Assignment 1: Essay Questions

Scientific communications are tailored to specific audiences. Literature can be directed to students, laypersons, or other scientists. When reading a paragraph in a textbook, you might take for granted that the information is clearly presented, free of ambiguities or conflicting data. You might be surprised to learn later that several years of work and many papers by professional scientists, post-doctoral fellows, and students contributed to that one paragraph. More often than not, that paragraph was the culmination of data coming from collaborating, or competing, research groups who didn't always agree about the interpretation of their results.

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

When conducting research in an unfamiliar field of biology, start with review articles and then move on to research articles. When reading research and review articles for the first time, it will be important to compare the two genres. Pay particularly close attention to the differences in titles, formats, and language, which will illustrate the purpose of writing in these different genres.

The assigned research articles were written over a course of a few years by five groups of researchers. The main focus of these studies is the preference that BoNT has for cholinergic cells and the discovery that gangliosides and a protein called synaptotagmin are involved in host cell binding. Since gangliosides and synaptotagmin are found on all nerve endings, it's not clear why the toxin preferentially binds to cholinergic neurons. To complicate matters, all seven serotypes of botulinum toxin exhibit different levels of toxicity with respect to length of the effect or potency, suggesting that each might bind to a different host cell receptor. Clearly this field has not yet resolved the identity of the host cell receptors for the botulinum serotypes. Given the increase in federal funding for Homeland Security and Defense, this area of research has gained more support and interest. Understanding how the toxin binds to neurons will lead the way for development of vaccines, inhibitors, and treatments for botulism as well as modifications for more efficient medical use of the toxin.

Instructions

1. Read the assigned text sections and primary research articles listed below (suggestions for textbook readings may be found in the **Teaching Notes** to Activity 3). For a detailed

overview in outline format visit the University of Washington web site listed under Review Articles. The reviews written by Schiavo and Pellizzari are rather difficult and very comprehensive and are recommended only if you are struggling with a particular aspect of the assignment.

2. A study guide, **Resource Seven: Worksheet for Reading Primary Literature,** is provided to help you decipher the meaning of the primary research articles and understand the relationships between the figures, tables, and text.
3. To help you place these findings in context, address the following questions in short essay format:
   - What is the dual receptor hypothesis? Which results support this hypothesis and which refute it?
   - Synaptotagmins have been implicated in neurotoxin binding. What other roles do synaptotagmins play in the cell?
   - There appear to be two schools of thought concerning the role that synaptotagmin plays in BoNT/B binding. Who make up these two schools of thought and which experiments support synaptotagmin's role in BoNT/B binding and which refute its involvement?
   - What experimental methods were used to identify protein receptors for botulinum toxin? Which of these do you feel address physiological conditions for toxin binding? Which of these do you feel need to be explored further?
   - How do these findings affect botulism vaccine development?
4. Submit answers for grading.

Readings

Review Articles:

1. Washington U. "Botulism" at the University of Washington Neuromuscular Disease Center Web Site. 2001. http://www.neuro.wustl.edu/neuromuscular/nother/bot.htm#protein
2. Schiavo G, Matteoli M, Montecucco C (2000). "Neurotoxins affecting neuroexocytosis." *Physiological Reviews* 80(2): 717-66. http://physrev.physiology.org/cgi/content/full/80/2/717
3. Pellizzari R, Rossetto O, Schiavo G, Montecucco C. (1999). "Tetanus and botulinum neurotoxins: mechanism of action and therapeutic uses." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences.* 354(1381): 259-68. http://dandini.ingentaselect.com/vl=3271893/cl=11/nw=1/rpsv/cw/rsl/09628436/v354n1381/s3/p259.idx

Primary Research Articles:

1. Bakry, N. M., Y. Kamata, et al. (1997). "Expression of botulinum toxin binding sites in Xenopus oocytes." *Infection and Immunity* 65(6): 2225-32. http://iai.asm.org/cgi/reprint/65/6/2225.pdf
2. Geppert, M., B. T. Archer, et al. (1991). "Synaptotagmin II. A novel differentially distributed form of synaptotagmin." *Journal of Biological Chemistry* 266(21): 13548-52. http://www.jbc.org/cgi/reprint/266/21/13548
3. Ginalski, K., C. Venclovas, et al. (2000). "Structure-based sequence alignment for the beta-trefoil subdomain of the clostridial neurotoxin family provides residue level information

about the putative ganglioside binding site." *Febs Letters* 482(1-2): 119-24. http://www.elsevier.com/febs/120/18/39/index.htt

4. Kozaki, S., Y. Kamata, et al. (1998). "Ganglioside GT1b as a complementary receptor component for Clostridium botulinum neurotoxins." *Microbial Pathogenesis* 25(2): 91-9.
5. Lalli, G., J. Herreros, et al. (1999). "Functional characterisation of tetanus and botulinum neurotoxins binding domains." *Journal of Cell Science* 112 (Pt 16): 2715-24. http://jcs.biologists.org/cgi/reprint/112/16/2715.pdf
6. Li, L. and B. R. Singh (1998). "Isolation of synaptotagmin as a receptor for types A and E botulinum neurotoxin and analysis of their comparative binding using a new microtiter plate assay." *Journal of Natural Toxins* 7(3): 215-26.
7. Nishiki, T., Y. Kamata, et al. (1994). "Identification of protein receptor for Clostridium botulinum type B neurotoxin in rat brain synaptosomes." *Journal of Biological Chemistry* 269(14): 10498-503. http://www.jbc.org/cgi/reprint/269/14/10498.pdf
8. Nishiki, T., Y. Tokuyama, et al. (1996). "The high-affinity binding of Clostridium botulinum type B neurotoxin to synaptotagmin II associated with gangliosides GT1b/GD1a." *Febs Letters* 378(3): 253-7. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T36-3Y0SKV5-MF-3&_cdi=4938&_orig=search&_coverDate=01%2F15%2F1996&_qd=1&_sk=996219996&view=c&wchp=dGLbVtz-zSkzS&_acct=C000050221&_version=1&_userid=10&md5=79fed76fdd3276dc9f6f2a7154490502&ie=f.pdf