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Germ band differentiation in the stomatopod *Gonodactylaceus falcatus* and the origin of the stereotyped cell division pattern in Malacostraca (Crustacea)

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ABSTRACT

We analysed aspects of the embryonic development of the stomatopod crustacean *Gonodactylaceus falcatus* focusing on the cell division in the ectoderm of the germ band. As in many other malacostracan crustaceans, the growth zone in the caudal papilla is formed by 19 ectoteloblasts and 8 mesoteloblasts arranged in rings. These teloblasts give rise to the cellular material of the largest part of the post-naupliar germ band in a stereotyped cell division pattern. The regularly arranged cells of the genealogical units produced by the ectoteloblast divide twice in longitudinal direction. The intersegmental furrows form within the descendants of one genealogical unit in the ectoderm. Hence, embryos of *G. falcatus* share some features of the stereotyped cell division pattern with that in other malacostracan crustaceans, which is unique among arthropods. In contrast to the other malacostracan taxa studied so far, stomatopods show slightly oblique spindle direction and a tilted position of the cells within the genealogical units. The inclusion of data on Leptostraca suggests that aspects of stereotyped cell divisions in the germ band must be assumed for the ground pattern of Malacostraca. Moreover, Stomatopoda and Leptostraca share the lateral displacement of cells during the mediolateral divisions of the ectodermal genealogical units in the post-naupliar germ band. The Caridoida within the Eumalacostraca apomorphically evolved the strict longitudinal orientation of spindle axes and cell positions, reaching the highest degree of regularity in the Peracarida. The phylogenetic analysis of the distribution of developmental characters is the prerequisite for the analysis of the evolution of developmental patterns and mechanisms.

1. Introduction

Segmentation is one of the main characteristics of Arthropoda. However, the way segments form varies to a high degree among arthropods and at different levels such as gene expression, cell division, and morphogenesis (e.g. Scholtz, 1997; Akam, 2000; Chipman, 2008). A very special case of segment formation occurs in malacostracan Crustacea. All malacostracan species for which the cellular level of segmentation has been studied exhibit a stereotyped cell division pattern in the ectoderm and mesoderm during formation and differentiation of segments in the post-naupliar part (the region posterior to the mandibular segment) of the ventral germ band. This phenomenon allows the identification of individual cells and the analysis of their fates (e.g. Dohle et al., 2004; Price and Patel, 2008; Ungerer and Scholtz 2008; Wolff and Scholtz 2008). This stereotyped cell division pattern of malacostracans is apparently unique amongst arthropods since a corresponding pattern has been found neither in non-malacostracan crustaceans nor in chelicerates, myriapods, or hexapods (Lawrence, 1992; Gerberding, 1997; Dohle et al., 2004; Koenemann et al., 2009).

The first detection of the regular arrangement and division of cells in the germ band dates back to the late 19th century. Pioneer studies like those of Reichenbach (1886), Bergh (1893, 1894), and McMurrich (1895) were the first to reveal various aspects of the differentiation of teloblasts (i.e. large cells which divide asymmetrically and produce progeny towards the anterior (Siewing, 1969)), in the growth zone, and the formation of regular rows in the ventral ectoderm and mesoderm in Decapoda, Mysidacea, Isopoda, and Amphipoda. Subsequent investigations by Manton (1928, 1934), Weygoldt (1958, 1961), and Scholl (1963) among others largely confirmed the previous results and included further species. In the 1970s, Dohle was the first who successfully reconstructed this cell division pattern in much more detail in representatives of Cumacea and Tanaidacea (Dohle, 1970, 1972, 1976). He provided a detailed analysis of the cell lineage starting with the formation of the ectoteloblasts up to the differentiation of ectodermal cells into ganglion

