The evolution of cell types in animals: emerging principles from molecular studies

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Abstract | Cell types are fundamental units of multicellular life but their evolution is obscure. How did the first cell types emerge and become distinct in animal evolution? What were the sets of cell types that existed at important evolutionary nodes that represent eumetazoan or bilaterian ancestors? How did these ancient cell types diversify further during the evolution of organ systems in the descending evolutionary lines? The recent advent of cell type molecular fingerprinting has yielded initial insights into the evolutionary interrelationships of cell types between remote animal phyla and has allowed us to define some first principles of cell type diversification in animal evolution.

Opsins
A family of G-protein-coupled receptors that function as light-sensitive photopigments.

Urbilaterian
The last common ancestor of all bilaterians.

Animals are composed of cell types that are specialized for functions as diverse as nutrient uptake, contraction, light perception and hormone secretion. Although some animals, such as sponges, have few cell types[1], there are hundreds of human cell types[2] and cell type number has been used as an index of complexity[3]. How did cell type diversity evolve? The identification of homologous cell types between species (those cells that evolved from the same precursor cell type in the last common ancestor), and of related cell types within a given species, the so-called sister cell types[4] (those that evolved from the same precursor cell type in the stem line of that species), is key to the study of cell type evolution (FIG. 1a).

Traditionally, cellular characteristics have been compared by light and electron microscopy. These techniques allowed the identification of homologous cell types between closely related species, but were more ambiguous across longer evolutionary distances, especially between distinct animal phyla[5,6]. It was often difficult to decide whether shared cellular features, such as the surface-enlarged photoreceptive cilium of a photoreceptor cell or the stripes of a striated muscle cell, reflected common ancestry or independent evolution[6]. These limitations can now be overcome by comparing cellular characteristics at the molecular level. For example, it was found that the surface-enlarged, light-sensitive cilia of ciliary photoreceptors in humans and worms harbour orthologous opsins[7], and that the stripes of striated muscle cells in various animals are composed of the same cytoskeletal proteins[8,9], which is indicative of homology. In addition, conserved, cell type-specific combinations of transcription factors have been identified that turn on cell type-specific differentiation genes. For the first time, comparing molecular fingerprints[10] allows us to identify homologous cell types over long evolutionary distances and thereby reconstruct systems such as the urbilaterian brain, eyes and immune system. Molecular fingerprinting also facilitates the identification of sister cell types within tissues and organs, as exemplified by the cell types of the vertebrate retina.

Now that cell type interrelationships have begun to be elucidated, this conceptual Review attempts to infer principles of cell type evolution. First, homologous cell types conserved over long evolutionary distances tend to exert multiple, distinct cellular functions, such as sensory, epithelial and contractile functions (the cnidarian epithelial muscle cell[11]) or sensory and neurosecretory functions (Vigh's protoneuron[12]). I will argue that multifunctionality has been a general feature of ancient cell types. Second, with increasing specialization during evolution, these multiple functions were then distributed in a complementary manner to sister cell types, as happened with the epithelial, sensory, neuronal or muscle cell type descendants of ancient epithelial muscle cells or with the functionally diverse cell types of the vertebrate retina. This principle, called segregation of functions, elegantly explains the emergence of axonal circuits in nervous-system evolution. At the regulatory level, functional segregation involves the cell type-specific loss of transcription factor expression. A third principle is divergence of functions, in which cellular functions are retained, but modified to different extents

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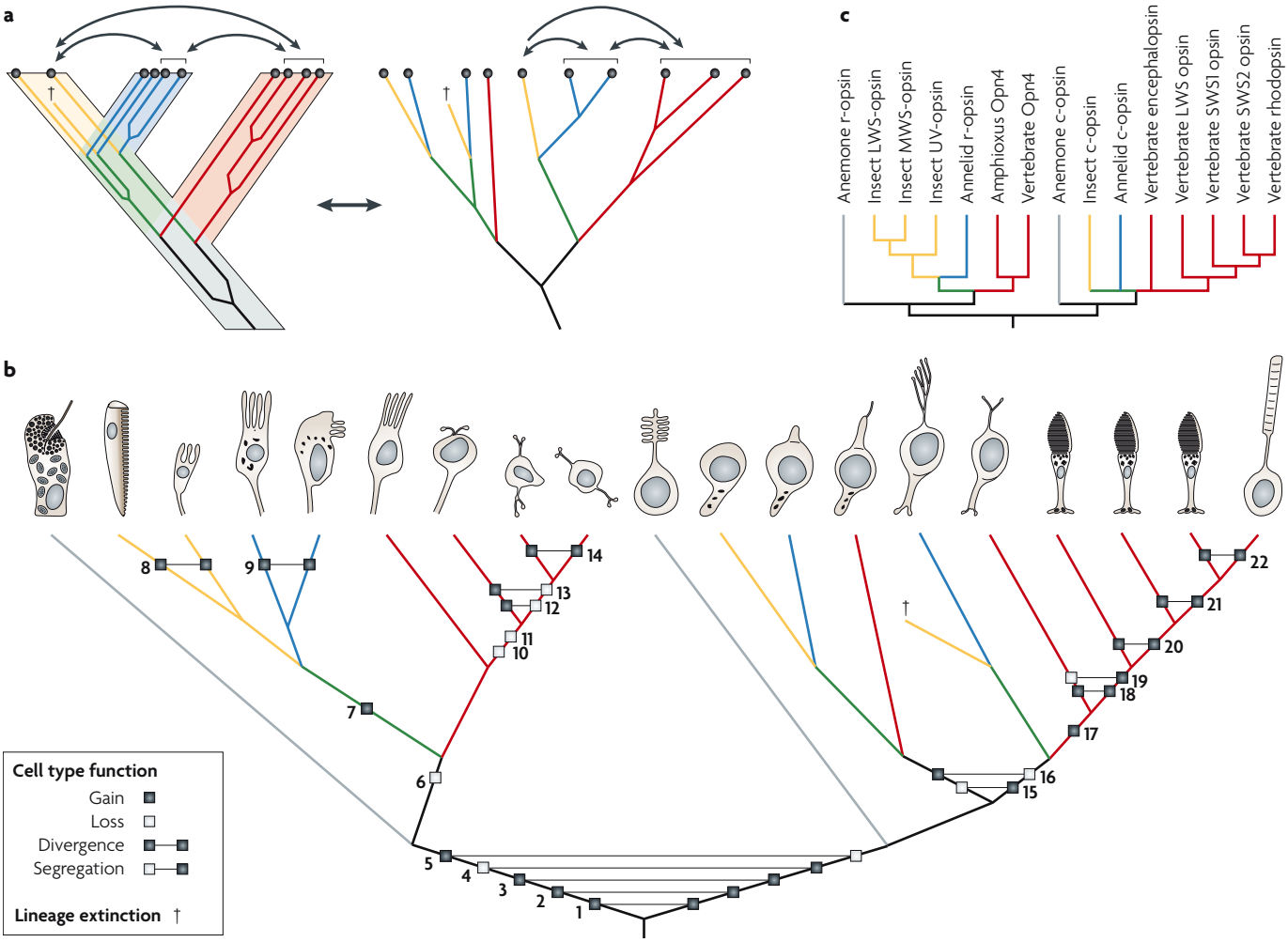

Figure 1 | **Cell typogenesis: homologous cell types and sister cell types. a** | The interrelationship between the phylogenetic tree and the typogenetic tree. On the left is a phylogenetic tree onto which the cell typogenetic tree has been superimposed. This illustrates the evolution of sister cell types within a given species (indicated by square brackets) and of homologous cell types between species (indicated by arrows). On the right is the same typogenetic tree alone. Cell types are ordered in a cladistic system that reflects their genealogy at the species level[114]. This cell type cladistic approach has been used in several pioneering studies[2,56,81,84,121,122]. **b** | The photoreceptor typogenetic tree. Yellow, blue, red and grey represent the evolution of cell types in Ecdysozoa, Lophotrochozoa, Deuterostomia and Cnidaria, respectively. Green represents evolution in the protostome stem line. Black represents evolution in the metazoan and bilaterian stem lines. It must be stressed that, at this stage of analysis with incomplete data sets, the precise order of branching in the typogenetic tree may still have to be refined at some positions, although the general degree of relatedness is well documented. Also, the list of characters is still incomplete. The photoreceptors from left to right are the cnidarian rhabdomeric eye[60], insect rhabdomeric compound eye, insect rhabdomeric larval eye[123], polychaete rhabdomeric adult eye[32], polychaete rhabdomeric larval eye[32], amphioxus rhabdomeric Hesse eyecup[124], vertebrate retinal ganglion cell, vertebrate amacrine cell, vertebrate horizontal cell, cnidarian ciliary photoreceptor, insect light-sensitive neurosecretory cell, annelid light-sensitive neurosecretory cell, vertebrate light-sensitive neurosecretory cell, annelid brain ciliary photoreceptor, vertebrate bipolar cell, vertebrate long wavelength-sensitive (LWS) cone, vertebrate short wavelength-sensitive 1 (SWS1) cone, vertebrate SWS2 cone and vertebrate rod. 1, opsin storage in the surface-extended apical membrane (rhabdomeric photoreceptor) or in the membrane of the cilium (ciliary photoreceptor); 2, deployment of the r-opsin of the c-opsin duplicate; 3, use of the Gq-α and phospholipase C or of the Gi-α and cGMP phototransduction cascade; 4, control of reproduction via neurosecretion; 5,6, locomotor cilium; 7, assembly of rhabdomeric photoreceptor cells into an eye: visual function; 8, specialization into larval or adult eye photoreceptors (for example, in arthropods); 9, specialization into larval or adult eye photoreceptors (for example, in annelids); 10, rhabdomeric photoreceptor structure; 11, direct association with pigment cell; 12, light sensitivity; 13, axonal projection to brain; 14, specialization into horizontal or vertical interneuron; 15, surface-extended cilium; 16, control of reproduction via neurosecretion; 17, visual function; 18, axonal projection or interneuron function; 19, light sensitivity via c-opsin; 20, deployment of the LWS or of the SWS1–2–rhodopsin duplicate. 21 deployment of the SWS1 or of the SWS27–rhodopsin duplicate; 22, deployment of the SWS2 or of the rhodopsin duplicate. **c** | Evolution of the opsin family of G-protein coupled receptors. c-opsin, ciliary opsin; MWS, medium wavelength-sensitive; Opn4, opsin4; r-opsin, rhabdomeric opsin; UV, ultra violet.

in the descendant sister cell types, as occurred during the development of light sensitivity of retinal rods and cones. In many cases, functional divergence is driven by gene duplication. Finally, evolving cell types can also acquire new functions. This involves either the expression of novel genes, for example, those that encode the structural proteins of the nematocyte in cnidarians, or the co-option of genes (or entire gene cascades) from other cell types. This mechanism of gene co-option explains the evolution of wing spots or the *de novo* evolution of ectopic eyes.

The expanding field of comparative molecular cell biology will elucidate the evolution of organ systems, such as the central nervous system, sensory organs, musculature and gut, thus revealing the emergence of the bilaterian body plan.

Cell type comparison via molecular fingerprints

What is a cell type and how can cell types be compared? By definition, any cell type has special physiological or structural characteristics. The aim of comparative study of cell type characteristics is to elucidate the evolutionary diversification of cell types (cell typogenesis) by detecting the similarities and differences between them. Physiological and structural characteristics will be reflected by cell type-specific gene expression at some point, and therefore comparisons can be based on differential expression profiling data. It is evident that these comparisons benefit from the increased availability of metazoan gene inventories[13].

