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# Madin-Darby canine kidney cells express multiple transmembrane serine proteases supporting trypsin-independent replication of various influenza viruses

## Inhalt

Hemagglutinin (HA) is a key determinant of influenza A virus (IAV) virulence. HA mediates host cell binding and membrane fusion, the latter requiring proteolytic cleavage into HA1 and HA2. Most IAVs contain a monobasic cleavage site recognized by trypsin-like proteases expressed only in specific cell types.

This study compared IAV replication in various cell lines, including MDCK-I, MDCK-II and MDCK-NBL2, with and without exogenous trypsin. All MDCK sublines supported trypsin-independent replication, although with delayed kinetics and reduced titers compared to trypsin-dependent replication. MDCK-NBL2 supported multiple IAV strains, including human H1N1, porcine H3N2, and avian H5Nx, H7N7, and H9N2 viruses. Analysis of H5Nx viruses revealed marked differences in the proteolytic activation despite identical HA cleavage motifs.

Serine protease inhibitors blocked trypsin-independent replication in MDCK-NBL2 cells, indicating involvement of endogenous serine proteases. RT-PCR identified TMPRSS1, TMPRSS2, TMPRSS4, and TMPRSS11D (HAT) expression in MDCK cells. Canine TMPRSS2 and TMPRSS4 were found to support the activation of H1N1 HA. Other canine TMPRSS proteases are under investigation.

Overall, these findings identify MDCK-NBL2 cells as a convenient cellular system for influenza virus isolation, screening of protease inhibitors as potential antiviral compounds, and vaccine production without the need for exogenous animal-derived trypsin.

## Keywords

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## Professional status of the speaker

Graduate student

## Junior scientist status

No, I am not a junior scientist.

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