IAV replication and co-infection dynamics analyzed by a versatile RNA viral genome labeling method

Dan Dou[1], Iván Hernández-Neuta[1], Hao Wang[1], Henrik Östbye[1], Xiaoyan Qian[1], Swantje Thiele[2], Patricia Resa-Infante[2], Nancy Mounogou Kouassi[2], Vicky Sender[3], Karina Hentrich[3], Peter Mellroth[3], Birgitta Henriques-Normark[3], Gülsah Gabriel[2], Mats Nilsson[1], and Robert Daniels[1]

1. Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden.
2. Heinrich Pette Institute, Leibniz Institute for Experimental Virology, 20251 Hamburg, Germany.
3. Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177 Stockholm, Sweden.

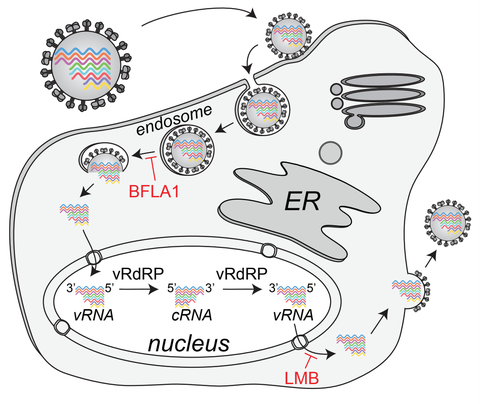
Abstract

Background: Viral infections are dependent on the delivery of the viral genome to the proper cellular compartment for transcription and replication. However, *in situ* methods for analyzing the localization of RNA viral genomes and differentiating genomes with high identity are lacking, which makes it difficult to investigate processes related to entry and cell co-infections.

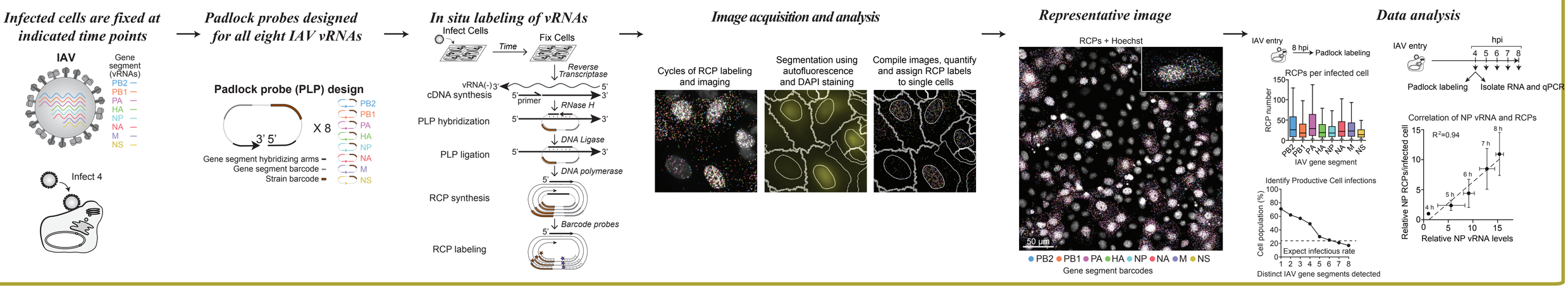
Methods: Recently, we developed a RNA labeling approach for single-cell analysis of RNA viral replication and co-infection dynamics *in situ*, that utilizes the versatility of padlock probes.

Results: The labeling approach was able to identify influenza A virus (IAV) infections in cells and lung tissue with single-nucleotide specificity and was used to classify viral entry and replication into different stages based on the gene segment localization. By extending the classification strategy to cells co-infected by IAVs with single nucleotide variations, we found that the dependence on intracellular trafficking places a time-restriction on secondary co-infections.

Conclusions: Our results demonstrate that this RNA viral genome labeling approach can help dissect the process of viral entry in a more defined manner and can distinguish between cell infections by viruses with high sequence precision. They also show that two IAVs must satisfy time as well as a spatial parameter to establish productive cell co-infections necessary for the process of reassortment.

