Biphasic Translation during Viral Infection

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***Short Abstract* — During viral infection cells have to execute transcription and translation programs to secret IFNβ and IL6 in order to inform other cells about pathogen threat. They should also degrade RNAs and inhibit translation in order to limit virus replication. By analyzing responses to RSV (respiratory syncytial virus) at the single-cell level we found that these two opposing programs are executed simultaneously within cell population.**

I. Background

THE innate immune responses are regulated by interlinked feedback loops mediated by three potent transcription factors: NF-κB, IRF3, and STAT1/STAT2. At the cell population level, cells are coordinated by IFNβ, which is secreted by infected or poly(I:C)-stimulated cells, and via paracrine signaling activate STAT1/2, triggering synthesis of antiviral proteins including RIG-I (sensor of dsRNA), PKR (kinase responsible for inhibition of protein translation) and OAS1A (responsible for mRNA degradation). In response to poly(I:C), IFNβ-primed cells rapidly produce antiviral responses and commit to apoptosis [1].

II. Results

Here, by analysis of A549 cell responses to RSV and poly(I:C) at small Multiplicities Of Infection (MOI=0.001–0.1), we found a distinct protein synthesis regulation in naïve and IFNβ-primed cells (Fig. 1).

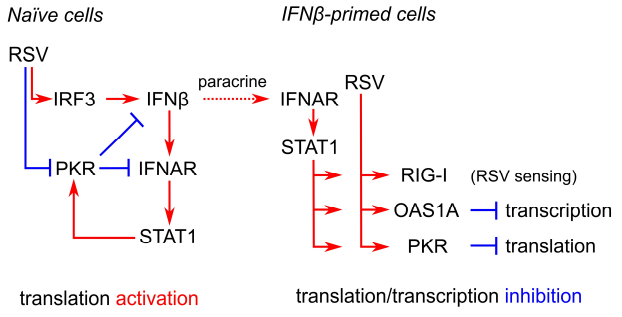

Fig. 1. Regulation of translation in naïve and IFNβ-primed cells upon RSV.

In naïve cells, RSV/poly(I:C) lead to downregulation of PKR (observed at both mRNA and protein level) and activation of IRF3 (and NF-κB), leading to massive IFNβ synthesis and secretion. By autocrine regulation IFNβ activates STAT1/2 allowing for PKR resynthesis. This in turn shuts down autocrine IFNβ signaling by depletion of IFNβ receptor (IFNAR) due to its endocytosis and inhibited translation.

In IFNβ-primed cells, STAT1/2 triggers synthesis of RIG-I, OAS1A and PKR. Upon subsequent contact with RSV, both OAS1A and PKR are activated enabling primed cells to degrade viral RNA and inhibit protein synthesis. In this way, at small MOI, almost all cells may can have their translation inhibited shortly after contact with virus. 16 h after infection at MOI=0.01, all but RSV infected cells show STAT1 activation (phosphorylation and nuclear translocation).

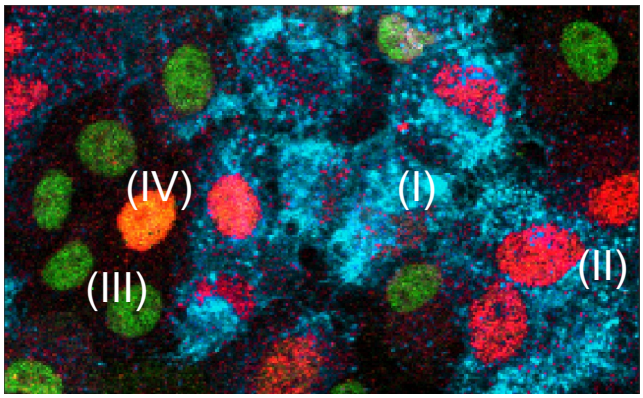

Fig. 2. Heterogeneous regulation of IRF3 (red) and STAT1 (green) 24 hours after RSV (blue) stimulation at MOI=0.1.

The discussed biphasic regulation can be observed through immunostaining. In Fig. 2, RSV-infected cells are stained blue (I). IRF3 (together with NF-κB, not stained here, whose activation is well-correlated with that of IRF3) is activated in a fraction of infected cells (II), leading to IFNβ secretion and STAT1 activation in neighboring cells (III). RSV-expressing cells show almost no STAT1 activation (due to IFNAR depletion). Some IFNβ-primed cells (IV) show IRF3 activation but no expression of viral proteins, suggesting the occurrence of RSV infection not followed by virus replication due to both its RNA degradation and inhibition of protein synthesis. Thus, differential translation regulation in naïve and IFNβ-primed cells slows down the spread of infection.

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References

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