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Short communication

On the ancestral recruitment of metalloproteinases into the venom of snakes

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ABSTRACT

Tracing the evolutionary history of proteins can reveal insights into gene alterations responsible for changes in structure and function. Here, the origin of snake venom metalloproteinases was rigorously reassessed using phylogenetics and the reconstruction of ancestral sequences. Basal SVMPs are most closely related to ADAM 7, 28 and decysin-1 proteins. Reconstructing the evolutionary history of these proteins and their hypothetical ancestors reveals progressive alterations in the amino acid composition and structural characteristics of ADAMs/SVMPs through evolutionary time.

Snake venom metalloproteinases (SVMPs) are large multi-domain proteins that are classified (P-I, P-II and P-III) based on the presence or absence of additional non-proteinase domains that extend the metalloproteinase domain (Fox and Serrano, 2005, 2008). A number of SVMPs exhibit evidence of post-translational modifications, such as the processing of domains and/or the formation of multimeric structures (Fox and Serrano, 2005, 2008). The generation of this large multi-locus gene family that encodes distinct SVMP scaffolds is likely the result of frequent gene duplication followed by adaptive evolution (Juárez et al., 2008; Casewell et al., 2011a). Notably, SVMPs can be major toxin components of snake venoms (particularly those of viperid snakes), with representation of functionally distinct isoforms from multiple sub-classes frequently observed (Junqueira-de-Azevedo and Ho, 2002; Gutiérrez et al., 2008; Casewell et al., 2009; Wagstaff et al., 2009). The diverse functions of these proteins (reviewed in Fox and Serrano, 2005) can cause a spectrum of severe local and cardiovascular pathologies that often manifest in victims of viper envenoming.

The SVMPs are classified as adamalysins, which, alongside the matrixins, astacins and serralysins, make up the large metizincin gene super-family (Bode et al., 1993; Huxley-Jones et al., 2007). Within the adamalysins, the SVMPs are grouped with ADAM (a disintegrin and a metalloproteinase) and ADAMTS (ADAM with thrombospondin motifs) proteins because of their sequence homology, particularly in the region of the metalloproteinase domain (Fox and Serrano, 2005; Huxley-Jones et al., 2007). ADAMs contain additional domains absent in SVMPs and are widely recognised as highly diverse proteins, with functional roles including the shedding of membrane-bound protein domains and acting as molecular switches that activate other proteins by proteolysis (Black et al., 1997; Srour et al., 2003; White, 2003; Blobel, 2005). In contrast, the ADAMTSs, which contain additional thrombospondin motifs and lack a disintegrin-like domain, can be broadly defined as substrate-specific secreted proteases (Apte,

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2004; Gerhardt et al., 2007). Evidence from molecular phylogenetics suggests that ADAM and ADAMTS proteins originate from a common ancestor (Andreini et al., 2005) and that SVMPs have evolved from an ADAM gene that was recruited into the 'venome' of snakes at the base of the advanced snake (Caenophidia) radiation (Moura-da-Silva et al., 1996; Fry, 2005; Fry et al., 2008). Previous analyses imply that this ancestrally recruited gene was likely an ADAM 7 or ADAM 28 ancestor (Moura-da-Silva et al., 1996; Fry, 2005). However, the inclusion of closely related outgroup sequences from species basal to the split of (i) the reptiles from all other vertebrates and (ii) the snakes from all other reptiles, are required to rigorously test this hypothesis and elucidate the origin of SVMP recruitment into snake venom. The recent publication of the *Anolis carolinensis* (the Carolina anole – a lizard) genome (Alföldi et al., 2011) presents a timely opportunity to test this hypothesis.

To investigate the evolutionary origin of SVMPs, phylogenetic analyses were undertaken on a comprehensive sequence dataset ($n = 122$; amino acid positions = 949) containing basal SVMPs (i.e. from the Elapidae and Atractaspidinae) identified by Casewell et al. (2011a), alongside ADAM and ADAMTS sequences isolated from the genomes of *Homo sapiens*, *Danio rerio* (zebrafish) and *A. carolinensis* (Huxley-Jones et al., 2007; Alföldi et al., 2011). Following translation of DNA sequences into amino acids and alignment in MEGA5 using MUSCLE (Edgar, 2004; Tamura et al., 2011), an adamalysin gene tree was produced using Bayesian inference and incorporating the WAG + Γ model of sequence evolution selected by ModelGenerator (Ronquist and Huelsenbeck, 2003; Posada and Buckley, 2004; Keane et al., 2006). Bayesian inference analyses were undertaken in duplicate, using four chains simultaneously (three heated and one cold) for 5×10^6 generations, sampling every 500th cycle from the chain, with default settings in regards to priors (cf. Casewell et al., 2011a, 2011b). Tracer v1.4 (Drummond and Rambaut, 2007) was used to estimate effective sample sizes and to verify the point of 'burnin' (cf. Casewell et al., 2011a, 2011b). The sequence alignment file can be accessed by its unique doi identifier (doi:10.5061/dryad.fd5q0p68) at the bioscience data repository Dryad (http://datadryad.org/).

