Gene Expression Patterns 14 (2014) 55–61

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Identification and embryonic expression of *Wnt2*, *Wnt4*, *Wnt5* and *Wnt9* in the millipede *Glomeris marginata* (Myriapoda: Diplopoda)

Ralf Janssen [a,*], Nico Posnien [b]

[a] Uppsala University, Department of Earth Sciences, Palaeobiology, Villavägen 16, 75236 Uppsala, Sweden
[b] Georg-August-University Göttingen, Department of Developmental Biology, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 26 December 2013
Accepted 29 December 2013
Available online 4 January 2014

Keywords:
Segmentation
Limb development
Tergite formation
Evolution
Arthropod development

ABSTRACT

The Wnt genes encode secreted glycoprotein ligands that are key players during animal development. Previous studies revealed the presence of 12 classes of Wnt genes in protostomes, although lineage specific losses of Wnt genes are common. So far, the gene expression profile of only two complete sets of arthropod Wnt genes has been studied; these are the Wnt genes of the fly *Drosophila melanogaster* and the beetle *Tribolium castaneum*. Insects, however, do not represent good models for the understanding of Wnt gene evolution because several Wnt genes have been lost in the lineage leading to the insects, or within the different orders of insects. Comparative gene expression data from non-insect arthropods are rare and restricted to a subset of Wnt genes.

This study aims to fill this gap and describes four newly detected Wnt genes from the millipede *Glomeris marginata* (Myriapoda: Diplopoda). Together with previous studies, now 11 *Glomeris* Wnt genes have been isolated and their expression has been studied. The only predicted but hitherto undetected Wnt gene is *Wnt10*. The new data provide a platform for the comparison of Wnt gene expression patterns in arthropods and reveal conserved as well as diverged aspects of Wnt gene expression in Arthropoda. Prominent expression of *Wnt4* in dorsal tissue implies a role in dorsal segmentation and suggests that *Wnt4* may be the predicted substitute for the previously reported missing expression of *wg/Wnt1* in dorsal tissue.

The Wnt genes represent key regulator molecules in metazoan development. They encode secreted glycoprotein ligands that bind to a number of different transmembrane receptors, and thereby activate various intracellular signaling cascades that regulate target gene transcription (Logan and Nusse, 2004; Murat et al., 2010 Perrimon et al., 2012; Swarup and Verheyen, 2012). The last common ancestor of the arthropods most likely possessed a set of 12 Wnt genes (Janssen et al., 2010a). At present, most knowledge about the expression and function of Wnt genes comes from studies in the fruit fly *Drosophila melanogaster*, which still is the main arthropod model organism. In *D. melanogaster*, however, as in many insects, the set of Wnt genes is reduced (summarized in Janssen et al., 2010a). Even in the conservatively developing beetle *Tribolium castaneum*, only nine Wnt genes are present (Bolognesi et al., 2008a). Data sets on non-insect arthropods are either incomplete or gene expression analysis has not been performed yet (summarized in Janssen et al., 2010a). To fully understand the importance of Wnt gene function during development and evolution it is imperative to investigate the expression patterns of all Wnt genes in various arthropod species representing all four (or five depending on the phylogenetic position of pycnogonids) main classes of arthropods: insects, crustaceans, myriapods and chelicerates. Today, however, exhaustive knowledge on Wnt gene expression is still restricted to insects (Bolognesi et al., 2008a, reviewed in Murat et al., 2010).

This study aims to complete earlier work on the myriapod *Glomeris marginata* to provide comprehensive insight into the Wnt gene expression landscape of a non-insect arthropod. In earlier studies the expression of *wg/Wnt1*, *Wnt6*, *Wnt7*, *Wnt8*, *Wnt11*, *Wnt16* and *WntA* has been studied. Based on the distribution of Wnt genes along the phylogenetic tree of arthropods the presence of further Wnt genes, such as *Wnt2*, *Wnt4*, *Wnt5*, *Wnt9* and *Wnt10* was predicted (Janssen et al., 2010a).

