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Specification of neural precursor identity in the geophilomorph centipede *Strigamia maritima*

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Abstract

Despite differences in the formation of neural precursors, all arthropod species analyzed so far generate about 30 single precursors (insects/crustaceans) or precursor groups (chelicerates/myriapods) per hemi-segment. In *Drosophila*, each precursor has a distinct identity conferred by segment polarity and dorso-ventral patterning genes that subdivide the ventral neuroectoderm into a grid-like structure. Temporal patterning mechanisms generate additional diversity after delamination from the neuroectoderm. Previous work shows that the genetic network involved in recruitment and specification of neural precursors is conserved in arthropods. However, comparative studies on generation of precursor diversity are few and partial. Here, we test whether aspects of the *Drosophila* model may apply in the geophilomorph centipede *Strigamia maritima*. We describe precursor formation, based on morphology and on *Delta* and *Notch* expression. We then show that in *S. maritima*, *hunchback* and *Krüppel* are expressed in subsets of neural precursors generating distinct temporal expression domains within the plane of the neuroectoderm. This expression pattern suggests that temporal changes in spatial patterning cues may result in the ordered production of different neural identities. We suggest that temporal patterning mechanisms were present in the last common ancestor of arthropods, although the regulatory interactions of transcription factors might have diverged in the lineage leading to insects.

Keywords: Neurogenesis; Precursor identity; Arthropod; Myriapod; Temporal identity

Introduction

Generation of neural precursor diversity is a crucial early stage in the patterning of the nervous system. Comparing this process in different taxa can shed light on the evolution of nervous systems, and help identify the direction of evolutionary change within and between major taxonomic groups. There are at present two competing views as to the relationship between the major arthropod groups. A group uniting the chelicerates and myriapods (the Myriochelata) has recently been suggested based on molecular data (Friedrich and Tautz, 1995; Hwang et al., 2001; Kusche and Burmester, 2001; Nardi et al., 2003; Mallatt et al., 2004; Pisani et al., 2004). This grouping is supported by comparative analysis of neurogenesis in the chelicerate *Cupiennius salei* (a spider) and the myriapods *Lithobius forficatus* (a centipede) and *Glomeris marginata* (a millipede) (Stollewerk, 2002; Stollewerk et al., 2001; Dove and Stollewerk, 2003; Stollewerk et al., 2003; Kadner and Stollewerk, 2004). Several characters have been described that cannot be found in equivalent form in the remaining arthropod groups, insects, and crustaceans. (1) Groups of neural precursors invaginate from the ventral neuroectoderm of chelicerates and myriapods, while single neural precursors are specified in insects and crustaceans. (2) In contrast to insects and crustaceans, neural precursors do not divide in a stem cell-like mode in chelicerates and myriapods. (3) The central region of the ventral neuroectoderm in chelicerates and myriapods generates exclusively neural cells, while in insects and crustaceans both neural and epidermal cells arise from the ventral neurogenic region. It is possible that these characters are shared derived characters (synapomorphies) of myriapods and chelicerates, providing the first morphological support for a clade uniting these two groups. However, they could also represent ancestral characters (sympleisiomorphies) retained in myriapods and chelicerates and lost in the more derived insects

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and crustaceans. This would be consistent with the competing (and traditional) view of insects and crustaceans forming a single clade, the Tetraconata (or Pancrustacea), with myriapods as a sister group to that clade (the Mandibulata hypothesis). Analyses of neurogenesis in outgroups to the Euarthropoda, the onychophorans, and tardigrades at the moment fail to resolve this conflict: invaginating neural precursor groups have not been described in these outgroups (Eriksson et al., 2003) (personal communication, A. Hejnol). However, only two, possibly derived, species have been analyzed that may not represent the ancestral state.

We aim to broaden our understanding of nervous development in basal arthropod groups and reconstruct the ancestral state of neurogenesis. It can be assumed that characters that are conserved in all arthropod groups were present in the last common ancestor. In this paper, we analyze an additional myriapod, the geophilomorph centipede *Strigamia maritima*. Unlike other species of myriapods that have been used previously for the study of neural development (Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004), *S. maritima* forms all of its segments during embryogenesis, a mode of embryogenesis known as epimorphic development. In contrast, in previously studied species, only a small number of segments are formed during embryonic development, and additional segments are added in successive moults (anamorphic development). *S. maritima* has 47–53 leg-bearing segments. Segments are added sequentially from a posterior undifferentiated disk throughout the segmentation process. Therefore, there is an anterior–posterior gradient in the developmental stage of individual segments, with anterior segments being more advanced than posterior ones. Due to this temporal gradient, we can follow dynamic processes by looking at a single specimen and observing a sequence of developmental events as they occur in successive segments.

