Received: 10 November 2017 | Revised: 20 December 2017 | Accepted: 21 December 2017

DOI: 10.1002/wdev.316

WILEY WIREs DEVELOPMENTAL BIOLOGY

ADVANCED REVIEW

Embryonic neurogenesis in echinoderms

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Funding information
NSF IOS, Grant/Award number: 1557431; NSF MCB, Grant/Award number: 1715721; Discovery Grant (NSERC Canada)

The phylogenetic position of echinoderms is well suited to revealing shared features of deuterostomes that distinguish them from other bilaterians. Although echinoderm neurobiology remains understudied, genomic resources, molecular methods, and systems approaches have enabled progress in understanding mechanisms of embryonic neurogenesis. Even though the morphology of echinoderm larvae is diverse, larval nervous systems, which arise during gastrulation, have numerous similarities in their organization. Diverse neural subtypes and specialized sensory neurons have been identified and details of neuroanatomy using neuron-specific labels provide hypotheses for neural function. The early patterning of ectoderm and specification of axes has been well studied in several species and underlying gene regulatory networks have been established. The cells giving rise to central and peripheral neural components have been identified in urchins and sea stars. Neurogenesis includes typical metazoan features of asymmetric division of neural progenitors and in some cases limited proliferation of neural precursors. Delta/Notch signaling has been identified as having critical roles in regulating neural patterning and differentiation. Several transcription factors functioning in pro-neural phases of specification, neural differentiation, and sub-type specification have been identified and structural or functional components of neurons are used as differentiation markers. Several methods for altering expression in embryos have revealed aspects of a regulatory hierarchy of transcription factors in neurogenesis. Interfacing neurogenic gene regulatory networks to the networks regulating ectodermal domains and identifying the spatial and temporal inputs that pattern the larval nervous system is a major challenge that will contribute substantially to our understanding of the evolution of metazoan nervous systems.

This article is categorized under:
Comparative Development and Evolution > Model Systems
Comparative Development and Evolution > Body Plan Evolution
Early Embryonic Development > Gastrulation and Neurulation

KEYWORDS
cellular signaling, deuterostomes, gene regulatory networks, larval nervous system, neurogenesis

1 | INTRODUCTION

Nervous systems are a prominent feature of an animal's morphology and behavior. They range in complexity from the nerve nets found in animals such as *Hydra* (Epp & Tardent, 1978) or *Nematostella* (Rentzsch, Layden, & Manuel, 2017), through to the extraordinarily complex and centralized nervous systems found in cephalopods (Abbott, Williamson, & Maddock,

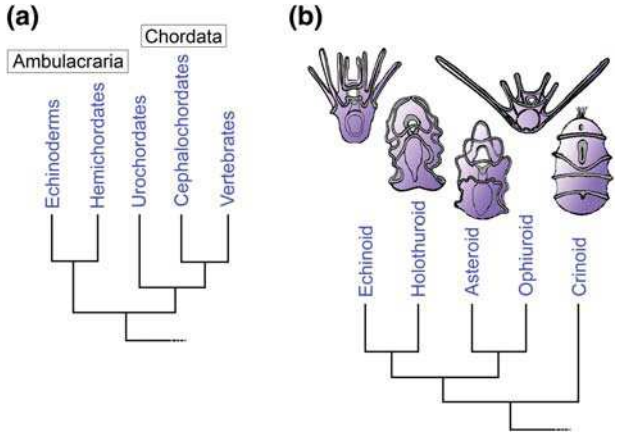

FIGURE 1 There are five classes of Echinoderm (Cameron, Garey, & Swalla, 2000; Smith et al., 2006), which all feature indirect development through a larval stage. Class Echinoidea (sea urchins) and Holothuroidea (sea cucumbers) are sister taxa, and along with Ophiuroidea (brittle stars) and Asteriodea (sea stars) comprise the grouping known as Eleutherozoa. The remaining class, Crinoidea (feather stars) are the clear outgroup. Although the disparity of the adults of these classes is well known, their larval forms are also morphologically very different. Larvae can be broadly characterized as plutei (i.e., the echinopluteus in the Echinoidea, and ophiopluteus in the Ophiuroidea) or dipleurula-like (i.e., auricularia in Holothuroidea and Crinoidea, or bipinnaria in Asteriodea). One of the most notable sources of morphological variation between these larval types stem from the presence, or not, of a biomineralized skeleton. Hemichordates when indirectly developing have a tornaria larva, which has many similarities to an echinoderm larva, and is one of the main features used to unite these taxa as Ambulacraria. (a) Deuterostome phylogeny. (b) Echinoderm classes with sketches of representative larval types

1995) and vertebrates. The developmental and evolutionary origins of nervous systems have thus been a source of intense interest. Perhaps no other grouping of animals is more intriguing than the deuterostomes. One branch of the deuterostome tree is the chordates, which includes the vertebrates, the other branch are the echinoderms and hemichordates, grouped together as the Ambulacraria (Figure 1a). The Ambulacraria, by comparison to their vertebrate cousins, have simpler, more diffusely organized nervous systems. Echinoderms, which as adults are radially symmetric and have radial nervous systems (Cobb, 1970, 1987), have often been viewed as atavistic (Box 1). In addition, the morphological diversity that exists even among the classes of echinoderms makes it difficult to draw comparisons with other animals. Thus, in spite of their phylogenetic position, echinoderms have proven challenging for understanding of the evolutionary origins, and common features, of deuterostome nervous systems.

The larval forms of echinoderms appear as morphologically diverse as the adults (Figure 1b). Yet, despite the outward morphological differences, a common feature of echinoderm larvae is a single oral territory that is circumscribed by a ciliary

BOX 1

ECHINODERM NEURAL ORGANIZATION

The echinoderm nervous system is probably the least well studied of all major phyla and persistent misunderstanding of echinoderm nervous systems has hampered discussions of neural evolution in deuterostomes. Their neuroanatomy is described by Hyman (1955) as "somewhat primitive" with "poor development of sensory organs." Often echinoderm neural architecture is described has being similar to neural organization in cnidarians (Hartenstein & Stollewerk, 2015). This is in part due to studies of neuroanatomy using reduced methylene blue to identify neurons, which are summarized by Smith (in Bullock & Horridge, 1965). These studies indicated that in echinoderms the epidermis is replete with what appeared to be bipolar sensory cells that contribute to a basiepithelial nerve net. However, these cells and the nerve net under the epidermis have not been substantiated by more precise molecular methods of identification of neurons (Burke et al., 2006; Diaz-Balzac, Lazaro-Pena, Vazquez-Figueroa, Diaz-Balzac, & Garcia-Arraras, 2016). Early studies of morphology provide the essential neuroanatomy, however, few conduction pathways are known because only a few neurophysiological studies have been reported. However, Cobb's neurophysiological studies of ophiuroid nervous systems established that sensory input in peripheral organs (tube feet, spines, and pedicellariae) project to ganglia within the appendages that integrate and control local motor responses (Cobb, 1987). Thus, like many invertebrate nervous systems, Echinoderm nervous systems appear to employ extensive peripheral integration and consequently have reduced central nervous systems. However, larval and adult nervous systems have conventional central and peripheral components similar to the nervous systems of other bilaterians.

band. The ciliary band is the principal swimming and feeding organ of larvae and the nervous system is closely associated with it in all larval types. In most echinoderm larvae, the ciliary band outlines the oral epidermis, a specialized epithelium that surrounds the mouth. In bipinnaria larvae of asteroids, the oral epidermis extends laterally and dorsally, and then fuses apically, producing separate pre-oral and post-oral bands (Lacalli, Gilmour, & West, 1990; Strathmann, 1971). All the larval groups have an anterior, bilaterally symmetric central nervous structure, the apical organ. Although, there are also specific differences in the neural organization of these apical organs across the classes, all contain distinctive clusters of serotonergic neurons (Byrne, Nakajima, Chee, & Burke, 2007). Descriptions of the neuroanatomy, stemming back from the beginning of the last century, noted commonalities. MacBride (1903), for instance, noted nerve fibrils extending from the anterior pole in Echinoids, which is derived embryonically from a thickened anterior ectoderm, suggested that these represented a similarity among the echinopluteus, bipinnaria and even tornaria larvae of the hemichordate. These similarities, among others, have been used to derive several hypotheses of the nature of the basal deuterostome and the origins of vertebrate nervous systems (Garstang, 1928), but for the most part, details of neuroanatomy and the localization of neurogenic transcription factors have not supported these ideas (Lacalli, 2005).