The specific set of differentiation genes that implements cell type-specific physiology or structure makes up the differentiation signature of the cell type, defined here as any combination of active differentiation genes that is unique to a particular cell type. At the regulatory level, these differentiation genes are turned on by a unique combination of differentially expressed transcription factors[14], which could be refined post-transcriptionally by the action of specific microRNAs[15]. This is the regulatory signature of the cell type. Together, the differentiation and regulatory signatures constitute the molecular fingerprint of a cell type[10,12]. Molecular fingerprinting is most easily accomplished at early differentiation stages, when the regulatory and differentiation genes are largely co-expressed and cell numbers are still low. We can identify homologous and sister cell types through their similar, or identical, molecular fingerprints (BOX 1). Together, morphological, physiological and developmental comparisons can unveil cell type interrelationships.

Comparative genomics recently pinpointed some limitations of the cell type comparative approach[13,16,17], revealing that important cell type-specific marker genes are often absent or strongly modified in fast-evolving species. In such cases, it is advantageous to include slow-evolving species that have retained these genes in the comparison. For example, *Drosophila melanogaster* and *Caenorhabditis elegans* must have lost (or modified beyond recognition) a number of ancient opsins, multiple hormones of the neurosecretory system and proteins involved in innate immunity, which are preserved in slower-evolving vertebrates and annelids[7,12,18,19].

Reconstructing ancient cell type inventories

The identification of homologous cell types between species implies that a similar precursor type existed in the last common ancestor of these species. We can therefore identify sets of cell types that existed at important evolutionary nodes, such as the node that separates the protostomes (insects, nematodes, annelids and molluscs) from the deuterostomes (starfish, amphioxus, ascidians and vertebrates). Pioneering studies revealed the homology of somatic motor neurons[20,21] between vertebrates, insects (*Drosophila* spp.) and nematodes (*Caenorhabditis* spp.) (FIG. 2a), and of peripheral sensory neurons between *Drosophila* spp. and vertebrates[22]. Accordingly, these cell types existed in the last common ancestor of the protostomes and the deuterostomes. Another more recent study revealed the homology of branchiomotor neurons between ascidians and vertebrates[23] (FIG. 2b). Below, I outline some examples that have been used to reconstruct ancient cell type inventories. In each case, the inclusion of slow-evolving species has been crucial for the comparisons.

Lines of photoreceptor evolution. In the past, the comparison of cell type molecular fingerprints has profoundly changed our view of photoreceptor evolution in the animal kingdom[4,7,24–27]. Morphologists had assumed that photoreceptor cells had independently evolved up to 50 times[6]. In striking contrast, an ancient evolutionary origin of two distinct types of photoreceptor cells, the ciliary and rhabdomeric cells (FIG. 1b), is now supported by the conserved deployment in protostomes and deuterostomes of two distinct opsin paralogues, ciliary opsin (c-opsin) versus rhabdomeric opsin (r-opsin), and of two distinct downstream phototransductory cascades, transducin–cGMP versus Gq-α–phospholipase C signalling. Accordingly, ciliary and rhabdomeric photoreceptors existed in urbilaterians[7,28]. The ciliary line of photoreceptor evolution seems to even date back to early metazoans, as indicated by the presence of c-opsin-related photopigment in the ciliary photoreceptors of jellyfish eyes[26,29]. In the bilaterians, an ancestral population of ciliary photoreceptors resides in the brain. This c-opsin-related photopigment was recently molecularly characterized in the marine annelid *Platynereis dumerilii*[7,12], but it also exists in mosquitoes[30] and in the vertebrate forebrain[31] (FIG. 2a). Because no shading pigment is associated with these cells, they have a non-visual function. Intriguingly, the molecular fingerprint comparison indicates that the vertebrate rods and cones have emerged from this ancient population of brain ciliary photoreceptors, which involved a change from non-visual to visual photoreceptor function[7] (number 17 in FIG. 1b). This observation strengthens the view that the vertebrate retina evolved as an outfolding from the brain, as recapitulated during the development of the present day vertebrate eye[32,33].

In contrast to vertebrate and jellyfish eyes, most animal eyes have rhabdomeric photoreceptors that use r-opsins. This holds true not only for the insect larval and compound eyes, but also for planarian eyes[34], larval and adult annelid eyes[28], and for the dorsal ocelli

Nematocyte
A venomous cell that evolved for catching prey and for predator defence by releasing the nematocyst, which is a miniature cellular weapon, in one of the fastest movements in the animal kingdom.

Cnidarian
Radially symmetrical animal that has a sac-like body with only one opening. The group includes jellyfish, corals, hydra and anemones

Amphioxus
The common name for the cephalochordate *Branchiostoma lanceolatus*, the most basal living invertebrate that is related to vertebrates.

Ascidian
A group of sessile animals with swimming larvae that are the closest living invertebrate relatives of the vertebrates.

Box 1 | **Principles of the molecular fingerprint approach**

The candidate gene approach

The candidate gene approach relies on comparative analysis of the expression of transcription factors and differentiation genes known to be important or specific for the specification of cell types.

Regionally expressed transcription factors respond to early signalling cascades that are involved in regional patterning. The signalling cascades subdivide the early embryonic or larval body into regions along the anterior–posterior and dorsal–ventral body axes, and often have a later role in the control of batteries of differentiation genes, as implied in the intercalary evolution scenario[106]. For example, the transcription factor sine oculis homeobox homologue 3 (Six3) has an early function in establishing regions[107], and has recently been identified as a putative direct stimulator of rhodopsin expression in rods and cones[108].

Downstream of the regional specification genes, additional transcription factors contribute to the specification of the outline, architecture and physiology of post-mitotic cells. In the developing nervous system, transcription factors specify transmitter phenotype, axonal outgrowth, dendritic arborization and other aspects of neuronal identity[109]. Given the combinatorial action of transcription factors, one factor is often active in different cell types, such as the LIM and POU homeodomain transcription factors, which show a widespread 'salt-and-pepper' pattern of expression.

Most useful for the candidate gene approach are the cell type-specific transcription factors that control a broad range of phenotypic traits in a given cell type[109]. For example, motor neuron and pancreas homeobox proteins (Hb9 and mnx, respectively) control various aspects of somatic motor-neuron identity, including cholinergic transmitter type and axonal projection[110], and the paired-like homeobox 2 (*Phox2*) genes have a conserved role in the broader control of chordate branchiovisceral motor-neuron fate[23,111]. The nocireceptor-specific runt-related transcription factor 1 (Runx1) coordinates neurotrophin receptor, ion channel and neuropeptide expression, as well as axonal target selection[112–114]. Typically, these cell type-specific transcription factors show highly restricted patterns of expression. Finally, the regulatory activity of transcription factors is complemented by the channelling activity[115] of microRNAs, which also show tissue and cell type-restricted activity[15].

The microarray approach

The microarray approach differs from the candidate gene approach in that it is more complete and unbiased. In microarray studies, the entire expression profile can be used to determine the regulatory and differentiation signature of the cell type under study, and no prior knowledge about the candidate genes expressed in this cell type is required. However, in many cases microarray studies require RNA sampling from single differentiating cells, which is demanding technically. Pioneering studies have succeeded in sampling RNA from GABA-containing and glutamate-containing neurons in the mammalian cortex[56], and for rods in the vertebrate retina[57], as well as for sensory and motor neurons of the gill-withdrawal reflex and serotonergic neurons in adult *Aplysia californica* (California sea slug)[116].

Putting molecular signatures to use

Once regulatory and differentiation signatures are determined for a given cell type, it is possible to identify sister cell types in the same species and homologous cell types in other species. Within a given species, sister cell types should present the most similar molecular fingerprint[56,57]. Between species, the fingerprint comparison is necessarily limited to orthologous genes. Putative homologous cell types are also identified by molecular fingerprint similarity. Note that as long as the fingerprint information is incomplete for the organism under study, the possibility remains that other cell types will be discovered that are even more similar. This would imply a change in the branching pattern of the typogenetic tree.

of the chordate amphioxus[25]. What happened to the rhabdomeric photoreceptor cell lineage during vertebrate evolution? Unexpectedly, molecular fingerprint comparisons indicate the existence of homology between invertebrate rhabdomeric photoreceptors and the ganglion cells of the vertebrate retina[4,24,25]. Despite their different morphological appearance, subsets of vertebrate ganglion cells have proved to be photosensitive and to use a phototransductory cascade that involves r-opsin, Gq-α and phospholipase C (FIG. 1b). This indicates that the retinal ganglion cells descended from a rhabdomeric type of photoreceptor that specialized in non-visual functions, such as circadian entrainment. The evolution of the vertebrate eye thus presents a puzzle: it seems that the previously non-visual brain ciliary photoreceptors evolved into the visual rods and cones, whereas the previously visual rhabdomeric photoreceptors persisted but perform non-visual functions. Therefore, any concept that explains vertebrate eye evolution will have to account for this apparent paradox.

Ancient origin of the hypothalamus and pituitary. The origin of the vertebrate endocrine system was previously obscure. Molecular studies now allow hormone and neuropeptide-secreting cell types of the hypothalamus and pituitary to be tracked from vertebrates to protochordates[35], annelids[12] and insects[36,37].

Hormones and neuropeptides released from the vertebrate hypothalamus were long considered to be vertebrate innovations; but recent genome and transcriptome comparisons indicate that several hormones and neuropeptides (pro-opiomelanocortin (POMC), gonadotropin, pituitary adenylate cyclase-activating polypeptide (PACAP), vasopressin and oxytocin) are shared with marine protostomes, and thus these molecules are representative of ancient bilaterian heritage[38]. Starting from these observations, cells have been identified in the annelid brain that, as supported by their regulatory signature and by the specific expression of vasotocin (the annelid orthologue of the vasopressin and oxytocin molecules) or FMRFamide, are related to cell types

