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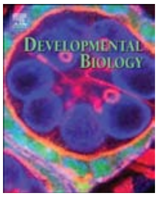

Evolution of Developmental Control Mechanisms

An anterior medial cell population with an apical-organ-like transcriptional profile that pioneers the central nervous system in the centipede *Strigamia maritima*

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ABSTRACT

The apical plate of primary marine larvae is characterized by a common set of transcription factors comprising *six3*, *rx*, *hbn*, *nk2.1* and *FoxQ2*. It harbours the apical organ, a neural and ciliary structure with neurosecretory properties. Recent studies in lophotrochozoans have found that apical organ cells form the anterior tip of the developing central nervous system.

We identify an anterior medial tissue in the embryonic centipede head that shares the transcriptional profile of the apical plate of marine larvae, including nested domains of *FoxQ2* and *six3* expression. This domain gives rise to an anterior medial population of neural precursors distinct from those arising within the segmental neuroectoderm. These medial cells do not express *achaete scute homologue* in proneural clusters, but express *collier*, a marker for post mitotic cells committed to a neural fate, while they are still situated in the surface ectodermal layer. They then sink under the surface to form a compact cell cluster. Once internalized these cells extend axons that pioneer the primary axonal scaffold of the central nervous system. The same cells express *phc2*, a neural specific prohormone convertase, which suggests that they form an early active neurosecretory centre. Some also express markers of hypothalamic neurons, including *otp*, *vtn and vax1*.

These medial neurosecretory cells of the centipede are distinct from those of the *pars intercerebralis*, the anterior neurosecretory part of the insect brain. The *pars intercerebralis* derives from *vsx* positive placodal-like invagination sites. In the centipede, *vsx* expressing invaginating ectoderm is situated bilaterally adjacent to the medial pioneer cell population. Hence the *pars intercerebralis* is present in both insect and centipede brains, whereas no prominent anterior medial cluster of pioneer neurons is present in insects. These observations suggest that the arthropod brain retained ancestrally an anterior medial population of neurosecretory cells homologous to those of the apical plate in other invertebrate phyla, but that this cell population has been lost or greatly reduced in insects.

Introduction

There is a long history of debate as to whether the arthropod head retains structures homologous to the anterior, unsegmented tissue of annelids and other invertebrates. Morphological studies have in recent years tended to reject this idea, suggesting that the entire arthropod head is segmentally derived (Budd, 2002; Haas et al., 2001) (Note here we use the term arthropod to include hexapods, myriapods, crustaceans and chelicerates, but exclude onychophorans). New phylogenies have made any close correspondence between arthropod and annelid head organisation seem less likely (Aguinaldo et al., 1997; Dunn et al., 2008). Against this however, the conservation of transcription factor expression in the anterior regions of the most diverse animals has recently lead to the proposal that aspects of anterior patterning are conserved across, and even beyond, the bilateria (Lowe et al., 2003; Posnien et al., 2011; Sinigaglia et al., 2013; Steinmetz et al., 2010).

The morphology of the head in adult arthropods, as in other animals, shows complex adaptions to behaviour and life style. If we are to find remnants of any ancestral organisation that underlies this diversity, and is shared between widely disparate groups, it seems likely that this will be most evident during early embryogenesis, and reflected in the molecular specification of the first distinct territories and cell types to arise during head patterning. This approach has already led to a better understanding of evolutionary

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Q3 conserved regions in axial patterning (Lowe et al., 2003; Schilling and Knight, 2001; Steinmetz et al., 2010), and to the identification of evolutionarily related cell types in distant animal taxa (Arendt, 2008; Tessmar-Raible et al., 2007; Tomer et al., 2010). We have taken this approach to study the organisation of the head in a centipede, as representative of an ancient but hitherto poorly studied lineage of the arthropods.

Debate about the nature of the anterior body region of arthropods has a long history, focussing on the number of segments in the head (*e.g.* Rogers and Kaufmann, 1996), the homology of the different head segments between arthropod lineages (Damen et al., 1998; Haas et al., 2001; Scholtz and Edgecombe, 2006; Telford and Thomas, 1998) and the nature of the arthropod brain (Lichtneckert and Reichert, 2005; Urbach and Technau, 2003). Molecular markers for segment patterning, and in particular, the analysis of Hox gene expression domains (Damen et al., 1998; Hughes and Kaufman, 2002; Telford and Thomas, 1998), have largely resolved controversies about segment homologies in the post antennal region, but the structure of the most anterior part of the head and brain remains controversial.

The arthropod brain is classically divided into three units: the tritocerebrum most posteriorly, deriving from the intercalary segment in insects and myriapods, and from the homologous 2nd antennal segment of crustaceans; the deutocerebrum, deriving from the antennal segment of insects (1st antennal of crustaceans), and the protocerebrum, positioned most anteriorly. The protocerebrum comprises the ocular lobes, the mushroom bodies and the central complex, which includes the pars intercerebralis (Scholtz and Edgecombe, 2006; Strausfeld, 2012). The embryonic origin of the protocerebrum is from the pre-antennal head, but it has not been clear whether the embryonic pre-antennal region is one large territory of segmental origin (the ocular region) or might additionally comprise an anterior medial tissue. The presence of an anterior medial tissue, giving rise to parts of the central complex and the labrum, has been proposed on the basis of recent molecular work in the beetle *Tribolium castaneum* (Kittelmann et al., 2013; Posnien et al., 2011, 2009).

