Molecular Neurobiology (2018) 55:8306–8327
https://doi.org/10.1007/s12035-018-0987-y

Transcriptional and Epigenetic Control of Mammalian Olfactory Epithelium Development

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Received: 7 January 2018 / Accepted: 5 March 2018 / Published online: 12 March 2018

Abstract

The postnatal mammalian olfactory epithelium (OE) represents a major aspect of the peripheral olfactory system. It is a pseudostratified tissue that originates from the olfactory placode and is composed of diverse cells, some of which are specialized receptor neurons capable of transducing odorant stimuli to afford the perception of smell (olfaction). The OE is known to offer a tractable miniature model for studying the systematic generation of neurons and glia that typify neural tissue development. During OE development, stem/progenitor cells that will become olfactory sensory neurons and/or non-neuronal cell types display fine spatiotemporal expression of neuronal and non-neuronal genes that ensures their proper proliferation, differentiation, survival, and regeneration. Many factors, including transcription and epigenetic factors, have been identified as key regulators of the expression of such requisite genes to permit normal OE morphogenesis. Typically, specific interactive regulatory networks established between transcription and epigenetic factors/cofactors orchestrate histogenesis in the embryonic and adult OE. Hence, investigation of these regulatory networks critical for OE development promises to disclose strategies that may be employed in manipulating the stepwise transition of olfactory precursor cells to become fully differentiated and functional neuronal and non-neuronal cell types. Such strategies potentially offer formidable means of replacing injured or degenerated neural cells as therapeutics for nervous system perturbations. This review recapitulates the developmental cellular diversity of the olfactory neuroepithelium and discusses findings on how the precise and cooperative molecular control by transcriptional and epigenetic machinery is indispensable for OE ontogeny.

Keywords Olfactory epithelium · Olfactory neural stem cell · Neurogenesis · Transcription factor · Chromatin remodeling factor · Epigenetic factor

Introduction

The olfactory epithelium (OE) and its underlying lamina propria make up the olfactory mucosa, which covers the nasal septum and turbinates in posterior aspects of the nasal cavity [1–3]. By embryonic day 9.5 (E9.5) in murine development, the rudimentary OE, which arises from thickening of the olfactory placode (OP), becomes distinguishable from other head structures and later on undergoes extensive infolding to form the olfactory pit and cavity [4, 5]. Rapid thickening of the OP alongside differentiation of pioneer precursor cells lead to establishment of the three (inner, intermediate, and apical) multicellular compartments of the OE after E12.5 ([5, 6]; Fig. 1a, b). From the inner/basal layer of the adult OE, which is closest to the basement membrane (basal lamina), is made up of two mitotic cell types: horizontal basal cells (HBCs) and globose basal cells (GBCs), which exhibit neural stem/progenitor cell characteristics ([3, 7–9]; Fig. 1c). The regenerative feature of the adult OE is linked to these HBCs and GBCs in the basal layer. The intermediate or middle layer is practically the neuronal differentiation zone as evident by a maturation gradient of olfactory sensory neurons (OSNs) in which nascent OSNs are more basally located while mature ones extend processes to the fringes of the apical layer. The apical layer, however, harbors the majority of cell bodies of the glial-like sustentacular (SUS) cells, microvillar cells (MCs), and cells lining ducts of bowman's glands (BGs) ([7, 10]; Fig. 1c).

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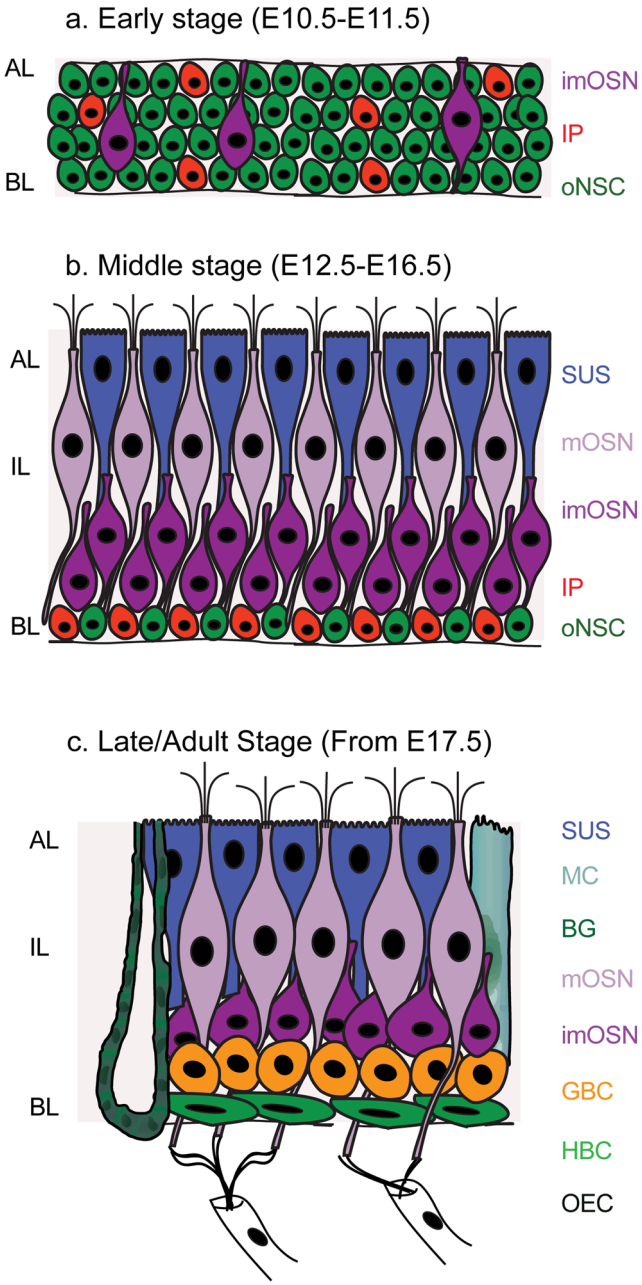

◀ **Fig. 1** Schematic illustration of major OE developmental stages. **a** During early embryonic stages (E10.5–E11.5), the OE has not changed much from its placodal stage, consisting mainly of oNSCs (in green); some of which transform to IPs (in red). Pioneer neurons (in purple) can also be seen at this stage. **b** By mid-stage (E12.5–E16.5) of OE development, the OE acquires its three layers: apical (containing somas of SUS cells), middle (containing mature and immature OSNs), and basal (contain oNSCs and IPs). **c** The late- or adult-stage OE has well-defined compartments (apical, middle, basal). In the oNSC-populated basal layer, round GBCs are distinguishably located on top of flattened HBCs that sit on the basal lamina of the lamina propria. In the middle layer, more basally located imOSNs undergo maturation events including basal axonal extension, apical dendritic elaboration, and relocate above immature ones as mOSNs. The apical layer mainly contains the cells bodies of SUS cells and MCs. BGs are typically deep in the OE tissue with ductal openings at the apical surface. The lamina propria contains OSN axons enwraped by ensheathing cells that project to the olfactory bulb. *AP* apical layer, *BP* basal layer, *oNSC* olfactory neural stem cell, *HBC* horizontal basal cell, *GBC* globose basal cell, *IP* intermediate progenitors, *imOSN* immature olfactory sensory cells, *mOSN* mature olfactory sensory cells, *SUS* cell sustentacular cells, *OEC* olfactory ensheathing cells, *MC* microvillar cell, *BG* Bowman's gland. Adapted with permission from Fig. S1 in [16]

The OE is known to offer an excellent model for probing the cellular and molecular factors involved in the systematic generation of neurons and non-neuronal cells that characterize nervous system development. The mammalian OE has a perpetual progenitor niche capable of ensuring lifetime neurogenesis, with the aim of furnishing and replenishing lost or injured OSNs [3, 11]. At both pre- and post-natal stages of development, some progenitors in the OE appear to be exclusively fated toward neuronal lineage while others display dual potency with capabilities of differentiating toward both neuronal and non-neuronal cell types (Fig. 2). Candidate progenitor cells (HBCs and GBCs) thus must have instructions to decipher which developmental commitment to assume [11–13].

Various studies have shown that OE progenitors respond to both endogenous and exogenous molecular cues to enable them to acquire specific cellular identities during embryonic or postnatal developmental stages. Key among these factors are transcriptional molecules that tightly orchestrate developmental dynamics in the OE. Transcription factors (TFs) such as Pax6, Mash1, Ngn1, and NeuroD are known to play delicate regulatory roles in gene expression patterns required for the establishment of cell fate and diversity in the embryonic and adult OE (reviewed in [14]; Table 1). These TFs and many more are typically recruited in a stepwise manner in specific areas of the developing OE (Fig. 2). However, just as in other neural tissues, TFs that drive OE development are no lone rangers. Phenomenal roles of epigenetic regulators in overall specification of neural tissue have extended our scope of understanding of the molecular and cellular control of neural development (reviewed in [15]). As such, the emerging perception of epigenetic regulators as being indispensable for OE developmental specifications is stimulating interest geared toward elucidating the precise molecular mechanisms involved [16]. Such epigenetic regulators include chromatin remodeling BRG1/BRM-associated factor (BAF) and polycomb repressor complexes, and activities of non-coding RNAs, whose functions are embedded in a broader regulator network that determines OE development. At least in part, these epigenetic players are known to critically harmonize regulatory networks including that of transcriptional machinery to effect proper development of the OE [16–18].

