Editorial: Molecule on a mission

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Popularity of Botox™ and concern about botulinum neurotoxins (BoNTs) as biothreat agents have kept up the research on product development, diagnostics, vaccine, and antidote design, in addition to more basic understanding of the mechanism of action and the anaerobic bacterium, *Clostridium botulinum*, which produces the toxin. While these areas are important and useful for public health, safety, and security, one may not forget the basic beauty of BoNT molecule which appears to have evolved for a mission. Its mission is to target the most critically evolved system, the nervous system, of animals and humans. Moreover, all the known serotypes and subtypes (currently numbering over 25) target a process of exocytosis as opposed to a molecule inside the nerve cell.

The fact that anaerobic organisms existed over 2 billion years ago always raises a question whether these toxins were part of the genome of bacteria for that long, and how these toxin genes may have been selected to be part of the evolution of the organism? How long has really *C. botulinum* been in existence? Strains of *C. botulinum* are divided into six types of organisms based on physiological and metabolic differences. How are they linked to each other during the evolution process? There are seven known serotypes (with over a dozen subtypes belonging to some of the serotypes) of BoNT which provide a rich source of information on their evolution. Did the toxin genes themselves evolve over a period of time? How long have these toxin genes been part of the organism?

The knowledge of the basic mechanism of BoNT action already turned this sword into a ploughshare over 30 years ago. What else can be learned from this very ancient organism which has gone through at least over 2 trillion generations involving changes and adaptations? Human beings have been in existence for about only one million years, and with their reproduction cycle of about 20 years would have gone through only about 500 iterations. The anaerobic bacteria thus have over 4 billion times advantage over humans to go through the adaptation and natural selection process. It is thus important to consider the bacteria being superior evolutionarily. This is especially relevant since the bacteria have survived in the nature throughout the time other organisms and species have appeared.

Modern genomic technology is opening many doors. One such technology, comparative genomic hybridisation microarray (CGH) technique (reviewed by Brian Raphael in this issue under 'exploring genomic diversity in *Clostridium botulinum* using DNA microarrays') is providing a more fundamental information on the nature of *Clostridium botulinum* and its promiscuity towards diversity. For example, CGH analysis of even BoNT serotype A from *C. botulinum* strain hall suggests significant differences

in the genomic sequences in terms of synteny and also single nucleotide polymorphisms (SNPs). This level of predisposition for change over a long period of time may provide a basis for understanding the mechanism of the BoNT evolution.

However, a very different level of complexity has been discovered for BoNT. In a recent publication, Gu et al. (2012) have shown that one of the neurotoxin associated proteins (NAPs) known also as non-toxin non-hemagglutinin (NTNHA) has the same general trimodular crystal structure as the BoNT/A despite having only 20% sequence homology. More importantly, the two structures are interlocked in their complex form involving pH-dependent binding to protect each other against low pH and proteases present in the gastro-intestinal (GI) tract.

NBPs (for neurotoxin binding proteins) as we refer to NTNHAs also have sequence homology to both BoNT (all types) and TeNT in light chain area. This area is also the conserved domain named peptidase_M27 (Singh et al., 2012) area. Although NBP has not been found to possess any peptidase activity it does contain the protein sequence similarity with a known Zn dependent peptidase (peptidase M27). It has even a truncated Zn binding motif in its M27 region. NBP is lacking of the consensus sequence 'abXHEbbHbc' therefore it loses the capability of binding with Zn. Based on this observation we suspect NBP to be in fact a pseudo-toxin, originated from the early genomic duplication events (also known as paralogous events), such as alpha-hemoglobin and beta-hemoglobin.

However, the most significant observation here is the structural evolution of the NBP and BoNT to interlock in a complex which provides protection against adverse environmental conditions of low pH and proteolysis, and lends suitable mechanism for the toxin's delivery across GI tract.

So, what is becoming apparent at this point from *C. botulinum* genes and proteins is that there may be a mechanism of evolution process that involves multiple proteins and domains targeted at structural stability and functional opportunity. In this regard, it should be noted that there are several unique features of BoNTs – a degree of specificity in botulism caused by different serotypes (e.g., A, B, and E mostly responsible for human botulism whereas C responsible for avian botulism), modular design of binding, translocation, and enzyme domains, specificity of receptors at the nerve membrane, specificity of intracellular targets of BoNT endopeptidase, and the very long lasting presence and effects inside poisoned nerve cells. Molecular basis of all these processes need to be examined. One additional and perhaps the most challenging observation to address is the basis of evolving different serotypes of BoNT evolution to target different components of SNARE proteins involved in a single process of exocytosis. Ordinarily, one would expect these serotypes to target one of these proteins or at least a common feature among the three SNARE proteins.

BoNTs are known to be maximally active under temperature conditions which transform them into molten globule, a structure traditionally not known to be associated with functionally active molecules. Therefore, it seems that the evolution process has introduced unique features in BoNT molecules that make them extremely effective physiologically. The flexibility of the molten globule may in fact be an evolutionary trait for maximum adaptability in specific target selection and survivability in the environment and inside the cell, reflecting indeed a molecule on mission.

References

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