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Decoupling brain from nerve cord development in the annelid *Capitella teleta*: Insights into the evolution of nervous systems

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ABSTRACT

In the deuterostomes and ecdysozoans that have been studied (e.g. chordates and insects), neural fate specification relies on signaling from surrounding cells. However, very little is known about mechanisms of neural specification in the third major bilaterian clade, spiralians. Using blastomere isolation in the annelid *Capitella teleta*, a spiralian, we studied to what extent extrinsic versus intrinsic signals are involved in early neural specification of the brain and ventral nerve cord. For the first time in any bilaterian, we found that brain neural ectoderm is autonomously specified. This occurs in the daughters of first-quartet micromeres, which also generate anterior neural ectoderm in other spiralians. In contrast, isolation of the animal cap, including the 2d micromere, which makes the trunk ectoderm and ventral nerve cord, blocked ventral nerve cord formation. When the animal cap was isolated with the 2D macromere, the resulting partial larvae had a ventral nerve cord. These data suggest that extrinsic signals from second-quartet macromeres or their daughters, which form mesoderm and endoderm, are required for nerve cord specification in *C. teleta* and that the 2D macromere or its daughters are sufficient to provide the inductive signal. We propose that autonomous specification of anterior neural ectoderm evolved in spiralians in order to enable them to quickly respond to environmental cues encountered by swimming larvae in the water column. In contrast, a variety of signaling pathways could have been co-opted to conditionally specify the nerve cord. This flexibility of nerve cord development may be linked to the large diversity of trunk nervous systems present in Spiralia.

1. Introduction

Where studied, neural development usually begins with a region of ectoderm receiving extrinsic signals instructing it to become neural (Stern, 2005). Our understanding of this process largely comes from data on two of three major bilaterian clades, deuterostomes (Alvarez et al., 1998; Bertrand et al., 2003; Lea et al., 2009; Muñoz-Sanjuán and Brivanlou, 2002; Pera et al., 2003; Rentzsch et al., 2004) and ecdysozoans (Gómez-Skarmeta et al., 2003; Skeath, 1998; von Ohlen and Doe, 2000). In vertebrates and insects, neural specification is linked to dorsal-ventral (D-V) axis specification and involves multiple signaling events. In vertebrates, neural tissue arises from future dorsal ectoderm and relies in part on inhibition of BMPs by graded antagonists secreted from the organizer (e.g. Chordin, Noggin, and Follistatin) as well as signaling by FGFs and Wnts (De Robertis and Kuroda, 2004; Stern, 2006, 2005; Wilson and Edlund, 2001). In *Drosophila melanogaster*, a ventral-to-dorsal gradient of nuclear Dorsal (NF-κB) protein in the early embryo helps establish the D-V axis, including the region of neurogenic ectoderm. This is achieved by regulating components of other signaling pathways including EGFR and Dpp (BMP2/4). Dorsal upregulation of *rhomboid* generates a gradient of EGFR signaling in the lateral (future ventral) ectoderm, which helps define the neurogenic domain. Dorsal also upregulates *sog* (*chordin*) in the lateral ectoderm, thus generating a dorsal gradient of Dpp (BMP2/4) signaling (Bier, 2011; Ferguson and Anderson, 1992; Irish and Gelbart, 1987; Lynch and Roth, 2011; von Ohlen and Doe, 2000; Wilson et al., 2014).

Very little is known about neural specification in the third major bilaterian clade, spiralians. Classic studies using blastomere isolation in several organisms, including spiralians, have contributed to our understanding of the role of autonomous and conditional fate specification of multiple tissues including neural ectoderm (Fig. 1A and B) (Costello, 1945; Goldstein, 1993; Novikoff, 1938; Reverberi, 1971; Thorpe et al., 1997; Wikramanayake and Klein, 1997). Many taxa within Spiralia have a stereotypical cleavage pattern called spiral cleavage, allowing cell fate and lineage to be compared across taxa (Hejnol, 2010; Lambert, 2010). Starting at the third round of divisions in these animals, the four blastomeres (A–D) cleave asymmetrically to generate a 'first-quartet' of smaller micromeres at the animal pole (1a–1d, also