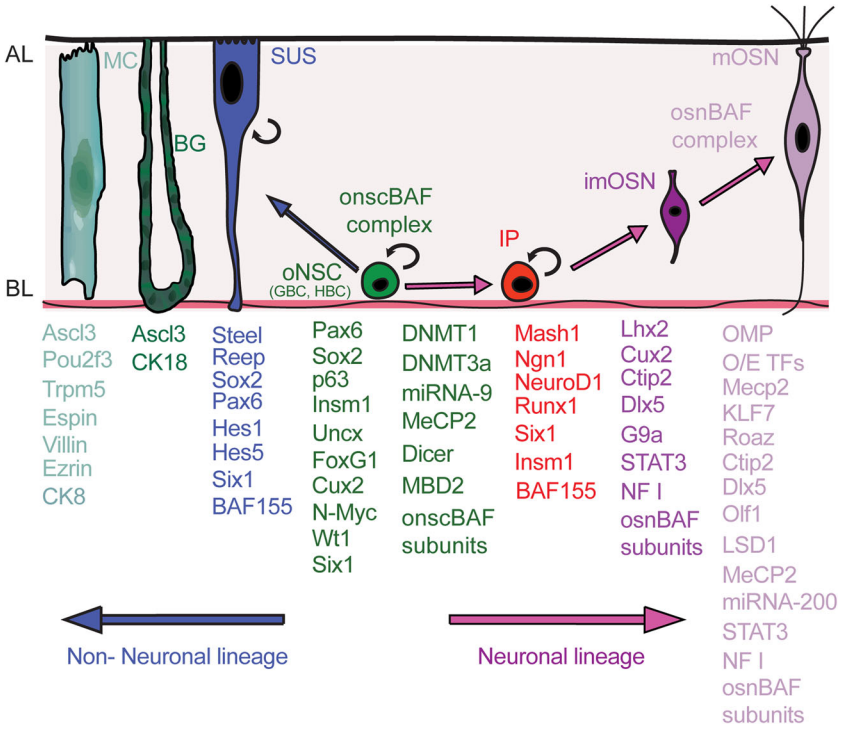

Fig. 2 Transcriptional regulation of cell specification in the olfactory epithelium. Progenitors including HBCs and GBCs require TFs marked in green to be able to proliferate and survive. The neuronal lineage pathway begins with the expression of proneural TFs (marked in red) that limit Sox2/Pax6+ progenitors to gain neuronal commitment to become intermediate progenitors (IP). IPs then differentiate under the control of differentiation TFs (in deep purple) to become immature olfactory sensory neuron (imOSN). imOSN subsequently undergo maturation by extension of single dendrite and axon toward the apical (AL) and basal layer (BL) respectively under the control of maturation TFs (in light purple) to become mature sensory neurons (mOSN). Depending on rate of imOSN-mOSN transition, TFs like STAT3 and NFI may be recruited to inhibit OSN maturation process. Sox2/Pax6+ progenitors can also choose a non-neuronal path by first blocking neuronal lineage tendencies via activity of TFs like Hes1 and Hes5, and likely other unknown factors to generate sustentacular (SUS) cells under the regulation of differentiation/maturation TFs (in blue), some of which ensure SUS cell self-renewal. Other non-neuronal derivatives of Sox2/Pax6+ progenitors include Bowman's glands (BGs) and microvillar cells (MCs) specified by TFs marked in deep and light green respectively. Name of onscBAF and osnBAF subunits is indicated in Fig. 3. Adapted with permission from Fig. S1 in [16]

In this review, we mainly focus on the transcriptional networks that determine cell diversity during development of the OE and juxtapose that with emerging findings implicating functionality of epigenetic machinery in embryonic and adult OE morphogenesis.

Cell Diversity in the Olfactory Epithelium

The pseudostratified post-embryonic OE is heterogeneous by cellular composition, although OSNs predominate the cell population. Cells in the intact adult main OE are typically organized into developmentally distinct areas that reflect some level of hierarchical placement. A basal lamina delimits the OE proper from its underlying loose areolar connective tissue space (lamina propria). Between the basal lamina and the apical surface of the postnatal OE are (basal) progenitor cells that reside in the basal compartment followed by OSNs [immature (im)OSNs and mature (m)OSNs] in the middle compartment and tall SUS cells whose somata are largely localized in the apical aspect of the OE. The underlying lamina propria contains cells such as olfactory ensheathing cells and mucus-secreting cells (BGs), together with traversing blood vessels and fascicles of OSN axons (reviewed in [3]; Fig. 1c).

On the other hand, the cellular composition of the embryonic OE is transitory and displays marked overall structural dynamics. Most of the early OE cells are derived from the OP progenitor cells [19]. Although these embryonic progenitors can be found in the apical and basal compartments of the developing OE, HBCs are excluded from the progenitor pool until perinatal stages. The future intermediate zone, which is flanked by these apical and basal progenitors, is made of only OP progenitor-derived imOSNs ([3, 20]; Fig. 1). Coupled with the absence of SUS cells, the early embryonic OE is considerably less diverse in cell type composition compared with the postnatal and adult stage OE (Fig. 1). Helpfully, cell diversification and identity in both embryonic and adult OE can be defined in terms of cell morphology, location, and antigen expression patterns.

Table 1 Transcription and epigenetic factors in olfactory epithelium development

Factor	Gene family	Mutant/model	Cell type	Phenotype	Reference
Transcriptional factors					
Pax6	Pax	Methimazole treatment following cKO	HBCs	Impaired OSN regeneration	[9]
		cKO following MeBr treatment	oNSCs, IPs	Reduced OSN regeneration	[236]
Sox2	Sox	cKO	oNSCs, IPs	Reduced production of OSNs and non-neuronal cells	[236]
		cOE		Enhanced OE neurogenesis	[88]
Sox10	Sox	KO	OEC	Disruption of OEC differentiation, reduction in number of mOSNs, defective mOSN axons targeting	[58]
Insm1	Insm	KO	Basal OE progenitors, IPs, imOSN	Reduced proliferation of OE progenitors and terminal differentiation, more Ascl1+ cells	[237]
Uncx	Paired homeobox	KO	Basal OE progenitors, imOSN	Reduced progenitor proliferation, decreased survival of OSNs	[238]
Ascl1/Mash1	bHLH	KO, MeBr treatment	GBCs, OSN precursors/IPs	Reduction in OSN population, increased SUS cells	[36, 57, 100]
Ascl3	bHLH	KO	BGs and MCs	Lack of BGs and MCs, reduced number of OSNs	[10]
Ngn1	bHLH	KO, RNA probe	GBCs, IPs	Decreased number of OSNs	[6, 239]
NeuroD1	bHLH	cKO	GBCs, IPs	Decreased number of mOSNs	[107]
FoxG1/BF1	Forkhead	KO	oNSCs	Delayed proliferation and differentiation of oNSCs	[240, 241]
Lhx2	LIM	KO	IPs, imOSN, mOSN	Increased population of NeuroD1+ cells, impaired terminal differentiation of OSNs	[110, 111]
Hes1	bHLH	KO	Apical OE progenitors, IPs	Increased number of Mash1+ cells and OSNs	[35]
Hes5		KO	oNSCs, IPs	Premature OSN differentiation	[108]
Runx1	Runx	KD	OSN precursors OECs	Increased proliferation of OECs	[242]
Cux2	Cut-like homeodomain	KD	IPs, OSN	Reduced OE neurogenesis	[243]
		cOE	precursors, OSN	Suppressed progenitor proliferation	[243]
N-Myc	MYC	cKO	imOSNs, Hes5+ progenitors	Decreased proliferation and neurogenesis, Lack of Hes5+ progenitors	[244]
Wt1 (+KTS)	WT1 (Zinc-finger)	KO	Basal OE cells including HBCs and GBCs	Thin OE, Few Mash1+ OSN precursors	[103]
Six1	Six-class homeobox gene	KO	Apical and basal OE progenitors	Lack of apical OE progenitors, SUS cells and mOSNs	[245]
p63	P53 tumor suppressor gene	cKO	HBCs	Reduced oSNC proliferation and maintenance	[99]
DIx5	DIx	KO	OSNS	Delayed ORN differentiation, altered axonal trajectory	[230, 233]
ARX	X-linked prd type homeobox	KO	IPs	Reduced proliferation, impaired OSN axonal targrting	[246]
Roaz/ZNF423	Kruppel-like C2H2 zinc finger	cOE	oNSCs, imOSNs	Impaired OSN differentiation	[126]

Table 1 (continued)

Factor	Gene family	Mutant/model	Cell type	Phenotype	Reference
O/E2, O/E3	Olf1/EBF	KO	OSNs, mOSNs	Abolished OSN axonogenesis	[120]
OMP	OMP	KO	mOSNs	Impaired functional maturation mOSNs	[119]
Skn-1a/Pou2f3	POU homeobox	KO	MCs	Complete lack of MCs	[138, 247]
Chromatin and epigenetic factors					
BAF155	BAF complex	cKO	oNSCs, mOSNs	Reduced oNSC proliferation and differentiation	[16]
BAF170	BAF complex	cKO	mOSNs	Reduced OSN axonogenesis and maturation	[16]
BAF100b/Ctip2	BAF complex	KO	mOSNs	Defective OSN maturation	[248]
CHD7	Chromodomain	Partial KO	ONSCs, OSN precursor cells	Reduced number of OSNs, disorganized OE ultrastructure	[151, 152]
LSD1	Histone demethylase	KO	OSN	Loss of OR expression, failed OSN maturation	[194]
DNMT1	DNMT	KD	OE progenitors, OSN	Increased neuronal differentiation	[212, 213]
DNMT3a	DNMT	KO, KD	OE progenitors	Disabled odorant-dependent gene activation in OSNs	[212, 214]
MBD2	MBD	KO	OE progenitors	Enhanced progenitor proliferation, reduced survival of OSNs	[176]
MeCP2	MeCP	KO	OE progenitors	Truncated terminal differentiation of OSNs, defective refinement of olfactory circuits	[175, 176]
		cKO	OSNs	Impaired circuitry refinement and activity-dependent transcriptional responses in OSNs	[249]
Dicer	miRNA	cKO	oNSCs, IPs, OSNs	Impaired OSN differentiation and survival of progenitors	[17]
miRNA-9 miRNA-200	miRNA	KD	oNSCs, IPs, imOSNs, mOSNs	Impaired OSN differentiation and axonal targeting	[17, 230]
miR-183	miRNA	KO	OE progenitors	Reduced number of mOSNs, irregular OSN morphology, reduced density of OSN dendritic knobs	[234]
G9a and GLP	Histone methyl-transferase	dKO	OSNs	Impaired OSN diversity	[199]

