The Platypus: A Venomous Mammal

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Abstract

The platypus is a monotreme, an egg-laying mammal, found only in Australia. Males are venomous. During the breeding season they are able to deliver venom through spurs located on their hind legs. Venom delivery is believed to provide individuals with an advantage over conspecifics throughout the breeding season. This paper reviews the current literature on platypus venom, focusing primarily on recent advances which have been made since the sequencing of the platypus genome and venom gland transcriptome. It first provides an overview of the genes and molecules involved in venom production and focuses on how these molecules explain the symptoms of envenomation: allodynia, hyperalgesia, swelling and changes to blood pressure. The paper concludes by providing insights into how these venom peptides could be developed into novel therapeutics for human use.

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Introduction

The Platypus

History

Since its discovery by Europeans in 1797, the venomous platypus (*Ornithorhynchus anatinus*) has remained an enigma. At first, when a dried skin was sent back to England, it was thought to be the trick of a taxidermist (Grant 2007), and even once it was proven to be real, controversy raged over its mode of reproduction and its classification. It was not until 1884 that questions about the reproductive mode of the platypus were definitively answered. Biologist William Caldwell, after the wholesale slaughter of hundreds of platypuses, declared in his famous telegram to the Montreal Meeting of the British Association for the Advancement of Science "Monotremes oviparous, ovum meroblastic" (Burrell 1927; Grant 2007). Platypuses, along with the echidnas (*Tachyglossus aculeatus* and *Zaglossus* sp.), became the monotremes (Grant 2007), the egg-laying mammals, true evolutionary oddities.

The platypus and echidnas are the only remaining members of mammalian subclass Prototheria, a group that once contained a diverse range of animals that are now extinct (Musser 2003). The living monotremes are sometimes referred to as "primitive" mammals. This is because subclass Prototheria was the first divergence from the mammalian lineage; it is estimated that this split occurred around 166 Mya (Warren et al. 2008). A schematic phylogenetic tree showing the evolutionary position of the monotremes is displayed in Fig. 1. The echidna family probably evolved from the platypus family, with the two groups diverging ~32 Mya (Phillips et al. 2009).

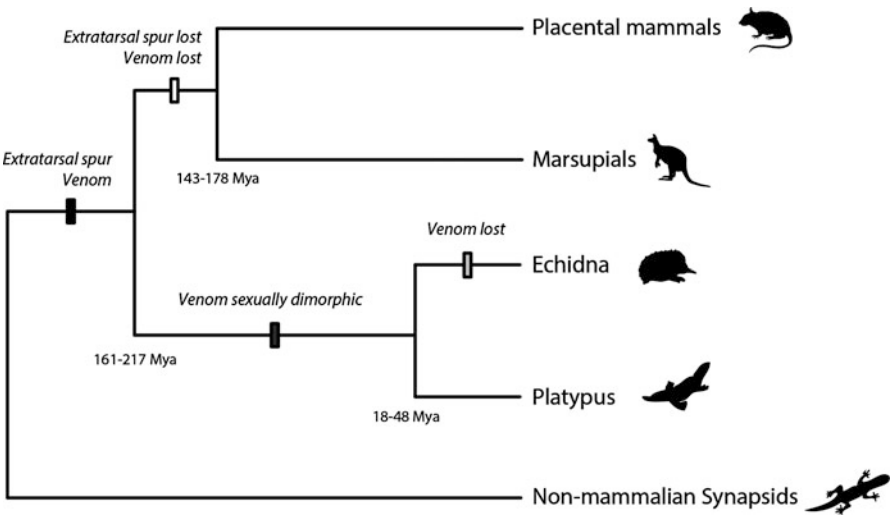

Fig. 1 Phylogenetic tree indicating the evolution of venom and extratarsal spur in monotremes (Divergence dates taken from Phillips et al. 2009)

