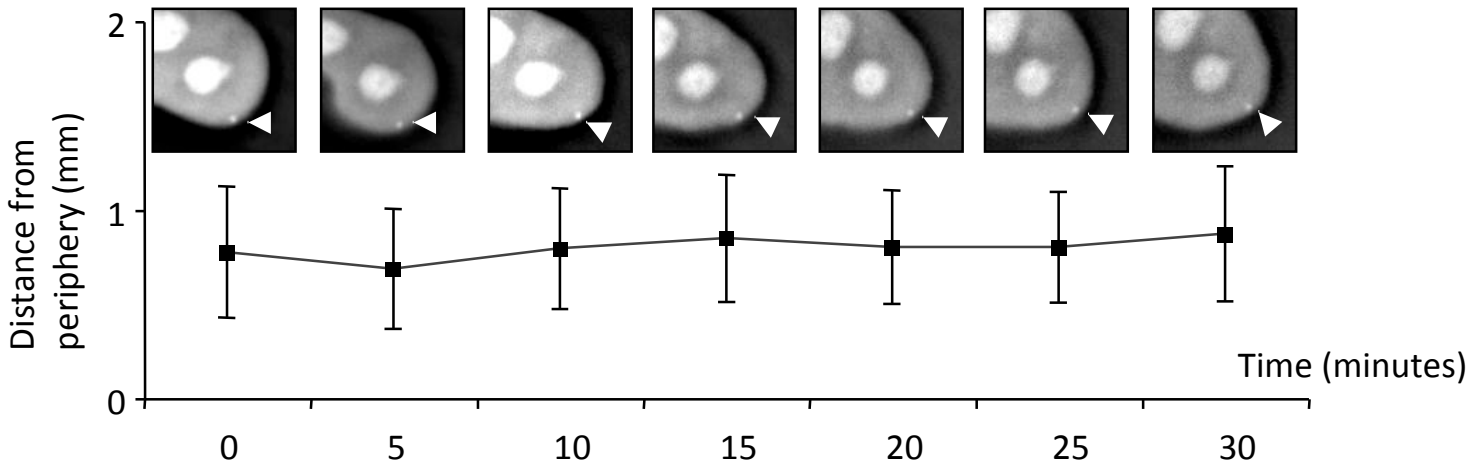
The nucleus is well organized in 4D ….

- Chromatin occupy specific positions (chromosome territories);
- Heterochromatin is more peripheral, euchromatin more central;
- Active genes loop out for activity;
- Nuclear structures are dynamically assembled for a specific function;

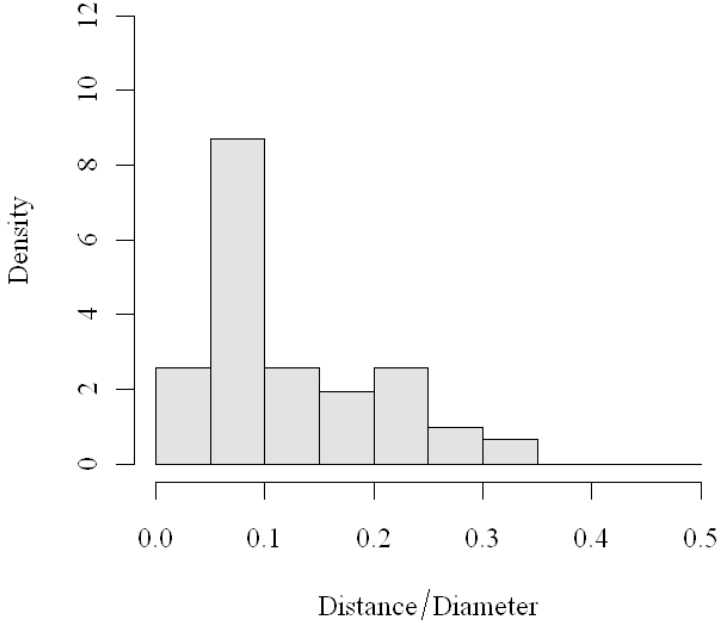
… 4D organization is functionally relevant.