We now have available a suite of sophisticated molecular tools established in multiple species of echinoderms that are used to examine gene expression, gene function, and systems level interactions of gene regulatory networks (GRNs). This work was especially strengthened after the sequencing of the sea urchin, *Strongylocentrotus purpuratus*, genome in 2006 (Burke et al., 2006; Sodergren et al., 2006), and the Asteroid, *Patiria miniata*, genome several years later (Cameron, Kudtarkar, Gordon, Worley, & Gibbs, 2015). There is a substantial literature investigating neurogenesis and neural patterning in echinoderm larvae, in particular for species of sea urchins and sea stars, which are the most studied organisms. These analyses have revealed a striking similarity in mechanisms of neurogenesis and neural patterning among echinoderms and deuterostomes. Here we provide an overview of neurogenesis in sea urchins and sea stars, highlighting similarities and differences. We also identify key objectives that will lead to a better understanding of how neurons are precisely positioned in these embryos.

2 | NEURAL ORGANIZATION IN ECHINODERM LARVAE

The larval nervous systems of the echinopluteus and bipinnaria are comprised of neurons and interconnecting tracts of axons associated with the ciliary bands, the larval mouth, and the digestive tract (Beer, Moss, & Thorndyke, 2001; Bisgrove & Burke, 1987; Nakajima, Kaneko, Murray, & Burke, 2004). The neurons are dispersed through several tissues and the organization appears dissimilar to that of other bilaterians. Although larval nervous systems have been described as a nerve net (Byrne, Cisternas, & Koop, 2001; Holland, 2015a, 2015b), nerve nets function in diffuse, nondirectional activation of sheets of effector cells (Bullock & Horridge, 1965; Satterlie, 2011); which is not how echinoderm larval nerves appear to function. Structural details are more consistent with larval nervous systems having typical bilaterian central and peripheral components with afferent and efferent pathways. The apical organ has ganglionic organization, and in echinoids is derived from a distinctive neurogenic epithelium and is considered the central nervous system of the larva. The neurons of the ciliary band are derived from broad ectodermal domains and have the features of peripheral neurons. The larval nervous system is typical of echinoderm nervous systems in general, where peripheral neurons in appendages integrate and control local motor responses and central integration is minimal (Cobb, 1987).

In sea urchins, the apical organ is a bilaterally symmetric aggregation of neurons at the anterior end of the larva between the preoral arms. In the early larva, the apical organ is composed of 4–6 bilaterally positioned sensory cells containing serotonin, a central cluster of 10–12 neurons, and nonneural supporting cells (Beer et al., 2001; Nakajima et al., 2004). Neurons continue to accumulate in the apical organ throughout larval life (Beer et al., 2001). The apical organ is organized as a ganglion; an epithelium of neurons and support cells overlying a neuropil (Burke, 1978; Moss, Burke, & Thorndyke, 1994). In asteroids the apical organ extends across the anterior end of the larva and most of the cells are concentrated in ganglionic clusters on either side of the mouth, between the ciliary bands (Burke, 1983; Byrne et al., 2007; Chee & Byrne, 1999; Moss et al., 1994; Murabe et al., 2008). The bipinnarian apical organ contains 30–50 serotonergic cells, and as the brachiolar attachment organ develops, the entire complex dominates the anterior portion of the larva (Murabe et al., 2008). The structure of apical organs of urchins and sea stars indicate they likely serve to integrate and coordinate larval neural responses. The peripheral components are the neurons that are within the tissues that they appear to control—the ciliary band, mouth, and gut. In all planktotrophic echinoderm larvae, neurons are associated with the ciliary band and arrayed at intervals along the length of the ciliary band. In asteroid and echinoid larvae, there are a small number of cells that are flask shaped and have basal axons that project along the length of the ciliary band. In echinoids the axons are bundled in tracts, but in asteroids, the axons often form anastomosing plexi (Murabe et al., 2008). In plutei and in bipinnariae, a class of neurons, which have been described as interneurons, have their axis aligned along the length of the ciliary band (Lacalli et al., 1990; Nakajima et al., 2004). Despite these many similarities in these disparate larvae, there is a clear distinction in neural organization:

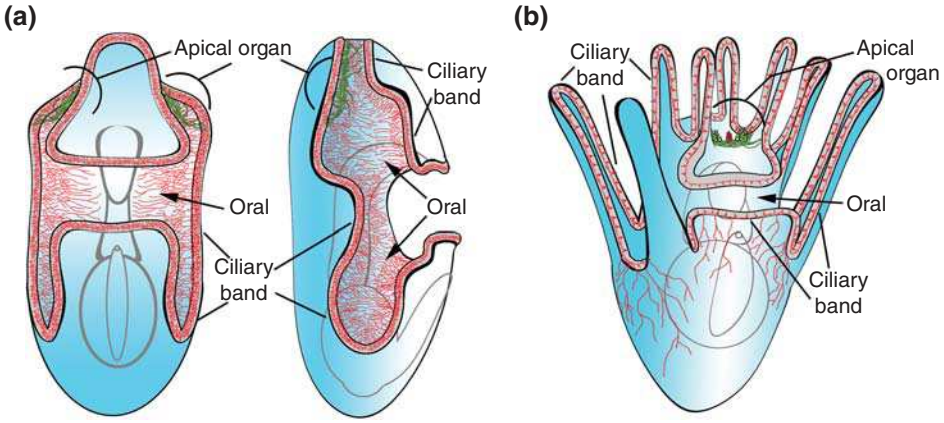

FIGURE 2 Simplified diagrams of the organization of larval nervous systems in bipinnaria (a, b) and a pluteus (c). The larval nervous systems have a small central ganglionic structure termed the apical organ, which is identified by serotonergic neurons (green), although neurons lacking serotonin are also present. There are extensive peripheral neurons and axonal tracts associated with the ciliary bands and extensive innervation of the esophagus and gut. In late stage sea star larvae, the attachment organ is extensively innervated. Key differences in neural organization relate to the neurites projecting from ciliary band neurons—in asteroids they extend underneath the oral epidermis toward the mouth, whereas in echinoids they extend posteriorly, beneath dorsal epidermis