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anlagen, limb buds, and other segmental structures. Since then, an increasing number of malacostracans has been analysed (Mysidacea: Scholtz, 1984; Isopoda: Dohle and Scholtz, 1988; Hejnol et al., 2006; Decapoda: Scholtz, 1992; Alwes and Scholtz, 2006; Amphipoda: Scholtz, 1990; Gerberding and Scholtz, 1999; Browne et al., 2005) allowing for detailed comparisons. However, to elucidate the origin and evolutionary changes of the cell division pattern of the post-naupliar germ band of malacostracans additional taxa have to be studied. These taxa have to be chosen based on their position in malacostracan phylogeny. Unfortunately, however, malacostracan phylogenetic relationships and evolution are controversial (see Schram and Hof, 1998; Watling, 1999; Scholtz, 2000; Richter and Scholtz, 2001; Spears et al., 2005; Jenner et al., 2009; von Reumont et al., 2009; Wirkner and Richter, 2009). Nevertheless, most authors agree that Malacostraca comprise Leptostraca and Eumalacostraca as sister groups. Siewing (1956) and Richter and Scholtz (2001) considered the Stomatopoda as the sister group of the remaining eumalacostracans, the Caridoida (Richter and Scholtz, 2001). This shows that, in particular, data on Stomatopoda and Leptostraca are crucial to address the question of malacostracan cell division pattern evolution. The presence of ectoteloblasts and mesoteloblasts is well established for Stomatopoda (e.g. Shiino, 1942; Nair, 1942) and Leptostraca (Manton, 1934). 19 ectoteloblasts and 8 mesoteloblasts arranged in rings around the caudal papilla are apomorphies of the Malacostraca (Scholtz, 2000; Richter and Scholtz, 2001; Dohle et al., 2004). However, the further fate of the progeny of these teloblasts is virtually unknown in the more basal groups.

This study aims to clarify these issues and to shed light on the question of whether a comparable stereotyped cell division pattern is part of the ground pattern of Eumalacostraca or even of the ground pattern of Malacostraca. In our analysis we follow Dohle et al. (2004) and consider the specific characteristics of mitoses like spindle orientation, temporal progression, size of the sister cells and the spatial organisation of the division products to evaluate the data.

2. Material and methods

Specimens of *Gonodactylaceus falcatus* (Forskål, 1775) were collected by G. Scholtz on Coconut Island (Hawaii, Oahu) in August 2003. Concretions of dead corals were collected by snorkelling in the lagoon around the island. The coral rocks were carefully destroyed with a hammer to get the specimens of *G. falcatus*, which inhabit the crevices. Three female adults with egg clusters were placed into a tank supplied with fresh seawater. Eggs were preserved daily or every second day. One female laid eggs during the stay in the seawater tank. The eggs were first preserved in 3.7% formaldehyde for 10 to 20 min and broached with a needle. Embryos were transferred to 100% methanol, washed three times, and kept in methanol at −20 °C. Some embryos were already dissected during preservation. The envelope of the egg and the yolk were removed in PBS (0.18 mmol/l NaH2PO4, 17.5 mmol/l NaCl, pH 7.4) before staining.

Embryos were washed 3 × 10 min in PBS, incubated with Hoechst (H33342, Invitrogen Molecular Probes®, Darmstadt, Germany) 0.1 μg ml^{-1} in PBS, and washed 6 × 10 min and 6 to 8 × 30 min in PBS. Embryos that were not stained successfully by Hoechst, were stained a second time with Sytox^{green} (Nucleic Acid Stain CS-7020, Molecular Probes, Darmstadt, Germany). They were first washed in TBS (0.65 mol/l NaCl, 1 mmol/l Tris, pH 7.5) 2 × 5 min, 4 × 30 min and 1 × 60 min, overnight and 1 × 5 min the next day, incubated with Sytox^{green} for 3 h and washed 2 × 10 min and 4 × 30 min in in TBS.

