MNB/DYRK1A: a multiple regulator of neuronal development

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ABSTRACT

MNB/DYRK1A is a member of the Dual-specificity tyrosine-regulated kinase (DYRK) family that has been strongly conserved across evolution. There is considerable data implicating MNB/DYRK1A in brain development and adult brain function, as well as in neurodegeneration and Down syndrome pathologies. In this article we review our current understanding of the neurodevelopmental activity of MNB/DYRK1A. We discuss how MNB/DYRK1A fulfills several sequential roles in neuronal development and the molecular mechanisms possibly underlying these functions. We also summarize the evidence behind the hypotheses to explain how the imbalance in *MNB/DYRK1A* gene dosage might be implicated in the neurodevelopmental alterations associated with Down syndrome. Finally, we highlight some research directions that may help to clarify the mechanisms and functions of MNB/DYRK1A signaling in the developing brain.

INTRODUCTION

MNB/DYRK1A[$] is a protein kinase that belongs to the Dual-specificity tyrosine-regulated kinase (DYRK) family. MNB/DYRK1A is highly conserved from insects to humans (Galcerán et al, 2003) and it displays characteristic properties that are discussed in detail in one of the three minireviews in this series (Becker and Sippl, 2010).

The evidence from diverse experimental systems has shown various possible functions of MNB/DYRK1A in CNS development including its influence on proliferation, neurogenesis, neuronal differentiation, cell death and synaptic plasticity (see Table 1). These data, together with the localization of the human *MNB/DYRK1A* gene on chromosome 21 (Guimera et al, 1996; Song et al, 1996) and its overexpression in the brain of fetuses with Down syndrome (DS, Trisomy 21) (Guimera et al, 1999), have provided support to several hypotheses implicating MNB/DYRK1A in neurodevelopmental alterations underlying the cognitive deficits of DS (previously reviewed by Hämmerle et al, 2003a and by Dierssen & Martinez de Lagran, 2006). These facts have certainly stimulated and conditioned the research into the neurobiological functions of MNB/DYRK1A. More recently, the observation that MNB/DYRK1A is overexpressed in the adult DS brain (Dowjat et al, 2007), along with biochemical data, also implicated MNB/DYRK1A in various neurodegenerative processes. This issue is extensively covered by Wegiel, Gong and Hwang in the second accompanying paper of this minireview series.

In this review we will focus on the neurodevelopmental functions of MNB/DYRK1A. We will discuss the data revealing the main roles interpreted by MNB/DYRK1A during brain development and their possible molecular mechanisms. Additionally, and given the extensive repertoire of putative substrates and proteins with which the MNB/DYRK1A kinase may interact, we will try to highlight the genes/proteins related to its neurodevelopmental activities. We will also discuss the possible implications of MNB/DYRK1A in the neurodevelopmental alterations associated with Down syndrome. Finally, we will highlight some directions for future research that we think may help to clarify the mechanisms and functions of MNB/DYRK1A signaling in the developing brain.

$ Footnote: Orthologous genes have been cloned independently in various organisms and named *Minibrain (Mnb)* or *Dyrk1A*

THE DIVERSE FUNCTIONS OF MNB/DYRK1A IN NEURONAL DEVELOPMENT

The initial evidence for the involvement of MNB/DYRK1A in neurodevelopment was provided by the analysis of *mnb* mutants of *Drosophila*. These flies develop a smaller adult brain, particularly in the optic lobes, which appears to be caused by altered proliferation in the neuroepithelial primordia of the larval CNS. This phenotype suggests a key function of MNB/DYRK1A in the regulation of neural proliferation and neurogenesis (Tejedor et al, 1995). The highly conserved structure of this kinase (Galceran et al, 2003) prompted extensive studies to be carried out on its vertebrate homologues. Indeed, a smaller brain with fewer neurons in certain regions was described in haploinsufficient *Dyrk1A* +/- mice (Fotaki et al, 2002), strongly suggesting an evolutionary conserved function of MNB/DYRK1A in brain development. This idea is also supported by the fact that truncation of the human *MNB/DYRK1A* gene causes microcephaly (Moeller et al, 2008).

Although in mammals, *Mnb/Dyrk1A* is expressed in most adult tissues (Guimera et al, 1999, Okui et al, 1999), its expression seems to be prevalent during embryonic brain development and it gradually decreases during postnatal periods to reach low levels in the adult (Okui et al, 1999; Hammerle et al, 2008). *Mnb/Dyrk1A* is specifically expressed in four sequential phases during the development of the mouse brain: transient expression in preneurogenic progenitors; cell cycle-regulated expression in neurogenic progenitors; transient expression in recently born neurons; and persistent expression in late differentiating neurons (Hammerle et al, 2008; summarized in Fig. 1). This rather dynamic cellular/temporal expression strongly suggests that MNB/DYRK1A plays several sequential roles in neuronal development, which we shall discuss in this section. These roles seem to be neuron specific since the analysis of the developing *Drosophila* (Colonques, Ceron and Tejedor, unpublished results), chick (Hammerle et al, 2002; *ibid* 2003b) and mouse CNS (Hammerle et al, 2008) show that MNB/DYRK1A expression is restricted to neuronal lineages, although its expression in glia has also been reported in primary cultures (Marti et al, 2003).

