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Beyond proneural: emerging functions and regulations of proneural proteins

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Proneural proteins, which include Ascl1, Atoh1 and Neurogenins épinière in vertebrates and Achaete-Scute proteins and Atonal in *Drosophila*, are expressed in the developing nervous system throughout the animal kingdom and have an essential and well-characterised role in specifying the neural identity of progenitors. New properties and additional roles of these factors have emerged in recent years, including the regulation of stem cell proliferation and the capacity to reprogram many types of cells into neurons. This review will focus on these recent findings. The review will also discuss the mechanisms that allow proneural proteins to induce the transcription of their target genes in different chromatin contexts and the phosphorylation events and other post-transcriptional mechanisms that regulate the proneural proteins themselves.

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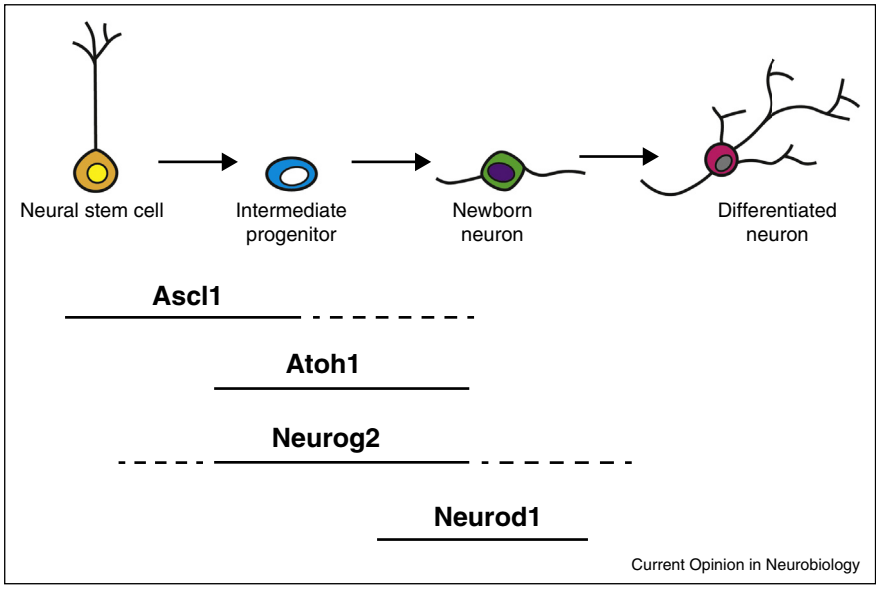
Introduction

Proneural proteins, first identified in *Drosophila* in the 1980s and later in vertebrates and other invertebrate species, are a small group of transcription factors of the basic-loop-helix class, which are necessary and sufficient to confer a neural (i.e. neuronal + glial) or neuronal fate to progenitor cells in the developing nervous system. This is an ancient function conserved through evolution from cnidarians to mammals, although variations exist between phyla regarding the exact stage in development at which these factors are expressed and function. Early expression in multipotent cells and a role in fate specification distinguish proneural proteins from neuronal differentiation factors such as members of the mammalian Neurod family, which are first expressed in cells already committed to a neuronal fate and promote their differentiation [1]. In addition to committing progenitor cells to a neuronal fate, proneural proteins also specify their identity, for example, sense organ identity in flies and the neurotransmission phenotype of mammalian neurons [1]. Although proneural proteins primarily act in progenitor cells, they sometimes remain expressed transiently in postmitotic neurons and regulate their migration and axonal and dendritic growth [2–4]. In this review, we will not discuss these well-established modes of expression and functions. We will focus instead on more recent findings, and specifically on a newly identified function of the proneural protein Ascl1 in neural stem cells, on a role for Ascl1 as pioneer factor, and on phosphorylation events that have recently been shown to greatly contribute to the regulation of the activity of proneural proteins.

