Super Resolution Microscopy Reveals that Caveolin-1 is Required for Antiviral Immune Response

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Abstract

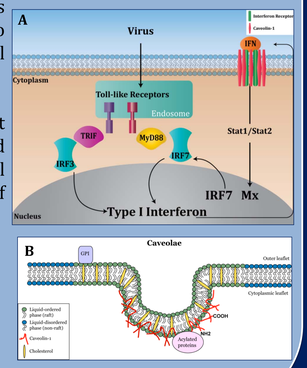
Caveolae are integral to numerous signaling pathways but their function in antiviral signaling is largely unexplored. Understanding spatial distribution and dynamics of receptors within unperturbed membranes is essential for elucidating the role of caveolae in antiviral signaling, but conventional studies of detergent-resistant membrane fractions cannot provide this information. While it is known that viruses exploit caveolae for entry into host cells, this study instead demonstrates an entry-independent mechanism for virus evasion of host cell defenses through disruption of clusters of signaling molecules organized within caveolae. Interferon (IFN) is crucial for mounting an innate antiviral response to infection, and *in vivo* knockdown of caveolin-1 (Cav-1) in zebrafish embryos showed disruption of this response, rendering the host more susceptible to infection. Super-resolution microscopy has enabled the first single-molecule imaging of interactions between type I interferon receptors (IFN-R) and caveolae. In particular, Cav-1 deficiency caused IFN-R clusters to disperse, suppressing antiviral immune response through abrogation of downstream signaling, a response strongly suggesting that IFN-R organization within caveolae is critical for IFN-mediated antiviral defense.

Introduction

Virus infections elicits an antiviral response. Pivotal to this is the IFN response, which is crucial for mounting a response to viral infection and triggers the induction of numerous antiviral genes[1] (A).

Caveolae represent a specialized morphologically distinct membrane domain (B). Critical cellular processes associated with caveolae include signal transduction, cholesterol homeostasis, and immune signaling[2-5]. Cav-1 serves as one of the structural components of caveolae.

We aim to:
- Elucidate the role of caveolae in the IFN response.
- Identify the spatial relationship between Cav-1 and IFN-R.
- Investigate the importance of Cav-1 for an antiviral response.



Fluorescence Photoactivation Localization Microscopy (FPALM)

- FPALM[6-9] relies on both the imaging of single molecules and the stochastic activation of sparse subsets of molecules to control the number of visible fluorescent molecules in the field of view.
- Reliable localization of single fluorescent emitters requires that molecules be spatially well-separated enough to be distinguished from one another.
- Images are subsequently reconstructed from the coordinates and intensities of the localized molecules.

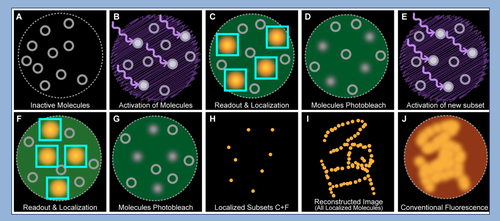


Figure 1. Principle of localization-based super-resolution microscopy techniques[9]. By controlling the number of fluorescent molecules emitting light at once such that only a sparse subset of molecules is activated at once, the images of single molecules become distinguishable. Molecules are initially in an inactive (non-fluorescent) state (A). Sparse subsets of fluorescent molecules are activated (B) and then imaged (C) until deactivated or photobleached (D). Molecules are localized by fitting the image with a two-dimensional Gaussian. Cycles of activation (B,E), readout and localization (C,F), and photobleaching (D,G) are repeated for many subsets of fluorescent molecules. Rendered images with few (H) and large number (I) of localized molecules show buildup of structural detail as density increases.
(J) Conventional image with diffraction-limited resolution.

IFN-R Colocalizes with Caveolin-1 in Caveolae Membrane Domains

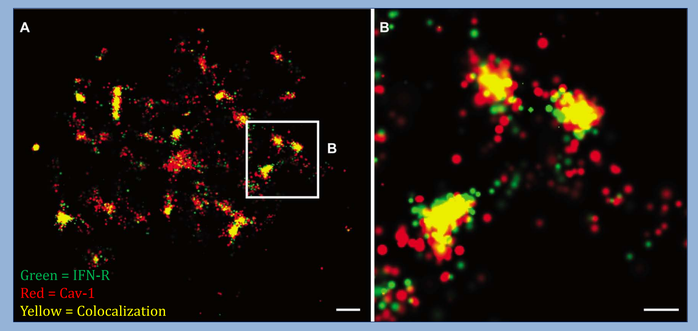
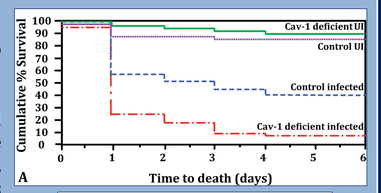


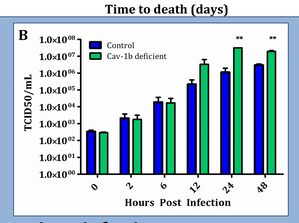
Figure 2. Cav-1 colocalizes with the zebrafish homolog of IFN-R. FPALM images of zebrafish cells expressing Cav-1-PA mCherry (red) and IFN-R-dendra2 (green). For all images, 60x objective/1.2 NA. Scale bars, 1 µm.
[A] Cav-1 molecules show significant co-localization with IFN-R molecules.
[B] Magnification of the white box in A. Cav-1 enriched domains cluster together with IFN-R molecules

IFN-R colocalizes and clusters within caveolae membrane domains.