Proliferation and neurogenesis

There is strong evidence that *Mnb/Dyrk1A* is transiently expressed during the single cell cycle of preneurogenic chick and mouse embryonic neuroepithelial progenitors that precedes the onset of neurogenesis (Hämmerle et al, 2002; Hämmerle et al, 2008). This expression is of particular interest since *Mnb/Dyrk1A* mRNA is asymmetrically segregated during cell division and it is

inherited by only one of the daughter cells (Hämmerle et al, 2002: Fig. 1). These data, together with its co-expression in preneurogenic mouse neuroepithelia with *Tis21* (Hammerle et al, 2002), an antiproliferative gene that is up-regulated in neural progenitors that make the switch from proliferative to neuron-generating divisions (Iacopetti et al, 1999), suggest that *Mnb/Dyrk1A* may act as a cell determinant of neurogenesis. Accordingly, *Mnb/Dyrk1A* could induce the switch from proliferative to neurogenic cell divisions in neuronal progenitors, a role for which genetic evidence has been obtained in *Drosophila* (Bieri, Colonques and Tejedor, unpublished data). Interestingly, the activity of Pom1p, a MNB/DYRK1A related kinase from *Schizosaccharomyces pombe,* is cell cycle regulated in relation to symmetric growth and division (Bahler and Nurse, 2001). However, *Pom1p* activity is high during symmetric cell division and when lost, cells undergo asymmetric growth and division, the opposite to what appear to occur with MNB/DYRK1A in neural progenitors (Hammerle et al, 2002; Hammerle et al, 2008; Colonques and Tejedor, unpublished data). Moreover, mutants of *mbk-1*, the closest *Mnb/Dyrk1A* related gene in *C. elegans*, do not show neurodevelopmental alterations (Raich et al, 2003). Thus, it seems likely that new functions have been acquired by DYRK kinases during evolution to adapt to the new morphogenetic requirements of complex nervous systems.

MNB⁄DYRK1A in also expressed in neurogenic progenitors in the *Drosophila* larval optic lobe (Colonques and Tejedor, unpublished data) and in the embryonic mouse brain (Hämmerle et al, 2008). Although this expression seems to occur throughout the cell cycle, it is possible that the intensity of *Mnb/Dyrk1A* expression might vary at different cell cycle stages. Indeed, the expression of *Mnb⁄Dyrk1A* can be regulated by E2F1 (Maenz et al, 2008), a transcription factor that plays a key role in the control of cell proliferation. Conversely, there is also evidence that MNB/DYRK1A may participate in the regulation of the cell cycle. For instance, it has been reported that MNB/DYRK1A interacts with SNR1 in *Drosophila* (Kinstrie et al, 2006), a chromatin remodeling factor with a relevant role in cell cycle regulation (Zraly et al, 2004). Interestingly, increased levels of Cyclin B1 have been detected in transgenic mice overexpressing *Mnb⁄Dyrk1A* (Branchi et al, 2004) and it has recently been proposed that MNB/DYRK1A regulates the nuclear export and degradation of Cyclin D1 in neurogenic mouse neuroepithelia (Yabut et al, 2010). There are also indications that MNB/DYRK1A is involved in the mitosis of non-neural cell lines (Funakoshi et al, 2003). These data establish a rather complex scenario with MNB/DYRK1A potentially fulfilling multiple actions in cell cycle regulation for which we have almost no understanding of the molecular details.

Interestingly, important evidence has emerged regarding the role of MNB/DYRK1A in terminating proliferation. Thus, based on the transient co-expression of MNB/DYRK1A with p27KIP1, the main cyclin-dependent kinase inhibitor in the mammalian forebrain (Nguyen et al, 2006), we proposed that MNB/DYRK1A is involved in the developmental signals that control cell cycle exit and early events of neuronal differentiation (Hämmerle et al, 2008). Indeed, it was recently reported that the over-expression of MNB/DYRK1A in the embryonic mouse telencephalon inhibits proliferation and induces premature neuronal differentiation of neural progenitors (Yabut et al, 2010). This gain of function (GOF) was proposed to be driven through Cyclin D1 nuclear export and degradation. Nevertheless, it has still to be proven whether the effect on Cyclin D1 is a direct effect of MNB/DYRK1A or an indirect consequence of cell cycle withdrawal. Thus, confirmation of this mechanism by loss of function (LOF) experiments would be important, especially since MIRK/DYRK1B, the closest homologue of MNB/DYRK1A, enhances Cyclin D1 turnover (Ewton et al, 2003).

Neuronal differentiation

In terms of the possible role of MNB/DYRK1A in early stages of neuronal differentiation, a recent report shows that the interaction and phosphorylation of the intracellular domain of NOTCH by MNB/DYRK1A attenuates NOTCH signaling in transfected neural cell lines (Fernandez-Martinez et al, 2009). NOTCH mediated lateral inhibition is a key mechanism to regulate neuronal differentiation in the vertebrate CNS (reviewed by Louvi and Artavanis-Tsakonas, 2006). During neurogenesis, the cells in which NOTCH signaling is activated remain as progenitors while those in which NOTCH activity diminishes differentiate into neurons. Thus, while the possible effects of MNB/DYRK1A kinase, as well as the underlying molecular mechanisms, need to be assessed in adequate models of the developing CNS, it is tempting to hypothesize that the MNB/DYRK1A kinase may regulate the onset of neuronal differentiation by inhibiting NOTCH signaling.

Another rather interesting possibility is that MNB/DYRK1A influences neuronal differentiation through the transcriptional regulator REST/NRSF. Using genetic approaches, transchromosomic models of DS, embryonic stem cells with partial trisomy 21 and transgenic *Mnb/Dyrk1A* mice, it has been shown that an imbalance in *Mnb/Dyrk1A* dosage perturbs *Rest/Nrsf* levels, altering gene transcription programs of early embryonic development (Canzonetta et al, 2009). REST/NRSF is expressed strongly during early brain development in non-neuronal tissues and in neural progenitors, cells in which it represses fundamental neuronal genes (Chong et al, 1995).