Proneural proteins and stem cell proliferation

Although the primary function of proneural proteins is to endow progenitors with a neuronal fate, they also often drive progenitors out of the cell cycle and initiate their differentiation [1]. A few exceptions exist however, including mouse Atoh1 promoting granule cell proliferation during cerebellar development and in medulloblastoma [5,6] and *Drosophila* Asense, which contributes to the self-renewal of embryonic neuroblasts [7]. Ascl1/Mash1 has also been shown to promote the proliferation of neural stem cells and/or progenitor cells in the ganglionic eminences of the embryonic telencephalon and in the neurogenic regions of the adult mouse brain (dentate gyrus and ventricular-subventricular zone) through direct induction of cell cycle regulators such as *Cyclin D* genes [8,9,10••]. In the adult dentate gyrus, Ascl1 expression is restricted to stem cells and early intermediate progenitors and the onset of differentiation and cell cycle exit are induced by other factors including Tbr2 and Neurod1 (Figure 1). Possibly reflecting this proliferation-promoting function, Ascl1 has been implicated in the tumorigenicity of glioblastoma and other tumours [11,12]. Moreover, Ascl1 is expressed in, and might promote the proliferation of, neuronal progenitors derived from parenchymal astrocytes following ischemia, neurotoxic injury or viral-mediated transduction of Sox2 [13,14•,15•,16], suggesting a broader role of Ascl1 in activation of neural stem cells in response to a variety of physiological and pathological stimuli.

Figure 1



Our current understanding of the timing of expression of proneural genes in different lineages. A generic neuronal lineage is represented on the top of the figure. Continuous lines indicate the portion of the lineage when the proneural protein indicated above the line is consistently expressed, while dashed lines indicate expression in some lineages but not others (e.g. Ascl1 is expressed in the embryonic ventral telencephalon in neural stem cells in the ventricular zone, in all intermediate progenitors in the subventricular zone, and transiently in a subset of neurons in the mantle zone, but only in neural stem cells and early intermediate progenitors in the adult dentate gyrus [1,9•]).

Proneural proteins and neuronal reprogramming

The pioneering work of Harold Weintraub established over 25 years ago that forced expression of a single transcription factor can be sufficient to convert a differentiated cell into another cell type [17,18]. Proneural proteins (e.g. Neurog1) and neuronal differentiation factor (e.g. Neurod1) were subsequently shown to have the capacity, when ectopically expressed in embryos, to convert non-neural ectoderm into neurons [19,20]. Magdalena Götz and colleagues extended this finding by showing that forced expression of Ascl1 and Neurog2 converts astrocytes in culture into fully differentiated and functionally mature neurons ([21,22]; Figure 2), and Marius Wernig and colleagues and others showed that Ascl1 in combination with other factors could directly reprogram (i.e. without an intermediate proliferative progenitor state) a variety of cultured cell types originating from mice, humans and other primates, into induced neuronal cells (iNs) [23–28]. Uniquely among proneural factors, Ascl1 alone can reprogram fibroblasts into iNs [29•]. Proneural proteins can also convert pluripotent cells into iNs, and expression of Neurog2 in human ESCs or iPSCs is currently the most efficient strategy to generate homogeneous populations of human neurons with a cortical-like identity [30•].

Given the efficiency of neuronal reprogramming of cultured cells, transcription factors have also been transduced into the mouse brain *in vivo* to circumvent the notoriously limited ability of the mammalian brain to replace lost neurons, by reprogramming glial cells, including parenchymal astrocytes, NG2 glia and retinal Müller glia, into neurons ([31–33]; Figure 3). The cocktail of three transcription factors including Ascl1 that was originally shown to reprogram fibroblasts *in vitro* [23] can also convert resident parenchymal astrocytes or transplanted human astrocytes into neurons [34•]. However, the extent to which Ascl1 alone is able to convert astrocytes in the brain into neurons is disputed, with one study reporting neuronal reprogramming of astrocytes in the midbrain, striatum and cerebral cortex [35], while other studies reported very little or no neuronal reprogramming by transduction of Ascl1 alone into glial cells of the spinal cord or cerebral cortex [13,36,37]. Neurog2 has the capacity to induce the conversion of glial cells into neurons only when cells are both activated by injury and exposed to exogenous growth factors, suggesting that the *in vivo* environment of glial cells limits their lineage plasticity [38•]. In contrast, Neurod1, a neuronal differentiation factor acting downstream of Neurog2 during neurogenesis, can efficiently reprogram on its own reactive astrocytes and NG2 glia into mature neurons [39].

Ascl1 as a pioneer factor

The ability of Ascl1 to convert multiple cell types into neurons suggests that it is able to activate target genes when these genes are not expressed and are actively