Convergent molecular evolution of toxins in the venom of advanced snakes (Colubroidea)

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Chapter 6. Conclusions

The findings of this study represent a significant advance in our knowledge of the broad-scale molecular evolution of snake toxin families. We have revealed novel patterns of expression of basal toxin types, including previously unrecognized instances of molecular and structural convergence. The new toxin encoding sequences from RFS and FFS that we have included in our analyses proved particularly valuable demonstration of the distribution of novel toxin classes derived from the propeptide domains of pre-existing toxin genes. These results provide a framework to help guide future bioactivity testing work and further evolutionary studies. Research into the evolutionary and selective forces that result in the instances of explosive diversification or molecular convergence will provide crucial insights into how venom evolves.

One interesting question raised by these data is why, despite all these toxin families being present in the last common ancestor of the advanced snakes, particular descendant lineages have specialized in the production and refinement of certain toxin families and isoforms. For example, kunitz peptides are fairly common in viperid venoms and have diversified to an extreme degree in the elapid family, but are largely absent in other lineages. It is unclear whether these lineage-specific differences are the product of chance or if they were constrained by the ecological contexts in which the progenitors of these snake families employed their venoms.

Other toxin families—such as the SVMPs—show broadly similar levels of duplication across multiple families. This mirrors the pattern of neofunctionalization where the P-III SVMPs have repeatedly evolved into potent procoagulant factor activating toxins. However, only in the viperids has the P-IIId multimeric form evolved, where a SVMP is covalently linked to a lectin dimer (which already possesses another interchain disulphide bond). The viperids show by far the greatest diversity of lectin dimers which may have provided a greater range of molecular opportunities for the P-IIId trait to evolve.

From a broad perspective, almost all toxin families we examined demonstrate a phylogenetic pattern of large clades belonging to snakes of the same family. This suggests that these toxins had not yet diversified in the common ancestor of Colubroidea. The only exception was the lectins, which suggests that this common ancestor likely possessed multiple copies of this toxin already including dimeric forms. Duplication of toxin genes in snakes is often associated with higher abundance of that toxin family in the final venom composition (Margres, et al. 2017; Jackson and Koludarov 2020), so this may constitute preliminary evidence that the common ancestor of Colubroidea would have possessed a primarily lectin-based venom to accompany the other innovations in the venom system such as partitioned oral glands and perhaps modified dentition. The pattern we see in the other families indicates that the vast majority of the variation in terms of composition, unconventional structures, and novel functions that we observe in extant snake venoms arose after the divergence between the families and during the diversification and specialization of those lineages.

While we have discussed many toxins which have rapidly diversified, this phenomenon is most extreme in the propeptide regions of the natriuretic and SVMP genes. Typically, the propeptide region is post-translationally cleaved and does not play a role in envenomation. However, in both these families, new

mutations have caused part of the propeptide region to be translated into protein to form entirely new toxins with novel functions. For the natriuretic peptides, the newly evolved toxins include repeating series of bradykinin potentiating peptides which increase the hypotensive effect of the venoms (Fry, Jackson, et al. 2015). The viperid genera *Azemiops* and *Tropidolaemus* have separately evolved novel neurotoxins derived from within the natriuretic propeptide domain, which share the unusual feature of creating multiple peptides that are translated from a single transcript and then separated during post-translational modification despite their independent origins. Another peptide type was first documented in *Dendroaspis* venom and the molecular evolutionary history remained enigmatic, but our analyses indicate these are members of yet another novel toxin class arising from the natriuretic gene propeptide region. The most explosive diversification of all toxin classes was that of the newly evolved toxin family that evolved in the SVMP propeptide domain within psammophiine snakes. The staggering sequence and structural diversity of these toxins makes it likely that other toxic activities in addition to the already documented novel neurotoxic forms (Brust, et al. 2013) will be documented as more bioactivity testing is undertaken.

Our analyses show that these shared colubroid toxin families exhibit remarkable instances of convergent evolution in terms of pathophysiological function and protein structure. For example, within two potently procoagulant lineages (the *Daboia* genus within the viperid snakes and the *Oxyuranus/Pseudonaja* clade within the elapid snakes), plasmin inhibiting kunitz peptides have evolved which would potentiate the procoagulant effects by increasing the half-life of the blood clots formed due to the inhibition of the blood clot destroying enzyme plasmin. Other taxa possess the arginine residue that is crucial for these plasmin inhibitors and may represent further instances of convergence if functional research confirms this hypothesized activity. Similarly, within the SVMP neofunctionalizationed procoagulant variants, which activate Factor X or prothrombin, have arisen on multiple independent occasions. The lectins may potentially contain further examples of functional convergence given the multiple origins of the QPD motif at a key functional location, but these have not been tested.

One of the most striking cases of structural convergence is the previously mentioned P-IIId derived form of the SVMP which form a covalent linkage to a lectin dimer. The novel cysteine crucial for the formation of these toxin complexes was shown to have evolved on three separate occasions within the viperids as structural modifications of forms that were themselves functionally derived (procoagulant). The selection pressures leading to this convergence have not been explored and the functional impacts are similarly uncharacterised.

The kunitz peptides have been the substrate for both levels of convergence. Structurally three out of the four neurotoxin types (MitTx, taicatoxin, and bungarotoxins) converge in their formation of heteromers with PLA_2 subunits, but diverge structurally in this regard by being non-covalently linked (MitTx and taicatoxin) or covalently linked (β-bungarotoxin), and also in the number of PLA_2 subunits associated with (MitTx = 2, taicatoxin = 1, β-bungarotoxin = 1). All four of the neurotoxic kunitz peptides converge in being ion-channel toxins, with dendrotoxins and β-bungarotoxins further converging on the same target (K_V channels). While taicatoxins affect a different ion channel type (L-type calcium channels) than dendrotoxins and β-bungarotoxins, they converge with bungarotoxin in the PLA_2 toxin facilitating a

secondary action that results in the net functional outcome in blocking the release acetylcholine, leading to flaccid paralysis. In contrast, dendrotoxins act upon the voltage-gated potassium channels to facilitate acetylcholine release, leading to spastic paralysis. β-bungarotoxin demonstrates another instance of convergent molecular evolution in the novel cysteines which allow for the formation of these complexes have evolved twice in the exact same location. The convergent evolution of novel cysteines at the same residue on two occasions within both β-bungarotoxin and the 3FTx dimers is strongly indicative of structural constraints in the formation of these dimers.

This study gives a broad overview of the diversity in the toxin families which are homologous in Colubroid venoms. It is this diversity that produces the wide range of clinical effects and variable responses to antivenom that contribute to the global problem of snakebite. However, such molecular diversity also provides fertile ground in the search for novel molecules as lead compounds for the discovery of new tools and medications. This diversity has also allowed these toxins to converge repeatedly on similar sequences, structures, and functions. This widespread convergence suggests that certain pathophysiological activities and certain configurations of proteins may be evolutionary 'good tricks' (Dennett and Dennett 1996) that are similarly effective across multiple taxa and may solve evolutionary problems that venoms encounter such as prey resistance.

References

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