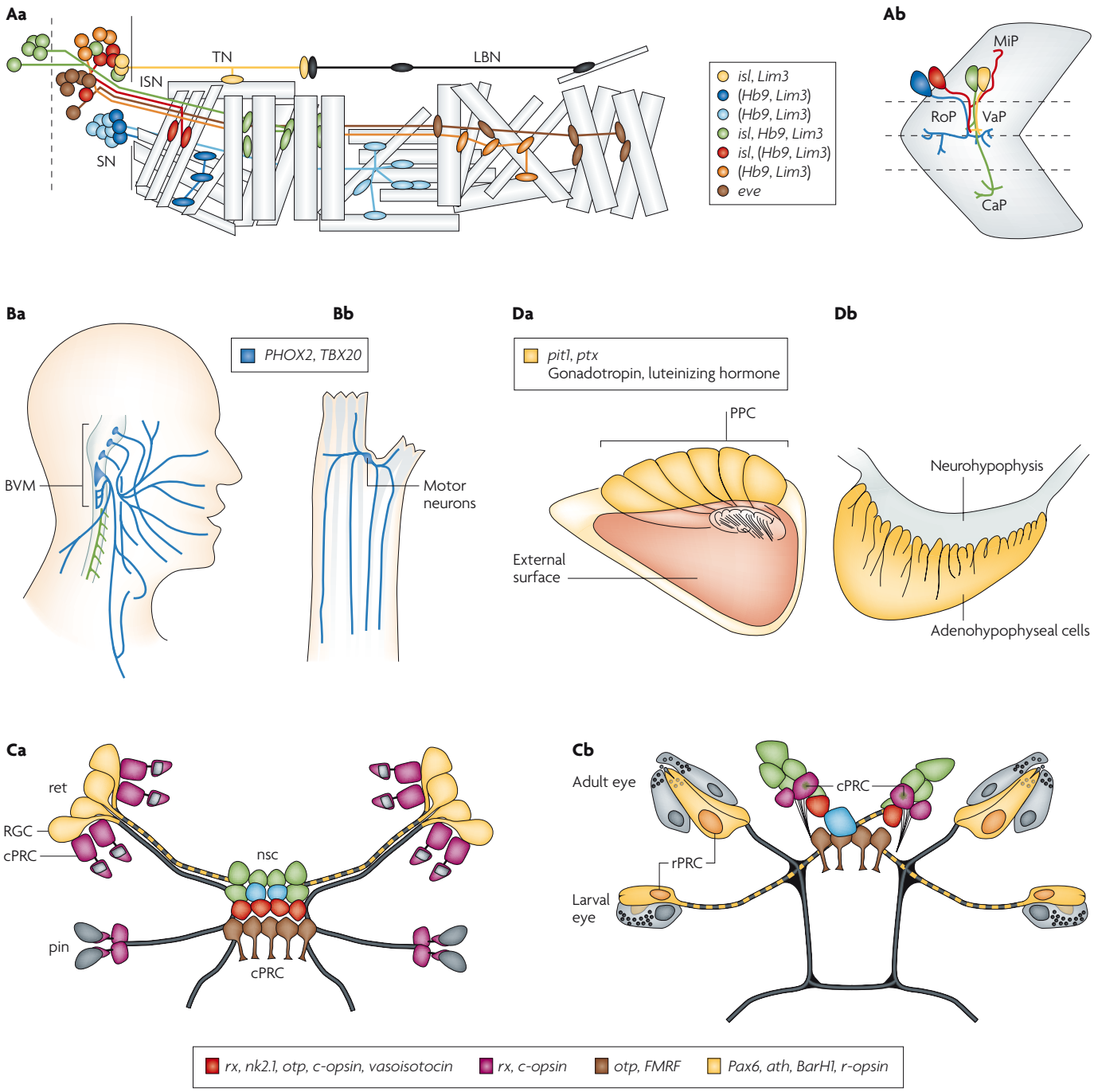

Figure 2 | **Homologous cell types identified by molecular fingerprints. a** | Somatic motor neurons in *Drosophila melanogaster* (**Aa**) and in fish (**Ab**). **b** | Branchiovisceral motor neurons (BVM) in the human hindbrain (**Ba**) and motor neurons in the ascidian cerebral ganglion (**Bb**). **c** | Forebrain cell types in vertebrates (**Ca**) and annelids (**Cb**). **d** | Early differentiating pre-oral pit cells (PPC), the later Hatschek's pit cells in amphioxus (**Da**) and adenohypophyseal cells in direct contact with the neurohypophysis in fish (**Db**). Regulatory and differentiation signatures are in the same colour as the corresponding cell type. Similar colours denote homologous cell types. *ath*, *atonal homeobox*; *BarH1*, *Bar homeobox 1*; CaP, caudal primary motor neuron; cPRC, ciliary photoreceptor cell; *eve*, *even-skipped*; *FMRF*, *FMRFamide*; *Hb9*, *homeobox 9*; *isl*, *islet*; ISN, intersegmental nerve; LBN, lateral bipolar neuron; *lim3*, *LIM-domain 3*; MiP, medial primary motor neuron; *nk2.1*, *NK homeodomain protein 2.1*; nsc, nucleus suprachiasmaticus; *otp*, *orthopedia*; *Pax6*, *paired-box 6*; *PHOX2*, *paired-like homeobox 2*; pin, pineal; *pit1*, *pituitary-specific positive transcription factor 1*; *ptx*, *pituitary homeobox*; Ret, retina; RGC, retinal ganglion cells; RoP, rostral primary motor neuron; rPRC, rhabdomeric photoreceptor cell; *rx*, *retinal homeobox*; SN, segmental nerve; *TBX20*, *T-box 20*; TN, transverse nerve; VaP, variable primary motor neuron. Part **A** is modified, with permission, from REF. 20 © (2002) Elsevier. Part **B** is reproduced, with permission, from REF. 23 © (2006) National Academy of Sciences USA. Part **C** is reproduced, with permission, from REF. 7 © (2004) American Association for the Advancement of Science. Part **Da** is modified, with permission, from REF. 39 © (2008) Blackwell Publishing.

in the vertebrate hypothalamus[12], which is indicative of cross-phylum homology (FIG. 2c). These conserved cell types apparently formed part of an ancient urbilaterian brain and allow first insights into its functionality. For example, the co-expression of vasotocin with c-opsin[12] indicates that vasotocin was secreted in direct response to changes in light conditions.

The vertebrate pituitary consists of two parts, the neurohypophysis, which is the neurosecretory release site of hypothalamal neurons, and the adenohypophysis, which is an independent endocrine gland. To elucidate the evolutionary origin of the adenohypophysis, comparative anatomists had long sought an adenohypophysis homologue in the protochordate amphioxus. From a long list of candidate structures, only one structure, the pre-oral organ (called Hatschek's pit after metamorphosis), has now been shown to be a possible homologue through molecular studies[35,39–41] (FIG. 2d). Pituitary-specific positive transcription factor1 (Pit1), a POU domain transcription factor that specifies the vertebrate adenohypophysis, is expressed exclusively in the pit cells of the pre-oral organ[35]. Pit1, together with the pituitary markers pituitary homeobox (Ptx)[42] and paired box protein 6 (Pax6)[40] constitute a unique regulatory signature. In addition, Hatschek's pit is also immunopositive for the hormones gonadotropin and luteinizing hormone, and Hatschek's pit extracts have a gonad-stimulating function in amphioxus and in toads (reviewed in REF. 43).

Haemocytes and innate immunity. Cell type molecular fingerprint comparisons suggest an early bilaterian origin of the immune system. The body cavity of many invertebrates harbours freely floating cell types, the so-called blood cells or 'haemocytes'. Of these, two types of haemocyte have a role in innate immunity: the 'plasmatocytes', which are named after their transparent cytoplasm and are generally recognized as macrophages, and the 'granulocytes', which are cells with densely packed, electron-dense lysosomes[44]. Classical cytological comparisons were unable to reveal whether these cell types were homologous between phyla and, if they were, how they related to the specialized cell types of the *Drosophila*[45] and vertebrate[46] immune systems. Molecular fingerprint comparisons have now partially solved this issue, indicating that at least one type of blood cell already existed in urbilaterians. This ancient cell type was macrophage-like and conveyed multiple steps of an ancient innate immune response[44,45]. It also expressed conserved molecules that were involved in apoptotic cell recognition[47–49], a surprisingly large repertoire of surface receptors, such as scavenger receptor cysteine-rich[50,51], and components of the complement system[19,52,53]. Conserved transcription factors related to GATA-binding factors 1–3, friend of GATA protein 1 (Fog1) and acute myeloid leukaemia (Aml) protein were involved in the specification of these urbilaterian haemocytes[44]. The urbilaterian immune system possibly also included an additional, macrophage-responsive cell type, specified by a member of the conserved Collier, olfactory 1 and early B cell factor (COE) family of transcription factors[54], which might have evolved into the specific cell types that are responsible for the adaptive immune response in vertebrates. It is not yet clear whether granulocytes already existed as a separate cell type, or whether they repeatedly diversified from the macrophage-like cells in independent evolutionary lines. Differential expression profiling of granulocytes and plasmatocytes in different animal groups should resolve this issue.

Evolution of tissues and organ systems

Once ancient cell type inventories are reconstructed for important evolutionary nodes, the next step is to unravel how these ancient cell types diversified into the many extant cell types that make up the tissues and organ systems of living animals. There are many questions that relate to the case studies discussed above. What was the contribution of cell types that diversified from the ancient ciliary and rhabdomeric photoreceptor precursors to the insect and vertebrate retinae? What cell types are derived from the hormone- and neuropeptide-secreting 'founder cells' of the urbilaterian brain in the vertebrate hypothalamus or insect neuroendocrine system? Finally, assuming that the three cell types of the *D. melanogaster* innate immune system (the phagocytic plasmatocytes, the highly specialized lamellocytes and the crystal cells) and most (if not all) of the vertebrate immune cell types evolved from one or two urbilaterian phagocyte-like cell types as recently speculated[44,45,55], how did this diversification arise? The way to trace this diversification is to resolve sister cell type relationships within tissues and organ systems. This is an ambitious task that requires expression profiling at a cellular resolution for all the cell types involved, which is only starting to be possible (BOX 1). Regarding the vertebrate immune system, a recent microarray survey[46] revealed that many genes are specifically shared between B- and T-lymphocytes, supporting their status as sister cell types (as compared with other cell types of the vertebrate immune system), but did not resolve the molecular fingerprints of the individual cell types of the myeloid lineage. Likewise, cell type-specific expression profiling data are not yet available for vertebrate and insect brains, with a few exceptions[56], leaving the sister cell type relationships of neuron types largely unresolved.

By contrast, expression profiling data are available on a large scale and at a cellular resolution for almost all cell types of the vertebrate retina. These data suggest that the vertebrate retina harbours two separate sets of sister cell types that have diversified from an ancient rhabdomeric and ciliary photoreceptor precursor type, respectively[4] (FIG. 1b). The set of cell types derived from ciliary photoreceptor precursors would comprise the non-visual bipolar cells as the sister cell type of the visual rods and cones[4,57], as indicated by their similar regulatory (*Otx2*, *Crx* and *Rx*) and differentiation signatures (for example, recoverin and potassium channels)[33], as well as from the large overlap in their gene expression profiles in microarray studies[57]. A sister cell type relationship of bipolar cells, rods and cones is also

