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Crosstalk among electrical activity, trophic factors and morphogenetic proteins in the regulation of neurotransmitter phenotype specification

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ABSTRACT

Morphogenetic proteins are responsible for patterning the embryonic nervous system by enabling cell proliferation that will populate all the neural structures and by specifying neural progenitors that imprint different identities in differentiating neurons. The adoption of specific neurotransmitter phenotypes is crucial for the progression of neuronal differentiation, enabling neurons to connect with each other and with target tissues. Preliminary neurotransmitter specification originates from morphogen-driven neural progenitor specification through the combinatorial expression of transcription factors according to morphogen concentration gradients, which progressively restrict the identity that born neurons adopt. However, neurotransmitter phenotype is not immutable, instead trophic factors released from target tissues and environmental stimuli change expression of neurotransmitter-synthesizing enzymes and specific vesicular transporters modifying neuronal neurotransmitter identity. Here we review studies identifying the mechanisms of catecholaminergic, GABAergic, glutamatergic, cholinergic and serotonergic early specification and of the plasticity of these neurotransmitter phenotypes during development and in the adult nervous system. The emergence of spontaneous electrical activity in developing neurons recruits morphogenetic proteins in the process of neurotransmitter phenotype plasticity, which ultimately equips the nervous system and the whole organism with adaptability for optimal performance in a changing environment.

1. Introduction

The genesis of a neuron starts with the neural progenitor exiting the cell cycle followed by the first phases of neuronal differentiation and the specialization of the newborn neuron. For a long time it was believed that the neurotransmitter phenotype was predetermined with the specification of the neural progenitor and that this fate was sealed and unique, meaning that the neuron born from the specified progenitor will permanently express a specific and single neurotransmitter phenotype. However, many studies spanning through the last decades have challenged these dogmas, demonstrating that neurotransmitter phenotypes may be multiple for a single neuron and that the identity of these phenotypes may change developmentally and upon changes of the intrinsic and extrinsic environments through adulthood (Spitzer, 2012, 2015).

The specialization of neural progenitors consists in the combinatorial expression of a specific set of transcription factors which will control expression of target genes related to the identity of the developing neuron, including genes associated with neurotransmitter phenotype. The expression of a specific neurotransmitter identity in the differentiating neuron depends on the transcriptional regulation of the biosynthetic and release machinery necessary for implementing the specific transmission in the chemical synapse. However, progenitor cells and developing neurons are sensitive to a myriad of signaling mechanisms that are spatiotemporally dynamic and may add to the genetic program triggered in progenitors, intercept it or even switch it.

Here we review studies in diverse species ranging from zebrafish and *Xenopus* to mice and rats that identify the mechanisms of neurotransmitter specification through neural progenitor specialization and neuronal differentiation with particular emphasis on the findings that demonstrate that acquisition of neurotransmitter identity is plastic and subjected to dynamic changes. We focused on classical neurotransmitters: acetylcholine, biogenic amines and the amino acid transmitters.

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The review is centered on the role of morphogenetic proteins and trophic factors in the specification of neurotransmitter identity and their interaction with electrical activity when mediating the changes in neurotransmitter phenotype.

1.1. Catecholaminergic phenotype

1.1.1. Preliminary specification

Expression of the noradrenergic and dopaminergic phenotypes starts with the recruitment of specialized progenitors. The sympathetic noradrenergic neurons originate from neural crest-derived progenitors that become fate-restricted mostly by bone morphogenetic protein (BMP) signal (Howard, 2005). Transcription factors necessary for the expression of dopaminergic and noradrenergic phenotypes include Mash1, Phox2a, Phox2b, Hand2 and Gata2/Gata3 (Stanke et al., 1999; Goridis and Rohrer, 2002). Regulatory regions in genes encoding the biosynthetic enzymes for catecholamines, tyrosine hydroxylase (TH) and dopamine β-hydroxylase, contain binding sites for these transcription factors. Alternatively, some of them are upstream of those transcription factors that bind to the neurotransmitter identity target genes like neurotransmitter biosynthetic enzymes and vesicular transporters, becoming necessary for the expression of the catecholaminergic phenotype. For instance, BMP2 supports the persistent expression of Mash1 in neural progenitors from the fetal rat gut (Lo et al., 1997), and Mash1, in turn, promotes the expression of proneuronal genes, but Mash1 expression terminates after neuronal differentiation when other transcription factors take over to promote expression of noradrenergic and dopaminergic phenotypes (Lo et al., 1999, 2002; Goridis and Rohrer, 2002).

