

## Watching Neurons Hand Off Molecules

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Since the discovery of nerve growth factor, it has been thought that neurotrophic factors are released or secreted from target cells. However, more recently it has been suggested that a specific neurotrophic factor known as brain-derived neurotrophic factor (BDNF) may reach target cells directly from presynaptic axons. It has not been known how these molecules get from the neuron in which they are produced to the target cells. Keigo Kohara, Akihiko Kitamura, Mieko Morishima, and Tadaharu Tsumoto have demonstrated that BDNF is transported anterogradely from presynaptic neurons to target neurons.

It would be technically difficult, if not impossible, to demonstrate transsynaptic transport in cortical neurons *in situ*. Kohara *et al.* got around this problem by using cultured cortical neurons extracted from the cortex of neonatal mice. A plasmid that contained the complimentary DNA for BDNF and green fluorescent protein (GFP) was injected directly into the nuclei of selected neurons under direct vision using a 40x objective on an inverted microscope. Green fluorescence was observed within these neurons within 24 hours, indicating that the BDNF-GFP molecule was adequately transcribed and translated within the neurons. The fluorescent pattern was seen as points of fluorescence grouped as clusters within nerve processes, whereas in the nerve cell bodies the fluorescent signal was dense and diffused. This pattern of fluorescence was quite similar to that previously reported for endogenous BDNF. To

further confirm that the fluorescent signal corresponded to BDNF in the plasmid-injected neurons, some neurons were fixed and stained immunocytochemically with an antibody to BDNF. Comparing the micrographs of the fluorescing cells to the pattern of BDNF localized immunocytochemically was almost identical within the same cell, as confirmed by superimposition of micrographs. There were a small number of points seen within the distal aspect of nerve cell processes that did not correspond to fluorescent points and these were interrupted to be endogenous BDNF. The plasmid-injected neurons were shown to be electrophysiologically the same as non-injected neurons.

The next challenge for Kohara *et al.* was to determine if fluorescence-tagged BDNF was localized within axons of the injected cells. To separate dendrites from axons, they used two approaches. One was to immunocytochemically detect microtubule associated protein 2 (MAP2) which is known to exist in dendrites but not axons. Other neurons were stained with an antibody against tau, which is known to exist almost exclusively in axons. Points of fluorescent signal were seen in the MAP2-negative and tau-positive nerve cell processes of these neurons. These two lines of evidence established that BDNF exists not only within the nerve cell body and dendrites of neurons, but also in the axon and its branches. Furthermore, the velocity of movement of these points representing BDNF could be measured and was approximately 0.3 mm per second, comparable to the velocity of a synaptic vesicle protein that had been previously measured.

The important question was to ask if the BDNF in the axons can move transsynaptically to the target or post-synaptic neurons. To answer this question, Kohara *et al.* simultaneously injected two different plasmids into the nucleus; one plasmid containing the



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GFP-tagged BDNF and another one containing another dye referred to as DsRed. These two distinct protein products within a single neuron was detected by changing the wave length of the fluorescent excitation. DsRed and GFP-BDNF were expressed together within the nerve cell body and nerve cell processes of these plasmid-injected neurons. Again using an antibody to map tau, it was demonstrated that DsRed-positive material was within terminals of an axon that surrounded the nerve cell body of a post-synaptic neuron. The points of DsRed were demonstrated to be at pre-synaptic sites by co-localizing the DsRed signal with an immunocytochemical stain for a protein known to be associated with synaptic vesicles, referred to as synapsin I. Of particular interest, it was shown in the plasmid-injected neurons that GFP-BDNF and DsRed had virtually the identical distribution, whereas the nerve cell body of the post-synaptic neuron was only labeled with GFP-BDNF. These results suggest that BDNF was transferred from the pre-synaptic axon to the post-synaptic neuron because only the pre-synaptic neuron received the injection of plasmid, and the DsRed was not similarly transferred.

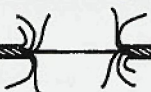
Next, Kohara *et al.* addressed the question whether the transfer of the GFP-BDNF was mediated through the BDNF receptor, referred to as TrkB. When the receptor was blocked with TrkB-immunoglobulin G, the GFP-BDNF was not transferred to the post-synaptic neurons. This suggested that TrkB mediates the transfer of BDNF. Finally, the relationship between neuronal activity and BDNF transsynaptic transfer was examined. When plasmid-injected neurons were paralyzed tetrodotoxin, the nerve cell bodies of the neuron adjacent to the DsRed-positive terminals did not show any GFP signal. The results indicated that the transsynaptic transfer of BDNF was dependent on neuronal activity. This was

further confirmed when picrotoxin, a molecule that excites neurons, was in the presence of plasmid-injected cells, the GFP signal almost doubled in the post-synaptic neurons, indicating that increased neuronal activity resulted in an increased transfer of BDNF.

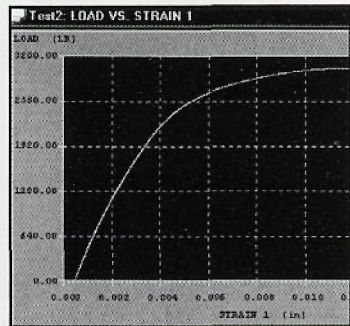
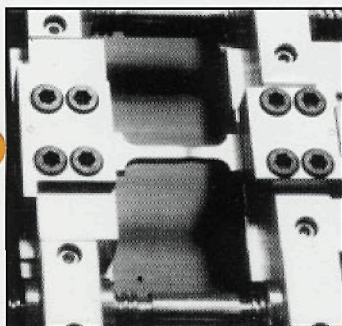
Kohara *et al.* have used an elegant, although technically challenging technique to demonstrate the direct transsynaptic transfer of a neurotrophic factor. This almost certainly occurred in an anterograde direction. It is possible that axon terminals of the post-synaptic neuron may have contacted the nerve cell body of the plasmid-injected neuron and that BDNF might have been transported retrogradely to the nerve cell body of the post-synaptic neuron but this possibility appears to be unlikely because fluorescent signal was not detected in axons that came from post-synaptic neurons. These results indicate transneuronal transfer of BDNF is dependent on neuronal activity and is not part of a general movement of protein between neurons because the DsRed was not transported to post-synaptic neurons. The co-expression of two fluorescent proteins in the study made it possible for Kohara *et al.* to directly observe the activity-dependent, transneuronal transfer of BDNF. **Quite an accomplishment!** ■

1. The author gratefully acknowledges Professor Tadaharu Tsumoto for reviewing this article.
2. Kohara, K., A. Kitamura, M. Morishima, T. Tsumoto, Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons, *Science* 291:2419-2423, 2001.

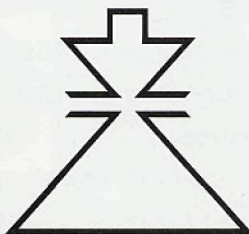
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