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Review

Venom evolution through gene duplications

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ABSTRACT

Venoms contain highly complex mixtures that typically include hundreds of different components and have evolved independently in a diverse range of animals including platypuses, shrews, snakes, lizards, fishes, echinoderms, spiders, wasps, centipedes, sea snails, cephalopods, jellyfish and sea anemones. Many venom genes evolved through gene duplication. Gene duplication occurs in all domains of life and provides the raw substrate from which novel function arise. In this review, we focus on the role that gene duplication has played in the origin and diversification of venom genes. We outline the selective advantages of venom gene duplicates and the role that selection has played in the retention of these duplicates. We use toxin gene intermediates to help trace the evolution of toxin innovation. We also focus on other genomic processes, such as exon and domain duplications, in venom evolution. Finally, we conclude by focusing on the use of high throughput sequencing technology in understanding venom evolution.

Contents

1. Introduction

Venoms are toxic substances that are delivered into the victim through a specialized apparatus. They contain a plethora of compounds including organic molecules, amines and alkaloids, salts and minerals, amino acids, peptides and proteins (Fry et al., 2009). As venom has evolved independently between phylogenetically divergent lineages and serves different purposes, the exact composition of venom, its delivery system and physiological target, can vary markedly between venomous species (Fry et al., 2009). Most commonly, venom is employed as a means for defense and predation, but also as a means of asserting dominance over conspecifics. Venomous animals include sea anemones, jellyfish, gastropods, cephalopods, centipedes, insects groups, echinoderms, some species of amphibians, fish, snakes, reptiles, and five species of mammals including shrew and the platypus.

Venom can be hemotoxic (act on the cardiovascular system and heart), neurotoxic (act on the nervous system and brain) and cytotoxic (localized and cellular) and produce symptoms that extend across the respiratory, muscular, renal and gastrointestinal systems (Gueron et al., 1992; Isbister and Fan, 2011; Reid and Theakston, 1983). Upon envenomation, or poisoning, victims may experience local pain, swelling, blisters, rashes, tissue necrosis in addition to systemic effects including vomiting, paralysis, nausea, diarrhea, fever,

Abbreviations: PLA2, phospholipase A2; Ig, immunoglobulin; aa, amino acids; bp, base pairs; EST, expressed sequence tag.

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headache, blurred vision, swollen lymph nodes, dizziness, muscle weakness or paralysis and a lack of muscle coordination. Sudden collapse and death may occur. Hemostatic and thrombotic effects such as hypotension, hemorrhage and blood coagulation are common.

These symptoms occur through a number of pathways (Sitprija and Suteparak, 2008). Ion channels are often targeted, for example, in sea anemone, cone snail, snake and scorpion venom. Disruption of ion channels and cell membrane receptors can occur through the release of neurotransmitters. Venom enzymes can cause lysis of cell membranes, pore formation, destruction of other cellular components and the release of inflammatory or cardiotonic bioactives. Enzymes that work in this manner include phospholipase A2 and proteases. Cellular destruction caused by such enzymes can trigger the release of immune molecules such as cytokines resulting in an inflammatory response causing pain and swelling (Sitprija and Suteparak, 2008). These responses are also triggered by the direct initial injury from envenomation.

On a molecular level, several features are common to all venom sequences — they are secretory and typically contain N-terminal signal peptides (Fry et al., 2009). Secretory proteins have a higher cysteine content and form cross-linking disulfide bonds that serve to provide stability and resistance to protease degradation (Fry et al., 2009). The stability of venom peptides combined with their potency, specificity and range of physiological effects has led to their development into valuable biomedical tools and therapeutics (King, 2011).

The complex cocktail of compounds that make up venom are believed to have arisen through gene duplication. The duplication of genes is a source of raw genetic substrate from which novel functions arise (Conant and Wolfe, 2008; Ohno, 1970). Eukaryotic gene duplicates are estimated to occur at a rate of 1 gene per 100 million years (Lynch and Conery, 2000), and result from backward strand slippage during DNA replication, retrotransposition and unequal crossing-over. Once duplicated, some duplicates may be retained by natural selection or genetic drift while the majority of duplicated genes are loss due to the accumulation of deleterious mutations (Lynch and Conery, 2000). Several models have been proposed to explain functional divergence following gene duplications through selective and neutral forces. The two major models are 1) neofunctionalization, whereby a new function is acquired in one gene copy following duplication 2) subfunctionalization, which is also known as the duplication–degeneration–complementation model. Subfunctionalization involves the partitioning of the original functional role of the ancestral gene through mutational changes. Mutations are allowed to accumulate due to the presence of an addition gene copy, which buffers against potential deleterious effects, allowing mutations to accrue over time and for adaptive changes to occur (Soskine and Tawfik, 2010).