This paper reports on the presence of all 'missing' *G. marginata* Wnt genes (except *Wnt10*) and describes their embryonic expression. The myriapod Wnt gene expression profiles are discussed in the light of possible redundant/combinatorial function during segmentation, brain regionalization and limb patterning. Beyond that, the results are compared with Wnt gene expression data from other arthropods in order to reconstruct the ancestral Wnt patterning landscape of Arthropoda. It is found that one of the Wnt genes, *Wnt4*, appears to be a good candidate to substitute for the classic segment polarity gene *wg/Wnt1* in dorsal tissue. This

* Corresponding author. Tel.: +46 18 471 2763.
E-mail address: ralf.janssen@geo.uu.se (R. Janssen).

1567-133X/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gep.2013.12.003

is an important finding since the existence of such a 'factor X', a *wg/Wnt1* substitute, has been predicted, but not as yet found (Janssen et al., 2004, 2008; Fusco, 2009).

1. Results

1.1. G. marginata Wnt genes

The embryonic transcriptome contains 11 Wnt genes. Seven of them, i.e. *wg/Wnt1*, *Wnt6*, *Wnt7*, *Wnt8*, *Wnt11*, *Wnt16* and *WntA* have previously been described and isolated by means of RT-PCR with degenerate primers (Janssen et al., 2004, 2010a). At least four Wnt genes, however, remained undetected in these PCR screens, but have been detected in a sequenced embryonic transcriptome (see Experimental Procedures Section). These new Wnt genes are *Wnt2*, *Wnt4*, *Wnt5* and *Wnt9*. In the conducted phylogenetic analysis these genes cluster with high reliability with their orthologs from other arthropods and an annelid (Fig. 1). The other previously studied Wnt genes also cluster with their orthologs supporting earlier phylogenetic analysis (Janssen et al., 2010a). Altogether, there is little doubt about Wnt gene orthology, and the newly isolated gene fragments where thus designated as *Glomeris Wnt2* (*Gm-Wnt2*), *Wnt4* (*Gm-Wnt4*), *Wnt5* (*Gm-Wnt5*) and *Wnt9* (*Gm-Wnt9*).

1.2. Expression patterns of G. marginata Wnt2, Wnt4, Wnt5 and Wnt9

1.2.1. Expression of G. marginata Wnt2

Wnt2 is exclusively expressed in two domains in the developing brain throughout all investigated embryonic stages (Fig. 2A and B). Expression starts at stage 1.1 as faint patches in the ocular fields (not shown). This expression persists until at least stage 6.1 (Fig. 2B).

1.2.2. Expression of G. marginata Wnt4

From the early blastoderm stage onwards *Wnt4* is strongly expressed the complete limb-less premandibular segment (Fig. 2C); this is reminiscent of the early expression of the head gap gene ortholog *collier* (*col*) in the same segment (Janssen et al., 2011a,b). Later, *Wnt4* expression covers most of the segment in the form of two ventral patches (Fig. 2D–F). These patches likely represent expression in the ventral nervous system. At later stages similar expression appears in an anterior to posterior order in the trunk segments (Fig. 2E and F). *Wnt4* is expressed prominently in transverse stripes in the middle of the dorsal segmental units (Fig. 2E–G). One single stripe of *Wnt4* is located in the dorsal portion of each embryonic diplosegment (see Janssen, 2011) in the posterior trunk. This pattern is reminiscent of that of the segment polarity genes *engrailed* (*en*) and *hedgehog* (*hh*) (Janssen et al., 2004, 2008). *Wnt4* is also expressed in the inside of the anal valves and the complete hindgut (Fig. 2C, D and H–J). Dichromatic double-hybridization experiments with *Wnt4* and either *en* or *hh* failed, but monochromatic double-hybridization experiments with *Wnt4* and *en* revealed co-expression of these two genes in the dorsal segmental units (Fig. 2K; the combined expression domain is of the same width as the single ones). At later stages, it is obvious that *en*, *hh* and *Wnt4* are all restricted to the dorsal grooves that demarcate the tergite boundaries (Fig. 2G) (cf. Janssen et al., 2004, 2006). *Wnt4* is weakly expressed in the distal part of the antennae, strongly in a ring close to the distal end of the antennae, and ventral to the basis of the antennae (Fig. 4A).

