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Review

Structures and properties of PAX linked regulatory networks architecting and pacing the emergence of neuronal diversity

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ABSTRACT

Over the past two decades, Pax proteins have received a lot of attention from researchers working on the generation and assembly of neural circuits during vertebrate development. Through tissue or cell based phenotypic analyses, or more recently using genome-wide approaches, they have highlighted the pleiotropic functions of Pax proteins during neurogenesis. This review discusses the wide range of molecular and cellular mechanisms by which these transcription factors control in time and space the number and identity of neurons produced during development. We first focus on the position of Pax proteins within gene regulatory networks that generate patterns of cellular differentiation within the central nervous system. Next, the architecture of Pax-linked regulatory loops that provide a tempo of differentiation to progenitor cells is presented. Finally, we examine the molecular foundations providing a "multitasking" property to Pax proteins. Amongst the Pax factors that are expressed within the developing nervous system, Pax6 is the most extensively studied and thus holds a dominant position in this article.

Contents

1. Introduction

The central nervous system serves as a tissue of choice to reveal the molecular and cellular mechanisms orchestrating vertebrate development. The temporally controlled emergence of patterns of cellular differentiation along the orthogonal axes of the embryo and the extraordinary diversity in the identity of mature cells generated have sparked enthusiasm amongst developmental biologists. This infatuation is also rooted in the fact that the functioning of neural circuits supporting thoughts, senses and movements during adulthood directly correlates with the stereotypical rearrangement and number of neuronal

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subtypes generated during embryogenesis [1–5]. In the light of many studies, the developmental programme guiding neuronal progenitors throughout their differentiation path appears to rely on complex dynamical interplays between a multitude of factors, including transcription factors (TFs), signalling molecules and core components of the cell cycle machinery [1,5,6]. Amongst the regulatory networks formed by these cross-interactions, those centred on the paired domain- and homeobox-containing Pax TFs harbour pivotal instructive properties that coordinate many cellular processes underpinning embryonic neurogenesis.

Most neuronal progenitors will express a Pax protein during the acquisition of their definitive traits and out of the 4 subclasses of Pax proteins indexed in vertebrates [7], only the Pax1 and Pax9 subgroup is excluded from embryonic tissues giving rise to the future central nervous system (Fig. 1) [8–15]. Pax are more likely to be found in cycling progenitor cells, yet a few post-mitotic neuronal populations have been shown to be Pax positive [12,16]. Accordingly, Pax proteins orchestrate various aspects of the neurogenic differentiation programme, ranging from neural commitment [17] to axonal pathfinding [18–21], including tissue patterning [22–24] and growth [25–27], as well as the establishment of neuronal subtype-specific traits [28–30]. Despite this wealth of knowledge of Pax cellular functions, the molecular framework underlying their pleiotropic regulatory activity is just starting to emerge. Based on studies carried out on the developing cortex, retina and spinal cord, we have aimed to describe in this primer the structural and functional features of discrete regulatory networks in which Pax proteins operate. We have focused on those by which Pax activity establishes patterns of gene expression in the developing neural tube or regulates the cycling activity of progenitor cells. We finish by summarising the identified biochemical foundations underlying the pleiotropic nature of Pax activity in neural progenitors.

2. Generation of patterned neuronal cell fates by Pax-centred transcriptional networks

The spatial and temporal determination of cell fates during embryogenesis involves the operation of regulatory networks composed largely of signalling cascades and TFs [6,31–33]. The dynamics in gene activation or repression underlying cell lineage specification constitute an emerging 'novel' property arising from the combination of individual interactions between these network components. Pax proteins are major components of network subcircuits underlying the establishment of functionally distinct territories within the embryonic neural tube [3,5,34–37]. Here we report studies that have shed light upon the structure and/or the information-processing functions of Pax-linked transcriptional circuits that generate and spatially arrange cellular diversity.