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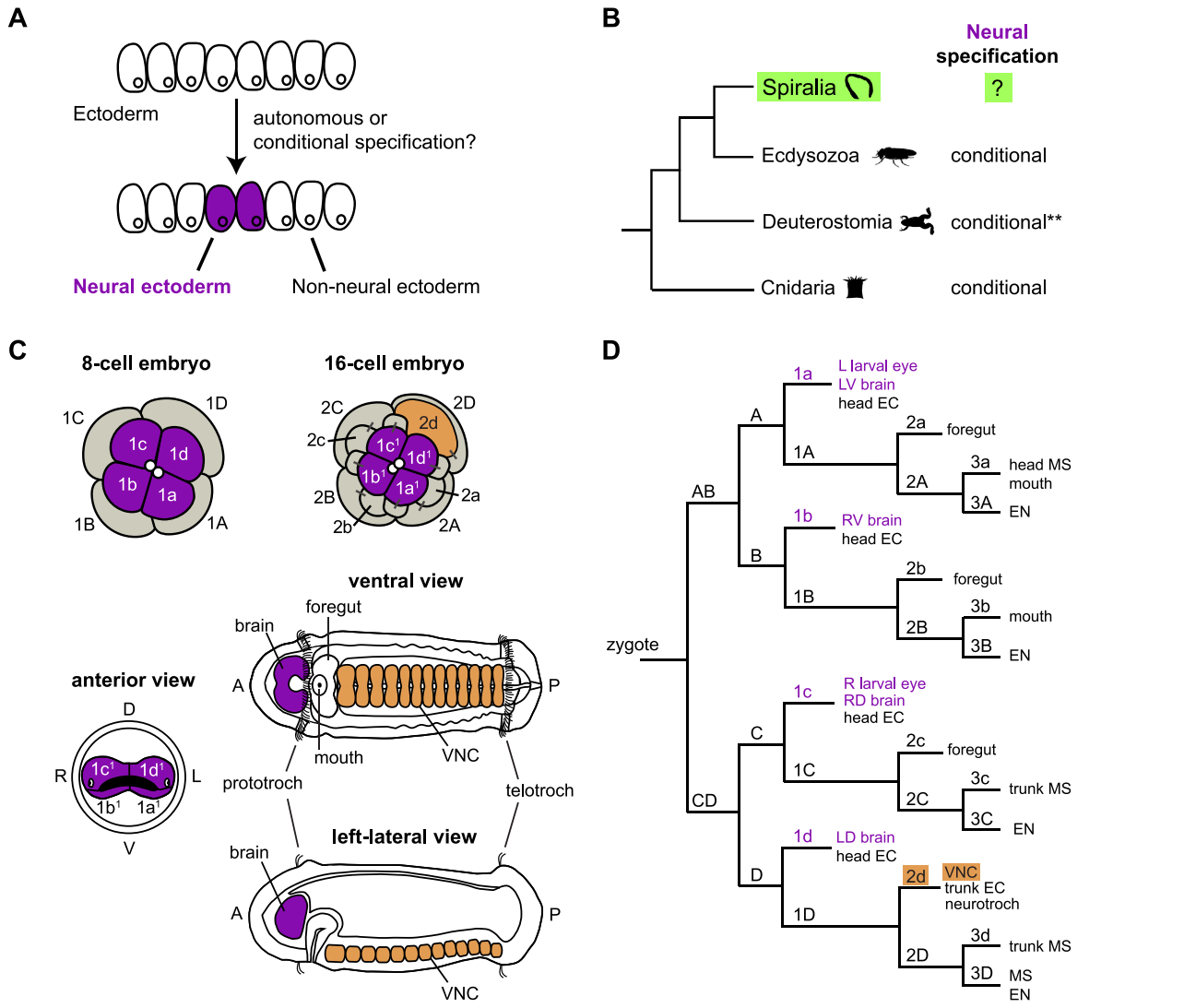

