Dev Genes Evol (2007) 217:235–239
DOI 10.1007/s00427-007-0136-5

SEQUENCE CORNER

Conserved and novel *Wnt* clusters in the basal eumetazoan *Nematostella vectensis*

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Received: 30 August 2006 / Accepted: 19 January 2007 / Published online: 20 February 2007

Abstract Evolutionarily conserved gene clusters are interesting for two reasons: (1) they may illuminate ancient events in genome evolution and (2) they may reveal ongoing stabilizing selection; that is, the conservation of gene clusters may have functional significance. To test if the *Wnt* family of signaling factors exhibits conserved clustering in basal metazoans and if those clusters are of functional importance, we searched the genomic sequence of the sea anemone *Nematostella vectensis* for *Wnt* clusters and correlated the clustering we observed with published expression patterns. Our results indicate that the *Wnt1–Wnt6–Wnt10* cluster observed in *Drosophila melanogaster* is partially conserved in the cnidarian lineage; *Wnt6* and *Wnt10* are separated by less than 4,500 nucleotides in *Nematostella*. A novel cluster comprised of *Wnt5–Wnt7/Wnt7b* was observed in *Nematostella*. Clustered *Wnt* genes do not exhibit *Hox*-like colinearity nor is the expression of linked *Wnt* genes more similar than the expression of nonlinked *Wnt* genes. *Wnt6* and *Wnt10* are not expressed in a spatially or temporally contiguous manner, and *Wnt5* and *Wnt7* are expressed in different germ layers.

Communicated by M.Q. Martindale

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Keywords *Wnt* · Gene clusters · Evolution · Development · Evo–devo

Introduction

In spite of the fact that only a small fraction of the genome of complex metazoans consists of coding regions (e.g., 5% in the human genome; International Human Genome Sequencing Consortium 2001), numerous clusters of evolutionarily related genes have been noted. A number of hypotheses have been proposed to explain the evolution and maintenance of these linked genes: (1) Linked genes are expressed under the influence of common enhancers, and in the absence of gene specific repressors should be expressed in a similar spatio-temporal pattern; (2) Linked genes are coordinately regulated by higher-order chromatin organization (Spitz et al. 2003), leading to spatial and/or temporal colinearity of expression, as seen in the expression pattern and genomic organization of the *Hox* family of transcription factors; and (3) Gene linkage may have no contemporary functional significance—it may simply represent a phylogenetic signature, the outcome of a relatively recent tandem duplication event or the remnant of an ancient gene cluster that has lost its functional significance.

One gene family which exhibits syntenic linkage of unknown functionality and origin is the *Wnt* family. The *Wnt* genes comprise an evolutionarily conserved group that is known to affect cell fate decisions during development and oncogenesis (Cadigan and Nusse 1997). Twelve *Wnt* subfamilies have been identified in deuterostomes (Nusse 2001). A total of 6 of these 12 subfamilies have been identified in *Drosophila,* whereas 11 have been recovered from the sea anemone *Nematostella vectensis*, a member of the basal metazoan phylum Cnidaria. This suggests (1) that

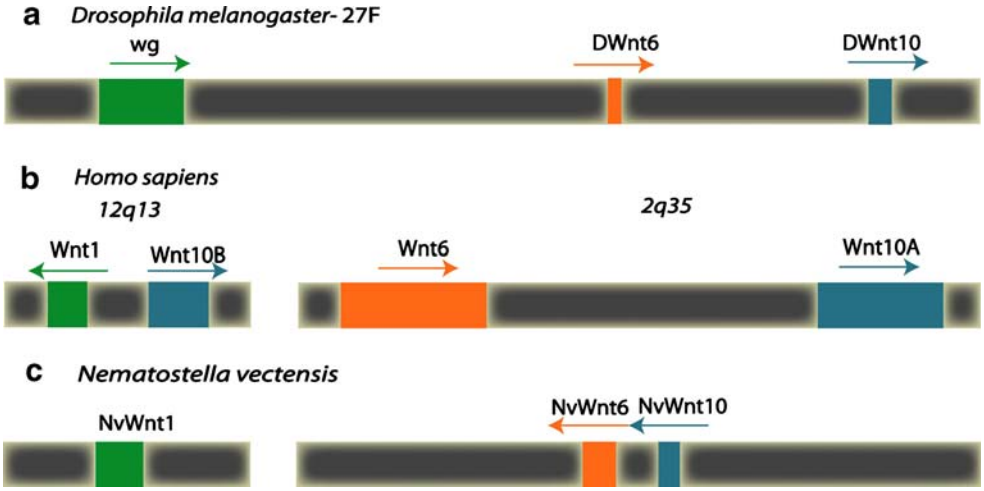

Fig. 1 *Wnt1–Wnt6–Wnt10* clusters. **a** and **b** reproduced from Nusse (2001)

the *Wnt* radiation preceded the cnidarian–bilaterian divergence and (2) that several *Wnt* genes have been lost in the fruit fly lineage (Nusse 2001; Kusserow et al. 2005; Lee et al. 2006).

