Nervous system centralization as the evolutionary scaffold for body segmentation: A review of the evidence

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Abstract

The evolutionary origins of two major bilaterian innovations, the centralized nervous system and the segmented body plan, have both been debated as cases of homology versus convergence, often using strikingly similar arguments. Here we review the comparative developmental genetics of both processes across the three segmented animal phyla (arthropods, annelids, and chordates) focusing on the overlapping toolkit used in both: Notch/Delta signaling, the segment-polarity gene *engrailed*, Wnt signaling, and the transcription factors *Krüppel* and *hunchback*. We show that each carries dual roles in neural specification and in segmentation, and that in several cases the neural function appears to be evolutionarily ancestral. We argue that the shared architecture of the motor unit, which couples segmentally reiterated neurons to their muscle targets, provides the mechanistic link that allowed neural patterning networks to be repeatedly co-opted for trunk segmentation. This scenario supports a "brain-first" hypothesis, in which nervous system centralization arose before, and served as the developmental scaffold for, segmented body plans. We conclude that segmentation is likely not a homologous character, but a convergent elaboration built repeatedly from a shared, ancestral neural toolkit, through the co-option of conserved neurogenic gene regulatory networks.

Key words: nervous system centralization; body segmentation; gene regulatory network co-option; convergent evolution; brain-first hypothesis; neuroblast timer; segment polarity

I. Introduction

Morphological complexity, its origin, drivers, and underlying mechanisms are central research topics in evolutionary biology. Complexity is strongly correlated with taxonomic diversity, as it provides more variable building blocks to generate diverse adaptations (Carroll, 2001). The repeated emergence of complex morphologies from simpler ancestors raises a key question: is similar complexity homologous across lineages, or did it evolve independently each time?

Body segmentation and nervous system centralization are two examples of overall morphological complexity in bilaterian animals. Segmentation is the organization of the body into a series of repeated units along the anterior-posterior axis, and is found in three distantly related animal phyla: Arthropoda, Annelida, and Chordata (Davis & Patel, 1999; Seaver, 2003; Minelli & Fusco, 2004; Tautz, 2004; Couso, 2009; Chipman, 2010; Hannibal & Patel, 2013). Centralization of the nervous system is the concentration of neuronal cell bodies in ganglia or a nerve cord along the body axis. These ganglia integrate information from the whole body and initiate whole-body responses (Denes *et al.*, 2007; Arendt *et al.*, 2008; Arendt, Tosches & Marlow, 2016).

Centralized nervous systems are found in many phyla. Their organization varies from an anterior brain governing most complex functions (e.g. in vertebrates) to more distributed processing centers that can act semi-independently (e.g. in arthropods). Both body segmentation and nervous system centralization contribute to the ability of an animal to move directionally and function in a complex three-dimensional environment (Arendt *et al.*, 2008; Chipman, 2026).

For many years, segmentation was viewed as a homologous trait, at least between Arthropoda and Annelida, this being the defining synapomorphy of the proposed clade Articulata, uniting the two phyla (Kimmel, 1996; De Robertis, 1997; Arthur, Jowett & Panchen, 1999; Scholtz, 2002; Seaver, 2003; Robertis, 2008). However, molecular phylogenetics and morphological evidence dismantled this grouping (Adoutte et al., 2000; Aguinaldo et al., 1997; Eernisse et al., 1992), suggesting that segmentation has evolved convergently in these two segmented phyla, as well as in the more distantly related chordates (Minelli & Fusco, 2004; Mayer, 2006; Blair, 2008; Chipman, 2019). Nonetheless, the source of the similarities in the genetic toolkit employed in the segmentation process by all three segmented phyla remains an intriguing question.

The debate surrounding the homology of the central nervous system (CNS) mirrors the debate on the origins of segmentation. The debate is still ongoing, with evidence supporting both a common origin for a centralized nervous system (Arendt *et al.*, 2008, 2016; Biffar & Stollewerk, 2014; Jung & Dasen, 2015; Sur *et al.*, 2017; Feuda & Peter, 2022) and multiple independent origins (Hejnol & Lowe, 2015; Martín-Durán *et al.*, 2018; Martín-Durán & Hejnol, 2021). As with the debate over segmentation, the crux of the discussion lies in identifying similar elements of the centralized nervous system in different phyla and determining whether their similarity provides evidence for a common origin.

In this review, we survey the evidence for the roles of a selection of genes in both segmentation and neurogenesis across the three segmented phyla. By collating existing data, we try to elucidate the ancestral role of these genes and formulate a hypothesis linking complex centralized nervous systems and segmented body plans. We propose that the evolution of complex trunk segmentation is linked to and dependent on the prior evolution of centralized nervous systems, and that the genetic toolkit driving both processes reflects a shared evolutionary history of co-option from neural to trunk patterning.

We focus on several elements of the developmental toolkit known to play a role both in subdividing the embryo and in neural development. We chose these elements because they have been studied in both nervous system development

and segmentation (Chipman, 2010; Stollewerk, 2016; Tidswell, Benton & Akam, 2021), or offer the most robust cross-phyla comparative data. The *Wnt* pathway is included although its ancestral role is believed to be in axial elongation rather than in the nervous system, since we wanted to explore its regulatory relationship with *engrailed*. We first detail the broad mechanisms of segmentation and neurogenesis across Arthropoda, Annelida, and Chordata. We then systematically review the specific functions of each selected group of genes within each of these phyla. Finally, we synthesize these findings into a discussion of how the two processes evolved and propose a scenario explaining their similarities.

II. Segmentation and neurogenesis across phyla

1. Arthropoda

Arthropod segmentation mechanisms exhibit significant variation both within and among the four Linnaean classes (Hexapoda, Crustacea, Myriapoda, Chelicerata). Despite these differences, the consensus, supported by fossil evidence and molecular data, is that the segmented body plan in Arthropoda is ancestral (Peel, Chipman & Akam, 2005; Clark, Peel & Akam, 2019; Chipman & Edgecombe, 2019). The specific modes of segment generation and patterning have evolved independently in each lineage.

The best-studied segmentation mechanism in arthropods is simultaneous segmentation; the mechanism found in the model fruit fly *Drosophila melanogaster*. In this segmentation mode, the entire anterior-posterior (A-P) axis is patterned and subdivided simultaneously within a syncytial blastoderm before cellularization. It is hierarchical, fast, and efficient, but generally accepted as derived. A comprehensive phylogenetic analysis of insect segmentation suggests simultaneous segmentation evolved independently in several holometabolous lineages (Peel, 2004; Peel *et al.*, 2005; Stahi & Chipman, 2016; Clark *et al.*, 2019).

The mode that is considered ancestral to Arthropoda is sequential segmentation (Davis & Patel, 2002; Peel *et al.*, 2005; Chipman & Akam, 2008). In this process, segments are formed sequentially in an anterior-to-

posterior progression from a posterior segment addition zone (Auman & Chipman, 2018). This process is observed in myriapods, chelicerates, most crustaceans, and most insects (Clark *et al.*, 2019).

