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DYRK1A and cognition: A lifelong relationship

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ABSTRACT

The dosage of the serine threonine kinase DYRK1A is critical in the central nervous system (CNS) during development and aging. This review analyzes the functions of this kinase by considering its interacting partners and pathways. The role of DYRK1A in controlling the differentiation of prenatal newly formed neurons is presented separately from its role at the pre- and post-synaptic levels in the adult CNS; its effects on synaptic plasticity are also discussed. Because this kinase is positioned at the crossroads of many important processes, genetic dosage errors in this protein produce devastating effects arising from DYRK1A deficiency, such as in MRD7, an autism spectrum disorder, or from DYRK1A excess, such as in Down syndrome. Effects of these errors have been shown in various animal models including *Drosophila*, zebrafish, and mice. Dysregulation of DYRK1A levels also occurs in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Finally, this review describes inhibitors that have been assessed *in vivo*. Accurate targeting of DYRK1A levels in the brain, with either inhibitors or activators, is a future research challenge.

Contents

Abbreviations:: APP, β-amyloid precursor protein; ASF1, alternative splicing factor 1; CGMC, group of kinases: CDK, CDKL, CK2, CLK, DYRK, GSK, MAPK, RCK, SRPK; CREB1, cAMP responsive element-binding protein 1; DREAM, dimerization partner, RB-like, E2F, and multivulval class B; DS, Down syndrome; DYRK1A, dual-specificity tyrosine phosphorylation regulated kinase 1A; EGCG, epigallocatechin gallate; EGFR, epidermal growth factor receptor; EPSP, excitatory postsynaptic potential; GAD1, glutamate decarboxylase 1; HSA21, human chromosome 21; HIP1, Huntingtin-interacting protein 1; IPSC, induced pluripotent stem cell; Mnb, minibrain (*Drosophila melanogaster*); MRD7, mental retardation, autosomal dominant 7; PEST, peptide sequence rich in proline (P), glutamic acid (E), serine (S), and threonine (T);; Ser, serine; SGZ, subgranular zone; SNCA, α-synuclein; SPRED1, sprouty-related EVH1 domain-containing protein 1; STAT1, signal transducer and activator of transcription 1; SYNJ1, synaptojanin 1; Thr, threonine; Tyr, tyrosine; SVZ, subventricular zone; VGLUT1, vesicular glutamate transporter 1; VZ, ventricular zone.

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1. Introduction

Protein kinases are vital for converting extracellular signals into biological responses. Functional alterations in kinases may directly contribute to age-dependent neuronal dysfunctions. Various kinases are positive regulators for brain development and memory function, and altered kinase signaling pathways can provoke memory disturbances. Many kinases associated with synaptic function are also age sensitive and are involved in neurodegenerative processes. Protein kinases are attractive drug targets because their ATP binding pocket is an excellent binding site for inhibitors, and many kinases are pivotal in pathological processes.

DYRK1A (dual-specificity tyrosine phosphorylation regulated kinase 1A), also designated as Minibrain (MNB) on the basis of its *Drosophila melanogaster* ortholog, is a protein kinase encoded on human chromosome 21 (HSA21). Compelling data implicate DYRK1A in the regulation of different cellular processes involved in brain development and function—ranging from early embryogenesis through late aging. Herein, we summarize existing data and highlight the roles of DYRK1A in healthy and affected brains. The molecular and physiological functions of DYRK1A are described in parts 2-5. We present the phenotypic consequences of DYRK1A genetic alterations in mental retardation autosomal dominant 7 (MRD7) (Part 6) and trisomy 21 (Down syndrome [DS]) (Part 7), and we discuss the DYRK1A-mediated functions that are associated with neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) (Part 8). Finally, the role of DYRK1A in several human diseases makes DYRK1A a potential target for therapeutic drugs; the use of several inhibitors is described in part 9. Because of the functional and molecular complexity of the DYRK1A protein kinase, the advantages and drawbacks of its possible use as a drug target for DS, MRD7, and AD must be carefully considered.

