PERSPECTIVE

Receptor-mediated increase in rabies virus axonal transport

Rabies virus (RABV) of the rhabdoviridae family is a prototype neurotropic virus that causes a fatal disease, and is still a major risk mostly in developing countries. A key step in the RABV infection process is its arrival into the central nervous system (CNS), for which it uses the cellular transport machinery. Neurons are irregular cells with a specialized anatomy, and often extend lengthy axons that may span over a meter long. In infected organisms, RABV virions enter the neuron periphery at the area of a bite and must overcome great distances in order to reach the peripheral neuron's cell body and from there, the CNS. To this end, RABV exploits the retrograde axonal transport machinery, a fast and directed route aimed for trafficking cargo from the neuron periphery to its soma. RABV's neuronal tropism and retrograde propagation, combined with the development of safe, labeled viruses in recent years (Klingen et al., 2008), have rendered it ideal for neural and synaptic tracing.

The p75 neurotrophin receptor ($p75^{NTR}$) and the neuronal cell adhesion molecule (NCAM) are both neuronal transmembrane proteins that were suggested to act as receptors for RABV. However, their exact role in RABV infection is not clearly understood, as neither was found imperative for RABV infection (Lafon, 2005; Tuffereau et al., 2007). In addition, RABV binds the α1 subunit of the nicotinic acetyl choline receptor (nAChR) located at the post-synaptic neuromuscular junction (NMJ). The function of this interaction is not yet clear as well, but may stimulate accumulation of RABV virions at the area of the NMJ, hence increasing the probability of RABV to infect the pre-synaptic motor neuron (Lafon, 2005). Interestingly, both $p75^{NTR}$ and NCAM are also known receptors for the nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF), respectively. Both factors are considered to promote sensory (NGF) and motor (GDNF) neuron survival. Similarly to RABV, trophic factors like NGF require receptor mediated internalization and long distance retrograde axonal transport to induce specific signaling. Another possible resemblance, as GDNF is secreted from muscles and acts on motor neurons, the NMJ might be its preferred binding/entry site. Considering these cellular receptor-ligand interactions, it is tempting to hypothesize that RABV not only hijacks the endogenous machinery aimed for transporting trophic factors, but also affects ligand-dependent downstream signaling. Using live total internal reflection fluorescence (TIRF) imaging, we have recently reported that like NGF, RABV binds $p75^{NTR}$ at the tip of primary sensory axons, followed by co-internalization of the receptor and its viral ligand. Subsequently, RABV was transported along the axon with $p75^{NTR}$ in a conjoint compartment, as seen in compartmentalized microfluidic neuronal cultures. Most of these transport compartments were acidic, indicating they might be a part of the signaling endosome machinery (Gluska et al., 2014). Our results sit well with those of Hislop et al. (2014) who reported that lentiviral vectors pseudotyped with RABV-G are localized with both $p75^{NTR}$ and NCAM in primary motor axons, after infection of the distal axon in compartmentalized cultures. In this study, the conjugation with RABV-G envelope proteins directed viral vectors into the endocytic pathway as seen from their colocalization with rab5 and rab7 proteins.

Upon characterization of RABV retrograde transport, we have found that the retrograde movement of RABV was faster than that of the endogenous $p75^{NTR}$ ligand NGF, although there was no significant difference in their internalization into the cell. This led us to look closer into the RABV trafficked compartments. When divided into two separate groups, we found most RABV-compartments were $p75^{NTR}$ positive. These compartments had higher instantaneous velocities and paused fewer and for shorter times along the way towards the cell body. Another interesting feature was these compartments' directedness: the $p75^{NTR}$ positive endosomes had a higher rate of retrograde movement in the direction of the cell soma. In short, compartments containing both RABV and $p75^{NTR}$ were more directed, their movement more continuous and had higher speeds when compared to RABV compartments negative for $p75^{NTR}$ (Gluska et al., 2014). It is not yet clear, whether RABV binding to NCAM plays a similar role in the virus transport or whether both receptors are synergistically involved in the fast retrograde transport.