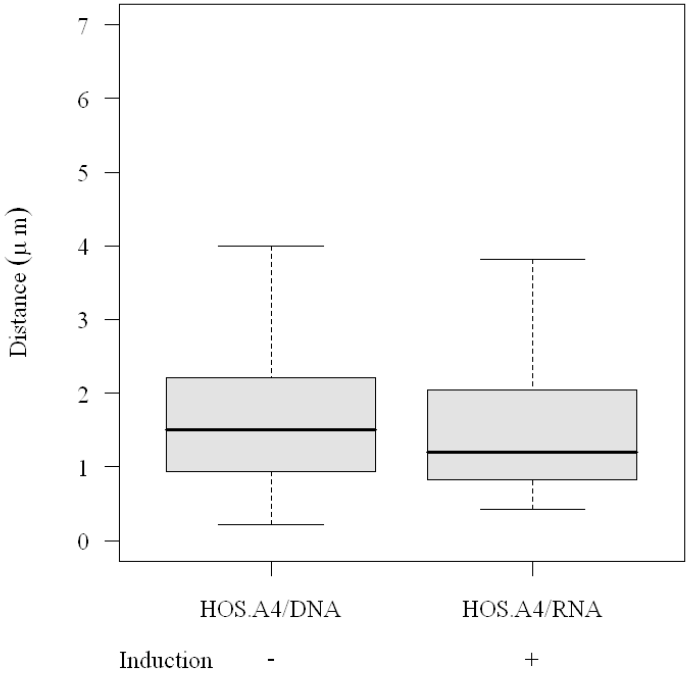


Detection of nascent HIV-1 RNA in living HOS_A4

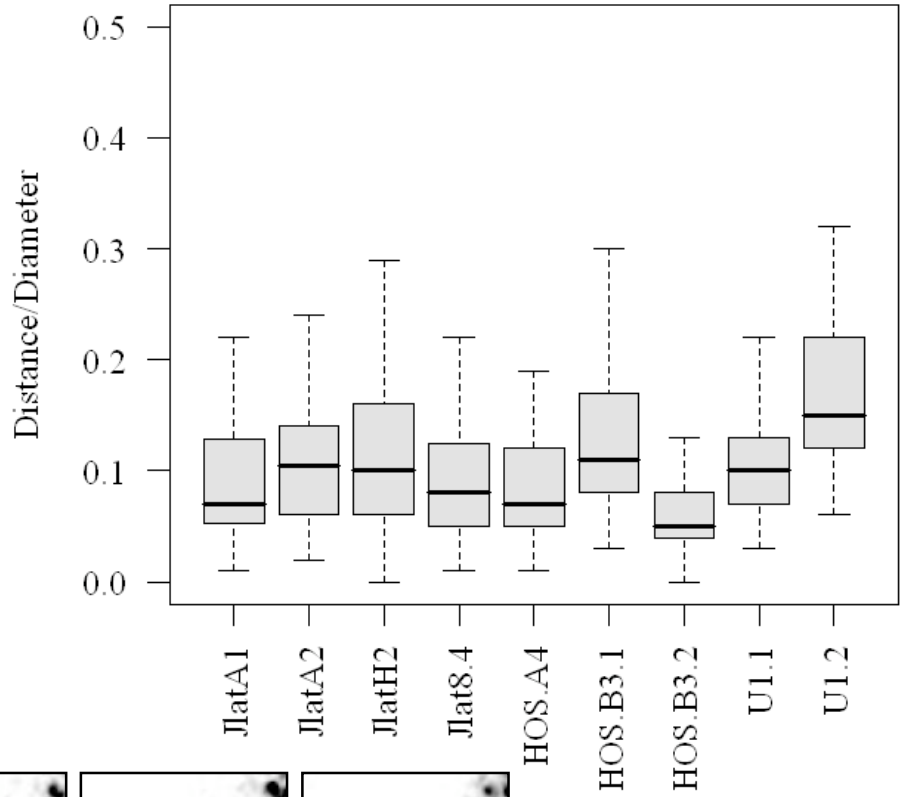


Detection of proviral HIV-1 DNA in HOS_A4 by ISH

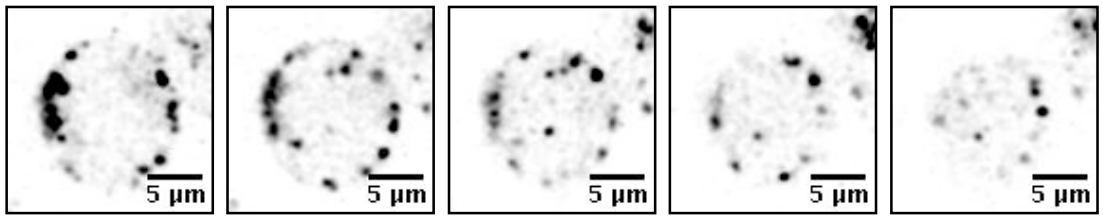




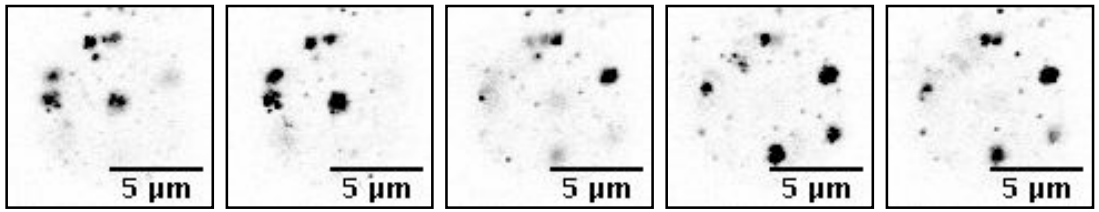
Localization to the nuclear periphery of the integrated provirus in various cell lines used to study HIV-1 post-integration latency



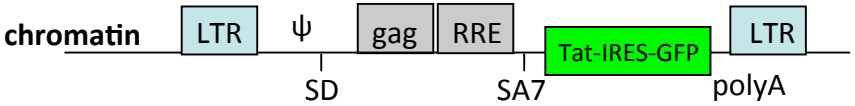
pan-a-satellite repeats probe (J-LatA1 z-stacks)

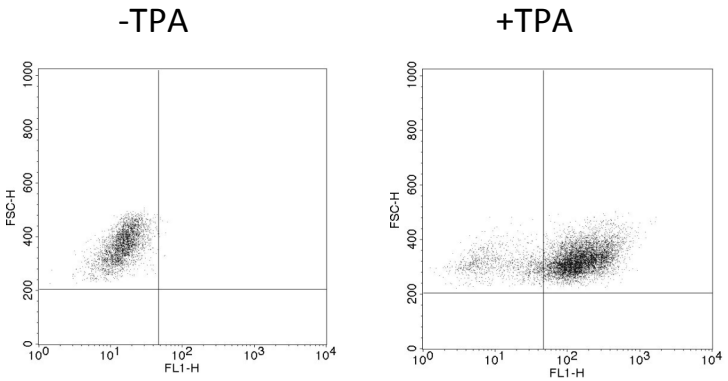


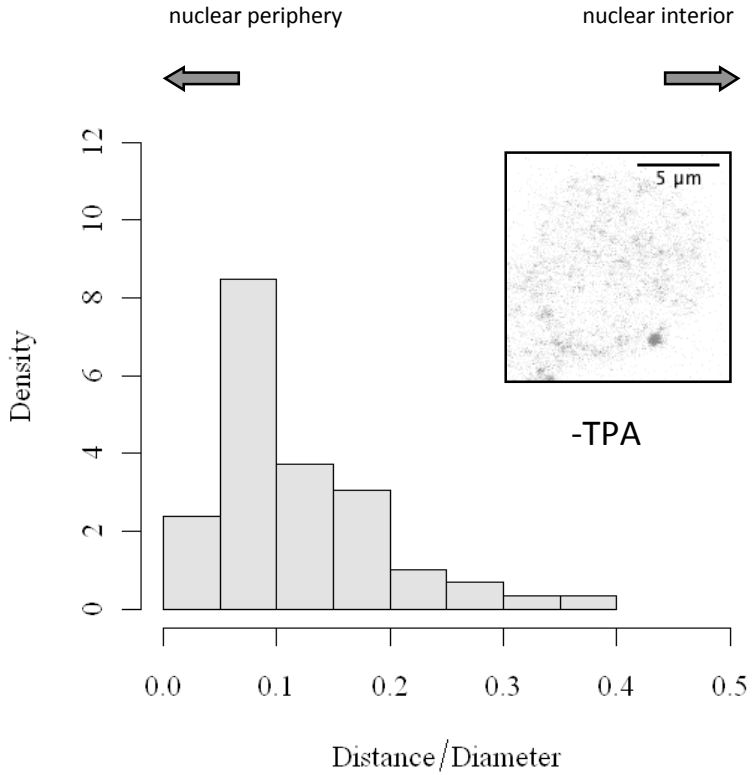
IF with the repressive marker H4K20me3 (J-LatA1 z-stacks)

