INFLUENZA A VIRUS PROPAGATION IN MDCK: INTRACELLULAR VIRUS REPLICATION, VIRUS RELEASE AND CELL-CYCLE PREFERENTIAL INFECTION ANALYSIS

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Cell culture-based processes for vaccine manufacturing offer advantages over egg-based processes in terms of product uniformity and sterility, production time and scaling up capacity[1,2]. Regarding influenza vaccines, MDCK cells are one of the host cell lines currently used to manufacture licensed products; however, virus titers remain lower compared to those obtained in eggs and further increase of specific and volumetric yields is required. To identify bottlenecks in influenza A virus (IAV) production, we thoroughly studied IAV replication in MDCK cells. For this, we analyzed different features of the infection process such as viral RNA replication, intracellular localization of viral components, virus release and morphology of the particles, and the preferential infection in different cell-cycle phases.

Using synchronous infections, we found that production of infectious particles dropped much earlier than the production of total particles. Furthermore, we found that the maximum virus release rate was reached when all viral RNA species attained their maximum intracellular concentration. Using qPCR we determined that the vRNA maximum concentration per cell was 10-fold higher than the specific viral titers obtained, indicating that vRNA concentration does not limit IAV particle assembly. When we evaluated the morphology of particles released using electron microscopy, we observed that a higher fraction of the viral particles produced at late times possess an abnormal morphology, concurring with the increased production of non-infectious viruses.

Using imaging flow cytometry, we determined that the export of influenza viral genome segments (ribonucleoprotein complexes, vRNPs) from the nucleus to the cytoplasm strongly correlated with the onset of virus release. However, our results also suggest that the induction of apoptosis caused that virus assembly became deficient producing more non-infectious particles at late infection times.

Lastly, using low MOI infections and imaging flow cytometry, we found that -in contrast to previous publications- IAV did not preferentially infect a specific cell cycle phase and no cell cycle arrest induction was observed during the time frame of the experiment (9 hpi).

In summary, the data presented here offers a comprehensive overview of the dynamics of IAV infection in MDCK and might contribute to the development of molecular or cell culture-based strategies to improve IAV production in MDCK cells.

[1] Gallo-Ramírez et al, 2015. Exp. Rev. Vaccines 14 (9).
[2] Pardue et al. 2011. Exp. Rev. Vaccines 10 (8).