Chapter 2
The Development and Evolution of Lateral Line Electroreceptors: Insights from Comparative Molecular Approaches

Clare V. H. Baker

Abstract In the jawless lampreys, most nonteleost jawed fishes, and aquatic-stage amphibians, the lateral line system has a mechanosensory division responding to local water movement ("distant touch") and an electrosensory division responding to low-frequency cathodal (exterior-negative) electric stimuli, such as the weak electric fields surrounding other animals. The electrosensory division was lost in the ancestors of teleost fishes and their closest relatives and in the ancestors of frogs and toads. However, anodally sensitive lateral line electroreception evolved independently at least twice within teleosts, most likely via modification of the mechanosensory division. This chapter briefly reviews this sensory system and describes our current understanding of the development of nonteleost lateral line electroreceptors, both in terms of their embryonic origin from lateral line placodes and at the molecular level. Gene expression analysis, using candidate genes and more recent unbiased transcriptomic (differential RNA sequencing) approaches, suggests a high degree of conservation between nonteleost electroreceptors and mechanosensory hair cells both in their development and in aspects of their physiology, including transmission mechanisms at the ribbon synapse. Taken together, these support the hypothesis that electroreceptors evolved in the vertebrate ancestor via the diversification of lateral line hair cells.

Keywords Ampullary organ · Electroreception · Electrosensory · Hair cell · Mechanosensory · Neuromast · Placode · Presynaptic ribbon · Ribbon synapse · RNA sequencing · Tuberous organ

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2.1 Introduction

Evidence from phylogenetic distribution, sensory receptor cell physiology, and innervation suggests that the ancestor of all living vertebrates had vestibular inner ears with mechanosensory "hair cells" detecting gravity and angular acceleration (see Fritzsch and Elliott 2017) plus a lateral line system comprising (1) a mechanosensory division, with sense organs containing hair cells that detect local water movement, and (2) an electrosensory division, with sense organs containing electroreceptor cells stimulated by low-frequency, cathodal (exterior-negative) electric fields (Bullock et al. 1983; Baker et al. 2013). The electroreceptors respond to minute direct-current standing electric fields around animals in water (arising from ions leaking across mucous membranes) that can be modulated by ventilation or limb movements, generating a low-frequency component (Bedore and Kajiura 2013).

The apical surface of lateral line hair cells, like that of vestibular hair cells, is characterized by a staircase array of actin-rich microvilli ("stereocilia" or "stereovilli") connected by tip links (the "hair bundle") and a primary cilium ("kinocilium") eccentrically positioned next to the tallest stereocilia (Fig. 2.1A; Jørgensen 2005). The apical surface of electroreceptor cells is more diverse, with a primary cilium and/or varying numbers of microvilli (Fig. 2.1B, C; see Sect. 2.1.2; Jørgensen 2005). Hair cells and electroreceptors all have basolateral presynaptic bodies ("ribbons") that tether many synaptic vesicles (Fig. 2.1; see Jørgensen 2005; Zanazzi and Matthews 2009). Depolarization of the hair cell or electroreceptor results in neurotransmitter release at these specialized "ribbon synapses" (see Zanazzi and Matthews 2009; Nicolson 2015) onto the terminals of afferent neurons whose cell bodies are collected in cranial ganglia. Innervation patterns in extant vertebrates (McCormick 1982; Bullock et al. 1983), including eptatretid hagfishes (Amemiya et al. 1985), suggest that in the vertebrate ancestor, the central targets of inner ear and lateral line afferent neurons were distinct nuclei in the octavolateral area in the rostral alar plate of the hindbrain. The octavolateral nuclei are (1) the ventral nucleus for inner ear afferents projecting via the eighth cranial nerve; (2) the medial nucleus for mechanosensory lateral line afferents projecting via the posterior lateral line nerve and the ventral root of the anterior lateral line nerve; and (3) the dorsal nucleus for electrosensory lateral line afferents projecting via the dorsal root of the anterior lateral line nerve (reviewed by Wullimann and Grothe 2014).

The inner ears and lateral line system are developmentally and evolutionarily independent (see Sect. 2.2.4.1). All vertebrates have inner ears, whereas the lateral line system was lost independently in the cyclostome lineage leading to myxinid hagfishes (Braun and Northcutt 1997) and with the transition to terrestrial life in the lobe-finned bony tetrapod lineage leading to amniotes (Fig. 2.2). (The lateral line system was also lost in a few direct-developing amphibian lineages without an aquatic larval stage; Schlosser 2002b.) The mechanosensory and electrosensory divisions are also independent. The mechanosensory division was lost in some

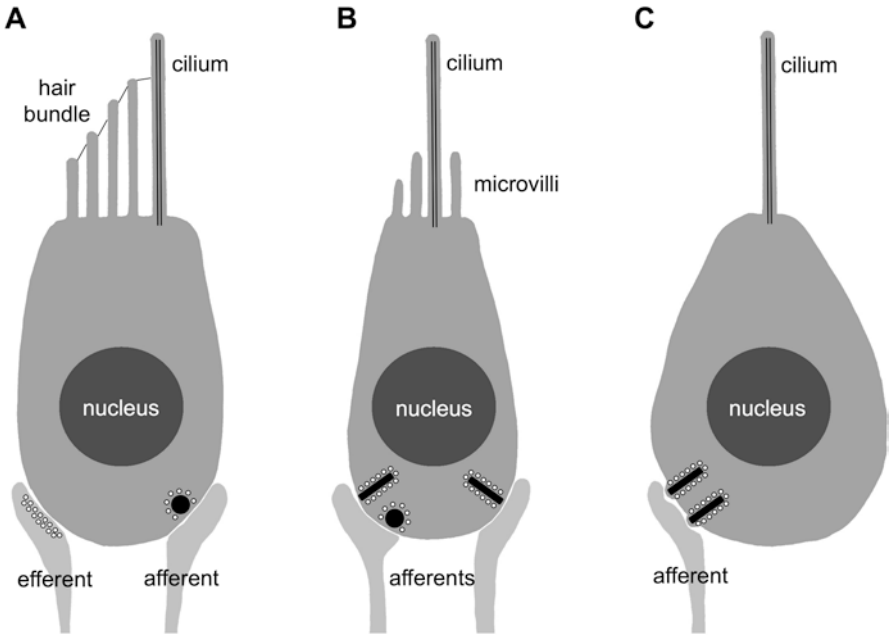

Fig. 2.1 Mechanosensory and electrosensory cells of the lateral line system. The apical surface varies, but they all have basolateral presynaptic bodies, surrounded by synaptic vesicles, opposite ribbon synapses with afferent lateral line nerve terminals. **A:** a hair cell characterized by a primary cilium (kinocilium) eccentrically positioned at the tallest edge of a staircase array of actin-rich microvilli (stereocilia) connected by tip links (hair bundle), with efferent as well as afferent innervation. **B:** an electroreceptor cell with a primary cilium and microvilli as found in, for example, ray-finned bony bichirs and lobe-finned bony lungfishes and amphibians. **C:** a pear-shaped electroreceptor cell with a primary cilium but without microvilli as found in, for example, cartilaginous fishes and ray-finned bony chondrostean fishes. Modified from Jørgensen (2011), with permission from Elsevier

aquatic caecilians that retain the electrosensory division (Schlosser 2002b). The electrosensory division was lost in the cyclostome lineage leading to eptatretid hagfishes (Braun and Northcutt 1997), in the lobe-finned bony tetrapod lineage leading to anuran amphibians (frogs and toads), and in the ray-finned bony fish lineage leading to neopterygian fishes (comprising gars, bowfin, and teleosts; Fig. 2.2; McCormick 1982; Bullock et al. 1983). A few teleost clades (see Sect. 2.1.2.3) possess an electrosensory division stimulated by anodal (exterior-positive) electric fields, with afferents projecting via both anterior and posterior lateral line nerves to distinct "electrosensory lateral line lobes" in the hindbrain (Bullock et al. 1983; Wullimann and Grothe 2014). The phylogenetic distribution suggests that teleost electroreception evolved independently at least twice (see Sect. 2.1.2.3.2; Bullock et al. 1983; Baker et al. 2013).

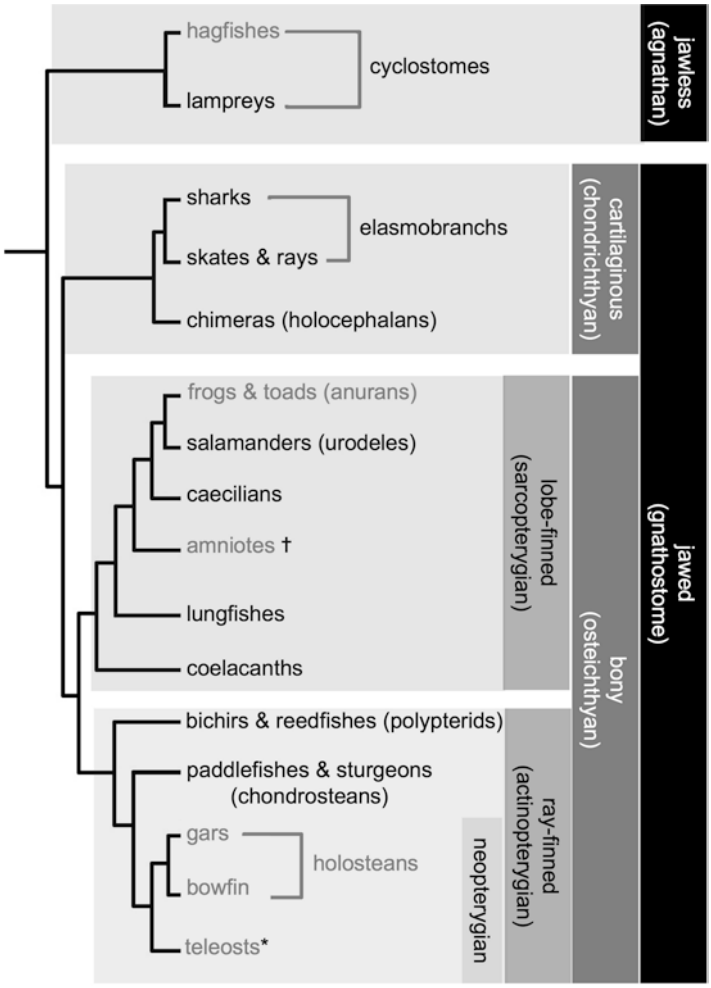

Fig. 2.2 Phylogenetic distribution of lateral line sensory divisions among living vertebrates. *Black text* indicates the presence of both the mechanosensory division, with afferents projecting to the medial octavolateral nucleus via the anterior and posterior lateral line nerves, and a low-frequency, cathodally sensitive electrosensory division, with afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve. *Gray text* indicates the presence of the mechanosensory lateral line only, except for amniotes (†), which lost the entire lateral line system with the transition to life on land and ray-finned teleost fishes (∗), where a few clades possess anodally sensitive lateral line electroreception, with afferents projecting to electrosensory lateral line lobes. Adapted from Baker and Modrell (2018), with permission from Oxford University Press

2.1.1 The Mechanosensory Division of the Lateral Line System

Lateral line neuromasts (Fig. 2.3) are distributed in characteristic lines over the head and body, either superficially or in canals connected to the surface via pores (see Webb 2014). Each neuromast contains a central cluster of hair cells (Fig. 2.1A) that project into a gelatinous cupula in jawed vertebrates (Fig. 2.3). In addition to basolateral ribbon synapses with afferent lateral line nerve terminals (Figs. 2.1A and 2.3; see Nicolson 2015), neuromast hair cells receive efferent innervation from medullary octavolateral efferent nuclei (see Chagnaud and Coombs 2014; Wullimann and Grothe 2014). The hair cells are surrounded and underlain by supporting cells that send processes between the hair cells, while a layer of mantle cells forms the outer rim of the neuromast (Fig. 2.3; see Webb 2014).