The arthropod nervous system is shaped by highly stereotyped patterns of neural precursor specification, arising from the neuroectoderm, in a strict pattern in register with the body segments. However, the differentiation of the neurons themselves follows a different pattern in different arthropod lineages. (Ungerer, Eriksson & Stollewerk, 2012; Hartenstein & Stollewerk, 2015; Stollewerk, 2016): Members of Pancrustacea (hexapods and crustaceans) utilize a mode of neurogenesis via neuroblasts, large specialized neural stem cells. These cells occupy stereotypical positions within the neuroectoderm and represent a highly efficient strategy for generating neural diversity from a limited progenitor pool (Hartenstein & Stollewerk, 2015; Stollewerk, 2016). In contrast, chelicerates and myriapods utilize a collective recruitment strategy. Rather than single stem cells, these embryos specify neural precursor groups (Stollewerk & Simpson, 2005; Chipman & Stollewerk, 2006; Hartenstein & Stollewerk, 2015). These recruit larger groups of cells that invaginate together. Unlike neuroblasts, these cells do not undergo prolonged proliferation, as most are already postmitotic or undergo very limited divisions before differentiating into neurons (Stollewerk & Simpson, 2005; Hartenstein & Stollewerk, 2015).

2. Annelida

Segmentation in Annelida is linked to the spiralian mode of development, originating with spiral cleavage and the very early determination of specific cell lineages. The 2d somatoblast generates the majority of the segmental ectoderm and the ventral nerve cord, while the 4d mesentoblast gives rise to the mesodermal bands that form the trunk musculature and coelom (Ackermann, Dorresteijn & Fischer, 2005; Ackermann *et al.*, 2005; Amiel, Henry & Seaver, 2013; Balavoine, 2014; Özpolat *et al.*, 2017; Zattara & Weisblat, 2020).

In Clitellata (leeches and oligochaetes), teloblasts – large stem cells located at the posterior of the embryo – drive segmentation. These cells undergo asymmetric divisions to produce linear columns of blast cells, which in turn generate specific descendant cells that contribute to segments (Davis & Patel, 1999; Zattara & Weisblat, 2020). This mode is considered derived, as it is not observed outside of this clade.

A mode more likely to be ancestral is observed in various polychaetes, such as *Platynereis dumerilii, Capitella teleta*, and *Alitta virens*. These animals undergo a larval period where a few initial segments are generated, with additional segments forming from a juvenile posterior growth zone (PGZ) that utilizes a ring of small, proliferative stem cells (Rivera & Weisblat, 2009; Dray *et al.*, 2010; Özpolat *et al.*, 2017; Zattara & Weisblat, 2020; Kairov & Kozin, 2023). Recent work in *Alitta virens* suggests that gene expression in this zone involves a distinct molecular signature where segment identity and elongation may be decoupled, hinting at a specific mechanism of segmentation (Kairov & Kozin, 2025).

Neurogenesis in Annelida is characterized by the formation of a CNS comprising a brain and a ventral nerve cord, which originates from a clearly defined neuroectodermal domain (Hartenstein & Stollewerk, 2015). Within the neuroectoderm, rapidly dividing neural progenitors are organized into specific subdomains (Meyer & Seaver, 2009, 2010; Hartenstein & Stollewerk, 2015).

In polychaetes, postmitotic cells delaminate from the neuroectoderm to form a stratified layer (Meyer & Seaver, 2009; Hartenstein & Stollewerk, 2015). These neural precursor cells (NPCs) are apically localized and maintain contact with the surface while undergoing multiple rounds of division (Sur *et al.*, 2020).

In clitellates, neurogenesis is primarily defined by a highly precise fixed-lineage mechanism driven by the teloblasts discussed above (Meyer & Seaver, 2009, 2010; Hartenstein & Stollewerk, 2015). Bilateral pairs of teloblasts generate the ventral nerve cord by budding off segmental blast cells

that undergo invariant, asymmetric divisions (Hartenstein & Stollewerk, 2015). This neural differentiation becomes prominent during organogenesis, as clitellates are direct developers (Koza *et al.*, 2006).

3. Chordata

Chordata (Cephalochordata, Tunicata, and Vertebrata) are characterized by the presence of a dorsal hollow nerve cord. Segmentation is most apparent in Vertebrata, with partial segmentation found in Cephalochordata. The process of segment generation in Chordata, particularly in vertebrates, is called somitogenesis (McGrew & Pourquié, 1998; Pourquié, 2001, 2007; Martin, 2020; Miao & Pourquié, 2024).

In cephalochordates, the paraxial mesoderm segments into somites, which are the precursors of the body musculature. Amphioxus somites form through distinct morphogenetic processes along the axis, in contrast with the mesenchymal-to-epithelial transition seen in vertebrate somites (see below). Anterior somites form via out-pocketing from the gut, while posterior somites form via schizocoely within the tail bud (Beaster-Jones *et al.*, 2008; Aldea *et al.*, 2019). These somites develop into myomeres (muscle blocks) that persist throughout the animal's life. Crucially, this segmentation maintains a direct physical correspondence between the myomeres and the dorsal nerve cord, as the muscle cells extend processes to contact the nerve cord directly, rather than nerves growing out to muscles (Rasmussen *et al.*, 2007; Aldea *et al.*, 2019).

In vertebrate somitogenesis, somites form sequentially from the unsegmented pre-somitic mesoderm (PSM). A molecular clock of oscillating gene expression, synchronized among neighboring cells, establishes the spatial pattern. The transition from oscillation to stable segmentation is controlled by a determination "wavefront". As the embryo elongates, the wavefront moves posteriorly. When cells drop below a certain gene expression threshold, they arrest the clock and differentiate into a somite (Pourquié, 2001; Saga & Takeda, 2001; Dequéant & Pourquié, 2008; Graham *et al.*, 2014).

The chordate CNS is unique in many regards. It is composed of a hollow, dorsal nerve cord, regionalized into a cerebral vesicle, a hindbrain, and a spinal cord (Holland & Holland, 1999; Candiani *et al.*, 2012). This is in contrast with most other phyla whose CNS is organized in solid ganglia. Neurogenesis in Cephalochordata occurs when individual cells are selected from the neuroepithelium, and cell division is primarily confined to the extreme anterior cerebral vesicle and the posterior tailbud (Lacalli, 2022; Gattoni *et al.*, 2025). This is considered to be the ancestral mode of neurogenesis in Chordata.