The observed stimulating effect of the RABV- $p75^{NTR}$ association on the retrograde transport of RABV suggests that binding and/or uptake of the receptor induce down-signaling processes that may facilitate RABV's axonal transport to the cell soma. Due to their high polarity and extensive lengths, neurons exert substantial effort in order to sustain a fast and efficient axonal transport machinery. This process is important for trafficking proteins, organelles and RNA from the cell body to its periphery, and on the other direction, for transporting internalized signals and cargo for recycling. These are crucial for neuronal health and survival, as evident from axonal transport deficiencies preceding several neurodegenerative and neurological disorders. Several levels of regulation over axonal transport are suggested. Among them are the quantity and stability of the microtubule tracks, regulation of the motor proteins activity by post-translational modifications such as phosphorylation, motor-cargo adaptors and energy (ATP) supply required for motor advancement. As our data demonstrate two separate effects on RABV transport, higher velocities on one hand and fewer, shorter pauses on the other, it is thus proposed that the virus-receptor downstream signaling may be involved in more than one level of axonal transport regulation. Moreover, viral particle dependent receptor/motor clustering might increase transport processes independently of signaling events. The different modes, by which viruses, as ligands, can regulate transport process, create a unique model for studying axonal transport mechanisms.

A possible explanation for the enhanced transport is that the RABV-$p75^{NTR}$ complex induces formation of faster, efficiently targeted signaling endosomes, which benefit from the abundance of either motor proteins/adaptors or available ATP. Indeed, we have seen that $p75^{NTR}$ positive compartments were larger in size and had a stronger RABV-derived fluorescent signal (Gluska et al., 2014), possibly indicating the presence of several RABV particles in a single compartment, or fusion of multiple virus-containing compartments.

Another contributing factor could be the recruitment and utilization of microtubule networks, as in the case of HIV1, which was recently shown to promote microtubule stabilization as part of its infectious cycle (Sabo et al., 2013). The exact function of either RABV receptor in the internalization and sorting process of RABV particles is still unclear. Nonetheless,

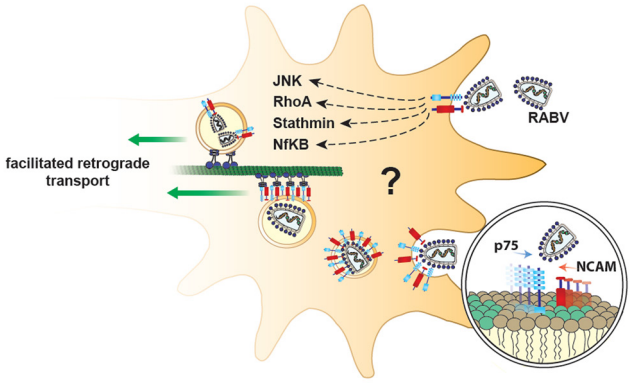

Figure 1 Receptor binding increases RABV axonal transport suggested model.
RABV facilitates its axonal transport by binding to its receptors, p75 neurotrophin receptor ($p75^{NTR}$) and neuronal cell adhesion molecule (NCAM) and altering signaling pathways (**The Signaling Theory**), or alternatively, by recruiting more motors *via* creating receptor clusters along the cell membrane (**The Receptor Clustering Theory**). RhoA: Ras homolog gene family, member A; JNK: c-Jun N-terminal kinases; NfKB: nuclear factor-kappaB; RABV: rabies virus; NCAM: neuronal cell adhesion molecule.

it is feasible that the binding of RABV to NCAM/$p75^{NTR}$ clusters at the cell membrane promotes the recruitment and tethering of both microtubules and dynein motors. GDNF for example, binds its receptors NCAM and GDNF family receptor α1 (GFRα1), to encourage phosphorylation of NCAM-associated Fyn kinases, which in turn induce cytoskeletal effects (Ibáñez, 2010). Other studies have shown that NCAM binds dynein directly, and the latter can tether microtubule tracks to the cell cortex (Perlson et al., 2013). We propose that RABV mimics trophic factors by binding to their receptors, then initiates a signaling cascade that regulates dynamic microtubules and forms tethering sites that provide a fast route from the cell cortex to the endocytic pathway and subsequently the cell soma (**The Signaling Theory**). To support this notion, we also recorded the mutual retrograde transport of RABV particles with fluorescently labeled GDNF and NGF in motor and sensory axons, respectively [unpublished data and fig. 5 from Gluska et al. (2014), respectively].

Although we have seen specifically that the transport of RABV particles is altered in the presence of $p75^{NTR}$, we have not ruled out that RABV has a global effect on axonal transport kinetics. Whether RABV enhances the transport of other cargos or the transport machinery as a whole remains to be seen. One such effect could be the induction of local protein synthesis. Indeed, it was recently reported that pseudorabies promotes local protein synthesis in order to undergo efficient retrograde axonal transport (Koyuncu et al., 2013). By enhancing the production of cytoskeletal elements, motor proteins, adaptors *etc.* RABV could increase its own probability and efficiency of transport, facilitating its route to the cell soma.