Neuromast hair cells respond to local water movement, mediating a sense of "distant touch" important for behaviors including feeding, avoiding predators and obstacles, and intraspecific communication (Dijkgraaf 1963; Montgomery et al. 2014). Hair cells are directionally sensitive (Flock 1965; Hudspeth and Corey 1977). Hydrodynamic stimuli that displace the hair bundle in the direction of the tallest stereocilia and kinocilium open mechanically gated cation channels at the stereociliary tips, resulting in hair cell depolarization and, ultimately, glutamate release, increasing the firing rate of the afferent fiber, whereas displacement in the opposite direction hyperpolarizes the hair cell, decreasing the firing rate (see Chagnaud and Coombs 2014). Oppositely oriented hair cells are intermingled in

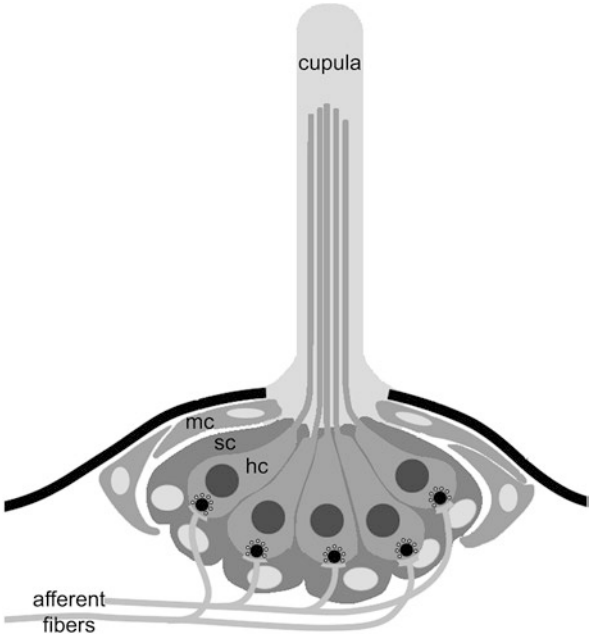

Fig. 2.3 A neuromast comprises a cluster of hair cells whose apical cilia project into a gelatinous cupula, surrounded and underlain by supporting cells, with an outer rim of mantle cells. Hair bundles and efferent fibers are not shown. hc, Hair cell; mc, mantle cell; sc, supporting cell. Modified from Ghysen and Dambly-Chaudière (2004), with permission from Elsevier

each neuromast (Flock 1965; López-Schier et al. 2004). During development and regeneration, hair cells of opposite orientation within the same neuromast become innervated by different afferent fibers (Nagiel et al. 2008; Dow et al. 2018).

2.1.2 The Electrosensory Division of the Lateral Line System

2.1.2.1 Electrosensory Organs in Jawless Fishes

Within the extant jawless fishes, lampreys have both electrosensory and mechanosensory lateral line divisions, although the neuromasts (all superficial) lack cupulae and efferent innervation (Braun 1996). Eptatretid hagfishes have a simple mechanosensory lateral line system only, whereas myxinid hagfishes lack even this (Bullock et al. 1983; Braun and Northcutt 1997). Molecular evidence overwhelmingly supports lampreys and hagfishes as a monophyletic clade, the cyclostomes (Fig. 2.2; see Shimeld and Donoghue 2012), suggesting that within hagfishes, the electrosensory division was secondarily lost in the eptatretid lineage, whereas the entire lateral line system was lost in the myxinid lineage, as previously suspected (Braun 1996).

Adult lampreys have both cranial and trunk epidermal "end bud" electroreceptor organs directly exposed at the surface, with supporting cells and electroreceptor cells lacking a primary cilium but with 80–90 short apical microvilli and basolateral spheroidal presynaptic bodies (Fig. 2.4; Jørgensen 2005). Both lateral line divisions are functional at ammocoete larval stages (Ronan 1988; Gelman et al. 2007). Ammocoetes lack electroreceptor organs, and their electroreceptor cells are thought to be lateral line-innervated epidermal "multivillous cells" with presynaptic bodies (Fig. 2.4; Jørgensen 2005).

2.1.2.2 Electrosensory Organs in Nonteleost Jawed Vertebrates

Within jawed fishes and amphibians, the "ancestral" electrosensory division (i.e., low-frequency, cathodally sensitive electroreceptors whose afferents project to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) is found in all lineages except the lobe-finned anuran amphibians (frogs and toads) and the ray-finned neopterygian fishes (teleosts and holosteans, i.e., gars and the bowfin), suggesting secondary loss of the electrosensory division within these lineages (Fig. 2.2; Bullock et al. 1983; Baker et al. 2013). Electroreceptor cells (see Leitch and Julius, Chap. 3) are found in "ampullary organs" (or "ampullae of Lorenzini"), named for their flask-like morphology. The sensory epithelium of electroreceptor cells and supporting cells is located at the base of a bulbous chamber from which a conductive jelly-filled duct (long in marine species; short in freshwater species) leads to a pore at the surface (Fig. 2.5; Jørgensen 2005). Each electroreceptor cell has an apical primary cilium, varying numbers of apical microvilli (from

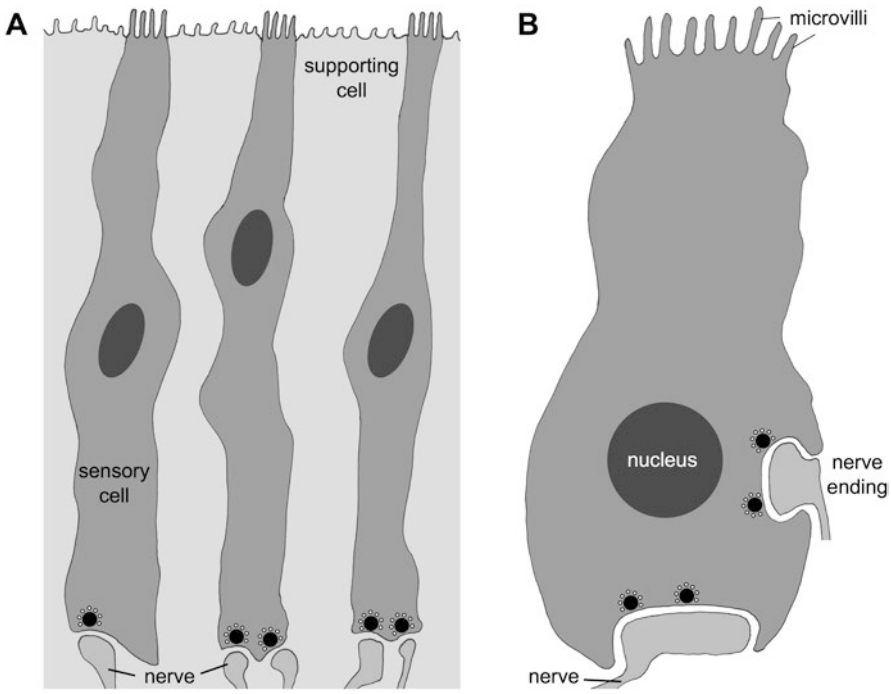

Fig. 2.4 Lamprey adult end bud electroreceptor cells (**A**) and ammocoete larval multivillous cells (not to scale; **B**). Individual supporting cells are not delineated. Redrawn after Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

none to a couple of hundred), and basal presynaptic ribbons opposite afferent lateral line nerve terminals (Fig. 2.5; Jørgensen 2005).

2.1.2.3 Electrosensory Organs in Teleost Fishes: Independent Evolution

2.1.2.3.1 Overview of Teleost Electroreception

Within the teleosts, electroreception is found in two related clades within each of two distinct lineages (Bullock et al. 1983; Baker et al. 2013). In the osteoglossomorph lineage (Fig. 2.6), the electroreceptive species are the African notopterids (featherbacks or knifefishes) and their sister group, the mormyroids, which comprise the mormyrids (freshwater elephant fishes) plus the gymnarchid *Gymnarchus niloticus* (the aba). In the ostariophysan lineage (Fig. 2.6), the two related electroreceptive clades are the siluriforms (catfishes) and gymnotiforms (South American knifefishes).

Teleost electroreception differs significantly from nonteleost electroreception (see Leitch and Julius, Chap. 3). Teleost electroreceptors are stimulated by anodal stimuli and inhibited by cathodal stimuli, and the basal membrane is the voltage

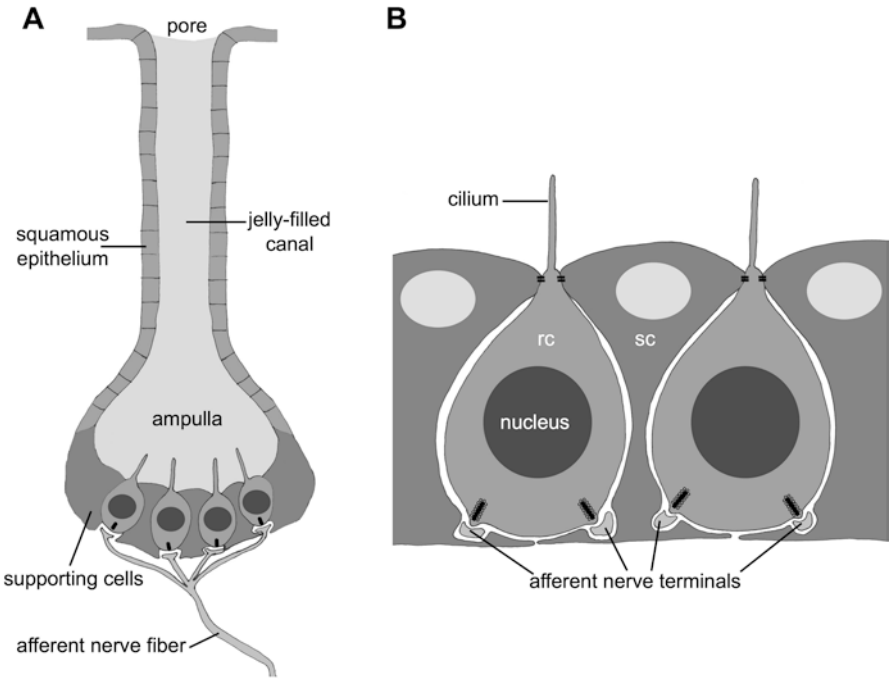

Fig. 2.5 **A:** a nonteleost jawed vertebrate ampullary organ. A surface pore opens to a conductive jelly-filled duct lined with squamous epithelium and ending in a bulbous chamber with a sensory epithelium at its base, comprising supporting cells (not delineated) and electroreceptor cells. Redrawn and modified from Sillar et al. (2016), with permission from John Wiley & Sons, Ltd. **B:** in the sensory epithelium, each electroreceptor cell has an apical primary cilium and variable numbers of microvilli (none in this example) with presynaptic ribbons opposite ribbon synapses with afferent lateral line nerve terminals. Apical tight junctions connect electroreceptor cells to neighboring supporting cells. rc, Receptor cell; sc, supporting cell. Redrawn and modified from Fields et al. (1993), with permission from Karger

sensor, whereas nonteleost electroreceptors respond to cathodal stimuli via the apical membrane (Bodznick and Montgomery 2005; see Leitch and Julius, Chap. 3). All electroreceptive teleosts have "ampullary" electroreceptors that respond to low-frequency environmental electric fields (passive electroreception). As in nonteleosts, the sensory epithelium containing ampullary electroreceptors is located at the base of a flask-like chamber, from which a mucus-filled duct leads to a surface pore (Fig. 2.7; Jørgensen 2005). Teleost ampullary electroreceptors have sparse microvilli, no primary cilium, and presynaptic ribbons (Fig. 2.7; Jørgensen 2005). The only teleost electroreceptors with a primary cilium are the ampullary electroreceptors of the osteoglossomorph African notopterid *Xenomystus nigri* (Jørgensen 2005). In contrast, all electroreceptors in nonteleost jawed vertebrates have a primary cilium (Jørgensen 2005).