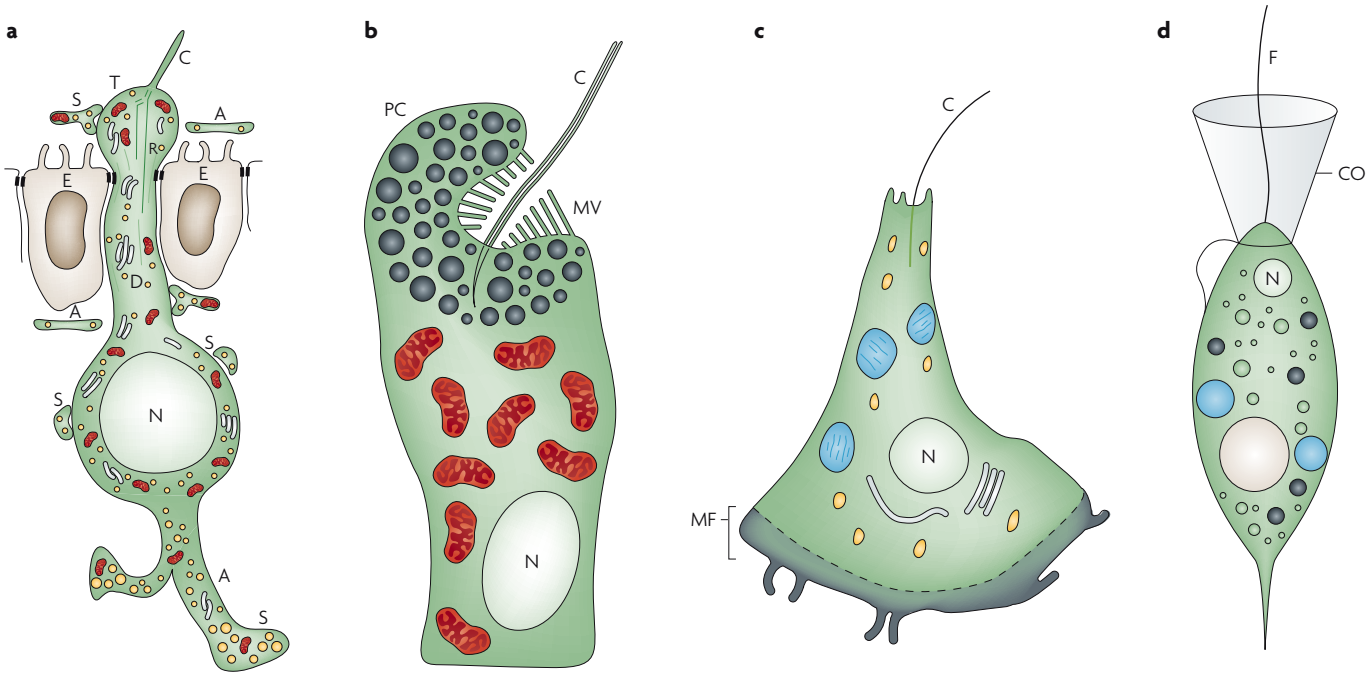

Figure 3 | **Ancient multifunctional cell types. a** | The central spinal fluid-contacting 'protoneuron' sends flask-shaped dendritic processes (D) into the brain vesicles, where they form terminal processes (T) bearing sensory cilia (C) of diverse modality. These neurons also release various hormones and neuropeptides from secretory vesicles (yellow). **b** | The one-celled ocellus of the cnidarian planula larva has a fully developed motile cilium (C), photoreceptive microvilli (MV) and a pigment cup (PC), all combined in the same cell. The striated ciliary rootlet, the nucleus and mitochondria are also indicated in the ocellus. Each cell operates as an independent sensory motor unit. **c** | The contractile myoepithelial (epithelial muscle) cell in cnidarian larvae, with myofibres (MF) and cilium (C). This cell also contains secretory vesicles (yellow) and exerts glandular functions. **d** | The choanoflagellate is a unicellular eukaryote characterized by a collar (CO) composed of microvilli and by a flagellum (F) and was a multifunctional cell type at the beginning of the Metazoa. A, axons; E, epithelial cell; N, nucleus; S, synapse. Part **b** is modified, with permission, from REF. 60 © (2003) Royal Society London. Part **c** is modified, with permission, from REF. 126 © (2006) UBC Press.

reflected by the common deployment of unique ribbon synapses, by the presence of a phototransduction-like G-protein cascade and an outer segment-like inflated cilium, Landolt's club, in bipolar cells, and also by the similar morphology of bipolar cells, rods and cones at early differentiation stages[33]. Therefore, bipolar cells, rods and cones exemplify how molecular fingerprinting, as well as complementary structural, functional, morphological and developmental evidence can be combined to unravel sister cell type relationships in tissue evolution.

Ancestral metazoan cell types are multifunctional

It is clear from the studies discussed above that the overall picture of cell type evolution in animals is only beginning to emerge. Yet from the limited insights gained so far, some first principles of cell type diversification are apparent. One first important observation is that ancient cell types tend to have multiple functions, as outlined above for the light-sensitive vasotocinergic cells (which resemble the multifunctional sensory and neurosecretory 'protoneurons' that are considered to be ancestral for the vertebrate brain)[58] (FIG. 3a) and for the presumed macrophage-like haemocytes in the urbilaterian blood that conveyed various aspects of an ancient innate immune response.

Multifunctionality also seems to be a characteristic of cell types that pre-date bilaterians. This is most evident from the recent sequencing of the cnidarian *Nematostella vectensis* genome[13] that revealed a surprisingly complex gene inventory, a large part of which was shared with the bilaterians, including genes encoding neuromuscular functions, such as enzymes important in synaptic transmission or vesicular trafficking, and various ion channels[13]. Given the apparent paucity of cell types in cnidarians and in other basal metazoans, this implies that the last common ancestor of cnidarians and bilaterians, the so-called eumetazoan, possessed few cell types that had many diverse functions. One example is the multifunctional, light-sensitive steering rudder cell that was recently described in ciliated sponge larvae and that functions as a photoreceptor, shading pigment and effector cell at the same time[1,59]. This cell type could be evolutionarily ancient because a similar cell exists in cnidarian larvae[60] (FIG. 3b). Another prominent example is the cnidarian epithelial muscle cell that functions as an epidermal cell, is capable of mechanosensory reception and transmission, and contracts like a muscle cell[11,61] (FIG. 3c). The multifunctionality of ancient cell types is even more obvious if we consider the first metazoan cell type, which most probably resembled a choanoflagellate[62–64] (FIG. 3d) and possessed, and most likely expressed, at least all

the genes and functions shared between the recently sequenced choanoflagellate *Monosiga brevicollis*[65] and any of the metazoans.

Consequently, we can rule out any scenario of cell typogenesis that views simple cell types at the beginning of animal evolution, with new functions being added continuously to newly emerging cell types, which would imply a constant rise in cell type complexity. Instead, from a cell type perspective, it is clear that a high degree of complexity was already in place at the beginning of metazoan evolution. As outlined below, cell type diversification did not necessarily increase the complexity of individual cell types, but rather triggered their stepwise specialization by functional segregation, functional divergence or the acquisition of new functions.

Segregation of functions in sister cell types

Starting from a few cell types with multiple functions at the beginning of metazoan evolution, subsequent cell type diversification involved a distribution of functions among emergent sister cell types, resulting in increasingly specialized descendants. This is referred to here as segregation of functions. It implies that one sister cell type specifically loses a function that is retained in the other (FIG. 4a). At the level of genes, functional segregation is reflected by the selective loss of expression of the corresponding effector genes encoding this function and of (at least some) upstream transcription factors (FIG. 4a). I reason here that functional segregation has been a major theme in cell type evolution.

An early functional segregation event in metazoan history was the split between the germ line and the soma, with the soma cell type retaining most of the functions of the initial choanoflagellate-like cell (except the function that leads to differentiation into germ cells). The subsequent segregation of functions produced more specialized cell types, such as external epithelial cells and primitive gut cells that retained and optimized protective and nutritive functions, respectively, and the above mentioned light-sensitive steering rudder cells that retained components of an ancient phototaxis system. Later in evolution, the rudder cells would have diversified further into separate, even more specialized cell types, such as photoreceptor, shading pigment and ciliated locomotor cells, as found in extant bilaterians. Likewise, the myoepithelial cells (which are still present in present-day cnidarians) would have given rise to separate epithelial, sensory neuronal and contractile muscle cell types, as proposed by Mackie[61] (FIG. 5a). Consistent with this scenario, Seipel and colleagues[11] have recently shown that jellyfish neurons and muscle cells share the specific expression of an atonal-like gene, *Atl1*, and of an RFamide neuropeptide, which is indicative of a sister cell type relationship. Also, the recent sequencing of the cnidarian *N. vectensis* revealed that ion channels involved in neuromuscular function and components of the dystrophin-associated protein complex in the sarcolemma are of a eumetazoan heritage[13], supporting the homology of cnidarian and bilaterian neuromuscular cell types.

Eumetazoa
All animals (metazoa) except sponges.

The vertebrate retina is a valuable case study for the segregation of functions between emerging sister cell types, which are traceable both at the cellular and at the molecular level. I propose here that in the retina, functional segregation occurred when the ancient urbilaterian rhabdomeric and ciliary photoreceptor precursors diversified into cell types that retained light sensitivity (ganglion cells, rods and cones) and interneuron types that have specialized to transmit and integrate the light signal (amacrine, horizontal and bipolar cells) (FIG. 1b). The comparative data suggest that the common evolutionary precursor of rods, cones and bipolar cells had a bipolar morphology, with dendrites that possessed a photosensitive cilium and with an axon that established direct synaptic contact to the ganglion cells (FIG. 5b), as can be deduced from the similar morphology of rods, cones and bipolar cells at early differentiation stages[33]. This precursor then gave rise to two sister cell types; one that retained the photoreceptor and lost the axonal connection to ganglion cells (rods and cones), and one that retained the interneuron function but lost the light-sensitive cilium (bipolar cells) (FIG. 5b). At the gene level, this is likely to have involved the selective loss of expression of ceh10 homeobox-containing homologue (*Chx10*) and visual system homeobox (*Vsx*), which encode two paralogous paired-type homeodomain transcription factors that are active in bipolar cells but not in rods and cones[66,67]. Chx10 promotes bipolar-cell differentiation at the expense of photoreceptor cell fate[68], which is at least partly achieved by directly targeting and silencing the expression of photoreceptor-specific genes[69]. Because a *Chx10* and *Vsx* orthologue is expressed in annelid photoreceptors (G. Jekely, R. Tomer and D.A., unpublished observations), it seems plausible that *Chx10* and *Vsx* expression was selectively turned off in rods and cones when these became functionally segregated from the bipolar cells.

Evolution of neuronal circuits. The principle of cell type functional segregation elegantly explains the evolution of neuronal circuits and could therefore allow us to understand nervous system evolution. If we assume that emergent (functionally segregating) neuron types are likely to move apart from each other according to the specializations they acquire, and if we further assume that, at the same time, these cell types maintain cellular contact for functional coordination, the evolution of axonal and dendritic extensions interconnecting these cells would be triggered. Such a scenario can explain how the evolutionary emergence of the first simple sensory-motor neuronal circuit took place, as originally proposed by Mackie[61] (FIG. 5a). This scenario would also explain the evolution of a retinal circuit, such that the newly evolving bipolar cells came to lie between the photoreceptor cells and the ganglion cells, maintaining dendritic contact with the photoreceptor cells and axonal contact with the ganglion cells[33] (FIG. 5b). Finally, we can envisage a scenario that explains the evolution of the axon tracts that interconnect the vertebrate pituitary, hypothalamus and nose (FIG. 5c). This scenario assumes that some endocrine cells of the vertebrate pituitary,