General Features

The platypus is semiaquatic and semi-fossorial, living and nesting in burrows in riverbanks and emerging into the river or stream to feed on benthic invertebrates. As a result of its membership of the earliest mammal group, it has a number of unusual features that are plesiomorphic (representative of the ancestral state) and more often associated with reptiles than with mammals. For example, platypuses lay eggs; however, they also possess mammary glands and suckle young (from milk patches rather than nipples) (Grant 2007). Like other mammals, the platypus is furred, but its skeleton has several reptilian features, including reptilian-like ribs and a pectoral (shoulder) girdle more similar to that of fossil reptiles than other modern mammals (Grant 2007). The platypus has a lower body temperature (32 °C) than other mammals, but it does regulate this and is able to maintain a constant body temperature even while feeding in water close to freezing point (Grant 2007). The karyotype of the platypus ($2n = 52$) is also unusual, as it contains macro-chromosomes as well as chromosomes resembling the micro-chromosomes seen in karyotypes of reptiles and birds (reviewed in Grützner et al. 2003). However, the most unusual feature of the platypus may be the fact that it is venomous. Venom in a mammal is extremely unusual, and the platypus possesses a complex venom system that is only just beginning to be understood.

Platypus Genomic Research

The unique characteristics of the platypus, along with the fact that many of these are poorly understood, make the animal an intriguing resource for genomic studies. The recent publication of the platypus genome sequence affords an unprecedented opportunity to do this (Warren et al. 2008). The platypus is shy and difficult to study, and genomic techniques can allow researchers to understand the basic mechanisms underpinning the unique biology of the platypus. Examinations of monotreme genomes also have the potential to increase current understanding of the evolution of mammals, providing insights into the genomes of extant mammals as well as ancestral mammals (e.g., Margulies et al. 2005). This is mediated by comparisons with genomes of other species, in a field known as comparative genomics. These studies of the similarities and differences between genomes can reveal genomic regions that are conserved between divergent organisms to provide clues as to their functions and evolutionary histories (e.g., Margulies et al. 2005; O'Brien 2009). Importantly, these conserved regions can reveal functionally important genes, gene products, and regions within noncoding areas; show changes in genome organization and the dynamics of gene families during evolution; reconstruct ancient genomes; and shed light on genomic regions important in inherited genetic disorders and even human disease (O'Brien 2009). The monotremes, as members of the earliest extant offshoot from the mammalian lineage, effectively straddle the phylogenetic gap between reptiles and therians (placental mammals) (O'Brien 2009). The platypus genome thus has the potential to allow valuable comparisons between lineages and represents a powerful tool for reconstructing the evolutionary history of mammals.

The draft platypus genome sequence was released in 2008 and represents the first monotreme genome sequence (Warren et al. 2008). It was constructed from

whole-genome shotgun sequence of the DNA of a single female platypus, collected at Glenrock Station, NSW. DNA segments were localized to chromosomes using fluorescence in situ hybridization (FISH). Around 18,000 protein-coding genes were predicted in the original assembly, similar to the number in the marsupial opossum (*Monodelphis domestica*) and human.

The platypus genome can be used to provide insight into the genetic development of mammalian characteristics such as lactation, along with the disappearance of traits such as egg laying, relevant to the basic biology of this animal. The genome paper examined, via homology, genes encoding some important biological traits. For example, it was found that the platypus genome includes two *ZPAX* genes, which encode egg envelope proteins in birds, amphibians, and fish, and one vitellogenin gene plus a pseudogene, present as three genes in chicken and none in therian mammals. However, it also includes four genes encoding mammalian zona pellucida proteins, indicating that the platypus has an amalgamation of reptilian and mammalian genes (Warren et al. 2008). The genome also encodes a cluster of casein genes involved in lactation, and tooth enamel matrix protein genes presumably involved in the presence of teeth in juvenile platypuses (Warren et al. 2008). In particular, as will be discussed here, genomics has allowed a much better understanding of platypus venom.