Vertebrates have innovated much in regard to neurogenesis. Perhaps the most significant from a developmental point of view is the neural crest, sometimes referred to as the fourth germ layer (Le Douarin & Dupin, 2014; Dupin & Le Douarin, 2014). The neural crest functions as a powerful stem-cell pool, generating a remarkably diverse array of cell types. In the context of this review, the neural crest is particularly relevant because dorsal root ganglia, peripheral sensory neurons derived from neural crest, form in strict register with somites, extending the segmental neuromuscular organization into the peripheral nervous system (Kahane & Kalcheim, 2021). Radial glial cells (RGCs) are another major novelty in the vertebrate lineage, derived from the dorsal neuroectoderm rather than the neural crest. RGCs act as primary neural stem cells that undergo symmetric and asymmetric divisions to expand the brain, and are used as scaffolding guiding newborn neurons to their destination as they migrate (Paridaen & Huttner, 2014; Gattoni *et al.*, 2025). This proliferation occurs across the entire neural axis, allowing for the significant enlargement and layering (e.g., in the neocortex) seen in the vertebrate lineage (Finlay, Hersman & Darlington, 1998; Paridaen & Huttner, 2014). Invertebrate chordates, such as amphioxus, possess neural plate border cells, but no true neural crest (Holland & Holland, 1999; Le Douarin & Dupin, 2014; Dupin & Le Douarin, 2014).

III. Notch/Delta signaling

1. Arthropoda

Notch/Delta signaling is one of the most evolutionary conserved

developmental mechanisms in metazoans, controlling cell fates by local interactions. These interactions are mediated by a membrane-bound ligand (Delta, as well as others) and a membrane-bound receptor (Notch). The interaction acts through a complex downstream pathway that produces a wide range of outcomes (Artavanis-Tsakonas, Rand & Lake, 1999). In most studied cases, the expression of *Delta* proves to be more informative about the role of the pathway, as its expression is more tightly modulated than that of the gene encoding its receptor, *Notch.*

In Arthropoda, *Notch/Delta* signaling has a role in both segmentation and neurogenesis in most studied species. *Delta* plays no role in segmentation in Onychophora, the sister group to Arthropoda (Janssen & Budd, 2016). Conversely, its role in arthropod segmentation seems ancestral, occurring in both chelicerates and mandibulates. In spiders, *Delta* functions as an integral component of the segmentation clock. Its oscillating expression in the segment addition zone (SAZ) suggests that a Notch-Delta signaling-based oscillator drives segmentation (Stollewerk, Schoppmeier & Damen, 2003a; Oda *et al.*, 2007; Schönauer *et al.*, 2016). In the centipede *Strigamia maritima*, *Delta* exhibits oscillatory expression in the posterior SAZ, while *Notch* is expressed in a stable segmental pattern (Chipman & Akam, 2008; Brena & Akam, 2013).

The role of *Delta* in crustacean segmentation is unclear. The expression patterns of *Delta* in *Artemia franciscana*, *Thamnocephalus platyurus* and *Daphnia magna* (Eriksson, Ungerer & Stollewerk, 2013) are consistent with a function in the posterior growth zone and segmentation. This suggests a mechanism akin to the oscillator described in other Arthropoda (Damen, 2007; Clark *et al.*, 2019) in at least some crustacean lineages.

In insects, Delta's role in segmentation is inconsistent across taxa. While it is required for axial elongation in *Gryllus bimaculatus* (Mito *et al.*, 2011) it isn’t required for segmentation (Kainz *et al.*, 2011). Similarly, evidence for a role in segmentation or axial elongation is also inconclusive in other hemimetabolous insects, including *Periplaneta americana* (Pueyo, Lanfear & Couso, 2008) and

Oncopeltus fasciatus (Stahi & Chipman, 2016), but the details vary from species to species.

In Holometabola, Delta's role in segmentation is variable. In *Tribolium castaneum* and *Bombyx mori* , Notch/Delta signaling is expressed in dynamic patterns in the posterior growth zone during segment addition (Sarrazin, Peel & Averof, 2012; El-Sherif, Averof & Brown, 2012; Liu, 2013), though whether it drives the segmentation clock or acts downstream in boundary formation is unresolved. However, it does not appear to have a role in segmentation in *Apis mellifera* (Wilson *et al.*, 2010b), or in *D. melanogaster* (Vässin & Campos-Ortega, 1987; Cabrera, 1990; Kooh, Fehon & Muskavitch, 1993; Muskavitch, 1994; Urbach & Technau, 2003a).

A role for Notch/Delta signaling in neurogenesis can be seen in both arthropods and onychophorans. It functions in the selection of neural progenitors in onychophorans (Eriksson & Stollewerk, 2010; Hartenstein & Stollewerk, 2015). Similarly, *Delta* presents a conserved neurogenic function in spiders, mediating lateral inhibition to single out neural precursors (Stollewerk, 2002; Hartenstein & Stollewerk, 2015). *Delta* is also expressed in the neuroectodermal clusters of *S. maritima*, where it facilitates the selection of neuroblasts during ventral nerve cord formation (Chipman & Stollewerk, 2006).

In Malacostraca, *Delta* is essential for specifying midline precursor cells and organizing the neuroectoderm. In other crustaceans, *Delta* cooperates via Notch/Delta signaling to restrict neural fate, consistent with a lateral inhibition model (Ungerer *et al.*, 2012; Eriksson *et al.*, 2013; Hartenstein & Stollewerk, 2015).

Unlike its segmentation role, the critical role of *Delta* for neurogenesis is conserved in insects, utilizing lateral inhibition to regulate neuroblast selection in all insect species studied so far (Jarvis, Bruce & Patel, 2012; Biffar & Stollewerk, 2014; Hartenstein & Stollewerk, 2015; Stollewerk, 2016).

The arthropod ancestor most likely employed a Notch/Delta-based oscillator mechanism for segmentation (Chipman, 2008; Clark *et al.*, 2019) that had been lost in species where axial elongation is no longer necessary (those with simultaneous segmentation). *Delta* expression in both myriapod and chelicerate neuroectodermal clusters facilitates the selection of neuroblasts via lateral inhibition and has a similar role in onychophorans. This supports the conclusion that lateral inhibition during neural precursor selection is a plesiomorphic and conserved role for *Delta* in Arthropoda.

2. Annelida

In polychaetes, there is evidence for the involvement of Notch/Delta signaling in segmentation. *Delta* is expressed in *C. teleta* in the posterior growth zone and segmentally in the nascent mesoderm. Nevertheless, while this suggests a role in segment addition, *Delta* does not strictly display oscillatory dynamics (Thamm & Seaver, 2008; Rivera & Weisblat, 2009; Meyer & Seaver, 2009). In *Helobdella robusta, Delta* and *notch* are expressed in specific subsets of blast cells, where they facilitate fate decisions between sister lineages derived from teloblasts (Rivera *et al.*, 2005; Rivera & Weisblat, 2009).