Several *Wnt* clusters have been identified in the Bilateria, including the *Wnt3–Wnt9B*, *Wnt3A–Wnt9A*, and *Wnt2–Wnt16* miniclusters that are found in vertebrates. An evolutionarily conserved cluster of three *Wnt* genes (*Wnt1–Wnt6–Wnt10*) is thought to have existed in the last common ancestor of arthropods and deuterostomes (Nusse 2001). In *Drosophila*, this compact cluster spans ~70 kb on chromosome 2 at position 27f (Fig. 1a). In human and mouse, *Wnt1* is linked closely to *Wnt10b*, and *Wnt6* is linked closely to *Wnt10a* (Fig. 1b). These two *Wnt* miniclusters are located on different chromosomes in both species (Nusse 2001).

To test the hypothesis that this cluster was present in the cnidarian–bilaterian ancestor, we consulted the recently completed genome sequence of *N. vectensis* (*Nematostella* genome sequencing project, Joint Genome Institute, D. Rokhsar, principal investigator; Miller et al. 2005). Additionally, we tested hypotheses of cluster origin and maintenance by searching for additional clusters of *Wnt* genes (both previously reported and novel) and correlating these with expression patterns of these genes in *Nematostella*. Recent research regarding gene expression patterns of homeobox clusters has been synthesized into the hypothesis that genes responsible for patterning each of the three germ layers of triploblasts are clustered such that the *Hox* cluster patterns the neuroectoderm, the *ParaHox* the endoderm, and the *NK* the mesoderm (Garcia-Fernàndez 2005). If colinearity is operative for *Wnt* genes in *Nematostella*, then those *Wnt* genes responsible for patterning each germ layer will be clustered, and the order of genes within clusters will mirror the order of *Wnt* expression territories along the body axis.

Materials and methods

A draft assembly of the *Nematostella* genome was produced using the Phusion Genome Assembler (Mullikin and Ning 2003) from genomic traces published on the National Center for Biotechnology Information by the Joint Genomes Institute. The assembly, comprising 81,401

Table 1 Genes linked to select *Wnt* clusters in *Homo sapiens* and *D. melanogaster*

Gene	Species	Accession no.	Cluster to which gene is linked	Location of gene relative to cluster
NinaC	Dm	NM_078779	*wg–Wnt6–Wnt10*	3′ of *Wnt10*
CG5149	Dm	NM_135266	*wg–Wnt6–Wnt10*	3′ of *Wnt10*
CG13785	Dm	NM_135263	*wg–Wnt6–Wnt10*	5′ of *wg*
Wnt4	Dm	NM_057624	*wg–Wnt6–Wnt10*	5′ of *wg*
CDK5R2	Hs	NM_003936	*Wnt6–Wnt10A*	3′ of *Wnt10A*
FEV	Hs	NM_017521	*Wnt6–Wnt10A*	3′ of *Wnt10A*
PRKAG3	Hs	NM_017431	*Wnt6–Wnt10A*	5′ of *Wnt6*
CYP27A1	Hs	NM_000784	*Wnt6–Wnt10A*	5′ of *Wnt6*
ARF3	Hs	NM_001659	*Wnt1–Wnt10B*	3′ of *Wnt10B*
FKBP11	Hs	NM_016594	*Wnt1–Wnt10B*	3′ of *Wnt10B*
DDN	Hs	NM_015086	*Wnt1–Wnt10B*	5′ of *Wnt1*
PRKAG1	Hs	NM_002733	*Wnt1–Wnt10B*	5′ of *Wnt1*