Platypus Crural System

Platypus Venom Gland and Spurs

Platypus venom is sexually dimorphic, as only males are venomous. Their venom (crural) system consists of paired venom glands on the dorsocaudal sides of the abdomen, each of which is connected via a venom duct to an extratarsal spur on each hind leg (Grant 2007). The spur is a hollow sheath made of keratin and is associated with a small articulating bone (*os calcaris*) that is attached to tendon and muscle to allow erection of the spurs prior to envenomation (Temple-Smith 1973). Juvenile females have a remnant spur sheath that is lost within a year of hatching, and spurs develop only in males (Grant 2007). The venom glands are thought to be derived from sweat glands and migrate during male development from the inner surface of the thigh to the dorsocaudal surface over the pelvis (Temple-Smith 1973). At maturity, during the spring breeding season, the venom gland increases to peak size and venom production increases (Temple-Smith 1973). Outside of the breeding season, the venom gland secretory epithelium becomes inactive and the gland regresses, and the behavior of the males changes to displaying little spurring activity (Temple-Smith 1973). This is the only known example of temporally variable (seasonal) venom production.

Function of Platypus Venom

Europeans first discovered the spur of the platypus in 1799; spurs were originally thought to be used by the male to hold the female during mating, but it was also

noted early on by some naturalists that the use of these spurs could cause serious wounding (Burrell 1927). The function of the platypus crural system is unclear. Although foxes and dogs can kill platypuses, the platypus has very few native predators (crocodiles, Tasmanian devils, and raptors being the only, occasional, exceptions) (reviewed in Whittington et al. 2009). This and the seasonal nature of venom production suggest that the crural system may have evolved to have a reproductive role such as territory defense [assertion of dominance over other male platypuses during the breeding season (Grant 2007)] rather than a defensive function. The trapping of male platypuses with healed spur marks suggests that intraspecific envenomation is not usually fatal (Grant 2007); envenomated male platypuses display edema and temporary limb paralysis (Temple-Smith 1973).

Evolution of the Crural System

Like the platypus, modern echidnas also have a crural system; male echidnas have spurs (0.5–1.0 cm) smaller than those of the male platypus, and females have vestigial spurs that are usually lost in later life (Griffiths 1978). The spurs are connected via a duct to a gland below the knee that is active during the breeding season (reviewed in Griffiths 1978; Krause 2010). The echidna spur cannot be erected or everted from beneath the covering protective skin flap. Echidna spurs are also in a different arrangement to platypus spurs with respect to the attachment of the *os calcaris* to the tarsal bones, meaning that the spurs cannot be rigidly locked into place for spurring (Hurum et al. 2006; Krause 2010). This suggests that the echidna possesses a nonfunctional, regressed venom system.

Unfortunately, as much of the monotreme fossil record is in the form of tooth and jaw fragments (e.g., reviewed in Musser 2003), it is not known whether the ancestral monotreme had a crural system and venomous spurs on the hind limbs. However, given the presence of either functional or regressed crural systems in modern monotremes, and recent molecular data indicating that at least one platypus venom component arose before to the divergence of platypuses and echidnas (Whittington et al. 2008a), the basal monotreme was probably also venomous (Fig. 1).

It is unclear why venom would have evolved in the monotremes, as unlike many other venomous species, modern monotremes do not use their venom systems to capture or digest prey (reviewed in Whittington and Belov 2007). The fact that spurs (functional or vestigial) are present in both sexes of platypuses and echidnas indicates a probable defensive function for the ancestral venom system. The divergence of monotremes occurred in the Jurassic ~166 Mya, and there are fossil monotremes from the Early Cretaceous period (~110–115 Mya, reviewed in Musser 2003). The first fossils of modern monotremes appear in the Pleistocene (1.78 Mya onwards, reviewed in Musser (2003)). The earth from the Jurassic to the Pleistocene was inhabited by dinosaurs and then a diversity of mammals, some of them large carnivores. These potential predators might have meant that defensive venom provided the ancient monotremes with a survival advantage. Perhaps once the

selective pressure of predation was lifted, the crural system was instead used in a reproductive context, and the energetically expensive venom then became a sexually dimorphic trait.