Furthermore, activation of REST/NRSF target genes is both necessary and sufficient for the transition from pluripotent embryonic stem cells to neural progenitor cells, and from these to mature neurons (Ballas et al, 2005). In addition, phosphorylation by MNB/DYRK1A also regulates the transcriptional activity of GLI1 (Mao et al, 2002), a major effector of SHH signaling that is a key pathway in the regulation of proliferation/differentiation during vertebrate CNS development (Ruiz i Altaba et al, 2002).

Given the roles played by MNB/DYRK1A in sequential steps of neurogenesis and its capacity to interact with and/or modulate different signaling pathways (FGF, NGF, SHH, NFAT, etc), it is tempting to hypothesize that MNB/DYRK1A plays a key role in coordinating neural proliferation and neuronal differentiation. Such coordination is crucial for proper brain development since premature differentiation or overproliferation can alter the balance between neuronal populations leading to mental disorders and neuropathologies.

MNB/DYRK1A has also been implicated in various aspects of late neuronal differentiation. Thus, MNB/DYRK1A kinase activity was upregulated in response to bFGF during the differentiation of immortalized hippocampal progenitor cells. Blockade of this upregulation inhibited neurite formation. The mechanism proposed implicates phosphorylation of the transcription factor CREB (Yang et al, 2001). MNB/DYRK1A overexpression also potentiates nerve growth factor (NGF)-mediated neuronal differentiation of PC12 cells by facilitating the formation of a Ras/B-Raf/MEK1 multiprotein complex in a manner independent of MNB/DYRK1A kinase activity (Kelly and Rahmani, 2005). Furthermore, the upregulation of MNB/DYRK1A expression and its translocation to the nucleus precedes the onset of dendrite formation in several differentiating neuronal populations (Hammerle et al, 2003b; Hammerle et al, 2008; see also Fig. 1). Indeed, the number of neurites developed by new born mouse hippocampal pyramidal neurons in culture is diminished when MNB/DYRK1A kinase activity is inhibited (Goeckler et al, 2009), indicating that MNB/DYRK1A kinase activity is required for neurite formation. So far, the mechanisms underlying this role of MNB/DYRK1A remain unclear. In addition, we observed that MNB/DYRK1A concentrates on the apical side of dendrites in differentiating neurons (Hammerle et al, 2003b; Hammerle et al, 2008), suggesting a possible role in dendrite growth. The fact that cortical pyramidal cells from haploinsuffcient *Dyrk1A*$^{+/-}$ mice were considerably smaller and less branched than those of control littermates further supports this idea (Benavides-Piccione et al, 2005).

Although the mechanisms underlying the effects of MNB/DYRK1A in dendritogenesis remain unknown, several possibilities might be considered in future studies. First, a kinome RNAi screen implicated MNB/DYRK1A in the regulation of actin-based protrusions in CNS-derived *Drosophila* cell lines (Liu et al, 2009). Thus, MNB/DYRK1A could be involved in regulating actin dynamics, an important process in the regulation of neuronal morphology. Second, it has been shown that MNB/DYRK1A primes specific sites of MAP1B for GSK3β phosphorylation, an event that seems to be associated with alterations in microtubule stability (Scales et al, 2009). It has also been sown that *Drosophila* MNB interacts with SNR1 (Kinstrie et al, 2006), a member of the SWI/SNF complex, which is involved in the morphogenesis of dendritic arbors in *Drosophila* sensory neurons (Parrish et al, 2006). Moreover, MNB/DYRK1A interacts with INI1 (the SNR1 mammalian orthologue) in transfected neural cell lines (Lepagnol-Bestel et al, 2009). In addition, the MNB/DYRK1A kinase has been shown to be a negative regulator of NFAT signaling (Arron et al, 2006; Gwack et al, 2006), which plays an important role in axonal growth during vertebrate development (Graef et al, 2003). Finally, it is worth mentioning that two known substrates of the MNB/DYRK1A kinase co-localize with MNB/DYRK1A on the apical side of growing dendrites in several groups of neurons (Hammerle et al, 2003b; Hammerle et al, 2008; Sitz et al, 2008): Dynamin 1 (Chen-Hwang et al, 2002; Huang et al, 2004), an important element in membrane trafficking; and SEPT4 (Sitz et al, 2008), a cytoskeletal scaffolding component implicated in neurodegeneration (Kinoshita et al, 1998).

There are also some indications that MNB/DYRK1A might be involved in synaptic funtions. At the molecular level, it has been shown that MNB/DYRK1A binds to, phosphorylates and/or modulates the interaction of several components of the endocytic protein complex machinery such as Amphiphysin, Dynamin1, Endophilin 1 and Synaptojanin 1 (Chen-Hwang et al, 2002; Huang et al, 2004; Adayev et al, 2006; Murakami et al, 2006; Murakami et al, 2009), suggesting that it is involved in synaptic vesicle recycling. Transgenic mice overexpressing *Mnb/Dyrk1A* exhibit altered synaptic plasticity associated to learning and memory defects (Ahn et al, 2006), while haploinsufficient *Dyrk1A* +/- mice have reduced number of spines in the dendrites of cortical pyramidal cells (Benavides-Piccione et al. 2005) and show alterations in the pre- and postsynaptic components of dopaminergic transmission (Martinez de Lagran et al, 2007). Thus, although these phenotypes may be due to changes in synaptic plasticity related to MNB/DYRK1A function in the adult brain, we should not rule out that these phenotypes might reflect impaired synapse formation during development, particularly since dendritogenesis and

synaptogenesis are two processes that are tightly co-ordinated during brain development (Cline, 2001).