1.2.3. Expression of G. marginata Wnt5

Wnt5 is expressed in the posterior segment addition zone (SAZ) and in transverse stripes in nascent segments (Fig. 3A and B). This expression is restricted to the ventral segmental units, while the expression in the SAZ extends into dorsal tissue (Fig. 3B). The ventral segmental pattern transforms into a refined pattern in older (i.e. more anterior) segments (Fig. 3B and C). *Wnt5* is also expressed in all appendages, except the antennae (Fig. 4B–E). The labrum expresses *Wnt5* as two dorsal patches (Figs. 3C and 4B). The complete mandibles express *Wnt5*, but in the maxillae the expression is mainly restricted to a sub-anterior region (Fig. 4C and D). From there, two thin offshoots of expression run to the distal tip of each maxilla leaving three distal *Wnt5*-negative regions in each tip. Expression in the walking limbs is restricted to the dorsal side (Fig. 4E). Two patches of weak expression are located in the brain lobes, possibly associated with the developing eyes (Fig. 3B–D).

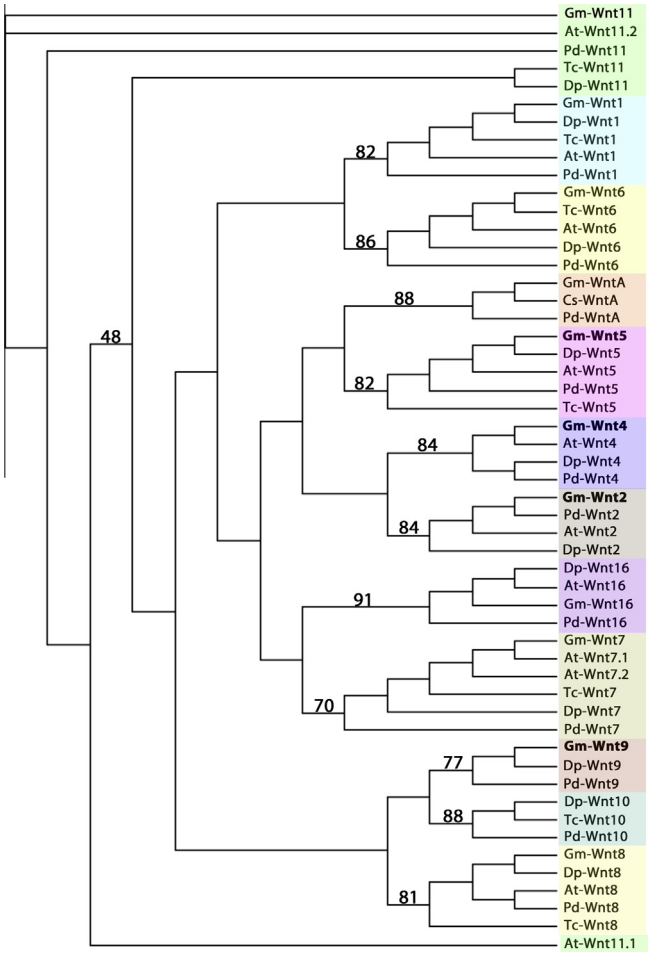

Fig. 1. Phylogenetic analysis of *Glomeris* Wnt genes. Unrooted majority rule consensus tree calculated from 1000 intermediate trees produced with the Quartet Puzzling method. Numbers represent reliability values. Newly discovered *Glomeris* Wnt genes are in bold. Subfamilies are color-marked. At, *Achaearanea tepidariorum* (aka *Parasteatoda tepidariorum*) (Chelicerata); Cs, *Cupiennius salei* (Chelicerata); Dp, *Daphnia pulex* (Crustacea); Gm, *Glomeris marginata* (Myriapoda); Pd, *Platynereis dumerilii* (Annelida); Tc, *Tribolium castaneum* (Insecta).

1.2.4. Expression of G. marginata Wnt9

Wnt9 is expressed in the tips of the limbs, including the labrum (Fig. 4J–M). Expression in the maxillae and antennae is stronger than expression in the walking limbs and the mandibles (Fig. 4J–M). In the antennae and the walking limbs the spot-like expression of *Wnt9* is not in the extreme distal end, but is slightly shifted ventrally (Fig. 4J and M). In contrast, expression in the labrum is shifted dorsally (Fig. 4J). Expression in all limb buds first appears shortly after they become morphologically visible, and in a strict