The adamalysin gene tree reveals that the ADAMTS proteins are non-monophyletic and basal to a strongly supported clade containing the ADAMs and SVMPs (Fig. 1). Considering ADAMTS and ADAM proteins have been identified in the genomes of *Drosophila melanogaster* (fruitfly) and *Ciona intestinalis* (tunicate), the split of ADAMs from ADAMTS proteins appears to predate the divergence of the protostome and deutrostome lineages (>1000 Mya) (Hausdorf, 2000; Huxley-Jones et al., 2007). Within the ADAM/SVMP clade, the SVMPs are monophyletic (Fig. 1), highlighting that their recruitment into snake venom was likely the result of a single origin following the divergence of the Pareatidae from the remaining Caenophidians (~60 Mya – Vidal et al., 2009), as previously described elsewhere (Moura-da-Silva et al., 1996; Fry, 2005; Fry et al., 2008; Casewell et al., 2011a). Adaptive evolution of SVMP genes duplicated from this ancestral scaffold is presumably responsible for driving the subsequent evolution and diversification of SVMP structures observed in the venom of Caenophidians (Casewell et al., 2011a).

Notably, the SVMPs form a strongly supported monophyly with mammalian ADAM 7, ADAM 28 and ADAM decysin-1 (ADAM DEC1) and reptilian ADAM 28 proteins (Fig. 1). Critically, incorporating ADAM sequences from non-mammalian species does not invalidate the previous hypothesis that the recruitment of the ADAM scaffold into the arsenal of venomous snakes was likely an ancestral form of an ADAM 7 or ADAM 28 protein (Moura-da-Silva et al., 1996; Fry, 2005). The closest ancestor to the SVMPs, a protein annotated as ADAM 28 isolated from the genome of the lizard *A. carolinensis* (Alföldi et al., 2011), has yet to be functionally characterised. Because this gene exhibits comparable sequence similarity to both mammalian ADAM 28 (56%) and basal SVMPs (54%), interpretation of the evolutionary history of the ADAM genes presented here will focus on the more characterised mammalian forms.

ADAM 7 is expressed in the epididymus of mammals and thought to enable sperm motility and fertilisation acquisition (Oh et al., 2009), whilst mammalian ADAM 28 is expressed in a variety of tissues and associated with integrin binding and facilitating enzymatic cleavage of the extracellular matrix and the transendothelial migration of lymphocytes (Fry, 2005; McGinn et al., 2011). Since mammalian ADAM DEC1 forms a monophyly with mammalian ADAM 7 and ADAM 28, it should also be considered when assessing the evolution of SVMPs. The function of ADAM DEC1 remains unclear – whilst it is expressed in macrophages and dendritic cells and associated with pathogenesis of the lungs during sarcoidosis (Shapiro, 2003; Crouser et al., 2009), the proteolytic function of this ADAM remains unknown. ADAM DEC1 is an atypical ADAM because it exhibits loss of the cysteine-rich domain and partial loss of the disintegrin domain (Bates et al., 2002). This is significant because the evolutionary history of the SVMPs is punctuated by domain loss (Moura-da-Silva et al., 1996; Casewell et al., 2011a) and strongly suggests that the ancestor of SVMPs and their related ADAMs (ADAM 7, 28 and DEC1) were predisposed to truncation. The close relationship between ADAM 7, ADAM 28 and ADAM DEC1 proteins has been noted previously – they cluster together on the same human chromosome and it has been suggested that they may have originally shared functionality, prior to partial gene duplication at this locus giving rise to their later diversification (Bates et al., 2002). Considering that the diversification of venom genes in the Caenophidians is thought to be largely the result of gene duplication (Kordiš and Gubenšek, 2000; Fry et al., 2003; Lynch, 2007; Casewell et al., 2011b), it will be interesting to see if future genome studies identify whether SVMP gene duplication events are also localised on the same chromosome and whether the ancestral SVMP gene exhibits synteny with its lizard and mammalian homologues.

Tracing the evolutionary history of venom gene families is a valuable tool for elucidating the timing and nature of the basal scaffolds that were originally recruited into venom. Subsequent analysis of predicted changes identified in derived proteins may reveal insights into amino acid substitutions (replacements) responsible for structural and