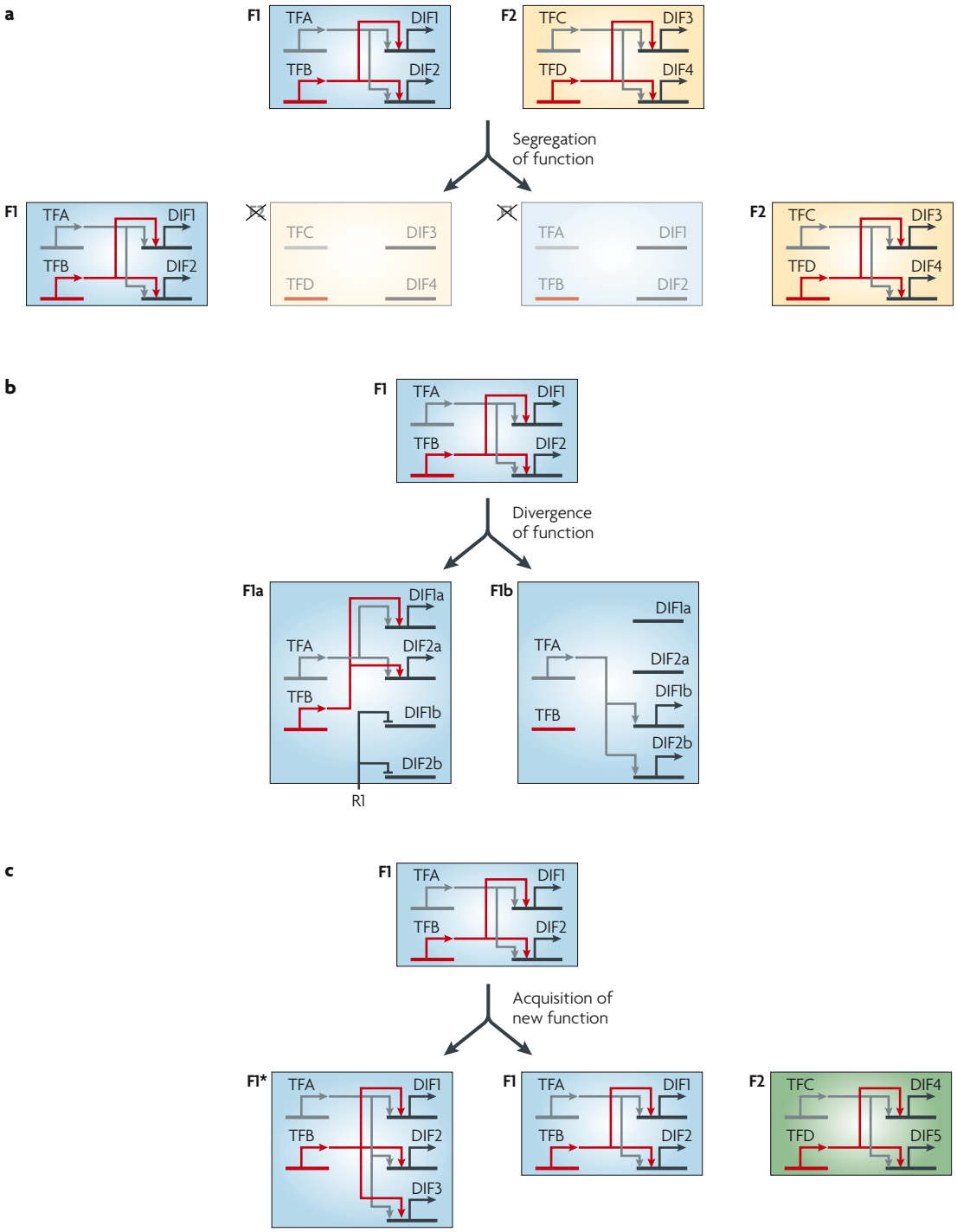

Figure 4 | **Modes of cell type diversification.** Ancient cell types diversify into sister cell type descendants (diversification is indicated from top to bottom). Cellular functions of evolving cell types are represented by boxes in different colours. Transcription factors (TFs) and differentiation genes (DIFs) that encode these functions are drawn in the boxes. Gene regulatory networks are indicated by lines. **a** | Functional segregation. Two functions (F1, blue box; F2, yellow box) are present in the multifunctional precursor but are segregated between the two descendant sister cell types. The schematic shows the loss of F2 in one cell type and of F1 in the other. **b** | Functional divergence. The initial cellular function (F1, blue box) diverges by gene duplication. Different paralogues (DIF1a or 1b and DIF2a or 2b) are expressed in the two descendant sister cell types that retain the modified functions F1a and F1b. This is achieved through the cell type-specific activity of a repressor (R1) in F1a. In F1b, DIF1b and DIF2b no longer depend on TFB activity (which is not expressed in the corresponding cell type). DIF1a and DIF2a are turned off because they still depend on TFB. **c** | Acquisition of new cellular functions. One ancient function (F1, blue box) is modified towards a new function F1* through the recruitment of novel differentiation genes into the network (DIF3). The descendant sister cell type on the right has acquired a new function F2, which was previously active in another unrelated cell type.

some neurons of the hypothalamus and the olfactory sensory neurons of the nose evolved by functional segregation of sister cell types, which emerged from a chemosensory–neurosecretory organ that was similar to Hatschek's pit in extant amphioxus (discussed above). These sister cells would then move away from each other according to their specialized functions, but would remain functionally interconnected, both by axons from the nose to the hypothalamus and by endocrine processes from the hypothalamus into the pituitary. The molecular and developmental evidence in favour of this scenario is summarized in BOX 2.

Divergence of functions in sister cell types

Another key principle of cell type evolution is the divergence of functions that acts in parallel to functional segregation, and likewise contributes to the specialization of cell types. During this process, in contrast to functional segregation, functions are retained in both sister cell types but are modified in different directions. For example, both rods and cones have retained light sensitivity but have been optimized for sensitivity to light of different wavelengths. Such functional divergence of cell types is mirrored by the functional divergence of duplicate genes, with complementary expression in the descendant sister cell types (FIG. 4b).

The resulting link between cell type functional divergence and gene duplication apparently played a pivotal part in cell type evolution. For example, teleost fishes possess more pigment cell types and a greater repertoire of duplicated pigment synthesis genes than any other group of vertebrates, which indicates that there is a link between cell type divergence and gene duplication[70]. In a similar manner, cellular diversification of the immune system in different animal groups seems to correlate with the duplication of genes involved in innate immunity, as shown by the expansion of Toll-like receptors in sea urchins, of leucine-rich repeat proteins in amphioxus[50] and of various genes of the major histocompatibility complex (MHC) in vertebrates[71,72]. Another example is the evolution of olfactory receptor sister cell types, which has been driven by the vast expansion of the olfactory G-protein-coupled receptor (GPCR) family[73]. From a more general perspective, up to 200 gene superfamilies have been identified, the size of which strongly correlates with the number of cell types in 38 species[74]. Among these superfamilies are families involved in cell adhesion (such as immunoglobulins, fibronectins, epidermal growth factors, laminins, cadherins and integrins) and immune responses (such as SCR domain-containing proteins, tumour necrosis factor (TNF)-like proteins, MHC domain-containing proteins, interleukins and cytokines)[74]. Moreover, a strong correlation between the rate of gene duplication and the rate of overall macroevolutionary change has been reported[75].

In retinal evolution, there is a particularly tight correlation between gene duplication and cell type diversification, and several rounds of opsin duplication triggered the diversification of an ancient cone-like photoreceptor into several subtypes that detected different wavelengths (short, medium and long), thereby triggering the evolution of colour vision in early vertebrates[76–79]. As a result, the typogenetic tree of vertebrate ciliary photoreceptor cells (numbers 20, 21 and 22 in FIG. 1b) is congruent with the vertebrate ciliary opsin family tree (duplication of long wavelength sensitive (LWS), short wavelength-sensitive 1 (SWS1), SWS2 and rhodopsin; FIG. 1c). For rod and cone evolution, other components of the phototransductory cascade, such as the G protein α-subunit, phosphodiesterase, cyclic nucleotide-gated ion channels and arrestin, apparently co-duplicated with the opsins[79,80]. However, gene duplication and the birth of sister cell types are not necessarily coupled, as has recently been argued for the duplication of rhabdomeric opsin and the emergence of separate larval and adult rhabdomeric photoreceptors in ostracods[81]. In this case study, gene duplication with subsequent complementary expression took place long after the birth of sister cell types, allowing their further functional divergence[81].

Gene subfunctionalization drives cell type functional divergence. A close link between gene duplication and cell type functional divergence is consistent with the duplication–degeneration–complementation model of gene subfunctionalization by Lynch and Force[82]. According to this model, duplicated gene pairs are evolutionarily stabilized by the complementary loss of regulatory elements that drive their spatially (or temporally) differential expression. Being a near-neutral event at first, this stabilization subsequently allows duplicate genes to become functionally optimized in different directions[83] (as occurred with opsins). At the cell type level, spatial gene subfunctionalization would therefore be equivalent to the birth of new sister cell types.

Acquisition of new cellular functions

Cell type functional segregation and divergence trigger specialization by the partitioning and modification of existing cellular functions. In addition, cell type evolution also involves the acquisition of new functions (FIG. 4c). This can occur via the extreme modification of existing functions beyond recognition or via the acquisition of entirely novel genes that start to be expressed in a given cell type (acquisition of F1* in FIG. 4c). Alternatively, cell types can turn on pre-existing regulatory modules and batteries of differentiation genes that were previously active only in other cell types (co-option) (F2 in FIG. 4c). The consequences of such lateral transfer of cell type characteristics (analogous to horizontal gene transfer) for cell typogenetic trees have recently been discussed[84].

The prototype for a highly specialized cell type that has acquired a novel function by the acquisition of novel genes is the cnidarian nematocyte[85]. Genes specific for nematocyte cell types have recently been identified. These encode: structural proteins essential for assembling the rigid capsule wall, the internal tubule and the spines; nematocyst toxins; poly-γ-glutamate, which is required for the explosive extrusion of the nematocyst; and proteins that make up the cnidocil, the sensory

Ostracod
The ostracoda are a group of crustaceans known as seed shrimps.

Subfunctionalization
The process whereby a pair of duplicated genes becomes permanently preserved because the two gene copies have reciprocally lost essential subfunctions by acquiring complementary degenerative mutations.

Horizontal gene transfer
The transfer of genetic material between the genomes of two organisms that does not occur through parent–progeny transmission.

apparatus of the nematocyte[86]. Less than 20% of these genes have orthologues in other species, indicating that the emergence of nematocytes was accompanied by the evolution of various novel structural proteins. Yet it seems plausible that the nematocyte did not evolve completely *de novo* but by extreme modification of a pre-existing cell type. The resemblance of the cnidocil to a sensory cilium may suggest that a sensory neuron with some degree of exocrine ability was present at the outset of nematocyte evolution[87].