In addition to specific effects on $p75^{NTR}$ and NCAM signaling, motor activity and cytoskeleton rearrangements, it is possible that virus particles with multiple copies of the receptor binding G-heterotrimers bind multiple $p75^{NTR}$ receptors or even a combination of different neuronal receptors (*e.g.*, NCAM). This binding of RABV particles to $p75^{NTR}$/NCAM clusters on the cell surface may result in recruitment of multiple dynein motor complexes to these RABV endosomes thatin turn could lead to faster and more processive retrograde transport (**The Receptor Clustering Theory**) (**Figure 1**).

The study of viral retrograde transport has progressed significantly over the past few years, shedding light on basic machineries of both viral infection/propagation and internalized cargo trafficking. In spite of this, we have still much to learn regarding the involvement of neuronal receptors and down-stream signaling as well as motor, adaptor and cytoskeleton regulation in the transport of viruses (Taylor and Enquist, 2015). A better understanding of how RABV and other viruses exploit and enhance the axonal transport machinery could allow researchers to intervene in early stages of neurodegenerative processes and perhaps restore transport abilities. Furthermore, it may allow manipulation of cellular receptors and/or cargo in order to direct the latter in a fast, efficient way to relevant neuron populations.

This work was supported by the German Israeli Foundation for Scientific Research and Development (GIF) grant G-1107-73.1/2010 to EP and SF. The European Research Council (ERC) grant 309377 and Israel Science Foundation ISF grant 614/11 to EP.

Shani Gluska, Stefan Finke*, Eran Perlson*

Department of Physiology and Pharmacology, Sackler Faculty of Medicine, and the Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 69978, Israel (Gluska S, Perlson E)
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Molecular Virology and Cell Biology, D-17493 Greifswald – Insel Riems, Germany (Finke S)

***Correspondence to:** Eran Perlson, Ph.D. or Stefan Finke, Ph.D., eranpe@post.tau.ac.il or stefan.finke@fli.bund.de.*
***Accepted:** 2015-04-28*
***doi:**10.4103/1673-5374.158337* ***http**://www.nrronline.org/*
Gluska S, Finke S, Perlson E (2015) Receptor-mediated increase in rabies virus axonal transport. Neural Regen Res 10(6):883-884.

References

Gluska S, Zahavi EE, Chein M, Gradus T, Bauer A, Finke S, Perlson E (2014) Rabies Virus Hijacks and accelerates the $p75^{NTR}$ retrograde axonal transport machinery. PLoS Pathog 10:e1004348.
Hislop JN, Islam TA, Eleftheriadou I, Carpentier DCJ, Trabalza A, Parkinson M, Schiavo G, Mazarakis ND (2014) Rabies virus envelope glycoprotein targets lentiviral vectors to the axonal retrograde pathway in motor neurons. J Biol Chem 289:16148-16163.
Ibáñez CF (2010) Beyond the cell surface: new mechanisms of receptor function. Biochem Biophys Res Commun 396:24-27.
Klingen Y, Conzelmann KK, Finke S (2008) Double-labeled rabies virus: live tracking of enveloped virus transport. J Virol 82:237-245.
Koyuncu OO, Perlman DH, Enquist LW (2013) Efficient retrograde transport of pseudorabies virus within neurons requires local protein synthesis in axons. Cell Host Microbe 13:54-66.
Lafon M (2005) Rabies virus receptors. J Neurovirol 11:82-87.
Perlson E, Hendricks AG, Lazarus JE, Ben-Yaakov K, Gradus T, Tokito M, Holzbaur ELF (2013) Dynein interacts with the neural cell adhesion molecule (NCAM180) to tether dynamic microtubules and maintain synaptic density in cortical neurons. J Biol Chem 288:27812-27824.
Sabo Y, Walsh D, Barry DS, Tinaztepe S, de Los Santos K, Goff SP, Gundersen GG, Naghavi MH (2013) HIV-1 induces the formation of stable microtubules to enhance early infection. Cell Host Microbe 14:535-546.
Taylor MP, Enquist LW (2015) Axonal spread of neuroinvasive viral infections. Trends Microbiol doi: 10.1016/j.tim.2015.01.002.
Tuffereau C, Schmidt K, Langevin C, Lafay F, Dechant G, Koltzenburg M (2007) The rabies virus glycoprotein receptor $p75^{NTR}$ is not essential for rabies virus infection. J Virol 81:13622-13630.