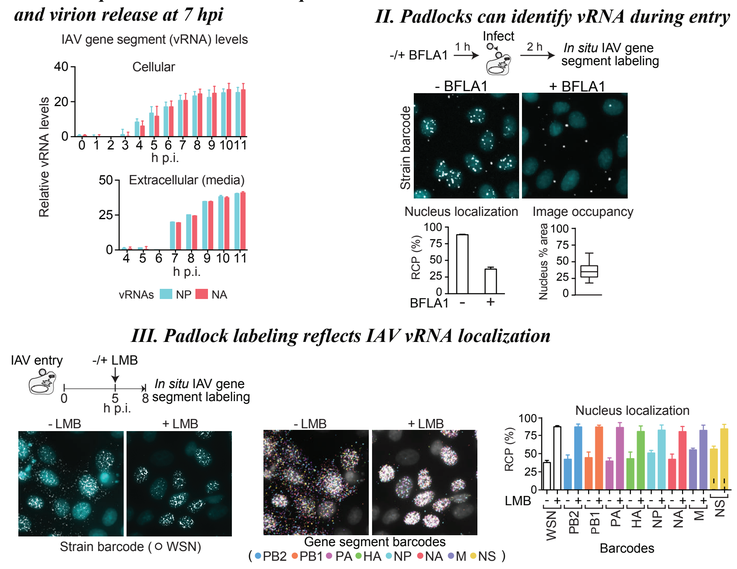


Method: *In Situ* Padlock Approach for IAV Gene Segment vRNA Labeling and Single-Cell Analysis

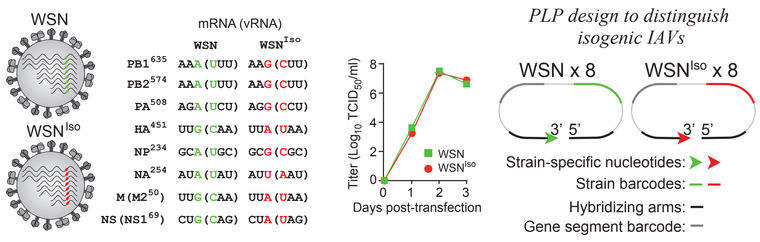


Results:

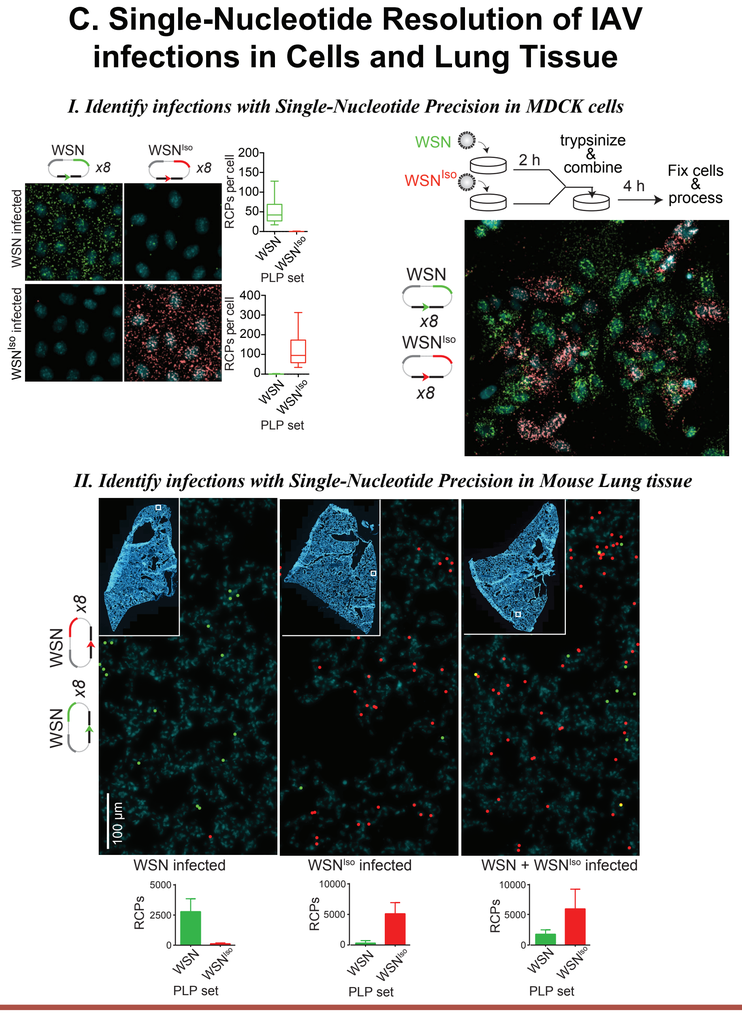
A. Padlock probes efficiently track IAV genome segment vRNA localization *in situ*