There is mixed evidence for the role of Notch/Delta signaling in annelid neurogenesis. In *C. teleta* it appears to have a neurogenic role (Thamm & Seaver, 2008; Meyer & Seaver, 2009; Sur *et al.*, 2017), however, in *P. dumerilii* it is not expressed in neurogenic epithelia, making it the only studied bilaterian species in which there is no evidence for *Delta* playing a role in neurogenesis (Gazave, Lemaître & Balavoine, 2017). *Delta* has a neurogenic function in the *H. robusta* ventral nerve cord; it is expressed in discrete clusters of neuroectodermal cells where it regulates the separation of neuroblasts via lateral inhibition, ensuring the precise architectural organization of the ganglionic primordia (Rivera *et al.*, 2005).

Notch and Delta appear to have a complex history in Spiralia (He *et al.*, 2021; Lv *et al.*, 2024). This makes the annelid sparse data difficult to interpret from an evolutionary perspective. However, one function that *Delta* seems to retain is its role in lateral inhibition, even when not applied to neuronal precursors

(Gazave *et al.*, 2017).

3. Chordata

In Cephalochordata, Delta is essential for both the periodic patterning of somites and for neural cell fate specification. In the amphioxus *Branchiostoma floridae*, *Delta* is transcribed in a dynamic, cyclic manner that correlates with somite formation, typical of a segmentation clock even as the “wavefront” is missing. This provides evidence for a Notch/Delta-based oscillatory clock mechanism during somitogenesis, without a vertebrate-style presomitic mesoderm or wavefront. This suggests that the clock component of Notch/Delta metamerism may be ancestral to Chordata (Beaster-Jones *et al.*, 2008).

In the Tunicate *Ciona intestinalis*, the function of *Delta* diverges from the segmentation clock mechanism. Rather than driving segmentation, *Delta* signaling from the prospective endoderm is required to induce secondary notochord specification and to define the neural plate boundary by laterally inhibiting neural fate in marginal cells (Hudson & Yasuo, 2006).

In vertebrates, the *Delta* gene family is a central component of the oscillating genetic network driving somitogenesis. Delta ligands are the output of the segmentation clock in the presomitic mesoderm, expressed in cyclic waves that propagate anteriorly to synchronize the clock and define somite boundaries (Lewis, 1998; Smithers *et al.*, 2000; Saga & Takeda, 2001; Isomura & Kageyama, 2025). Disruption of this function leads to a loss of somite coherence and severe skeletal defects (de Angelis, Mclntyre & Gossler, 1997).

In *B. floridae*, *Delta* is also expressed in scattered cells within the neural plate, where it functions via lateral inhibition to regulate the density and spacing of developing neurons (Rasmussen *et al.*, 2007; Lu, Luo & Yu, 2012). In *C. intestinalis* during late larval development, *Delta* expression becomes restricted to specific sensory precursors in the trunk and tail, ensuring the correct number of sensory neurons through lateral inhibition (Pasini *et al.*,

2006).

Delta in vertebrates acts as a key regulator of neurogenesis. In the developing CNS, Delta maintains the neural progenitor pool and regulates the timing of differentiation via lateral inhibition (Henrique *et al.*, 1995; Haddon *et al.*, 1998; Grandbarbe *et al.*, 2003; Kageyama *et al.*, 2008, 2009).

4. Summary

The role of Notch/Delta signaling in selecting individual neural precursors via lateral inhibition is conserved across Arthropoda, Annelida, and Chordata, with only a single studied species as an exception. The involvement of Notch/Delta in segmentation, while present in all three phyla, exhibits significant differences, as well as lineage-specific loss. It is reasonable to hypothesize that lateral inhibition in the neuroectoderm is an ancestral role of the Notch/Delta signaling pathway in Bilateria. This regulatory module was subsequently co-opted to regulate the timing and spacing of segmentation during axis elongation.

IV. Engrailed

1. Arthropoda

Engrailed encodes a widely conserved homeodomain transcription factor, part of a GRN that establishes compartment borders by partitioning cells into distinct populations. This function is coordinated with temporal markers and other spatial signals to drive morphogenesis (Joyner, Ortigão-Farias & Kornberg, 2024).

In Onychophora, *engrailed* (*en*) acts as a segment polarity gene, maintaining posterior segment boundaries. It is expressed in stripes in all segments before morphological segmentation is visible. There is *en* expression in both mesoderm and ectoderm, with slight differences in expression hinting at different functions in the two germ layers (Wedeen *et al.*, 1997; Eriksson *et al.*, 2009).

In Arthropoda, as in Onychophora, *en* is a highly conserved segment-polarity gene marking each segment's posterior border. The expression of *en* is one of the most conserved features of arthropod development, appearing in segmentally reiterated stripes as well as in the developing nervous systems in chelicerates, myriapods, crustaceans and hexapods (Patel, Kornberg & Goodman, 1989; Hughes & Kaufman, 2002; Kettle *et al.*, 2003; Peel, Telford & Akam, 2006; Janssen, Turetzek & Pechmann, 2022).

Due to a whole genome duplication event in chelicerates, some species have retained two copies of *en*. In spiders, *en1* is expressed in both segmental stripes and neural progenitors, while en2 is restricted to segmental stripes without a neurogenic function (Janssen *et al.*, 2022). In centipedes, en is expressed in the transition zone where segments are emerging, before they are morphologically distinct (Hughes & Kaufman, 2002; Kettle *et al.*, 2003; Green & Akam, 2013). In the centipede *Lithobius peregrinus*, *en* continues to function after hatching as segments are added post-embryonically (Bortolin, Benna & Fusco, 2011). In insects, *en* has undergone a tandem duplication, creating the paralog *invected*. The two paralogs are often expressed in an almost identical pattern of segmental stripes (Peel *et al.*, 2006; Nakagaki, Sakuma & Machida, 2015; Janssen *et al.*, 2022; Blunk *et al.*, 2023).

Beyond its conserved role in boundary maintenance, *en* is expressed in a stereotyped subset of neuronal precursors across all studied members of Arthropoda. In Onychophora, ectodermal *en* becomes limited to a transversal row of neural precursors and neurons. Later expression suggests that these *en*-expressing cells play a role in shaping the brain commissures (Eriksson *et al.*, 2005; Whitington & Mayer, 2011).

In the spider nervous system, *en1* is primarily expressed in apical neuroepithelial cells of the head and along the body axis, including neural progenitors that give rise to the optic ganglia and specific interneuron populations (Stollewerk, Weller & Tautz, 2001; Stollewerk & Simpson, 2005; Doeffinger, Hartenstein & Stollewerk, 2010; Janssen *et al.*, 2022). In the centipede *Strigamia maritima*, *en* is expressed in a subset of neuronal

precursors, probably playing a role in specifying neural precursor identity and the formation of axonal pathways (Whitington, Meier & King, 1991; Stollewerk & Chipman, 2006; Hunnekuhl & Akam, 2017).