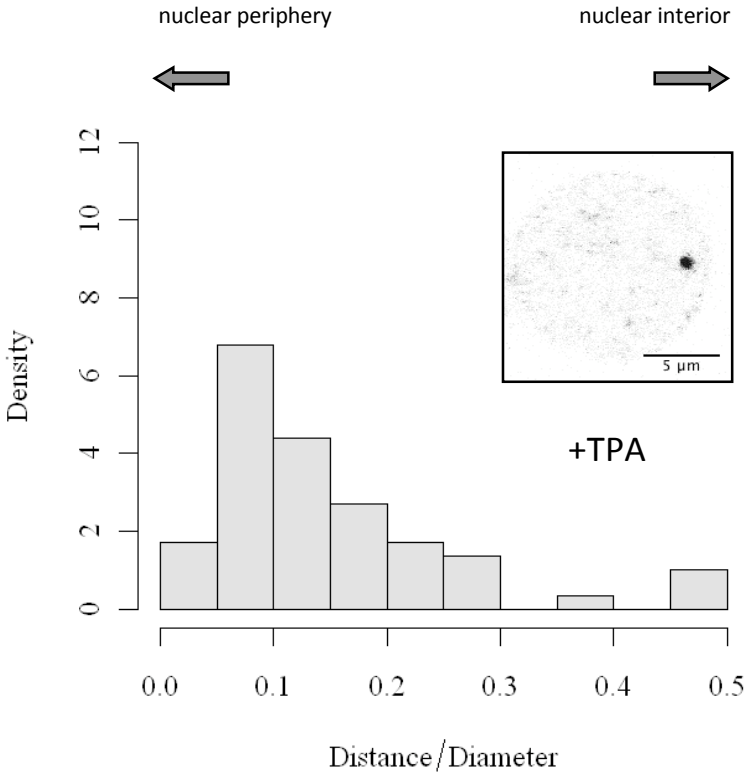


The J-LatA1 model

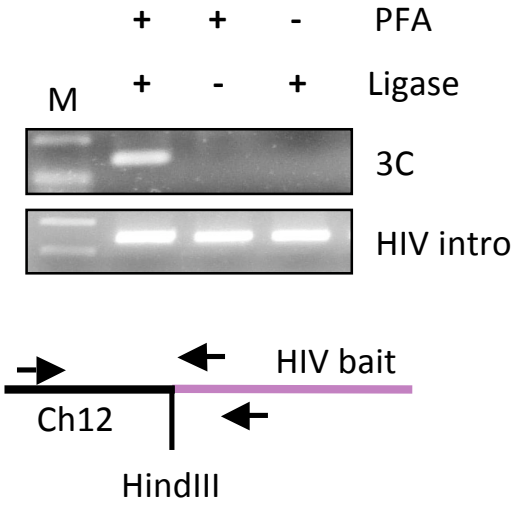


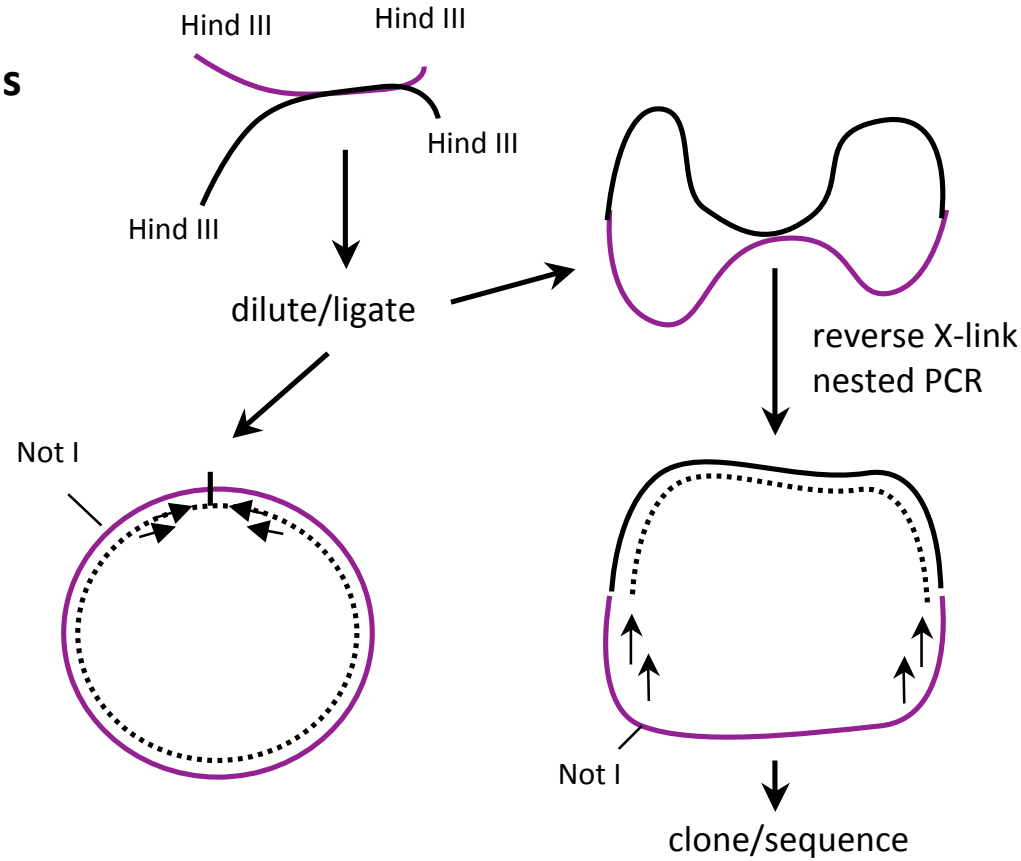


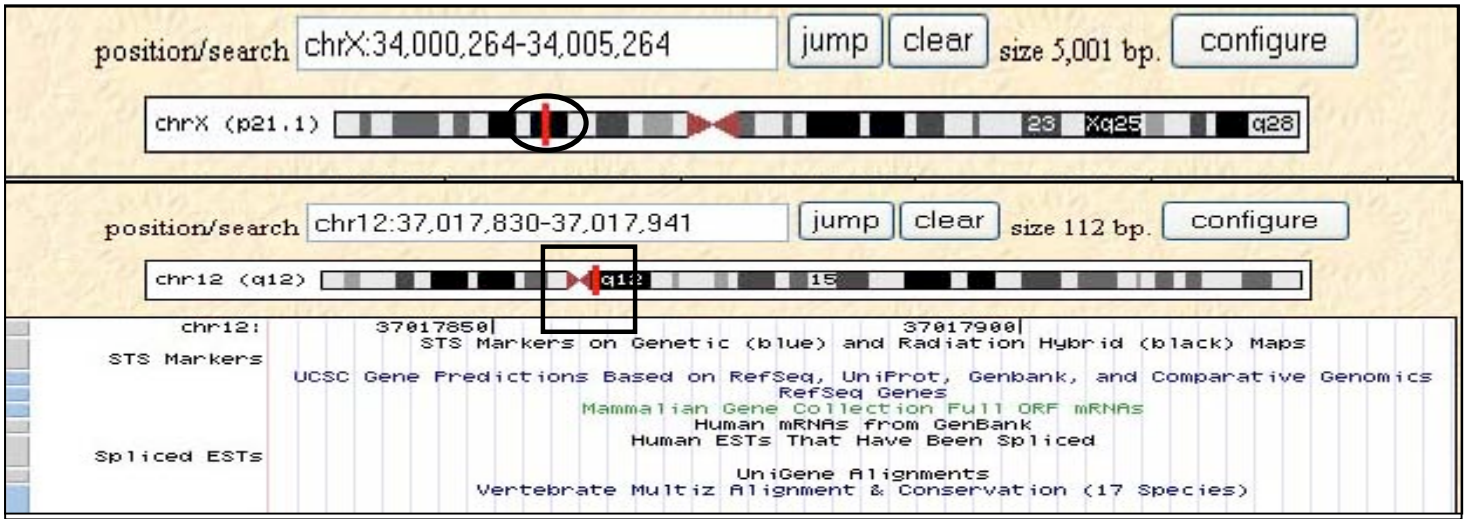




Circular Chromosome Conformation Capture (4C) analysis of the HIV-1 provirus identify pericentromeric Ch12q12 association in trans (J-LatA1)