BOX 2

NERVOUS SYSTEM FUNCTIONS

The ciliary band is the principal swimming and feeding organ of echinoderm larvae and it appears to be controlled by the nervous system. In feeding forms, the ciliary band outlines the sinuous perimeter of the larva or the edges of larval arms (Strathmann, 1971, 1975). Cilia of the ciliary band normally beat away from the mouth and provide propulsion, however, the cilia are able to reverse the direction of their effective stroke, and beat toward the mouth. As food particles approach the ciliary band, localized reversal of ciliary beat redirecting the particle toward the circumoral epithelium (Strathmann, 1971, 2007). Larvae use reversals of the direction of beat of the entire ciliary band and muscular contractions to change direction (Strathmann, 1971). During particle rejection, or obstacle avoidance, there is coordination of the direction of ciliary beating, esophageal musculature, and various other muscles (Strathmann, 1971, 1975). The reversal of direction of beat is associated with a biphasic depolarization of cells of the ciliary band (Mackie, Spencer, & Strathmann, 1969; Satterlie & Cameron, 1985). It is most likely that the recordings are of Ca++ potentials of the ciliated cells, and the function of the larval nervous system is likely one of evoking the ciliary reversal potentials. In the case of the localized reversal of feeding, neurons on the upstream side of the ciliary band with specialized branched microvilli, or elongate surface villi could potentially respond to presence of food particles to induce a localized reversal.

in plutei, axons from peripheral neurons project aborally, or away from the mouth, whereas in bipinnariae, ciliary band neurons project axons beneath the oral epidermis, toward the mouth (Figure 2). This difference in neural organization may be a reflection of the substantial differences in feeding and locomotion seen in urchin and sea star larvae (Strathmann, 1975) (Box 2).

3 | NEURONAL SUBTYPES AND SPECIALIZED SENSORY CELLS

Diverse neuronal subtypes in echinoderm larval nervous systems are reflected in the diversity and distribution of neurotransmitters. Although neurons release more than a single neurotransmitter, there appear to be some distinct neuronal subtypes that express different neurotransmitters in echinoderm larvae. The analysis of genomic data appears to give the fullest picture of the range of neurotransmitters employed, and there is supporting evidence for expression in subsets of echinoderm larval neurons (Burke et al., 2006).

Serotonin containing neurons of the apical organ are the best-studied neuronal cell type, principally owing to the availability of high quality antibodies. Serotonergic neurons are the first neurons to differentiate in most species and they form two clusters of neurons at the anterior end of developing larvae (Byrne et al., 2007). Larvae from all five classes have apical

organs and similar structures have been described for direct developing forms (Barbaglio et al., 2012; Bisgrove & Raff, 1989; Bishop & Burke, 2007; Dupont, Thorndyke, Thorndyke, & Burke, 2009; Hirokawa, Komatsu, & Nakajima, 2008; Katow, Elia, & Byrne, 2009; Nakano, Murabe, Amemiya, & Nakajima, 2006; Nakano, Nakajima, & Amemiya, 2009). The cells containing serotonin are variously described, but they appear to be primary sensory neurons with short apical dendritic poles and basal axonal projections.

The serotonergic neurons are most abundant in the apical organ and the lower lip of the larval mouth and esophagus and the number of cells increases throughout larval development (Bisgrove & Burke, 1987; Chee & Byrne, 1999; Murabe et al., 2008). Bipolar and multipolar neurons containing serotonin have also been described in most echinoderm larval forms.

Dopaminergic neurons have been identified only in echinoid larvae. The immunolocalizations of dopamine and dopamine associated enzymes is supported by hybridization studies using high-density oligonucleotide microarrays (Bisgrove & Burke, 1987; Wei, Angerer, & Angerer, 2006). Larvae express dopamine synthesis enzymes and at least four dopamine receptors (Burke et al., 2006). Dopamine localizes to neurons in the larval mouth and esophagus and to the postoral neurons, which are a set of neurons at the bases of the postoral arms. The postoral neurons appear distinct from the other neural types for several reasons: their location at the base of the arms in the oral epidermis, they express the transcription factor orthopedia during specification (Cavalieri, Spinelli, & Di Bernardo, 2003), and they differentiate earlier than other peripheral neurons (Burke, Moller, Krupke, & Taylor, 2014). The postoral neurons also appear to have distinctive functions, for example, Adams, Sewell, Angerer, and Angerer (2011) showed that dopamine is part of a neural pathway in which sensing algal density regulates larval arm length and thus can control feeding according to food supply.

In addition to monoamine neurotransmitters, there is evidence of GABAnergic neurons in echinoid plutei. Antibodies to GABA localize to neurons in the esophagus and mouth (Bisgrove & Burke, 1987) and glutamate decarboxylase has been reported to co-localize to serotonergic neurons within the ciliary band (Katow, Katow, Yoshida, Kiyomoto, & Uemura, 2016). Expression of glutamic acid decarboxylase and two GABA transporters was revealed by oligonucleotide array hybridization studies (Wei et al., 2006). Enzymes associated with acetylcholine synthesis and degradation are expressed in echinoid larvae (Wei et al., 2006) and there is evidence from neuropharmacological studies that acetylcholine agonists and antagonists regulate ciliary reversals and muscular contractions (Gustafson, Ryberg, & Treufeldt, 1972; Lacalli et al., 1990). Expression of catecholamines has been reported using histochemical methods, and although the genes necessary for synthesis and degradation are present in the genome, there is no evidence for expression in early echinoid larvae. Similarly, genes for histamine synthesis appear not to be expressed in early plutei, but there is immunolocalization data indicating histamine containing neurons in the apical organ, lateral ganglia and ciliary bands of plutei nearing metamorphosis (Sutherby et al., 2012).

Neuropeptides and their receptors are critical mediators of neuronal signaling that form evolutionarily related families with bilaterian origins (Jekely, 2013; Mirabeau & Joly, 2013). Echinoderm neuropeptides are diverse and many are expressed by neurons in larvae (Byrne & Cisternas, 2002; Moss et al., 1994; Semmens et al., 2016; Thorndyke, Crawford, & Burke, 1992). Sea urchin transcriptomes have at least 28 candidate neuropeptide/peptide hormone precursors and sea star transcriptomes have identified at least 40 candidate neuropeptides (Rowe & Elphick, 2012; Semmens et al., 2016). In plutei the SALMFamide peptide, S1 colocalizes with serotonin in the apical organ neuropil, but has a broader distribution in several other tissues, suggesting diverse functions in the larval nervous system (Thorndyke et al., 1992). Additional localizations of neuropeptides identify distinct subpopulations of neurons and suggest there are distinct subsets of neurons defined by neuropeptides.

Ultrastructural studies have identified several specialized cells thought to function as sensory cells (Burke, 1978, 1983; Lacalli et al., 1990). Many of the neurons in the apical organ and the ciliary bands have the form of primary sensory neurons. The neurons have short apical, dendritic poles, project axons basally into axonal tracts or neuropil, and are distinct from bipolar and multipolar inter-neurons. The apical dendritic projections of sensory cells are varied. In plutei and bipinnaria cells sensory cells with short, branched microvilli are situated on the oral, or upstream margin of the ciliary band (Burke, 1978, 1983). Lacalli et al. (1990) provide detailed descriptions of multipolar neurons that are distributed throughout the ciliary band. These cells have extensive apical projections in sea star larvae there are 4–6 slender projections that span the width of the ciliary band and extend several cell diameters. The projections are on the surface of the cells at the base of the microvilli, beneath the hyaline layer (Lacalli et al., 1990; Lacalli & West, 1993). In echinoplutei there are similar projections that extend along the length of the ciliary band (Katow et al., 2016; Lacalli & West, 1993; Nakajima, 1986a). In addition to microvillar projections, sensory cells with modified cilia have been described in plutei. Nakajima (1986a, 1986b) described cilia associated with the apical organ that are coiled, lie underneath the hyaline layer and have axonemes lacking dynein arms.