The conservation of *en* in specific, identifiable neurons across both insects and malacostracan crustaceans provides a primary line of evidence for neuronal homology across the Mandibulata (Patel *et al.*, 1989; Duman-Scheel & Patel, 1999; Ahzhanov & Kaufman, 2000; Hughes & Kaufman, 2002; Harzsch, 2003; Stollewerk & Simpson, 2005; Sintoni, Fabritius-Vilpoux & Harzsch, 2007; Loesel *et al.*, 2013; Nakagaki *et al.*, 2015). In several cases, late en expression shifts from the ectoderm to future muscle junctions, contributing to axon guidance and the formation of neuron–muscle and interneuron – sensory-neuron junctions.

The conserved role of *en* in segmentation in all Arthropoda, as well as their sister group Onychophora, indicates that at the base of Arthropoda it was already involved in generating segments. Its neuronal function appears related to selecting specific progenitor lineages, marking neuroblast identity by spatial position early in development. Later developmental evidence supports a role in the formation of nerve-muscle junctions and the correct formation of motor units, which is where the connection to the mesoderm comes into play.

2. Annelida

In nereidid polychaetes, *en* expression is strongly associated with the formation and maintenance of segment boundaries. It is expressed in ectodermal stripes, defining the anterior border of each forming segment, during both larval and post-larval growth (Prud'homme *et al.*, 2003). However, in other polychaetes and in leeches, *en* expression does not support a role in body axis segmentation: it is not expressed in the ectodermal boundary stripes characteristic of nereidids, but instead shows expression restricted to subsets of neurons in the ventral nerve cord (Seaver *et al.*, 2001; Seaver & Kaneshige, 2006; Kairov & Kozin, 2025).

However, *en* plays an important and highly conserved role in annelid neurogenesis, specifically in the specification of neuronal cell types within the central nervous system. The primary function of *en* in the annelid nervous system is to specify cell fate for a subset of neurons. Its expression is often linked to cell-type differentiation events within already established segments. In most studied annelids, *en* is expressed in a segmentally reiterated pattern in the ventral nerve cord, in a subset of populations of neuronal precursors (Wedeen & Weisblat, 1991; Seaver & Kaneshige, 2006).

The variable role of *en* in annelid segmentation leads to two equally parsimonious reconstructions: either *en* was independently recruited into segmentation in nereidid polychaetes, or it was present ancestrally in annelids and secondarily lost in clitellates and non-nereidid polychaetes. This question remains to be tested with broader taxon sampling. However, the role of *en* in neuron identity specification is more likely to reflect the ancestral state for annelids, as similar neuronal expression exists in the mantle lobes of Brachiopods (Shimizu *et al.*, 2017).

3. Chordata

In Chordata, there is no direct link between *en* and segmentation. In amphioxus, *en* is expressed in the posterior margin of the first eight somites prior to morphological segmentation; however, this expression is not accompanied by evidence of a role in boundary formation, as no loss-of-function data demonstrate disrupted somitogenesis (Holland *et al.*, 1997; Benito-Gutiérrez, 2006). In ascidians, *en* expression is restricted to a small cluster of cells in the anterior nervous system with no metameric repetition (Jiang & Smith, 2002; Beaster-Jones, Schubert & Holland, 2007). The restriction of *en* to neural contexts in ascidians, and its somite-associated expression without a demonstrated segmentation function in amphioxus, together suggest that *en* in Chordata has a main role in neural or regional identity specification rather than a role in segment boundary formation.

In vertebrates, *en* has no apparent segmentation role. It is, however, involved in the correct patterning of the nervous system. Specifically, it determines the

midbrain-hindbrain boundary (Danielian & McMahon, 1996; Krumlauf & Wilkinson, 2021; Joyner *et al.*, 2024), but also other unique cell populations along the body axis, such as certain types of motor neurons and inhibitory spinal interneurons (Loomis *et al.*, 1996; Higashijima *et al.*, 2004; Ahmed *et al.*, 2017). This suggests that in vertebrates, En is utilized to specify identities of individual cells or groups of cells. Specifically, in axon direction, and forming morphological boundaries, ensuring correct innervation within an already segmented framework (Ahmed *et al.*, 2017; Joyner *et al.*, 2024).

4. Summary

The conservation of *en* expression in defined neuronal subsets across arthropods and vertebrates, in contexts without segmentation function, is consistent with a hypothesis that neuronal identity determination is the ancestral role of *en*. The recruitment of *en* into the segmentation machinery may have occurred through the co-option of a pre-existing module used to determine neuronal identities and axon formation. In arthropods and in some annelids, this module was integrated into the early ectodermal patterning system to define segment boundaries. In chordates, the gene largely retained its ancestral role in neural identity determination.

V. The *Wnt* pathway

1. Arthropoda

Wnt ligands are a large family of glycoproteins that activate an important and widely conserved signaling pathway. An estimated 13 *Wnt* subfamilies can be traced to the common ancestor of Cnidaria and Bilateria (Cho *et al.*, 2010). The best-studied member, *wingless (wg/Wnt1)*, is mostly known as a segment-polarity gene in Arthropoda, where it has a tight regulatory relationship with *en*. Members of the *Wnt* gene family are integral to both the maintenance of segment boundaries and to the patterning of the neuroectoderm.

In onychophorans, *Wnt1* shows posterior embryonic expression, in addition to segment polarity like stripes, making both axial elongation and segmentation-related roles likely (Eriksson *et al.*, 2009; Hogvall *et al.*, 2014; Mundaca-Escobar, Cepeda & Sarrazin, 2022). The *wg* segment polarity pattern is conserved in myriapods (Hayden & Arthur, 2014; Janssen & Posnien, 2014), crustaceans (Constantinou *et al.*, 2016; Du *et al.*, 2018), and insects (Bolognesi *et al.*, 2008; Martin & Kimelman, 2009; Murat, Hopfen & McGregor, 2010). In chelicerates, *Wnt16* and *Wnt8* often substitute for or complement *wg* in its axial elongation and segmentation role, appearing in a stereotyped segment-polarity-like pattern during both anterior and posterior segment formation (Damen, 2002; McGregor *et al.*, 2008; Leite & McGregor, 2016; Janssen, Pechmann & Turetzek, 2021).

The picture is more complex for neurogenesis. In Onychophora, *Wnt1* is expressed in a subset of neural progenitors after segmentation has ended (Eriksson *et al.*, 2009; Hogvall *et al.*, 2014; Mundaca-Escobar *et al.*, 2022). There is no evidence supporting *Wnt* genes playing a role in the neurogenesis of myriapods or chelicerates. However, in insects and crustaceans, *wg* is expressed in neural precursors (Baker, 1987, 1988; Murat *et al.*, 2010; Oberhofer *et al.*, 2014; Constantinou *et al.*, 2016; Du *et al.*, 2018), playing a role in the development of these neuroblast lineages (Chu-LaGraff & Doe, 1993).