Finally, we must stress that although MNB/DYRK1A is widely expressed in the developing CNS, there are clear indications that MNB/DYRK1A does not affect neuronal proliferation/differentiation in all CNS structures. For instance, regional morphological phenotypes have been reported in the brain of *Mnb/Dyrk1A* mutant flies (Tejedor et al, 1995) and mice (Fotaki et al, 2002). Furthermore, the effect of *Mnb/Dyrk1A* LOF and GOF in the developing mouse retina indicates that the main role of MNB/DYRK1A in this tissue may be related to cell death/survival rather than to cell proliferation/differentiation (Laguna et al, 2008).

POSSIBLE IMPLICATIONS OF *MNB/DYRK1A* IN THE NEURODEVELOPMENTAL ALTERATIONS ASSOCIATED WITH DOWN SYNDROME.

The human *MNB/DYRK1A* orthologue was initially localized in the so called DS Critical Region (DSCR) (Guimerá et al., 1996; Song et al, 1996), the minimal region of Chromosome 21 that when triplicated confers most DS phenotypes (Delabar et al, 1993). This finding together with its overexpression in fetuses with DS (Guimera et al., 1999) initially suggested the implication of *MNB/DYRK1A* in a broad range of DS phenotypes. However, a recent more refined genetic analysis of numerous HSA21 segmental trisomies has generated a high-resolution genetic map of DS phenotypes (Korbel et al, 2009). According to this study, there is not a single DSCR but rather different ones for the diverse phenotypic features. Thus, the extra dosage of *MNB/DYRK1A* appears to be associated to a more restricted repertoire of DS phenotypes than previously thought, including mental retardation but excluding congenital heart disease.

The brains of individuals with DS are characterized by their reduced size and a decrease in neuronal density in certain regions (reviewed by Coyle et al, 1986). This neuronal deficit most probably originates through alterations in neurogenesis during development since it is already detected in fetuses and children with DS (Wisniewski et al, 1984; Schmidt-Sidor et al, 1990). Accordingly, altered neural proliferation and neurogenesis have been found in the forebrain of fetuses with DS and in trisomic DS mouse models (Chakrabarti et al, 2007; Contestabile et al, 2007; Guidi et al, 2008).

Based on the previously described functions of MNB/DYRK1A in the transition from proliferation to differentiation during neurogenesis, we predict that overexpression of MNB/DYRK1A in the developing brain of fetuses with DS could contribute to this neuronal deficit in several ways. Firstly, through its role as an asymmetric determinant of neurogenesis, the overexpression of MNB/DYRK1A may cause the precocious onset of neurogenesis in progenitors and the concomitant depletion of the proliferating progenitor pool (Fig. 2). Secondly, due to its role in regulating the cell cycle exit of neurons, the overexpression of MNB/DYRK1A may induce premature cell cycle arrest of neurogenic progenitors leading to a decrease in the number of neurons generated by each progenitor. Thus, the combined effects of impairing these two activities could result in a decrease in the production of neurons (Fig. 2). Considering the effect of MNB/DYRK1A on cell cycle regulators like Cyclin D1 (Yabut et al, 2010), a third possible effect of the overexpression of MNB/DYRK1A might be to modulate the cell cycle of neuronal progenitors. For instance, extended cell cycles have been found in a DS mouse model (Chakrabarti et al, 2007; Contestabile et al, 2007). This may be relevant since neurogenic progenitors have a longer cell cycle than proliferative progenitors, and lengthening cell-cycle could contribute to a switch from proliferative to neurogenic divisions (Calegary et al, 2005). Further work will be required to assess these hypotheses.

Surprisingly, despite all the evidence pointing to various roles of MNB/DYRK1A in neural proliferation, neurogenesis and neuronal differentiation, no strong CNS developmental phenotypes have so far been described for most transgenic mice overexpressing *Mnb/Dyrk1A*. Nevertheless, all these transgenic mice exhibit learning/memory impairments (Smith et al, 1997; Altafaj et al, 2001; Branchi et al, 2004; Ahn et al, 2006). It is possible that moderate increases of MNB/DYRK1A could produce subtle phenotypes that would require a more detailed analysis to detect. However, we should not rule out the possibility that that due to the activities of MNB/DYRK1A in several sequential phases in proliferation/neurogenesis/differentiation, a maintained overexpression in the trangenic mice could result in compensatory phenotypes. Strikingly, the brains of 152F7 mice, which carry a YAC mouse line with three copies of at least two neighboring HSA21 genes in addition to MNB/DYRK1A, are enlarged (Smith et al, 1997; Branchi et al, 2004), a phenotype that apparently contradicts with the expected antiproliferative effect of MNB/DYRK1A (Yabut et al, 2010).

It is also well known that cortical neurons of brains with DS exhibit dendritic shortening or atrophy (reviewed by Kaufman and Moser, 2000). Thus, another developmental process that

could be impaired through the overexpression of MNB/DYRK1A in DS is dendritogenesis. Indeed, cultured cortical neurons of *Mnb/Dyrk1A* transgenic mice exhibit poorer dendrite arborization (Lepagnol-Bestel et al, 2009). Moreover, overexpression of MNB/DYRK1A in *wild type* primary mouse cortical neurons leads to similar changes (Lepagnol-Bestel et al, 2009), strongly suggesting that *MNB/DYRK1A* triploidy can impair dendrite development in DS.