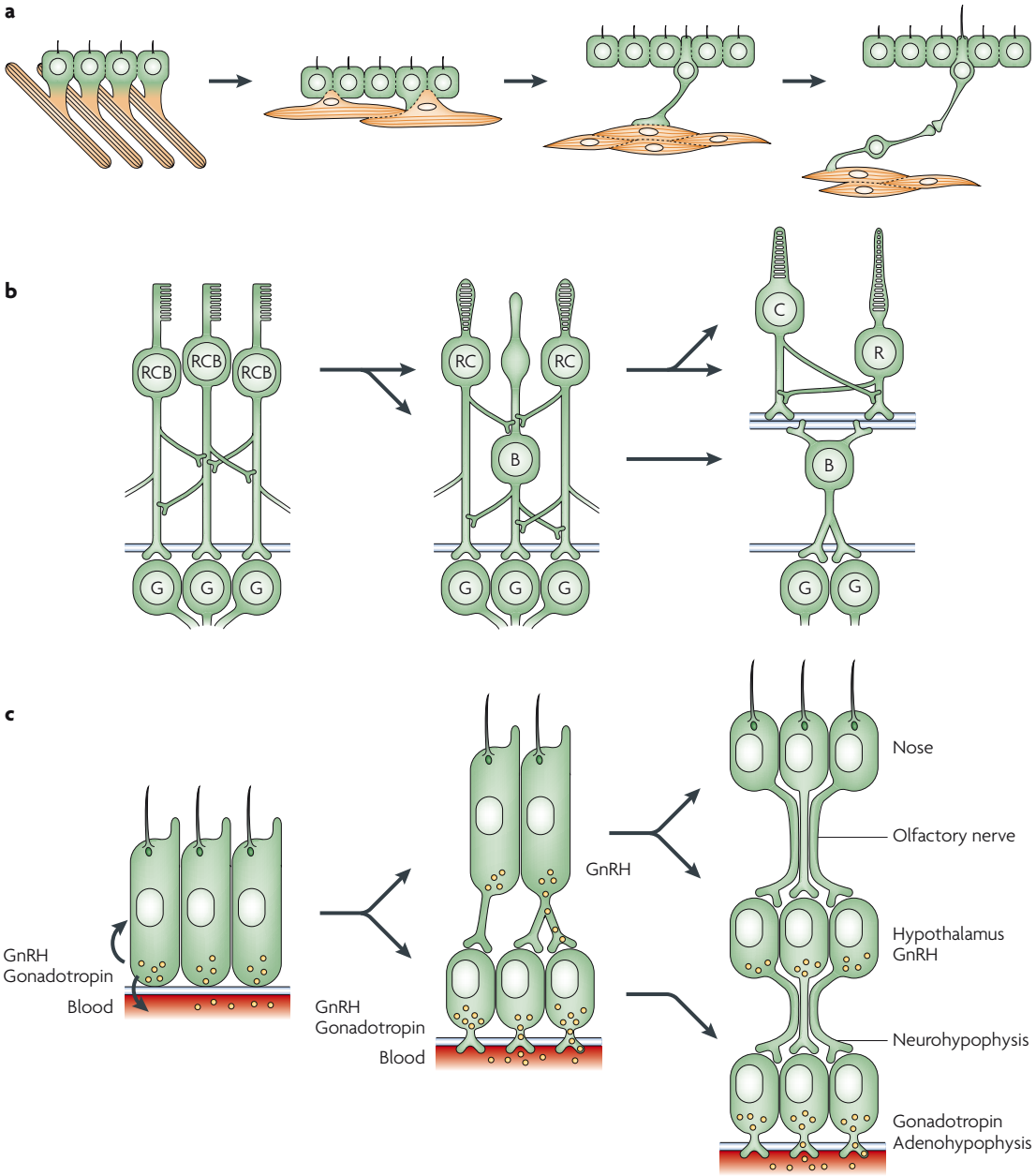

Figure 5 | **The evolution of neuronal circuits by functional segregation. a** | Diversification of the cnidarian myoepithelial cell into the sensory cell, motor neuron and muscle cell. **b** | Segregation of function in the evolution of rods, cones and bipolar cells. Individual neurons are interconnected by axon collaterals. In the middle panel, the bipolar precursor gradually loses the photoreceptor function. In the right panel, rods, cones and bipolar cells are fully segregated. **c** | Segregation of olfactory, integrative and neurosecretory functions during the diversification of the olfactory–hypothalamal–pituitary axis. Functionally segregating cell types retain axonal contact and this contact evolves into a neuronal circuit that interconnects the nose, hypothalamus and pituitary. The most downstream endocrine cells relocate to the pituitary, where they directly release gonadotropin into the blood. The gonadotropin-releasing hormone (GnRH)-releasing cells that are located more upstream come to lie in the hypothalamus, where they integrate information from other neurons, and the most upstream olfactory sensory neurons form part of the peripheral sensory organ, the nose, which must be in direct contact with the environment. B, bipolar cell; C, cone cell; G, ganglion; R, rod cell; RC, rod and cone precursor cell; RCB, rod, cone and bipolar evolutionary precursor cell with both photoreceptor and interneuron functions. Part **a** is modified, with permission, from REF. 61 © (1970) University of Chicago Press.

Microevolutionary studies have unravelled repeated *cis*-regulatory changes in the *yellow* gene that trigger the gain or loss of pigmentation spots in wing epithelial cells in different *Drosophila* species[88]. Given that the wing of the *Drosophila* common ancestor was unspotted[88], this is a case in which a pre-existing cell type, the wing epithelial cell, acquired a new cellular function, pigmentation, by co-option. More profound changes in cellular identity can arise when an upstream transcription factor that positively regulates whole batteries of genes is turned on in a given cell type. We are currently exploring cases of 'ectopic' eyes, such as the tentacular crown eyes or pygidial eyes of sedentary polychaetes[89], in which batteries of phototransduction genes, including opsin, seem to be turned on in former epithelial cells that lie far away from their 'normal' site of activity, possibly by the ectopic activity of key transcription factors.

Cell type development: recapitulating evolution?

Functional segregation, divergence and the acquisition of new functions (as outlined above) are evolutionary processes. However, it is evident that similar processes occur during cell type development. Here, I give a brief account of the interrelationship between cell type evolution and development. As discussed in the above case studies, developmental evidence often facilitates the identification of sister cell types and corroborates the molecular fingerprinting data. Examples that underscore sister cell type relationships include: the resemblances in cell shape and projection pattern between bipolar cells, rods and cones at early differentiation stages; the common origin of olfactory, hypothalamal and adenohyophyseal cells from a common placode; and the emergence of B and T cells from a common lymphoid progenitor. Hence, in some cases, cell type development seems to recapitulate cell type evolution (as defined by Haeckel[90]) and sometimes developmental peculiarities can only be understood from an evolutionary viewpoint; for example, the migration of hypothalamal gonadatropin-releasing hormone (GnRH)-positive cells along the olfactory nerve into the hypothalamus. Considering the principles of cell type diversification outlined above, this recapitulation is not surprising. Immediately after an evolutionary diversification event, the two nascent sister cell types should follow an almost identical course of development, except for the differential use of at least one effector gene that makes the cell types distinct.

However, as on the organismal level, the resemblance between development and evolution can be blurred or disappear completely[90], owing to modifications in cell type development with evolutionary time. Indeed, numerous developmental pathways have been altered by co-option of signalling pathways and of entire regulatory modules even though the identities of the resulting differentiating cell types have not been affected. For example, comparative studies indicate that in nematodes the developmental mechanisms that give rise to similar vulval cell types have changed rapidly during evolution[91]. Also, Hudson and Yasuo have shown that in ascidian notochord development, remarkably different upstream strategies can converge on the same regulatory sequences to generate the same cell type[92,93]. The different ways of producing homologous somatic motor neurons in insects, nematodes, vertebrates and annelids, as mentioned above, is another example. In addition, the mesoderm specification network differs substantially between vertebrates[94,95] and *D. melanogaster*[96], whereas the heart specification network that occurs later in development and directly turns on muscular differentiation genes is highly conserved[97], as are the somatic muscle cells[98]. In all these examples, the differentiating cell types seem to be islands in a sea of developmental change. Modification of cell type development may be either neutral, or driven by a selective advantage, such as the faster development of ephemeric substrates that occurred in flies and nematodes.

The most prominent example of profound changes in cell type development is the evolution of the neural crest, which represents a key novel feature of the vertebrate lineage[99,100]. Neural crest cells migrate from the neural-plate border to various locations in the developing vertebrate body, where they give rise to many different cell types, which compose over 10% of approximately 400 human

Notochord
A rod-shaped structure that runs along the dorsal axis of the embryo, separating the muscle blocks. The notochord is one of the defining features of the phylum Chordata, which vertebrates belong to.

Neural crest
A migratory cell population that arises at the lateral extremities of the embryonic neural plate, and which differentiates into various cell types, depending on the location. These cells include endothelial cells, smooth and skeletal muscle cells, bone, adrenal medulla, and cells of the sensory and autonomic nervous systems.

Box 2 | The enigmatic evolutionary link between the hypophysis, hypothalamus and nose

It has been argued that the cells that compose Hatschek's pit in amphioxus resemble the multifunctional precursors of the olfactory, hypothalamal and adenohypophyseal sister cell types that evolved into an 'olfacto-hypothalamo-adeno-hypophyseal' circuit in vertebrates. Hatschek's pit cells are open and exposed to environmental water, and secrete mucus to facilitate food uptake[39]. At the same time, they are considered to be chemosensory and neuroendocrine cells[43], with basal contact to blood spaces[117]. In addition to gonadotropin and luteinizing hormone, another hormone secreted by these pit cells may be related to vertebrate gonadotropin-releasing hormone (GnRH), as shown by immunocytochemistry (reviewed in REF. 43). Therefore, from the vertebrate perspective, Hatschek's pit cells seem to combine olfactory, hypothalamal (GnRH-secreting) and adenohypophyseal (gonadotropin- and luteinizing hormone-secreting) functions. A sister cell relationship of vertebrate olfactory, hypothalamal and adenohypophyseal cells is further supported by some intriguing observations. First, the adenohypophysial and olfactory placodes are initially congruent[118], and the olfactory organs and adenohypophysis co-develop for a long period of time and remain connected in the lamprey[117], consistent with a common evolutionary origin of the adenohypophyseal and olfactory cell types. In addition, the hypothalamal GnRH-secreting cells originate from the olfactory placode, which has provoked the view that they originated from a peripheral endocrine organ that was associated with the olfactory system[119]. Furthermore, immunoreactive GnRH has been detected in the olfactory placode, olfactory organ, olfactory tract, nervus terminalis and in axons projecting from these regions to the hypothalamus[43], adding to the similarities in the differentiation signatures. Finally, direct olfactory projection pathways from a discrete population of olfactory neurons to the GnRH neurons in the hypothalamus that control reproduction and fertility have recently been identified[120].

cell types. These include melanocytes, sensory neurons, sympathetic and parasympathetic neurons, various endocrine cells, pharyngial cartilage and bone[2]. From the cell type-evolution perspective, the multipotent neural crest cells are unique, as they have proved to be incredibly adept in 'taking over' the development of pre-existing cell types (that previously originated from other sources), reflecting an enormous developmental plasticity[101]. The best evidence for this comes from the mixed embryonic origin of cell types, such as dermal bones, gland cells, oligodendroglia or epithelial cells[2], as the developmental 'takeover' of these cells by the neural crest apparently remained incomplete. Other cell types experienced a total developmental takeover by the neural crest, which was coupled to the acquisition of evolutionary novelty. For example, the vertebrate pharyngeal skeleton is older than the neural crest[102], and thus its neural crest origin in extant vertebrates must have been a secondary event[103–105]. However, the neural crest-derived cartilage differs substantially from its evolutionary predecessor owing to the acquisition of novel cartilage-specific genes[105].

Conclusions and perspectives

The segregation, divergence and subsequent modification of functions may account for a large part of the cell typogenesis that has occurred in animal evolution. To elucidate the full story of cell type specialization, we will need an almost complete molecular account of the cell type inventory in conventional developmental models, and in a handful of slow-evolving evolutionary model species, such as amphioxus, or *Saccoglossus, Platynereis* and *Nematostella*. This will require cell type-specific expression profiling on a large scale for as many conserved genes as possible. Once the molecular fingerprint is established for a number of cell types in various organisms, sister cell types within species and homologous cell types between species can be unravelled on a larger scale. When the resulting typogenetic tree of homologous cell types is congruent between species, we will be able to infer the evolutionary history of cell type diversification for entire organ systems, such as the central nervous system, the musculature, the immune system and finally the entire animal body.