The osteoglossomorph mormyroids and the unrelated ostariophysan gymnotiforms (Fig. 2.6) are described as "weakly electric" teleosts. This is because they

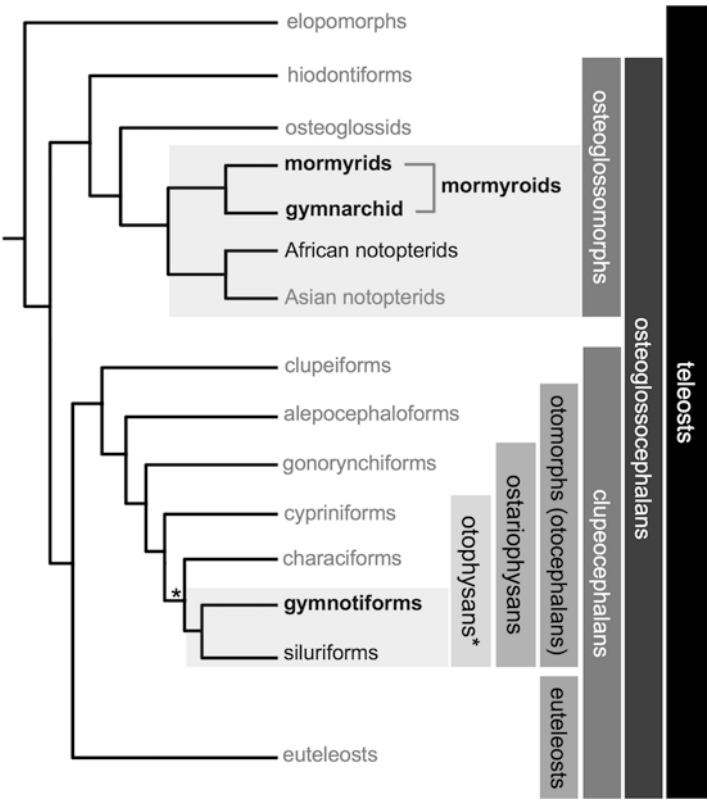

Fig. 2.6 Phylogenetic distribution of lateral line electroreception within teleosts. *Gray text* indicates the presence of the mechanosensory lateral line only. *Black text* indicates the presence of ampullary electroreceptors stimulated by low-frequency, anodal electric fields (passive electroreception) and electrosensory lateral line lobes in the hindbrain. *Bold black text* indicates the weakly electric fish clades, which also have electric organs and tuberous organs responding to high-frequency electric organ discharges (active electroreception). In otophysans (∗), there is a continuing debate over the sister-group relationships among characiforms, gymnotiforms, and siluriforms. The phylogeny follows that in Betancur-R et al. (2017)

possess not only ampullary electroreceptors but also electric organs (modified muscle or nerve cells) that generate high-frequency electric fields (see Gallant, Chap. 4), and "tuberous" electroreceptors that respond to these high-frequency electric organ discharges (see Leitch and Julius, Chap. 3). Tuberous organs are morphologically varied but united in lacking ducts and being plugged by loosely packed epidermal cells (Fig. 2.8; Jørgensen 2005). Tuberous electroreceptor cells, which are characterized by many microvilli apically and presynaptic ribbons basally, are located within an intraepidermal cavity (Fig. 2.8; Jørgensen 2005).

Teleost electroreceptor organs are found on both the trunk and head, innervated by posterior and anterior lateral line nerves, respectively, projecting to hindbrain electrosensory lateral line lobes (Fig. 2.9; see Bullock et al. 1983; Wullimann and

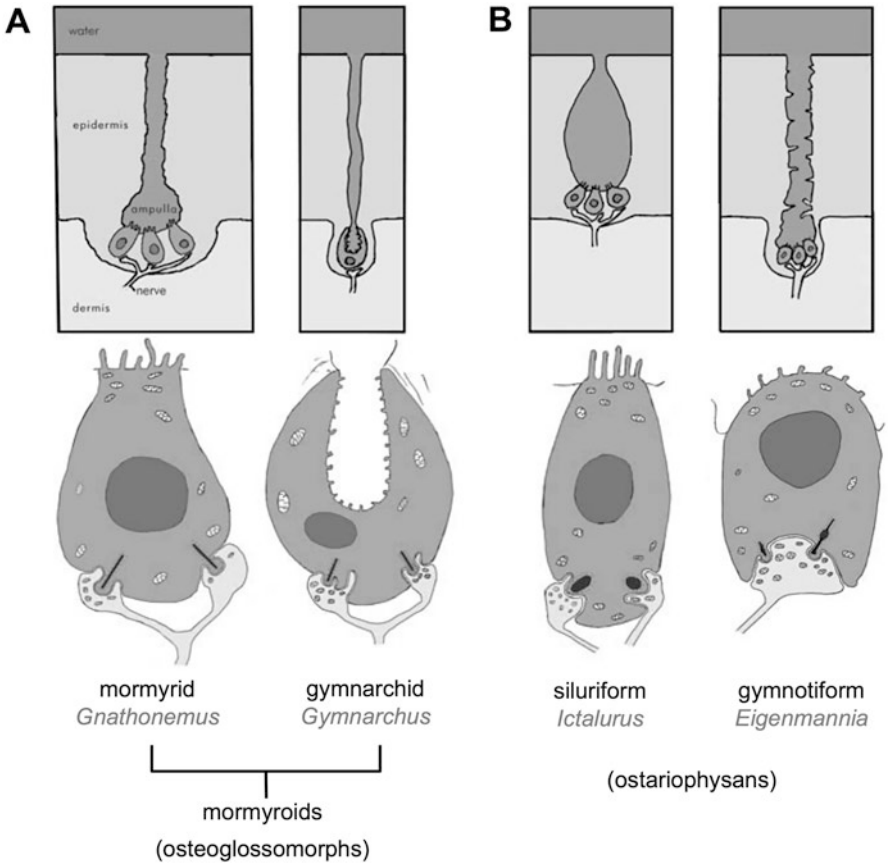

Fig. 2.7 Teleost ampullary organs (*top*) and electroreceptor cells (not to scale; *bottom*) from representative osteoglossomorph mormyroids (mormyrids plus the gymnarchid *Gymnarchus niloticus*; **A**) and ostariophysan siluriforms and gymnotiforms (**B**). Adapted from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

Grothe 2014). These share a cerebellum-like organization and circuitry with the medial octavolateral nuclei (the targets of mechanosensory lateral line afferents) and the dorsal octavolateral nuclei of nonteleosts (Fig. 2.9; (Bell et al. 1997; Bell and Maler 2005).

2.1.2.3.2 Electroreception Evolved Independently At Least Twice in Teleosts

Within the ray-finned bony fishes, "ancestral" electroreception, (i.e., stimulated by low-frequency, cathodal electric fields, with afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) is present in the basally branching lineages, namely, polypterids (bichirs and reedfishes) and

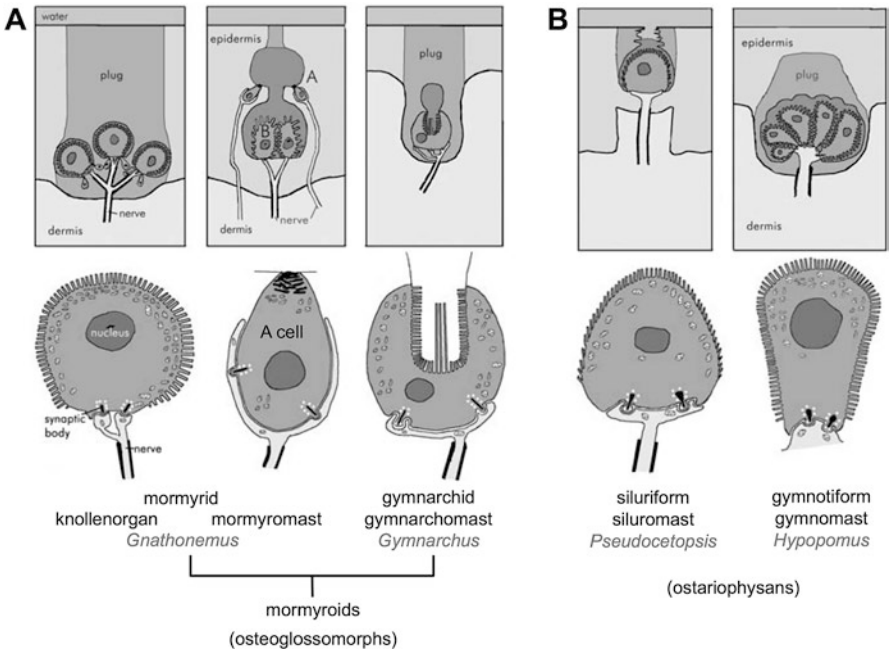

Fig. 2.8 Teleost tuberous organs (*top*) and electroreceptor cells (not to scale; *bottom*) from representative osteoglossomorph mormyroids (**A**) and from ostariophysan siluriforms and gymnotiforms (**B**). **A:** a mormyrid knollenorgan (*left*); a mormyrid mormyromast (*center*) containing both pear-shaped type A electroreceptor cells and knollenorgan-electroreceptor-like type B electroreceptor cells; and a gymnarchid gymnarchomast (*right*). Adapted from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

chondrosteans (paddlefishes and sturgeons), but is absent from neopterygians (Fig. 2.2). Given this phylogenetic distribution and the very different characteristics of teleost electroreception, as described in Sect. 2.1.2.3.1, the simplest hypothesis is that electroreception was lost in the ray-finned bony fish lineage leading to the neopterygian clade (Fig. 2.2) and evolved independently at least twice within teleosts (see Bullock et al. 1983; Baker et al. 2013). Less parsimoniously, ancestral electroreception could have been lost independently in each lineage.

In the osteoglossomorph lineage, the most parsimonious hypothesis is that anodally sensitive ampullary electroreception (with afferents projecting to a novel electrosensory lateral line lobe in the hindbrain) evolved along the stem leading to the common ancestor of mormyroids and notopterids (and was lost in the lineage leading to Asian notopterids) and that electric organs and tuberous electroreceptors subsequently evolved in the lineage leading to mormyroids (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013). The less parsimonious hypothesis (assuming that novel trait evolution is less likely than trait loss) is that ampullary electroreception evolved independently in the lineage leading to African notopterids, and in the lineage leading to mormyroids (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013).

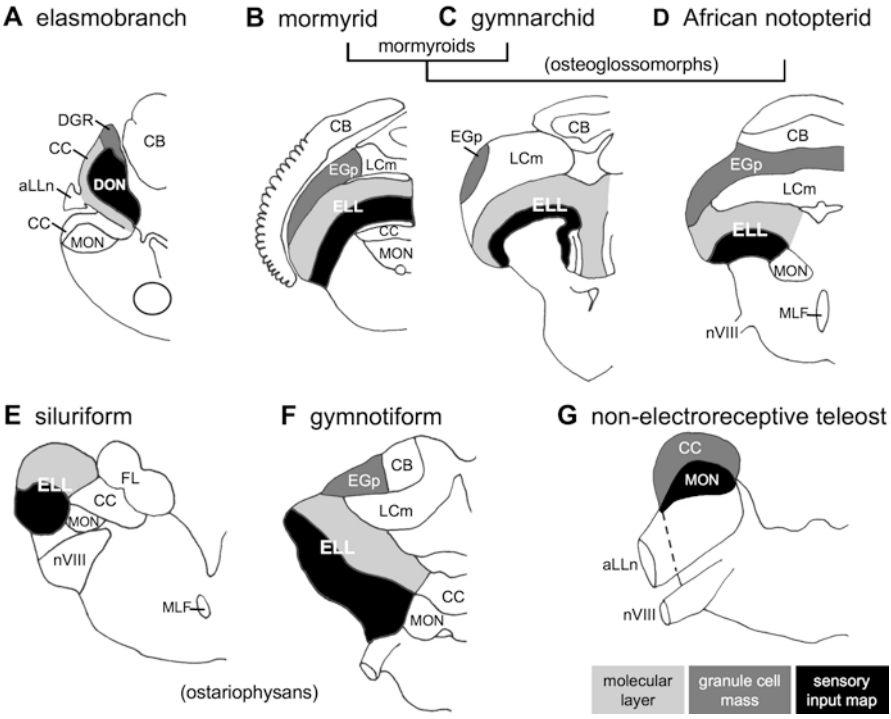

Fig. 2.9 First-order cerebellum-like electrosensory hindbrain structures in a nonteleost fish (an elasmobranch; **A**), osteoglossomorph teleost fishes (**B–D**), ostariophysan teleost fishes (**E** and **F**), and, for comparison, the cerebellum-like mechanosensory hindbrain structures from a nonelectroreceptive teleost (**G**). *Black areas* indicate where primary afferent fibers terminate (i.e., the sensory input map). *Dark gray areas* indicate the mass of granule cells whose parallel fibers form the molecular layer (*light gray areas*) of these structures. aLLn, anterior lateral line nerve; CB, cerebellum; CC, cerebellar crest; DGR, dorsal granular ridge; DON, dorsal octavolateral nucleus; EGp, eminentia granularis posterior; ELL, electrosensory lateral line lobe; LCm, molecular layer of the caudal lobe of the cerebellum; MLF, medial longitudinal fasciculus; MON, medial octavolateral nucleus; nVIII, eighth cranial nerve. Redrawn and modified from Bell et al. (1997), with permission from Karger and from Bell and Maler (2005), © Springer Science+Business Media, Inc., with permission

In the ostariophysan lineage, the picture is complicated by continued debate over the sister-group relationships among the siluriform, gymnotiform, and characiform clades within the otophysans (Fig. 2.6; Betancur-R et al. 2017; Dai et al. 2018). Under all scenarios, however, the most parsimonious hypothesis is that anodally sensitive ampullary electroreception (with afferents projecting to a novel electrosensory lateral line lobe in the hindbrain) evolved along the stem leading to the common ancestor of siluriforms and gymnotiforms. Subsequently, electric organs and tuberous electroreceptors evolved in the gymnotiform lineage, with ampullary electroreception being lost independently in any other lineages falling

within the clade containing siluriforms and gymnotiforms. The alternative would be that ampullary electroreception evolved independently in the lineage leading to siluriforms and in the lineage leading to gymnotiforms (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013).