OPs olfactory placodes, *OE* olfactory epithelium, *oNSCs* olfactory neural stem cells, *IPs* intermediate progenitors, *GBCs* globose basal cells, *HBCs* horizontal basal cells, *im* immature, *m* mature, *OSNs* olfactory sensory neurons, *OR* olfactory receptor, *SUS* sustentacular cells, *OECs* olfactory ensheathing cells, *cKO* conditional knock-out, *dKO* double knock-out, *cOE* conditional overexpression, *KD* knock-down, *IUE* in utero electroporation, *GBCs* globose basal cells, *HBCs* horizontal basal cells, *MCs* microvillar cells, *BG* Bowman's gland, *MeBr* methyl bromide

Horizontal Basal Cell Progenitors

The HBCs are morphologically flattened cells organized as a monolayer that abut the basal lamina of the olfactory mucosa and are molecularly identified by their marked expression of cytokeratin 5/6 and 14, ICAM1, and conventional neural stem cell markers such as Pax6 and Sox2 [9, 16, 21–25]. Recently, they have been found to possess unique primary cilia that may be important in collecting differentiation cues to stimulate OSNs regeneration after lesions [26].

Albeit they are progenitor cells, HBCs infrequently divide: especially during normal generation or renewal turnover of OSNs; thus indicating their unique quiescent stemness feature making them serve as OSN progenitor depot that affords lifelong constitutive neurogenesis and lesion-induced neuronal regeneration characteristic of the adult OE [9, 27–30]. Indeed, by employing a HBC-specific Krt5-Cre construct in cell fate mapping investigation, strong evidence has been provided to support the idea that HBCs are multipotent stem cells that are capable of producing GBCs, which in turn can generate neuronal (OSNs) or non-neuronal cells [26, 28]. Other lines of evidence that support the stem-like nature of HBCs and the ancestral link of GBCs to them include their expression of Pax6 and Sox2, the existence of cells that have intermediate morphologies between HBCs and GBCs, and antigenic co-expression similarity between both basal cells following the olfactotoxin methyl bromide (MeBr)-induced OE lesion [7, 9, 16, 23, 25, 31, 32]. Due to their classic expression of integrins, HBC can be positively selected based on adhesion receptor expression profiles to allow for manipulative studies of their multipotency and/or stemness in vitro.

Globose Basal Cell Progenitors

GBCs are polyhedral in shape and have high mitotic figures. Some of them are known to exist as basal precursors of OSNs [7, 21, 22, 31, 33, 34]. They have striking resemblance to OP progenitor cells in terms of transcription factor profile, even though the latter are mainly responsible for generating cells in the early embryonic OE [6, 25, 35, 36]. Unlike HBCs, GBCs are bipotent stem cells that undergo several rounds of mitosis to produce either neurons or SUS cells [37–40]. Neurogenic GBCs express Mash1 and Neurogenin1, and are present in both embryonic and adult OE [5, 41, 42]. Their subsequent differentiation can be initiated by neuronal turnover stimuli.

Intriguingly, it has been found that the GBC pool in OE is rather made up of two mixed population of cells: early transit amplifying cells and immediate (neuronal) precursors (INPs). Transit amplifying cells are considered to be direct descendants of stem cells (say HBCs). Even though being more committed progenitors with restricted propensity of self-renewal, they still are able to undergo a few rounds of cell division before terminally differentiating, thus making them serve the purpose of amplifying differentiated cell outcome from a single stem cell during normal or lesion conditions of cell replacement in the OE. INPs on the other hand directly differentiate into OSNs after one or two cell cycles [21, 41]. Depletion of GBCs under any condition is thus expected to result in neurogenic cell exhaustion that can jeopardize olfactory epitheliopoiesis.

Olfactory Sensory/Receptor Neurons

Right atop GBCs are recently differentiated bipolar imOSNs generated from the INPs in the adjoining upper basal layer ([7, 16, 21]; Fig. 1c). They can be identified by their expression of proteins such Lhx2, Cux2, HuC/D, β-tubulin, NCAM, GAP43, and SCG10 ([21, 43–45]; Fig. 2). Together with their fully differentiated kind (mOSNs), they form the intermediate layer that is not obvious at early embryonic stages of OE development. The developmental transition of imOSN to mOSN involves axonal and single dendritic elaboration by imOSN to basal and apical aspects of the OE, respectively (Figs. 1b, c and 2). The dendrites of mOSNs terminate with a bulbar protrusion, which bears 10–20 cilia for binding odorants in the OE mucus layer to initiate intracellular signal transduction cascade, whereas their axons fasciculate in the lamina propria before traversing the cribriform plate as cranial nerve I [CNI] [1, 46–48]. mOSNs express mature neuronal markers including olfactory marker protein (OMP) and form the bulk (75–80%) of cells in the adult OE ([16, 49]; Fig. 2).

Sustentacular Cells

SUS cells are non-neuronal glial-like cells that generally span the whole height of the OE. They have their bulged somata arranged in a single row to form the luminal surface (apical layer) of the OE via tight junction connections and possess branched end feet that make contact with the basal lamina [7, 50, 51]. They typically express the antigen Sus4 [52].

Although SUS cells form only 15% of the OE parenchyma, they provide support and generally enhance OSN function. They also perform detoxification of xenobiotics, degradation of odorants, regulation of ionic composition, and phagocytosis of exhausted OSNs [53–55]. When fully differentiated, SUS cells look elongated and have reduced mitotic activity with no demonstrable progenitor characteristic, despite still expressing proliferative factors such as Pax6, Sox2, and Steel ([25, 56, 57]; Fig. 2).

Olfactory Ensheathing Cells

Another important glial cell type essential for OE function and development are the olfactory ensheathing cells (OECs). They are specialized cells found in the lamina propria that chiefly enwrap and support bundles of OSN axons as they project to

the olfactory nerve layer of the olfactory bulb (OB) [58, 59]. It implies that OECs are able to migrate from peripheral to central nervous system [60–62]. They express a variety of molecular markers depending on their location along the OSN axonal trajectory [63–65]. Although much is yet to be known about their exact origin, especially in mammals, OECs have been said to likely have dual origin: from the olfactory placode and neural crest [58, 66, 67]. As such, OECs are reported to be pluripotent cells capable of assuming astrocyte- or Schwann cell-like characteristics in vitro [68], although they do not normally execute myelination in the peripheral olfactory system.

Nonetheless, when experimentally transplanted into myelin-deficient segments in the spinal cord, OECs are able to re-establish peripheral myelination schemes similar to that of Schwann cells to augment action potential transmission [69–71]. These and other findings have made it tangible to assign critical role(s) to OECs in terms of OSN replacement and axon regrowth that continuously occur in the adult OE throughout life [71, 72]. These functional properties of OECs make them potential candidates for clinical rescue of neural degeneration [73, 74].

Microvillar Cells and Bowman's Glands

Also distributed throughout the OE are non-neuronal (supporting) MCs and BGs that originate from progenitors in the OE and generally function to provide proper development, regeneration, and function of the OE [10]. BGs are made of cluster of branched tubuloalveolar structures found in the mesenchyme of the OE lamina propria (reviewed in [75]). The acinar cells of the gland are known to produce mucus with immunologic and homeostatic functions that gets transported to the apical OE surface via narrow duct-like conduits that traverse the epithelium [76, 77]. Like SUS cells, BGs are rich in xenobiotic enzymes important for detoxification in the OE [78].

MCs are on the other hand mainly located close to the olfactory epithelial surface. They are flask-shaped with a tuft of microvilli located in the OE mucus layer and cytoplasmic extensions that enter the OE lamina propria, hence giving them an apparent overall bipolar morphology [79]. Three types of microvillar cells in the OE can be distinguished based on morphology and receptor expression [80–82]. One such type is known to be the main source of neuropeptide Y, which stimulates basal progenitor proliferation and neurogenesis in the OE [83, 84].

Transcriptional Control of OE Development

Our understanding of the transcriptional regulatory networks that control OE development and plasticity has increased over time. Yet, there is still a vast number of TFs expressed in the OE whose precise role in OSN production and regeneration is unclear. Interestingly, transcriptional control during OE development seems to be consistent throughout life. This stems from the fact that during OE neurogenesis, say in murine, the expression profile of key TFs is mainly similar at both embryonic and postnatal developmental stages and even during lesion-induced OE regeneration [6, 36, 85]. The sheer tissue plasticity and high fidelity in expression pattern of TFs throughout life make the OE ideal for exploring potential cell repair and reprogramming options for damaged neural tissue.