Bishop has described neurons within regions of the ciliary band on the ventral surface of echinoplutei suggested to function in olfaction (Bishop & Brandhorst, 2007; Bishop & Hall, 2009). The neurons contain nitric oxide synthase, the enzyme responsible for formation of the gaseous cellular signaling molecule, nitric oxide. The neurons are within the ciliary bands of vibratile lobes, arm-like extensions of the ciliary band that lack skeletal elements. The neurons arise late in larval life, and

project axons to the apical organ. Removal of the vibratile lobes or inhibition of nitric oxide synthesis suggests these neurons may function in detecting olfactory stimuli that mediate metamorphosis.

Sea urchin larvae have bilateral clusters of ciliary photoreceptors anterior to the mouth, adjacent to the apical organ (Valero-Gracia, Petrone, Oliveri, Nilsson, & Arnone, 2016). Small clusters of neurons express opsin3.2 on short, nonmotile, apical cilia and the cells increase cytoplasmic calcium in response to light stimulation. Pigment cells on the dorsal surface are suggested to provide shading pigmentation that may facilitate direction photoreception (Valencia et al., submitted).

Although most of the studies of neuronal subtype diversity have been on echinoid larvae, it seems reasonable to conclude that all echinoderm larvae have diverse neuronal cell types that sense various aspects of the larval environment and coordinate a range of larval behaviors. The diversity of subtypes and their nonrandom distribution is central to questions of neurogenic patterning and studies of neurogenesis in echinoderms will provide novel insights into the molecular mechanism of their formation and positioning.

4 | THE ANIMAL VEGETAL AXIS AND THE SPECIFICATION OF THE ANIMAL POLE DOMAIN

The work of many researchers has revealed the mechanisms of axis formation in various species of echinoderms. Our summary infers that despite the morphological differences and deep time of divergences of these larvae, there are many similarities in the mechanism of axial patterning. This summary also notes several commonalities between echinoderms and other animals.

The larvae of echinoids and asteroids have an animal-vegetal axis regulated by an interplay between Wnt and Wnt antagonists and a dorsal-ventral axis regulated by TGFβ signaling pathways; Nodal and BMP. The animal-vegetal axis, which becomes the embryonic anterior–posterior axis, of the primary axis. In both taxa, β-catenin is nuclearized in the vegetal pole of the early embryo (Logan, Miller, Ferkowicz, & McClay, 1999; McCauley, Akyar, Saad, & Hinman, 2015; Miyawaki et al., 2003), by cortical localization of Disheveled in the sea urchin (Weitzel et al., 2004), and by a yet unknown mechanism in sea stars. Several Wnt ligands are subsequently expressed in overlapping vegetal territories by blastula formation in urchins and sea stars (McCauley, Akyar, Filliger, & Hinman, 2013; Stamateris, Rafiq, & Ettensohn, 2010). Canonical Wnt (cWnt) signaling is needed for the specification of the site of gastrulation and thus cWnt is also needed for the specification of endoderm and mesoderm. Wnt signaling is key to specification of the primary axis in many metazoans and may represent a deep homology (Niehrs, 2010). Short range Wnt5 signaling from the endoderm then specifies the endoderm/ectoderm boarder region, which induces expression of several transcription factors, including, *nk1* and *pax2/5/8* (McIntyre, Seay, Croce, & McClay, 2013). It is not known whether this mechanism exists in sea star, but expression of *wnt5*, *nk1* and pax2/5/8 is expressed similarly in the two taxa (McCauley et al., 2013; Yankura, Martik, Jennings, & Hinman, 2010). The remaining more anterior ectoderm is a Wnt negative zone, and therefore when cWnt signaling is experimentally abrogated, the resultant embryos fail to gastrulate, do not develop endoderm or mesoderm, and form permanent blastulae in which the neuroectoderm is expanded and unpatterned (Cheatle Jarvela, Yankura, & Hinman, 2016; Logan et al., 1999; Range, Angerer, & Angerer, 2013; Yaguchi, Yaguchi, & Burke, 2006).

A series of elegant experiments in sea urchins, explains the regulatory and signaling interactions that further subdivide the primary axis (Range & Wei, 2016). Posterior Wnt signaling (notably Wnt 8 and Wnt1) establish a posterior to anterior gradient, which activates Frzl5/8 and Jnk in the more anterior ectoderm Range et al., 2013). pJNK in turn functions to suppress the expression of animal pole domain (APD) markers, for example, the transcription factors *foxq2* and *six3* from the posterior. The expression of these genes in the anterior, APD, is needed to activate the GRN for neurogenesis in the apical organ (Range & Wei, 2016). Fzl5/8 in turn activates the Wnt inhibitor dkk1, which limits the activity of Wnt signaling anteriorly, while Foxq2 positively regulates the diffusible sFRP1/5 and dkk3 Wnt inhibitors. Thus, a series of positive–negative feedback loops are used to establish apposing anterior and posterior territories along the primary axis, which leads to the precise control of the APD boundary, and the specification and positioning of the anterior animal pole domain and apical organ.

In sea stars, the expression of orthologous Wnt and Fzl genes is similarly positioned along the AP axis (McCauley et al., 2013). *Foxq2* is expressed in the anterior most ectoderm, where it is needed to activate the expression of *dkk3*, and is necessary for the formation of serotonergic neurons (Cheatle Jarvela et al., 2016). These data are therefore consistent with sea star APDs being established through interplay between Wnt and Wnt antagonists although the specific interactions observed in sea urchins have not been tested. Differences in patterning between these embryos however, are seen in specifics of transcription factor expression and some of their interactions. For example, in sea urchins, *foxq2* and *six3* are initially co-expressed, and Six3 maintains the expression of foxq2 (Wei, Yaguchi, Yaguchi, Angerer, & Angerer, 2009). As development proceeds, Foxq2 functions to repress *six3*, so that their expressions resolve into an inner territory of *foxq2* surrounded by an outer ring of *six3* (Range & Wei, 2016) (Figure 3). In sea stars, *foxq2* and *six3* remain co-expressed in the APD, although *six3*

