VIRAL INFECTION

Entry through tunnel vision

Entry of simian virus 40 (SV40) particles into a host cell depends on the interaction between the major capsid protein (VP1) and a glycolipid receptor, monosialotetrahexosylganglioside (GM1). Ewers *et al.* now show that SV40 particles induce the formation of tubular invaginations in the host cell membrane in a manner that is dependent on the acyl-chain length of GM1 but independent of active host cell trafficking machinery.

The SV40 capsid is composed of 72 icosahedrally organized VP1 pentamers, each of which possesses 5 GM1-binding sites. SV40 particles probably bind multiple GM1 molecules and enter the cell through clathrin-independent endocytosis. To assess the importance of the glycolipid structure of GM1 and the multivalent binding of SV40 during cell entry, Ewers *et al.* incorporated a range of GM1 lipid species differing in the composition of the lipid backbone into a mutant mouse melanoma cell line (GM95) that lacks all endogenous glucose-based glycolipids. Although SV40 particles were unable to bind untreated GM95 cells, they bound GM95 cells supplemented with native GM1 to a similar extent as they bound wild-type mouse cells. Consistent with this observation, GM95 cells were resistant to infection by SV40, whereas up to 76% of cells supplemented with native GM1 could be infected. When these cells were supplemented with GM1 species bearing acyl chains of varying lengths and saturations, binding of SV40 could be restored in all cases. However, SV40 infection was only supported in GM95 cells supplemented with GM1 species bearing long acyl chains, indicating that the glycolipid receptor tail structure is crucial for SV40 infection.

Using transmission electron microscopy, the authors observed viral particles residing in membrane invaginations as well as tight-fitting vesicles. Blocking host cell trafficking processes led to the formation of numerous SV40-containing membrane tubules of varying lengths. Similar tubules were also observed in mouse embryonic fibroblasts lacking caveolin 1, suggesting that SV40 induces the formation of membrane invaginations in the absence of active cellular trafficking machinery and caveolar coats. Indeed, SV40 particles could stimulate the formation of membrane tubules in cytosol-free giant unilamellar vesicles (GUVs) containing native GM1 but not in those either lacking GM1 or containing GM1 species with short acyl chains. Furthermore, a recombinant VP1 pentamer that could not form viral particles could also induce tubulation in GUVs, although, in comparison with SV40 particle-induced tubulation, a time lag between VP1 addition and the formation of membrane tubules was observed.

Finally, the authors propose a thermodynamic model of the tubulation process that, together with these data, suggests that although multivalent binding to GM1 by the VP1 pentamer alone can be sufficient for membrane deformation, it is the curved, colloidal organization of GM1-binding sites in the SV40 particle that makes this process highly efficient. Different pathogens and pathogenic factors, such as the GM1-binding cholera toxin, have probably evolved a common mechanism based on glycosphingolipid clustering to drive the coat-independent bending of the plasma membrane as an initial step for their uptake into cells.

Andrew Jermy

> “SV40 particles induce the formation of tubular invaginations in the host cell membrane”

ORIGINAL RESEARCH PAPER Ewers, H. *et al.* GM1 structure determines SV40-induced membrane invagination and infection. *Nature Cell Biol.* 20 Dec 2009 (doi:10.1038/ncb1999)