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Unraveling the antiviral activity of plitidepsin by subcellular and morphological analysis

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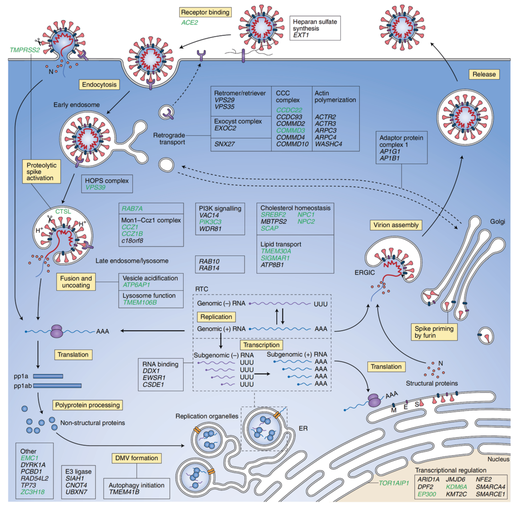
1. Background

The use of compounds against highly conserved cellular host factors required to complete the replication cycle of distinct viruses such as SARS-CoV-2 offers a common solution to diverse viral threats. This approach is especially relevant for pan-antiviral effects given that viruses converge at intracellular steps such as viral genome replication and protein production.

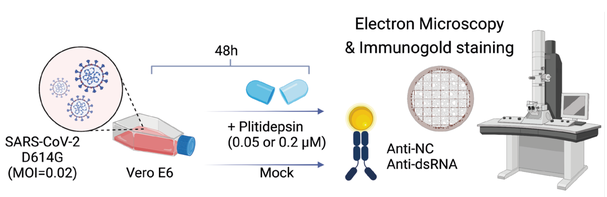


Baggen, J., et al., 2021. Nat Microbiol. https://doi.org/10.1038/s41564-021-00958-0

Plitidiepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A. However, how plitidepsin exerts its antiviral activity remains unknown.

Here we aimed to explore the antiviral effect of plitidepsin at the cellular level

2. Materials & Methods



https://www.biorxiv.org/content/10.1101/2021.12.16.472880v1

3. Results

Ultrastructural analysis allowed to recapitulate the SARS-CoV-2 infectious life cycle, where evident viral double membrane vesicle (DMV) formation was observed (**Fig. 1A-B**; **Fig. 2A-C**) as well as viral budding events (**Fig. 1C**) along with cell-associated viruses (**Fig. 1A**, inset). However, in cells treated with plitidepsin at different non-toxic concentrations (0.2 or 0.05 μM), there was a lack of viral DMV formation and a complete absence of viral particles (**Fig. 1D-F** and **Fig. 2D-G**).

Figure 1

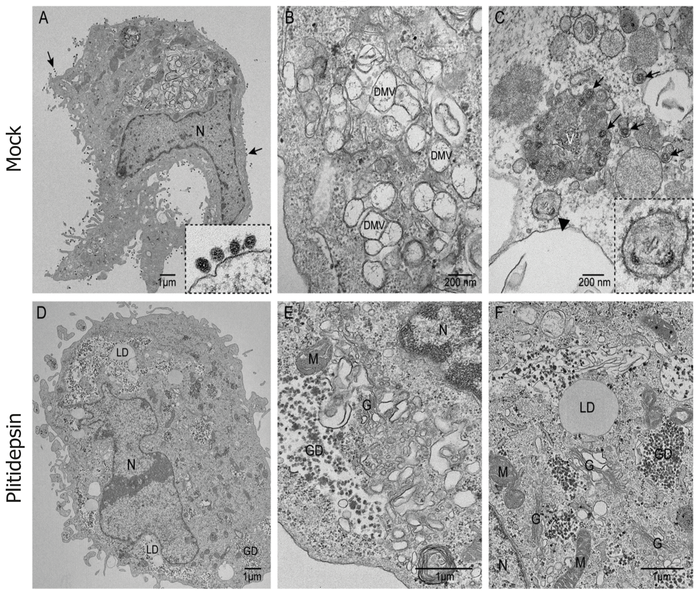
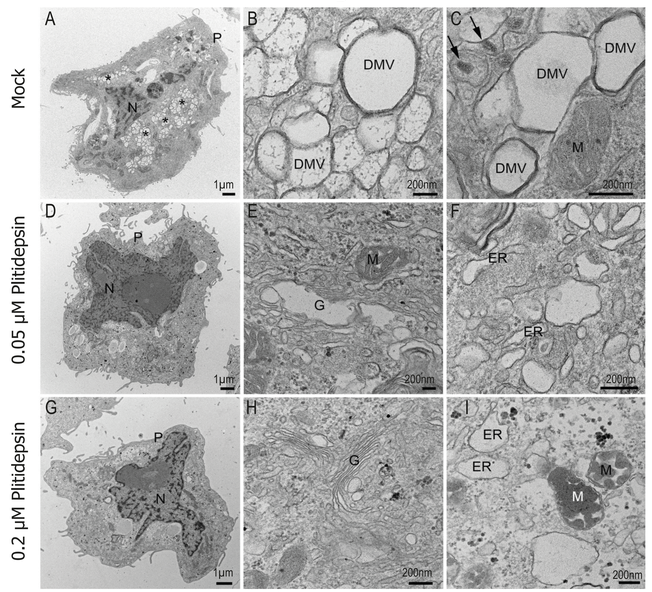