Hs Homo sapiens, Dm Drosophila melanogaster

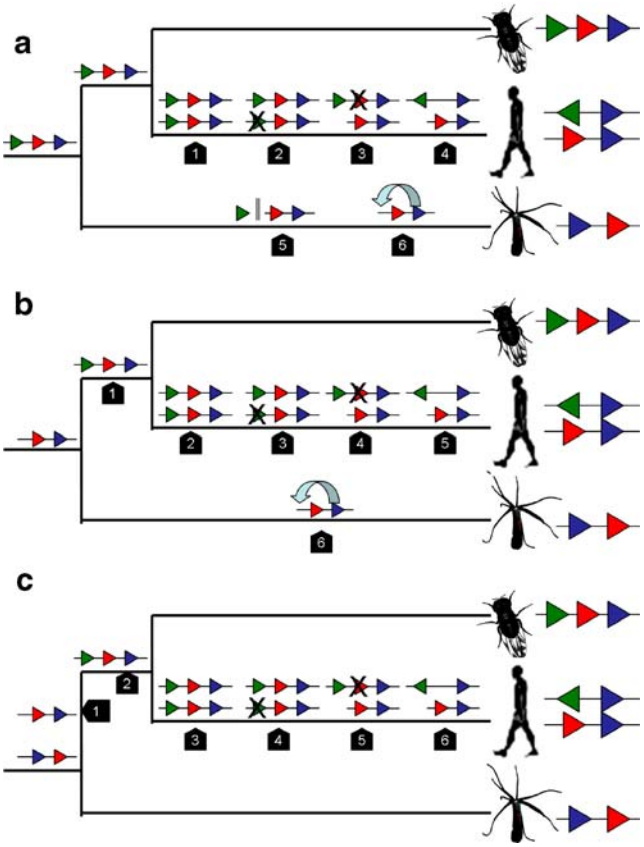

Fig. 2 Three plausible scenarios for the evolution of the *Wnt1–Wnt6–Wnt10* cluster. *Wnt1/wg* is represented by *green triangles*, *Wnt6* by *red triangles*, and *Wnt10* by *blue triangles*. Potential ancestral states are shown at the *nodes* representing the bilaterian ancestor and the cnidarian–bilaterian ancestor. In scenario **a**, *Drosophila* retains the condition present in the cnidarian–bilaterian ancestor. The lineage leading to humans experiences (*1*) a cluster duplication, (*2*) the loss of *Wnt1* from one cluster, (*3*) the loss of *Wnt6* from the other cluster, and (*4*) an inversion of *Wnt1*. In the lineage leading to *Nematostella*, (*5*) *Wnt1* becomes dispersed from the cluster, and (*6*) the relative order of *Wnt6* and *Wnt10* becomes reversed. In scenario **b**, only *Wnt6* and *Wnt10* are clustered in the cnidarian–bilaterian ancestor. In the line leading to bilateria, (*1*) *Wnt1* joins the *Wnt6–Wnt10* cluster. Subsequently, the line leading to humans undergoes the same changes as in scenario **a**. In the line leading to *Nematostella*, (*6*) the relative order of *Wnt6* and *Wnt10* becomes reversed. In scenario **c**, *Nematostella* retains the condition found in the cnidarian–bilaterian ancestor. In the line leading to bilateria, (*1*) the relative order of *Wnt6* and *Wnt10* becomes reversed, and (*2*) *Wnt1* joins the *Wnt6–Wnt10* cluster. The line leading to humans experiences the same changes as in scenarios **a** and **b**

contigs spanning ~3.6 Mb, has been described in detail elsewhere (Ryan et al. 2006). Previously published coding sequences for *Nematostella Wnt* genes (Kusserow et al. 2005) were blasted against the assembled genome at *StellaBase* (Altschul et al. 1990; Sullivan et al. 2006). Gene alignments were created using ClustalX (Thompson et al. 1997). The neighbor-joining method (Saitou and Nei 1987) was applied to the PAM–Dayhoff-matrix-based distances using the Phylip software package (Felsenstein 1989). Reliability of internal branches was assessed using the bootstrap method with 1,000 replicates.