2. Protein structure and interactors

2.1. Expression patterns

DYRK1A is encoded on HSA21 in 21q22.2 (Guimera et al., 1996; Hattori et al., 2000; Patil et al., 1995). The gene comprises 151 kb and 15 exons (Ensemble release 90); it encodes two main protein isoforms of 763 and 754 amino acids. DYRK1A belongs to the DYRK family of dual-specificity protein kinases (CMGC group: DYRK family: DYRK subfamily). The kinase domain is located centrally in the primary structure of the protein. In mammals, the DYRK subfamily members (DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4) share a conserved motif N-terminal to the kinase domain known as the DYRK homology (DH)-box. DYRK1A also harbors a functional bipartite nuclear localization signal (NLS) N-terminal to the DH-box, a second NLS between subdomains X and XI within the kinase domain, a C-terminal PEST motif, and a polyhistidine tract that acts as a nuclear speckle targeting signal (Alvarez, Estivill, & de la Luna, 2003; Aranda, Laguna, & de la Luna, 2011; Becker & Joost, 1999) (Fig. 1A). DYRK1A and its closest family member DYRK1B are 73.9% identical (89.4% similar), and their kinase domains are 85.0% identical (95.6% similar). They share no sequence similarity within their C-terminal domains. *DYRK1A* is ubiquitously expressed in human and mouse tissues, but *DYRK1B* exhibits a more restricted pattern, with the highest mRNA levels in the testes and muscles.

DYRK1A and other human kinases from the CMGC group share a similar fold, the kinase domain, comprising an N-terminal lobe (N-lobe) with five antiparallel β-strands and a conserved regulatory αC-helix and a larger C-terminal (C-lobe) consisting of α-helices. The N- and C-lobes are connected by a hinge region, an important part of the ATP-pocket. The "gatekeeper" is a vital residue in the ATP pocket responsible for the selectivity of inhibitors. DYRKs have phenylalanines as gatekeepers, F238 in DYRK1A and F190 in DYRK1B, located at the beginning of their hinge regions. These features have been described in crystallographic studies (Alexeeva, Aberg, Engh, & Rothweiler, 2015; Anderson et al., 2013; Falke et al., 2015; Ogawa et al., 2010; Rothweiler et al., 2016; Tahtouh et al., 2012) mostly performed on complexes of the N-terminal fragment of DYRK1A, including the kinase domain, and competitive inhibitors targeting the ATP pocket (Fig. 1B). The functional specificity of each DYRK family member likely arises from the distinct C-terminal domain of that member. These domains have only recently been considered in crystallographic studies.