In Arthropoda, Wnt signaling regulates axial elongation and segmentation, and is not required for the patterning of the nervous system. When the Wnt pathway is involved in neurogenesis, its role seems to be specifying neural progenitor populations within the neuromeres (Stollewerk, 2016). This might be an effect of the relationship between Wnt and *en*, as a relatively recent recruitment event. The evidence suggests that the role of Wnt in axial elongation is an ancestral feature that was subsequently refined.

2. Annelida

In Annelida, members of the *Wnt* gene family serve as key molecular coordinators for axial patterning, segmentation, and organogenesis. Primarily, the *Wnt* signaling pathway acts as a conserved regulator of posterior axial identity and growth across the phylum (Cho *et al.*, 2010).

In polychaetes, *Wnt* genes coordinate both metameric patterning and central nervous system organization. In *A. virens*, *Wnt1* is expressed in the posterior growth zone and nascent segment boundaries, marking axial elongation and segment polarity (Kairov & Kozin, 2025). In *C. teleta*, the pathway is highly active in the blastema of regenerating fragments, and its activation rescues posterior regeneration in normally non-regenerative tissues by stimulating cell division and stem-cell marker expression (Kunselman & Seaver, 2025). In the context of neurogenesis, the Wnt signaling pathway is required for the transition of neural progenitor cells from a state of proliferation to terminal differentiation within the neuroectoderm (Demilly *et al.*, 2013).

Within the clitellate annelids, *Wnt* genes are pivotal for early cell-fate specification. In the leech *Helobdella triserialis*, the expression of *Wnt-A* within specific micromeres and epithelia suggests a regulatory role in specifying segmental, neural and ectodermal lineages (Kostriken & Weisblat, 1992). In *Whitmania pigra*, *Wnt* components are differentially expressed through organogenesis, acting as a core module driving neural differentiation (Liu *et al.*, 2023), and other organogenesis processes (Žídek, Machoň & Kozmik, 2018). This suggests that the pathway's involvement in controlling neural cell proliferation and the maturation of the segmented nervous system is a conserved feature across annelids (Demilly *et al.*, 2013).

The data point to a conserved role for Wnt-signaling in body patterning, specifically axis elongation, with some role in lineage specification within the nervous system. Because Wnt signaling is known to broadly function in axial elongation and body patterning, predating the lineage-specific neural functions described here, and because its role in neural fate specification varies considerably between taxa, convergent co-option into neural cell-fate

determination is more parsimonious than a single ancestral neurogenic function.

3. Chordata

In Chordata, *Wnt* ligands are primary regulators of embryonic axis specification and tissue morphogenesis, best-known in the vertebrate segmentation clock. Canonical *Wnt* signaling is essential for antero-posterior (A-P) patterning of the ectoderm, where it functions to specify posterior neural and epidermal fates while inhibiting anterior identities (Feinberg *et al.*, 2019).

Wnt signaling controls the specification of mesoderm from the early endomesoderm in amphioxus (Holland & Onai, 2011; Onai, 2018). During gastrulation, the shift of the expression of *Wnt1* from the blastopore lip to the neurenteric canal suggests it has a role in establishing posterior identity (Holland, Holland & Schubert, 2000). As the larvae undergo posterior elongation, a distinct combinatorial code of *Wnt* genes is expressed within the tail bud to coordinate somitogenesis and axial extension (Schubert *et al.*, 2001). During ascidian morphogenesis, *Wnt* signaling orchestrates the extension of notochord cells (Sasakura & Makabe, 2001; Jiang & Smith, 2007). In vertebrates, *Wnt* signaling is responsible for the formation of the paraxial mesoderm and the periodic specification of somites, as it coordinates the molecular clock required for sequential segmentation (Parr & McMahon, 1995; Huelsken & Birchmeier, 2001).

Regarding neurogenesis, in the ascidian *Halocynthia roretzi, Wnt* genes exhibit localized expression in the dorsal and ventral ependymal cells of the neural tube, suggesting a role in the regional specification of the central nervous system (Sasakura & Makabe, 2001). In vertebrates, *Wnt* signaling is required from neural induction through synaptic maturation. Within the developing central nervous system, *Wnt* ligands maintain the balance between the expansion of the neural progenitor pool and the induction of neuronal differentiation (Huelsken & Birchmeier, 2001; Mulligan & Cheyette, 2012). In addition, the spatial information of *Wnt* paralogs patterns the neural tube by specifying neuronal posterior identity and establishing coordinates in

the spinal cord (Parr & McMahon, 1995; Mulligan & Cheyette, 2012; Hikasa & Sokol, 2013).

4. Summary

The common element of *Wnt* signaling across the three phyla is its role in axial elongation and A/P patterning. This is often also applied to neural patterning but is not unique to it. The very different roles in segmentation seen in the segmented phyla, from segment-polarity stripes in Arthropoda, to the reinforcement of boundaries in Annelida, and the segmentation clock in Vertebrata point to independent co-option events. In each case, a pre-existing axial patterning module (the *Wnt* gradient) was recruited to provide the periodic timing or spatial spacing required for segmented body plans. The tight connection to *engrailed* could have driven multiple co-options from the patterning of the body axis to the patterning of the nervous system.

VI. Krüppel and *hunchback*

1. Arthropoda

The best-known role of *Krüppel* (*Kr*) and *hunchback* (*hb*) is as gap genes involved in the progressive subdivision of the embryo in *D. melanogaster* (Nüsslein-Volhard & Wieschaus, 1980; Jäckle *et al.*, 1986). Both are transcription factors, and are mostly known to function in defining distinct developmental regions via expression-level gradients and mutual regulatory interactions, together with the other members of the gap-gene network (Jaeger, 2011). Mutations in gap genes famously result in body parts either forming with the wrong identity or not forming at all ("gap" phenotypes) (Nüsslein-Volhard & Wieschaus, 1980).

This gap function is broadly conserved across insects. *Kr* and *hb* are essential for patterning the central region of the embryo along the anterior-posterior axis, and knockdown of either gene produces a characteristic "gap" phenotype in which thoracic and anterior abdominal segments are deleted or transformed to a different tagma identity, a role maintained across insect species with otherwise divergent modes of development, despite differences

in detail and specific regulatory interactions (Nüsslein-Volhard & Wieschaus, 1980; Tautz *et al.*, 1987; Schröder, 2003; Liu & Kaufman, 2004a, 2004b; Mito *et al.*, 2006; He *et al.*, 2006; Ben-David & Chipman, 2010; Nakao, 2015, 2016; Tidswell *et al.*, 2021). Within this network *Kr* acts high in a hierarchical cascade, translating maternal gradients into broad zygotic domains and subsequently regulating *hb*, other gap genes, and pair-rule genes (Patel *et al.*, 2001; Liu & Kaufman, 2004a, 2004b; Mito *et al.*, 2006; He *et al.*, 2006; Marques-Souza, Aranda & Tautz, 2008; Wilson, Havler & Dearden, 2010a; Mao, Liu & Zeng, 2013).