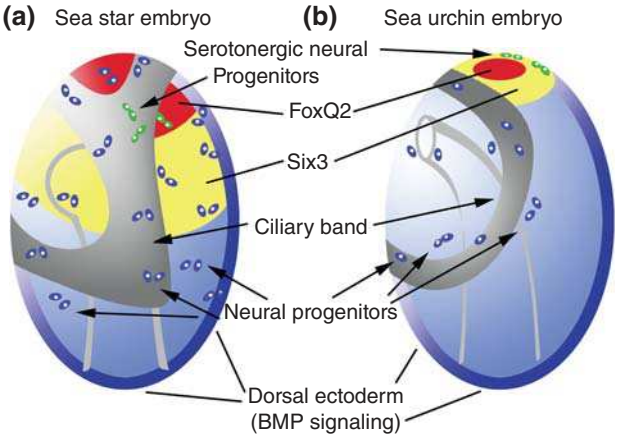

FIGURE 3 Sites of neurogenesis mapped onto domains of gastrula stage ectoderm. A field of ectoderm surrounding the mouth (ventral ectoderm) circumscribed by a ciliary band is a shared feature of the two larval forms. The concentric domains of FoxQ2 and Six3 expression define the animal pole domain and the ectoderm that has activated BMP signaling (dorsal ectoderm) is identified. Neural progenitors are identified by expression of SoxC or Brn1/2/4. Serotonergic neural progenitors arise in the animal pole domain and peripheral neural progenitors arise outside this domain. In sea urchins, neurons arise in ectoderm that has not been influenced by BMP signaling, whereas in asteroid embryos, neurons appear to arise throughout the ectoderm

expression extends further into the posterior domain (Yankura et al., 2010). There are no identified regulatory interactions between Foxq2 and Six3; as *six3* expression remains in a Foxq2 morphants, and vice versa (Cheatle Jarvela et al., 2016). There is a further difference in the expression of *rx*, which in sea stars is expressed in an additional domain, that extends beyond that of *foxq2* but is within that of *six3*. It has been speculated that neural precursors may proliferate to produce additional neurons in this *rx+, foxq2−* domain, whereas in the *rx+* and *foxq2+* zone neural precursors appear to differentiate (Cheatle Jarvela et al., 2016). In urchins *rx* is expressed in the animal pole domain (Burke et al., 2006), but it is unclear if *rx* regulates neurogenesis.

A future direction of research will be to determine the phenotypic consequences of the differences in the regulatory interactions of APD transcription factors in sea urchins and sea stars. Lessons from work comparing endomesoderm GRNs between sea urchins and sea stars indicate that some network rewiring may have little or no consequence for later gene expressions and functions, and can reflect a type of neutral, or compensatory evolution (Hinman & Davidson, 2007; McCauley, Weideman, & Hinman, 2010). As an example, change in regulation between Six3 and Foxq2 may have minor functional consequence, if other mechanisms maintain *foxq2* expression in the APD in sea stars. Conversely some of the differences in the size of the embryonic territories, for example, the large domain of *rx* expression in sea stars, has been suggested to provide a larger territory for progenitor proliferation (Cheatle Jarvela et al., 2016). Many of the common features observed among echinoderms are also present in axial organization and neural specification in other animals. Foxq2 is not present in vertebrate genomes, yet appears to have an common role in anterior neural specification and in maintaining a Wnt free zone in many invertebrates (Kitzmann, Weisskopf, Schacht, & Bucher, 2017; Marlow et al., 2014; Santagata, Resh, Hejnol, Martindale, & Passamaneck, 2012). In vertebrates, Wnt and Wnt antagonist interactions regulate formation of the anterior neuroectoderm through many of the same orthologs described in echinoderms (Range et al., 2013). Therefore broad functional regulatory mechanisms that establish primary axis, and consequently specification of neuroectoderm found throughout metazoa, are features of neurogenesis in echinoderm embryos.

4.1 | The dorsal ventral axis and the positioning of the ciliary bands

Significant neural patterning is also observed along the dorsal-ventral axis. In all echinoderm larvae, the ventral domain is marked by the formation of the mouth, which can be identified by mid-gastrula when the archenteron begins to lean toward this territory. Thus, oral ectoderm is referred to as ventral ectoderm and aboral ectoderm is referred to as dorsal ectoderm. The ciliary band is an obvious morphological feature that is patterned along the dorsal-ventral axis. In sea urchins, the ciliary band is a thickened strip about four cells wide that loops around the mouth and lies at the junction between the ventral and dorsal ectodermal territories. Neurons differentiate within the ciliary band (Garner et al., 2016; Nakajima et al., 2004) and project neurites within the ciliary band and toward the dorsal ectoderm (Figure 2). Molecularly, the band is marked by the expression of transcription factors such as *foxg* and *onecut* (Poustka et al., 2004; Tu, Brown, Davidson, & Oliveri, 2006). Sea star embryos have two loops of ciliary band, a preoral band which loops anteriorly, thereby enclosing an anterior-ventral ectoderm, and a band that crosses under the mouth and loops dorsally over the anterior pole (Figure 2). *Foxg* is first expressed in a broad oral ectoderm region and during gastrulation expression extends dorsally and toward the anterior pole (Yankura, Koechlein, Cryan, Cheatle, & Hinman, 2013). Expression then becomes restricted to two bands at the edges of this region. Sea star neurons associate with the ciliary band, but project neurites within the ciliary band and toward the mouth. Thus, sea urchins and sea stars form a ciliary band around an oral ectodermal field, but because of the complex *foxg* patterning in sea stars, the appearance of the ciliary bands appears dissimilar in larval stages.

The TGFβ gene, *nodal*, is expressed in the territory of the future mouth during cleavage stage in sea urchins (Duboc, Rottinger, Besnardeau, & Lepage, 2004) and at least by blastula in sea stars (Yankura et al., 2013), thus preceding emergence of morphological makers of the axis. In sea urchin, *nodal* expression is restricted to the ventral ectoderm through a combination of redox-sensitive and insensitive responses. Mitochondria are asymmetrically localized in eggs and are suggested to produce a redox gradient (Coffman, Coluccio, Planchart, & Robertson, 2009; Range et al., 2007), which may then lead to the asymmetric stabilization of the hypoxia inducible factor HIFα, which restricts *nodal* ventrally (Chang et al., 2017). Additionally, the maternal TGFβ ligand, Panda (Haillot, Molina, Lapraz, & Lepage, 2015) localizes dorsally to restrict *nodal* to ventral ectoderm. The mechanism of *nodal* restriction in sea stars remains unknown. Thus, unlike chordates where *nodal* is expressed dorsally, nodal in sea stars and sea urchins is localized to ventral ectoderm.

In sea urchins, Nodal then autoregulates and activates the expression of its own antogonists, BMP2/4, lefty, *chordin,* and *admp* (Chang et al., 2016; Duboc et al., 2004; Lapraz, Besnardeau, & Lepage, 2009; Lapraz, Haillot, & Lepage, 2015). Nodal also activates the key transcription factors needed for mouth formation, *goosecoid* and *foxa.* Whereas BMP2/4 is expressed in the ventral ectoderm, BMP2/4 protein localizes to dorsal ectoderm, possibly after being shuttled by Chordin (van Heijster, Hardway, Kaper, & Bradham, 2014). Nodal therefore acts to establish ventral and dorsal territories. Further dissection of these interactions in sea urchins, reveals an exquisite mechanism by which a Nodal (ventral) and BMP2/4 (dorsal) establish a network of activators and repressors producing ventral to dorsal gradients of TGFβ proteins across the ectoderm (Duboc et al., 2004; Lapraz et al., 2015; Saudemont et al., 2010; Yaguchi, Yaguchi, Angerer, Angerer, & Burke, 2010). It is thought that this interplay between molecules operating on the ventral and dorsal ectoderm sets up a boundary in which the ciliary band can form (Yaguchi et al., 2010). A knock down of Nodal leads to the formation of the ciliary band domain throughout the ectoderm, presumably because ciliary band formation is the default regulatory state, when no dorsal-ventral patterning occurs. Knock down of only BMP2/4 leads to a loss of dorsal ectoderm, and an expansion of the ciliary band into this territory. A recent experiment, takes this model as step further. Overexpression of *nodal* in one dorsal blastomere reorganizes the embryo into conjoined twins, with complete duplication of two ventral sides, two fused dorsal sides, and two ciliary bands (Lapraz et al., 2015). This suggests an organizer like property for Nodal, and has been used to support the idea of that the Nodal territory in sea urchin acts akin to the vertebrate Spemann organizer (Lapraz et al., 2015).

