

## Activity 2: Identification of Botulinum Toxin Molecular Targets

### Assignment 4: Identification of syntaxin as target for BoNT/C

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 articles assigned to you. Begin with the review article and then use **Resource Seven: Worksheet for Reading Primary Literature** to assist in reading the primary research article.
3. Watch for the main ideas and experimental methods listed under the Key Ideas heading. Define the Scientific Terms.

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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:** Huttner, W. B. (1993). "Cell biology. Snappy exocytosis." *Nature* 365(6442): 104-5.

**Research Article:** Blasi, J., E. R. Chapman, et al. (1993). "Botulinum neurotoxin C1 blocks neurotransmitter release by means of cleaving HPC-1/syntaxin." *Embo Journal* 12(12): 4821-8.

**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:** Syntaxin is one the plasma membrane bound SNAREs (soluble NSF-attachment protein receptors) that mediates the fusion of vesicles with the plasma membrane. BoNT/C is capable of cleaving (cutting) syntaxin and disrupting this process, leading to a block of acetylcholine release in neurons.

**Experimental Methods:** Synaptic transmission assays, Western analysis, dose response assays, cell fractionation analysis, site directed deletion mutant analysis, *in vitro* translation studies.

### Scientific Terms:

endopeptidase and protease	serotype, immunoreactivity, antibody,
synaptobrevin and syntaxin	affinity-purified antibody,
depolarization, presynaptic, and	immunoblotting
postsynaptic cells	recombinant DNA and site-directed
SDS-PAGE	mutagenesis
subcellular fractionation	<i>in vitro</i> translation
cell free assay	protein domain
NSF and SNAP	chelator

### 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?

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2. How do the results in this research article affect the field in general?
3. Based on these results what questions remain? Can you propose experiments for the future?
4. In "Cell biology. Snappy exocytosis" Huttner reviews the process of neuronal regulated exocytosis over the last few years and highlights several important findings. In your own words, compare and contrast the process of regulated exocytosis with that of constitutive exocytosis. What components are common to these systems? In regulated exocytosis, vesicle docking need not be followed immediately by membrane fusion. What other cell types would benefit from a delayed fusion event? What cargo might these vesicles carry? What types of signals would initiate fusion in these examples?
5. Since botulism is a result of blocked acetylcholine release at the neuromuscular junction, why are Blasi, et al. measuring glutamate release in the presence of BoNT/C in Figure 1a?
6. Why were SV2 and synaptophysin used by Blasi, et al. in the experiment represented by Figure 2b? During the same year, 1993, the authors published a paper in *Nature* revealing that SNAP-25 is the molecular target for BoNT/A. Why do you think that SNAP-25 was neglected from this current study as a potential target for BoNT/C?
7. At first glance, Figure 4 in Blasi, et al. looks very similar to that of Figure 2b. What questions were the authors trying to address in the experiment depicted by Figure 4? How did this experiment vary from that shown in Figure 2b?
8. The results represented by Blasi, et al. Figure 5 suggest that BoNT/C is capable of cleaving syntaxin protein that contains the native transmembrane domain, suggesting that syntaxin membrane association is necessary for recognition by BoNT/C. In Figure 6a, the authors address this hypothesis by conducting an *in vitro* translation of syntaxin in the presence and absence of microsomes. They conclude that cleavage does not occur in the absence of microsomes. Do you agree with their findings? How might they have been more convincing in the presentation of this last set of results? Do the results in Figure 6b alleviate any reservations you might have about the conclusion drawn from Figure 6a?
9. The lack of complete degradation of syntaxin proteins in the presence of BoNT/C suggests that a "toxin resistant pool" of syntaxin exists in the synaptosomes, liposomes, and microsomes. Blasi, et al. provide possible explanations for this result found *in vitro*. Can you suggest an experiment that could be done *in vivo* to address whether the incomplete digestion is an artifact of working in an *in vitro* environment?