

The evolutionary origin of hedgehog proteins

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Animal development is orchestrated largely by diffusible ligands of the Wnt, TGF- β , hedgehog (Hh) and FGF signaling pathways, as well as cell-surface molecules, such as Notch, cadherins, integrins and the immunoglobulin-like proteins [1,2]. Here, we show that Hh proteins are likely to have evolved very early in metazoan evolution by domain shuffling. We identify in sponges and cnidarians a transmembrane protein, Hedgling, that contains the amino-terminal, signalling domain of Hh (hedge-domain), as well as cadherin, EGF and

immunoglobulin domains. While Hedgling appears to have been lost in bilaterians, the likely capture of a hedge-domain by the more ancient, intein derived hog-domain may have given rise to the Hh proteins.

Components of the Hh signaling pathway have been recently reported in basal metazoans [3,4] (D.Q.M. and M.Q.M., unpublished). A Hh-related protein has also been discovered in a close unicellular relative of the Metazoa, the choanoflagellate *Monosiga ovata*. The *M. ovata* protein contains a carboxy-terminal hog-domain but lacks a hedge-related domain [5]. Instead, the amino-terminus of this protein consists of a large threonine-rich domain and two cellulose-binding domains. In addition, a hedgehog-like sequence has been identified in a shallow EST survey of a homoscleromorph sponge [4]. However, this sequence is missing the first ~70 amino acids of the hedge domain, contains at least one large insertion of ten amino acids that is absent

in all other hedge-domain genes, and sequence outside the hedge-domain bears no resemblance to hedgling, Hh or anything in the NCBI database, thus casting doubt on its relationship to a true *Hh* gene.

In the sequenced genome of the demosponge *Amphimedon queenslandica*, we identified a single hedge-domain. It is not associated with the five hog-domains found in the genome, at least four of which are associated with laccase-encoding sequences (Supplemental Data). We call this hedge-domain protein 'Hedgling'. Hedgling is a large transmembrane protein that contains a signal sequence followed by the definitive hedge domain, a von Willebrand domain, 11 complete and one incomplete cadherin repeats, two immunoglobulin domains, two EGF domains, a transmembrane domain and a cytoplasmic tail of approximately 150 amino acids that does not display significant similarity to any known protein (Figures 1 and

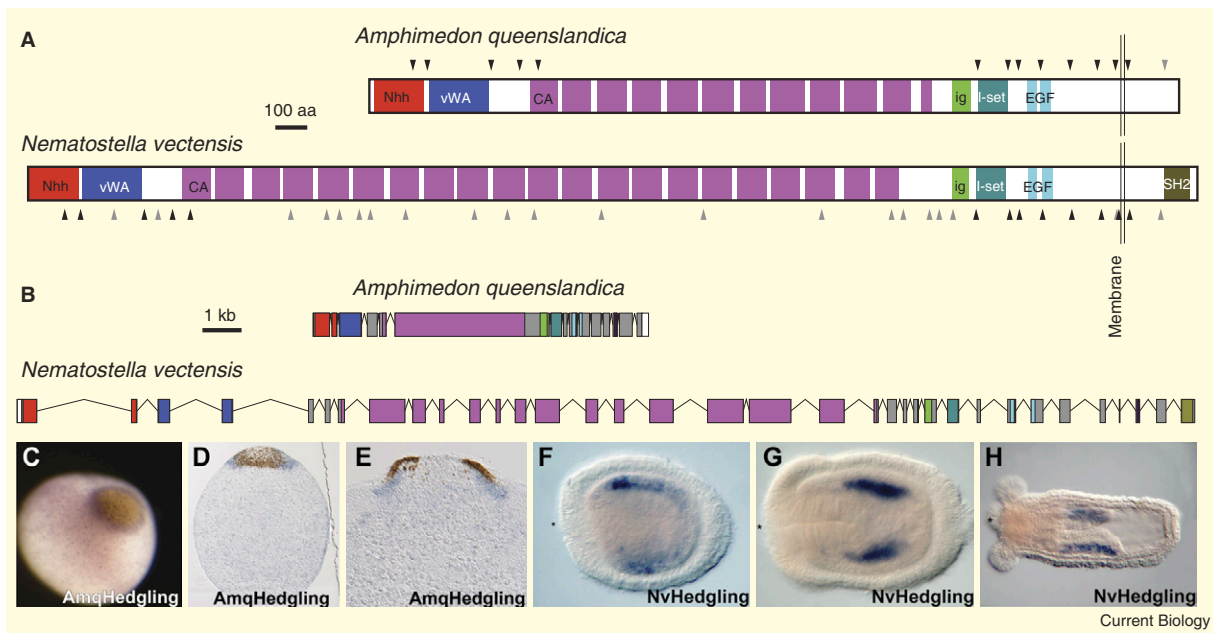


Figure 1. Domain structure and expression of Hedgling.