Fig. 1. Early neural ectoderm specification across Bilateria and neural fate map in the annelid *Capitella teleta*. (A) Ectoderm receives early signals instructing formation of neural versus non-neural ectoderm. (B) Generalized phylogeny of Bilateria and its sister group Cnidaria, showing that in the organisms studied so far, neural specification is achieved via extrinsic signals (conditional specification). ** Within Deutorostomia, autonomous specification of the nerve cord has been reported in two species of ascidians (Hudson, 2003; Minokawa et al., 2001). In Spiralia, the mechanisms involved in neural specification are unknown. (C) Diagrams (modified from Meyer et al. (2010)) showing spiral cleavage in 8- and 16-cell embryos and the larval body plan at stage 6 in the annelid *Capitella teleta*. Blastomeres that generate the brain (purple) and ventral nerve cord (orange) are indicated. Blastomere nomenclature is as in Conklin (1897). (D) Lineage diagram (modified from Meyer et al. (2010)) showing the fates of early blastomeres in *C. teleta*. The cells that make the brain (micromeres 1a–1d) are in purple, and the cell that makes the vast majority of the ectoderm in the trunk, including the ventral nerve cord, (micromere 2d) is in orange. A: anterior; D: dorsal; EC: ectoderm; EN: endoderm; L: left; LD: left-dorsal; LV: left-ventral; MS: mesoderm; P: posterior; R: right; RD: right-dorsal; RV: right-ventral; VNC: ventral nerve cord.

referred to as 1q) and larger macromeres at the vegetal pole (1A–1D, also referred to as 1Q) (Conklin, 1897) (Fig. 1C). Daughters of 1q micromeres ($1q^1$) generate anterior neural ectoderm in many spiralians (Ackermann et al., 2005; Dictus and Damen, 1997; Hejnol et al., 2007; Henry et al., 2004; Henry and Martindale, 1998; Huang et al., 2002; Render, 1991; Wilson, 1892). Furthermore, the 1q blastomeres and their descendants asymmetrically inherit maternal mRNAs (Henry et al., 2010; Kingsley et al., 2007; Lambert and Nagy, 2002; Nakamura et al., 2017), although the functions of most of these mRNAs remain unknown and none have yet been identified as neural determinants. One maternal mRNA, *IoLR5*, is necessary for development of the eyes and ciliary bands in the episphere of the snail *Tritia obsoleta* (previously known as *Ilyanassa obsoleta*) (Rabinowitz and Lambert, 2010).

The lineages that contribute to trunk neural ectoderm are more variable in spiralians (Boyer et al., 1998, 1996; Dictus and Damen, 1997; Hejnol et al., 2007; Henry et al., 2004; Henry and Martindale, 1998; Meyer et al., 2010; Render, 1997). In annelids, one cell at the 16-cell stage, the 2d micromere, generates the entire trunk ectoderm and ventral nerve cord (VNC) (Fig. 1C) (Ackermann et al., 2005; Anderson, 1959; Huang et al., 2002; Meyer and Seaver, 2010; Wilson, 1892). In contrast, 2d in mollusks makes only portions of the foot and shell gland (Dictus and Damen, 1997; Hejnol et al., 2007; Render, 1997). Furthermore, the D-quadrant, including 2d, is conditionally specified in some spiralians (Henry and Perry, 2008; Martindale et al., 1985), and subsequently acts as an organizer, specifying the dorsal-ventral (D-V) axis and certain tissues in both the head and the trunk (reviewed in Henry (2014) and Lambert (2008)).

Comparisons of fate maps across annelids and mollusks suggests that there may be differences in neural fate specification between the brain and ganglia in the trunk as these tissues are generated from different lineages. To begin to address this question, we examined neural specification in the annelid *Capitella teleta*, whose brain is derived from daughters of 1q micromeres ($1q^1$) and whose VNC is derived from daughters of 2d ($2d^{1121}$ and $2d^{1122}$; Fig. 1C and D) (Meyer et al., 2010; Meyer and Seaver, 2010). In addition to generating the VNC, the 2d micromere in *C. teleta* has been hypothesized to act as the organizer. However, laser ablation of 2d did not block expression of