What then of the formation of the ciliary bands in sea stars? Many of these TGFβ genes are similarly expressed; *nodal*, *lefty*, and *BMP2/4* are expressed in ventral ectoderm by blastula-stage (Yankura et al., 2013), and just as in sea urchins, the BMP2/4 effector, pSmad1/5/8 is localized in the dorsal ectoderm, consistent with BMP2/4 functioning in the dorsal. The regulatory genes expressed in the single sea urchin ciliary band, *foxg* and *onecut*, are also expressed in the oral domain that forms the two sea star ciliary bands (Yankura et al., 2013). Knockdown of Nodal function also leads to a loss of *foxa* and *goosecoid*, and significantly, to loss of *foxg* expression. Hence, the ciliary band does not form in these embryos (Yankura et al., 2013). This again is in contrast with sea urchins where Nodal knockdown leads to an expansion of the ciliary band throughout the ectoderm (Duboc et al., 2004; Saudemont et al., 2010; Yaguchi et al., 2006; Yaguchi et al., 2010). An explanation for this difference comes from the ontogeny of *foxg* expression. In sea stars, *foxg* is first expressed in the ventral ectoderm, co-incident with nodal expression, and then expands dorsally and anteriorly to form a broad territory, which only by late gastrulation will resolve into two ciliary bands—one forming at each edge of *foxg* expressing territory. Thus, the marker of ciliary bands is initially expressed in the ventral ectoderm, and may initially require Nodal function. BMP2/4 knockdown leads to radialization and formation of two ciliary bands, which circumscribe the ectoderm (Yankura et al., 2013). This may be viewed as a loss of the dorsal-most ectoderm and similar to the phenotype in sea urchin in which BMP2/4 knockdown expands the ciliary band into the dorsal.

Understanding how opposing gradients of TGFβ proteins are formed will require understanding how the networks of inductive and repressive interactions form, which will require a knowledge of the biochemical properties that allow diffusion and stabilization of these proteins. A further challenge is to understand how these networks and gradients evolved; that is, what properties of the proteins and their interactions lead to changes in the gradients. Finally, unraveling how *cis* regulatory modules of the key transcriptional factors interpret these signals, and how *cis* regulatory modules evolve will be critical for understanding how ectodermal patterning has changed through time. As we show for the anterior–posterior axis, there are also common features of dorsal-ventral axis formation in echinoderms that are found broadly throughout the metazoans. Although there is an inversion between molecules on the dorsal and ventral axis (Bier & De Robertis, 2015), Nodal and BMP signaling also shapes neurogenic territories across the dorsal-ventral axis of vertebrates.

5 | A GENE REGULATORY NETWORK THAT PATTERNS ECTODERM

Researchers using sea urchins and sea stars have been at the forefront of the emerging trend to elucidate GRNs that explain developmental processes (reviewed in Cary & Hinman, 2017; Davidson, 2009). GRNs portray all known regulatory

interactions between transcription factors and their target genes, and regulation by signaling pathways, to provide a systems-level description of a given developmental process. Studies of GRNs in sea urchins initially focused on understanding the specification of the embryonic endomesoderm. This GRN is now sufficiently complete that a Boolean model of the network predicts outcomes of specific perturbations (Peter, Faure, & Davidson, 2012). GRNs explaining specification of the ectoderm are less well known, but there have been efforts by several groups to generate these models, which are beginning to explain aspects of the mechanisms that establish and delineate ectodermal territories (Cui, Siriwon, Li, Davidson, & Peter, 2014; Li, Cui, Peter, & Davidson, 2014; Saudemont et al., 2010). It is these territories that are the source of cells that give rise to neurons.

Members of the TGFβ and Wnt signaling pathways serve as anchor points for ectodermal GRNs as these molecules establish a coordinate system. Saudemont et al. (2010) performed an extensive analysis of gene expression when a variety of Nodal and BMP pathway components are perturbed. Their work shows how feedback circuits stabilize Nodal and BMP function, and activate the transcription factor *gsc* in the oral, and *tbx2/3* in the dorsal ectoderm (Ben-Tabou de-Leon, Su, Lin, Li, & Davidson, 2013; Saudemont et al., 2010). *Gsc* and *tbx2/3* are repressors, and thus are predicted to activate specification of the ventral or dorsal territories through repression of a more global repressor. A similar double negative gate operates to specify the skeletogenic lineage (Oliveri, Tu, & Davidson, 2008; Revilla-i-Domingo, Oliveri, & Davidson, 2007). Such double negative gates are predicted to activate the expression of many genes in a coordinated way through simultaneous relief from repression. This, however, has not been formally demonstrated for these repressors in the ectodermal GRN.

The goal of a GRN is to explain how maternally deposited regulatory molecules control the activation of regulatory genes, and how over time this leads to the expression of distinct sets of genes in each cell type. Indeed, the cell type can be defined by the set of expressed regulatory genes, or regulatory state of a cell. A GRN thus explains how the regulatory state of a cell changes over time. The work of several groups (Howard-Ashby et al., 2006; Li et al., 2014; Li, Materna, & Davidson, 2013; Su et al., 2009) examining the expression of many transcription factors from blastula through gastrula have revealed a surprising regulatory state complexity. For example, the sea urchin ectoderm (not including the most posterior ectoderm) could be partitioned into at least six territories by early gastrula-stage, including apical, lateral animal, animal aboral, near apical, central, and stomodeal (Li et al., 2014). These do not necessary correspond to known morphological territories, although it is assumed that cells with different properties are formed in these molecularly distinct territories. Perturbation and time series expression data reveal how these territories are formed, and critically the mechanisms that lead to the reinforcement of territory boundaries that are thought to be needed for their precise, robust partitioning (Li et al., 2014). For instance, the transcription factor Not is expressed downstream of Nodal, and represses *foxq2* in the oral ectoderm. Another transcription factor, Emx represses *foxq2* in the lateral ectoderm. Foxq2 in turn represses *emx* and *nodal* expression (and by extension *not*). This dual repression serves to sharpen and reinforce the boundary between the *foxq2*+ apical ectoderm and adjacent ectodermal types, thereby reinforcing this boundary and controlling the size of the apical ectoderm where neurons will form.

While still a work in progress, the ectodermal GRN begins to explain how the signaling pathways first carve out broad territories across the ectoderm and how these are further refined and reinforced by the interactions between transcription factors. In particular, the repressors are used to refine boundaries and to coordinately activate sub territories downstream of the signaling. This will eventually provide an understanding the regulatory state of the neural precursors that form in these territories.