This gap role, however, does not appear to be ancestral. In *Euperipatoides rowelli* (Onychophora), *hb* shows no typical "gap-like" expression and is instead found predominantly in neural progenitors (Franke & Mayer, 2015), suggesting that the region-definition function arose at the base of Arthropoda or later. Consistent with this, the neural expression of *Kr* and *hb*, not their gap function, is conserved broadly across the arthropod groups.

Across Chelicerata and Mandibulata, *Kr* and *hb* are expressed in the developing nervous system in patterns consistent with the neuroblast timer function described in insects (Stollewerk, Tautz & Weller, 2003; Stollewerk & Chipman, 2006; Janssen, Budd & Damen, 2011). In the spiders *Achaearanea tepidariorum* (Schwager *et al.*, 2009) and *Cupiennius salei* (Stollewerk *et al.*, 2003), *hb* is expressed in a wide early domain, particularly in the neuroectoderm and neural progenitor populations. In the centipede *Strigamia maritima*, both genes are expressed in neural progenitors of the ventral neuroectoderm (Chipman & Stollewerk, 2006), and in the millipede *Glomeris marginata*, *Kr* expression extends anteriorly from the posterior segment addition zone up to the post-maxillary segment, broadly complementary to *hb* in a manner consistent with a conserved *hb -Kr* regulatory interaction (Janssen *et al.*, 2011).

In the crustacean *Artemia franciscana*, *hb* is expressed in the nervous system in a pattern suggesting conservation of the neural-identity function (Kontarakis, Copf & Averof, 2006). This neural role is shared with insects,

where the gap-gene cascade, and especially its first tier, *Kr* and *hb* ,closely resembles the neuroblast timer series that specifies neuronal identity (Romani *et al.*, 1996; Isshiki *et al.*, 2001; Urbach & Technau, 2003b; Urbach, Jussen & Technau, 2016; Tidswell *et al.*, 2021; Pollington & Doe, 2025). Together, these data indicate that *Kr* and *hb* assign specific temporal identities to neuronal precursor lineages across the major arthropod groups and not only in insects (Romani *et al.*, 1996; Novotny, Eiselt & Urban, 2002; Stollewerk *et al.*, 2003; Urbach & Technau, 2003b; Kontarakis *et al.*, 2006; Chipman & Stollewerk, 2006; Janssen *et al.*, 2011; Urbach *et al.*, 2016; Tidswell *et al.*, 2021; Pollington & Doe, 2025). In *D. melanogaster*, both genes additionally affect the innervation of motor circuits and muscles (Hartmann *et al.*, 1997).

The ancestral role of *Kr* and *hb* in arthropods is most likely the specification of neural progenitor-lineage temporal identity. This function was subsequently co-opted into the broader gap-gene function, recruiting at least the first two members of the neuronal-identity network and adding further regional genes to build the gap-gene network seen today.

2. Annelida

The role of *hb* in annelid segmentation varies within the clade. In *P. dumerilii*, *hb* is expressed in the mesodermal bands and the posterior growth zone, implying a role in the formation of segmented somatic mesoderm (Kerner *et al.*, 2006).

In the polychaete *Capitella capitata* and the leech *H. triserialis*, the *hb* orthologs (*Cc-hb* and *LZF2*, respectively) are not expressed in the segmental precursor lineages (teloblasts or posterior growth zone) during the formation of the body axis, nor do they display an anterior-posterior gradient, suggesting they do not have a function in segment determination (Savage & Shankland, 1996; Werbrock *et al.*, 2001). Finally, in the oligochaete *Tubifex*, *hb* is transiently expressed in a subset of ectodermal teloblasts, an expression pattern that is probably derived within the oligochaete lineage (Shimizu & Savage, 2002).

Conversely, expression of *hb* orthologs in the developing central nervous system (CNS) appears to be a conserved feature among annelids, suggesting an ancestral role in neurogenesis. In *P. dumerilii*, *hb* is expressed in a broad ventral ectodermal domain as well as in specific precursor cells of the CNS, indicating a role in early neural specification (Kerner *et al.*, 2006). A segmentally restricted neuronal expression pattern is also observed in the polychaete *C. capitata*, where *hb* is expressed in a subset of differentiated neurons in the adult CNS, although it is notably absent from the nerve cord during earlier organogenesis (Werbrock *et al.*, 2001). In the leech *H. triserialis*, the ortholog *LZF2* is expressed both during organogenesis in the ventral nerve cord, specifically in inter-ganglionic muscle cells and in subsets of neurons within each segmental neuromere (Savage & Shankland, 1996; Iwasa, Suver & Savage, 2000).

The conservation of *hb* expression in the CNS across these diverse annelid species supports the hypothesis that its involvement in neurogenesis is an ancestral role of this gene (Shimizu & Savage, 2002; Kerner *et al.*, 2006). We could not find any published reports on the expression or function of *Kr* in Annelida.

3. Chordata

Kr vertebrate orthologs, known as *Krüppel*-like factors (KLFs) are not involved in segmentation but rather function in diverse processes in chordates (Oates *et al.*, 2001; Antin *et al.*, 2010). KLFs play integral roles in the specification of neural tissues and the peripheral nervous system of Chordata.

In the ascidian *C. intestinalis*, *klf*s are expressed in the dorsal and ventral epidermal midlines, specialized neurogenic regions that give rise to epidermal sensory neurons (Pasini *et al.*, 2006). In the frog *Xenopus laevis* and the lamprey, *Klf7* and *Klf2* are expressed in the neural plate border and neural crest, where they restrict expansion of these domains (Rigney, York & LaBonne, 2025). In *Gallus domesticus (Chicken)*, *klf7* is expressed in the neuroepithelium and dorsal root ganglia, suggesting a conserved involvement in neuronal development (Antin *et al.*, 2010).

Orthologs of *hb* (*Ikaros*/*Ikzf1*) are present in Chordata, where they specify early temporal identity in neural progenitor cells of the vertebrate retina and cerebral cortex (Elliott *et al.*, 2008), suggesting that the neuronal temporal identity function of *hb* is conserved in chordates. However, there is no evidence for involvement of *Ikaros* in somitogenesis. The most likely scenario is that at least *Kr* was initially a neuronal identity-specifying gene, although the original network it belonged to is unknown. We suggest that in the lineage leading to Chordata, *Kr* went through multiple duplications and neofunctionalization events resulting in the creation of multiple KLFs.

4. Summary

The function of *hb* and *Kr* as gap genes appears to be an evolutionary novelty restricted to the insect lineage (Pinnell *et al.*, 2006). More broadly, *Kr* and *hb* have an ancestral role in temporal identity specification of neuroblast lineages.

VII. Discussion

Arguments supporting the homology of segmentation often mirror those used to support the homology of centralized nervous systems. While the data leads us to conclude that both are convergent characters, the data also suggests a deep connection between the development of a centralized nervous system and a segmented body plan. This similarity points toward a shared evolutionary logic in the organization of these complex systems under similar constraints.