1. Leys, S. P. & Degnan, B. M. Cytological basis of photoresponsive behavior in a sponge larva. *Biol. Bull.* **201**, 323–338 (2001).
2. Vickaryous, M. K. & Hall, B. K. Human cell type diversity, evolution, development, and classification with special reference to cells derived from the neural crest. *Biol. Rev. Camb. Philos. Soc.* **81**, 425–455 (2006).
3. Valentine, J. W. in *Keywords and Concepts in Evolutionary Devlopmental Biology* (eds Hall, B. K. & Olson, W. M.) 35–53 (Harvard Univ. Press, Cambridge, 2003).
4. Arendt, D. Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571 (2003).
5. Willmer, E. N. *Cytology and Evolution* (Academic Press, New York and London, 1970).
6. Salvini-Plawen, L. V. & Mayr, E. in *Evolutionary Biology* Vol. 10 (eds Hecht, M. K., Steere, W. C. & Wallace, B.) 207–263 (Plenum, New York, 1977).
7. Arendt, D., Tessmar-Raible, K., Snyman, H., Dorresteijn, A. W. & Wittbrodt, J. Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* **306**, 869–871 (2004).
8. Royuela, M., Fraile, B., Arenas, M. I. & Paniagua, R. Characterization of several invertebrate muscle cell types: a comparison with vertebrate muscles. *Microsc. Res. Tech.* **48**, 107–115 (2000).
9. Seipel, K. & Schmid, V. Evolution of striated muscle: jellyfish and the origin of triploblasty. *Dev. Biol.* **282**, 14–26 (2005).
10. Arendt, D. Genes and homology in nervous system evolution: comparing gene functions, expression patterns, and cell type molecular fingerprints. *Theory Biosci.* **124,** 185-197 (2005).
A conceptual review that discusses the different levels of homology in evolutionary developmental biology research; it introduces the concept of molecular fingerprints for cell type comparisons.
11. Seipel, K., Yanze, N. & Schmid, V. Developmental and evolutionary aspects of the basic helix–loop–helix transcription factors Atonal-like 1 and Achaete-scute homolog 2 in the jellyfish. *Dev. Biol.* **269**, 331–345 (2004).
12. Tessmar-Raible, K. *et al.* Conserved sensory–neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. *Cell* **129**, 1389–1400 (2007).
This paper unravels striking similarities in the regional specification and cell type inventory of the developing brain neurosecretory system in annelids and vertebrates.
13. Putnam, N. H. *et al.* Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86–94 (2007).
14. Lee, S. K. & Pfaff, S. L. Transcriptional networks regulating neuronal identity in the developing spinal cord. *Nature Neurosci.* **4**, S1183–S1191 (2001).
15. Wienholds, E. *et al.* MicroRNA expression in zebrafish embryonic development. *Science* **309**, 310–311 (2005).
16. Raible, F. *et al.* Vertebrate-type intron-rich genes in the marine annelid *Platynereis dumerilii*. *Science* **310**, 1325–1326 (2005).
17. Kortschak, R. D., Samuel, G., Saint, R. & Miller, D. J. EST analysis of the cnidarian *Acropora millepora* reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr. Biol.* **13**, 2190–2195 (2003).
18. Koyanagi, M., Terakita, A., Kubokawa, K. & Shichida, Y. Amphioxus homologs of Go-coupled rhodopsin and peropsin having 11-*cis*- and all-*trans*-retinals as their chromophores. *FEBS Lett.* **531**, 525–528 (2002).
19. Altincicek, B. & Vilcinskas, A. Analysis of the immune-related transcriptome of a lophotrochozoan model, the marine annelid *Platynereis dumerilii*. *Front. Zool.* **4**, 18 (2007).
20. Thor, S. & Thomas, J. Motor neuron specification in worms, flies and mice: conserved and 'lost' mechanisms. *Curr. Opin. Genet. Dev.* **12**, 558–564 (2002).
21. Denes, A. S. *et al.* Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129**, 277–288 (2007).
22. Rusten, T. E., Cantera, R., Kafatos, F. C. & Barrio, R. The role of TGFβ signaling in the formation of the dorsal nervous system is conserved between *Drosophila* and chordates. *Development* **129**, 3575–3584 (2002).
23. Dufour, H. D. *et al.* Precraniate origin of cranial motoneurons. *Proc. Natl Acad. Sci. USA* **103**, 8727–8732 (2006).
A pioneering study that elucidates the homology between motor neurons in the brain of adult ascidians and in the hindbrain of vertebrates, exemplifying the cell type comparative approach.
24. Isoldi, M. C., Rollag, M. D., Castrucci, A. M. & Provencio, I. Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. *Proc. Natl Acad. Sci. USA* **102**, 1217–1221 (2005).
25. Koyanagi, M., Kubokawa, K., Tsukamoto, H., Shichida, Y. & Terakita, A. Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. *Curr. Biol.***15**, 1065–1069 (2005).
26. Plachetzki, D. C., Degnan, B. M. & Oakley, T. H. The origins of novel protein interactions during animal opsin evolution. *PLoS ONE* **2**, e1054 (2007).
27. Suga, H., Schmid, V. & Gehring, W. J. Evolution and functional diversity of jellyfish opsins. *Curr. Biol.* **18**, 51–55 (2008).
28. Arendt, D., Tessmar, K., de Campos-Baptista, M. I., Dorresteijn, A. & Wittbrodt, J. Development of pigment-cup eyes in the polychaete *Platynereis dumerilii* and evolutionary conservation of larval eyes in Bilateria. *Development* **129**, 1143–1154 (2002).
29. Eichinger, L. *et al.* The genome of the social amoeba *Dictyostelium discoideum*. *Nature* **435**, 43–57 (2005).
30. Velarde, R. A., Sauer, C. D., Walden, K. K., Fahrbach, S. E. & Robertson, H. M. Pteropsin: a vertebrate-like non-visual opsin expressed in the honey bee brain. *Insect Biochem. Mol. Biol.* **35**, 1367–1377 (2005).
31. Blackshaw, S. & Snyder, S. H. Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain. *J. Neurosci.* **19**, 3681–3690 (1999).
32. Arendt, D. & Wittbrodt, J. Reconstructing the eyes of Urbilateria. *Phil. Trans. R. Soc. Lond. Biol. Sci. B* **356**, 1545–1563 (2001).
33. Lamb, T. D., Collin, S. P. & Pugh, E. N. Jr. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nature Rev. Neurosci.* **8**, 960–976 (2007).
An essential read for those interested in vertebrate eye evolution. The authors discuss the evolution of retinal cell types with a special emphasis on opsins and on bipolar cells.
34. Salo, E. *et al.* Genetic network of the eye in Platyhelminthes: expression and functional analysis of some players during planarian regeneration. *Gene* **287**, 67–74 (2002).
35. Candiani, S., Holland, N. D., Oliveri, D., Parodi, M. & Pestarino, M. Expression of the amphioxus *Pit-1* gene (AmphiPOU1F1/Pit-1) exclusively in the developing preoral organ, a putative homolog of the vertebrate adenohypophysis. *Brain Res. Bull.* **75**, 324–330 (2008).
36. de Velasco, B. *et al.* Specification and development of the *pars intercerebralis* and *pars lateralis*, neuroendocrine command centers in the *Drosophila* brain. *Dev. Biol.* **302**, 309–323 (2007).
37. Hartenstein, V. The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J. Endocrinol.* **190**, 555–570 (2006).
A comprehensive review of the anatomy and hormone release of neuroendocrine systems in various invertebrates. It also provides a detailed comparison of neuroendocrine-system specification in vertebrates and insects.
38. Tessmar-Raible, K. The evolution of neurosecretory centers in bilaterian forebrains: insights from protostomes. *Semin. Cell Dev. Biol.* **18**, 492–501 (2007).
39. Lacalli, T. Mucus secretion and transport in amphioxus larvae: organization and ultrastructure of the food trapping system, and implications for head evolution. *Acta Zool. Stockholm* **89**, 219–230 (2008).