In the central nervous system, another morphogenetic protein, Wnt, specifically regulates the number of progenitors specified for the dopaminergic phenotype of diencephalic neurons, early during neural ectoderm patterning in zebrafish (Russek-Blum et al., 2008). Wnt activity restricts the number of dopaminergic neurons in the developing diencephalon by negatively regulating expression of the transcription factor Fezl (Russek-Blum et al., 2008), which in turn regulates the development of monoaminergic neurons (Levkowitz et al., 2003). In zebrafish forebrain and anterior hindbrain catecholaminergic neuron specification depends strongly on Nodal signaling and to a lesser extent on Sonic hedgehog (Shh) and fibroblast growth factor (FGF) 8 (Guo et al., 1999; Holzschuh et al., 2003). Specification of midbrain dopaminergic neurons is strongly regulated by Shh signaling through the transcriptional regulation of target genes at different stages of these neurons' development (Abeliovich and Hammond, 2007). The recruited transcription factors include the canonical Shh pathway effectors Gli, which are required for neural progenitor proliferation (Zervas et al., 2004) and the expression of Phox2a (Blaess et al., 2006) as demonstrated in the developing mouse midbrain. Lmx1a is necessary for chick and mouse midbrain dopaminergic neuron specification and its expression is also dependent on Shh signaling (Andersson et al., 2006). On the other developmental end, expression of Nurr1 also mediated by Shh in postmitotic midbrain precursors induces TH expression allowing for the maturation of the midbrain dopaminergic phenotype (Wallen and Perlmann, 2003). Many other transcription factors contribute to the specification of the midbrain dopaminergic phenotype and it is through the complex interaction among these factors that the mature phenotype is established (Abeliovich and Hammond, 2007; Panman et al., 2011).

1.1.2. Plasticity

The catecholaminergic phenotype has become a classic paradigm for the switch in neurotransmitter identity. Noradrenergic sympathetic axons innervating the rat sweat glands experience a switch to the cholinergic phenotype when they reach their target (Landis and Keefe, 1983; Schotzinger and Landis, 1990; Francis and Landis, 1999). This target-dependent switch in neurotransmitter phenotype is dependent on the expression of gp130 receptor in mouse sympathetic neurons and cytokine release from the sweat glands (Stanke et al., 2006). The transcriptional mechanism of this switch involves the upregulation of the expression of Satb2, chromatin architecture protein, when noradrenergic nerves contact the rat sweat glands, which in turn binds to responsive elements in the choline acetyltransferase (ChAT) locus becoming necessary and sufficient for the switch from noradrenergic to cholinergic phenotype (Apostolova et al., 2010). Moreover, Satb2 expression is regulated by the mitogen-activated protein kinase p38α/β activity, which is necessary for the upregulation of the cholinergic phenotype in noradrenergic sympathetic rat neurons grown *in vitro* and in mice *in vivo* (Loy et al., 2011). The participation of p38 both in neurotransmitter phenotype switching (Loy et al., 2011) and activity-dependent synaptic plasticity (Thomas and Huganir, 2004) poses it as a key signaling factor in the integration of short-term transcription-independent and long-term transcription-dependent plastic responses to the changing environment, challenging the presumed rigidity of the early neurotransmitter phenotype specification.

Indeed, the role of electrical activity in the specification of the catecholaminergic phenotype manifests in the central nervous system. A change in environmental stimuli changes the number of neurons expressing the dopaminergic phenotype of the ventral suprachiasmatic nucleus in *Xenopus* larva (Dulcis and Spitzer, 2008) and in the adult rat brain (Dulcis et al., 2013), suggesting a universal mechanism. In the amphibian the switch to the dopaminergic trait results in the expression of a dual neurotransmitter phenotype, NPY- and TH-expressing neurons (Dulcis and Spitzer, 2008). In the rat the increase in number of dopaminergic neurons happens at the expense of the decrease in the somatostatin phenotype (Dulcis et al., 2013).

The role of morphogens in contributing to the neurotransmitter phenotype specification continues beyond morphogenesis and early patterning. The transcription factors Engrailed 1 and 2 necessary for the midbrain dopaminergic phenotype are first expressed during mouse brain patterning by the action of FGF8, and then they induce expression of this morphogen to maintain the dopaminergic phenotype in already differentiated neurons (Alberi et al., 2004; Simon et al., 2004). Also, BMP4 induces the dopaminergic phenotype in cultured GABAergic neurons derived from the mouse cortical striatum during a sensitive period *in vitro* (Stull et al., 2001). Considering that BMPs (Swapna and Borodinsky, 2012), Shh (Belgacem and Borodinsky, 2011), Wnts (Varela-Nallar et al., 2010) and FGF modulate Ca^{2+} dynamics and kinase activity in developing neurons together with the electrical activity-dependent plasticity of neurotransmitter phenotype, the potential role of morphogenetic proteins in postmitotic neurons participating in neurotransmitters respecification through Ca^{2+}-mediated signaling becomes apparent.

1.2. GABAergic/Glutamatergic phenotypes

1.2.1. Preliminary specification

GABAergic interneurons represent a diverse population in the central nervous system. Differentiation of GABAergic phenotypes is thought to be a default fate of differentiating neuronal precursors (Furmanski et al., 2009), which depends on the expression of particular transcription factors. For instance, the Dlx transcription factors promote differentiation of olfactory GABAergic interneurons in mice by regulating the expression of Wnt5a (Paina et al., 2011). Interestingly, the Wnt signaling switches from canonical, β-catenin-mediated, to non-canonical,