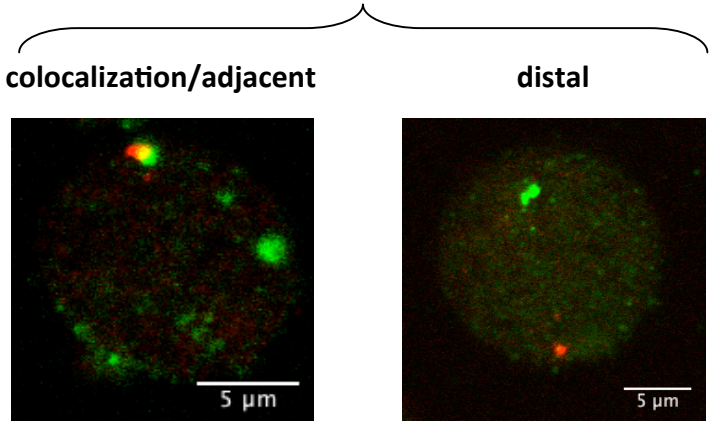


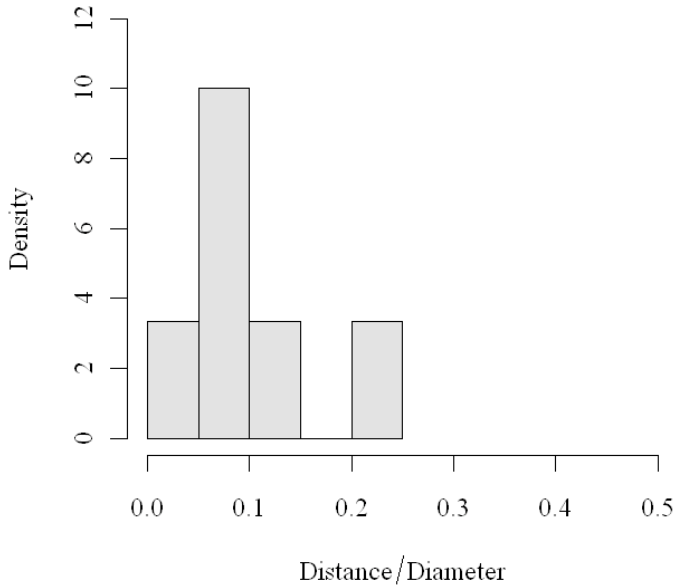


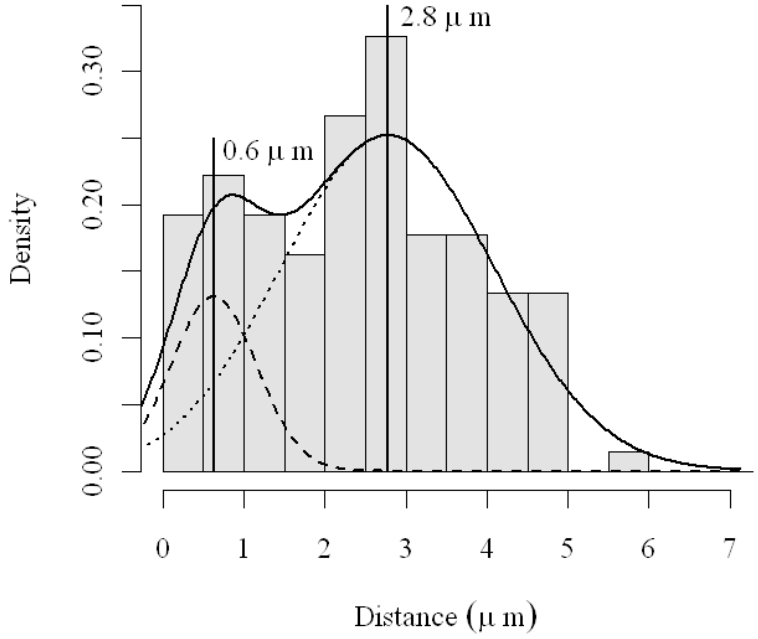


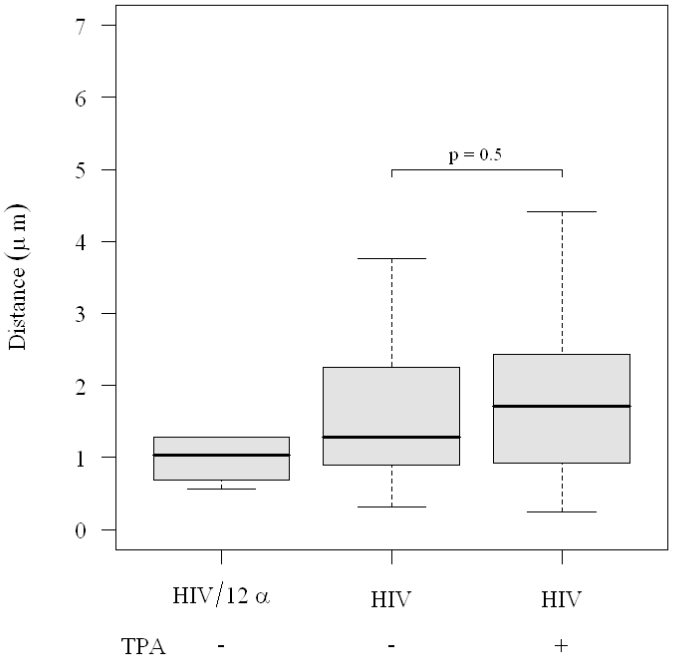
DNA FISH detection of the interaction of ChXp21.1 and Ch12q12 in J-lat A1

Not induced, J-lat A1 (Ch12q12 & ChXp21.1)

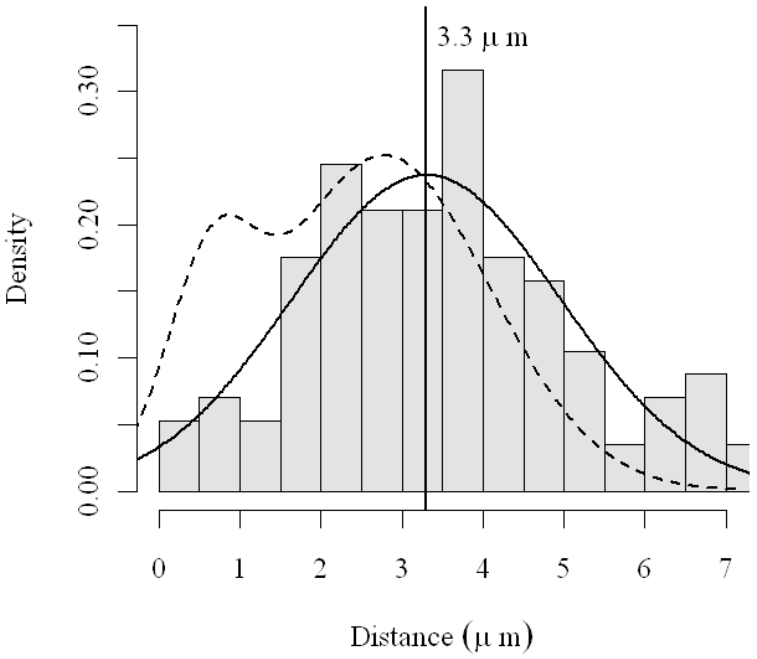


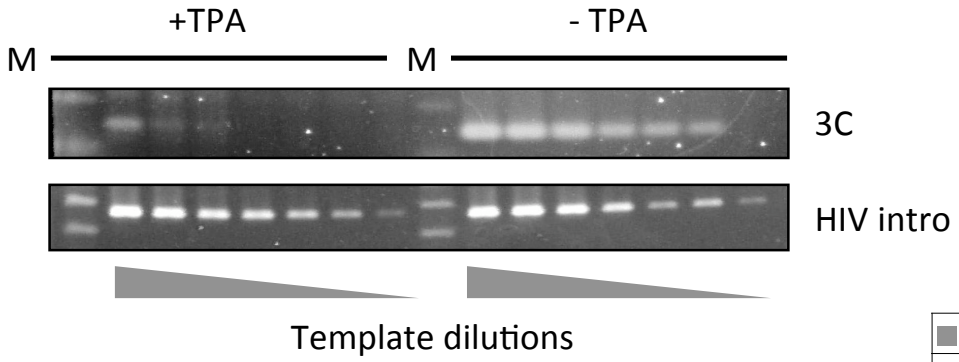




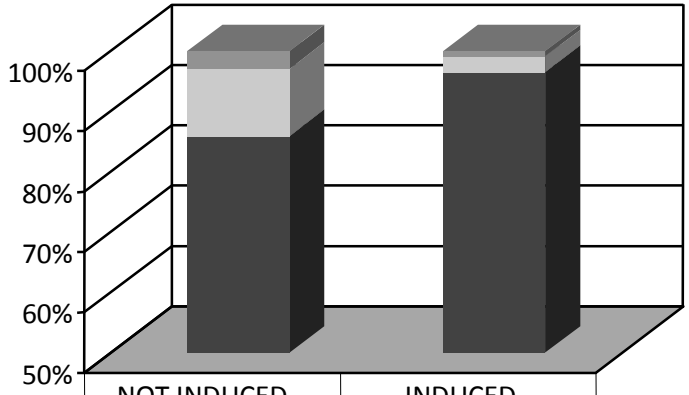


Dissociation of the HIV-1 provirus from the pericentromeric heterochromatin of Ch12q12 upon TPA treatment