Caveolin-1 Is Critical For Survival Upon Viral Challenge

Figure 3. *Cav-1* Deficient Embryos Are Unable to Clear Virus Infection.
[A] *Cav-1 deficiency leads to greater mortality.*
Embryos were infected with 1 x 10^6 $TCID_{50}$/ml virus and monitored for mortality. Results are representative of three separate experiments. Statistical analysis (Wilcoxon test) was performed ($p<0.01$).
[B] *Cav-1 deficiency leads to greater viral burden.*
Fish were infected as above. Error bars are standard error of the mean for three experiments. (*, p=0.01).



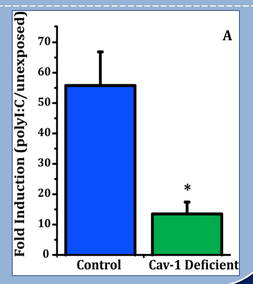


Cav-1 deficiency leaves embryos more susceptible to virus infection.

Caveolin-1 Deficiency Leads to Impaired Antiviral Response

Figure 4. Decrease of Cav-1 expression negatively affects the IFN pathway.
[A] *IFN activity is dampened when Cav-1 is depleted in zebrafish cells.*
Figure represents three experiments and shows fold change of cells exposed to poly(I:C) compared to unexposed cells. Error bars are representative of standard error of the mean for three experiments (*, $p<0.05$).

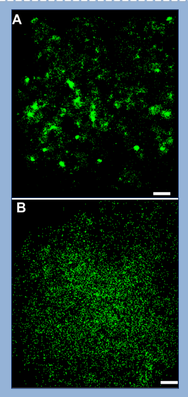
IFN activity is dampened due to Cav-1 deficiency.



Virus Infection Leads to Dispersed IFN-R Molecules

Figure 5. Virus Infection Reduces IFN-R Clustering. FPALM images of zebrafish cells expressing IFN-R-dendra2. For all images, 60x objective, 1.2NA. Scale bars, 1 µm.
[A] *IFN-R molecules are clustered in the cell membrane.* Uninfected cells overexpressing IFN-R demonstrate that the receptor exists in clusters indicative of caveolae**.**
[B] *Infection leads to dispersal of IFN-R molecules.* Cells overexpressing IFN-R and infected with virus demonstrate that IFN-R becomes dispersed as a result of infection.

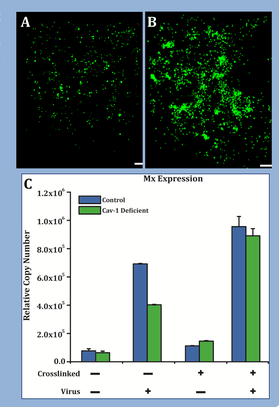
IFN-R molecules in cells become dispersed as a result of whole virus infection.



IFN-R Clustering is Crucial for Antiviral Signaling

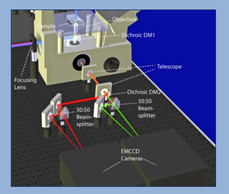
Figure 6. Expression of Mx, an IFN-inducible gene, is rescued with crosslinking, despite Cav-1 depletion. Zebrafish cells were transfected, allowed to recover, and crosslinked with BS^3 reagent.
- Cav-1 deficient cells with no BS^3 treatment show dispersed IFN-R molecules [A], while Cav-1 deficient cells with BS^3 treatment show that clusters of IFN-R are maintained [B].
- When Cav-1 is depleted and cells are not crosslinked, minimal Mx expression is measured. When Cav-1 is depleted and IFN-R is clustered, Mx expression remains equal to controls [C].

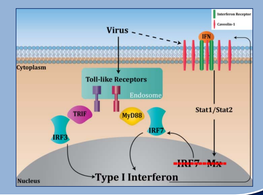
Clustering of the IFN-R is critical for downstream signaling and caveolae membrane domains play a critical role in maintaining clusters of IFN-R molecules.



Conclusions

- Caveolin-1 Deficiency Leads to:
  - Increased mortality *in vivo*
  - Increased viral burden *in vivo*
  - Dampened IFN response *in vitro*
- FPALM enabled visualization of the intricate interaction between Cav-1 and IFN-R molecules:
  - IFN-R colocalizes with Cav-1
  - Caveolae domains maintain IFN-R clusters
  - IFN-R clusters are dispersed upon virus infection
  - Antiviral signaling through the IFN signaling pathway depends on IFN-R clusters
- These results lead to an expanded view of the biological function of caveolae in the cellular response to virus infection





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