Published in final edited form as:
Biol Bull. 1999 October ; 197(2): 257–258.

Squid Axoplasm Supports the Retrograde Axonal Transport of Herpes Simplex Virus

E. L. Bearer,

Brown University

M. L. Schlief[1], **X. O. Breakefield**[2], **D. E. Schuback**[2], **T. S. Reese**[1], and **J H. LaVail**[3]

[1] NINDS, National Institutes of Health, Bethesda, Maryland [2] Massachusetts General Hospital, Boston, Massachusetts [3] University of California, San Francisco

Neurotropic viruses, such as Herpes simplex virus type 1 (HSV1), first enter the axon terminal and are transported to the cell body where they replicate in the nucleus. Based on previous studies using whole animal assays (1), as well as assays of whole cells *in vitro* (2,3), we know that HSV enters axons and travels as an unenveloped particle–capsid plus associated tegument proteins–in a retrograde direction toward the cell body. The rate of transport has been estimated to be 3–5 mm/hr (2).

Although these earlier studies provided important insight into the delivery of virus to the nucleus, they were limited. What has been needed is an experimental system in which HSV can be applied to a particular region of a neuron at a known concentration and time, and the transport of the virus assayed quantitatively within the axon. With such an assay one could address questions about the real time motility of the viral particle and about the viral and cellular proteins that are essential for this behavior.

The giant axon of the squid, *Loligo pealei*, serves as a powerful model for the molecular mechanisms of axoplasmic transport. The microtubule-based motor, kinesin, was discovered in squid (4); and microtubule-based transport in both anterograde and retrograde directions has been extensively characterized. Recently, actin-based transport of squid axoplasmic organelles has also been described (5,6). Organelles move in either direction in the giant axon at 2–4 μm/s. Organelles isolated from squid axons move towards the barbed ends of actin filaments (6,7) at 1.6 μm/s (8) and in either direction on microtubules at 2 μm/s (9). The axon contains long tracks of microtubule-actin filament bundles that appear to serve collectively as tracks for axonal transport (10). Thus, the squid axon contains all the requisite molecular machinery for transport. In this report, we describe the use of the squid giant axon as an alternative to previous approaches and present for the first time direct observation of the movement of HSV in living axons.

To image the virus in the axon, we used a viral HSV strain in which a major tegument protein, viral protein (VP) 16, was generated as a fusion protein with GFP at the C terminal. This virus was grown in Vero cells, purified, and concentrated to a titer of about 1.0×10^9 pfu/ml. Different aliquots of the viral stock were treated in various ways to remove the viral envelope. This mimics the first step in viral infection in which the viral envelope fuses with the cell membrane, releasing the capsid together with its tegument into the cytosol. *In vitro* removal of the envelope was necessary for these experiments because injection of virus into the axon by-passes the normal membrane fusion step. Several treatments were found that produced motile, fluorescent particles in the axon after injection.

The viral preparation was co-injected with non-fluorescent oil. The oil droplet was used to determine an appropriate focal plane and as a fixed reference marker, since it remains stationary after injection (11). The movement of viral particles was recorded on a laser scanning confocal microscope. Movement was sampled ~1 frame/3 s, and transport rates were determined by analyzing sequential frames.

When we examined fields upstream from the oil droplet, *i.e.*, closer to the cell body, we identified rapidly moving particles. One of these moved ~36 μm in 18 s for an overall rate of 1.9 μm/s in the retrograde direction (Fig. 1). At the end of this sequence, this particle went out of the plane of focus. Of 113 moving particles examined, all were found in the region of the axon between the oil droplet and the cell body, moving in the retrograde direction.

The effect on the virus of the pre-injection treatment was determined by electron microscopy of negatively stained preparations. Of the virus in those samples that produced motile particles when injected in the axon, ~90% had lost their surface envelope. Since the GFP-labeled protein, VP16, is a tegument protein, the moving particles we observed must include at least tegument, either as whole virus, tegument plus capsid, or simply as tegument aggregates. Since neither capsid nor membrane are GFP-labeled, we cannot know from these studies whether they also are capable of moving.

We have demonstrated the rapid and preferential retrograde axonal transport of HSV particles in the living axon. The rate of movement of these viral particles in the squid giant axon is similar to the estimated rates of retrograde transport of this human virus in rat dorsal root ganglia in culture (2). Furthermore, this rate is consistent with the rates of retrograde transport of endogenous organelles in the squid axon and of isolated organelles on either microtubules or actin filaments. Both the direction and rate of movement suggest that a host cell motor molecule, such as the retrograde microtubule motor, dynein, may be co-opted by invading virus. Thus, by combining the power of the squid axon with a biochemical and genetic dissection of the virus, we expect to be able to identify the viral proteins required for transport, as well as the cellular transport machinery that they recruit.