Increased cell death is also associated with DS. For instance, cultured human cortical DS neurons exhibit intracellular oxidative stress and increased apoptosis (Busciglio and Yankner, 1995). Furthermore, increased cell death has been observed in the forebrain of fetuses with DS (Guidi et al, 2008). The involvement of MNB/DYRK1A in the regulation of Caspase 9-mediated apoptosis in differentiating neurons of the developing retina has generated some speculation about the effects of MNB/DYRK1A gene-dosage imbalance in deregulating the apoptotic response in DS (Laguna et al, 2008). However, it seems unlikely that the overexpression of MNB/DYRK1A can contribute to the neuronal deficit of DS by stimulating developmentally regulated cell death since several studies have related increased MNB/DYRK1A levels to anti-apoptotic or cell survival effects rather than to the induction cell death (Chang et al, 2007; Laguna et al, 2008; Guo et al, 2010). As a matter of fact, we recently found that the overexpression of MNB/DYRK1A does not induce cell death during vertebrate CNS neurogenesis (Hammerle and Tejedor, unpublished results).

CONCLUDING REMARKS AND PERSPECTIVES

As already discussed, there is evidence accumulating regarding several key functions performed by MNB/DYRK1A in brain development. Now the major goal is to determine the underlying molecular mechanisms. This is going to be a complex task since there are clear indications that MNB/DYRK1A might act at the crossroads of several signaling pathways, probably helping to integrate various cellular processes (for example, proliferation and differentiation). Thus, we would like to propose some directions for future research that we think will provide insight into these relevant molecular mechanisms.

As summarized in Table I, many proteins have been identified as possible substrates and/or interacting proteins of the MNB/DYRK1A kinase through very diverse approaches, and various signaling pathways have been associated with MNB/DYRK1A. Nevertheless, we know very little about the actual physiological substrates/interacting partners of MNB/DYRK1A in neuronal development. In large, this is due to the fact that most molecular studies have been carried out in

non-neuronal cells. Thus, efforts should be made to address the true specificity of these putative MNB/DYRK1A related proteins in adequate neuronal systems and in suitable functional contexts. Also, given the wide molecular repertoire of substrates (transcription factors, translation factors, cytoskeletal proteins, membrane receptors, regulators of membrane dynamics, etc), it is possible that MNB/DYRK1A kinase could act at several levels in a multifaceted manner, integrating several cellular responses within a given neuronal process. For example, by acting on Cyclin D1 to stop the cell cycle as well as on NOTCH signaling to initiate differentiation, thereby co-ordinating the transition of neuronal precursors from proliferation to differentiation.

In agreement with its capacity to phosphorylate such a wide repertoire of substrates, MNB/DYRK1A also displays a rather varied subcellular distribution during neurodevelopment (Hammerle et al, 2002, 2003b, 2008). Thus, the early literature classified MNB/DYRK1A as a nuclear protein kinase because it contained a bipartite nuclear translocation signal and MNB/DYRK1A-tagged peptides indeed localized in the nucleus of transfected cell lines (Becker et al, 2008). However, immunocytochemical analysis by high resolution confocal microscopy has since shown that the endogenous MNB/DYRK1A protein has a mainly cytoplasmic and perinuclear localization in differentiating mammalian neurons (Hammerle et al, 2008). Nevertheless, MNB/DYRK1A has also been detected in the form of speckles in neuronal nuclei at given developmental stages (Hammerle et al, 2003b; Hammerle et al, 2008). Thus, a working hypothesis is that MNB/DYRK1A is normally concentrated in the perinuclear area and that it translocates into the nucleus to regulate transcription factors in response to certain stimuli. It will therefore be very interesting to study the mechanisms that regulate this translocation process (see also the interesting comments about the distribution of MNB/DYRK1A in the adult mammalian brain in the accompanying review by Weigel et al, 2010).

As previously discussed, there is also compelling evidence for the very precise spatio-temporal regulation of *Mnb/Dyrk1A* expression during brain development (Okui et al, 1999; Hammerle et al, 2002, 2003b, 2008), which appears to be crucial for MNB/DYRK1A function. For example, it has been reported that the transient expression/activation of MNB/DYRK1A induces neuronal differentiation (Yang et al, 2001; Kelly and Rahmani, 2005) but this is impaired by its stable over-expression (Park et al, 2007). Furthermore, it should be noted that the only well known mechanism to activate the MNB/DYRK1A kinase is through a transient Tyr-kinase activity that autophosphorylates tyrosine residues in the activation loop during protein translation (Lochhead,

et al, 2005). This implies that the up-regulation of MNB/DYRK1A kinase can be indirectly controlled by regulating its expression, making the observed transient expression of MNB/DYRK1A in specific neurodevelopmental contexts (Fig. 1) even more relevant functionally. However, only a few molecules have been found to modulate *Mnb/Dyrk1A* gene expression in cell lines (reviewed by Becker and Sippl, 2010, see also Table I) and almost nothing is known about the mechanisms regulating its expression during brain development. Thus, studies in true neurodevelopmental systems will be required to dissect out the mechanisms that actually regulate *Mnb/Dyrk1A* expression and their implication in brain development.