Platypus Venom

Symptoms of Envenomation

To envenomate, the male platypus wraps the hind legs around the victim, drives the spurs into the flesh, and injects the venom. The spurs and attachments are very strong, and males are able to support their full weight by the spurs (Fenner et al. 1992). Up to 4 mL of venom is available to be injected (Temple-Smith 1973), although the small diameter of the duct and the high pressure required for injection means that much smaller amounts would be injected each time (Whittington et al. 2009).

Platypus envenomation has been known to kill dogs (e.g., Burrell 1927), and although no human fatalities have been reported, the venom produces swelling and immediate and excruciating pain that cannot be relieved through normal first-aid practices including morphine administration (Fenner et al. 1992). It also produces nausea, gastric pain, cold sweats, and lymph node swelling (Temple-Smith 1973). One case of platypus envenomation has been clinically reported, recording a high erythrocyte sedimentation rate and low total serum protein and albumin levels in an envenomated human patient, as well as generalized and persistent localized pain and muscle wasting of the spurred limb (Fenner et al. 1992).

As humans rarely fall victim to platypus envenomation and fatalities have never been recorded, no antivenom has been developed.

The Contents of Platypus Venom

The unusual nature of the symptoms of platypus envenomation may be due to the complex mixture of many different peptides and proteins in the venom, some of which have unknown function (de Plater et al. 1995) and some of which may be potentially novel and clinically useful. However, platypus venom research has been hindered because of the limited quantities of venom available for study (platypuses cannot be easily bred in captivity and produce little venom for collection) (Whittington et al. 2009). The research that has been done on platypus venom can be broadly separated into two categories: pharmacological studies of the crude venom and proteomic studies. More recently, genomic techniques have also been used to elucidate the venom components.

Pharmacological Studies of Crude Platypus Venom

The venoms of other species such as snakes are often tested in rodent models, but this type of platypus venom research has been very limited.

Venom injection into rabbits produces coagulation, lowered blood pressure (probably due to vasodilation), hemorrhagic edema, and death (Martin and Tidswell 1895; Kellaway and Le Messurier 1935).

These effects may be a result of disruption of cell membrane ion transport pathways or the creation of abnormal ion channels, which occurs when platypus venom is applied onto cell membrane-mimicking artificial lipid bilayers (Kourie 1999b; Torres et al. 2002a) and onto putative nociceptors (sensory neurons able to send pain signals to the brain) (de Plater et al. 2001). This would disrupt ion concentrations and cause symptoms such as edema (Kourie 1999b); disrupted ion concentrations could cause nerve firing in vivo and thus the pain that is characteristic of platypus envenomation (Kourie 1999b; de Plater et al. 2001).

The venom has mild proteolytic activity. In laboratory animals, injections of venom cause histamine release (rabbits) and cutaneous anaphylaxis (localized skin allergic reaction) and rapid death following intravenous doses of 75–90 mg kg^{-1} (mice) (Temple-Smith 1973). In vitro, the venom causes smooth muscle relaxation (Kellaway and Le Messurier 1935; de Plater et al. 1995), feeble hemolysis (red blood cell breakdown) (Kellaway and Le Messurier 1935), and calcium-dependent nonspecific cation current into neuronal cells, which in vivo may produce edema, nerve firing, and pain (de Plater et al. 2001).