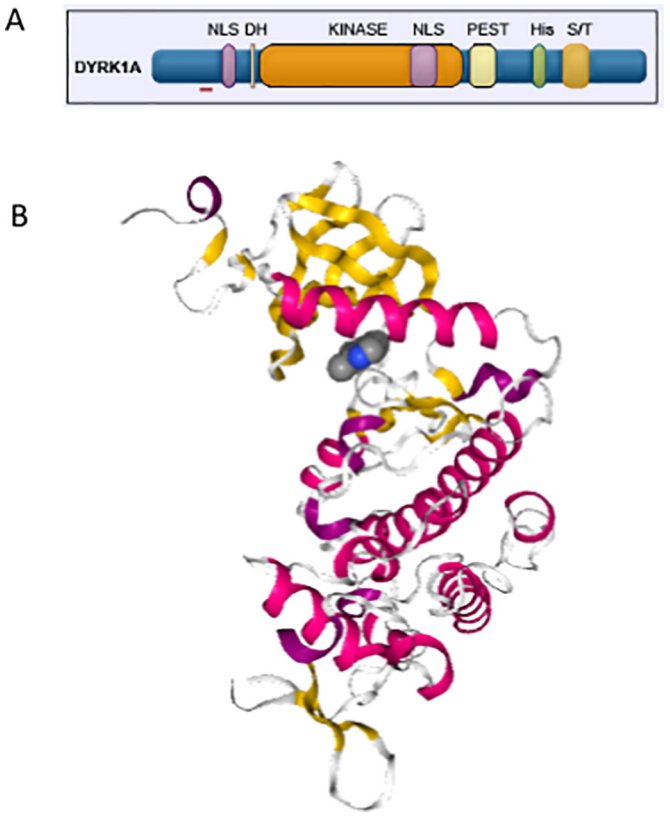


Fig. 1. (A) Representation of the domain structure of DYRK1A: NLS, nuclear localization signal; DH, DYRK homology domain; PEST, domain enriched in proline, glutamic acid, serine, and threonine residues; His, histidine repeats; S/T, serine and threonine-rich region. (B) Structure of the DYRK1A–harmine complex. The kinase domain (amino acids 126–490) of human DYRK1A was expressed in *E. coli* and co-crystallized with harmine (3ANR in ProteinDataBase) (NGL view with harmine in the spacefill mode) (from Ogawa, 2010).

DYRKs are an evolutionarily conserved family, and homologs have been found in yeast, for example, Yak1p from *Schizosaccharomyces pombe* (Garrett & Broach, 1989), and in nematodes, for example, the two kinases MBK-1 and MBK-2 (homologs of the MNB kinase) from *Caenorhabditis elegans* (Raich et al., 2003). MNB dysfunction leads to reduced optic lobe size in the brain of adult flies, thus highlighting the role of MNB in neurogenesis (Tejedor et al., 1995). In zebrafish, gene dosage modifications of *dyrk1aa* induce alterations in the brain development. (O. H. Kim, et al., 2017). Comparison of the modern human genome with Neanderthal and Denisovan genomes identified modern human *DYRK1A* variants, thus suggesting that this gene was influenced by late selective pressures (Mozzi et al., 2017).

On the basis of its RNA expression profile, *Dyrk1a* is ubiquitous during rodent development but has stronger reactivity in central nervous system (CNS) regions (Okui et al., 1999; Rahmani, Lopes, Rachidi, & Delabar, 1998). The *Dyrk1a* gene in chick and mouse embryos is expressed in proliferative regions of the nervous system at a very early stage during development, before the onset of neurogenesis. Expression of the *Dyrk1a* gene in the developing CNS shows a dynamic spatio-temporal pattern, and the protein is detected in cycling neurogenic progenitors and in differentiated neurons (Hammerle et al., 2002; Hammerle, Elizalde, & Tejedor, 2008). Immunofluorescence

studies performed on the developing brain and retina of the mouse indicate that the location of DYRK1A in both neuronal progenitors and differentiated neurons is mainly cytoplasmic (Hammerle et al., 2002; Laguna et al., 2008). Expression of DYRK1A during brain development peaks around birth and is maintained at lower levels until adulthood (Okui et al., 1999). In the adult mouse brain, varying levels of DYRK1A immunoreactivity are detected throughout the neuropil (Marti et al., 2003). Biochemical fractionation of the mouse brain confirmed that DYRK1A partitions between the nucleus and the cytoplasm (Aranda, Alvarez, Turro, Laguna, & de la Luna, 2008), which correlates with histological studies showing various levels of DYRK1A in the soma and dendrites as well as in the nuclei of different adult brain neuronal types (Hammerle et al., 2008). Consistently, the distribution of DYRK1A in primary cell cultures revealed the presence of this protein in the cytoplasm and the nucleus of neurons and astrocytes (Marti et al., 2003). In the human brain, DYRK1A is also found in the cytoplasm and the nucleus of various neuronal types as well as in astrocytes, ependymal, and endothelial cells. The distribution of DYRK1A in these cells indicates cell type- and brain structure-specific patterns of trafficking and utilization of this kinase (Wegiel et al., 2004).

Two NLSs contribute to DYRK1A nuclear translocation, and the histidine stretch is responsible for the accumulation of the protein in the splicing factor compartment. The C-terminal region of DYRK1A interacts with a brain-specific protein, phytanoyl-CoA hydroxylase-associated protein 1 (PAHX-AP1, also named PHYHIP), which may induce relocalization of nuclear DYRK1A to the cytosol (Bescond & Rahmani, 2005).