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Table I. Substrates and proteins that interact with MNB/DYRK1A in relation to its neuronal functions

This is a list of proteins that possibly interact with or serve as substrates for the MNB/DYRK1A kinase. Since the spatio-temporal regulation of its expression appears to be critical to understand the MNB/DYRK1A's roles in neuronal development, we have also included possible regulators of *Mnb/Dyrk1A* expression and of MNB/DYRK1A kinase activity. For each protein we show: its main molecular properties, the molecular relationship with MNB/DYRK1A, the experimental system used to define this relationship, the possible function in neuronal development (if any), and the literature showing the relationship to MNB/DYRK1A. This list has been restricted to those genes/proteins for which there is evidence in the literature of a neuronal related activity. Additionally, we highlight (*****) those cases in which there is evidence (or strong indications) that the interaction with MNB/DYRK1A is involved in neuronal functions.

Abbreviations. AD= Alzheimer Disease; Molecular relationship with MNB/DYRK1A: ActR = Regulator of Activity; ExpR= Regulator of Expression; I = Interacting protein; S= Substrate; ($) MNB/DYRK1A kinase primes the phosphorylation of several substrates by GSK3. Experimental system: CultNeu= Cultured neurons; ivCNS= CNS in vivo; NCL= Neural cell line; nNCL= non-Neural cell line. Functions: Dif= Differentiation; Other= Non developmental neuronal function; Prol= Proliferation; Syn= Synapse related; UF= Unknown

Protein or Signalling pathway	**Molecular nature**	**Molecular Relation with MNB/DYRK1A**	**Experimental system**	**Function**	**Literature**
Amphiphysin	Protein associated with the cytoplasmic surface of synaptic vesicles	S	NCL, ivCNS	Syn	Murakami et al. 2006
β-Amyloid	Peptide derived from APP. Main component of amyloid plaques in AD	ExpR	ivCNS, NCL	Other	Kimura et al. 2007
Arip4 (Androgen receptor interacting protein 4)	Steroid hormone receptor cofactor	I	nNCL, CultNeu, ivCNS	UF	Sitz et al. 2004
APP	Amyloid precursor protein	S	nNCL	Other	Ryoo et al, 2008
ASF (Alternative splicing factor)	Splicing factor	S, I	NCL, nNCL	Other	Shi et al. 2008
bFGF	Growth factor	ActR	NCL	Dif	Yang et al. 2001
***** Caspase 9	Cystein aspartyl protease	S	nNCL, ivCNS	Cell death	Seifert et al. 2008, 2009 Laguna et al 2008
***** Cyclin D1	Cell cycle regulator	?	ivCNS, NCL	Prol	Yabut et al. 2010
CREB (cAMP responsive element binding protein)	Transcription factor	S	NCL	Dif	Yang et al. 2001
CRY2 (Chryptochrome2)	Flavoprotein, involved in circadian rhythm	S	nNCL, ivCNS	Other	Kurabayashi et al. 2010
***** DNM1 (Dynamin1)	Cytoplasmic protein, involved in membrane trafficking	S	nNCL, ivCNS	Dif	Chen-Hwang et al. 2002; Huang et al. 2004; Hämmerle et al.

					2003b; Hämmerle et al. 2008
Endophilin 1	Cytoplasmic protein involved in membrane trafficking	I	ivCNS	Syn	Murakami et al. 2009
E2F1	Transcription factor, involved in cell cycle regulation	ExpR	NCL, nNCL	Prol, Dif	Maenz et al. 2008
FKHR (Forkhead in rhabdoyosarcoma,)	Transcription factor	S, I	nNCL	UF	Woods et al. 2001b; v Groote-Bidlingmaier et al. 2003
GLI1 (Glioma – associated oncogene 1)	Transcription factor involved in SHH signaling	S	NCL, nNCL	Prol/Dif	Mao et al. 2002 Morita et al. 2006
* GSK-3 (Glycogen Synthase Kinase 3)	Protein kinase involved in multiple cellular processes	$	nNCL, CultNeu, ivCNS	Dif, other	Woods et al. 2001a; Skurrat and Dietrich, 2004, Morita et al. 2006 ; Scales et al, 2009
Hip1 (Huntingtin interacting protein1)	Accessory protein of the clathrin-mediated endocytosis pathway	S	NCL	Dif	Kang et al. 2005
INI1//SNF5; SNR1	Chromatin modifying proteins	I	NCL, CultNeu, ivCNS	Prol	Kinstrie et al., 2006; Lepagnol-Bestel et al, 2009
* MAP1B	Microtulbule associated protein	S	nNCL, CultNeu	Dif	Scales et al, 2009
NFAT (Nuclear factor of activated T-cells)	Transcription factor	S	ivCNS, NCL	Dif	Arron et al. 2006; Gwack et al. 2006
* Notch	Cell-cell signalling transmembrane receptor protein	S	NCL, nNCL, ivCNS	Prol, Dif	Fernández-Martínez et al. 2009;
NRSF/REST (neuron-restrictive silence factor)	Transcriptional repressor	ExpR	nNCL, ivCNS	Prol/Dif	Canzonetta et al. 2008
PAHX-AP1	Phytanoyl-CoA α-hydroxylase associated protein 1, brain specific protein	I	NCL	UF	Bescond and Rahmani, 2005
Presenilin1	catalytic subunit of γ-secretase	S	NCL, nNCL, ivCNS	UF	Ryu et a. 2010
Ras/ Map Kinase signaling	Transmemenbrane signaling pathway	I	NCL	Dif	Kelly and Rahmani 2005
* SEPT4 (Septin4)	GTPases and cytosketal scafolding protein	S	nNCL, ivCNS	Syn	Sitz et al. 2008
SIRT1	NAD_-dependent protein deacetylase	S	nNCL	Cell death	Guo et al. 2010
* SPRY2 (Sprouty 2)	negative modulator of growth factor-mediated MAPK signaling	S	CultNeu, ivCNS	Prol, Dif	Aranda et al, 2008
STAT3	Signal transducer and activator of transcription	S	nNCL	UF	Matsuo et al. 2001; Wiechmann et al. 2003
SJ1 (synaptojanin1)	phosphoinositide phosphatase	S	ivCNS	Syn	Adajev et al. 2006