Proteomic Studies

The venom includes nineteen different peptide fractions along plus non-peptide components (de Plater et al. 1995; Kourie 1999b). The identification of these components began with protein characterization techniques. The reverse-phase high-performance liquid chromatography (HPLC) chromatogram of platypus venom reveals many novel polypeptides ranging in size from 4 to 6 kDa (Torres et al. 1999, 2000) and proteins with sizes above 12 kDa. Until recently, three types of peptides had been identified and fully sequenced. These were named after peptides with which they share homology: C-type natriuretic peptides (OvCNPs, de Plater et al. 1998a), defensin-like peptides (OvDLPs, Torres et al. 1999), and nerve growth factor (OvNGF; A. Torres and P.W. Kuchel, unpublished data; described in de Plater 1998). Their exact functions are not known. A venom peptide isomerase and hyaluronidase have also been discovered, but their full sequences have not been determined, and the venom has protease activity (de Plater et al. 1995). The isomerase catalyzes the conversion of the second L-amino acid from the *N*-terminus to a D-form in at least two of the platypus venom components (reviewed in Whittington et al. 2009).

OvDLPs

OvDLPs (*Ornithorhynchus* venom defensin-like peptides) are the most abundant peptides in platypus venom (Torres et al. 2000). They share structural and sequence similarities with antimicrobial beta-defensins. They are characterized by having six paired cysteine residues (Torres and Kuchel 2004) in a similar arrangement to those of the beta-defensins. For example, five cysteine residues match those found in bovine beta-defensins (Torres et al. 2000). This suggests that their tertiary structures are similar. The OvDLPs also have some similarity in folding structure to ShI, a sea anemone (*Stichodactyla helianthus*) sodium channel neurotoxin, but OvDLP-C, unlike ShI, does not affect rat vas deferens or dorsal root ganglia sodium channels

(Torres et al. 1999); the OvDLPs show similarity to two snake venom peptides [rattlesnake myotoxin a and crotamine (Torres and Kuchel 2004)]. In addition, defensin peptides have been isolated from the other venoms, e.g., from the ectoparasitic wasp *Nasonia vitripennis* (Ye et al. 2010).

The side chains of OvDLPs and the homologous venom peptides in other species are different, suggesting that they may have different modes of activity (Torres et al. 1999; Torres and Kuchel 2004). The similarity of OvDLPs to the beta-defensins suggests that they may have similar functions, but there is no evidence that OvDLP-C acts antimicrobially, nor has any myotoxic (muscle toxicity) activity been found (Torres et al. 1999).

OvCNPs

OvCNP (*Ornithorhynchus* venom C-type natriuretic peptide) is present in two isomeric forms in platypus venom, OvCNPa and OvCNPb, which have identical amino acid sequences (Torres et al. 2002b), the significance of which is discussed below. They are the most biologically active peptides identified in the venom so far (Torres et al. 2002a). Natriuretic peptides [atrial (ANP), brain (BNP), and C-type (CNP)] are normally produced in mammalian non-venom tissues, where they are believed to play a role in blood pressure regulation (de Plater et al. 1998b). OvCNP in platypus venom shares homology with C-type natriuretic peptides (in particular, CNP-53 and CNP-22), which are produced in the brain and endothelium but lack natriuretic activity (de Plater et al. 1998a). CNPs have also been found in the venom of some snakes, e.g., the rattlesnake *Crotalus durissus collilineatus* (Boldrini-França et al. 2009).

OvCNP has been shown to form fast cation-selective channels in lipid bilayer membranes (Kourie 1999a) and causes relaxation of smooth muscle (de Plater et al. 1995), uterus, and vas deferens (de Plater et al. 1998b). It produces mast cell degranulation (histamine release) (de Plater et al. 1998b), which may cause swelling. OvCNP also activates calcium-dependent cationic currents which may lead to nerve depolarization (firing) (de Plater et al. 2001) and thus the intense pain that is also characteristic of platypus envenomation (Fenner et al. 1992); 11 analogues of fragments of OvCNP have recently been found in platypus venom, and these also cause calcium influx into neuroblastoma (neuroendocrine tumor) cells (Kita et al. 2009). It has been suggested that OvNGF may cause allodynia (pain due to a stimulus that is not normally painful) and hyperalgesia (increased sensitivity to pain) (de Plater et al. 2001), but the role of the OvDLPs is unclear.