How might teleost electroreceptors have evolved? Like nonteleost electroreceptors, hair cells release neurotransmitter in response to sufficiently large anodal stimuli, which presumably directly depolarize the basal presynaptic membrane (e.g., Bodznick and Preston 1983; Münz et al. 1984). It has been suggested that greater sensitivity of the basal membrane to electrical stimuli, potentially achieved by increasing the density of basal voltage-gated calcium channels, could have been selected for in a subpopulation of hair cells (Bullock et al. 1983; Bodznick 1989). It seems plausible, therefore, that teleost ampullary electroreceptors evolved via the modification of neuromast hair cells such that their basal membranes responded to increasingly smaller electrical stimuli, and they lost the apical mechanosensory hair bundle (and cilium). The independent evolution of tuberous electroreceptors in the osteoglossomorph mormyroids and ostariophysan gymnotiforms could have involved the modification of either ampullary electroreceptors or neuromast hair cells. Furthermore, the evolutionary pathway could be different in the two lineages. Future comparative transcriptomic approaches, ideally at the single-cell level (e.g., Haque et al. 2017), would enable the transcriptomes of neuromast hair cells, ampullary and tuberous electroreceptors to be compared directly both within and across species. This could reveal the extent to which the evolution of different electroreceptor types in different teleost groups involved similar or wholly distinct molecular pathways and mechanisms.

2.1.3 Trigeminal Nerve-Mediated Electroreception in Monotremes and Dolphins

Monotreme mammals (the duck-billed platypus, *Ornithorhynchus anatinus*, and the echidnas, Tachyglossidae) and at least one fully aquatic eutherian mammal (the Guiana dolphin, *Sotalia guianensis*) independently evolved electroreception, mediated via naked afferent trigeminal nerve endings associated with accessory structures in the snout (Czech-Damal et al. 2013). In monotremes, the accessory structures are mucous or serous glands in the bill/snout (Fig. 2.10), whereas in the Guiana dolphin, they are whiskerless vibrissal crypts on the upper jaw (Czech-Damal et al. 2013). Given the independent evolution of trigeminal electroreception, it will not be considered further here. Future molecular work, should this prove feasible, may reveal whether there is any convergence with lateral line electroreception, for example, in the ion channels involved.

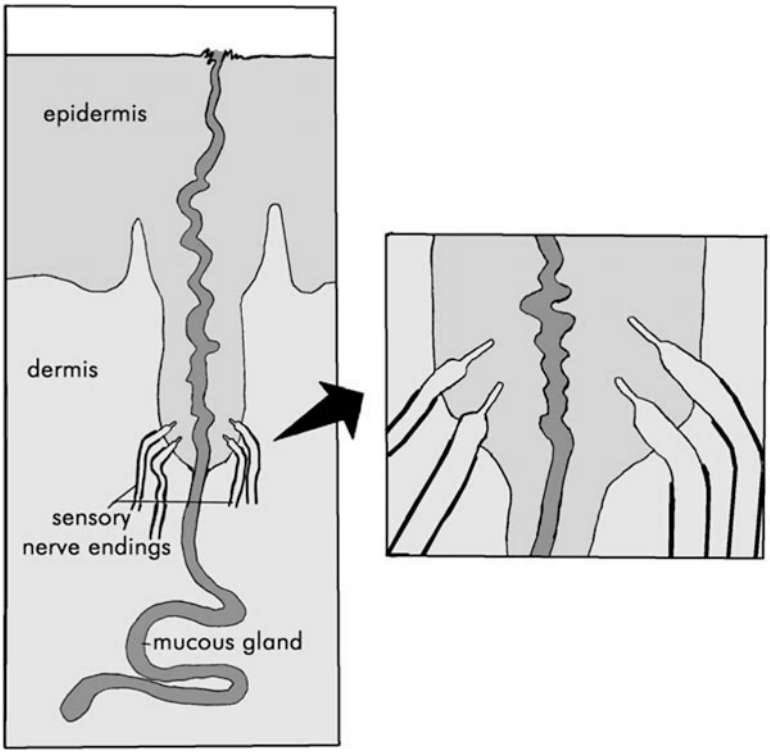

Fig. 2.10 A mucous gland from the bill of the duck-billed platypus (*Ornithorhynchus anatinus*) containing naked electroreceptive trigeminal nerve endings. Reproduced from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

2.2 Electroreceptor Development

2.2.1 An Introduction to Cranial Placodes

Following descriptions in the late nineteenth century of lateral line development in teleost (ray-finned bony) fish and urodele amphibian (lobe-finned bony tetrapod) embryos, a series of experimental grafting and ablation studies in both urodele and anuran amphibian embryos showed that lines of lateral line neuromasts, together with their afferent neurons in lateral line ganglia, originate from cranial lateral line placodes (LLPs; see Schlosser 2002a; Piotrowski and Baker 2014). The migrating posterior lateral line primordium of the zebrafish (*Danio rerio*, a cypriniform ostariophysan teleost; Fig. 2.6), which can be manipulated genetically to enable live imaging of migrating and differentiating cells and to study gene function, has become a key model for understanding the molecular mechanisms underlying collective cell migration, organ morphogenesis, and hair cell specification and regeneration (see Kniss et al. 2016; Dalle Nogare and Chitnis 2017). More recently,

ablation and fate-mapping studies revealed that individual LLPs in electroreceptive jawed vertebrates form ampullary organs as well as neuromasts and afferent neurons (Sect. 2.2.2; Baker et al. 2013).

LLPs are a subset of the cranial placodes. These simple patches of thickened columnar ectoderm form in characteristic positions on the embryonic vertebrate head and give rise (after, in some cases, extensive morphological changes) to a diverse array of different organs and cell types, which are critical not only for extero- and interoception but also for homeostasis and fertility (Schlosser 2010). All the hair cell-forming placodes develop as bilateral, paired structures from a "posterior placodal area" adjacent to the hindbrain (see Schlosser 2010). The otic placodes form the inner ears and their afferent neurons, which are located in the ganglia of cranial nerve VIII (Schlosser 2010). Phylogenetic analysis suggests that in the lineage leading to jawed vertebrates, there were three preotic LLPs and three postotic LLPs (Fig. 2.11; see Northcutt 2005a). The anterodorsal, anteroventral, and otic LLPs (the latter not to be confused with the inner ear-forming otic placode) are preotic, whereas the middle, supratemporal, and posterior LLPs (the latter forming the trunk lateral line) are postotic (Fig. 2.11; see Northcutt 2005a). The posterior placodal area also includes precursors of the epibranchial placodes, which form at

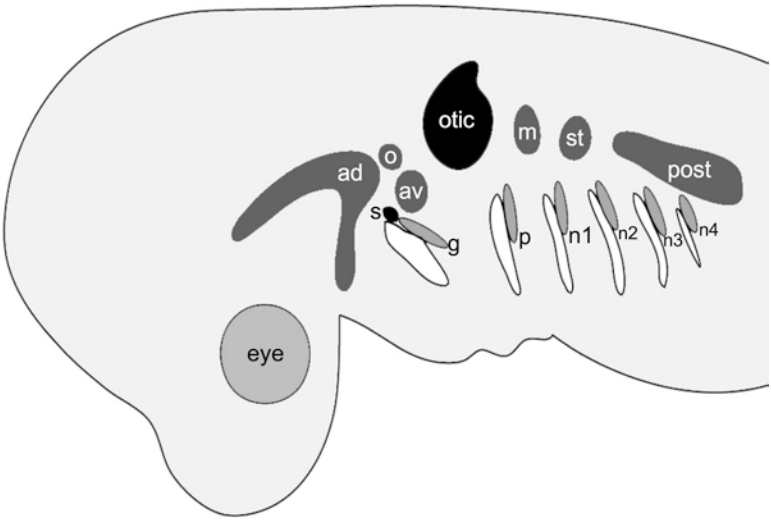

Fig. 2.11 An idealized cartilaginous fish embryo showing the relative positions of the placodes arising from the posterior placodal area. Dorsally, these are the placodes that form hair cells and their afferent neurons: the otic placode (which forms the inner ear, shown here at the otic vesicle stage), three preotic lateral line placodes (LLPs; anterodorsal, shown here as elongating to form the supraorbital and infraorbital sensory ridges, plus anteroventral and otic LLPs), three postotic LLPs (middle, supratemporal, and posterior), and the spiracular/paratympanic organ placode. The latter forms immediately dorsal to the geniculate placode, the first in the series of epibranchial placodes that develop dorsocaudal to each pharyngeal cleft (geniculate, petrosal, and nodose). ad, Anterodorsal; av, anteroventral; g, geniculate; m, middle; n, nodose; o, otic; p, petrosal; post, posterior; s, spiracular; st, supratemporal. Redrawn and modified from O'Neill et al. (2012)

the dorsolateral edge of each pharyngeal cleft (Fig. 2.11; see Schlosser 2010) and give rise to the gustatory and viscerosensory afferent neurons located in the distal ganglia of cranial nerves VII (geniculate), IX (petrosal), and X (nodose). Finally, in some extant jawed vertebrates, an additional "spiracular/paratympanic organ" placode develops immediately dorsal to the first epibranchial (geniculate) placode (Fig. 2.11); this is distinct from the LLP series and forms the hair cell-containing spiracular organ of nonteleost fishes and paratympanic organ of amniotes and associated afferent neurons (O'Neill et al. 2012).

Fate-mapping studies in chicken and African clawed frog (*Xenopus laevis*) embryos have shown that the cranial placodes originate from an "inverted U"/"horseshoe"-shaped ectodermal territory surrounding the rostral (anterior) neural plate, the "preplacodal ectoderm" (see Saint-Jeannet and Moody 2014; Schlosser 2014). This is defined by coexpression of members of the Six family of homeodomain transcription factors (encoded by vertebrate homologues of *Drosophila sine oculis*) and its transcriptional coactivator of the Eya family (encoded by vertebrate homologues of *Drosophila eyes absent*), whose expression is maintained in individual cranial placodes (Saint-Jeannet and Moody 2014; Schlosser 2014). The preplacodal ectoderm is likely to be a domain of competence to form placodes in response to local signals (see Schlosser 2010). Current models suggest that signals secreted from surrounding tissues (neural plate and endomesoderm) lead to the subdivision of the preplacodal ectoderm into three "multiplacodal" domains along the rostrocaudal axis, distinguished by the expression of different combinations of transcription factors (with some species-specific differences), within which individual cranial placodes are specified in response to more localized signaling from adjacent tissues (see Saint-Jeannet and Moody 2014; Schlosser 2014). The adenohypophysis, olfactory, and lens placodes develop from an anterior (rostral) Pax6/Otx2-positive domain; the profundal/trigeminal placodes develop from an intermediate Pax3/Otx2-positive domain, whereas the hair cell-forming placodes (otic, lateral line, and spiracular organ/paratympanic organ placodes, where present) and the epibranchial placodes develop from the Pax2/Sox2/Sox3/Gbx2-positive posterior placodal area (Saint-Jeannet and Moody 2014; Schlosser 2014).