(A and B) Domain architecture and genomic structure of demosponge (*Amphimedon queenslandica*) and sea anemone (*Nematostella vectensis*) hedgling proteins (A) and genes (B). Domain homology was assigned by InterProScan search, with following PFAM domains identified: Nhh (PF01085: Hedgehog amino-terminal signaling region, red), vWA (PF00092: Willebrand factor, type A, navy blue), CA (PF00028: Cadherin, pink), ig (PF00047: Immunoglobulin, light green), I-set (PF07679: Immunoglobulin I-set, teal), EGF (PF00008: EGF-like, light blue), SH2 (PF00017: SH2 motif, brown). Black triangles indicate conserved intron positions and grey triangles intron positions different between sponge and sea anemone. (C–E) Expression of *Amq-hedgling* in a narrow band surrounding and partially underlying (C and D) the pigment spot and (E) the developing pigment ring in the *Amphimedon* embryo. (F–H) Expression of *NvHedgling* in (F and G) the planula stage in a bilateral pattern in the endoderm. (H) This pattern persists into the polyp, where transcripts are detectable in the endodermal component of the directive mesenteries. (C) and (F–H) are whole mounts; (D and E) are sections. (See Supplemental data for experimental details.)

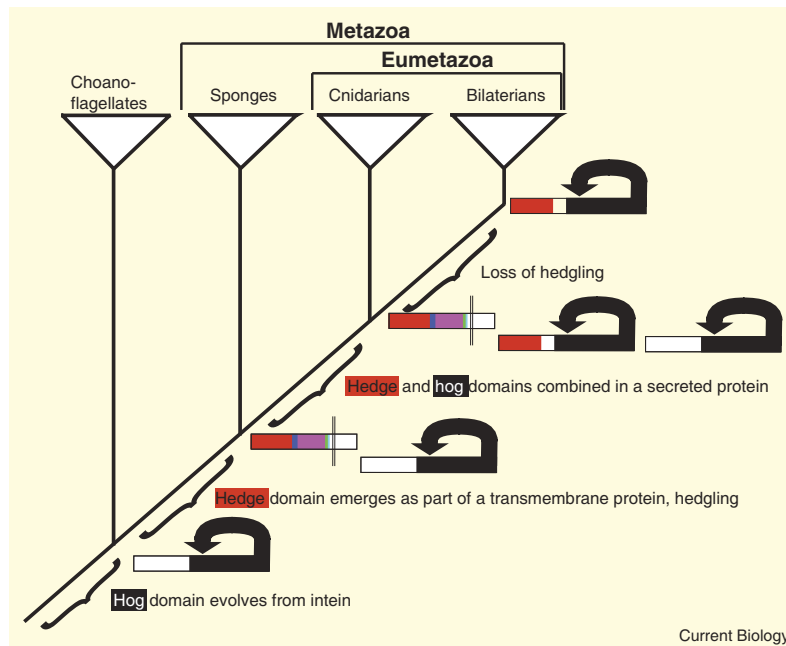


Figure 2. A model of Hedgehog protein evolution.

The intein-related hog domain (black) predates multicellularity and can be associated with various unrelated proteins (white). The hedge-domain (red) arose in the ancestor of sponges and eumetazoans, and was originally part of Hedgling (red, pink and blue), but only became associated with the hog-domain in the common ancestor of cnidarians and bilaterians. Alternatively, this conventional hedgehog may have evolved earlier and been lost in the sponge lineage.

Supplemental data). A *hedgling* orthologue is also present in the cnidarian *Nematostella vectensis* but not in any bilaterian genomes. The *Nematostella* Hedgling (NvHedgling) displays striking domain structure and sequence similarity to the *Amphimedon* Hedgling (Figure 1 and Supplemental data); 13 of the 14 *Amphimedon* intron sites are conserved in *Nematostella* (Figure 1). The *Nematostella* protein contains 21 cadherin repeats and a Src homology, SH2 motif in the cytoplasmic tail. The domain configuration of sponge and cnidarian Hedglings resembles that of the cadherins – transmembrane proteins involved in cell-cell adhesion – and shows high similarity to the non-classical cadherins FAT and Flamingo [6]. This suggests that Hedgling may be involved in cell–cell communication [1].

Sponge and cnidarian *Hedgling* genes are expressed during development (Figure 1). *Amq-hedgling* is expressed after gastrulation in a narrow band

of posterior cells associated with pigment ring formation [7]. After gastrulation, *NvHedgling* is expressed in two bilateral bands of aboral endoderm (the directive mesenteries) overlapping with the *Wnt* gene *NvWnt* [8].

We propose that the hedge-domain arose in the common ancestor of sponges and other multicellular animals as a part of a transmembrane protein involved in direct communication between neighboring cells (Figure 2). Early in metazoan evolution, domain shuffling resulted in the emergence of the conventional Hh, composed of the hedge-domain and the more ancient hog/intein-domain. The autocatalytic activity of the hog/intein-domain in Hh could have allowed the release of the hedge ligand and hence longer range signaling that could be used to control more complex morphogenetic patterning. True *Hh* genes have only been detected in cnidarian and bilaterian genomes and *Hedgling* appears to have been lost in the bilaterian lineage. While *Hh* might have

evolved before the divergence of sponge and eumetazoan lineages and been lost in the *Amphimedon* lineage, domain swapping is likely to have played a crucial role in the origin of the Hh family of signaling molecules. We have found a Hh signalling domain in the recently sequenced genome of the choanoflagellate *Monosiga brevicollis*, raising the possibility that Hedgling may predate the origin of the metazoans.

Supplemental data

Supplemental data including acknowledgements and experimental procedures are available at <http://www.current-biology.com/cgi/content/full/17/19/R836/DC1>

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