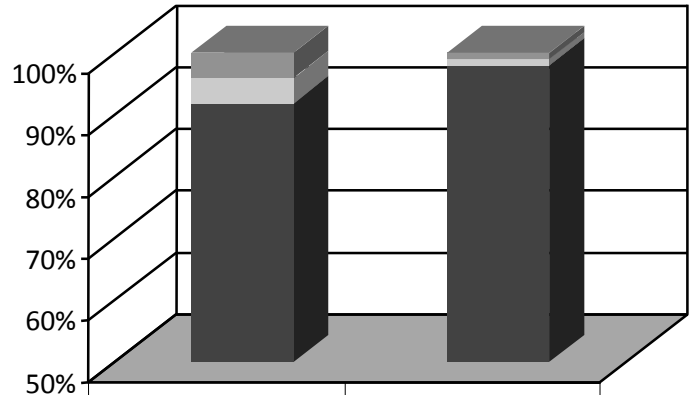


FISH Chr12 + ChrX

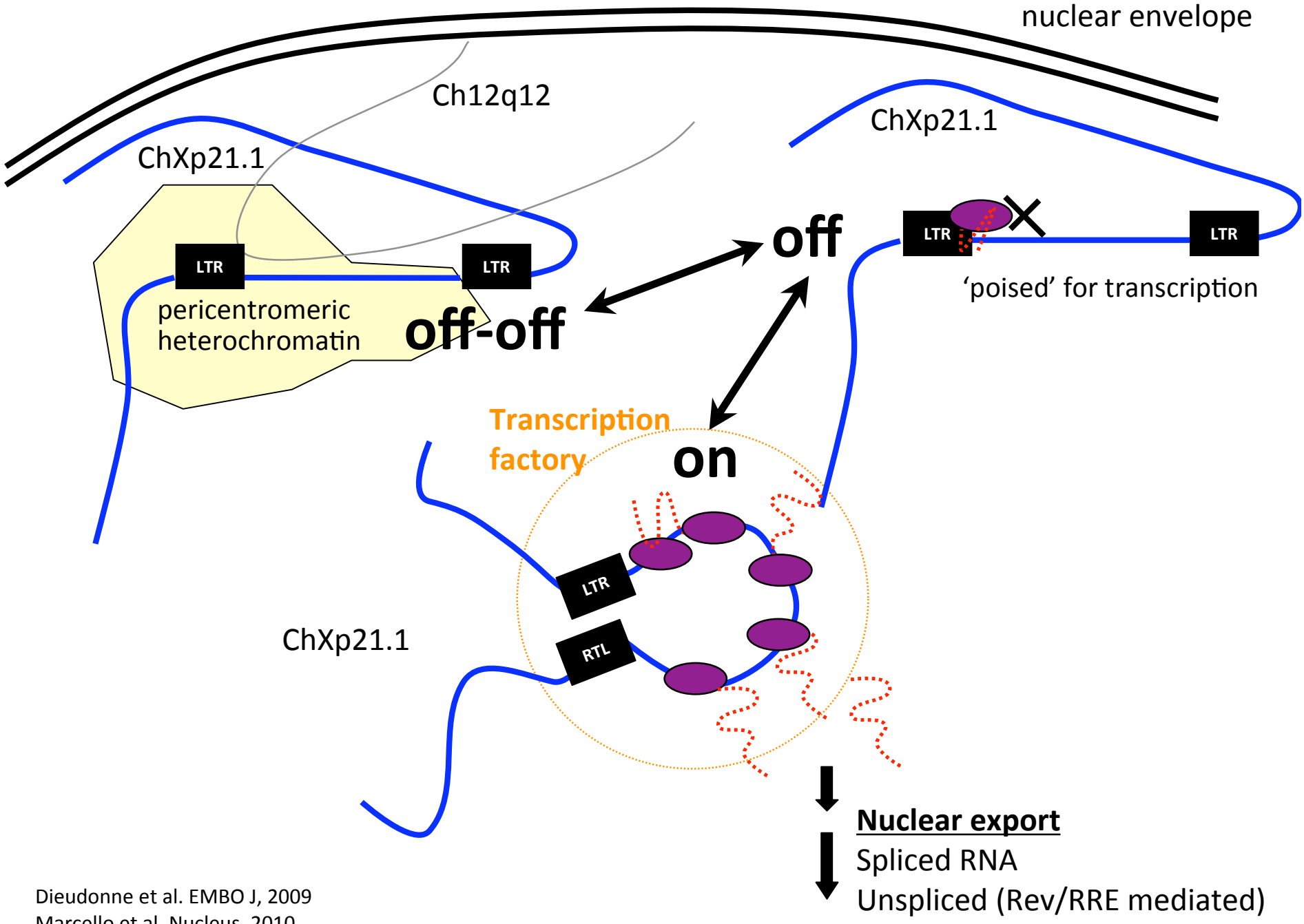


	NOT INDUCED	INDUCED
Colocalization	2.9±0.7%	0.8±0.8%
Adjacent	11±4%	3±2%

FISH Chr12alpha + HIV

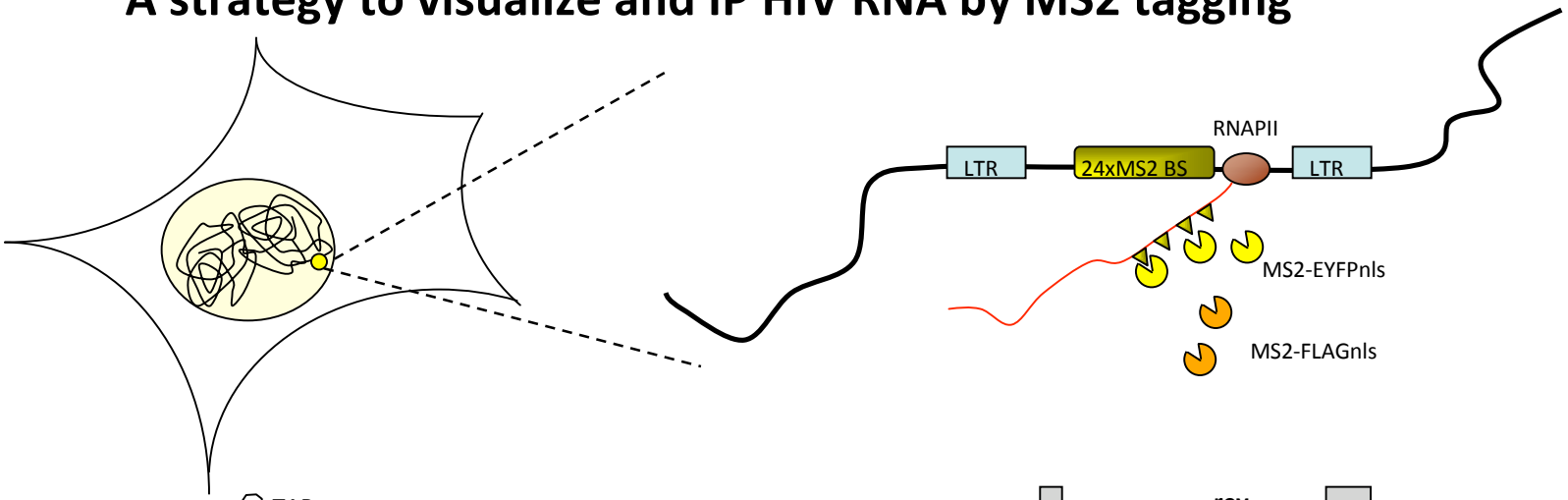


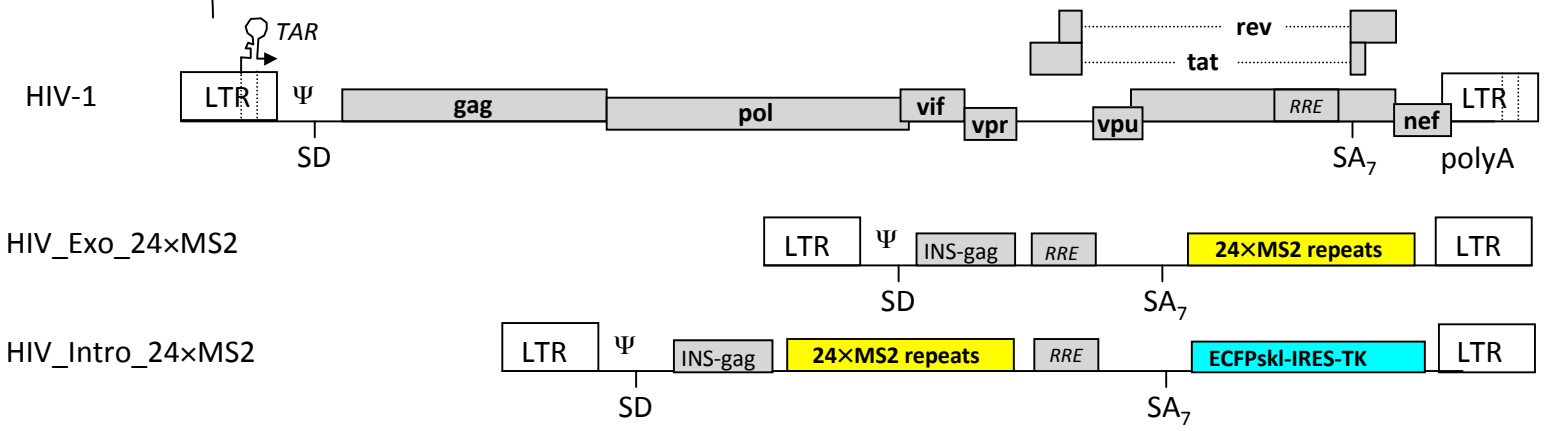
