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Short communication

Discovery of α-defensins in basal mammals

David J. Lynn*, Daniel G. Bradley

Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland

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Abstract

α-Defensins are essential molecules of the innate immune system that have broad spectrum antimicrobial activity against a range of bacteria and viruses. To date, α-defensins have only been identified in the *Euarchontoglires* branch of the mammals. This has led to speculation that α-defensins may be specific to this group, a somewhat surprising finding, given their importance in the immune system. The mammalian genome project provided us with the opportunity to search for α-defensins in previously unexamined mammalian superorders. Using hidden Markov model (HMM) profile searching, we report the discovery of α-defensins in the African savanna elephant, the lesser hedgehog tenrec, and the nine-banded armadillo genomes representing two of the most basal mammalian superorders, *Afrotheria* and *Xenarthra*. Furthermore, we identify an α-defensin-like gene in the gray short-tailed opossum, suggesting that α-defensins may have evolved much earlier than previously thought, before the divergence of placental mammals and marsupials approximately 130 mya.

Keywords: α-Defensins; Mammal; Elephant; Tenrec; Armadillo; Opossum; Evolution; Hidden Markov model profile

Defensins are cationic antimicrobial peptides that are abundant in neutrophils, intestinal Paneth cells, and epithelial cells. In humans, α- and β-defensins are two genetically distinct groups, which differ in size and in their spacing of a six-cysteine motif and in their tissue distribution [1]. Compared with the α-defensins, which are primarily expressed in neutrophils [2] and intestinal Paneth cells [3], β-defensins have a much broader expression pattern, and are expressed in epithelial cells of a range of tissues including human skin, lung, and urogenital tract [4,5]. β-defensins also have a wide species distribution, and have been reported in birds [6,7], fish [8] and throughout all mammals studied to date [9].

A third type of defensin, the theta- or mini-defensin, was first identified in granulocytes of the Rhesus macaque (*Macacca mulatta*) [10]. These theta-defensins are actually encoded by two separate truncated α-defensin-like precursors that are fused to form a cyclic peptide using a previously unknown post-translational modification [11]. Theta-defensins are found only in primates and are pseudogenes in humans [12].

α-defensins are cationic antimicrobial peptides that are critical effector molecules of the innate immune system. In humans, α-defensins have been shown to block papillomavirus infection [13], reduce HIV transmission in breastfed infants [14] and are implicated in Crohn's disease [15]. Despite their obviously important roles in the immune response

Abbreviations: HMM, hidden Markov model; ELAD, elephant α-defensin; TNAD, tenrec α-defensin; ARMAD, armadillo α-defensin; OPAD, opossum α-defensin

*Corresponding author. Tel.: +353 1 896 1265; fax: +353 1 679 8558.
E-mail address: david.lynn@tcd.ie (D.J. Lynn).

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and in comparison with β-defensins, to date, α-defensins have only been identified in the *Euarchontoglires* branch of the mammals [16], a mammalian superorder that includes the rodents, lagomorphs and primates (Fig. 1). Analysis of the zebrafish, pufferfish, dog [16], chicken [5] and cow (Btau1.0) (DJ Lynn, unpublished data) genomes failed to identify any α-defensins outside of this group.

Previous searches for novel mammalian α-defensins have been restricted to the *Boreoeutheria*. Recently, low-coverage genomic sequences from representatives of several mammalian orders have become available as part of the mammalian genome project (http://www.broad.mit.edu/mammals/). The relatively small size and poor sequence conservation of α-defensins mean that commonly used similarity-search tools, such as BLAST [17], are likely to miss genes encoding these peptides. Fortunately, the presence of the conserved six-cysteine motif makes these peptides ideal for HMM profile searching [18]. To investigate the occurrence of α-defensins outside of *Euarchontoglires*, we have performed HMM profile searches of α-defensins on the six-frame translated genomes of several previously unexamined mammals.

Mammalian α-defensins are usually encoded by two exons. The first exon encodes the α-defensin pre-pro region, which is cleaved to release the active mature antimicrobial peptide containing a conserved cysteine motif (exon 2) [19]. To create an α-defensin HMM profile, protein sequences representing known α-defensins were aligned using T-coffee [20]. A core region containing the conserved cysteine motif was identified and used to construct a profile HMM using hmmbuild from HMMER 2.1.1 (http://hmmer.wustl.edu/) [18]. hmmcalibrate was used to calibrate *E*-value scores. Similarly, a HMM profile was constructed for the α-defensin pre-pro region. As the HMMER programs are not capable of directly searching DNA sequences, genomic sequence from each species was translated in six reading frames. The core α-defensin HMM profile was used to search the six frame translations using hmmsearch. Genomic contigs that contained the consensus α-defensin motif were extracted. The HMM profile of the α-defensin pre-pro region was then searched against these contigs to identify full-length genes encoding the α-defensin proteins.

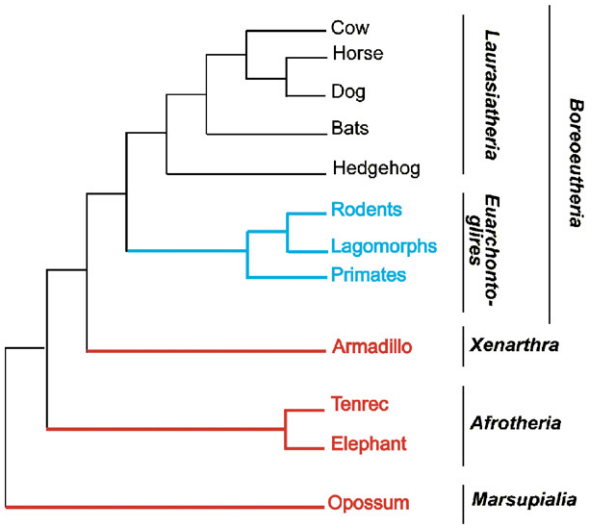

Fig. 1. Phylogeny of the mammals based on the Bayesian phylogenetic tree of Murphy et al. [28]. The branches shown in blue represent the previously known occurrence of α-defensins in the *Euarchontoglires*. The red branches indicate the species representing Afrotherian, Xenarthran and marsupial mammals, in which we have identified novel α-defensins.

The presence of α-defensins has not been investigated in two of the most basal Eutherian mammal superorders, the *Afrotheria* and the *Xenarthra*. The *Afrotheria* are an ancient group of mammals, including elephants, tenrecs, aardvarks and hyraxes that evolved when the African continent was isolated by plate tectonics [21]. Genomic sequence of two representative species of this group, the African savanna elephant (*Loxodonta africana*) and the lesser hedgehog tenrec (*Echinops telfairi*), was available for analysis. A HMM profile search of the elephant genome identified three distinct α-defensin cysteine motif domains on three separate genomic contigs, which we have named elephant α-defensin 1–3 (ELAD1-3) (Supplementary Fig. 1). The contigs encoding ELAD1 and ELAD2 both contain regions upstream of the cysteine domain with significant homology to the α-defensin pre-pro region. Full-length gene predictions for both of these genes were generated using the GenomeScan program [22]. We were unable to identify a pre-pro region for ELAD3, possibly due to fragmented nature of the low-coverage elephant genome. Six distinct α-defensin cysteine domain motifs were identified in another Afrotherian mammal, the tenrec. We have named these tenrec α-defensins 1–6 (TNAD1–6) (Supplementary Fig. 2). TNAD1–3 also had regions showing significant homology to the α-defensin pre-pro region upstream of the cysteine domain. GenomeScan [22] generated full-length gene predictions for TNAD1 and TNAD2. TNAD3 contained