Results and discussion

Wnt6 and *Wnt10* are more closely linked in *Nematostella* than in the fruit fly or human—the intergenic distance is only 4,474 nucleotides. The relative orientation of these two genes is reversed in *Nematostella* (5′-*Wnt10*-*Wnt6*-3′) versus bilaterian animals (5′-*Wnt6*-*Wnt10*-3′). Unlike in the fruit fly, *Wnt1* is not closely linked to *Wnt6* or *Wnt10* in *Nematostella* (Fig. 1c). To help resolve the evolutionary events leading to this organization in human, fruit fly, and *Nematostella*, we searched for a conserved syntenic linkage of protein-coding regions adjacent to the *Wnt* clusters. The two protein-coding regions in closest proximity to each cluster in humans and *Drosophila*, both upstream and downstream, were used to query the *Nematostella* genome (tblastn) to determine if any of these protein-coding regions are present on the same assembled contig as *Nv–Wnt1* or *Nv–Wnt6–Nv–Wnt10* (Table 1). No genes which are neighbors to *Wnt* clusters in *Drosophila* or human are closely linked to either *Wnt6–Wnt10* or *Wnt1* in *Nematostella*. This clustering is suggestive of one of three evolutionary scenarios (Fig. 2). Assuming *Nv–Wnt1* was not lost from a primordial *Wnt1–Wnt6–Wnt10* cluster (Fig. 2a), the cnidarian–bilaterian ancestor likely possessed a cluster comprised of *Wnt6–Wnt10*. Based on the assembly, *Wnt10* and *Wnt1* can be no closer than 59 kb in *Nematostella* (Fig. 1).

We noted a novel cluster comprised of *Wnt5* and *Wnt7* in *Nematostella*. The 3′ end of *Wnt5* is 9,458 nucleotides from the 3′ end of *Wnt7* (Fig. 3). The published *Wnt7* and *Wnt7b* transcripts (Kusserow et al. 2005) map to a single locus in *Nematostella* and share 533 nucleotides (Fig. 3). Only a single *Wnt7* transcript is known from *Drosophila*, but diverse bilaterians (e.g., human and common house spider, *Achaearanea tepidariorum*) express at least two distinct

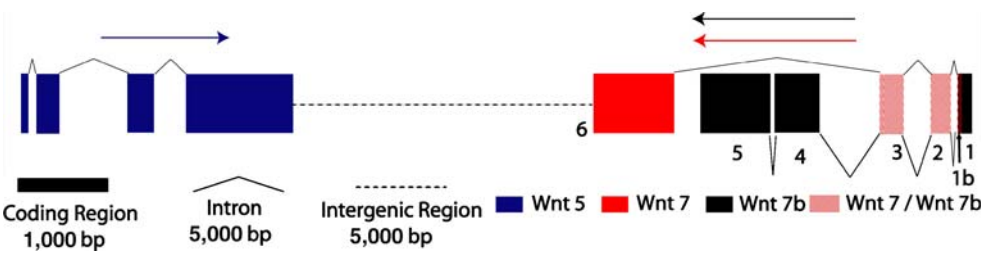

Fig. 3 A novel *Wnt* cluster of *Wnt5*/*Wnt7* is observed in *N. vectensis* with alternative splicing resulting in distinct *Wnt7* and *Wnt7b* transcripts. *Wnt7* is composed from exons 1b, 2, 3, and 6 and *Wnt7b* from exons 1, 1b, 2, 3, 4, and 5