6 | ASYMMETRIC DIVISION OF NEURAL PROGENITORS AND NOTCH SIGNALING

Neural progenitors in sea urchins and sea stars are identified by expression of the transcription factor SoxC (Cheatle Jarvela et al., 2016; Garner et al., 2016; Mellott, Thisdelle, & Burke, 2017; Wei, Angerer, & Angerer, 2016; Yankura et al., 2013) (Figure 3). The distribution of cells expressing SoxC provides an indication of the regions that are neurogenic. In sea urchins, SoxC expressing cells are clustered in the animal pole domain and apical organ. There are also SoxC expressing cells scattered throughout the ventral ectoderm and in the dorsal ectoderm adjacent to the ciliary band (Garner et al., 2016). Wei, Angerer, and Angerer (2011) demonstrated that neural progenitors also arise in the foregut and give rise to neurons associated with the esophagus and gut. Thus, the only region of the sea urchin embryo that is not neurogenic appears to be the dorsal ectoderm, the tissue most clearly affected by BMP signaling. The patterning of neurogenic domains in sea urchin embryos is sensitive to manipulations of BMP signaling (Saudemont et al., 2010; Yaguchi et al., 2010). In sea star embryos, *soxC* is expressed in gastrula stage embryos in cells scattered throughout the ectoderm. The cells expressing *soxC* are characteristically in pairs that are more abundant in the anterior part of the embryo (Cheatle Jarvela et al., 2016; Yankura et al., 2013). Thus in sea star embryos, some neural progenitors form in regions of high BMP2/4. As neural progenitors differentiate, there is an apparent redistribution as cells expressing markers such as *ELAV* or *synB*; the neural precursors align along

the edge of the ciliary bands (Nakajima et al., 2004; Yankura et al., 2013). Similarly, in sea urchins neurons differentiate in the ciliary bands and in the animal pole domain, suggesting that in both forms neural progenitors migrate prior to differentiation.

A key aspect of neurogenesis relates to the cell divisions of neural progenitors (Hartenstein & Stollewerk, 2015). The patterns vary widely, but commonly there is an asymmetric division in which a neural progenitor divides and one of the resulting cells differentiates as a neuron. In some situations the second cell retains neurogenic potential and serves to renew the progenitor. In addition, the neural progenitor may undergo one or more proliferative divisions before differentiating. In many bilaterians there are dedicated self-renewing neural progenitors, which persist throughout development of the nervous system.

There are several lines of evidence in sea urchins that indicate neural progenitors expressing SoxC are mitotically active. Wei et al. (2016) used an antibody that identifies cells in prophase and anaphase and Garner et al. (2016) used a short pulse of a thymidine analogue (EdU) and concluded that some neural progenitors are dividing. Garner et al. (2016) used birthdating with EdU pulses to determine the stage at which identified neurons completed their last cell cycle. The first serotonergic neurons to differentiate in the animal pole domain complete their final cell division as late blastulae. In sea star embryos short pulses of EdU will label cells expressing *soxC* or *lhx2/9* (Cheatle Jarvela et al., 2016). Lineage tracing of neural progenitors indicate they divide asymmetrically. In sea urchins a *soxC:GFP* transgene was used to determine the fate of progeny of the final division (Mellott et al., 2017). Some neural progenitors appear to divide giving rise to one cell that persists and a second cell that undergoes apoptosis. In sea star embryos, pairs of cells are interpreted to be the product of a recent cell division and in some instances one of the pair of cells in the animal pole domain can be labeled with EdU (Cheatle Jarvela et al., 2016). Additionally, in pairs of cells expressing *soxC*, one of the two cells can also expresses *lhx2/9*. Instances were identified in which pairs of cells express *lhx2/9* and only one of the cells express the differentiation marker *ELAV*. Thus, in echinoderms neural progenitors in the animal pole domain and in the peripheral nervous system appear to undergo asymmetric divisions. The sequence of divisions in the animal pole domain appears to be more complex. The neuroectoderm of the animal pole domain is patterned as concentric rings of cells and the resulting apical organ has bilateral symmetry and in urchins, dorsal-ventral patterning. Thus, it is possible that within the animal pole domain there are dedicated neural progenitors, although at this time we lack direct evidence for their existence. Neural progenitors for the peripheral nervous system are suggested to arise directly from the larval epidermis, which is speculated to be in a regulatory state that has the potential to become neurogenic (Mellott et al., 2017). As SoxC localizes to epidermal cells throughout larval life, it is suggested that patches of epidermal cells respond to localized signaling and initiate neurogenesis.

Notch signaling during early neurogenesis is a conserved feature in many bilaterians (Artavanis-Tsakonas, Rand, & Lake, 1999). *Delta* is expressed in the animal pole domain and in cells scattered throughout the ventral ectoderm and ciliary band of sea urchin embryos (Lapraz et al., 2009; Rottinger et al., 2006; Saudemont et al., 2010). In sea star embryos *Delta* expression is in scattered cells during early neurogenesis, consistent with expression in neural progenitors. DAPT, which inhibits γ-secretase and Notch signaling, has been shown to increase the number of neurons in the endoderm and animal pole domain of sea urchin embryos (Wei et al., 2011; Yaguchi et al., 2011; Yaguchi, Angerer, Inaba, & Yaguchi, 2012). As well, Yaguchi et al. (2012) report that a morpholino that suppresses *Delta* expression produces supernumerary serotonergic neurons. Similarly, in sea star embryos, injection of a morpholino that suppresses *Delta* expression results in an increase in the number of pairs of neural progenitors (Yankura et al., 2013). Mellott et al. (2017) examined the role of Notch signaling in urchin neurogenesis and demonstrated that inhibition of γ-secretase, injection of Sp-Delta morpholinos, or CRISPR/Cas9-induced mutation of Sp-Delta results in supernumerary neural progenitors and neurons. They also used a Notch reporter to show Notch signaling is activated in cells adjacent to cells expressing SoxC. By examining the initial stages of SoxC expression they concluded that Notch signaling has a role in restricting the number of neural progenitors recruited, most likely through a mechanism of classical lateral inhibition. In addition, their data indicates a second distinct function of Notch signaling in regulating the fate of progeny of the asymmetric division. Suppression or enhancement of Notch signaling indicates a role in determining which of the progeny of the final mitosis will become a neuron (Mellott et al., 2017). Thus, Delta/Notch signaling, functioning at different levels, regulates and patterns neurogenesis, and may provide a sufficient mechanism to explain the appearance of scattered neural progenitors in broad ectodermal domains.

7 | LINKING ECTODERMAL PATTERNING WITH NEUROGENESIS

Echinoderms have proven to be useful models for experimentally determining GRNs, which has enabled detailed comparisons that have changed how we think about evolution and development. GRN studies to date have focused on understanding progressive subdivision of regions of the embryo, most recently how domains of ectoderm become different, and boundaries become distinct (Ben-Tabou de-Leon et al., 2013; Li et al., 2014; Su et al., 2009; Li et al., 2013; Howard-