In this section, we present information on the transcriptional networks that regulate the establishment of the OP and the subsequent sequential events leading to proliferation, fate specification, differentiation, and maintenance of cells in the OE (Table 1).

Progenitor Proliferation and Maintenance

Transcription factors that control the self-propagation and survival of progenitor/stem cells are among the early onset master gene expression products that ensure supply of appropriate cell derivatives within a critical time window or even continuously throughout life as in the case of the OE. Sox2 (SRY-box containing gene 2) and Pax6 (Paired box 6) are two such powerful TFs that are indispensable for the maintenance of neuro-competent multipotent stem cells in the embryonic and adult OE, and more generally in germinal zones of the entire nervous system. These key TFs are frequent cooperative partners whose regulatory functions are critical for the formation of the olfactory placode and the later-stage sensory OE [25, 56, 86–88].

Generally, cells in the central nervous system that express Sox2 are considered as early progenitor/stem cells that are capable of giving rise to neurogenic or gliogenic precursor cells [87, 89]. Some cells of the olfactory pit inherit and/or maintain expression of Sox2 even after OP induction. Later, cells that form the upper (apical) layer of the developing OE also acquire Sox2 immunoreactivity [86, 90] and may arguably be the pool from which basally located Sox2-expressing cells migrate to reside in the basal layer of the embryonic and adult OE. Sox2 expression is thus detectable in the apical and basal OE layers where cell proliferation is prominent. Experimental OE lesion paradigms in adult mice have revealed that Sox2-expressing cells are multipotent and capable of producing neurons and SUS cells that mediate regeneration of the injured OE [3, 25, 28, 91]. Interestingly, when neuronal cell lineage is interrupted in the OE, there is an exaggerated expression of Sox2 even beyond the usual zonal limit of detection [86, 92]. Sox 2 is thus essential for OE neuronal and non-neuronal stem cell activity needed for development and regeneration of the OE.

Another master transcription factor involved in progenitor proliferation and survival is Pax6. Like Sox2, it modulates activity of multipotent stem cells in many germinative regions of developing and post-natal systems including the CNS [93]. Pax6 is known to be indispensable for OE development and maintenance [25, 32, 56]. It is therefore not surprising that the OE and the entire nose absolutely failed to form in Pax6 mutant animal models [94]. Specifically, Pax6 is expressed in both neuronal (GBCs) and non-neuronal progenitors such as HBCs and SUS cells [56]. Intriguingly, the Pax6 expression needed for olfactory placode formation is regulated by synergistic activity of the TFs Sox2 and Oct-1 [86].

The TF p63, which belongs to the p53 tumor suppressor gene family, has been recently implicated as a key player in maintenance of multipotency in OE stem cells. Traditionally, p63 is responsible for stem cell proliferation and survival in other stratified epithelial tissues including the skin [95–97]. By employing complete knockout models (p63-null mice), it has been found that p63 regulates stem cell dynamics in the OE [98]. The transcriptional activity of p63 is necessary for the proliferation and maintenance of HBCs. Notably, OE lesions, especially involving SUS cell death, trigger downregulation of p63 expression leading to acquisition of multipotency by quiescent OE stem cells like HBCs to set the stage for cell regeneration [8, 98, 99].

Given that conditional knockout of p63 results in disturbance of Sox2 and Pax6 expression [99], it is possible to envisage plausible cooperation of several cognate factors in the control of progenitor dynamics during OE development. Sox2, Pax6, and p63 may thus putatively form a transcriptional regulatory network that subtly regulates the proliferation and differentiation equilibrium in multipotent progenitor cell population in the developing and adult OE. These TFs potentially also can be manipulative targets for at least OE tissue re-engineering to rectify clinical conditions related to loss of the sense of smell and probably other associated limbic functions.

Neuronal fate Determination

Sox2 and Pax6 expressing progenitor/stem cells in the OE normally acquire neuronal fate at the onset of neurogenesis, which coincides with the time of expression of another important TF called Mash1 (Mammalian Achaete Scute Homolog 1), belonging to the basic helix-loop-helix (bHLH) family. Expression of this proneural gene is known to be responsible for initiating molecular and cellular programs to afford attainment of neuronal identity and further differentiation. That implies that Mash1+ progenitors including some GBCs are committed to neuronal lineage [6, 35, 57, 85, 100].

Homozygous loss of Mash1 ($Mash1^{-/-}$) rendered the OE thin. This was attributed to the drastic reduction in the population of OSNs in such mutants, which instead had increased expression of Steel, a marker of SUS cells [57, 85, 100, 101]. The reduction in the number of OSNs in $Mash1^{-/-}$ may be due to loss of the neuro-competent mitotic basal progenitor pool, probably as a result of abnormal non-exit from the cell cycle leading to their susceptibility to apoptosis [85, 102]. On the contrary, early born (pioneer) SCG10+ neuron generation seems unperturbed by blockage of Mash1 expression, signifying their possible non-dependence on Mash1 transcriptional functionality [85].

During embryonic development of OE, Mash1 expression has been shown to be explicitly controlled by two other TFs, namely Wilms' tumor zinc-finger protein (Wt1) and Hairy and Enhancer of split (Hes1) [35, 89, 103]. Wt1 is mainly expressed in the basal layer of the pre-natal OE. Its pattern of expression overlaps with Mash1 expression in some OE cells during development [103]. Wagner et al. (2005) have provided strong proof of Wt1 as Mash1 upstream effector based on the fact that there is striking loss of Mash1 expression in the embryonic OE after selective deletion of the Wt1 (+KTS) isoform. As could have been expected, in vitro overexpression of Wt1 (+KTS) in HEK293 cells led to upregulation of Mash1 transcripts and proteins. Contrary to these outcomes, which are the reasons we believe there may be other modulatory factors in play, co-transfection of HEK cells with a construct bearing Mash1 promoter and Wt1 is insufficient to facilitate Mash1 activity [103]. To solve this conundrum, further investigations must be carried out to identify the precise role of Wt1 in the expression or function of Mash1 during neurogenesis in the OE and possibly identify other collateral factors that may be involved.

Unlike Wt1, Hes1 is prominently expressed in the apical aspect of the early developing OE and despite its dramatic downregulation in the $Mash^{-/-}$ mouse mutant OE at E12.5, it is undisturbed at earlier embryonic stages in the absence of Mash1; implying Hes1 as a candidate player in the so-called pioneer neurogenesis [6, 35]. Other findings also reveal that Hes1 may play restrictive function in neurogenesis, given that in its absence there is increase in Mash1+ cells and SCG10+ OSNs at E10.5 and E12.5, respectively [35]. Studies have identified Hes1 as a Notch signaling pathway downstream effector, which requires Mash1 for appropriate expression of associated signaling ligands [6, 104]. Indeed, it has been found that Mash1 expression is regulated via Notch family receptor-based signaling to orchestrate specification and generation of OSNs and also olfactory glial cells [6, 36, 85, 105]. That notwithstanding, it is also possible to consider involvement of other TFs in modulating the expression of Mash1 through the Notch signaling axis. For instance, Hes5, another downstream effector in Notch signaling [104], may play substitutive or autonomous roles during OE development.

The acquisition of neuronal fate and subsequent differentiation of Mash1+ progenitors into OSNs require expression of various neurogenic bHLH proteins such as neurogenin 1 (Ngn1), and neuronal differentiation 1 (NeuroD1). These

TFs act sequentially and in concert with other cofactors to promote Mash1+ progenitors with neuronal commitment to exit the cell cycle and assume full neuronal identity. In the absence of Mash1, the aforementioned bHLH proteins are misexpressed [6, 57, 85]. In the next section, we will discuss the transcriptional function of these Mash1-dependent bHLH proteins and other TFs in OSN differentiation.

Neuronal (OSN) Differentiation

Usually, upon exit from the cell cycle, intermediate neural precursors activate a battery of transcription factors that will ultimately ensure their differentiation and maturation. As already mentioned, some bHLH proteins are well noted players in neuronal differentiation. In general, a key feature of these TFs is that they act downstream of Mash1. An immediate acting TF that comes in play to initiate differentiation of Mash1+ progenitors is the bHLH protein Ngn1. This factor in itself is regulated by Mash1, hence its similarity to Mash1 in terms of regional expression in the embryonic OE. Ngn1+ cells also known as intermediate neural precursors finally reside in the basal OE layer, after their migration from their initial location in the apical layer [85, 106].

Without any disruption in Mash1 expression, and hence progenitor pool, $Ngn1^{-/-}$ mutant mice show considerable decrease in number of OSNs that results in a thin OE. Strikingly, pioneer neurons in the olfactory pit are the most affected in the absence of Ngn1. Also, there is a dramatic gradient of reduction in expression of the neuronal marker SCG10, with the most severity in the caudal $Ngn1^{-/-}$ mutant OE [6]. This signifies that Ngn1 is a very powerful TF for neuronal differentiation.

Interestingly, the Notch ligands Hes1 and Hes5 negatively regulate Ngn1 expression, although the overall Notch signaling cascade is unaffected in $Ngn1^{-/-}$ mutants [35]. It has been reported that Hes6, which is downstream Ngn1, may antagonize Hes1 to promote neuronal differentiation in the OE [6, 35]. A notable consequence of Ngn1 deletion is the reduction in NeuroD1 expression, which usually follows Ngn in the sequence of transcriptional events during neuronal differentiation [6]. Like Ngn1, NeuroD1 is expressed in cells located in the basal OE compartment and maintained throughout development of the OE [6, 85]. Although the exact role of NeuroD1 is quite unclear, studies by Packard et al. reveal its importance in neural precursor transition to mOSNs in postnatal OE. A likely dual role of NeuroD1 in olfactory neuronal differentiation and maturation is suggested by the fact that the number of OMP+ cells was reduced in some aspects of postnatal $NeuroD1^{-/-}$ mutant OE [107].