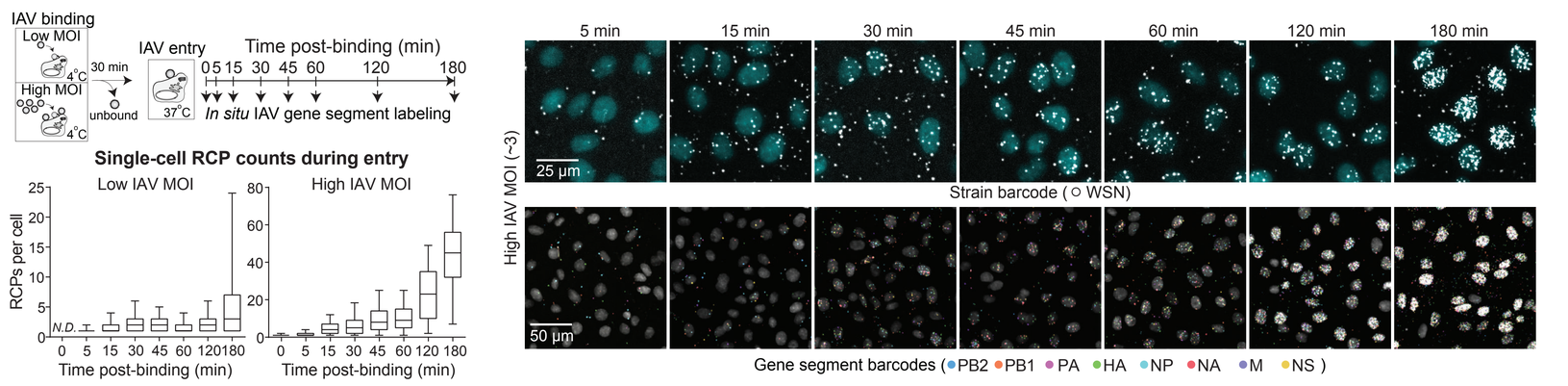
B. Design for Labeling IAV Gene Segments with Single-Nucleotide Substitutions



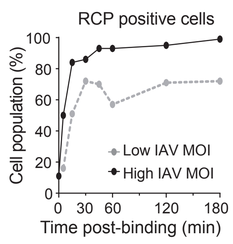
C. Single-Nucleotide Resolution of IAV infections in Cells and Lung Tissue



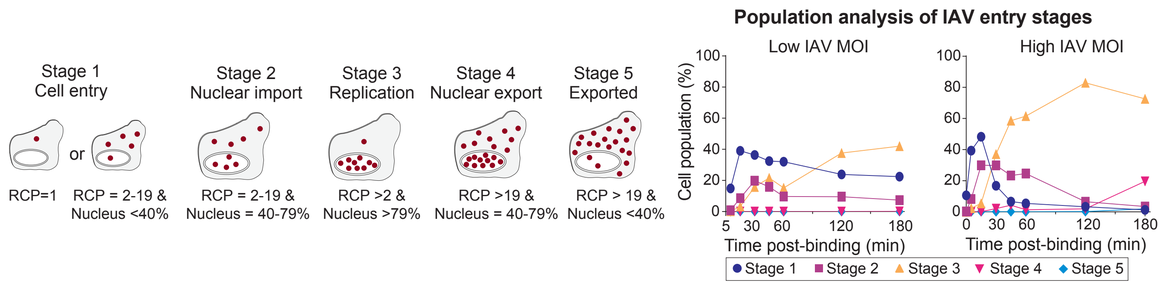
D. Analysis of IAV Entry Kinetics by Gene Segment Labeling



Entry half-time for one viral particle



E. Classification of IAV Infection Stages by Gene Segment Labeling



F. Co-labeling of IAV Genomes Reveals the Time Window for Productive Cell Co-infections