40. Kozmik, Z. *et al.* Pax–Six–Eya–Dach network during amphioxus development: conservation *in vitro* but context specificity *in vivo*. *Dev. Biol.* **306**, 143–159 (2007).
41. Welsch, L. T. & Welsch, U. Histologische und elekronmikroskopische Untersuchungen an der präoralen Wimpergrube von *Saccoglossus horsti* (Hemichordata) und der Hatschekschen Grube von *Branchiostoma lanceolatum* (Acrania). Ein Beirag zur phylogenetischen Entwicklung der Adenohypophyse. *Zool. Jahrb. Anat.* **100**, 564–578 (1978).
42. Yasui, K., Zhang, S., Uemura, M. & Saiga, H. Left–right asymmetric expression of *BbPtx*, a Ptx-related gene, in a lancelet species and the developmental left-sidedness in deuterostomes. *Development* **127**, 187–195 (2000).
43. Nozaki, M. & Gorbman, A. The question of functional homology of Hatschek's pit of amphioxus (*Branchiostoma belcheri*) and the vertebrate adenohypophysis. *Zool. Sci.* **9**, 387–395 (1992).
44. Hartenstein, V. Blood cells and blood cell development in the animal kingdom. *Annu. Rev. Cell Dev. Biol.* **22**, 677–712 (2006).
An excellent survey of blood cell types in various bilaterians. Hartenstein speculates on how the diverse cell types of the immune system in extant animals have evolved from common evolutionary precursors.
45. Evans, C. J., Hartenstein, V. & Banerjee, U. Thicker than blood: conserved mechanisms in *Drosophila* and vertebrate hematopoiesis. *Dev. Cell* **5**, 673–690 (2003).
46. Palmer, C., Diehn, M., Alizadeh, A. A. & O'Brown, P. Cell-type specific gene expression profiles of leukocytes in human peripheral blood. *BMC Genomics* **7**, 115 (2006).
47. Li, J. *et al.* cDNA microarray analysis reveals fundamental differences in the expression profiles of primary human monocytes, monocyte-derived macrophages, and alveolar macrophages. *J. Leukoc. Biol.* **81**, 328–335 (2007).
48. Franc, N. C., Heitzler, P., Ezekowitz, R. A. & White, K. Requirement for croquemort in phagocytosis of apoptotic cells in *Drosophila*. *Science* **284**, 1991–1994 (1999).
49. Fadok, V. A. *et al.* A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85–90 (2000).
50. Pancer, Z. & Cooper, M. D. The evolution of adaptive immunity. *Annu. Rev. Immunol.* **24**, 497–518 (2006).
51. Pancer, Z. Dynamic expression of multiple scavenger receptor cysteine-rich genes in coelomocytes of the purple sea urchin. *Proc. Natl Acad. Sci. USA* **97**, 13156–13161 (2000).
52. Smith, L. C., Shih, C. S. & Dachenhausen, S. G. Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. *J. Immunol.* **161**, 6784–6793 (1998).
53. Gross, P. S., Clow, L. A. & Smith, L. C. SpC3, the complement homologue from the purple sea urchin, *Strongylocentrotus purpuratus*, is expressed in two subpopulations of the phagocytic coelomocytes. *Immunogenetics* **51**, 1034–1144 (2000).
54. Crozatier, M., Ubeda, J. M., Vincent, A. & Meister, M. Cellular immune response to parasitization in *Drosophila* requires the EBF orthologue collier. *PLoS Biol.* **2**, E196 (2004).
55. Hartenstein, V. & Mandal, L. The blood/vascular system in a phylogenetic perspective. *Bioessays* **28**, 1203–1210 (2006).
56. Sugino, K. *et al.* Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nature Neurosci.* **9**, 99–107 (2006).
Pioneering work on the classification of neuron types in the vertebrate telencephalon, based on expression profiling.
57. Blackshaw, S., Fraioli, R. E., Furukawa, T. & Cepko, C. L. Comprehensive analysis of photoreceptor gene expression and the identification of candidate retinal disease genes. *Cell* **107**, 579–589 (2001).
58. Vigh, B. *et al.* The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. *Histol. Histopathol.* **19**, 607–628 (2004).
This is one of the many excellent reviews by Vigh on the system of cerebrospinal fluid-contacting neurons in vertebrates and the supposed evolutionary origin of such neurons from an ancient 'protoneuron'.
59. Leys, S. P., Cronin, T. W., Degnan, B. M. & Marshall, J. N. Spectral sensitivity in a sponge larva. *J. Comp. Physiol. A. Neuroethol. Sens. Neural Behav. Physiol.* **188**, 199–202 (2002).
60. Nordstrom, K., Wallen, R., Seymour, J. & Nilsson, D. A simple visual system without neurons in jellyfish larvae. *Proc. R. Soc. Lond. B* **270**, 2349–2354 (2003).
61. Mackie, G. O. Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.* **45**, 319–332 (1970).
62. Clark, J. Note on the infusoria flagellata and the spongiae ciliatae. *Am. J. Sci.* **1**, 113–114 (1866).
63. Philippe, H. *et al.* Phylogenomics of eukaryotes: impact of missing data on large alignments. *Mol. Biol. Evol.* **21**, 1740–1752 (2004).
64. Lang, B. F., O'Kelly, C., Nerad, T., Gray, M. W. & Burger, G. The closest unicellular relatives of animals. *Curr. Biol.* **12**, 1773–1778 (2002).
65. King, N. *et al.* The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**, 783–788 (2008).
66. Liu, I. S. C. *et al.* Developmental expression of a novel murine homeobox gene (*Chx10*): evidence for roles in determination of the neuroretina and inner nuclear layer. *Neuron* **13**, 377–393 (1994).
67. Chow, R. L. *et al. Vsx1*, a rapidly evolving paired-like homeobox gene expressed in cone bipolar cells. *Mech. Dev.* **109**, 315–322 (2001).
68. Livne-bar, I. *et al.* Chx10 is required to block photoreceptor differentiation but is dispensable for progenitor proliferation in the postnatal retina. *Proc. Natl Acad. Sci. USA* **103**, 4988–4993 (2006).
69. Dorval, K. M., Bobechko, B. P., Fujieda, H., Chen, S. & Zack, D. J. Chx10 targets a subset of photoreceptor genes. *J. Biol. Chem.* **281**, 744–751 (2006).
70. Braasch, I., Schartl, M. & Volff, J. N. Evolution of pigment synthesis pathways by gene and genome duplication in fish. *BMC Evol. Biol.* **7**, 74 (2007).
71. Kasahara, M., Suzuki, T. & Pasquier, L. D. On the origins of the adaptive immune system: novel insights from invertebrates and cold-blooded vertebrates. *Trends Immunol.* **25**, 105–111 (2004).
72. Kasahara, M. The 2R hypothesis: an update. *Curr. Opin. Immunol.* **19**, 547–552 (2007).
73. Buck, L. B. The molecular architecture of odor and pheromone sensing in mammals. *Cell* **100**, 611–618 (2000).
74. Vogel, C. & Chothia, C. Protein family expansions and biological complexity. *PLoS Comput. Biol.* **2**, e48 (2006).
75. Ding, G. *et al.* Insights into the coupling of duplication events and macroevolution from an age profile of animal transmembrane gene families. *PLoS Comput. Biol.* **2**, e102 (2006).
76. Collin, S. P. & Trezise, A. E. The origins of colour vision in vertebrates. *Clin. Exp. Optom.* **87**, 217–223 (2004).
77. Collin, S. P. *et al.* Ancient colour vision: multiple opsin genes in the ancestral vertebrates. *Curr. Biol.* **13**, R864–R865 (2003).
78. Plachetzki, D. C., Serb, J. M. & Oakley, T. H. New insights into the evolutionary history of photoreceptor cells. *Trends Ecol. Evol.* **20**, 465–467 (2005).
79. Plachetzki, D. C. & Oakley, T. H. Key transitions during the evolution of animal phototransduction: novelty, "tree-thinking", co-option, and co-duplication. *Integr. Comp. Biol.* **47**, 759–769 (2007).
An account of the excellent conceptual work of Oakley and colleagues on cell type evolution. Cell type is one of many hierarchical levels of character evolution that evolves according to common principles.
80. Nordstrom, K., Larsson, T. A. & Larhammar, D. Extensive duplications of phototransduction genes in early vertebrate evolution correlate with block (chromosome) duplications. *Genomics* **83**, 852–872 (2004).
81. Oakley, T. H., Plachetzki, D. C. & Rivera, A. S. Furcation, field-splitting, and the evolutionary origins of novelty in arthropod photoreceptors. *Arthropod Struct. Dev.* **36**, 386–400 (2007).
82. Force, A. *et al.* Preservation of duplicate genes by complementary degenerative mutations. *Genetics* **151**, 1531–1545 (1999).
83. Lynch, M. The frailty of adaptive hypotheses for the origins of organismal complexity. *Proc. Natl Acad. Sci. USA* **104** (Suppl. 1), 8597–8604 (2007).
84. Serb, J. M. & Oakley, T. H. Hierarchical phylogenetics as a quantitative analytical framework for evolutionary developmental biology. *Bioessays* **27**, 1158–1166 (2005).
85. Nuchter, T., Benoit, M., Engel, U., Ozbek, S. & Holstein, T. W. Nanosecond-scale kinetics of nematocyst discharge. *Curr. Biol.* **16**, R316–R318 (2006).
86. Hwang, J. S. *et al.* The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of Hydra. *Proc. Natl Acad. Sci. USA* **104**, 14735–14740 (2007).
87. Miljkovic-Licina, M., Gauchat, D. & Galliot, B. Neuronal evolution: analysis of regulatory genes in a first-evolved nervous system, the hydra nervous system. *Biosystems* **76**, 75–87 (2004).
88. Prud'homme, B. *et al.* Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene. *Nature* **440**, 1050–1053 (2006).
89. Purschke, G., Arendt, D., Hausen, H. & Müller, M. C. M. Photoreceptor cells and eyes in Annelida. *Arthropod Struct. Dev.* **35**, 211–230 (2006).
90. Haeckel, E. Die Gastraea-Theorie, die phylogenetische Classification des Thierreiches und die Homologie der Keimblätter. *Jena Z. Naturwiss.* **8**, 1–55 (1874).
91. Tian, H., Schlager, B., Xiao, H. & Sommer, R. J. Wnt signaling induces vulva development in the nematode *Pristionchus pacificus*. *Curr. Biol.* **18**, 142–146 (2008).
92. Hudson, C. & Yasuo, H. A signalling relay involving Nodal and Delta ligands acts during secondary notochord induction in Ciona embryos. *Development* **133**, 2855–2864 (2006).
93. Yasuo, H. & Hudson, C. *FGF8/17/18* functions together with *FGF9/16/20* during formation of the notochord in *Ciona* embryos. *Dev. Biol.* **302**, 92–103 (2007).
94. Smith, J. C. Making mesoderm — upstream and downstream of Xbra. *Int. J. Dev. Biol.* **45**, 219–224 (2001).
95. Penn, B. H., Bergstrom, D. A., Dilworth, F. J., Bengal, E. & Tapscott, S. J. A. MyoD-generated feed-forward circuit temporally patterns gene expression during skeletal muscle differentiation. *Genes. Dev.* **18**, 2348–2353 (2004).
96. Sandmann, T. *et al.* A core transcriptional network for early mesoderm development in *Drosophila melanogaster*. *Genes. Dev.* **21**, 436–449 (2007).
97. Cripps, R. M. & Olson, E. N. Control of cardiac development by an evolutionarily conserved transcriptional network. *Dev. Biol.* **246**, 14–28 (2002).
98. Oota, S. & Saitou, N. Phylogenetic relationship of muscle tissues deduced from superimposition of gene trees. *Mol. Biol. Evol.* **16**, 856–867 (1999).
99. Gans, C. & Northcutt, R. G. Neural crest and the origin of vertebrates: a new head. *Science* **220**, 268–273 (1983).
100. Sauka-Spengler, T., Meulemans, D., Jones, M. & Bronner-Fraser, M. Ancient evolutionary origin of the neural crest gene regulatory network. *Dev. Cell* **13**, 405–420 (2007).
101. Martinez-Morales, J. R., Henrich, T., Ramialison, M. & Wittbrodt, J. New genes in the evolution of the neural crest differentiation program. *Genome Biol.* **8**, R36 (2007).
102. Rychel, A. L. & Swalla, B. J. Development and evolution of chordate cartilage. *J. Exp. Zoolog. B Mol. Dev. Evol.* **308**, 325–335 (2007).
103. Couly, G. F., Coltey, P. M. & Le Douarin, N. M. The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. *Development* **117**, 409–429 (1993).
104. Donoghue, P. C. J. & Sansom, I. J. Origin and early evolution of vertebrate skeletonization. *Microsc. Res. Tech.* **59**, 352–372 (2002).
An exceptional review on the evolution of skeletal systems in the deuterostomes.
105. Meulemans, D. & Bronner-Fraser, M. Insights from amphioxus into the evolution of vertebrate cartilage. *PLoS ONE* **2**, e787 (2007).
The authors define a hierarchy of gene regulatory networks that specifies cell types in the neural crest. The conservation of each level of this network hierarchy is investigated in the basal chordate amphioxus.
106. Gehring, W. J. & Ikeo, K. Pax6: mastering eye morphogenesis and eye evolution. *Trends Genet.* **15**, 371–377 (1999).
107. Carl, M., Loosli, F. & Wittbrodt, J. Six3 inactivation reveals its essential role for the formation and patterning of the vertebrate eye. *Development* **129**, 4057–4063 (2002).
108. Manavathi, B. *et al.* Repression of Six3 by a corepressor regulates rhodopsin expression. *Proc. Natl Acad. Sci. USA* **104**, 13128–13133 (2007).

109. Ma, Q. Transcriptional regulation of neuronal phenotype in mammals. *J. Physiol.* **575**, 379–387 (2006).
110. Tanabe, Y., William, C. & Jessell, T. M. Specification of motor neuron identity by the MNR2 homeodomain protein. *Cell* **95**, 67–80 (1998).
111. Pattyn, A. *et al.* Coordinated temporal and spatial control of motor neuron and serotonergic neuron generation from a common pool of CNS progenitors. *Genes Dev.* **17**, 729–737 (2003).
112. Chen, C. L. *et al.* Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron* **49**, 365–377 (2006).
113. Kramer, I. *et al.* A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron* **49**, 379–393 (2006).
114. Marmigere, F. *et al.* The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. *Nature Neurosci.* **9**, 180–187 (2006).
115. Bushati, N. & Cohen, S. M. microRNA functions. *Annu. Rev. Cell Dev. Biol.* **23**, 175–205 (2007).
116. Moroz, L. L. *et al.* Neuronal transcriptome of aplysia: neuronal compartments and circuitry. *Cell* **127**, 1453–1467 (2006).
117. Gorbman, A. Olfactory origins and evolution of the brain-pituitary endocrine system: facts and speculation. *Gen. Comp. Endocrinol.* **97**, 171–178 (1995).
A review on the comparative anatomy and evolutionary origin of the vertebrate pituitary. The pituitary is deduced from an olfactory precursor structure in vertebrate ancestors.
118. Toro, S. & Varga, Z. M. Equivalent progenitor cells in the zebrafish anterior preplacodal field give rise to adenohypophysis, lens, and olfactory placodes. *Semin. Cell Dev. Biol.* **18**, 534–542 (2007).
119. Muske, L. E. Evolution of gonadotropin-releasing hormone (GnRH) neuronal systems. *Brain Behav. Evol.* **42**, 215–230 (1993).
120. Yoon, H., Enquist, L. W. & Dulac, C. Olfactory inputs to hypothalamic neurons controlling reproduction and fertility. *Cell* **123**, 669–682 (2005).
121. Hennig, W. *Phylogenetic Systematics* (Univ. of Illinois Press, Urbana, 1966).
122. Page, R. D. & Charleston, M. A. From gene to organismal phylogeny: reconciled trees and the gene tree/species tree problem. *Mol. Phylogenet. Evol.* **7**, 231–240 (1997).
123. Green, P., Hartenstein, A. Y. & Hartenstein, V. The embryonic development of the *Drosophila* visual system. *Cell Tissue Res.* **273**, 583–598 (1993).
124. Ruiz, M. S. & Anadon, R. Some considerations on the fine structure of rhabdomeric photoreceptors in the amphioxus, *Branchiostoma lanceolatum* (Cephalochordata). *J. Hirnforsch* **32**, 159–164 (1991).
125. Leonhardt, H. in *Handbuch der mikroskopischen Anatomie des Menschen* Vol. 4 (eds Oksche, A. & Vollrath, L.) 177–666 (Springer, Heidelberg, 1980).
126. Seipel, K. & Schmid, V. Mesodermal anatomies in cnidarian polyps and medusae. *Int. J. Dev. Biol.* **50**, 589–599 (2006).

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FURTHER INFORMATION
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