Engrailed staining: the embryos were first washed with 3 × 5 min PBS followed by 4 × 30 min in PBT (0.5% BSA Bovine Serum Albumin, (Sigma, St. Louis, MO, USA), 0.3% Triton, 1.5% DMSO in PBS), and 2 × 30 min in PBT + N (PBT+ 5% Normal goat serum, Dako Cytomation, Hamburg, Germany). Primary antibody staining (monoclonal antibody Mab 4D9, 46.8 μl/ml, produced by the Harald Saumweber group, HU-Berlin, 1:1 in PBT + N) was performed overnight at 4 °C. Embryos were washed 3 × 5 min and 4 × 30 min in PBT, 2 × 30 min in PBT + N and incubated overnight in the secondary antibody (Cy™ 3 conj. AffiniPure, goat anti-mouse IgG 115-165-003, Dianova, Hamburg, Germany, 1:200 in PBT + N). Afterwards they were washed again 3 × 5 min and 4 × 30 min in PBT and stained with Hoechst as described above.

Embryos were mounted in DABCO-Glycerol (2.5% DABCO (1.4-Diazabicyclo-[2.2.2.]-octane, Merck, Darmstadt, Germany), 70% Glycerol in PBS) and scanned with a confocal laser scanning microscope (Leica, True Confocal Scanner SP2, Wetzlar, Germany,) using a UV-Laser (405 nm) for Hoechst, using He/Ne-Laser (543 nm) for Cy3 and an Argon-Laser (488 nm) for Sytox^{green}, with 0.4–0.5 μm focal planes. The data were analysed with the Imaris Software (Bitplane AG, Zurich, Switzerland), using the Slice-mode, Section-mode, Easy-3D-mode, and the Surpass-mode.

The caudal papillae of 35 individuals were analysed on the ventral side. Out of these, 30 individuals were also analysed on the dorsal side.

The nomenclature follows Dohle (1970, 1976):

ET: ectoteloblasts, median ET_0, beginning from there ET_1, ET_2 and so on in both directions
e-rows: rows of cells produced by the ETs, also called abcd-rows. They are chronologically numbered eI, eII, eIII and so on.

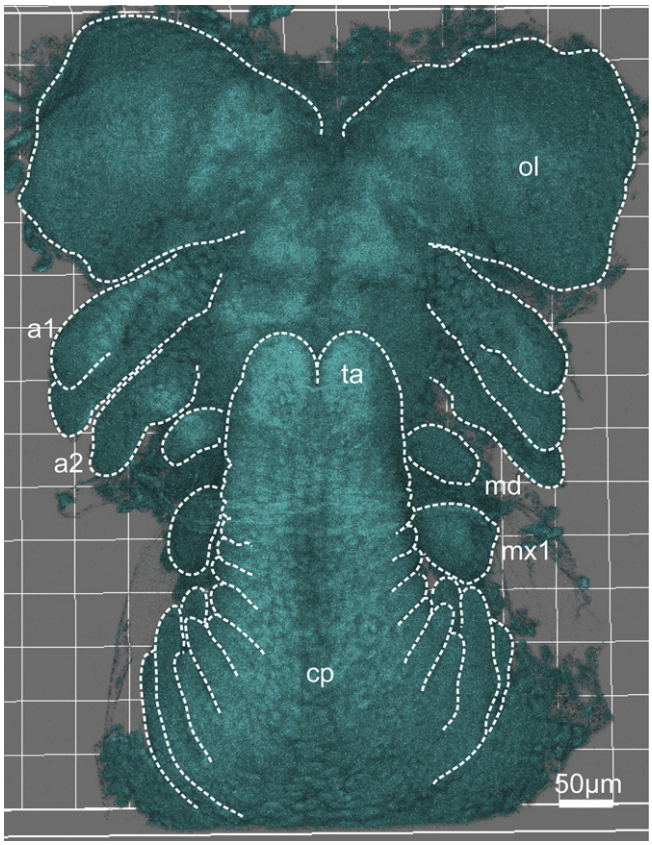

Fig. 1. Morphology of an advanced embryo of *Gonodactylaceus falcatus*: Z-projection (blend mode) of an embryo stained with Hoechst. The optical lobes (ol), the anlagen of the first antennae (a1), second antennae (a2), mandible (md) and the first maxilla (mx1) are clearly visible. The caudal papilla (cp) is folded naturally in a jack-knife manner, so that the telson anlage (ta) points towards the anterior.