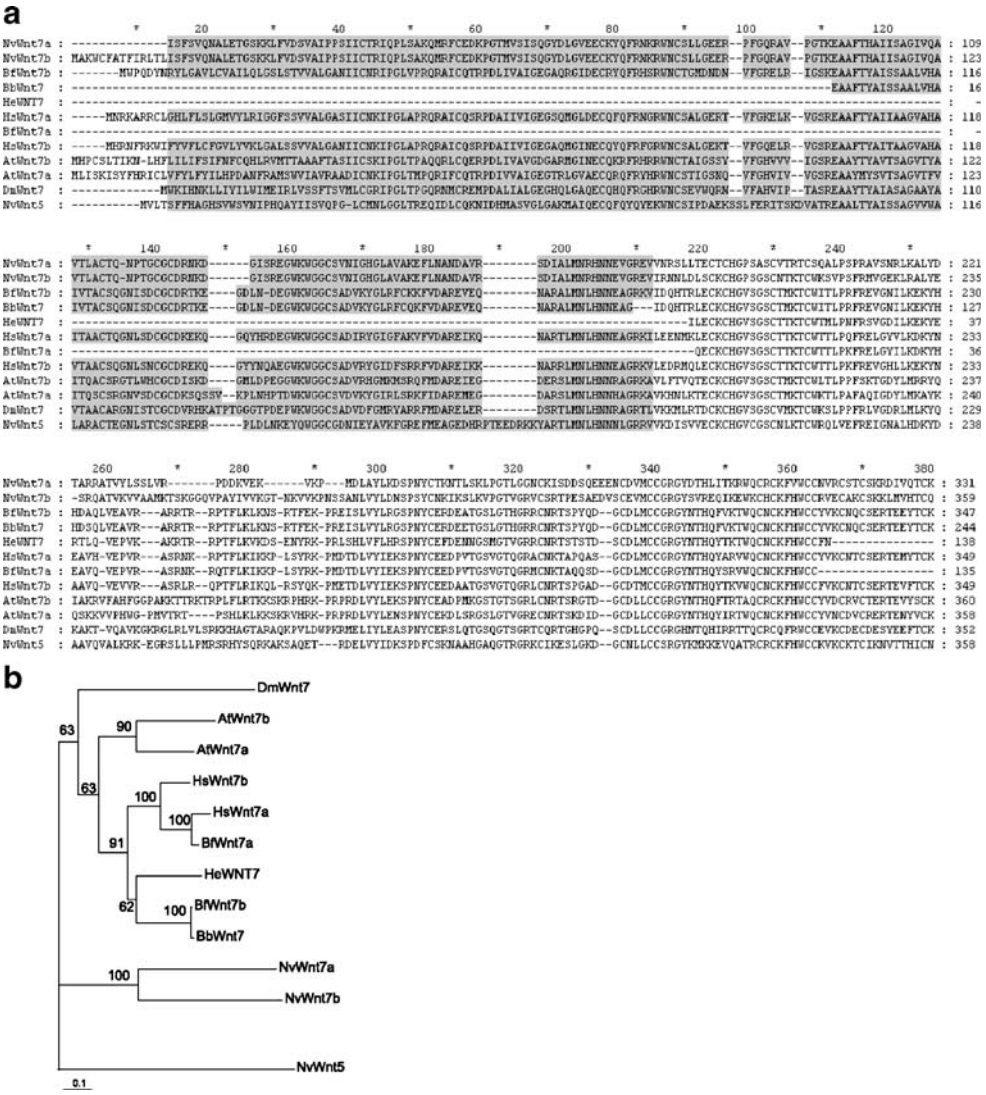

Fig. 4 Evolutionary relationships of *Wnt7* variants from diverse taxa. **a** An alignment of *Wnt7* genes was generated using ClustalX (Thompson et al. 1997). The nucleotides aligned to the exons shared by *Nematostella Wnt7* and *Wnt7b* are highlighted in gray. **b** A neighbor-joining tree generated from a PAM–Dayhoff matrix comparison of *Wnt7*, excluding those portions of each sequence which align to the shared exons of *NvWnt7* and *NvWnt7b* (Felsenstein 1989). *Labels* in the nodes represent the percentage of times each branch occurred in 1,000 trees. The *scale bar* represents the number of substitutions per site. *At*, *Achaearanea tepidariorum* (*AtWnt7a*—AB167809, *AtWnt7b*—AB167811); *Bb*, *Branchiostoma belcheri* (AF206499); *Bf*, *Branchiostoma floridae* (*BfWnt7a*—AF100739, *BfWnt7b*—AF061975); *Dm*, *Drosophila melanogaster* (NM_023653); *He*, *Heliocidaris erythrogramma* (AY532158); *Hs*, *Homo sapiens* (*HsWnt7a*—NM_004625, *HsWnt7b*—NM_058238); and *Nv*, *Nematostella vectensis* (*NvWnt5*—AY725202, *NvWnt7a*—AY687350, *NvWnt7b*—AY725204)

Wnt7 transcripts. In human and mouse, these are encoded by separate loci. It is possible that the ancestral bilaterian possessed two *Wnt7* loci and that one of these has been lost in the fruit fly lineage.

Each *Wnt7* transcript in *Nematostella* could be more closely related to a different *Wnt7* locus in bilaterians if the *Wnt7* duplication in Bilateria occurred by retroposition of a *Wnt7* splice variant present in the cnidarian–bilaterian ancestor. To test this possibility, we performed a phylogenetic analysis of *Wnt7* genes from *Nematostella* and various bilaterian taxa. Unlike a previously published *Wnt* phylogeny (Kusserow et al. 2005), we excluded from the alignment those amino acid positions aligned with the 533-nucleotide stretch that is shared by the *Wnt7* and *Wnt7b* transcripts of *Nematostella* (Fig. 4a). Our results suggest that the *Wnt7* locus underwent independent gene duplications in the ecdysozoa and the deuterostomia and that these duplicate genes bear no direct relationship to the alternate splice variants of *Nematostella* (Fig. 4b).