Recent support for the idea that the most anterior part of the head in arthropods and annelids may be homologous comes from studies of a homeobox transcription factor, *six3*, which is widely conserved across the animals. *Six3* is expressed in the apical plate of the annelid trochophore and the anterior medial head of several arthropods (Steinmetz et al., 2010), as well as in the anterior ectoderm of other bilaterian animals, and even in the larva of the cnidarian *Nematostella vectensis.* This suggests that a *six3* expressing anterior territory may have been inherited from the bilaterian ancestor (Sinigaglia et al., 2013; Steinmetz et al., 2011). If so, parts of the central nervous system that derive from the anterior medial head, and express *six3,* are likely to have a deep evolutionary origin.

Further studies on gene expression in free-swimming larvae of marine organisms have elucidated a conserved set of transcription factors characterising this most anterior (apical) region, comprising *six3*, *FoxQ2*, *nk2.1*, *rx* and *hbn* (Santagata et al., 2012; Sinigaglia et al., 2013; Steinmetz et al., 2010; Takacs et al., 2004; Tessmar-Raible et al., 2007; Wei et al., 2009; Yaguchi et al., 2008). Orthologues of *six3*, *rx* and *nk2.1* have also been shown to be involved in development of the vertebrate forebrain and hypothalamus (Lagutin et al., 2003; Lu et al., 2013; Muranishi et al., 2012; Ohuchi et al., 1999; Oliver et al., 1995; Tessmar-Raible et al., 2007), whereas *hbn* is missing from the vertebrate genomes (Mazza et al., 2010) and *FoxQ2* is not present in mammals (Shimeld et al., 2010) and has so far not been characterised in any vertebrate.

This apical territory of marine larvae harbours the apical organ, which is positioned centrally within nested domains of *FoxQ2* and *six3* (*e.g.* Santagata et al., 2012; Sinigaglia et al., 2013). Apical organs are larval sensory structures that include neurosecretory cells (Conzelmann et al., 2011; 2013). A recent study argues for homology of larval apical organs among animals that develop free-swimming marine larvae, including cnidarians (Marlow et al. 2014). In species that undergo a dramatic change of body plan during metamorphosis the apical organ is completely lost at the transition to the adult form (see for example Nielsen, 2005). By contrast, the polychaete annelid *Platynereis dumerilii* undergoes a gradual mode of metamorphosis and cells of the apical organ are partially maintained into late larval and adult stages. They produce pioneer neurons and are thought to form a nucleation centre for the developing nervous system of the animal (Marlow et al. 2014, Fischer et al. 2010).

Until now it has not been clear what parts of the arthropod brain derive from an anterior medial territory. One candidate is the *pars intercerebralis*, which has been shown to derive from the *six3*+ territory in the insects *Drosophila* and *Tribolium.* The *pars intercerebralis* constitutes the anterior neurosecretory part of the central complex in insects (Boyan and Reichert, 2011; De Velasco et al., 2007; Posnien et al., 2011) and so might plausibly be homologous to the anterior neurosecretory brain centres in the lophotrochozoans and the hypothalamus of vertebrates (Hartenstein, 2006; Tessmar-Raible, 2007).

Both the annelid apical organ and the insect *pars intercerebralis* are located within the *six3*+ territory at the anterior end of the the axonal scaffold. but the pars intercerebralis develops from bilateral ectodermal placodes at the lateral edges of the *six3*+ territory (De Velasco et al., 2007; Steinmetz et al., 2010). The central anterior medial head of insects mainly gives rise to the labrum, a non-neural and probably appendicular structure (Posnien et al., 2011, 2009) (though others have interpreted the labrum as appendicular but of segmental origin [*e.g.* Boyan et al. 2002]). So far it is not clear whether the *pars intercerebralis* bears any further developmental or transcriptional similarity with the anterior neurosecretory cells that are part of the apical organ and connect to the anterior axonal scaffold of the marine larvae (Conzelmann et al., 2013; Fischer et al., 2010; Santagata et al., 2012; Tessmar-Raible et al., 2007).

Studies of the anterior medial head in insects are complicated by the fact that the insect head undergoes major structural rearrangements during development. This process is carried to an extreme in *Drosophila* and other *Diptera,* where the whole anterior head undergoes the process of head involution (Turner and Mahowald, 1979). In *Tribolium*, which is currently the major model for the genetic control of head development in insects (Kittelmann et al., 2013; Posnien et al., 2011, 2010; Schinko et al., 2008), the anterior medial region is a small region of tissue which comes to lie between the ocular lobes in early development, and forms the labrum anlagen, the anterior-most portion of the medial head (Kittelmann et al., 2013; Posnien et al., 2011).

Little work has been done on head regionalization and molecular specification of anterior brain structures in non-insect arthropods. Here we study these processes in a myriapod, the centipede *Strigamia maritima.* Myriapods are now recognised as an early branch of the mandibulate arthropods, which emerged basal to the pancrustacean (*i.e.* crustacean and insect) radiation (Regier et al., 2010; Rota-Stabelli et al., 2011) (but see (Friedrich and Tautz, 1995; Mayer and Whitington, 2009; Pisani et al., 2004) for alternative views). *Strigamia* is the first myriapod for which a sequenced genome is available (〈http://www.ncbi.nlm.nih.gov/assembly/322118/〉). The gene content of *Strigamia* is conservative; the genome contains a number of factors that have been lost from insect genomes. We could for example identify a clear homologue of *vax1* (Chipman et al., in preparation), a gene involved in development of anterior neurosecretory organs in vertebrates (Bertuzzi and Hindges, 1999; Bharti et al., 2011; Wataya et al., 2008), which is not present in insects (Tessmar-Raible, 2007).

The head field of *Strigamia* condenses during early development on the egg surface (Brena and Akam, 2012), allowing gene

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