

Activity 2: Identification of Botulinum Toxin Molecular Targets

Assignment 2: Biochemical identification of SNAP receptors

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.
3. Watch for the main points and experimental methods listed under the Key Ideas heading. Define the Scientific Terms.

Activity 2: Identification of Botulinum Toxin Molecular Targets

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: Warren, G. (1993). "Cell biology. Bridging the gap." *Nature* 362(6418): 297-8.

Research Article: Sollner, T., S. W. Whiteheart, et al. (1993). "SNAP receptors implicated in vesicle targeting and fusion." *Nature* 362(6418): 318-24.

Recommended: NIH. (2004) "The Brain: Understanding Neurobiology Through the study of Addiction, Lesson 2, Neurons, Brain Chemistry, Neurotransmission."

http://science.education.nih.gov/supplements/nih2/addiction/activities/lesson2_neurotransmission.htm

Key Ideas

Main Points: SNAREs (soluble NSF-attachment protein receptors) aid in and confer specificity to vesicular trafficking. The identification of SNAREs was accomplished by using NSF affinity chromatography.

Experimental Methods: cell free vesicular assays, recombinant DNA techniques to tag proteins, affinity chromatography, density gradient assays, Western analysis.

Scientific Terms:

cell-free assay	recombinant DNA
ER, cisterna, and golgi	epitope, IgG, immunoblotting
SDS-PAGE	protein sequencing
eluate (chromatography)	protein domain
NSF and ATPase	synaptobrevin, syntaxin, SNAP-25
SNAP, SNAREs, and rab (small GTP-binding protein)	presynaptic and postsynaptic cells
	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?
2. How do the results in this research article affect the field?
3. Based on the results what questions remain? Can you propose experiments for the future?

Activity 2: Identification of Botulinum Toxin Molecular Targets

4. In “Bridging the Gap,” Warren mentions that vesicle docking need not be followed immediately by membrane fusion and offers regulated exocytosis in neurons as an example. Name another type of cell that would benefit from a delayed fusion event. What cargo might vesicles in this cell carry? What types of signals would initiate fusion in this example?
5. In “SNAP receptors implicated in vesicle targeting and fusion,” Sollner, et al. refer to previous work demonstrating that NSF will only associate with SNAPs that are already bound to membrane-bound SNAREs. Later work indicated that NSF is an ATPase that triggers the disassembly of SNAREs to prepare them for another round of vesicle fusion. How did this enzymatic activity affect the method of affinity chromatography chosen to isolate the SNAREs in this paper? Consider both the construction of the column and solutions used to elute proteins from the column.
6. Sollner et al. purified three bovine brain SNAREs via affinity column chromatography, by assembling NSF/SNAP/SNARE complexes *in vitro* and eluting the SNARE/SNAP complexes. The proteins were microsequenced and found to be SNAP-25, synaptobrevin/VAMP, and syntaxin. What prior evidence suggests that these three proteins are in fact involved in exocytosis *in vivo*?
7. Figure 4 in Sollner et al. is a composite panel showing the results of four different 20S particle (the NSF/SNAP complex) assembly reactions and the location of NSF, SNAPs, and SNAP-25 during these reactions. Each symbol in the graph represents different experimental conditions. For instance, in the case in which the SNAPs were examined, labeled SNAPs were incubated in the presence (a) or absence (b) of labeled NSF. When NSF was examined, it was in the presence (a) or absence (b) of brain extract. Panel c and d represent the experimental reactions of 20S assembly while panel a and b represent the control reactions, or those in which 20S assembly will not occur. The X-axis indicates that the sedimentation reactions are fractionated in 1 ml aliquots and each aliquot is analyzed for the presence of NSF, SNAPs SNAP-25 and the 20S particle. How is each protein detected in the reaction and fractionation experiments? What is the significance of this figure?