2.2.2 Nonteleost Ampullary Organs Develop from Lateral Line Placodes that Elongate to Form Sensory Ridges

As noted in Sect. 2.2.1, extensive experimental evidence from grafting and ablation studies in urodele and anuran amphibian embryos plus recent genetic lineage-tracing work in zebrafish embryos has shown that neuromasts and their afferent neurons originate from LLPs (see Schlosser 2002a; Piotrowski and Baker 2014). Researchers only turned their attention to the developmental origin of ampullary organs in the last decade of the twentieth century. Histology and scanning electron microscopy in the axolotl (*Ambystoma mexicanum*, a urodele amphibian) suggested that all LLPs

except the posterior (which migrates onto the trunk) elongate to form "sensory ridges" (Northcutt et al. 1994). A row of neuromasts forms along the center of each ridge whereas ampullary organs differentiate later, on the flanks of the ridge (Fig. 2.12; Northcutt et al. 1994). LLP ablations and grafting experiments between pigmented and albino axolotl embryos subsequently confirmed that an individual-elongating LLP forms ampullary organs, neuromasts, and their afferent neurons (Northcutt et al. 1995). Focal labeling experiments using the fluorescent lipophilic dye DiI yielded the same results in a chondrostean ray-finned bony fish (the Mississippi paddlefish, *Polyodon spathula*; Modrell et al. 2011) and a cartilaginous fish (the little skate, *Leucoraja erinacea*; Gillis et al. 2012). Hence, ampullary organs originate from elongating LLPs in all major jawed vertebrate groups (Fig. 2.2).

Before LLPs begin elongating or migrating, neuroblasts delaminate from the pole nearest the otic vesicle and coalesce into individual ganglia that may fuse with other lateral line and/or other nearby ganglia (see Piotrowski and Baker 2014). The axons of these afferent neurons (and associated Schwann cells) track the primordium and innervate neuromast hair cells as they form (Fig. 2.12). First studied in amphibian embryos (see Piotrowski and Baker 2014), this has been investigated in depth for the migrating posterior LLP in zebrafish, where different transgenic lines and vital dye labeling can be combined to label hair cells, axons, and Schwann cells with different fluorescent reporters (e.g., Gilmour et al. 2004; Pujol-Martí et al. 2014). Efferents for neuromast hair cells, originating from hindbrain motor nuclei, reach their targets by following the sensory lateral line nerve (see Piotrowski and Baker 2014).

2.2.3 Experimental Evidence Is Lacking for the Embryonic Origin of Lamprey and Teleost Electroreceptors

In contrast to nonteleost jawed vertebrates, experimental evidence is lacking for the embryonic origin of both lamprey electroreceptors (see Sect. 2.1.2.1) and the various independently evolved teleost electroreceptors (see Sect. 2.1.2.3). There is strong support for the homology of lamprey and nonteleost jawed vertebrate electroreceptors from both innervation (projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) and physiology, namely, stimulation by low-frequency cathodal electric fields (Bullock et al. 1983; Baker et al. 2013). Given this, it is likely that lamprey electroreceptors are also LLP derived, but this remains to be tested. The contribution of lamprey cranial placodes to neurons in cranial ganglia, including lateral line ganglia, has been fate mapped by vital dye labeling in the sea lamprey, *Petromyzon marinus* (Modrell et al. 2014). However, neuromasts have only been reported much later, at ammocoete larval stages (Gelman et al. 2007). Ammocoete larvae respond to weak cathodal electric fields (Ronan 1988), suggesting that electroreceptors, thought to be epidermal multivillous cells (see Sect. 2.1.2.1; Jørgensen 2005), are also present. It will be important to identify

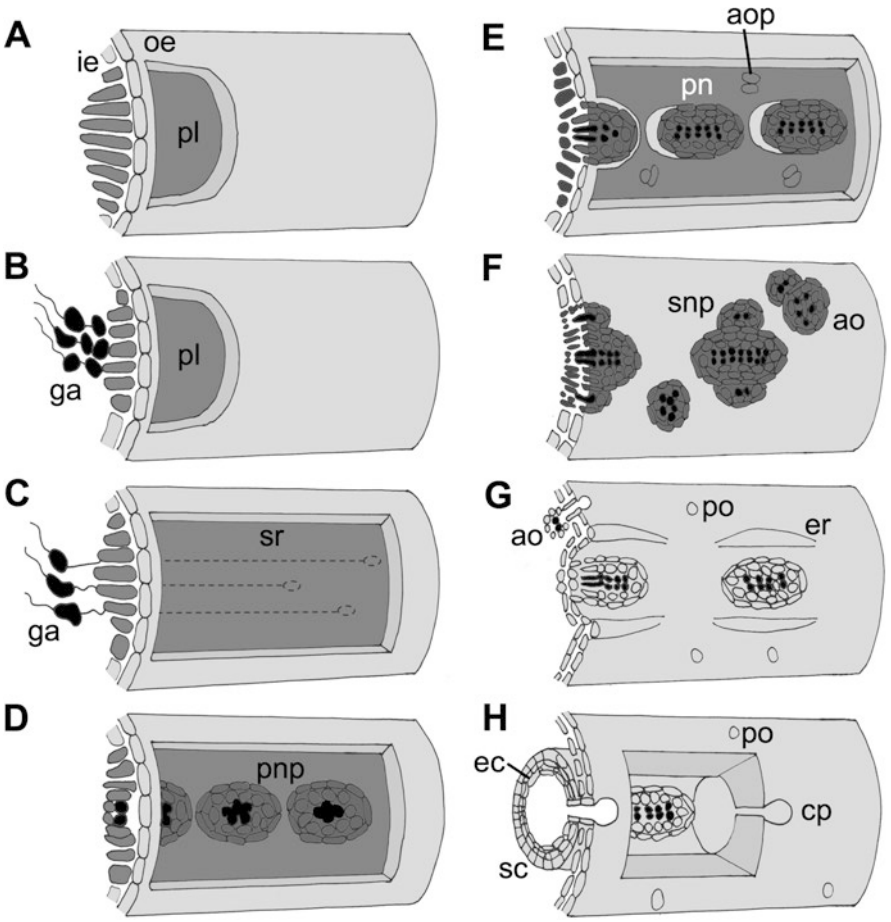

Fig. 2.12 Stages in the development of a LLP that forms ampullary organs as well as neuromasts. **A:** formation of the placode, i.e., a patch of columnar ectoderm within the inner ectodermal layer (in species with a bilayered ectoderm). **B:** neuroblasts delaminate from the placode and differentiate to form the afferent neurons of the lateral line ganglion. **C:** the placode elongates to form a sensory ridge, accompanied by the axons of lateral line afferent neurons. **D:** primary neuromast primordia form in a line along the center of the ridge. **E:** ampullary organ primordia form later, on the flanks of the ridge. **F:** first neuromasts and then ampullary organs erupt to the surface, following which secondary organs form by budding from the mantle zones of the primary organs. **G:** ectodermal ridges develop parallel to the neuromast lines, while ampullary organs invaginate. **H:** eventually, the neuromasts are enclosed within a primary ectodermal canal surrounded by a secondary connective tissue canal, with pores at the surface between adjacent neuromasts. ao, Ampullary organ; aop, ampullary organ primordium; cp, canal pore; ec, epithelial canal; er, ectodermal ridge; ga, ganglionic cells of lateral line nerve; ie, inner layer of ectoderm; oe, outer layer of ectoderm; pa, placode; pn, primary neuromast; pnp, primary neuromast primordium; po, ampullary pore; sc, secondary connective tissue canal; snp, secondary neuromast primordium; sr, sensory ridge. Redrawn and modified from Northcutt et al. (1994), ©1994 Wiley-Liss, Inc., with permission from John Wiley & Sons, Ltd

when lamprey neuromasts and electroreceptors form and to undertake longer term fate-mapping studies.

Similarly, fate-mapping experiments are needed to confirm the embryonic origin of teleost electroreceptors (see Sect. 2.1.2.3). Descriptive studies in mormyrid (osteoglossomorph) embryos suggested that all electroreceptors develop directly from basal epidermal cells (see Kirschbaum and Denizot 2011). However, apart from the large posterior (trunk) LLP, teleost LLPs are difficult to identify without molecular markers (see Northcutt 2005a). Lateral line nerves can always be identified before lateral line organs, leading some to suggest that nerves locally induce both cranial neuromasts and electroreceptors from surface ectoderm in ostariophysan (siluriform and gymnotiform) fishes (see Northcutt 2005a). Roth (2003) reported that unilateral ablation of the posterior lateral line nerve in siluriform (Wels catfish, *Silurus glanis*) embryos prevented electroreceptor development in trunk and tail skin without affecting neuromast development. In contrast, the only confirmed role for innervation in the mechanosensory lateral line is for postembryonic organ maintenance and for the "budding" of secondary neuromasts from primary neuromasts (see Piotrowski and Baker 2014). In the gymnotiform *Eigenmannia* (unidentified species), both ampullary and tuberous electroreceptors develop "adjacent to" the neuromast lines after they have formed, whereas in the channel catfish, *Ictalurus punctatus*, ampullary organs develop within the "lateral zones" of the sensory ridges formed by elongating LLPs on the head (see Northcutt 2005a). This was precisely what was observed using the same methods in axolotl embryos in which the LLP origin of ampullary organs was subsequently confirmed by ablation and grafting studies (see Northcutt 2005a). Fate-mapping studies of electroreceptive teleost LLPs are long overdue.

2.2.4 The Molecular Control of Lateral Line Placode Formation

2.2.4.1 Lateral Line and Otic Placodes Are Developmentally Independent

The Pax2/Sox2/Sox3/Gbx2-positive posterior placodal area adjacent to the hindbrain, within which the otic, lateral line, and epibranchial placodes develop (Fig. 2.11; see Sect. 2.2.1), is induced by fibroblast growth factor (FGF) signaling from endomesoderm and/or the hindbrain (see Saint-Jeannet and Moody 2014; Schlosser 2014). Within the posterior placodal area, Wnt signaling from the caudal hindbrain is required to specify an otic placode fate dorsally, whereas sustained FGF signaling from outpocketing pharyngeal pouch endoderm, which contacts the overlying ectoderm, specifies an epibranchial placode fate more ventrally (Fig. 2.11; Saint-Jeannet and Moody 2014; Schlosser 2014). In contrast, relatively little is known about the molecular control of LLP formation.

Transplantation studies in amphibian embryos showed that LLP induction is experimentally separable and temporally distinct from otic placode induction and

likely involves both the underlying mesoderm and the adjacent hindbrain (Schlosser 2002a). Recent experiments in zebrafish showed that a higher level of FGF signaling was needed for preotic LLP formation than for otic placode formation and that Wnt signaling (which, as noted earlier, specifies an otic placode fate) blocked the formation of both "anterior" (preotic) LLPs and the postotic posterior LLP (Nikaido et al. 2017). (Other postotic LLPs were not examined.) The specific FGF ligands required for preotic LLP formation remain unknown, as does their source, although possible candidates are FGF3 and FGF8, which are produced at the midbrain-hindbrain boundary and by the underlying mesendoderm at relevant stages (Nikaido et al. 2017).

The developmental independence of lateral line versus otic placodes plausibly underlies the evolutionary loss of LLPs in amniotes and some direct-developing frogs. The failure of LLPs to form in the common coqui (*Eleutherodactylus coqui*), for example, was shown (by reciprocal heterospecific grafting experiments between this direct-developing frog and the axolotl) to result not from the loss of LLP-inducing signals but from the loss of competence in head ectoderm to respond to such signals (Schlosser et al. 1999).