Another TF that potentially regulates NeuroD1 is the Runt-related transcription factor 1 (Runx1). This Runx family protein helps prevent untimely differentiation of neuronal progenitors in the OE. Its expression is mainly in the basal layer of the OE where it displays an overlapping expression pattern with Mash1 and NeuroD1 [108]. In the absence of Runx1, the number of NeuroD1-labeled cells is diminished, OSN precociously and ectopically differentiate, however, without accompanying alteration in Mash1 expression [6, 108, 109].

The LIM homeobox 2 (Lhx2) protein is also an important TF that acts downstream of Mash1 to most likely regulate OSN differentiation [110–112]. It is expressed in both basal and intermediate compartments of the OE and when genetically ablated in mice ($Lhx2^{-/-}$), leads to increased numbers of NeuroD1 expressing cells, thus implying its possible role in augmenting cell cycle exit of neurogenic OE progenitors [111–113]. Nonetheless, the exact role of Lhx2 in OSN differentiation is quiet puzzling. This is stemming from conflicting outcomes of SCG10, GAP43, and OMP expression in Lhx2 mutant OE, which particularly make Lhx2 function in OSN differentiation somewhat indecipherable [111, 112]. In any case, it has been reported that Lhx2 participates in terminal differentiation of OSNs during olfactory system development. Notably, deletion of Lhx2 in the OE abolished OSN axonal projection to targets in the OB and to the olfactory cortex by second-order neurons in the OB [110, 114]. In all, further studies are needed to elucidate the precise function of Lhx2 in OSN differentiation during OE development.

Maturation of Olfactory Sensory Neurons

A class of transcription factors called O/E (Olf/Ebf) has been shown to be critical for maturation of OSNs to ensure their ultimate functional integration. Widely recognized molecular markers of mOSNs such as OMP, Golf, OcNc, and ACIII have enrichment of the O/E TFs binding sites in their promoter regions. It implies that O/E TFs may play key roles in enhancing the expression of these markers, which are known to be necessary for neuronal maturation and functional integration in the peripheral olfactory system [115–119]. Strangely, however, the absence of O/E TFs seems not to affect OMP expression [120]. This probably means that other TFs may be involved in OSN maturation during OE development. For instance, the TFs nuclear factor I (NFI) and signal transducer and activator of transcription 3 (STAT3) are known suppressors of genes required for neuronal maturation [121, 122]. When the NFI binding sites in the promoter region of OMP were mutated, it resulted in elevated activation of OMP promoter by O/E TFs, making NFI a plausible antagonist of O/E TFs [121, 123].

The Kruppel-like TF, KLF7, is also of importance for OSN maturation. Without any noticeable change in progenitor pool and cell apoptosis, mutation of KLF7 in mice resulted in reduction of the number of NCAM+ OSNs with attendant truncation of axonogenesis and subsequent denervation of the OB [124, 125].

Other TFs such as methyl CpG binding protein 2 (MeCP2) and a rat C_2H_2 zinc finger protein (Roaz) studied under various experimental conditions in OE development have been shown to play key roles in OSNs maturation, although the exact mechanisms involved are yet to be determined [126–129].

Non-Neuronal Differentiation in the OE

Typically, during neural development, multi-/bipotent progenitors have the option or predisposition to activate transcriptional programs that will determine their fate choice between neuronal and glial lineages. Similarly, such molecular decision is also required in the generation of OSNs and non-neuronal cell types in the embryonic and adult OE. While transcriptional activators (e.g., Mash1, Ngn1, and NeuroD) drive OSN fate, transcriptional suppressors (e.g., some Hes family members of TFs) block neuronal fate in favor of non-neuronal lineage acquisition [35, 36].

Hes1 for instance is critical for non-neuronal differentiation of precursors in the peri- and postnatal OE. Its expression thus seems to be a vital signal for promoting glial cell fate but a repressive signal for Mash1-directed neuronal commitment (Fig. 2). In the adult OE, and even following olfactory bulbectomy (removal of the OB), Hes1 is expressed by SUS cells whose somata form the apical layer of the OE [36, 130, 131]. However, MeBr-induced OE lesion activates expression of Hes1 in basal progenitor cells [35], probably to promote restorative processes for SUS cells. Interestingly, the TF Hes5, another repressor of neuronal differentiation, interactively synergizes with Hes1 to promote non-neuronal differentiation [35, 132]. Fully differentiated SUS cells retain their ability to express Pax6, Sox2, Otx2 (reviewed in [12]), and Steel [57], even though they have extremely low capacity to proliferate. SUS4-expressing cells also appear to be fated toward the generation of SUS cells [40].

Neural crest-derived OECs have been shown to express the TF Sox10 from E10.5 [58, 133], when there is emergence of axons and migratory mass of neurons from the OE [134, 135]. As such, loss of Sox10 achieved in homozygous $Sox10^{lacZ/lacZ}$ mutant mouse embryos resulted in disruption of OEC differentiation, reduction in number of OMP+ OSNs, and accumulation of olfactory axons in the ventromedial aspect of the olfactory nerve layer of the OB [58].

The TF Runx1 has also been shown to be important in the developmental regulation of OECs. Its selective expression is seen in OECs residing in the inner olfactory never layer and their precursors that form the migratory mass. Runx1 expression in this population of OECs seems to be essential for their proliferation, as in vivo knockdown of mouse Runx1 led to their increased proliferation, whereas Runx1 overexpression in OEC primary cell culture hampered proliferation. Interestingly, however, Runx1+ OEC precursors increase in number in the presence of reduced Runx1 activity, albeit with incidental reduction in the number of mature OECs [242].

Generally, OECs express many transcriptional and signaling factors that have been implicated in the regenerative capacity of the OE (reviewed in [74]). However, their precise roles need to be investigated further to clearly define the bona fide regulatory networks that ensure development of OECs in the peripheral olfactory system.

MCs and BGs are other important non-neuronal components of the OE, which have been shown to likely possess specific regulatory programs during OE development. Like the other aforementioned non-neuronal cells, MCs and (cells of) BGs are among the less investigated of the OE. As shown in Fig.. 2, a few TFs have been found to be expressed by both MCs and cells of BGs, albeit the exact functional role played by most of them in OE development is yet to be elucidated. For example, proteins like Trpm5 [80], Ezrin, Cytokeratin 8 (CK8), Espin, and Villin [136] predictably regulate MC formation, whereas CK18 is probably involved in formation of cells in BGs [137].

A couple of detailed studies have provided more information on how MC and BG development is transcriptionally regulated. The TF Ascl3 (Achaete-Scute Family BHLH Transcription Factor 3), which has been demonstrated to be specifically expressed in precursors of MCs and BGs, is known to be necessary for their development and regeneration [10]. By using lineage tracing strategies, it has been shown that both MCs and BGs are solely derived from OE progenitors, including a subset of HBCs that express Ascl3. As such, there is activation of Ascl3 expression in this subpopulation of HBCs immediately after methimazole-induced OE lesion. Interestingly, following genetic ablation of Ascl3+ cells, no MC or BG was regenerated, although other regenerative events remained unaffected. The number of OSNs was also reduced as a result of Ascl3 misexpression, which was linked to depletion of neurogenic GBCs and/or increased apoptosis in such methimazole-insulted OE [10].

In another study, Skn-1a/Pou2f3, a member of the POU (Pit-Oct-Unc) TFs, was identified to be essential for the generation of a population of MCs known to express Trpm5 (transient receptor potential channel M5) [80, 138]. Notably, Skn-1a was observed to be expressed in some basal, including Mash1+ IPs, and apical non-neuronal cells, a few of which were co-labeled with Trpm5 in the embryonic and adult OE. In Skn-1a null mice, most OSN differentiated normally; however, Trpm5+ MCs were phenomenally absent in the Skn-1a-deficient OE. Additionally, the density of superficial MCs was reduced in the absence of Skn-1a functionality [138]. This implies that the TF Skn-1a may be important in specifying a subset of MCs that express Trpm5.

Overall, not too much is known about the transcriptional regulation during generation of non-neuronal supporting cells as compared with other cells type in the OE. Given the

relatively limited information on the molecular and cellular control of non-neuronal glial-like cell formation in the OE, further investigations into the molecular instructions involved in the generation of supporting/non-neuronal OE cells will consolidate our knowledge of how these classes of cells are produced and maintained in the OE.

Role of Chromatin Remodeling and Epigenetic Regulation in OE Development

Over the past two decades, our understanding of how some factors alter gene expression without an underlining change in DNA sequence has increased tremendously. The term epigenetic has thus been universally adopted as second code of inheritability of such non-gene-related phenotype. Events that result in epigenetic control of (neuro)developmental processes include DNA methylation, histone modification, chromatin remodeling, and activity of noncoding RNAs (reviewed in [139]). The realization of the role of chromatin remodeling and sophisticated epigenetic regulators in various aspects of nervous system development is pervasive, with increasing comprehension and interest among neurobiologists. Typically, chromatin and epigenetic factors are able to establish non-genetic programs such as chromatin structure dynamics, recruitment of specific regulatory elements to gene loci, and targeted shifts in cellular stoichiometry that have great impact on gene expression profiles, say in neural cells. These non-genetic mechanisms are thus capable of directing neurodevelopmental and functional events such as proliferation and survival of neural progenitors, neural fate specification, differentiation of neural cells, and functional integration of neurons [15, 140].

