Antagonism between viral infection and innate immunity at the single-cell level

Marek Kochańczyk[1], Frederic Grabowski[1], Maciej Czerkies[1], Zbigniew Korwek[1], Wiktor Prus[1] and Tomasz Lipniacki[1]

***Abstract*— Recognition of viral RNA initiates a signaling cascade culminating in synthesis of interferons (IFNs). Secreted IFNs, by activation of transcription factors STAT1/2 in surrounding cells, prompt them to prepare for viral infection. Viruses, in turn, convey non-structural proteins to impede the innate immune response. Based on results obtained using single-cell techniques, we proposed an agent (single cell)-based, stochastic, computational model and used it to explain how a population of cells can spontaneously stratify into IFN producers and IFN responders. The model reproduces the experimentally observed complex spatial patterns of respiratory syncytial virus (RSV) spread and dichotomous cell responses.**

***Keywords*— virus–host interactions, infection dynamics, cell fate decision making, stochastic cellular automaton, bistability, interferon-stimulated genes, microscopic image analysis**

I. Background

When viral infection progresses, IFNs reach most cells before they come into contact with the proliferating virus. Consequently, a population of infected cells is stratified into three functionally distinct subpopulations: (*i*) primary infected cells that may produce IFNs, (*ii*) not-yet-infected cells that respond to IFNs, and (*iii*) IFN-primed cells that become infected due to infection spread. The emergence of these subpopulations is spatially organized and markedly stochastic (Fig. 1A).

Computational modeling of viral spread requires an agent-based approach, wherein individual cells execute their internal programs and communicate via IFNs, and virus spreads by infecting neighboring cells.

II. Results

By analyzing Western blots for multiple components involved in innate immune signaling in A549 WT cells and five A549 KO cell lines — subjected to: IFN stimulation, RSV infection, and both these agents subsequently — we were able to delineate the structure and determine dynamics of the regulatory network (Fig. 1B).

Single cell-level analysis of fluorescence microscopy images from immunostaining allowed to capture the spatial organization of the system (cell neighborhood was established according to Voronoi tessellation). Spatial regulation of signaling was expressed in terms of signed Kolmogorov–Smirnov (KS) statistics for RSV fusion protein-conditioned phospho-STAT1 and, auxiliarily, for IRF3-conditioned phospho-STAT1 (KS for neighboring cells and KS for the same cell were compared). A bistable single-cell switching between the innate immune response and viral replication was characterized by means of the risk ratio of IRF3 *vs*. RSV fusion protein and of IFNβ *vs*. RSV fusion protein (all these being quantized as binary observables).

The model-implicated bistable switch between virus replication and the host system immune response was validated using live cell microscopy of A549 cells expressing GFP under IFNβ promoter and a fluorescently tagged RSV.

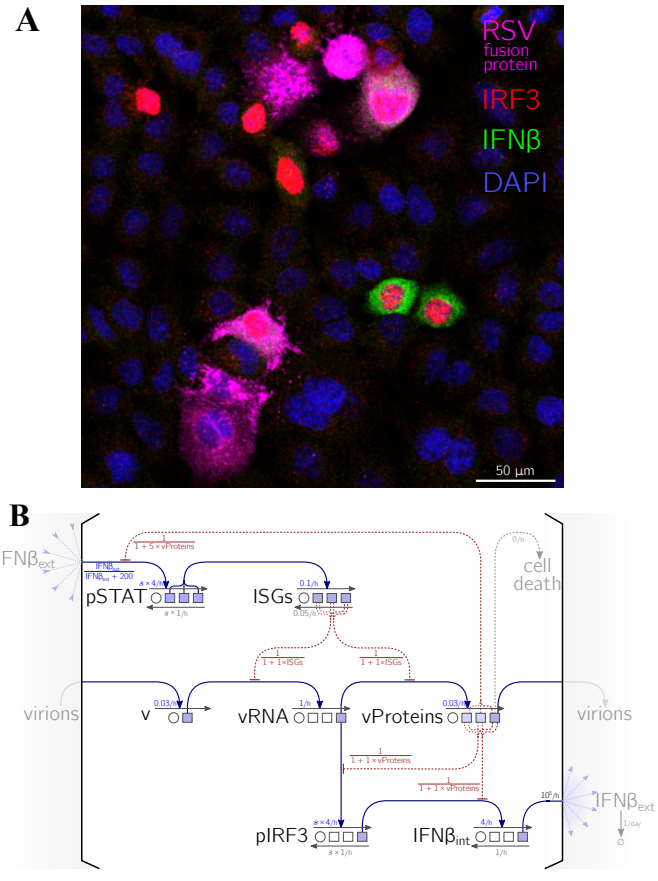

Fig. 1. (**A**) Immunostained monolayer of A549 cells infected with RSV at MOI = 0.01. (**B**) Scheme of transitions and interactions simulated in each cell-agent (hexagonal node of a triangular lattice). Intracellular stochastic dynamics is coupled with deterministic diffusive IFNβ propagation.

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[1]Department of Biosystems of Soft Matter, Institute of Fundamental Technological Research of the Polish Academy of Sciences, Warsaw, Poland. {mkochan,fgrabows,mczerkie,zkorwek,wprus,tlipnia}@ippt.pan.pl.