2.2.4.2 Different Lateral Line Placodes Have Different Molecular Requirements

Experiments in both zebrafish and axolotl have revealed significant heterogeneity in the molecular mechanisms underlying the formation of different LLPs. FGF signaling, although necessary for the formation of preotic LLPs in zebrafish (Sect. 2.2.4.1), inhibits the formation of the postotic posterior LLP (Nikaido et al. 2017). (Other postotic LLPs were not examined.) Retinoic acid treatment of late blastula-stage zebrafish embryos resulted in only one-third the normal number of LLP-derived neurons forming in the (preotic) anterior lateral line ganglion, but threefold more neurons in the posterior lateral line ganglion (Holder and Hill 1991). (Neuromasts were not examined.) This suggested that excess retinoic acid signaling reduced the preotic LLPs but expanded the posterior LLP. Similarly, experiments in which retinoic acid synthesis was blocked showed that retinoic acid is required in zebrafish at late gastrulation stages for the formation of the posterior LLP (Sarrazin et al. 2010; Nikaido et al. 2017) but not the preotic LLPs (Nikaido et al. 2017). (Other postotic LLPs were not examined.) In the axolotl, in which all LLPs except the posterior form ampullary organs as well as neuromasts and neurons (Northcutt et al. 1994), retinoic acid treatment at late gastrula/early neurula stages resulted in the loss of all ampullary organs and significantly fewer neuromasts but a larger posterior lateral line ganglion (Gibbs and Northcutt 2004b). This suggested that excess retinoic acid signaling reduced all ampullary organ-forming LLPs (i.e., all LLPs except the posterior) but expanded the posterior LLP. Taken together, these data suggest that the response to retinoic acid differs between the postotic posterior LLP (which migrates on the trunk) and all the other LLPs (which migrate/elongate on the head) rather

than differing between preotic and postotic LLPs. However, this hypothesis must be tested further.

Molecular differences in the induction of individual LLPs could explain the evolutionary loss of specific LLPs in different lineages. Within amphibians, the (preotic) otic LLP is missing in all anurans, some urodeles, and some caecilians (Schlosser 2002a; Northcutt 2005a). Within the teleosts, the postotic supratemporal LLP is missing in the channel catfish (Northcutt 2005a). Although neither otic nor supratemporal LLPs have been identified in the zebrafish (Andermann et al. 2002), they presumably exist because both the otic and supratemporal neuromast lines form (Raible and Kruse 2000). In axolotl embryos, the homeobox transcription factor gene *Hoxb3* is expressed specifically in the postotic middle LLP and the immediately adjacent hindbrain region (Metscher et al. 1997), although it is unknown whether Hoxb3 plays any role in LLP development.

Another question relates to the mechanisms underlying the formation of ampullary organs by different subsets of LLPs in different species. For example, ampullary organs are formed only by the preotic LLPs in the Mississippi paddlefish (Modrell et al. 2011), by the preotic LLPs plus the postotic supratemporal LLP in another chondrostean, the sturgeon *Scaphirhynchus platorynchus* (Gibbs and Northcutt 2004a), and by all LLPs except the posterior LLP in the axolotl (Northcutt et al. 1994).

2.2.5 Investigating the Molecular Basis of Nonteleost Electroreceptor Development

2.2.5.1 The Candidate Gene Approach

The candidate gene approach, based primarily on knowledge of the molecular basis of LLP development in nonelectroreceptive species, has identified some markers for developing ampullary organs and electroreceptors in nonteleost jawed vertebrates and signaling pathways likely to be important for ampullary organ development. The limited results obtained suggest significant parallels, but also some differences, across different vertebrate groups.

2.2.5.1.1 Transcriptional Regulators

In nonteleost ray-finned bony fishes, the first molecular marker identified for developing ampullary organs was the high mobility group (HMG) domain transcription factor gene *Sox3* in a chondrostean, the Mississippi paddlefish (see Baker et al. 2013). *Sox3* is expressed in the LLP-forming posterior placodal area (see Sects. 2.2.1 and 2.2.4.1) and throughout LLP development in the nonelectroreceptive African clawed frog (Schlosser and Ahrens 2004). *Sox3* is also expressed in the LLPs of two unrelated nonelectroreceptive teleosts, the medaka (*Oryzias latipes*, a

percomorph euteleost) and the zebrafish (a cypriniform ostariophysan; Fig. 2.6; Köster et al. 2000; Nikaido et al. 2007). In the Mississippi paddlefish, *Sox3* also proved to be expressed in the posterior placodal area and maintained throughout LLP development, including in developing ampullary organ fields and ampullary organs as well as in neuromasts (see Baker et al. 2013).

In an attempt to clone the "pan-placodal" marker *Eya1* (see Sect. 2.2.1) in a cartilaginous fish (the small-spotted catshark, *Scyliorhinus canicula*), the related family member *Eya4* was cloned; this was fortuitous because *Eya4* proved to be a specific marker for LLPs (and the otic placode) throughout their development, including in ampullary organs as well as in neuromasts (O'Neill et al. 2007). This expression pattern is conserved across all jawed vertebrates, i.e., in a ray-finned bony fish (the Mississippi paddlefish) and a lobe-finned bony tetrapod (the axolotl), as well as in another cartilaginous fish, the little skate (see Baker et al. 2013). Furthermore, immunostaining for the calcium-buffering protein "Pv3" (Heller et al. 2002), an oncomodulin-related β-parvalbumin (Pvalbβ1/Ocm; Modrell et al. 2017a) that is thought to be the major Ca^{2+} buffer in hair cells (Heller et al. 2002), revealed that *Eya4* expression in neuromasts and ampullary organs is restricted, respectively, to hair cells and electroreceptor cells in the Mississippi paddlefish, axolotl, and little skate (see Baker et al. 2013).

The pan-placodal homeodomain transcription factor gene *Six1* and its transcription coactivator gene *Eya1*, which are expressed from preplacodal stages and maintained in all cranial placodes and their derivatives except the lens in the African clawed frog (Schlosser and Ahrens 2004), are also expressed, as might be expected, in paddlefish LLPs, neuromasts, and ampullary organs, as well as in other cranial placodes (see Baker et al. 2013). Indeed, *Six1, Six2, Six4*, and all four *Eya* family members are expressed throughout the development of paddlefish LLPs, including in lateral line organs (see Baker et al. 2013).

Conserved expression across vertebrate groups is not seen for all genes, however. A study of homeobox gene expression in axolotl embryos, undertaken to test the hypothesis that a "*Hox* code" might pattern cranial placodes at different dorsoventral and rostrocaudal axial levels, identified *Msx2* and *Dlx3* expression throughout the development of all LLPs, including in neuromasts and ampullary organs, and *Hoxb3* expression specifically in the postotic middle LLP (Metscher et al. 1997). However, it was noted (although without showing any data) that *Msx2* and *Dlx3* are not expressed during lateral line organ development in the Mississippi paddlefish (Modrell and Baker 2012). Similarly, expression of the T-box transcription factor gene *Tbx3*, reported specifically in LLPs in the African clawed frog (Schlosser and Ahrens 2004), is restricted to LLP-derived neurons in the small-spotted catshark (O'Neill et al. 2007).

Overall, perhaps the most significant finding of the candidate gene approach was the conserved expression of *Eya4* throughout LLP (and otic placode) development specifically, and its maintenance in hair cells and electroreceptors, across the three major groups of jawed vertebrates (see Baker et al. 2013). *Eya4* encodes one of the four members of the Eya family of transcription coactivators, which also have phos-

phatase activity and cytoplasmic roles (see Rebay 2015). In the African clawed frog, high levels of the pan-placodal family member Eya1 and its transcription partner Six1 promote placode cell proliferation, whereas lower levels promote neuronal and sensory differentiation (Schlosser et al. 2008; Riddiford and Schlosser 2016, 2017). Mutations in human *EYA4* underlie nonsyndromic sensorineural hearing loss *DFNA10* (Schönberger et al. 2005). Together, these data suggest that Eya4 is likely to play both early and late roles in LLP development and in the differentiation of both electroreceptors and neuromast hair cells. Testing this hypothesis will require blocking Eya4 function in an experimentally tractable electroreceptive species, such as the axolotl. Genome editing using CRISPR/Cas9, which efficiently yields mutant phenotypes in axolotl and lamprey embryos injected at the one-cell stage (Flowers and Crews 2015; Square et al. 2015), is an exciting possibility. CRISPR/Cas9 could, in principle, also be used to mutate genes of interest in other electroreceptive species, provided that many fertilized eggs can be obtained during a sufficiently long spawning season to optimize the conditions for that species.

2.2.5.1.2 Signaling Pathways

As described in Sect. 2.2.4, Wnt signaling blocks the formation of both preotic LLPs and the postotic posterior (trunk) LLP in the zebrafish, whereas FGF signaling is required for the formation of preotic LLPs and blocks the formation of the posterior LLP (Nikaido et al. 2017). Nevertheless, both Wnt and FGF signaling are critical during later stages of posterior LLP development, for both neuromast formation and hair cell differentiation. Briefly, during the migration of the posterior lateral line primordium, Wnt activity in the leading domain results in the expression and secretion of FGF3 and FGF10, which activate FGF receptor 1 (FGFR1) in the trailing domain (for detailed reviews, see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). Signaling through FGFR1 drives expression in the central cell of the proneural transcription factor gene *Atoh1* (required for hair cell formation in both the inner ear and lateral line; Millimaki et al. 2007; Costa et al. 2017) and the Notch ligand gene *DeltaA*. Atoh1 expression specifies the central cell as a hair cell progenitor and drives expression of a second Notch ligand gene, *DeltaD*, plus *Fgf10*. FGF10 secreted by the hair cell progenitor activates FGFR1 in its neighbors. This maintains the expression of Notch3, which, in turn, is activated by the Notch ligands expressed by the hair cell progenitor, resulting in the inhibition of *Atoh1* expression and thus of a hair cell fate ("lateral inhibition") in its neighbors. Furthermore, Notch and FGF signaling promote cell adhesion and apical constriction in the supporting cells, leading to the formation of "protoneuromasts," namely, epithelial rosettes of supporting cells around a central hair cell progenitor (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017).

Only the migrating posterior LLP of the zebrafish has been studied in such detail. It is not known to what extent the roles played by these pathways are conserved even within the other LLPs of the zebrafish, let alone across the LLPs of

other teleost and nonteleost species. A recent study using small-molecule inhibitors took the first steps in this endeavor by investigating the roles of FGF and Notch signaling during the development of ampullary organs and neuromasts from elongating preotic lateral line primordia in the Mississippi paddlefish (Modrell et al. 2017b). During zebrafish posterior LLP development, inhibiting FGF signaling blocks *Atoh1* expression and hence blocks hair cell differentiation and also protoneuromast (epithelial rosette) formation (Thomas et al. 2015; Dalle Nogare and Chitnis 2017). In the Mississippi paddlefish, inhibiting Fgf signaling during placode elongation stages resulted in the formation of fewer neuromasts, but with more hair cells than usual, and accelerated (rather than blocked) the formation of ampullary organs, each of which had many more electroreceptor cells than during normal development (Modrell et al. 2017b). Although more work is needed to clarify the specific mechanisms involved, these results nevertheless suggest significant differences in the roles of FGF signaling in neuromast versus ampullary organ formation from elongating LLPs and also in neuromast formation from elongating versus migrating primordia.

In the migrating zebrafish posterior LLP, inhibiting Notch signaling expands the domain of *Atoh1* expression, which, in turn, causes a reduction in FGF signaling that blocks protoneuromast maturation (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). In the Mississippi paddlefish, blocking Notch signaling just before and during sense organ formation resulted in the formation of irregularly spaced neuromasts with supernumerary hair cells and in the clustering of ampullary organs (sometimes resulting in large domains of adjacent ampullary organs) with supernumerary electroreceptors (Modrell et al. 2017b). The supernumerary sensory receptor cell phenotype suggests that Notch signaling normally prevents supporting cells from adopting a sensory receptor cell fate in both ampullary organs and neuromasts, consistent with the data from zebrafish (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). However, the formation of neuromasts and ampullary organs with abnormal spacing after blocking Notch signaling in the Mississippi paddlefish differs from the failure of protoneuromast (epithelial rosette) maturation seen after blocking Notch signaling in zebrafish (Thomas et al. 2015; Dalle Nogare and Chitnis 2017).

Overall, the limited data gathered thus far from small-molecule inhibitor experiments in the Mississippi paddlefish (Modrell et al. 2017b) suggest that, apart from the importance of Notch signaling for preventing supporting cells from differentiating as sensory receptor cells, both FGF and Notch signaling may play different roles in the development of ampullary organs versus neuromasts from elongating LLPs and in neuromast development from elongating versus migrating LLPs. Before any conclusions can be drawn about conservation of mechanisms, it is essential to gather experimental evidence from more species as outgroups, both for the migrating posterior lateral line primordium (e.g., are the mechanisms identified in zebrafish conserved in the African clawed frog and/or the axolotl?) and for preotic LLPs, both from nonelectroreceptive species like the zebrafish and the African clawed frog as well as electroreceptive species like the Mississippi paddlefish and axolotl.