Immediately following a duplication event, duplicated toxins increase venom dosage and accelerate venom replenishment (Fry et al., 2009). Duplicates increase the functional diversity of venom through drift and selection allowing an animal to target a diverse range of molecules (e.g. different ion channels) (Kordis and Gubensek, 2000) in many species (Duda and Palumbi, 1999). For example, cone snail toxins are able to discriminate between two ligand-binding sites on a nicotinic acetylcholine receptor (Olivera, 1997) and between different subtypes of voltage-gated $Ca2^{+}$ channels (Olivera et al., 1994). In fact, the ability of conotoxins to discriminate between closely related targets has led to the success of a ω-contoxin as an FDA-approved drug to treat intractable pain (ziconotide) (Atanassoff et al., 2000; McIntosh et al., 1982). Akin to the 'arms-race' relationship used to describe immune co-evolution between host and pathogen, variability in toxin specificity can also reduce the likelihood of resistance developing in predator or prey species (Duda and Palumbi, 1999).

Duplicated genes can act cooperatively to induce synergistic effects in victims (Kordis and Gubensek, 2000). For example, *Conus purpurascens* venom causes a two-phase response in envenomated fish (Terlau et al., 1996). First, a subgroup of peptides induces an excitotoxic shock, which prevents voltage-gated sodium channels from closing, thereby increasing sodium influx. The second phase suppresses the motor circuitry of the prey by inhibiting potassium efflux. The first group of toxins causes huge membrane depolarizations at the injection site, which has the combined effect of almost instantaneous immobilization of the fish, while the second group causes irreversible paralysis by neuromuscular block (Olivera et al., 1985; Terlau et al., 1996).

2. Toxin multigene families

In platypus, we have found that three venom defensin-like peptides, a major component of platypus venom, have evolved through tandem duplications from antimicrobial beta-defensin genes (Torres et al., 1999; Torres et al., 2000; Whittington et al., 2008a, 2008b). The absence of antimicrobial activity in these venom peptides suggests that the duplicated copies have taken on specialized roles in venom (Whittington et al., 2008b). Analogous toxins containing the beta-defensin fold have also been found in snake venom (Rádis-Baptista et al., 2003). Known as crotamines, these neurotoxins, which have an analgesic effect, are believed to have evolved through a similar route of repeated gene duplications in the snake lineage (Mancin et al., 1998; Oguiura et al., 2009). The defensin scaffold has also been identified in toxins from other reptiles, scorpions and ticks; these toxins have also evolved through independent evolutionary paths (Fry et al., 2009).

Larger venom-specific multigene families have been identified in snake, cone snails, spiders and scorpions. These include phospholipases A2 (PLA2s), serine proteases, Kunitz-type serine protease inhibitors, metalloproteinases, and in particular, cysteine-rich families that comprise of the three-fingered toxins in snakes, cone snail conotoxins and scorpion neurotoxins (Table 1). PLA2s are enzymes that hydrolyze the sn-2 acyl bond of phospholipids releasing fatty acids. In snakes, they are one of the best-studied examples of a toxin

Table 1
Venom-specific multigene family expansions.

Name	Function	Animal	Reference
Venom defensin-like peptide/crotamines	Neurotoxin in snake, analgesic, myonecrotic	Snake, platypus	Oguiura et al. (2009), Torres et al. (2000), Whittington et al. (2008a, 2008b)
Serine proteases	Perturb hemostasis	Snake, platypus	Pahari et al. (2007), Whittington et al. (2010)
Serine protease inhibitors	Inhibit blood coagulation enzymes, neurotoxin	Snake, platypus	Whittington et al. (2010), Zupunski et al. (2003)
Cysteine-rich secretory proteins	Neurotoxin	Snake, cone snail, scorpion, spider	Fry et al. (2003), Fujimi et al. (2003), Jiang et al. (2011), Olivera et al. (1999), Sollod et al. (2005), Zhijian et al. (2006)
C-type lectins	Act on blood coagulation pathways	Snake	Jiang et al. (2011), Ogawa et al. (2005)
Phospholipases A2	Anti-platelet activity, myotoxin, neurotoxin	Snake	Deshimaru et al. (1996)
Metalloproteinases	Act on blood coagulation pathways	Snake	Juárez et al. (2008), Pahari et al. (2007)