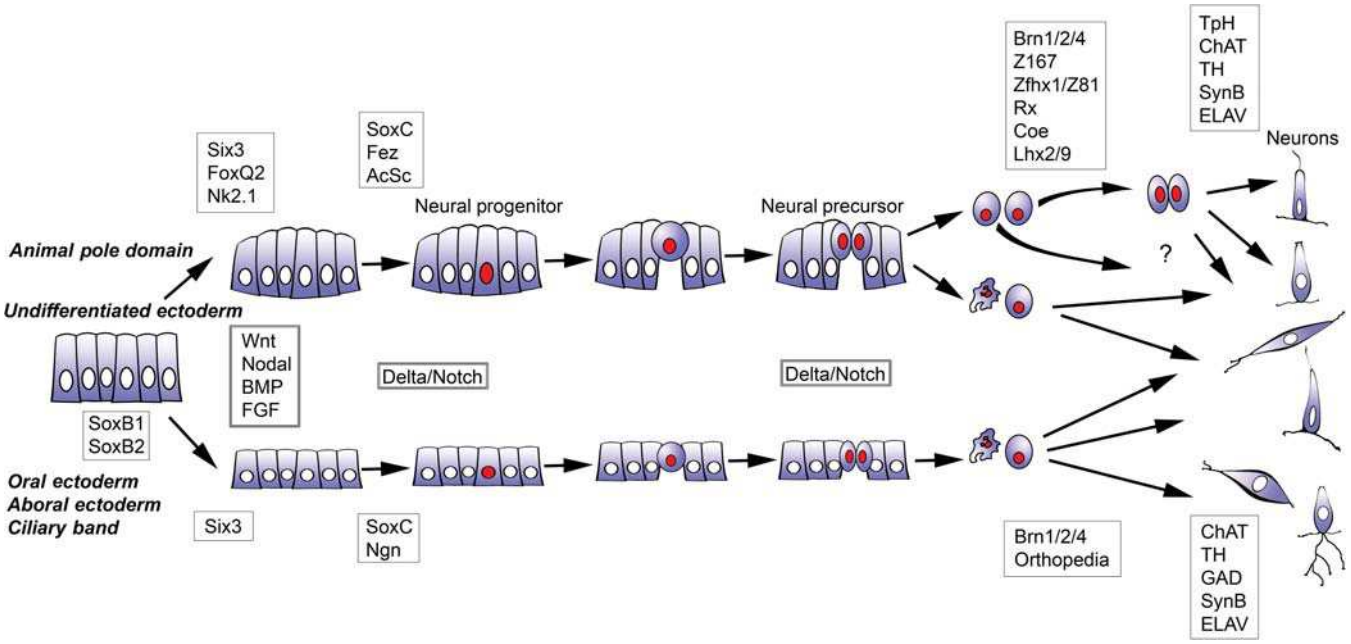

FIGURE 4 Diagrammatic summary of the developmental sequence of neural specification, asymmetric divisions, and differentiation in echinoderms. Domains with distinct regulatory states are established for the animal pole domain (top row) and other domains of ectoderm (bottom row). Neural progenitors are specified (red nucleus) and neural progenitors initiate an asymmetric division. In the animal pole domain, asymmetric divisions may produce progenitors that also divide, or division give rise to a neuron and an apoptotic cell. In other regions of ectoderm, divisions appear to give rise to only a single neuron. Throughout the specification and differentiation a gene regulatory network controls the expression of a series of regulatory genes that control neurogenesis (single box). Cellular signaling (double box) mediates and regulates specification of ectodermal domains and restricts neurogenesis. Diverse neuronal subtypes, with stereotypic positions and patterns result

Ashby et al., 2006; Saudemont et al., 2010). It is also clear that GRNs mediate specification of neurons (Figure 4). Wei et al. (2009) identified a large number of regulatory genes expressed in the animal pole domain of sea urchins and showed that expression of most of these genes is dependent on expression of Six3. Six3 expression is necessary for neurogenesis throughout the embryo and sufficient to expand a patterned animal pole domain. SoxC and Brn1/2/4 were subsequently identified as Six3 dependent genes that function sequentially and are required for neurogenesis (Garner et al., 2016; Wei et al., 2016). Serotonergic neurons are the only subtype for which there is data, but it is clear that the regulatory state of differentiating serotonergic neural progenitors involves genes such as *z167* and *zfhx/Z81*. A similar set of genes has been identified in sea star animal pole domain neurogenesis in which *six3*, *foxq2*, *rx*, *SoxC*, and *lhx2/9* have been shown to be hierarchically necessary for specification and differentiation of serotonergic neurons (Cheatle Jarvela et al., 2016; Yankura et al., 2013). Although details of the epistatic relationships and direct interactions of the regulatory elements are not yet available, it is clear that neurogenesis in echinoderms is mediated by a GRN that shares several key genes. In addition, the neurogenic GRN of echinoderms shares many features of the neurogenic GRNs of bilaterians and chordates, as many of the genes expressed in the animal pole domain have counterparts expressed in early development of the vertebrate forebrain and eye field (Wei et al., 2009).

A key question is how do the GRNs that pattern ectoderm interface with the GRNs regulating neurogenesis. We know from sea urchins that the initial patterning of neurogenesis is achieved in part through Wnt-mediated down-regulation of neurogenic potential along the anterior–posterior axis. The resulting patterning of the animal pole domain clearly regulates aspects of neurogenesis in sea star embryos (Cheatle Jarvela et al., 2016). However, we know only a little of the regulatory state of cells in the animal pole domain and the diverse regions of ectoderm that give rise to peripheral neurons. The ectoderm is patterned into several regulatory states, yet there is no direct correspondence of ectodermal territory and formation of neurons. Different neural cell types appear to form in a single ectodermal territory, and similar neural cell types appear to form in different ectodermal territories. Potentially, neurogenesis is a direct consequence of the regulatory state of a region of ectoderm and localized signaling restricting neurogenesis, providing the spatial information to specify subtype, or guiding migration of subtype precursors. An implication of this is that that the regulatory state of a neural progenitor, plus the regulatory state of surrounding cells cooperatively regulates the genes that specify subtypes of neurons. New themes will emerge from these GRN studies, as this situation is quite different from studies in which large domains of contiguous cells acquire a similar developmental identity. As these are a common developmental process for almost all metazoans, studies of the GRNs in echinoderm neural specification are likely to be enormously fruitful.

8 | COMPARING ECHINODERM NEUROGENESIS

In spite of the overall morphological disparity of echinoderms, and a nervous system that appears unrelated to chordate nervous systems, there are many underlying similarities in ectodermal patterning, neural specification and the underlying GRNs. There are also many similarities to chordate nervous system development. Thus, studies of nervous systems that have been dismissed as irrelevant to discussions of the origins of chordate nervous systems (Hartenstein & Stollewerk, 2015; Holland, 2015a, 2015b) reveal instead how neurogenesis has changed during the evolution of deuterostomes and allows inferences of neurogenesis in common ancestors. We have also highlighted the differences in these attributes, such as neurites projecting ventrally from the ciliary band in sea stars and dorsally in the sea urchin. A difference that suggests substantial changes in axonal pathfinding. Another notable distinction is the insensitivity of sea star neural differentiation to BMP; in sea stars some neurons arise in high BMP domains, whereas in urchins they arise only in low BMP domains. This appears similar to that noted in the direct developing hemichordate (Lowe et al., 2006). There are still enormous gaps in our understanding of echinoderm neurogenesis. We have only indirect evidence for dedicated neural progenitors in the animal pole domain, so we do not know if there are cells with stem-like properties that give rise to neurons. As researchers working with different organisms have focused on different genes, detailed studies of gene function with direct comparisons of neurogenesis are an important future direction. Additional studies can also determine how phenotypic changes in spatial organization of neural types in larval forms confer changes in nervous system function (Box 2) or determine the functions of specific neural subtypes. Echinoderms are an exceptional system for linking GRN evolution with the evolution of neural function and behavior, which is essentially impossible to determine for complex nervous systems. The tools available for perturbing function, tracing cells, and determining regulatory states when combined with the simplicity of echinoderm larval nervous systems will drive significant innovation in understanding of nervous system development and evolution.

ACKNOWLEDGMENTS

Supported by a Discovery Grant (NSERC Canada) to RDB, NSF MCB 1715721 and NSF IOS 1557431 to VFH.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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How to cite this article: Hinman VF, Burke RD. Embryonic neurogenesis in echinoderms. *WIREs Dev Biol*. 2018;7: e316. https://doi.org/10.1002/wdev.316