2.2.5.2 Insights from an Unbiased Transcriptomic (Differential RNA Sequencing) Approach

The candidate gene approach described in Sect. 2.2.5.1, i.e., studying in electroreceptive species the genes and signaling pathways identified in nonelectroreceptive species as being important for LLP and/or neuromast formation, can and has been fruitful. However, this approach is less likely to identify the molecular mechanisms required specifically for the development of ampullary organs/electroreceptors. For this, an unbiased transcriptomic approach holds more promise. Differential next-generation RNA sequencing (RNA-seq) analysis in late-larval stages of the Mississippi paddlefish generated a dataset of several hundred candidate genes that are putatively enriched in lateral line organs (Modrell et al. 2017a). Validation of a subset of these candidates in the Mississippi paddlefish revealed that critical components of the transcription factor network essential for hair cell development (see Costa et al. 2017), in particular, the basic helix-loop-helix (bHLH) transcription factor gene *Atoh1* and the POU-domain transcription factor gene *Pou4f3* (*Brn3c*), were expressed in developing ampullary organs as well as in neuromasts (Modrell et al. 2017a). Atoh1 is essential for the differentiation not just of hair cells but also, for example, of cerebellar granule neurons, Merkel cells and proprioceptive neurons, and intestinal secretory cells (Costa et al. 2017). Hence, the developmental context within which Atoh1 acts (for example, which other transcription factors are expressed) is critical for the phenotypic outcome; however, relatively little is known about how Atoh1 acts to promote hair cell development (Costa et al. 2017).

As a class II bHLH transcription factor, Atoh1 binds DNA as a heterodimer with a class I bHLH ("E-protein") binding partner for which it competes with other class II bHLH transcription factors (see Costa et al. 2017). Intriguingly, mouse embryonic stem cells develop as neurons when forced to express Atoh1 but form hair cell-like cells when forced to express Atoh1 plus Pou4f3 and the zinc-finger transcriptional repressor Gfi1 (see Costa et al. 2017). Pou4f3 and Gfi1 are each required for normal hair cell differentiation and survival (see Costa et al. 2017). Gfi1 is the vertebrate ortholog of *Drosophila* Senseless, which directly binds (via its zinc fingers) to proneural bHLH transcription factors, including the Atoh1 ortholog Atonal, modulating the transcriptional activity of both proteins (see Costa et al. 2017). The mouse embryonic stem cell data suggest that Gfi1 and Pou4f3 together somehow transform Atoh1 from a neuronal determinant to a hair cell determinant (see Costa et al. 2017). *Gfi1* is present in the lateral line organ-enriched dataset from the Mississippi paddlefish (Modrell et al. 2017a), although its expression has not yet been examined. The LIM homeodomain transcription factor Lhx3, which is expressed in all inner ear hair cells and regulated by Pou4f3 (Hertzano et al. 2007), was also expressed in developing ampullary organs as well as neuromasts in the Mississippi paddlefish (Modrell et al. 2017a).

Similarly, the HMG domain transcription factor Sox2, which interacts with Six1 (and/or Six4) and its transcriptional coactivator Eya1 in a physical complex that is sufficient to induce *Atoh1* in mouse cochlear explants (Ahmed et al. 2012; Zhang et al. 2017), was expressed in both developing ampullary organs and neuromasts in

the Mississippi paddlefish (Modrell et al. 2017a). In the mouse cochlea, Six1 activity is also required later to downregulate Sox2 expression (Zhang et al. 2017), enabling Atoh1 to drive hair cell differentiation (Dabdoub et al. 2008; Zhang et al. 2017). *Six1*, *Six4,* and *Eya1* (together with *Six2*, *Eya2*, *Eya3,* and *Eya4*) had previously been reported as being expressed throughout LLP development in the Mississippi paddlefish, including in developing ampullary organs as well as in neuromasts (see Baker et al. 2013). Furthermore, the *miR-183* family of microRNAs (*miR-183*, *miR-96,* and *miR-182*, processed from a single transcript), which are important for hair cell development and maintenance (Soukup 2009; Weston and Soukup 2009), are Atoh1 dependent in hair cells and may also be involved in downregulating *Sox2* expression (Weston et al. 2011, 2018) and fine-tuning the transcriptional response to Atoh1 in favor of hair cells (Ebeid et al. 2017). This family of microRNAs is expressed by axolotl electroreceptors as well as hair cells (Pierce et al. 2008).

Taken together, these data suggest that the molecular mechanisms underlying electroreceptor development are highly conserved with those underlying hair cell development, although functional experiments are needed to confirm this. The level of conservation also begs the question of how electroreceptors are specified as opposed to hair cells. The lateral line organ-enriched dataset from the Mississippi paddlefish provided one candidate: the proneural bHLH transcription factor gene *Neurod4* (*Ath3*, *NeuroM*), which was expressed in developing ampullary organs but not in neuromasts (as well as in sites expected from other species, including the brain, olfactory epithelium, eyes, and trigeminal ganglion; Modrell et al. 2017a). Neurod4 could specify an electroreceptor fate given its role in specifying other cell fates. In the retina, Neurod4 cooperates with the bHLH transcription factor Ascl1 (Ash1) and the homeodomain transcription factor Vsx2 (Chx10) to determine bipolar cell fate and is required together with the related bHLH transcription factor Neurod1 to specify amacrine cells (Hatakeyama and Kageyama 2004). Furthermore, different Neurod family members may be important for specifying different subtypes of hair cells. Neurod1 prevents otic neurons from expressing Atoh1 and adopting a hair cell fate and is required for the maturation of outer hair cells in the cochlea (Jahan et al. 2010), whereas Neurod6 is enriched in cochlear but not in vestibular hair cells (Elkon et al. 2015). Further studies are needed to determine the role(s) played by Neurod4 in electroreceptor development, the identity of its transcriptional partners, and whether or not this is conserved in developing ampullary organs outside chondrostean ray-finned fishes.

Overall, both the candidate gene (see Sect. 2.2.5.1.1) and unbiased transcriptomic (RNA-seq) approaches suggest that the molecular mechanisms underlying nonteleost electroreceptor development are likely to be highly conserved with those that underlie hair cell development. In particular, essentially all the transcription factor genes known to be important for hair cell development are also expressed in developing ampullary organs in the Mississippi paddlefish (Modrell et al. 2017a). This very close developmental relationship may also support a close evolutionary relationship between these cell types, as discussed in Sect. 2.3.

2.3 Electroreceptor Evolution

The homology of electroreceptors in lampreys and nonteleost jawed vertebrates is supported by both physiology and innervation: they are stimulated by weak, low-frequency cathodal (exterior-negative) electric fields (and inhibited by anodal fields) and innervated by lateral line afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve (Bullock et al. 1983; Baker et al. 2013). As described in Sect. 2.2.2, fate-mapping experiments have shown that in representatives of the three major clades of jawed vertebrates (Fig. 2.2) individual LLPs give rise to ampullary organs as well as to neuromasts and lateral line neurons (see Baker et al. 2013). Furthermore, within these three clades, nonteleost ampullary electroreceptors and neuromast hair cells maintain expression of the transcriptional coactivator gene *Eya4* and express the calcium-buffering protein "Pv3" (see Baker et al. 2013), an oncomodulin-related β-parvalbumin (Pvalbβ1/Ocm; Modrell et al. 2017a). It will be important to extend the LLP fate-mapping and molecular studies to lampreys. Nevertheless, the shared physiology and innervation of lamprey and nonteleost jawed vertebrate electroreceptors support their being homologous, i.e., that the electrosensory division of the lateral line system evolved once, in the lineage leading to the common ancestor of all living vertebrates. The independent evolution of teleost electroreception is discussed in Sect. 2.1.2.3.2.

As proposed by Jørgensen (1982), electroreceptors could have evolved via the modification of hair cells. Alternatively, electroreceptors and hair cells could have evolved independently from a ciliated secondary sensory cell, which itself likely evolved via the diversification of an ancestral primary sensory neuron (Jørgensen 1982; also see Fritzsch and Elliott 2017). Sections 2.3.1 and 2.3.2 discuss the similarities between nonteleost electroreceptors and hair cells, then Sect. 2.3.3 brings these together to discuss hypotheses for electroreceptor evolution.

2.3.1 Morphological and Physiological Similarities Between Hair Cells and Nonteleost Electroreceptors

As noted in Sect. 2.1, hair cells and nonteleost ampullary electroreceptors are secondary sensory cells (i.e., lacking an axon), with basolateral presynaptic ribbons and a single apical primary cilium surrounded by varying numbers of actin-rich microvilli and basolateral ribbon synapses (Fig. 2.1; also see Sect. 2.3.2; Jørgensen 2005). Lamprey adult end bud electroreceptors and ammocoete-stage multivillous cells share all these characteristics except for the primary cilium (Fig. 2.4; Jørgensen 2005). However, the development of lamprey electroreceptors has not been characterized, so it is possible that an apical cilium forms but is subsequently lost, as occurs during mammalian cochlear hair cell development (Lu and Sipe 2016).

During hair cell maturation, the primary cilium (kinocilium) moves eccentrically and the apical microvilli (stereocilia) elongate in a graded fashion such that they

become organized into rows in a staircase array; they are connected at their distal tips by tip links and to the kinocilium by kinociliary links (Fig. 2.1A; see Lu and Sipe 2016). This stepped, linked array of stereocilia comprises the "hair bundle" that characterizes hair cells. Deflection of the hair bundle in the direction of the kinocilium (or of the tallest stereocilia in mammalian cochlear hair cells) increases tension on the tip links, which triggers the opening of cation-selective mechanoelectrical transducer channels (Nicolson 2017; Cunningham and Müller 2019). Cation entry depolarizes the hair cell, opening L-type voltage-gated Ca^{2+} ($Ca_v1.3$) channels clustered in the basolateral membrane at presynaptic ribbons (Safieddine et al. 2012; Nicolson 2015). Ca^{2+} entry via these $Ca_v1.3$ channels leads to synaptic vesicle exocytosis and neurotransmitter release (see Safieddine et al. 2012; Nicolson 2015).

Until 2017, the most detailed information about how nonteleost electroreceptors work had been gathered using ampullary organ preparations from various skate species (Bennett and Obara 1986; Bodznick and Montgomery 2005; also see Leitch and Julius, Chap. 3). Briefly, the electroreceptors are partially depolarized at rest by an inward "bias current," resulting in constant neurotransmitter release and tonic activity of the afferent fibers. Weak cathodal (exterior-negative) stimuli open apical voltage-gated Ca^{2+} channels, depolarizing the apical membrane and, in turn, depolarizing the basal membrane. This opens basal voltage-gated Ca^{2+} channels, leading to Ca^{2+} entry and neurotransmitter release, thus increasing spike frequency. Apical Ca^{2+} entry ultimately triggers a Ca^{2+}-activated outward K^+ current, repolarizing the apical membrane and terminating the depolarization of the basal membrane.

In both the little skate and the chain catshark (*Scyliorhinus retifer*), the $Ca_v1.3$ channel was identified as the apical low-threshold voltage-sensing Ca^{2+} channel (Bellono et al. 2017, 2018; also see Leitch and Julius, Chap. 3). In the little skate, as predicted from earlier work (Bennett and Obara 1986; Bodznick and Montgomery 2005), the Big Potassium (BK) channel is the large-conductance Ca^{2+}-activated K^+ channel working with the $Ca_v1.3$ channel to mediate electroreceptor membrane oscillations (Bellono et al. 2017). Although chain catshark electroreceptors express *Kcnma1*, which encodes BK, oscillations in this species are mediated by the voltage-gated K^+ channel $K_v1.3$, encoded by *Kcna3* (Bellono et al. 2018).

Specific channels involved in electroreceptor function have not been identified in bony fishes. However, analysis of the lateral line organ-enriched RNA-seq dataset from the Mississippi paddlefish showed that *Cacna1d*, encoding the $Ca_v1.3$ channel, is expressed in ampullary organs as well as in neuromasts (see also Sect. 2.3.2) and that *Kcna5*, encoding the $K_v1.5$ channel, and *Kcnab3*, encoding the β-subunit $K_vβ3$, are ampullary organ-specific (Modrell et al. 2017a). These expression data suggest the hypothesis, which remains to be tested, that the $Ca_v1.3$ and $K_v1.5$ channels mediate electroreceptor membrane oscillations in the Mississippi paddlefish, like the $Ca_v1.3$ and $K_v1.3$ channels do in the chain catshark (Bellono et al. 2018).