OvNGF

The role of OvNGF (*Ornithorhynchus* venom nerve growth factor), which is homologous to endogenous nerve growth factors (NGFs), is also unknown, and little research has been carried out. NGFs have also been found in snake venoms, and when recombinant human NGF has been injected into humans, some of the symptoms produced (such as allodynia and hyperalgesia) (Dyck et al. 1997) are similar to those produced by platypus envenomation. Other researchers have suggested that OvNGF and OvDLPs may act synergistically to produce pain.

For example, it is possible that NGF renderers cells more susceptible to other venom components, in part because during RP-HPLC, DLP-3 co-elutes with NGF (Torres et al. 2000), although this has not been investigated further.

L-to-D-Peptide Isomerase

As previously mentioned, OvCNP is found in two isomeric forms in platypus venom. These have identical amino acid sequences and differ only by a D-amino acid residue at position two (OvCNPb) that is in L-form in OvCNPa (Torres et al. 2002b). DLP-2 and DLP-4 also have identical amino acid sequences, with a D-form amino acid residue at position two of DLP-2 (Torres et al. 2005). Torres et al. began searching for an isomerase in platypus venom that was responsible for posttranslational conversion of the second amino acid from L- to D-form (Torres et al. 2002b). The isomerase (referred to as L-to-D-peptide isomerase) was partially purified and found to be ~55–65 kDa in size (Koh et al. 2009). Subsequently, a two-base mechanism of isomerization has been proposed, in which two histidine residues of the active site of the isomerase are positioned on either side of the second amino acid of the venom peptide substrate and act as proton donors and acceptors to cause isomerization (Koh et al. 2009).

D-Amino acid residues do not occur frequently in nature and have previously been found only in prokaryotes, yeasts, and some invertebrates (crustaceans, mollusks, spiders) (Torres et al. 2002b, 2006). In some cases, these residues are also present at the second amino acid position (Torres et al. 2002b), raising the possibility that there are similar isomerases between these species. Work is currently underway to search for the presence of similar isomerases in higher mammals (P.W. Kuchel, pers. comm.), and isomerase activity has been found very recently in mouse heart (Koh et al. 2010). The effect of the D-form amino acids in OvCNPb and DLP-2 is unknown, but in other species, the D-form is more biologically active (reviewed in Torres et al. 2006). It has been suggested that the D-form peptides may be more resistant to protease degradation during storage in the venom gland, and perhaps also in the tissues of the victim, thus prolonging envenomation symptoms (Torres et al. 2002b, 2006, reviewed in Koh et al. 2009); this could account for the persistent pain that is characteristic of platypus envenomation (Fenner et al. 1992). Recently, similar isomerase activity has been discovered in mouse heart (Koh et al. 2010), raising the possibility that this isomerase is far more widespread and biologically significant than previously thought.

Genomic Studies

The platypus genome has provided a new avenue for platypus venom research, resulting in a leap forward in the current understanding of platypus toxins. From the genome, researchers decoded the gene sequences for the toxins identified by previous proteomics research, allowing them to investigate the tissue expression patterns of these genes (Whittington et al. 2008b; Whittington and Belov 2009). From here, it was discovered that a number of platypus toxin genes are expressed in non-venom tissues in both males and females, raising the possibility that these genes function in broader, non-venom roles including OvDLP-mediated immunological protection of

the venom gland, which is open to the external environment via the venom duct and spur (Whittington et al. 2008b), and suggesting that these genes may be processed posttranslationally in the venom gland (possibly by the venom isomerase) to confer toxin function (Whittington and Belov 2009).