In the insect *Drosophila melanogaster*, the competence to adopt neural fate depends on the presence of the proneural genes *achaete*, *scute*, and *lethal of scute*. At the beginning of neurogenesis, these genes are expressed in clusters of cells in each hemi-segment. During specification of neuroblasts, proneural gene expression becomes restricted to a single cell of the cluster, the future neuroblast (Cabrera et al., 1987; Romani et al., 1987; Skeath et al., 1992). This process is called lateral inhibition and is mediated by the neurogenic genes *Notch* and *Delta* (Simpson, 1990; Martin-Bermudo et al., 1995; Heitzler et al., 1996; Seugnet et al., 1997). The remaining cells of the cluster become epidermal, so that in *D. melanogaster*, both neural and ectodermal cells arise from the ventral neuroectoderm (Jiménez and Campos-Ortega, 1979; Cabrera et al., 1987; Jiménez and Campos-Ortega, 1990). Although there are differences in the formation of neural precursors, all arthropod species analyzed generate about 20 to 30 single neural precursors (insects/crustaceans) or precursor groups (chelicerates/myriapods) per hemi-segment that are arranged in 7 rows (Bate, 1976; Scholtz, 1992; Broadus and Doe, 1995; Gerberding, 1997; Harzsch, 2001; Stollewerk, 2002; Stollewerk et al., 2001, 2003; Dove and Stollewerk, 2003; Harzsch, 2003; Kadner and Stollewerk, 2004; Withington, 2004; Wheeler and Skeath, 2005; Wheeler et al., 2003). Furthermore, the genetic network involved in recruitment and specification of neural precursors is conserved in all arthropods that have been analyzed (Hartenstein and Campos-Ortega, 1984; Cabrera, 1990; Cabrera et al., 1987; Jiménez and Campos-Ortega, 1990; Simpson, 1990; Martin-Bermudo et al., 1991, 1995; Goodman and Doe, 1993; Heitzler et al., 1996; Seugnet et al., 1997; Stollewerk, 2002; Stollewerk et al., 2001, 2003; Dove and Stollewerk, 2003; Wheeler and Skeath, 2005; Wheeler et al., 2003; Kadner and Stollewerk, 2004). These data suggest that the regular arrangement of neural precursors as well as the genetic interactions that lead to recruitment of neurectodermal cells for neural fate were present in the last common ancestor of the arthropods.

However, comparative studies of the events that generate neural precursor diversity, following the recruitment of neural precursors, during early development of the ventral nerve cord in the different arthropod groups are few and incomplete (Stollewerk and Simpson, 2005). It has been shown in the insect *D. melanogaster* that once the neural precursors are selected they divide in a unique and invariant pattern generating a stereotyped sequential series of ganglion mother cells (GMC) (Doe and Goodman, 1992). Each GMC divides once to give rise to two neurons. Neural precursor diversity in *Drosophila* is achieved by both spatial and temporal patterning mechanisms. During neurogenesis, segment polarity and dorso-ventral patterning genes subdivide the ventral neuroectoderm into a grid-like structure (reviewed by Skeath, 1999). Each proneural cluster thus expresses a unique set of genes giving rise to neuroblasts with spatial heterogeneity. After delamination from the ventral neuroectoderm, neuroblasts become independent of spatial patterning cues. Subsequently, temporal patterning mechanisms generate additional diversity among the cell-lineages of individual neuroblasts. Kambadur et al. (1998) and Isshiki et al. (2001) demonstrated that temporal identity in neuroblasts is regulated by sequential expression of Hunchback, Krüppel, Pdm, and Castor. The temporal expression profile is maintained in the progeny of the neuroblasts leading to expression of transcription factors in mutually exclusive cell layers in the ventral neuromeres. *Hunchback* is expressed in early-born neurons that are located in the deepest layer, while *Krüppel* is expressed at low levels in the *Hunchback* layer and in a distinct layer between *Hunchback* and *Pdm*. *Castor* transcripts accumulate in the late-born superficial layer neurons.

We have shown previously that the expression of the segment polarity gene *engrailed* in neural precursors is conserved in chelicerates and myriapods suggesting that spatial patterning mechanisms similar to those of *Drosophila* generate heterogeneity among the neural precursors in the ventral neuroectoderm (Stollewerk and Chipman, in press). However, it is obvious that spatial information from segment polarity and dorso-ventral patterning genes alone cannot account for the high complexity of cell types in both groups. Counting mechanisms of the sort used by *Drosophila* neuroblasts could not operate in chelicerates and myriapods, since stem cell-like neuroblasts seem to be absent in these groups. Furthermore, the neural precursors are mainly postmitotic after invagination.