Developmental Biology 344 (2010) 363–376

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Evolution of Developmental Control Mechanisms

Evolutionary plasticity of *collier* function in head development of diverse arthropods

Nina D. Schaeper [a], Matthias Pechmann [a], Wim G.M. Damen [b], Nikola-Michael Prpic [a], Ernst A. Wimmer [a,*]

[a] *Georg-August-Universität, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Abteilung Entwicklungsbiologie, GZMB, Ernst-Caspari-Haus, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany*

[b] *Friedrich-Schiller-University, Department of Genetics, Philosophenweg 12, 07743 Jena, Germany*

ARTICLE INFO

Article history:
Received for publication 2 December 2009
Revised 29 April 2010
Accepted 3 May 2010
Available online 10 May 2010

Keywords:
Arthropod head evolution
Intercalary segment
Collier
Tribolium castaneum
Oncopeltus fasciatus

ABSTRACT

The insect intercalary segment represents a small and appendage-less head segment that is homologous to the second antennal segment of Crustacea and the pedipalpal segment in Chelicerata, which are generally referred to as "tritocerebral segment." In *Drosophila*, the gene *collier* (*col*) has an important role for the formation of the intercalary segment. Here we show that in the beetle *Tribolium castaneum col* is required for the activation of the segment polarity genes *hedgehog* (*hh*), *engrailed* (*en*) and *wingless* (*wg*) in the intercalary segment, and is a regulatory target of the intercalary segment specific Hox gene *labial* (*lab*). Loss of *Tc col* function leads to increased cell death in the intercalary segment. In the milkweed bug *Oncopeltus fasciatus*, the loss of *col* function has a more severe effect in lacking the intercalary segment and also affecting the adjacent mandibular and antennal segments. By contrast, *col* is not expressed early in the second antennal segment in the crustacean *Parhyale hawaiensis* or in the pedipalpal segment of the spider *Achaearanea tepidariorum*. This suggests that the early expression of *col* in a stripe and its role in tritocerebral segment development is insect-specific and might correlate with the appendage-less morphology of the intercalary segment.

Introduction

The arthropod phylum comprises four large groups (classes), the chelicerates (e.g. spiders, scorpions, mites), myriapods (e.g. millipedes, centipedes), crustaceans (e.g. crabs, shrimps, lobsters), and insects (e.g. bugs, beetles, flies). Despite this huge diversity, the general morphology of the anterior body region ("head") is conserved in the arthropods and the head segments can be homologized using the staggered expression of the Hox genes (Damen et al., 1998; Telford and Thomas, 1998). The most anterior region is termed protocerebral region, because it contains the major portion of the brain (protocerebrum) that innervates a number of anterior sensory organs including the eyes. Behind the protocerebral region follow two segments that are named after the part of the central nervous system they contain: the deutocerebral segment and the tritocerebral segment. Although this general head architecture is the same in all arthropods, the specific morphology of these segments differs substantially between the four arthropod classes (see schematic drawings of selected arthropod embryos in Fig. 1, where the tritocerebral segment is denoted by red coloring). In the chelicerates the deutocerebral segment bears a pair of fangs, the chelicerae, and is therefore also called cheliceral segment, whereas the tritocerebral segment bears a pair of pedipalps (appendages of a mixed sensorial and gnathal function) and is thus called pedipalpal segment. In myriapods, crustaceans and insects the deutocerebral segment bears a pair of sensorial appendages, the antennae (in crustaceans often called antennules). The tritocerebral segment bears antennae in crustaceans as well, but in myriapods and insects this segment underwent a significant evolutionary change: the tritocerebral segment does not bear any appendages and is very much reduced in size. It is therefore difficult to discern in the adult head capsule and is evident only during embryogenesis. The tritocerebral segment in myriapods and insects (often termed intercalary segment) is thus an interesting case of evolutionary change of segment morphology. In addition, since both myriapods and insects are terrestrial arthropods, the evolution of the special morphology of the tritocerebral segment in these groups might correlate with terrestrialization, a key event in arthropod evolution.