In 2010, knowledge of platypus venom was vastly increased with the publication of platypus venom gland transcriptome sequence (Whittington et al. 2010). Using technologies similar to those employed to sequence the platypus genome, researchers were able to sequence all of the genes that are expressed in a breeding season platypus venom gland, gaining a snapshot of the sequences of toxins produced in the gland during this time. This new technique identified 83 new putative toxins classified into 13 different toxin families: serine protease, stonustoxin-like, Kunitz-type protease inhibitor, zinc metalloproteinase, latrotoxin-like, CRiSP (cysteine-rich secretory protein), sea anemone cytolytic toxin-like, unknown (IG domains), mamba intestinal toxin-like, C-type lectin domain containing, sarafotoxin-like, VEGF, and DNAse II (Whittington et al. 2010). The putative toxins, which require further functional testing to confirm toxin activity, were identified on the basis of DNA sequence similarity to toxins in other venomous species (including arachnids, marine invertebrates, fish, snakes and lizards, and insectivores). Based on this similarity, the researchers were also able to speculate about the functions of these putative toxins in the venom, which included inflammation, pain, edema, muscle wasting, and coagulation (Whittington et al. 2010).

A limitation to the methodology of the 2010 transcriptomic study is that identifying platypus toxins on the basis of similarity to venom toxins in other species would miss completely novel platypus toxins. In order to discover completely novel toxins, researchers sequenced the genes expressed in both a breeding season and an out-of-breeding season venom gland and compared the levels of each expressed gene. In tandem with this, they also utilized protein sequencing techniques to directly sequence the toxins in platypus venom samples. In this way, they were able to identify novel toxin genes that were strongly upregulated during the breeding season as well as toxin proteins present in the venom (Wong et al. 2012). This uncovered five new platypus toxins: growth differentiation factor 15, nucleobindin-2, CD55, a CXC-chemokine, and corticotropin-releasing factor-binding protein (Wong et al. 2012).

Venom Gene Evolution

The genomic studies of platypus venom have not only revealed a large number of new toxins, as they have also allowed a glimpse into the evolutionary history of platypus toxins. One important factor in platypus venom gene evolution is gene duplication. Gene duplication occurs when a gene is replicated, for example, by unequal crossing over during meiosis, to form two copies. One copy is able to fulfill the original function of the gene, effectively removing the functional constraints from the other copy and leaving it free to vary and take on new functions (e.g., Nei and Rooney 2005). Gene duplication is an important source of possibilities for adaptive evolution, as is evident in the evolution of platypus venom genes, where, as in other animals, venom peptides have been derived via duplication from genes

for nontoxic peptides. For example, the OvDLPs were found to have evolved via gene duplication from antimicrobial beta-defensins ~192 Mya, after which they underwent neofunctionalization to become toxins (Whittington et al. 2008a). Gene duplication has also been responsible for venom gene diversification, generating large multigene families of platypus toxins that may serve to increase expression levels of a particular toxin type (Whittington et al. 2010).

Another important feature of platypus toxin development is convergent evolution. The similarity of platypus venom toxins to the venom toxins of other species (Whittington et al. 2008a, 2010) adds to previous studies finding independent recruitment of venom components in such widely divergent species as cephalopods, cnidarians, cone snails, fish, insects, scorpions, shrews, spiders, reptiles, Hymenoptera, and ticks, in fact across all of the major phyla of venomous animals (e.g., reviewed in Fry et al. 2009). The concept of convergent toxin evolution is important, as it raises the idea that there are certain peptide motifs that appear to be preferentially selected for evolution to venom peptides, possibly due to structural or functional constraints (e.g., reviewed in Fry et al. 2009). This allows speculation into the features that predispose a protein to evolve into a venom toxin. These features include proteins from families with extensive cysteine cross-linkages, secretory proteins, protein scaffolds that allow diversification into multimember toxin families with slightly differing activities, and protein scaffolds that have similarities to endogenous proteins, allowing a toxin to take advantage of one of the three different mechanisms for immediate disruption of the victim's homeostasis (physical damage, agonistic targeting, or antagonistic targeting) (e.g., Fry et al. 2009). Many of the platypus venom peptides satisfy these conditions: cysteine cross-linkages (e.g., OvDLPs), multigene toxin families (e.g., Kunitz-type protease inhibitors), and similarities to existing nontoxin proteins (e.g., OvDLPs and beta-defensins) (Whittington et al. 2008a, 2010).

