Assignment: Structure, Function and Dynamics Molecular Skit

Group Number: _____ Serotype: _____ Assigned Role: _____

To understand the molecular mechanisms of botulinum neurotoxin (BoNT) action, you will role-play the natural process of acetylcholine release and then demonstrate how this process is disrupted by BoNT. By acting out the process in the form of a skit with other students, you will experience the dynamic and three-dimensional nature of cell biological processes and visualize how the cell responds to extracellular signals and environmental variables. You will also begin to see why endocytosis and exocytosis require a fluid membrane, receptors and ligands with varying binding affinities, and regulated signaling cascades that allow for specific protein interactions. The background reading list at the end of this assignment sheet will provide you with the necessary background and detail to write and act out a skit.

Instructions

Day 1: The Assignment and Character Development

1. The class will be broken down into small groups of 10-20. Each group will be assigned one of the seven botulinum serotypes and background reading.
2. Take 10 minutes to assign group members a role such that each member will play the part of a relevant cell structure or molecule; host molecule, host organelle, toxin or narrator. Your group will use pieces of paper to depict the acetylcholine molecule (Ach). Your instructor may choose to add more, combine some, or remove a few roles.
3. To prepare for the skit, outside of class review the textbook assignments and the readings and websites listed at the end of this Assignment (suggestions for textbook readings maybe be found in the Teaching Notes to Activity 4). Pay close attention to the role that you will be playing in this skit.

Day 2: Group Rehearsal

4. Assemble your group and work on skit. The skit should last for approximately ten minutes. Members will perform their individual roles, everyone should work to highlight the temporal and spatial relationships of the molecules, protein domains, and subunits at play while the narrator introduces and describes the events.

Day 3: Performance Time

5. During this class session, your instructor will ask groups to perform portions of their skits and audience members will try to identify which BoNT serotype or inhibitor/vaccine is being depicted.

Molecular Skit Roles

SNAP-25	BoNT receptor (BoNTRe)	narrator

synaptobrevin/VAMP	4 phospholipids of presynaptic membrane	vesicles
syntaxin	4 phospholipids of postsynaptic membrane	inhibitor
BoNT heavy chain (H)	acetylcholine receptor (AchRe)	vaccine
BoNT light chain (L)	acetylcholinesterase	RAB GTPase

Key Events

1. Release of acetylcholine from presynaptic neurons of the neuromuscular junction. (For this skit, assume that the presynaptic neuron has been stimulated and the influx of calcium has initiated the process of acetylcholine release).
   a. Binding, docking, and fusion of secretory vesicles containing acetylcholine with the host cell membrane.
   b. Release of acetylcholine from the presynaptic cell.

2. Block of acetylcholine release in the presence of BoNT.
   a. Binding of BoNT to the host cell membrane.
   b. Internalization of BoNT by receptor-mediated endocytosis.
   c. Translocation of BoNT from the endosome compartment into the host cytoplasm.
   d. Proteolytic cleavage of proteins involved in the secretory process.
   e. Block of Ach release.

3. Block of BoNT action by either an inhibitor or vaccine. Indicate which of the following steps is affected and repeat processes above and the new outcome.
   a. Binding of BoNT to the host cell membrane.
   b. Translocation of BoNT from the endosome compartment into the host cytoplasm.
   c. Proteolytic cleavage of proteins involved in the secretory process.

Group Work Tips: (Also see Resource One: Group Role Profiles)

- Remember that each member of your group may have a slightly different interpretation of the biological process or the effect of the toxin on the host. Be respectful of these interpretations and work towards a representation that encompasses these variations.
- Since 10-20 people will be moving around during the group skit, you may choose to use large signs that have the title of each member's role written on them. Since there may be contortions and bending, it is best if signs are displayed on both the front and backside of an individual.
- Conduct the skit as a time-lapse demonstration, so that the sequence of events is clearly delineated.

Character Sketch

To help you prepare for your role, your instructor may ask you to write a short character sketch for your molecule. This sketch should:

- Describe your molecule or organelle both physically and functionally.

- Explain how this character fits into the process depicted by the skit.
- Highlight the most important point and activity in the skit with respect to your role.
- Use creative language as well as computer-generated or hand-drawn illustrations to describe shape, location, and timing.
- The narrator for the group may choose any molecule in the process.
- These sketches may be turned into the instructor and graded based on creativity and scientific accuracy.
- The instructor may choose to share a few with the rest of the class.

Reading and Animations

1. WEMOVE (2002). "Botulinum Toxin Type A Mechanism of Action." http://www.mdvu.org/multimedia/videoclips/btx_moa.html
2. Allergan. Mechanism of BOTOX Action. Click on the side menu titled "Product Information" and review all the sections in the pull down menu. http://www.botox.com/site/professionals/product_info/mechanism_of_action.asp
3. Arnon, S. S., R. Schechter, et al. (2001). "Botulinum toxin as a biological weapon: medical and public health management." *JAMA* 285(8): 1059-70. This article is a complete review of the toxin, its uses and abuses. Focus on the sections titled "Therapy," "Prophylaxis," and "Research Needs," found on the menu at the left hand side of this page. See particularly Figure 1. http://jama.ama-assn.org/cgi/content/full/285/8/1059
4. Terry T. (2000)."Botulinum Toxin Mechanism of Action" as found in Biology 102: Lecture Notes: The Nervous System. University of Connecticut. November 13, 2000. View the animation of native neurotransmitter release and the block of this release by BoNT. http://www.microvet.arizona.edu/Courses/MIC420/lecture_notes/clostridia/clostridia_neurotox/movie/botulinum_movie.html
5. Lawrence, E. (1998). "Getting to Grips With Botulinum Toxin." One page article reviews treatments and vaccine strategies for botulism. http://www.nature.com/nsu/981029/981029-2.html
6. Gerritson V. (2002). "From Sausages to Wrinkles." Protein Spotlight Online. Feb, (19). One-page article reviews history, molecular mechanisms, and applications of botulinum toxin. http://www.expasy.org/spotlight/articles/sptlt019.html
7. Groleau R. (2001). "Making Vaccines" as seen in the NOVA Bioterror Companion Website. http://www.pbs.org/wgbh/nova/bioterror/vaccines.html#
8. 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
9. Pellizzari, R., O. Rossetto, et al. (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

10. Schiavo, G., M. Matteoli, et al. (2000). "Neurotoxins affecting neuroexocytosis." *Physiological Reviews* 80(2): 717-66. http://physrev.physiology.org/cgi/content/full/80/2/717
11. Washington, U. (2001). ""Botulism" at the University of Washington Neuromuscular Disease Center Web Site." A detailed website in the form of an outline. It also contains links to more resources. http://www.neuro.wustl.edu/neuromuscular/nother/bot.htm#protein