The BK channel has been localized to the primary cilium of both olfactory receptor neurons and principal cells in the rabbit nephron (Delgado et al. 2003; Carrisoza-Gaytán et al. 2017). Furthermore, the primary cilium of kidney cells is a specialized calcium-signaling organelle containing calcium-permeant channels at a high density, within which the Ca^{2+} concentration is effectively insulated from changes in

cytoplasmic Ca^{2+} (DeCaen et al. 2013; Delling et al. 2013). Other than in lampreys, all nonteleost electroreceptors bear a primary cilium (Jørgensen 2005). It seems plausible, therefore, that the $Ca_v1.3$ and BK channels may be localized to the primary cilium of electroreceptors, although this remains to be tested.

The importance of the $Ca_v1.3$ and BK channels for little skate electroreceptor function (Bellono et al. 2017) further emphasizes similarities between hair cells and nonteleost electroreceptors because basolateral $Ca_v1.3$ channel activity triggers neurotransmitter release at hair cell (but not photoreceptor) ribbon synapses (see Safieddine et al. 2012; Nicolson 2015; also see Sect. 2.3.2), whereas $Ca_v1.3$ and BK channel interaction in the basolateral hair cell membrane mediates electrical "tuning" (Fettiplace and Fuchs 1999). It may also be worth noting that in skate electroreceptors, weak anodal (lumen-positive) stimuli inhibit the resting discharge, but large anodal stimuli directly depolarize the basal membrane, resulting in neurotransmitter release (Bennett and Obara 1986). Similarly, neuromast hair cells release neurotransmitter in response to large anodal stimuli (e.g., Münz et al. 1984; Barry et al. 1988).

2.3.2 RNA Sequencing Data Suggest Nonteleost Electroreceptors Share Synaptic Transmission Mechanisms with Hair Cells

The specific mechanisms underlying transmission at the hair cell ribbon synapse are thought to be unique (see Zanazzi and Matthews 2009; Safieddine et al. 2012). As described in Sect. 2.1.2, all vertebrate electroreceptors have ribbon synapses (see Jørgensen 2005) as do vertebrate retinal photoreceptors, retinal bipolar cells, and pineal photoreceptors (see Zanazzi and Matthews 2009; Safieddine et al. 2012).

The main structural constituent of the presynaptic ribbon (and the only ribbon-specific protein known) is the protein Ribeye, which is generated via an alternative start site for the reduced nicotinamide adenine dinucleotide (NADH)-sensitive transcriptional corepressor gene *Ctbp2*, producing an N-terminal A-domain unique to Ribeye (see Zanazzi and Matthews 2009; Nicolson 2015). In mice lacking Ribeye, ribbons are abolished in the retina (Maxeiner et al. 2016) and cochlear hair cells (Becker et al. 2018; Jean et al. 2018) and synaptic transmission is impaired, confirming that the ribbon is important for rapid synaptic vesicle replenishment. In zebrafish neuromast hair cells, Ribeye protein depletion by morpholino injection and genetic mutation also showed its importance in clustering $Ca_v1.3$ channels at the presynaptic membrane (see Nicolson 2015; Lv et al. 2016).

There are significant differences in the mechanisms underlying synaptic transmission at the hair cell ribbon synapse versus other ribbon synapses (see Zanazzi and Matthews 2009; Safieddine et al. 2012). Hair cell synaptic vesicles are loaded with glutamate by the vesicular glutamate transporter Vglut3, whereas Vglut1 and Vglut2 are used at retinal photoreceptor and bipolar cell ribbon synapses and at central glutamatergic synapses (see Zanazzi and Matthews 2009). As noted in Sect.

2.3.2, synaptic vesicle exocytosis is triggered in hair cells by activation of the $Ca_v1.3$ channel, whose abundance and function is regulated by the auxiliary subunit $Ca_v\beta2$ (Neef et al. 2009), whereas retinal photoreceptors depend on the $Ca_v1.4$ channel (see Zanazzi and Matthews 2009; Nicolson 2015). Finally, synaptic vesicle exocytosis in hair cells is uniquely mediated by the multi-C2 domain transmembrane protein otoferlin, a calcium-sensitive type II ferlin (Hams et al. 2017; Michalski et al. 2017), rather than by neuronal soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs; see Safieddine et al. 2012).

Candidate gene analysis from the lateral line organ-enriched gene set generated by differential RNA-seq analysis in the Mississippi paddlefish (described in Sect. 2.2.5.2) revealed that in this species, late-larval ampullary organs as well as neuromasts express *Slc17a8* (encoding Vglut3), *Cacna1d* (encoding the pore-forming α-subunit of the $Ca_v1.3$ channel), *Cacnb2* (encoding $Ca_v\beta2$), *otoferlin,* and the Ribeye-specific A domain of *Ctbp2* (Modrell et al. 2017a). These expression data suggest that transmission mechanisms at the electroreceptor ribbon synapse are conserved with hair cells, although this remains to be tested.

2.3.3 Hypotheses for Electroreceptor Evolution: The Importance of Ribbons

Recent hypotheses about neurogenic placode evolution take as their starting point the variety of sensory and neurosecretory cell types found in the epidermis of invertebrate chordate filter feeders (i.e., the tunicates, the closest living invertebrate relatives of the vertebrates, and the cephalochordates, the outgroup to the tunicates and vertebrates, representing the most basally branching chordate lineage) and suggest how the evolution of ectodermal patterning in the vertebrate lineage may have enabled the concentration of such sensory and neurosecretory cells in patches on the head (see Patthey et al. 2014; Schlosser et al. 2014). This would be consistent with the cephalization and elaboration of placode-derived sense organs in the vertebrate ancestor, in the transition from filter feeding to predation, as originally proposed by Northcutt and Gans in their "New Head Hypothesis" (see Northcutt 2005b). Similarly, rather than focusing on the evolution of individual hair cell-forming placodes (otic before lateral line or lateral line before otic?), a "hair cell first" hypothesis has been proposed in relation to inner ear evolution, incorporating molecular evidence relating to the development of inner ear hair cells and their afferent neurons (see Fritzsch and Elliott 2017). As noted in Sect. 2.3.1, electroreceptors and hair cells could have evolved as separate, independent diversifications of a ciliated secondary sensory cell or hair cells could have evolved first, with electroreceptors subsequently evolving via the diversification of lateral line hair cells (Jørgensen 1982). (Electroreceptor evolution via lateral line hair-cell diversification most likely also occurred independently at least twice within teleosts; see Sect. 2.1.2.3.2.)

Intriguingly, in tunicates (the sister group of vertebrates), secondary sensory cells with microvilli, one or more apical cilia, afferent glutamatergic synapses, and

at least some gene expression patterns shared with hair cells have been described in the mechanosensory coronal organ of the ascidian oral siphon and in the appendicularian circumoral ring (see Burighel et al. 2011; Rigon et al. 2018). It is feasible that these tunicate secondary sensory cells and vertebrate hair cells evolved from the same mechanosensory cell type (whether a primary sensory neuron or a secondary mechanosensory cell) in the common ancestor of tunicates and vertebrates, i.e., that they are homologous.

A key difference between these tunicate secondary sensory cells and both hair cells and electroreceptors in vertebrates, however, is the absence of presynaptic ribbons (Burighel et al. 2011), which have not been reported in any cells in invertebrate chordates (Petralia et al. 2016). Because cephalochordates (amphioxus species) have homologues of vertebrate retinal and pineal photoreceptors, this suggests that ribbon synapses evolved independently in vertebrate retinal cells, pineal photoreceptors, and hair cells (see Baker and Modrell, 2018). As noted in Sect. 2.3.2, synaptic vesicle loading and exocytosis are mediated by different proteins at hair cell versus retinal ribbon synapses (Zanazzi and Matthews 2009; Safieddine et al. 2012). Electroreceptors are more closely related to hair cells in all these respects, at least as determined by gene expression in the Mississippi paddlefish (Modrell et al. 2017a), as well as in the molecular mechanisms underlying their development (see Sect. 2.2.5). If electroreceptors and hair cells evolved independently in the vertebrate ancestor via separate diversifications of a ciliated secondary sensory cell, then either this cell had already evolved ribbon synapses dependent on $Ca_v1.3/Ca_v\beta2$ channels, otoferlin, and Vglut3 and its development already involved all the molecular mechanisms that seem likely to be shared by hair cells and electroreceptors (see Sect. 2.2.5), or these features evolved independently in both hair cells and electroreceptors. It seems more parsimonious to suggest that electroreceptors evolved in the vertebrate ancestor via the diversification of lateral line hair cells to form a "sister cell type" (*sensu* Arendt et al. 2016). The selection pressure in early vertebrate evolution for the modification of a hair cell such that it depolarizes in response to low-frequency cathodal electric fields, perhaps involving the expression of $Ca_v1.3$ (and BK) channels at a high density in the primary cilium as well as in the basolateral membrane, could reflect the advantage of being able to detect not only local water movement but also nearby living prey items and/or predators.

2.4 Summary

Significant progress has been made in the understanding of electroreceptor development in nonteleost jawed vertebrates. This includes the experimental confirmation of the embryonic origin of ampullary organs (together with neuromasts and afferent neurons) from LLPs in representatives of all three major jawed vertebrate groups (lobe-finned bony fishes/tetrapods, ray-finned bony fishes, and cartilaginous fishes) and the identification of the transcriptional regulator *Eya4* and an oncomodulin-related β-parvalbumin as conserved markers of electroreceptors across all

nonteleost jawed vertebrates. The first inroads have been made into identifying signaling pathways involved in ampullary organ development and, more generally, into the molecular basis of LLP development, including heterogeneity of mechanism among different LLPs. The trickle of genes reported as expressed in developing ampullary organs in different species, based on candidate genes, has turned into a stream with the advent of differential RNA-seq analysis, enabling an unbiased approach. In the Mississippi paddlefish, at least, this has revealed very high levels of conservation of gene expression between developing ampullary organs and neuromasts, including essentially all the transcription factor genes known to be important for hair cell development plus genes required specifically for transmission at the hair cell ribbon synapse. This degree of conservation also suggests that electroreceptors most likely evolved in the vertebrate ancestor via the diversification of lateral line hair cells as opposed to the independent evolution of electroreceptors and hair cells from a secondary ciliated cell. The unbiased transcriptomic approach also identified the first-reported transcription factor gene expressed in developing ampullary organs but not in neuromasts, which could be involved in specifying electroreceptors rather than hair cells.

However, these advances are, for the most part, descriptive. Experimental evidence is still lacking for the embryonic origin of lamprey electroreceptors and the various independently evolved teleost electroreceptors. Nothing is yet known at the molecular level about these electroreceptors. More experimental evidence, from multiple species, is needed to understand the molecular mechanisms underlying the development of the elongating LLPs that form both ampullary organs and neuromasts, and how these differ from the mechanisms underlying the development of the migrating zebrafish posterior LLP, on which most LLP research is currently focused. Gene expression patterns are indicative, but experimental studies are needed to test hypotheses about gene function.

Technical advances in the second decade of the twenty-first century make the future of experimental research into electroreceptor development very bright. First, the reduced cost of next-generation transcriptome sequencing (RNA-seq), including from relatively small amounts of extracted RNA, plus software that assembles RNA-seq data without a genome sequence, together make unbiased transcriptomic approaches feasible in any species. Furthermore, single-cell RNA-seq should allow electroreceptor-specific transcriptomes to be generated (as opposed to tissue-level or electrosensory organ-level transcriptomes), enabling direct comparison to identify conserved and divergent features of different nonteleost electroreceptors and of ampullary versus tuberous electroreceptors in and between different electroreceptive teleosts. The results should also shed light on electroreceptor evolution, including in teleosts. Finally, genome-editing CRISPR/Cas9 technology (already used in the axolotl and lamprey) should enable gene function to be tested in both nonteleost and teleost electroreceptive species. Overall, these new technologies should enable spectacular future advances in our understanding of electroreceptor development and evolution.

Compliance with Ethics Requirements Clare Baker declares that she has no conflict of interest.

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