

Activity 2: Identification of Botulinum Toxin Molecular Targets

Assignment 3: Identification of SNAP-25 as target for BoNT/A

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 points and experimntnal 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 A selectively cleaves the synaptic protein SNAP-25." *Nature* 365(6442): 160-3.

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>.

WEMOVE (2002). "Botulinum Toxin Type A Mechanism of Action." http://www.wemove.org/spa_mbmoa.html

Key Ideas

Main Points: SNAP-25 is one of the plasma membrane bound SNAREs (soluble NSF-attachment protein receptors) that mediates the fusion of vesicles with the plasma membrane. BoNT/A is capable of cleaving (cutting) SNAP-25 and disrupting this process, leading to a block of acetylcholine release in neurons.

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

Scientific Terms:

endopeptidase
synaptobrevin, syntaxin, rab
depolarization, presynaptic, and
postsynaptic cells
SDS-PAGE
subcellular fractionation
NSF and SNAP

serotype, immunoreactivity, antibody,
affinity-purified antibody, immunoblotting
recombinant DNA and site-directed
mutagenesis
in vitro translation
protein domain
chelator

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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 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 neuronal regulated exocytosis research 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/A in Fig. 1a?
6. In Figure 1b, two antibodies are used to detect the molecular target of the BoNT/A endopeptidase. Since an antibody directed against the C-terminal portion of SNAP-25 was readily available for this study, why did the authors affinity purify another antibody from sera for SNAP 25? What does this decision tell you about the BoNT/A cleavage site in SNAP 25? What other data did the authors collect to support this assumption about the cleavage site?
7. In Figure 3 of Blasi, et al., we see the results of experiments using *in vitro* translated full-length BoNT/A and the light chain of BoNT/A. The bands in the gel appear due to [³⁵S]methionine labeling. Which proteins were translated *in vitro* and why? Which proteins are labeled?
8. BoNT/A is the only botulinum neurotoxin serotype whose *in vivo* effects can be overridden by black widow spider venom, α -latrotoxin. It has also been shown that α -latrotoxin binds to neurexin, synaptotagmin, and syntaxin. Neurexins are single transmembrane proteins which bind to α -latrotoxin in a calcium-dependent manner and also interact with the synaptic vesicle protein synaptotagmin. Synaptotagmin II is thought to be the host cell receptor for BoNT/B, while syntaxin is one of the molecular targets of BoNT/C. Given this information, propose a hypothesis which explains α -latrotoxin's ability to overcome BoNT/A neurotoxicity but not the effects of BoNT/B or BoNT/C.