No other clusters of *Wnt* genes were observed. Nonetheless, the widely conserved clustering of *Wnt6* and *Wnt10* and the presence of a *Wnt5–Wnt7* cluster in *Nematostella* suggest that selection may be acting to maintain a close linkage. Neighboring *Wnt* genes could be under the influence of common *cis*-regulatory elements, as has been observed in other developmentally important genes such as the *Hox* family of transcription factors whose expression is

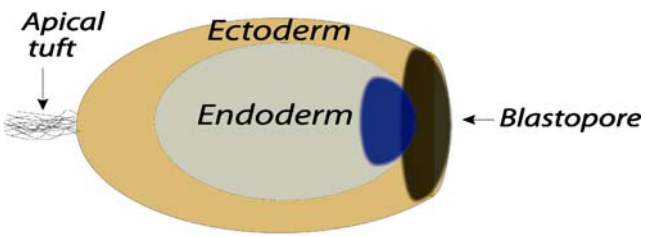

Fig. 5 *Wnt5* (*blue*) and *Wnt7*/*7b* (*black*) are expressed at the oral pole of developing *Nematostella* planulae in the endoderm and ectoderm, respectively (Kusserow et al. 2005)

collinear with their genomic organization (Kondo and Duboule 1999). In *Nematostella*, *Wnt* genes are expressed in distinct domains along the anterior–posterior axis of the developing larva, and they are presumably critical for patterning this axis (Kusserow et al. 2005). Similarities in expression suggest possible common regulation for *Wnt5* and *Wnt7* but not for *Wnt6* and *Wnt10*. *Wnt6* is expressed in body wall endoderm in the center of the body column, whereas *Wnt10* is expressed in scattered pharyngeal cells. These expression territories should not be regarded as adjacent—the pharynx is an inversion of the body wall at the oral end of the animal and as such should be regarded as the animal's oral extremity (Stephenson 1926). *Wnt5* and *Wnt7/7b* are expressed in adjacent axial locations, at the junction of the mouth and the pharynx. However, *Wnt5* is expressed in the endoderm, whereas *Wnt7/7b* is expressed in the ectoderm (Kusserow et al. 2005; Fig. 5). It is conceivable that one or more shared *cis*-regulatory elements impact the axial expression of both genes, but support for collinear germ-specific gene clusters is not provided.

Acknowledgements This research was supported by NSF grant IBN-0212773 (JRF) and by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health (JCM and JFR). We would like to acknowledge the helpful comments provided by two anonymous reviewers.

References

Altschul SF, Gish W, Miller W, Myers E, Lipman D (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Cadigan K, Nusse R (1997) Wnt signaling: a common theme in animal development. Genes Dev 11:3286–3305

Felsenstein J (1989) PHYLIP—Phylogeny Inference Package. Cladistics 5:164–166

Garcia-Fernàndez J (2005) The genesis and evolution of homeobox gene clusters. Nat Rev Genet 6:881–892

International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature 409: 860–921

Kondo T, Duboule D (1999) Breaking colinearity in the mouse HoxD complex. Cell 97:407–417

Kusserow A, Pang K, Sturm C, Hrouda M, Lentfer J, Schmidt HA, Technau U, von Haeseler A, Hobmayer B, Martindale MQ, Holstein T (2005) Unexpected complexity of the *Wnt* gene family in a sea anemone. Nature 433:156–160

Lee PN, Pang K, Matus DQ, Martindale MQ (2006) A Wnt of things to come: Wnt signaling in basal metazoans. Semin Dev Biol 17:157–167

Miller DJ, Ball EE, Ulrich T (2005) Cnidarians and ancestral genetic complexity in the animal kingdom. Trends Genet 21:536–539

Mullikin JC, Ning Z (2003) The phusion assembler. Genome Res 13:81–90

Nusse R (2001) An ancient cluster of Wnt paralogues. Trends Genet 17:443

Ryan JF, Burton PM, Mazza ME, Kwong GK, Mullikin JC, Finnerty JR (2006) The cnidarian–bilaterian ancestor possessed at least 56 homeoboxes: evidence from the starlet sea anemone, *Nematostella vectensis*. Genome Biology 7:R64

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Spitz F, Gonzalez F, Duboule D (2003) A global control region defines a chromosomal regulatory landscape containing the HoxD cluster. Cell 113:405–417

Stephenson TA (1926) The British sea anemones. The Ray Society, London

Sullivan JC, Ryan JF, Watson JA, Webb J, Mullikin JC, Rokshar D, Finnerty JR (2006) StellaBase: the *Nematostella vectensis* genomics database. Nucleic Acids Res 34:D495–D499

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882