	NOT INDUCED	INDUCED
Colocalization	4±2%	1±1%
Adjacent	4±2%	1±1%

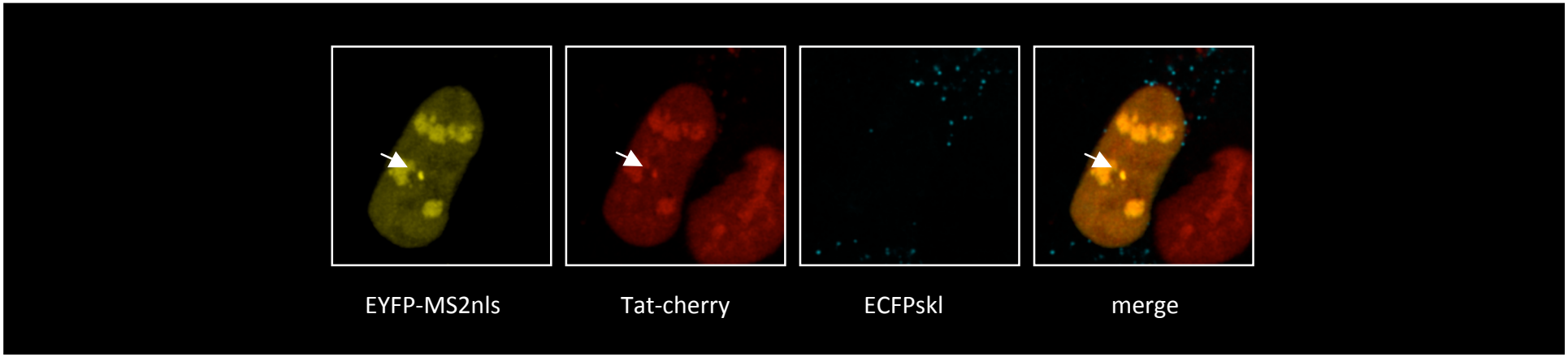


Dieudonne et al. EMBO J, 2009
Marcello et al. Nucleus, 2010

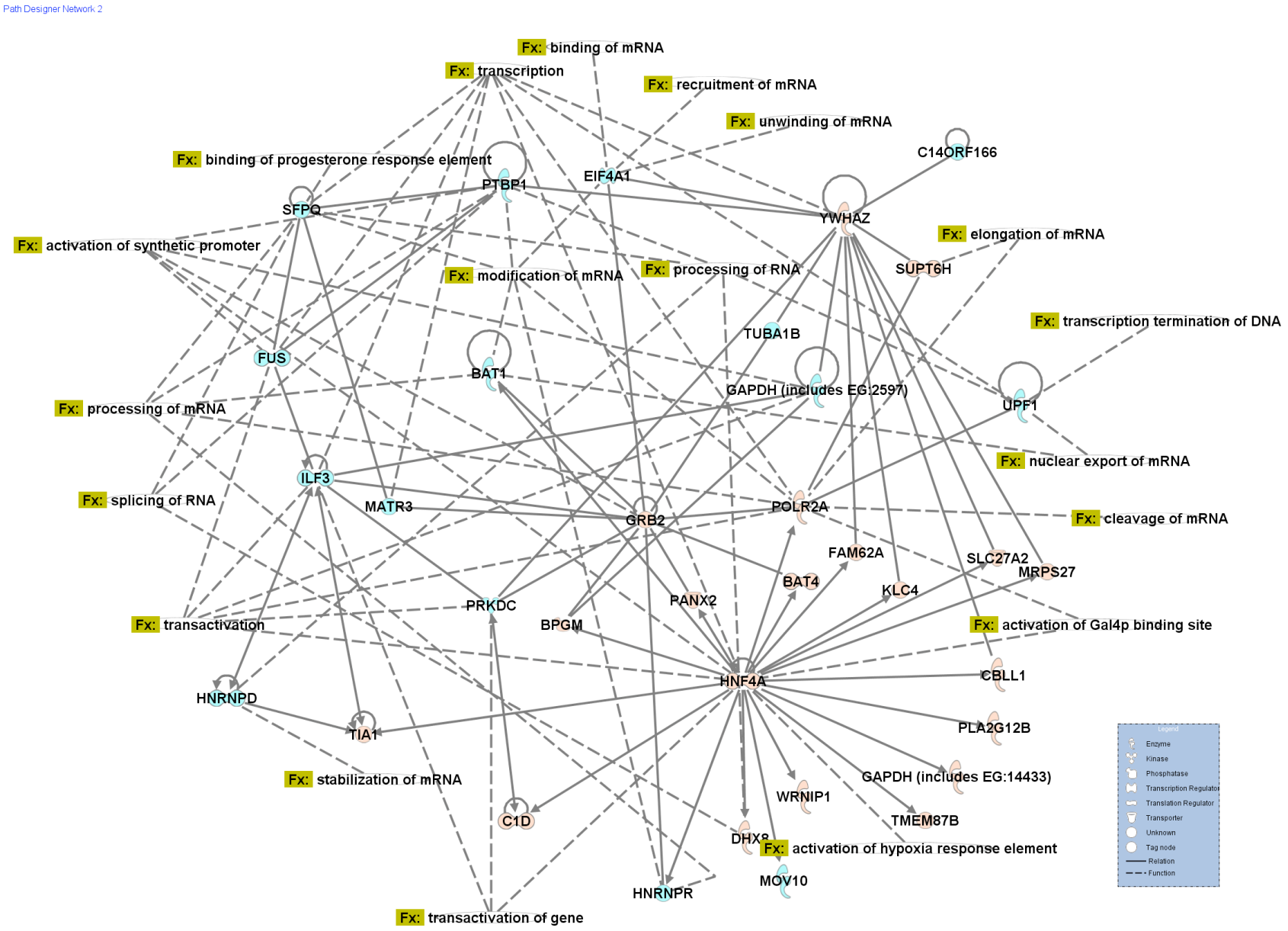
A strategy to visualize and IP HIV RNA by MS2 tagging