When overexpressed in mammalian cells, the DYRK1A protein mainly localizes to the nucleus with a punctate pattern of immunostaining consistent with accumulation in nuclear speckles (Alvarez et al., 2003; Becker et al., 1998). Nuclear speckles containing DYRK1A can be detected in neural progenitors and differentiating neurons *in vivo* (Hammerle et al., 2008; Laguna et al., 2008). In differentiating neurons (pyramidal neocortical neurons), DYRK1A is mainly found in the growing dendrites and developing neuropil (Hammerle, Elizalde, Galceran, Becker, & Tejedor, 2003b).

DYRK1A autophosphorylates on tyrosine, serine, and threonine residues but phosphorylates its substrates only on serine and threonine residues. Autophosphorylation of Tyr 312/321 (754/763 in variants) in the activation loop of DYRK1A is required for the full catalytic activity of the protein (Himpel et al., 2001). This tyrosine autophosphorylation is an intramolecular reaction that takes place during or immediately after translation (Lochhead, Sibbet, Morrice, & Cleghon, 2005). This reaction is due to an intrinsic ability of the catalytic domain of DYRK1A and does not require chaperone proteins (Gockler et al., 2009). Comparative analysis of inhibitors suggests that during DYRK1A autoactivation, tyrosine autophosphorylation in the activation loop stabilizes a conformation of the catalytic domain with enhanced serine/threonine kinase activity without disabling tyrosine phosphorylation (Walte et al., 2013). Further, DYRK1A autophosphorylates serine residues such as Ser520, which modulates binding to 14-3-3 proteins and kinase activity (Alvarez, Altafaj, Aranda, & de la Luna, 2007). Mass spectrometry analysis disclosed different phosphorylation patterns according to the intracellular distribution of DYRK1A and supports the hypothesis that compartment-specific functions of DYRK1A may depend on its phosphorylation status (Kaczmarski et al., 2014).

2.2. Regulation of DYRK1A transcription, translation, and enzymatic activity

Dyrk1a transcription is regulated by multiple proteins. P44 (a short isoform of the p53 start site in exon 4 of p53) binds to the mouse promoter of *Dyrk1a* and activates transcription; by contrast, p53 induces the expression of miR-1246, which, in turn, reduces the level of *Dyrk1a* mRNA (Pehar, Ko, Li, Scrable, & Puglielli, 2014; Zhang, Liao, Zeng, & Lu, 2011). miR-199b, which is under the control of NFATc, is another microRNA that targets *DYRK1A* in cardiac hypertrophy but has not been investigated in the CNS (da Costa Martins et al., 2010). The transcription factor E2F1 modulates the choice between two *Dyrk1a* promoters, which differ strongly depending on reporter gene activity (Maenz, Hekerman, Vela, Galceran, & Becker, 2008). OLIG2, an HSA21 protein, may also regulate *Dyrk1a* gene expression (W. Liu et al., 2015). Mena (ENAH), which is highly expressed in the developing and adult CNS and is a member of the Ena/VASP protein family, is part of a ribonucleoprotein complex that modulates DYRK1A translation and synthesis, both under steady state conditions and after brain derived neurotrophic factor (BDNF) stimulation (Vidaki et al., 2017). REST can activate *DYRK1A* transcription through a neuron-restrictive silencer element in the human *DYRK1A* promoter (Lu et al., 2011).

DYRK1A may also be regulated post-translationally. Dephosphorylation of its activation loop by a phosphatase, yet to be identified, may inactivate its kinase activity. In addition, phosphorylation events outside the activation loop may modulate DYRK1A activity. Phosphorylation of DYRK1A by LAST2, a component of the hippo pathway, enhances the ability of DYRK1A to phosphorylate the DREAM complex subunit LIN52 (Tschop et al., 2011). DYRK1A also contains a PEST sequence that allows truncation of the protein by Calpain and may increase kinase activity (Jin et al., 2015). Interacting proteins such as 14-3-3 and SPRED1 may also modulate DYRK1A kinase activity (Alvarez et al., 2007; Li, Jackson, Yusoff, & Guy, 2010).