2.1. Feed-forward regulatory loops involving several paralogue Pax proteins to establish and maintain patterns of gene expression

One of the gene regulatory networks in which the place of Pax TFs has been well characterised is that governing the establishment of the midbrain–hindbrain boundary (MHB) organiser (Fig. 2A) [38,39]. In this network, all three members of the vertebrate Pax2/5/8 family operate (Fig. 2A), with the exception of Pax8 in frog [40]; providing a first level of functional redundancy to this circuit [41–45]. Pax2 is one of the first network components to appear in the presumptive mesencephalic and metencephalic areas of the neural plate at the gastrulation stage [13]. This induction occurs in response to the transient activity of Pou5f1 (Oct3/4) TF [46,47]. Upon somitogenesis, Pax2 triggers Pax5 and Pax8 expression [48] and the combined activity of the 3 paralogue proteins engages an intricate gene circuit, promoting and stabilising the expression of key determinants of the MHB identity (Fig. 2A) [38,39]. These include the secreted molecule Fgf8 and the TFs En1 & En2 [43,49–52]; the latter being directly regulated by Pax2 [53]. In turn, Fgf8 and En1/2 activity either sustains or further extends the Pax induced-gene expression profiles, including that of *Pax2* (Fig. 2A) [38]. Thus, these feedback loops represent a second level of functional redundancy within the network.

The central position of Pax2/5/8 within this network explains why altering their activity impacts on cell fate decisions. Forced expression of either Pax2 or Pax5 ectopically induces midbrain markers at the expense of the diencephalic ones. The diencephalic brain area then adopts a tectum-like morphology [42,44,52]. Conversely, in zebrafish or mouse mutants, in which the expression of Pax2/5/8 is down-regulated, markers of the anterior midbrain shift posteriorly at the expense of MHB specific genes. The cerebellum and posterior midbrain of these mutants display severe reduction, while the diencephalic and anterior midbrain structures expand caudally [14,41,45,50,54,55].

Finally, the identification and functional analyses of the *cis*-regulatory modules (CRM) in the vicinity of *Pax2*/*5*/*8* gene loci have revealed the existence of a third level of redundancy amongst these modules and their regulators; likely providing robustness to the wiring between network components (Fig. 2B) [39,47,48,56]. Pax2 induction and maintenance is most likely ensured by the combined activity of an incredibly high number (≈60) of evolutionarily conserved CRMs [39]. Amongst them, one in particular stands out, as it recapitulates the early spatial and temporal profiles of Pax2 expression in mouse embryos [47]. Its activity depends on several DNA binding motifs for the Pou and other types of homeodomain TFs and could account for the Pou5f1 dependent induction of Pax2. Pax2 expression is then sustained by the activity of distinct proximal CRMs [47,56]. In one of them, functional Pax recognition sites have been identified, to which only the Pax2/5/8 subtypes can bind [47]. Thus, not only does this enhancer serve as a platform for an auto-regulatory activation of Pax2, but it is also subject to cross-regulatory control by Pax5 and Pax8. Intriguingly, one of the identified *Pax5* CRMs contains highly similar Pax binding sites to that found in the latter *Pax2* enhancer [47,48], despite the usual divergence of Pax binding sites (Fig. 2B) [57,58]. This supports the idea that this regulatory network, including multi-layered feed-forward loops between members of the Pax2/5/8 subfamily, probably arose from the two rounds of whole genome duplication that have occurred during vertebrate evolution [39]. Interestingly, highly similar genomic architecture is also the foundation of *Pax3* expression promotion and refinement in the developing dorsal spinal cord (Fig. 2B) [59]. In both the MHB organiser and the developing spinal cord, the activities of two distinct sets of CRMs segregate the regulation of the induction and maintenance of Pax expression. Inputs from transient cues onto the first set trigger gene expression. This is then converted into a sustained pattern by the second set, which integrates the activity of several paralogue proteins.

2.2. Subdivision of the embryonic brain by reciprocal repressive states induced by Pax6 linked regulatory network

The territorial segmentation of the brain neuroepithelium emerges, in part, in response to reciprocal repressions between Pax6 and several TFs induced in progenitor domains complementary to that of Pax6. The ventral and dorsal domains of optic neuroepithelium are segregated by Pax2 and Pax6, respectively [23]. Similarly, the cross-repressive activity of Pax2/5 and Pax6 promotes the establishment of a neat diencephalon–mesencephalon border [42,44,60–64]. Finally, antagonistic activity of Pax6 and Gsx2 establishes the dorso-ventral telencephalic domains by delineating a pallial–subpallial boundary (Fig. 3A) [24,65–69]. The