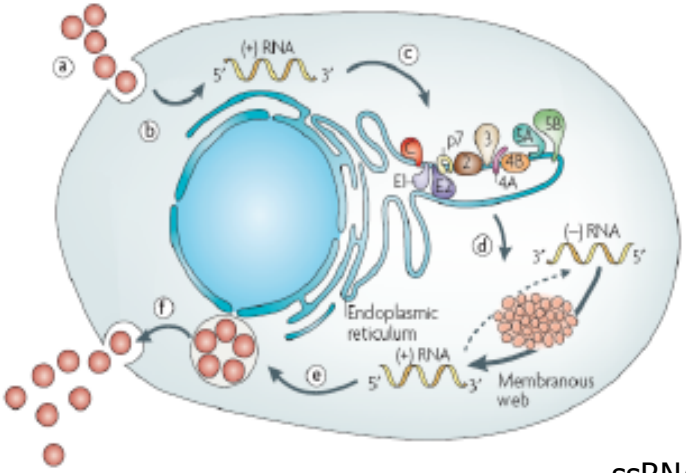




Proteomic analysis of HIV RNA cellular binding factors



TBEV dynamics in single live cells



a) Virus binding and internalization

b) Uncoating

c) Translation and polyprotein processing

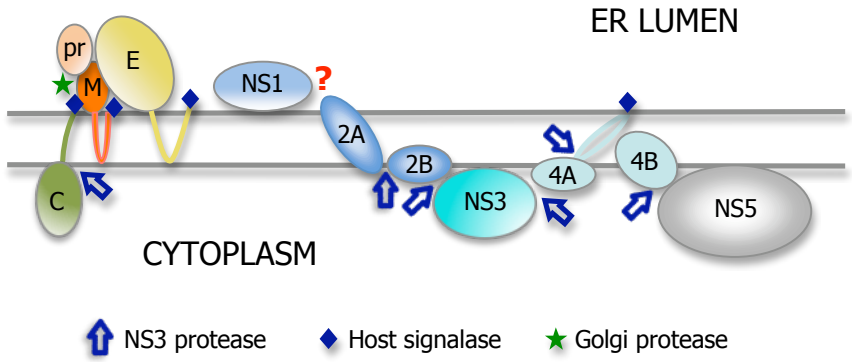
d) RNA replication

e) Packaging and assembly

f) Maturation and release

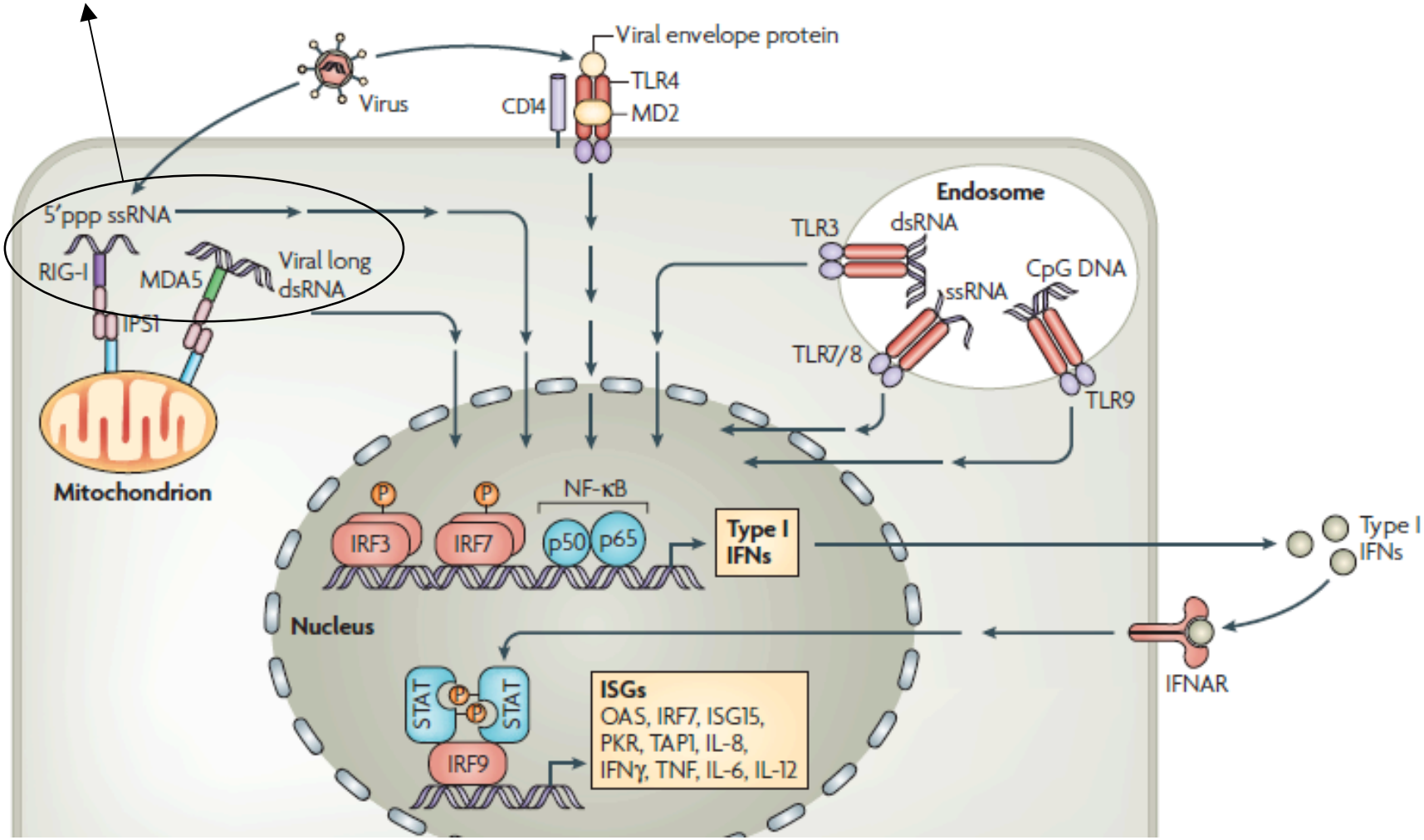
ssRNA(+) _ single ORF

TRANSLATION
&
POLYPROTEIN PROCESSING



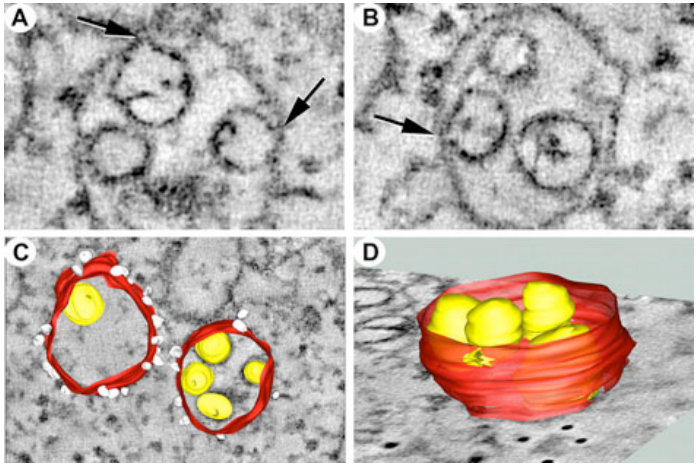
RLR-mediated signal transduction

Sequestering viral RNA from RLR detection

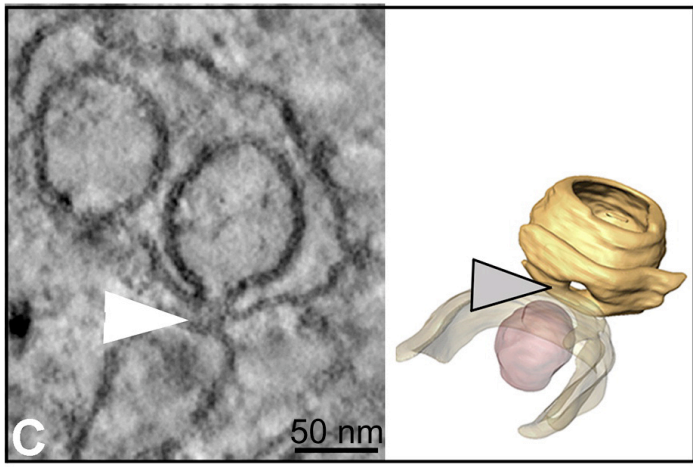


Katze et al. Nature Reviews Immunology 2008

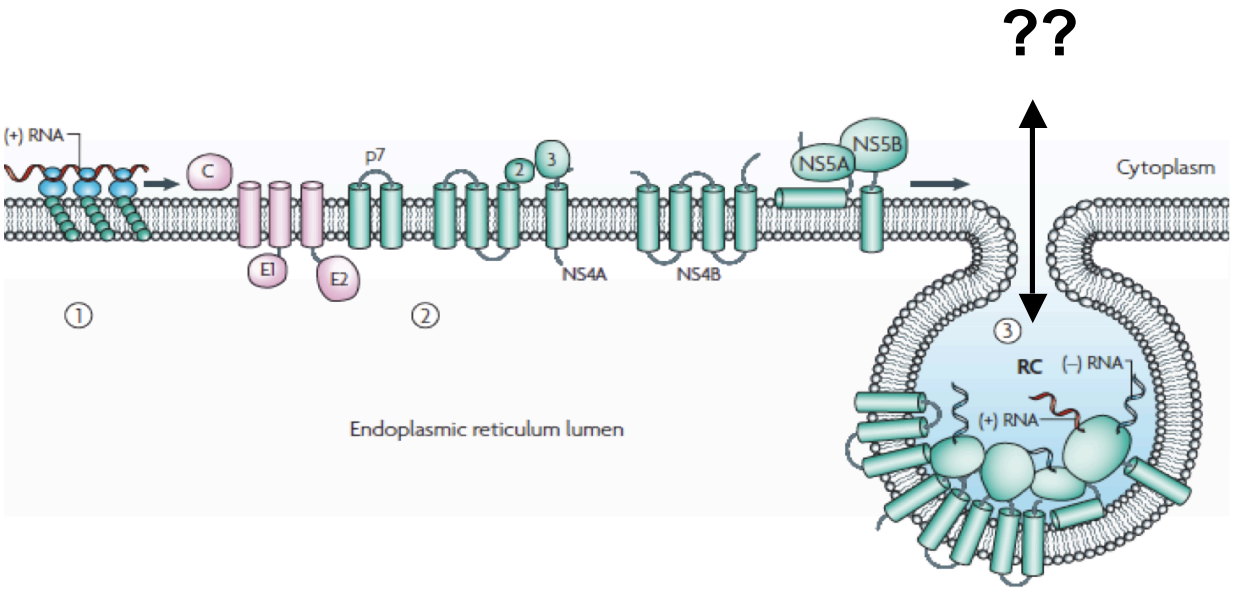
Flaviviruses replicate in vesicles



WNV ER vesicles (Gillespie JVI 2010)

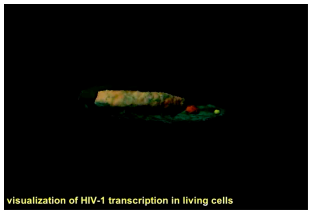


DENV ER vesicles (Welsch CH&M 2010)



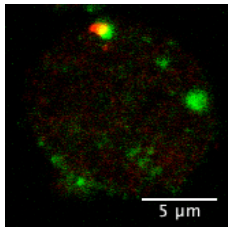
Conclusions (1): HIV-1

Visualization of HIV-1 RNA



- We generated a robust system to track HIV-1 RNA in living cells
- We measured the rate of transcribing RNAPII in living cells