Although comparatively not so many epigenetic studies have been conducted to expound mechanisms involved in development of the OE, a few have elegantly provided strong evidence indicating involvement of DNA/histone modifiers, chromatin remodelers, and microRNAs in orchestrating embryonic- and adult-stages of OE development. In this section, we have put together information from such key studies and other investigations that fit into the frame of epigenetic regulation of OE development.

ATP-Dependent Chromatin Remodelers

ATP-dependent chromatin remodelers are capable of using energy from ATP to re-order the structure of chromatin in the cell nucleus via mobilization of nucleosomes, leading to conversion of condensed chromatin state (heterochromatin) to de-compacted (eurochromatin) state. Normally, the balance between heterochromatin and euchromatin is a key factor to conserve genome stability and a cell's functional integrity; hence, ATP-dependent chromatin modulators are also considered as very influential regulators in the establishment and maintenance of the transcriptomic landscape [140, 141]. Several studies have indicated the importance of such non-covalent chromatin modification in development of the nervous system and imply their dysregulation in some neural disorders [15, 140]. The role of ATP-dependent chromatin remodeling factors in neural development extends to the domains of OE histogenesis. Of keen interest are the emerging roles of the SWI-like ATP-dependent chromatin remodelers: Chromodomain helicase DNA-binding protein 7 (CHD7) and BRG1/BRM-associated factor (BAF) complexes in determining development of the OE.

Chromodomain Helicase DNA-Binding Protein 7

CHD7 is a member of the subclass III of the CHD family proteins and the largest (252.5 kDa) in size of all known members that can form complex protein structures with likely tissue-specific assemblage. Similar to other eight members of its protein family, CHD7 possesses two truncated chromodomains in the N-terminus for methylated histone binding, a centrally placed SNF2-like ATPase/helicase motif for chromatin remodeling, and a DNA-binding domain in its C-terminus. With its SNF2-like helicase/ATPase domain, CHD7 is able to cause conformational stress that remodels chromatin, leading to either activation or repression of gene expression [142, 143]. Arguably, CHD7 seems to be the most extensively investigated protein among its family members, probably due to the interesting clinical consequence of its de novo heterozygote mutation in humans leading to CHARGE syndrome, with cardinal symptoms such as ocular defects (coloboma), heart defects, choanal atresia (nasal cavity malformations), severe growth retardation, genital hypoplasia, and auditory abnormalities [144–148].

Various staining techniques applied to human and mouse embryos have revealed that CHD7 is ubiquitously expressed, with high amounts at an early developmental stage, which then takes a restrictive pattern especially in tissues such as the OE [145, 147, 149–152]. Following the discovery of CHARGE syndrome, various rodent models have been used to study many phenotypic aspects of CHD7 mutation, in an attempt to elucidate the mechanisms involved.

By using human subjects and electro-olfactogram recordings in CHD7 mutant mouse models for CHARGE syndrome, it was shown that CHD7 is expressed in parts of the brain involved in olfaction and the peripheral olfactory tissue during development [149, 151–153]. Hence, loss of CHD7 expression has been strongly linked to impaired sense of smell (hyposmia), although with reduced penetrance in animal models when compared with similar symptoms in humans [152].

In a quest to dissect the mechanistic basis of loss of smell due to CHD7 haploinsufficiency, Layman et al. employed a CHD7 mouse model ($CHD7^{Gt/+}$) heterozygous for a gene-trapped lacZ allele [151]. In wildtypes, they found high expression of CHD7 in OMP-immunonegative proliferating basal NSCs and basal cells with neuronal commitment in the adult OE, whereas with downregulated expression in mOSNs and the OB. Along that logic, it was observed that loss of CHD7 results in considerable reduction in the OE stem cell pool with attendant depletion of the OSN population and an overall distortion the OE ultra histoarchitecture. The reduced number of OSNs may partly explain the hypoplastic OB observed in CHD7 mutants ($CHD7^{Gt/+}$) and rationalizes the importance of OSN projection in OB development alike.

Intriguingly, CHD7 deficiency did not perturb the non-neuronal SUS cell population in the mature OE, implying that CHD7 mostly functions to regulate OSN generation, differentiation, and perhaps regeneration [152]. Mechanisms including (i) keeping specific sites of chromatin open for TF accessibility [154], (ii) recognition of specific patterns of methyl (H3K4me) signatures [155], and (iii) repressive binding of p53 [156] may mediate CHD7-dependent specific modulation of processes like transcription, cell cycle, apoptosis, DNA and histone binding (reviewed in [157]), plausibly being involved in the establishment and maintenance of cell diversity during OE development. In any case, investigating other interaction partners of CHD7 will help deepen our understanding of the exact mechanisms with which it orchestrates OE development.

SWI/SNF (BAF) Complex

A close functional relative of CHD proteins in epigenetic chromatin regulation is the multimeric BAF complex, which belongs to the SWI/SNF family proteins. It is made up of about 15 subunits that can be assembled combinatorially to form various distinct complexes, based on developmental stage demands. These subunits include core proteins such as interchangeable ATPases (Brg1/Brm), two known scaffolding subunits (BAF155 and BAF170), and BAF47 and other variant proteins [158–160]. Like CHD proteins, BAF complexes are able to regulate chromatin structure by using energy from ATP hydrolysis to cause nucleosomal mobility, leading to increased accessibility of TFs to genomic regulatory elements that drive specific gene expression programs during development [160–164].

The BAF complex subunits are highly and dynamically expressed in the developing OE [16], as it is in other neural tissues [15]. From E10.5 onwards, it was found that the BAF155 subunit is expressed in most cell types: oNSCs (GBCs and HBCs), Mash1+ neuronal precursors, Lhx2+ OSNs, and proliferative SUS cells in the developing OE [16]. However, consistent with the expression pattern in embryonic stem cells [165, 166] and cortical neural progenitors [167, 168], BAF170 is downregulated in cells residing in the basal and apical layers of the early embryonic OE while its expression is high in the intermediate layer, which harbors im- and mOSN [16].

By means of Foxg1-driven Cre recombinase, the effect of loss of BAF155 in OE formation has been investigated. At E10.5, BAF155 knockout mutants displayed smaller OP that likely underline an observed thin or small OE at mid embryonic stages. Loss of BAF155 was observed to impair proliferation and maintenance of Pax6+ and Sox2+ oNSCs, leading to depletion of progenitor population in the E10.5 OE, although the early/pioneer OSN population and SUS cell numbers were unaffected. The effect of oNSCs depletion due to BAF155 deletion was noticeable in the OE at E13.5 as neurogenesis was drastically reduced. Also, Ctip2 expression, one of the TFs needed for OSN maturation, was low and hence OMP+ mOSNs were significantly reduced in the E13.5 and E15.5 OE [16]. Normally, mOSNs extend their N-CAM/Tuj+ axons, which form fascicles in the lamina propria and finally project to the OB, as first-order fibers/cranial nerve I (CNI), whereas second-order neurons in the OB project their axons to the olfactory cortex as the lateral olfactory tract [16, 169]. Interestingly, this pathway was observed to be truncated in BAF155 mutants [16], meaning that BAF155 may be essential for axonogenesis of OSN in the OE and further projections to higher brain centers.

Measurements of luciferase activity, via Western blot, from cultured mutant (BAF155cKO_CAG-CreER) oNSCs transfected with a Pax6-dependent reporter plasmid (pCON/P3) revealed that BAF155 is needed for the transcriptional activity of Pax6. To consolidate the synergistic role of both factors in OE neurogenesis and differentiation, double conditional mutants with homozygous loss of BAF155 and heterozygous loss of Pax6 (BAF155fl/fl_Pax6fl/+_FoxG1-Cre) were created. It was observed that the associated OE phenotype (i.e., perturbation of neurogenesis) in BAF155 mutants was exacerbated in the double conditional knockout mutants, which mechanistically implies that loss of BAF155 probably leads to reduction in Pax6 recruitment and targeting at genomic loci, needed for neurogenesis in the OE [16].

On the other hand, BAF170 deletion partially phenocopies BAF155 knockout in terms of regulating maturation of OSN, although much more Ctip2 expression and OMP+ mOSNs were observably lost in the OE of BAF170 mutants at E15.5. Proliferative Sox2+ oNSCs and SUS cells, and Lhx2+ intermediate progenitors were unaffected by the loss of BAF170, although comparably generation of the later cell population in the developing and adult brain is reported to be regulated by BAF170 [16, 167, 168, 170].

The difference in the effect of loss of BAF155 versus BAF170 is explainable in the sense that both may differently be working toward the establishment of some cell or tissue

specificity, as already described in the cortex: where neural progenitor-specific BAF complex (npBAF) has been identified to be functionally distinct from the neuronal BAF complex (nBAF) known to specifically drive progenitor genesis and differentiation of neurons, respectively [171, 172]. Likewise, olfactory neural stem cell BAF complex (onscBAF) and olfactory sensory neuron BAF complex (onBAF) have been identified in the OE that regulate oNSCs and OSNs, respectively, in the embryonic OE ([16]; Fig. 3). This suggests that due to the dynamics of BAF complex in the OE, it is conceivable that its role in neural development may be largely stereotypic and conserved in the entire nervous system.