Because of this special nature of the tritocerebral (intercalary) segment in myriapods and insects, this segment has received special attention in developmental studies aiming at understanding the development and evolution of this reduced state of a head segment (reviewed in Rogers and Kaufman (1997)). Studies in the fly *Drosophila melanogaster* have identified a small number of genes that are expressed more or less exclusively in the intercalary segment. One of these genes is the Hox gene *labial* (*lab*). This gene is expressed throughout the intercalary segment but is required for the development of a larger portion of the head exceeding its domain of expression, because the loss of *lab* function leads to the loss of the intercalary segment as well as adjacent head segments (Merrill et al., 1989). The expression pattern of *lab* in the tritocerebral segment is conserved throughout the arthropods (see review by Hughes and Kaufman (2002)), but only few functional

---

* Corresponding author. Fax: +49 551 39 5416.
E-mail address: ewimmer@gwdg.de (E.A. Wimmer).

0012-1606/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2010.05.001

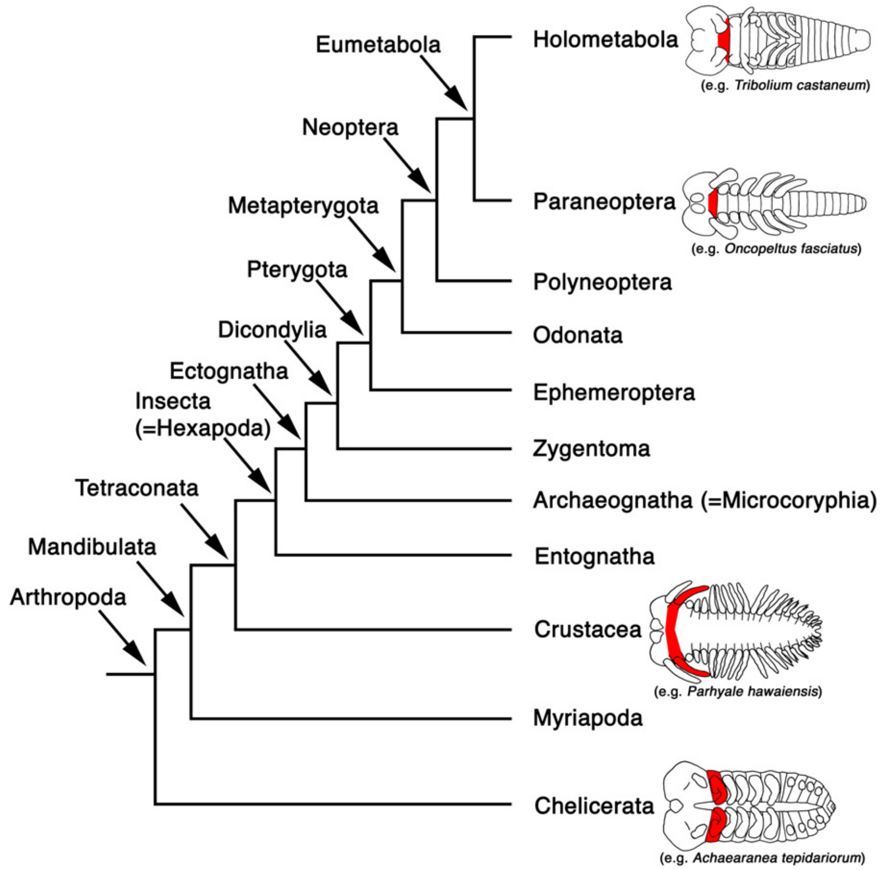

Fig. 1. Overview of the position of the species used in this study on the arthropod phylogenetic tree. Shown is the phylogeny based on morphological characters based on the analyses of Hennig (1981) and Klausnitzer (2007). For a current molecular view, please see the recent study by Regier et al. (2010). Please note that we prefer the name Insecta for the clade comprising the entognathous and ectognathous insects, because this is the oldest name given to this group and has already been used by Linnaeus (1758). Please also note that the Entognatha comprise the following recent orders: Collembola, Protura and Diplura, the Polyneoptera comprise the following recent orders: Plecoptera, Notoptera (=Grylloblattodea), Mantophasmatodea, Embioptera, Dermaptera, Mantodea, Blattoptera (=Blattariae), Isoptera, Caelifera, Ensifera, and Phasmatodea (incl. Timematodea), the Paraneoptera comprise the following recent orders: Zoraptera, Psocoptera (=Psocodea, Corrodentia, Copeognatha), Phthiraptera, Thysanoptera, Auchenorrhyncha, Sternorrhyncha, Heteroptera, Coleorrhyncha, and the Holometabola comprise the following recent orders: Strepsiptera, Raphidioptera, Megaloptera, Neuroptera (=Planipennia), Coleoptera, Hymenoptera, Trichoptera, Lepidoptera, Mecoptera, Siphonaptera, and Diptera. The species used in this study are represented by drawings of embryos to the right of the tree. The beetle *Tribolium castaneum* (Coleoptera) and the fly *D. melanogaster* (Diptera) both belong to the holometabolous insects (Holometabola) that are characterized by the existence of a dormant stage, the pupa, between the last larval stage and the adult. The milkweed bug *O. fasciatus* belongs to the Heteroptera (true bugs). Like several other hemimetabolous insect orders, this insect order differs from the Holometabola by not having a pupal stage in its developmental cycle. The amphipod shrimp *P. hawaiensis* belongs to the Crustacea. The spider *A. tepidariorum* finally represents the Chelicerata, the most basal branch in the arthropod phylogenetic tree. The four representative species studied in this work