α-synuclein	Cytoplasmic proteín, major component of Lewy bodies	S	NCL, ivCNS	Other	Kim et al. 2006
* TAU	Cytoskeletal protein, microtubule associated	S	nNCL, ivCNS	Other	Woods et al. 2001a; Kimura et al.2007; Ryoo et al, 2007
14-3-3	14-3-3 family of regulating proteins	I, ActR	NCL, nNCL	UF	Kim et al, 2004 Alvarez et al 2007

FIGURE LEGENDS

Fig. 1. Schematic representation of the sequential expression of *Mnb/Dyrk1A* during the transition from neural proliferation to neuronal differentiation.
In the vertebrate neuroepithelia, *Mnb/Dyrk1A* mRNA is first transiently expressed in preneurogenic progenitors, before it is asymmetrically segregated during cell division and it is inherited by only one of the daughter progenitor cells, triggering the onset of neurogenic divisions. Its expression is maintained in neurogenic progenitors although at a lower level. Later, *Mnb/Dyrk1A* is also transiently upregulated in postmitotic precursors (newborn neurons) and downregulated as the neuron begins to migrate away form the ventricular zone (VZ). Once the migrating neuron reaches its target position, *Mnb/Dyrk1A* is again expressed and it translocates transiently into the nucleus preceding the onset of dendrite formation. As dendrites begin to grow, MNB/DYRK1A localizes to the apical side of the growing dendrites.

Fig. 2. A working model for the involvement of MNB/DYRK1A overexpression in the neuronal deficit of Down syndrome.
Schematic representation of the pattern of progenitor division and neuronal generation in a normal brain, and the possible consequences that MNB/DYRK1A overexpression might cause during neurogenesis in the DS brain. During normal neurogenesis, the transient expression of *Mnb/Dyrk1A* in preneurogenic progenitors triggers the onset of neurogenic divisions and consequently, the production of neurons. The increase in the level of *Mnb/Dyrk1A* expression in DS may produce the precocious onset of neurogenic progenitors and a concomitant loss of proliferating progenitors, leading to a reduction in the total number of neurogenic lineages. Additionally, the overexpression of MNB/DYRK1A might induce premature cell cycle arrest of neurogenic progenitors leading to a decrease in the number of neurogenic divisions undertaken by each neurogenic progenitor. Thus, the consequences of these alterations in neurogenesis would be a decrease in the production of neurons.