2.3. Interactants/substrates

A consensus phosphorylation sequence for DYRK1A serine/threonine kinase activity has been proposed (RPXS/TP) (Himpel et al., 2000), although some phosphorylation sites do not fit with the consensus, for instance, LTAT(434)P, SF3B1/SAP155, or RPAS(640)V in glycogen synthase (de Graaf et al., 2004; Skurat & Dietrich, 2004). Analysis of *in vitro* phosphorylated synthetic peptide substrates established the preference of DYRK1A for an arginine residue in the -2 or -3 position and for a proline at the +1 position (Himpel et al., 2000). Substrates are related to the subcellular localization of DYRK1A (nuclear or cytosolic).

Chromatin regulators and transcription factors are an important group of DYRK1A substrates (Table 1 and Fig. 2), thus suggesting that DYRK1A plays a role in regulating gene expression. DYRK1A is directly associated with transcriptional control: DYRK1A phosphorylates the C-terminal domain (CTD) of RNA polymerase II (RNAPII) at Ser2 and Ser5 (Di Vona et al., 2015). Phosphorylation of histone H3 by DYRK1A antagonizes transcriptional repression mediated by heterochromatin protein 1 and participates in the activation of a subset of genes, including those encoding cytokines (Jang, Azebi, Soubigou, & Muchardt, 2014). When tested in gene reporter assays, DYRK1A acts as a transcriptional activator of cAMP responsive element-binding protein 1 (CREB1) (E. J. Yang, Ahn, & Chung, 2001), GLI1 (Ehe et al., 2017; Mao et al., 2002), forkhead box protein O1 (FOXO1/FKHR) (von Groote-Bidlingmaier et al., 2003; Woods et al., 2001b), and androgen receptor-interacting protein 4 (ARIP4/RAD54L2) (Sitz, Tigges, Baumgartel, Khaspekov, & Lutz, 2004) and as an inhibitor of Notch-dependent transcription (Fernandez-Martinez et al., 2009). DYRK1A is also a negative regulator of NFAT transcription factors in distinct cellular environments and acts by phosphorylating NFATs and retaining them in the cytoplasm (Arron et al., 2006; Fernandez-Martinez et al., 2009; Lee et al., 2009).

DYRK1A is proposed as a regulator of splicing on the basis of the localization of kinase in nuclear speckles (Alvarez et al., 2003) and the identification of several splicing factors as DYRK1A substrates (de Graaf et al., 2006). DYRK1A phosphorylation of the alternative splicing factor (ASF) prevents ASF-mediated inclusion of the alternatively spliced exon 10 in tau mRNA (Shi et al., 2008). There is evidence that DYRK1A phosphorylates other proteins involved in mRNA splicing, including the SR proteins SC35, SRp55, and 9G8, at several serine residues (Ding et al., 2012; Qian et al., 2011; Yin et al., 2012).

Table 1
DYR1KA interactors, partners, and targets.