have been chosen to represent at least some of the most fundamental branches of the tree. In addition, they have been chosen because they are amenable to molecular techniques e.g. whole-mount in situ hybridization or RNA interference. Most importantly, the selected species represent the different morphologies of the tritocerebral segment. The tritocerebral segment is denoted in red in the drawings of the embryos of the four species. In Chelicerata and Crustacea the tritocerebral segment is a proper segment with a pair of appendages, whereas in Insecta the tritocerebral segment is reduced and does not have any appendages (intercalary segment).

tests have been performed. Recently, the function of *lab* has been studied in other insects, the beetle *Tribolium castaneum* and the milkweed bug *Oncopeltus fasciatus* (Angelini et al., 2005; Posnien and Bucher, 2010). In *O. fasciatus* the RNA interference (RNAi) with *lab* function did not result in any visible phenotype. However, the results from *T. castaneum* showed a loss of lateral head structures and thus indicate a conserved role of *lab* in head development although unlike in *Drosophila* the role of *lab* appeared to be restricted to its domain of expression.

Another set of genes has been identified in *Drosophila* that is expressed in the head in a staggered and overlapping manner, similar to the Hox genes in the trunk. These genes, *orthodenticle* (*otd*), *empty spiracles* (*ems*) and *buttonhead* (*btd*), are required for the development of the head region in which they are expressed and the region is malformed or lost if the gene is mutated (e.g. Cohen and Jürgens, 1990). Thus, the spatially restricted expression patterns correlating with loss-of-segments phenotypes resemble the gap genes in the trunk, and *otd*, *ems* and *btd* are therefore often referred to as head gap genes. Two of these genes, *ems* and *btd*, are also expressed in the *D. melanogaster* intercalary segment (e.g. Dalton et al., 1989; Wimmer et al., 1993), but despite their importance for the development of this segment in *D. melanogaster*, this function does not seem to be evolutionarily conserved, not even in other insect species. Recent work in *T. castaneum* demonstrated a role for *ems* in the head but only in the antennal segment and thus different than in *D. melanogaster*, whereas for *btd* no visible phenotype could be observed (Schinko et al., 2008).

Another gene that is also expressed in the intercalary segment in *D. melanogaster* is *collier* (*col*) (also known as *knot* (*kn*)). This gene is required for the development of the nervous system (e. g. Crozatier et al., 1996; Baumgardt et al., 2007; Crozatier and Vincent, 2008), wing (Nestoras et al., 1997; Vervoort et al., 1999; Mohler et al., 2000; Crozatier et al., 2002; Hersh and Carroll, 2005), and somatic muscles (Crozatier and Vincent, 1999; Dubois et al., 2007). But its most relevant function in the context of the present work is its role in head development. The *col* gene is expressed in parasegment 0 and is thus spanning the boundary between the intercalary segment and the posteriorly adjacent mandibular segment. Detailed studies of the role of *col* in *D. melanogaster* have shown that it controls the activation and pattern formation of the