So, what then is the consequence of complete abolishment of the BAF complex in OE development? Notably, in the absence of the scaffolding BAF subunits BAF155 and BAF170, the whole BAF complex is aberrantly assembled leading to its dissociation and subsequent degradation/abolishment by the ubiquitin-proteasome system [16, 173]. Interestingly, after conditional deletion BAF155 and BAF170 under the control of the Foxg1-Cre recombinase, it was observed that there were no Sox2+ oNSCs and HuCD+/Tuj+/Ctip2+ OSNs present in the OP, hence the OE was mostly not specified. The reduction in the number of these cells in BAF complex-deficient OE was, however, not due to cell death, since caspase immunoreactivity was no different from that observed in control OE. It means that the malformation of the OE in double conditional BAF155/BAF170 knockout mutants is imputable to a lack of whole BAF complex functionality needed to orchestrate cell development in the mouse OE [16].

The function of the BAF complex in OE development may further be indicated by the role played by the BAF complex subunit BAF57 in interacting with MeCP2 and contributing to MeCP2-dependent gene repression programs [174], known to be important in the differentiation and maturation of OSNs in the OE [175, 176].

It is know that the BAF complex is antagonized by the polycomb repressor complex (PRC), which leads to the formation of (repressive) heterochomatin signatures, including trimethylation of H3 at lysine position 27 (H3K27me3) in health or disease [177–179]. PRC has also been reported to be important in limiting neurogenic tendencies of NSCs to pave way for the specification of other cell types like astrocytes during brain development [180].

The expression of PRC 1/2 proteins is highly localized in the nuclei of cultured GBCs and in basal cells of the adult OE [18]. After pharmacological treatment with the chemical GSK343, known to inhibit an essential component of the PCR2 complex, it was observed that in vitro (cultured) and in vivo GBCs rapidly lost their proliferative potential [18], a phenotype reminiscent of BAF complex inactivation in the embryonic OE. It therefore means that despite their opposing functions, the BAF and PRC complexes may play specific unsubstituted roles in OE development that in any case requires some stoichiometric balance of the two for normal morphogenesis and lifelong maintenance of the OE.

Covalent Histone (Chromatin) Modifiers

Post-transcriptional modifications (e.g., acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation) of amino acid residues can occur at the N-terminus of histone tails which can result in local or regional chromatin changes [181–186].

These covalent modifications of histone tails serve as docking signals and/or sites for the recruitment of other epigenetic chromatin remodelers and TFs. They can thus act as switches for altering chromatin architecture (i.e., heterochromatin-euchromatin state). In effect, histone

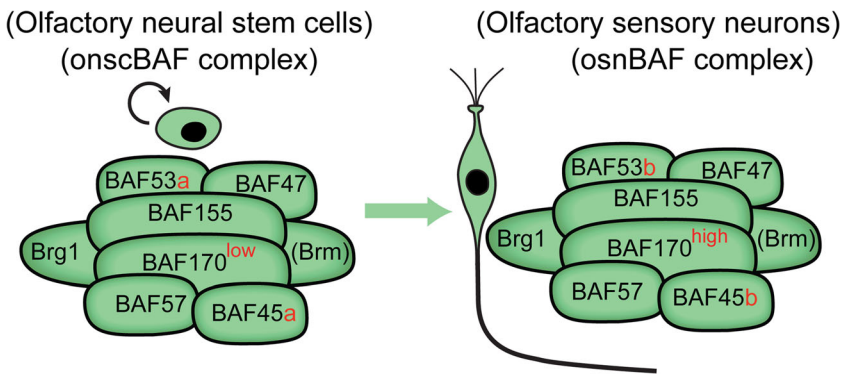

Fig. 3 Olfactory epithelium specific assembly and function of BAF complexes during OE development, specific BAF complex in olfactory neural stem cells (onscBAF complex) contain BAF53a together with other core and variant subunits but with low expression level of the scaffolding subunit BAF170. Transition/differentiation of olfactory neural stem cells to sensory neurons however require replacement of BAF53a with BAF53b, additional incorporation of another subunit BAF47, and high expression of BAF170 leading to the formation of a olfactory sensory neuron-specific BAF complex (osnBAF complex). Slightly modified with permission from Fig. 1e in [16]

modifications affect the accessibility of TFs to their binding sites, thereby modulating gene expression.

Typically, in order to ensure the systematic generation of cells during neural development, histones at or around various neuronal and non-neuronal gene promoters are modified on demand and probably under the control of other subtle control mechanisms. Perhaps the most investigated histone modification mechanism is the addition (methylation) and removal (demethylation) of methyl groups to lysine residues of histone tails. Mono-, di, and tri-methylated forms of lysine residues are achieved by specific enzymes called methyltransferases, whereas specific opposing enzymes, demethylases, are capable of removing such repressive methyl marks in a regulated manner [187].

The role of histone modifiers in OE development has not been extensively investigated. So far, most studies have focused on the function of the histone-modifying enzyme, lysine-specific demethylase 1 (LSD1), a nuclear amino oxidase homolog, which removes only mono- or dimethyl marks on H3 at lysine positions 4 (H3K4me1/2) and 9 (H3K9me1/2) [188]. Normally, LSD1-driven demethylation of H3K4me1/2 results in transcription repression, whereas that of H3K9me1/2 leads to transcription activation, the choice of which may depend on its interacting co-regulators [188–192]. One of the early studies to investigate the role of LSD1 in neural tissue reported its importance in regulating proliferation of neural stem cells [193]. Following this finding, it has been shown that LSD1 is crucial for oNSC proliferation, OSN maturation, and olfactory receptor (OR) gene expression [194, 195]. By using various molecular and cellular probes, it was found that LSD1 expression is strongest in GBCs compared to other OE cells, indicating an early action of LSD1 during OE development [194–197]. Consistent with this, by using an OP-derived immortalized cell line, it was apparent that LSD1 expression is under the control of the cell cycle with highest occurrences in early G1 phase [197].

Deletion of LSD1 in mice using various Cre lines indicates that early loss of LSD1 via Foxg1-Cre activity perturbs OSN differentiation and OR expression, a phenotype which could not be established in late-acting MOR28- and OMP-Cre drivers in mature OSNs [194]. In order to study the effect of LSD1 in the adult OE, tamoxifen-induced Cre activation strategy was employed to knockout LSD1 in specific populations of adult OE basal progenitor cells. In support of previous studies, it was found that LSD1 expression is mainly found in early mitotic OE cells and occurs well ahead of OR expression and OSN maturation [195]. Interestingly, co-repressor for repressor element 1 silencing transcription factor (CoREST), histone deacetylase 2 (HDAC2), and Lhx2, a TF involved in OR regulation, were found to be interacting partners of LSD1 [195, 197]. Putatively, these factors may mechanistically interact with LSD1 as part of the molecular machinery driving the generation of OSN and their typical expression of a single specified OR gene to attain a fully functional mature state.

Methyltransferases that act in opposition to demethylases like LSD1 have also been identified to play critical role in OE development. Generally, they are involved in the formation of constitutive heterochromatin known to cause silencing of pericentromeric and telomeric repeats, independent of cell cycle and differentiation processes [198]. In the mouse OE, constitutive heterochromatin marks like H3K9me3 and H4K20me3 dynamically label OR genes to permit a singular and stochastic choice for their monogenic and monoallelic expression [198].

Another study specifically showed that the methyltransferases G9a (KMT1C) and Glp (KMT1D) are indispensable in setting OSN nuclear topology to allow selection and subsequent expression of OR genes [199]. In this study, it was observed that the overall olfactory neuron transcriptome complexity and the phenomenal one-OSN-one-OR expression rule is distorted in the absence of G9a and Glp, and cell diversification in the OE seemed to be strongly dependent on the dual activities of G9a and Glp in a dose-dependent manner. Mechanistically, it has been proposed that after G9a and Glp generate the repressive gene silencing mark H3K9me3 on all OR genes, LSD1 comes into play to randomly demethylate H3K9 on a single selected OR allele to allow its expression among the lot [199].

The unknown role of other existing histone modifiers in OE development provokes further studies to deepen our understanding thereof. For example, how is the crosstalk (if any) between histone demethylases other than LSD1, say those that belong to the JmjC-domain-containing family (e.g., KDM6 A and B), and histone acetylases or deacetylases, resolved to establish the right epigenetic landscape that ensures proper OE development? Dissecting these possible epigenetic interrelationships should contribute to elucidating how histone modifications precisely regulate OE formation.

DNA Methylation

DNA methylation is an important cellular event that is indispensable for embryogenesis and normal development; its dysregulation has been implicated in various neurodevelopmental disturbances [200–203]. Generally, it is considered as a stable epigenetic mark established through the transfer of a methyl group (-CH3) to the C5 position of the cytosine residues of DNA [204].

Classically, the process is linked to repressive (condensed) heterochromatin state and silencing of proximal promoter activity through inhibition of some TFs and/or via the recruitment of methyl-CpG-binding proteins (MBPs) [205]. However, methylation of distal promoter regions is also possible, although it atypically leads to profound augmentation of

some specific gene expression patterns [206]. In any case, because promoter silencing is a common consequence of DNA methylation, it has been implicated in playing a key role in regulating cell-type-specific gene expression.

Enzymes called DNA methyltransferases (DNMTs) mediate the process of DNA methylation [207]. Four DNMTs with a common conserved domain have been identified in mammals. Among them, DNMT1 (the founding member) is said to maintain DNA methylation during replication [208]. Whereas DNMT3a and DNMT3b are in charge of de novo methylation via targeting of unmethylated CpG sites [200].

DNMTs are well expressed in most neural cells [209, 210]; for this reason, DNA methylation has been reported to play significant roles in the epigenetic regulation of nervous system development, including self-renewal and differentiation of NSCs (reviewed in [211]).