We suggest a stepwise evolution of centralized nervous systems linked to that of segmented body plans. Early in the evolution of Bilateria, bilateralization coevolved with cephalization – the concentration of sensory organs and nervous tissues at the anterior end of an animal – leading to the development of an anterior sensory center; a prototypic “brain” (Chipman & Edgecombe, 2019; Chipman, 2024). This simple brain, patterned by simple developmental networks, was probably a feature of early bilaterians.

Later, during the Cambrian, the increase in ecological complexity led to an increase in sensory input complexity, which selected for increased neural processing, forming more complex brains by complex network patterns (Chipman, 2026). This probably occurred several times in different lineages (Hejnol & Lowe, 2015; Martín-Durán & Hejnol, 2021). Networks involved in neural development were consequently co-opted for body regionalization and for creating the segmented body plan (Chipman, 2010, 2026; Tidswell *et al.*, 2021).

Based on the reviewed data, we hypothesize that neural patterning GRNs, which specialize in the precise spatial and temporal placement of neurons and their connectivity, provided an ancestral scaffold that could be readily co-opted for body segmentation. The mechanistic basis for this connection lies in the organization of motor units. A motor unit, comprising a motor neuron and the muscle fibers it innervates, is the elementary functional unit coupling neural circuits to body movement (Heckman & Enoka, 2004).

This tight link between neural networks and repeated muscular units provided the selective pressure for co-opting neural patterning networks from the ancestral nervous system into the trunk. In all three segmented phyla, motor units are organized in strict register with segmental boundaries: each body segment has a defined complement of motor neurons whose axons innervate the muscles of that segment (Landgraf *et al.*, 1997; Loesel *et al.*, 2013; Starunov *et al.*, 2015; Kahane & Kalcheim, 2021; Verasztó *et al.*, 2024; Büschges & Ache, 2025). Thus, any system specifying neural progenitor identity in the trunk must do so relative to segment position. We suggest that this functional requirement, the need for each segment to have correctly specified motor neurons innervating the correct muscle targets, led to the co-option of neural patterning and neural identity genes into the segmentation machinery (Figure 1).

Notch/Delta signaling was ancestrally used for lateral inhibition, controlling the number and spacing of neural progenitors through intercellular feedback. The inherent negative feedback in this pathway easily leads to oscillating dynamics. The combination of oscillatory dynamics and the boundary-forming capacity was independently repurposed to coordinate trunk extension and segment patterning

in both Arthropoda and Vertebrata. In vertebrates the dynamics were elaborated into the sustained oscillations of the segmentation clock (Pourquié, 2001; Aulehla & Herrmann, 2004), whereas in arthropods the pathway drives periodic boundary formation (Chipman, 2008; Clark *et al.*, 2019).

Engrailed, participates in nerve–muscle junction formation in arthropods (Patel *et al.*, 1989; Stollewerk & Simpson, 2005), and also specifies neuronal and compartment identities. It was likely recruited to define morphological segment boundaries in arthropods and in certain annelids, due to its tight regulatory relationship with the *Wnt* pathway. This adds a layer of spatial refinement on top of the periodicity established by *Notch/Delta*, acting through its regulatory feedback loop that stabilizes segment boundaries during axial elongation.

The *Wnt* pathway has an almost definite ancestral role in axial elongation. Its recruitment into neural specification may have arisen from its regulatory relationship with *en* during boundary formation in some lineages. This led to a transition from general axial patterning toward the patterning of specific, segmentally repeated tissues.

Krüppel and *hunchback* probably functioned ancestrally in the temporal determination of neural identity. At least in *D. melanogaster*, *Kr* and *hb* have an additional role in defining motor-neuron innervation patterns (Hartmann *et al.*, 1997). In the lineage leading to Insecta, their spatio-temporal role was co-opted to establish broad regional identities, forming the anterior tier of the gap-gene cascade.

The proposed framework suggesting a functional link between segmentation and neurogenesis remains a model requiring experimental testing, particularly experiments that dissociate the neurogenic from the segmentation functions of these genes. Our current ability to generalize these findings across Bilateria is hampered by the annelid dataset, which remains limited compared to other taxa. Furthermore, the possibility of reciprocal co-option, regulatory crosstalk, and overlap between networks suggests a complexity we do not yet fully understand. Nonetheless, this model provides a coherent and consistent explanation for the

non-homologous similarities in both segmentation and neurogenesis across phyla.

Table 1: Summary of the different developmental components discussed and their role in neurogenesis and segmentation in the three phyla.

	Function in Arthropoda	**Function in Annelida**	**Function in Chordata**	**Ancestral Role (Hypothesized)**	**link to Segmentation (Hypothesized)**
Notch/ Delta	Segmentation - clock Neurogenesis (lateral inhibition)	Neurogenesis; Chaetogenesis (*Platynereis*); Segmentation (*Leech*)	Segmentation clock (somitogenesis); Neurogenesis (stem cell maintenance)	Neurogenesis (Lateral Inhibition)	Boundary formation. Oscillatory dynamics.
engrailed (en)	Segment boundary definition (posterior compartment); Neuroblast identity	Neuronal identity (ganglia); Segment boundary (*Platynereis*)	Midbrain-Hindbrain boundary; Interneuron identity; Somite differentiation (late)	Neuronal Specification / Regionalization	Regionalization by boundary formation.
Wnt (Wg)	Segment polarity (boundary maintenance); Posterior Growth	Posterior Growth; Segment polarity (*Platynereis*)	Posterior Growth (Tailbud); Segmentation clock regulation; D-V neural patterning	Posterior Growth Zone / Axial Elongation	Axis elongation. Boundary stabilization in tandem with *engrailed*.
Gap Genes *(Hb, Kr)*	Segmentation (spatial subdivision); Neuroblast temporal identity	Neuronal identity and organization (*Hb* only, *Kr* unknown)	Neural temporal identity	Temporal Identity in Neural Progenitors	Adding temporal component to spatial divisions of identity determination.

VIII. Conclusions

1. A complex, centralized nervous system arose before the segmented body plan, making segmentation a secondary elaboration that arose when those pre-existing neural GRNs were redeployed to regionalize the trunk. This is consistent with the “Brain first hypothesis” (Chipman, 2026). As Cambrian ecological complexity raised sensory and locomotor demands, the ancestral bilaterian brain was elaborated into a centralized processing center built on precise, repeated neural patterning networks.
2. A centralized nervous system seems to be a prerequisite for the development of segmentation, but not the reverse, as several unsegmented phyla have complex centralized nervous systems (e.g. cephalopods, flatworms).
3. The original simple centralized bilaterian nervous system is likely homologous across Bilateria, but the subsequent emergence of complex, centralized systems, and the segmented architectures they likely scaffolded, are a case of convergent evolution driven by environmental and functional necessity, using similar developmental toolkits.

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Figure 1: Schematic summary of the evolution of nervous systems and segmented body plans.