The independent origin of venom molecules in platypuses and other species also appears to coincide with the independent origin of venom glands, for example, in snakes and platypuses. Snake venom glands are specialized salivary glands, which may have been derived from the pancreas (Kochva 1987), whereas platypus venom glands evolved from modified sweat glands (Temple-Smith 1973). These origins appear to be reflected in tissue expression patterns of venom genes; many of the platypus venom genes identified are derived from endogenous genes that are expressed in the skin, such as kallikreins and defensins (Whittington et al. 2008a, b, 2010). In contrast, there are several examples of snake venom molecules that have evolved from peptides expressed in the salivary gland and pancreas (Kochva 1987).

The Importance of Platypus Venom Research

The platypus is one of Australia's unique native species. Identifying the constituents of platypus venom has constituted an improvement in knowledge of the basic biology of this animal. For effective conservation measures, it is essential to understand all aspects of the biology of the species in question, and so any further

progress in this area would be beneficial. One example is that the effect of intraspecific platypus envenomation is unknown; knowledge of the platypus venom toxins and their functions could provide key improvements in this area.

The utility of platypus venom research should also extend to having human benefits. An understanding of mammalian venoms may improve knowledge of intra- and intercellular signaling in mammals. For example, further research into the platypus venom peptide isomerase may lead to its identification in higher animals including humans, where D-amino acids possibly participate in the etiology of altered-protein-turnover diseases like Alzheimer's and amyloidosis (Shanmugam et al. 2005).

Even more promising is the possibility of discovering novel therapeutic agents in platypus venom. Many venoms have already been used in pharmaceutical applications (reviewed in Escoubas and King 2009), and platypus venom is an untapped resource. The identification of new platypus venom toxins now allows their synthesis and subsequent functional characterization, and it is anticipated that this will provide another rich source of new molecules for pharmaceutical design. Of course, drug development from the platypus toxins is a long way off; not only do the putative toxins need to be confirmed by expression or synthesis of the toxin and then testing in functional assays, but the road to drug development, testing, and regulatory approval is a long one. However, this research is an important first step toward identifying sources of new molecular scaffolds in platypus venom for possible drug design.

The unusual symptoms of platypus envenomation suggest that platypus venom does contain many substances which may be clinically useful. For example, given that D-amino acid-containing peptides and proteins are more stable in vivo, medically useful therapeutic peptides with greater stability in vivo may eventuate from platypus venom peptide isomerase research. The platypus stonustoxin-like toxins, serine proteases, and protease inhibitors, like their functional analogues in snake venom, have potential applications for treating vascular disease in humans (Felicori et al. 2003). The platypus protease inhibitors with similarities to bikunin could be used to gain further insight into the inflammatory process and develop anti-inflammatories to treat organ injuries, cancers, and inflammatory disorders (Kobayashi et al. 2003). The platypus VEGF-like toxin could be used to increase permeability of tumor cells to anticancer drugs without increasing angiogenesis in the proliferating cells (Takahashi et al. 2004). The platypus toxins that cause the extreme pain could be used to probe new pain pathways and thus identify targets for novel painkillers.

Conclusion and Future Directions

The platypus holds an important position at the base of the mammalian phylogenetic tree. Its genome provides a treasure trove of novel evolutionary innovations that may yield unique human therapeutics. The first steps have been taken – the genome and transcriptome are now available. The door is now open for understanding the functional role of these genes and ultimately for the development of novel therapeutics.

Cross-References

► Snake Venom Phospholipase A_2: Evolution and Diversity

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