During development of the OE, specific DNA methylation schemes are known to sculpt the epigenetic landscape therein to allow development of OSNs through cell-type restriction of gene expression [212]. MacDonald and colleagues identified induction and expression of DNMT1, DNMT3a, and DNMT3b at specific developmental stages in OSN lineage that are synchronous with changes in developmental gene expression. They reported that DNMT1 expression is induced in proliferating OE progenitors and maintained after their exit from the cell cycle as post-mitotic OSNs. Also, while DNMT3a expression is limited to dividing OE progenitors, expression of its isoform, DNMT3b, is restricted to post-mitotic imOSNs, before they assume terminal maturation. It was found that the expression pattern of the latter paralleled that of the HDAC2, which is critically involved in methylation-dependent heterochromatin formation [212]. Given this unique developmental stage-specific expression pattern of DNMTs in olfactory neuron lineage, it was suggested that DNA methylation may provide progressive lineage restrictions through regulating gene expression programs in establishing OSN cell lineage in the OE [212].

Through in vitro treatment of rodent and human OE stem cells with procainamide, a specific chemical inhibitor of DNMT1, the function of DNMT1 in maintaining stemness or proliferative capacity of OE progenitors was recapitulated. Loss of DNMT1 seems to abolish the differentiative restrictions in OE progenitors like GBCs and HBCs created by related DNA methylation schemes. Hence, increase in neuronal differentiation/maturation was observed in OE progenitors pharmacologically treated with the said DNMT1 inhibitor [213].

Ordinarily, during differentiation, DNMT3a is known to play key function in reorganizing DNA modification patterns in the neuronal genome. In relation to that, deletion of DNMT3a globally distorts gene expression through the de-repression of silenced genes and concomitant decrease in mOSN-expressed transcripts including those whose activation is triggered by odorants [214]. In effect, knockout of DNMT3a disables odorant-dependent gene activation in OSN mostly as a result of disruption in DNA modification state and related inducibility of gene expression patterns in olfactory receptor neurons in the OE [214].

Normally, DNMT catalyzes methylation of DNA through the mediation of MBDs known to in turn recruit HDACs to ultimately silence DNA during development [205]. The MBD proteins methyl-CpG-binding domain protein 2 (MBD2) and MeCP2 regulate discrete transitional stages of OSN differentiation [176]. Knockout of MBD2 in OE progenitors enhanced their proliferative capacity and reduced survival of their (MBD2 null) OSN derivatives. However, MeCP2-deficient OSNs lose their capacity to progressively mature but temporarily halt at terminal differentiation stage with aberrantly sustained expression of the imOSN marker Gap43, despite having initiated the expression of mOSN genes. Unlike embryonic stages, GAP43 promoter is heavily methylated in the mature OE and so signifies the role of DNA methylation in regulating OSN differentiation in the developing and adult OE [176]. MBD2 and MeCP2 may thus sequentially regulate the transition of imOSNs from nascent to fully mature and functional stage [176], hence making them essential for OSN functional maturation in the OE, at least per the evidential requirement of MeCP2 in activity-dependent refinement of olfactory circuitry [175].

Micro RNAs

Micro RNAs (miRNAs or miRs) are endogenously formed small non-coding RNAs, made up of ~20–22 nucleotides and capable of binding to their complementary mRNA transcripts. That way, miRNAs are able to cause mRNA instability/decay and ultimately suppress translation [215–217]. They are thus considered as powerful epigenetic regulators of gene expression.

The emerging roles of miRNAs in the development and function of various tissues in the body has intrigued biologists since its relatively recent discovery. Notably, they are reported to regulate development of the nervous system through modulation of processes like NSC proliferation and differentiation, cell death and survival of neurons, neural patterning, and connectivity of mature neurons [17, 218–224].

Realizing their phenomenal roles in the development of various sensory receptors in invertebrates [225–228], Choi and colleagues [17] asked whether miRNAs are also involved in the generation of OSN in the OE and development of the olfactory system. Indeed, after experimental analyses, they found a repertoire of miRNAs expressed in mouse tissues, with regional enrichments that included the embryonic and mature olfactory system.

Specifically, and of interest in this review, miRNA expression (including miR-9 and -200) is markedly detectable in the

neuroepithelium of the OE proper [17, 229, 230]. Strikingly, at embryonic stages in mouse, the predominant expression of miR-200 family members is already noticeable in the OE at the placodal stage (E9.5), with persistent expression in the posterodorsal part of the OE primordium at E11.5 and further into E13.5 where the expression of miR-200b is of even distribution throughout the OE but not detected in the supporting cell layer. In adult OE, the expression of miR-200 family members is limited to the intermediate cell layer, containing im- and mOSN but absent in the basal cells and apical cells [17].

By downregulating miR-9 and -200 via anti miR-morpholino oligonucleotide injection [17, 230], and conditional inactivation of Dicer (the enzyme for functional miRNA processing) in oNSCs and mOSNs under the control of Foxg1 and OMP promoters, respectively [17, 231], it has been revealed that both miR-9 and 200-class are required for OSN differentiation. While miR-9 and -200 inhibition by morpholino injection caused olfactory placode disorganization and defective OSN fiber trajectory/targeting, that caused by Dicer deletion impaired differentiation and survival of progenitors in the OE. However, loss of miR-9 leads to a less severe OE phenotype compared to lack of the miR-200 family, which is strongly linked to aberrant differentiation of oNSCs into mOSNs and increased apoptosis [17, 230]. Probably, the loss of miR-9 and -200 may disturb pro-apoptotic miRNAs together with other apoptosis-associated factors like caspase and p53 that normally cooperate to effect mammalian NSC differentiation [232].

Since key microRNAs, including miR-9 and 200, are significantly reduced in mice null for the distal-less homeobox 5 (Dlx5) gene, but with concomitant increase in Foxg1, it has been proposed that, mechanistically, a putative Dlx5-Foxg1 pathway mediated by miR-9 and -200 may exist in regulating OE development—especially since Dlx5 seems to be important for differentiation, axon trajectory, and connectivity of OSN [230, 233]. Choi et al. [17] have further posited that Notch and TGFβ signaling cascades and Foxg1 are plausible regulatory targets of the miR-200 family in OE development.

It has also been show that the polycistronic miRNA gene that encodes for the miR-183 cluster (miR-183/96/182) is important for the development of sensory receptor neurons including OSNs [234]. Although overall tissue architecture and imOSN number were not significantly perturbed, the mOSN population was more than halved (~ 60% reduction) in the OE of miR-183 cluster knockout mice, leading to thinning of the OE. Phenotypic details of the miR-183 cluster mutant OE included compact and irregular OSN morphology and reduced density of dendritic knobs, attributable to olfactory ciliopathy that is most likely stemming from temporal dysregulation of mechanisms of gene network interaction, including chromatin remodeling during terminal differentiation of OSNs [234].

Conclusion and Future Perspectives

The OE has been a favorite neuroepithelial tissue used to investigate many aspects of neural development, reminiscent of what does occur in more complex neural structures like the spinal cord and brain. With its heterogeneous cell population, being dominated by olfactory sensory neurons, the embryonic and adult OE is able to establish, maintain, and coordinate a regulatory microenvironment with a plethora of interconnected molecular events geared toward formation and maintenance of structural and functional tissue integrity.

Key among these regulators are TFs, epigenetic chromatin, histone and DNA-modifying factors, and non-coding RNAs. Many studies in the development and regeneration of the OE have unraveled numerous TFs that are important for the establishment and sustenance of cell diversity and ultimate physiology of the OE. Most of these TFs exhibit a phenomenal stepwise activity, with one setting the appropriate developmental milieu for its downstream counterpart(s) to act properly. For instance, sequential expression of Sox2/Pax6, Mash1, Ngn1/NeuroD1, and OMP is required for oNSC proliferation, neuronal fate commitment, differentiation, and maturation respectively. However, this is not a unidirectional regulatory developmental cascade as it may appear. There are other critical collateral regulatory factors that are recruited to finely modulate (positively or antagonistically) such developmental programs. For example, whereas some TFs like Hes1 limit neuronal commitment in favor of non-neuronal cells like SUS cells (Fig. 2), many other TFs have unclear or unidentified functions in OE genesis.

Epigenetic control has emerged as another powerful level of regulating OE development. Being capable of controlling the transcriptome, epigenetic factors like chromatin remodelers [16, 195] and microRNAs [17, 230] seem to exert robust effects on gene expression patterns, leading to demonstrable phenotypes in the developing and adult OE. However, we still do not fully understand how specific epigenetic regulators interact with TFs and signaling pathways to orchestrate OE development.

Given that till date the knowledge on the precise role and mechanism of operation of most OE regulatory factors is incomplete, the challenge persists in completing our understanding of the control mechanisms in the proliferation, differentiation, maturation, and functional integration of cell types in the OE and the olfactory system at large. The application of more targeted genetic tools, such as gene editing techniques and single cell probes, promise disentanglement of the transcriptional and epigenetic regulatory networks involved in OE formation; hence, consolidating our current understanding of OE development and regeneration that potentially have bearing on efforts to understand developmental dynamics in the entire nervous system and in designing plausible clinical interventions for neurological perturbations [235].

Acknowledgments We apologize to colleagues whose work was not cited due to unintentional oversight. We thank H. Sebesse for preparing illustrations. This work was supported by the Research Program at the Faculty of Medicine, Georg-August University Göttingen, TU432/1-1, TU432/1-3 DFG grants, DFG-CNMPB, and Schram-Stiftung to TT. The authors declare no competing financial interests.

Author Contributions GS, EA, JFS, and TT all contributed to writing and editing the manuscript.

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