

## Activity 2: Identification of Botulinum Toxin Molecular Targets

### Assignment 1: Identification of Synotobrevin as a Target of BoNT/B

When reading a paragraph in a textbook, it is easy to take for granted that the information presented is clear and free of ambiguities and conflicting data. It can come as a surprise that several years of research--conducted by many professional scientists, post-doctoral fellows and students--contributed to the information in that one paragraph. Still more surprising can be that just one paragraph articulates a final consensus regarding data that came from collaborating or even competing research groups that disagreed about the interpretation of their results. The debate leading up to such a consensus can be long and range over many kinds of scientific literature. Some articles are written for scientists researching within the same field, while others are written for those with only a general scientific background or no formal scientific training at all. In this assignment, you will read a "News and Views" review article from the widely-read journal *Nature* and a more technical research article.

Research articles are written by scientists in a specific area of research for other scientists working in that area. Research articles aim to report a unique contribution to the field and focuses on the technical details of a particular experiment, the experimental results and the wider implications of those results. Collectively, research articles document the incremental process of scientific discovery and present alternative points of view. The body of research articles from a particular field are referred to as the "primary literature" of the field.

Review articles, by contrast, are written to attract the interest of scientists and students who are not experts within a particular field. These articles usually provide a synopsis of the state of affairs within that field, and give the non-expert an overview of recent advances and controversies. These review articles are usually considered part of the "secondary literature" of the field. When conducting research in an unfamiliar field of biology, then, it is useful to start with review articles and then move on to research articles.

In this assignment, you will learn how to approach a field of scientific research for the first time. You will read both a review article and a research article and be able to compare the two genres. Notice particularly the differences in titles, formats and language that reflect the purposes of the different genres. The research articles in this activity are among the seminal papers demonstrating that botulinum toxin (BoNT) is a zinc-dependent protease that is highly specific for a set of host proteins (SNAREs) involved in secretion of the neurotransmitter acetylcholine (ACh). They show that when the toxin is present, SNAREs are cleaved, leading to a block of neurotransmission, paralysis, and in some cases death.

### Instructions

1. Review the assigned textbook sections (suggestions may be found in the Teaching Notes to Activity 2).
2. Read the assigned articles. Begin with the review article and then use **Resource Seven: Worksheet for Reading Primary Literature** to assist in reading the primary research article.

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3. Watch for the main points and experimental methods listed under the Key Ideas heading. Define the Scientific Terms.
4. Answer the questions listed below. They are designed to help you decipher the meaning of these papers and understand the relationships between the figures, tables, and text. Some of the questions are challenging, and the answers may not be in the reading. Apply your skills and knowledge to construct the answer.
5. Your instructor may ask you to submit your answers for grading or ask you to discuss or present what you have learned to the rest of the class.

### Readings

**Review Article:** Cutler, D. (1992). "Cell biology. Progress by poisoning." *Nature* 359(6398): 773.

**Research Article:** Schiavo, G., F. Benfenati, et al. (1992). "Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin." *Nature* 359(6398): 832-5.

**Recommended:** Washington, U. (2001). "Presynaptic Proteins, Synaptic Vesicle Docking and Membrane Fusion" at the University of Washington Neuromuscular Disease Center Web Site." <http://www.neuro.wustl.edu/neuromuscular/pathol/snare.htm>

### Key Ideas

**Main Points:** A protein on the surface of vesicles is cleaved (cut) by tetanus toxin and botulinum neurotoxin B (BoNT/B). This cleavage event does not allow the vesicle to reach the plasma membrane. Since the vesicle can no longer fuse with the plasma membrane, the contents of the vesicle cannot be released, and this leads to paralysis of muscles, and sometimes death.

**Experimental Methods:** SDS-PAGE, protease assays, voltage clamp assays, inhibition studies using peptides.

### Scientific Terms:

SSV	EDTA , captopril and chelator
protease	Synaptobrevin/VAMP
electrophoresis	protein domain
presynaptic cell and postsynaptic cell	immunoblotting, antibody
SDS-PAGE and Commassie Blue	

### Questions

1. What is the precedent for the work described in this research article? What work does it build it on? Is this paper an extension of previous work or does it aim to present an alternative point of view?
2. How do the results in this research article affect the wider field?

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3. Based on these results what questions remain? Can you propose experiments for the future?
4. In “Progress by Poisoning,” Cutler ends the review article with this statement: “In the meantime, the sales of tetanus toxin and botulinum-B toxin will surely rise.” What is he implying?
5. Given your understanding of synaptic vesicle fusion, name the proteins with which the cytoplasmic region of synaptobrevin/VAMP interacts. Draw a schematic diagram of this interaction.
6. In Figure 1 in Schiavo, et al., there are two sets of three terms used to describe the molecular state of the toxins. The first set of terms refers to: sc-NT, NT, and L and H. The second set of terms refers to: single chain, di-chain or 2-chain, and reduced di-chain or reduced 2-chain. Define these acronyms and match the corresponding terms from the two sets. How does the reduction reaction affect toxin activity?
7. In Figure 3, Schiavo, et al. demonstrate that there are two isoforms of synaptobrevin in the rat small synaptic vesicle (SSV) fractions. What prompted them to investigate the possibility that there might be two isoforms? Why weren’t the two bands representing the two isoforms in Figure 3 seen in Figure 1? In other words, what were the “appropriate conditions” needed to resolve these two components.
8. What is the experimental rationale behind this statement by Schiavo, et al. on p. 834: “If the specific proteolytic cleavage of SYB-2 is the cause of the inhibition by TeTx and BoNT/B of neurotransmitter release, then a peptide sequence encompassing the cleavage site should inhibit the intracellular poisoning that results when the two neurotoxins are injected into neurons of the buccal ganglion of *Aplysia californica*, a well characterized electrophysiological system.”
9. In Figure 4, synthetic peptides delay the TeTx inhibition of acetylcholine release in *Aplysia* neurons. In the text, the Schiavo, et al. mention that when synthetic peptides are pre-incubated with the TeTx and added to synaptic vesicles *in vitro*, synaptobrevin/VAMP cleavage is completely blocked. Why then doesn’t preincubation of the peptides with TeTx, completely restore acetylcholine release in the *Aplysia* neurons? The same results are described in the text of the article for BoNT/B in the presence of these peptides. Would you feel comfortable if these peptides were synthesized and injected to treat human botulism? Explain the reasoning for your decision (hint: remember that BoNT/B has endopeptidase activity).