Acknowledgments

Supported by NIH EY08773 (JHL), GM47638 (ELB), NINDS (TSR), NINDS24279 (XDB), and by the Frederik B. Bang Fellowship Fund and the Evelyn and Melvin Spiegel Fellowship Fund, Marine Biological Laboratory, Woods Hole, MA, and by a predoctoral NINDS fellowship (MLS).

Literature Cited

1. Topp KS, Meade LB, LaVail JH. J Neurosci. 1994; 14:318–325. [PubMed: 8283239]
2. Lycke E, Kristensson K, Svennerhoim B, Vahlne A, Zeigler R. J Gen Viral. 1984; 65:55–64.
3. Sodeik B, Ebersold MW, Helenius A. J Cell Biol. 1997; 136:1007–1021. [PubMed: 9060466]
4. Vale RD, Reese TS, Sheetz MP. Cell. 1985; 42:39–50.0. [PubMed: 3926325]
5. Kuznetsov SA, Langford GM, Weiss DG. Nature. 1992; 356:722–725. [PubMed: 1570018]
6. Bearer EL, DeGiorgis JA, Bodner RA, Kao AW, Reese TS. Proc Natl Acad Sci USA. 1993; 90:11252–11256. [PubMed: 8248236]
7. Langford GM, Kuznetsov SA, Johnson D, Cohen DL, Weiss DG. J Cell Sci. 1994; 107:2291–8. [PubMed: 7527056]
8. Bearer EL, DeGiorgis JA, Medeiros NA, Reese TS. Cell Motil Cytoskeleton. 1995; 33:106–114. [PubMed: 8635200]
9. Schnapp BJ, Vale RD, Sheetz MP, Reese TS. Cell. 1985; 40:455–462. [PubMed: 2578325]
10. Bearer EL, Reese TS. J Neurocytol. 1999; 28:85–98. [PubMed: 10590510]

11. Galbraith JA, Reese TS, Schlief ML, Gallant PE. Proc Natl Acad Sci USA. 1999 in press.

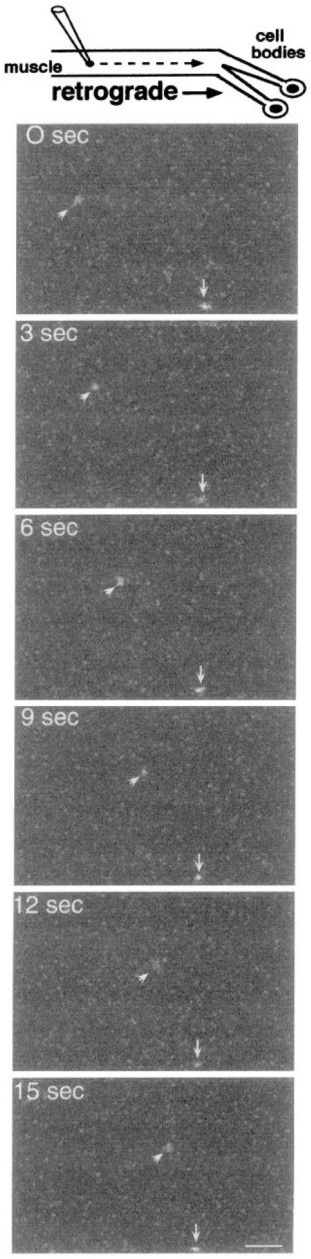

Figure 1.

Retrograde transport of a GFP-labeled viral particle in a living squid axon. The giant axon was dissected in Ca^{++}-containing seawater and injected with >100pfu of GFP-VP16 labeled and extracted Herpes simplex virus. Still frames were taken from a BioRad confocal microscope sequence captured at 2–3 set intervals and processed with NIH Image software. The diagram shows the orientation of the giant axon and site of injection relative to observed movements (dashed arrow). In this preparation, the cell body is to the right. GFP-labeled particle (oblique arrowhead) moves at 1.9 μm/s towards the cell body, contrasting with a stationary fluorescent spot (vertical arrow). Axons were dissected away from cell bodies and synapses, and their two ends labeled with color coding string. Bar = 10 μm.