- We analysed the dynamic recruitment of factors at the HIV-1 transcription site in living cells

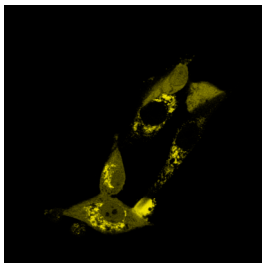
Positional control of HIV-1 transcription in 4D



- The HIV-1 provirus is associated to the nuclear periphery in latent cells
- Transcriptional activation of the provirus does not affect its sub-nuclear localization
- Transcriptional silencing is an effect with many causes

Conclusions (2): TBEV

Visualization of TBEV RNA



- We generated a robust system to track flavivirus RNA in living cells
- We characterized TBEV replication sites
- We define the sub-cellular localization of TBEV replicated RNA

The Molecular Virology lab at the ICGEB

http://www.icgeb.org/molecular-virology.html

Funding:

HFSP: Young Investigator Grant
EU: FP6 STREP
MIUR-FIRB, Italy
ISS-AIDS, Italy
Beneficentia Stiftung, Lichtenstein

Initiatives:

ICGEB-IUBMB Workshop on Human RNA Viruses, from 10-12 February 2010, New Delhi, India.

Advanced Summer School in Africa on the "Molecular Mechanisms of Viral Infection and Propagation" from 6-14 March 2010, Hermanus, South Africa.