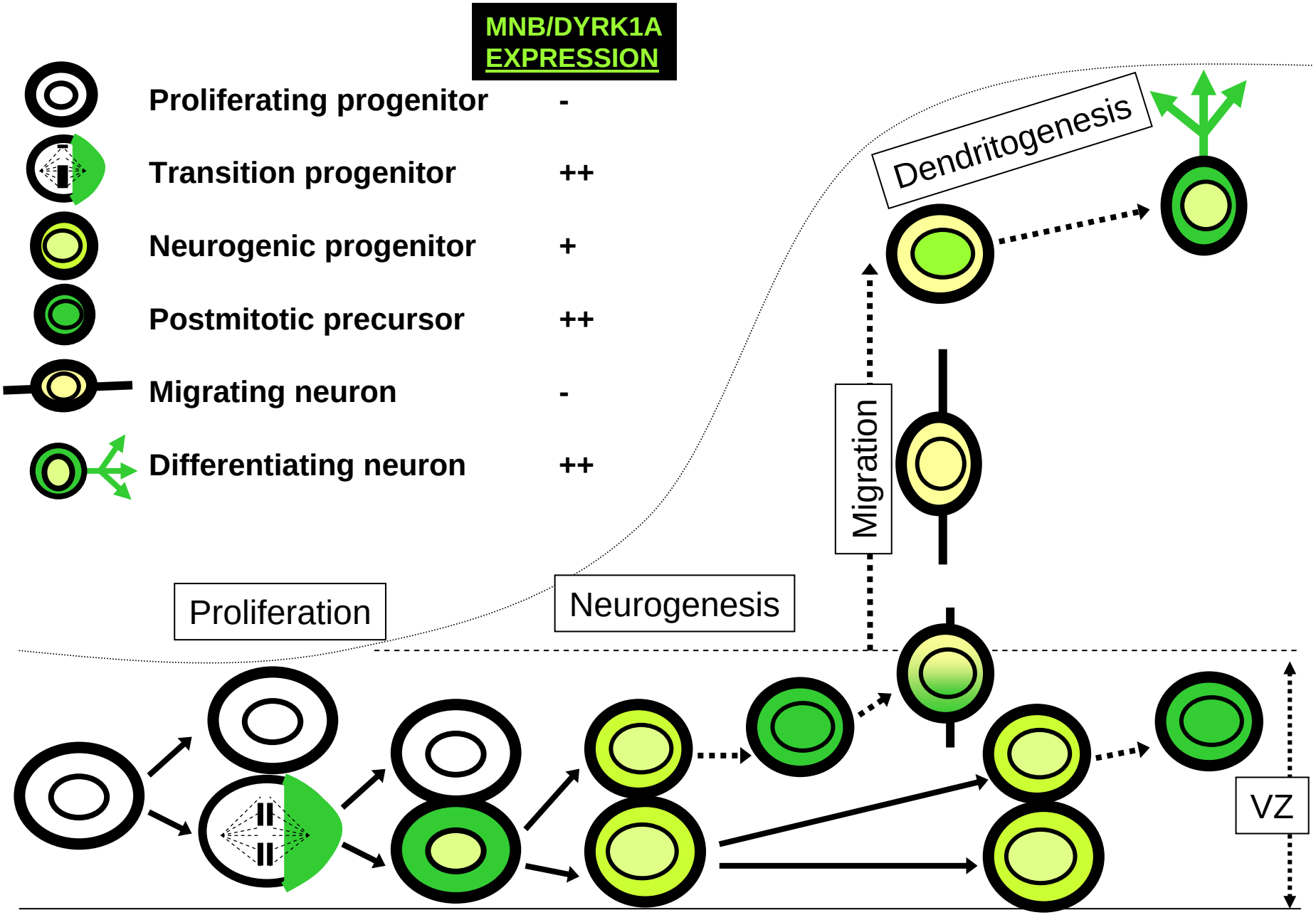
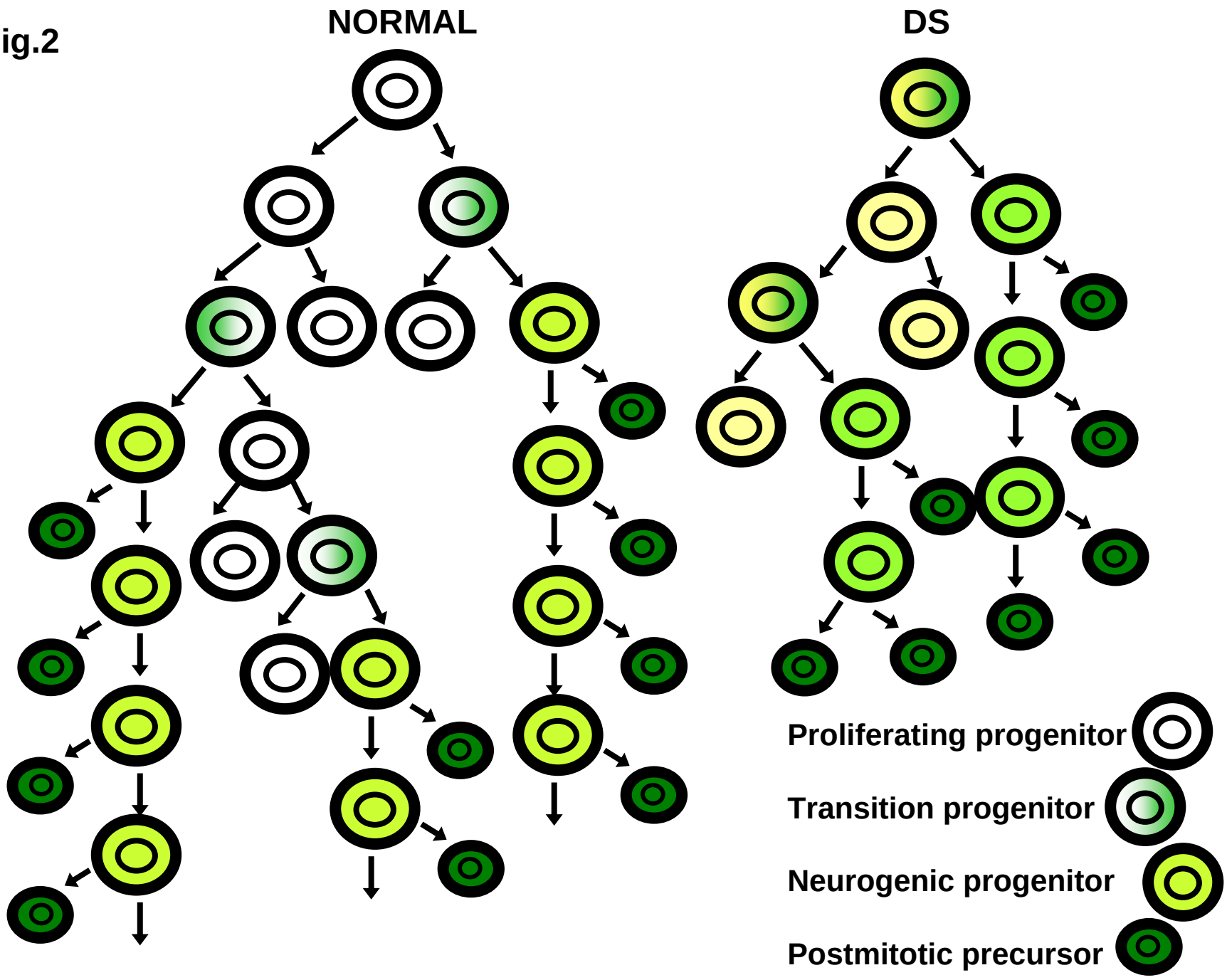

MNB/DYRK1A
EXPRESSION
Proliferating progenitor -
Transition progenitor ++
Neurogenic progenitor +
Postmitotic precursor ++
Migrating neuron -
Differentiating neuron ++
Dendritogenesis
Migration
Proliferation
Neurogenesis
VZ

Fig.1

Fig.2


NORMAL
DS
Proliferating progenitor
Transition progenitor
Neurogenic progenitor
Postmitotic precursor