Figure 2



Arrows; viral particles; N: Nucleus; DMV: Double membrane vesicle; V: Single membrane vesicles with viruses; LD: Lipid droplet; M:Mitochondria;G:Golgi stacks; GD; Glycogen deposits.

Asterics:DMV; P: Plasma membrane.

SARS-CoV-2 nucleocapsid and dsRNA immunogold staining detected viral products in mock treated cells (**Fig. 3 A-C** and **Fig. 4 A-C**, respectively). In sharp contrast, plitidepsin abrogated the detection of nucleocapsid or dsRNA (**Fig.3 D-E** and **Fig. 4 D-F**).

Figure 3

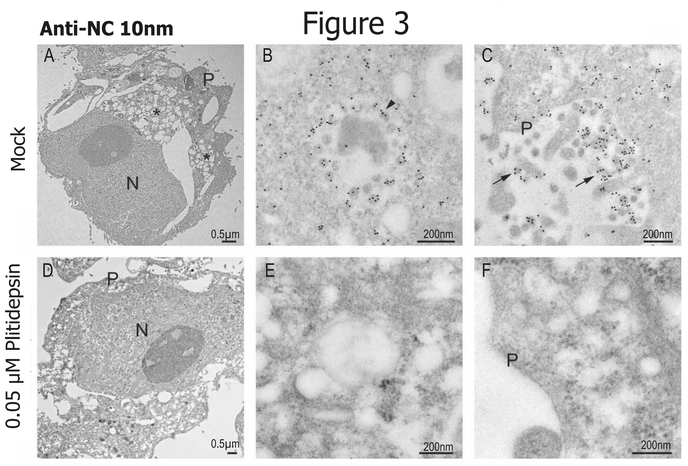
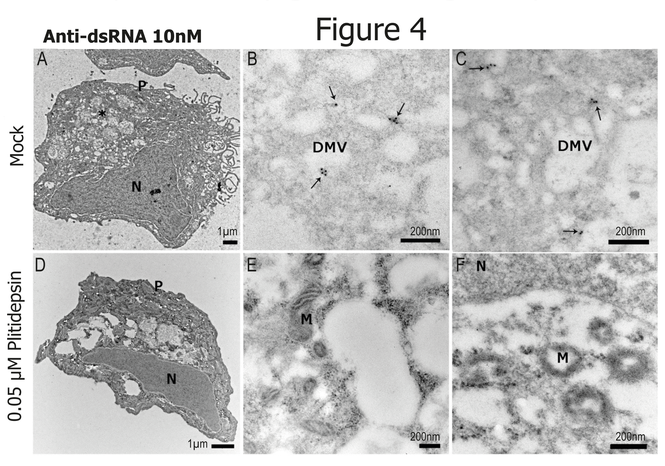
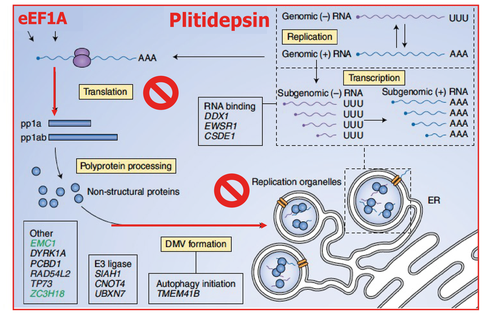


Figure 4



4. Conclusions

- Plitidepsin-mediated anti-SARS-CoV-2 activity at low concentrations (50 nM) may be the result of a highly specific inhibition of genomic RNA viral translation.
- Protein synthesis inhibition in eukaryotic cells is achieved at higher concentrations of plitidepsin (e.g. >450 nM).
- Inhibition of the translation may affect viral non-structural proteins, whose predicted transmembrane domains are key for DMV formation during the replication of distinct coronaviruses.



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Electron microscopy ultrastructural analysis coupled to immunogold staining of SARS-CoV-2 products offer a unique approach to understand how antivirals work.

5.Acknowledgments

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