Functions	Proteins	Subcellular localization	References
Chromatin regulator and Transcription	Arip4	Nucleus	(Sitz et al., 2004)
	Creb1	Nucleus	(E. J. Yang et al., 2001)
	CRY2	Nucleus, cytosol	(Kurabayashi et al., 2010)
	FOXO1	Nucleus	(von Groote-Bidlingmaier et al., 2003)
	Gli1	Nucleus	(Ehe et al., 2017)
	HIST1H3E	Nucleus	(Jang et al., 2014)
	NFAT	Nucleus/cytosol	(Arron et al., 2006; Lee et al., 2009)
	NOTCH	Nucleus	(Fernandez-Martinez et al., 2009)
	P53	Nucleus	(J. Park et al., 2010)
	RNAPII	Nucleus	(Di Vona et al., 2015)
	SIRT1	Nucleus	(Guo et al., 2010)
	STAT3	Nucleus/cytosol	(Wiechmann et al., 2003)
Splicing	SC35	Nucleus	(Qian et al., 2011)
	SF2/ASF	Nucleus	(Shi et al., 2008)
	SRp55	Nucleus	(Yin et al., 2012)
	9G8	Nucleus	(Ding et al., 2012)
Cell cycle, cell signaling, and pathways	BRAF	Cytosol	(Kelly & Rahmani, 2005)
	CASP9	Nucleus/cytosol	(Laguna et al., 2008)
	CCND1	Nucleus/cytosol	(Soppa et al., 2014)
	DCAF7	Cytosol	(Skurat & Dietrich, 2004; J. Xiang et al., 2017)
	GSK3B	Nucleus/cytosol	(Song et al., 2015)
	LIN52	Nucleus	(Guiley et al., 2015)
	MEK1	Cytosol	(Kelly & Rahmani, 2005)
	NEP	Membrane	(Kawakubo et al., 2017)
	PAHX-AP1	Cytosol	(Bescond & Rahmani, 2005)
	PSEN1	Membrane	(Ryu et al., 2010)
	PRKN	Cytosol	(Im & Chung, 2015)
	PROX1, ASCL1	Nucleus	(Shaikh et al., 2016)
	SPRED1	Cytosol	(Li et al., 2010)
	SPRY2	Cytosol	(Aranda et al., 2008)
	YWHAE	Cytosol	(Alvarez et al., 2007)
Synaptic plasticity, endocytosis, and trafficking	SNCA	Cytosol, membrane	(Murakami et al., 2012)
	AMPH	Cytosol, membrane	(Murakami et al., 2006)
	APP	Cytosol, membrane	(Ryoo et al., 2008)
	CLTC	Cytosol, membrane	(Murakami et al., 2012)
	DNM1	Cytosol	(Chen-Hwang et al., 2002)
	SH3GLB1	Cytosol, membrane	(Murakami et al., 2009)
	GRB2	Nucleus, cytosol	(Abekhoukh et al., 2013; Shin et al., 2007)
	GRIN2A	Membrane	(Grau et al., 2014)
	HAP1	Cytosol	(J. Xiang et al., 2017)
	HIP1	Cytosol, membrane	(Kang et al., 2005)
	N-WASP	Cytosol	(Park, Sung, et al., 2012a)
	SEPT4	Cytosol	(Sitz et al., 2008)
	SYNJ1	Cytosol	(Adayev, Chen-Hwang, Murakami, Wang, & Hwang, 2006a)
	MAPT (TAU)	Cytosol	(Kimura et al., 2007; Woods, Cohen, et al., 2001a)

DYRK1A inhibits apoptosis by phosphorylating the NAD-dependent deacetylase Sirtuin-1 (SIRT1), an inhibitor of p53 (Guo, Williams, Schug, & Li, 2010), and HIP1 (Kang, Choi, Park, & Chung, 2005), a protein normally associated with Huntingtin. SIRT1 together with HIPPI (HIP1 protein interactor) forms a complex with caspase-8 and induces apoptosis through components of the extrinsic cell death pathway (Gervais et al., 2002). Phosphorylation of p53 was demonstrated in neuronal cells in the context of proliferation; DYRK1A-mediated deacetylation did not affect proliferation but instead inhibited cell death. Moreover, DYRK1A restrains the activity of the intrinsic cell death pathway by phosphorylating Thr125 in caspase-9 (CASP9), a phosphorylation event that prevents CASP9 cleavage and subsequent activation of the pathway (Laguna et al., 2008; Seifert, Allan, & Clarke, 2008).

Increasing evidence indicates that DYRK1A is a negative regulator of cell cycle progression. DYRK1A phosphorylation of LIN52 is required for the assembly of the DREAM complex (Litovchick, Florens, Swanson, Washburn, & DeCaprio, 2011). This complex mediates gene repression during the G0 phase and coordinates periodic gene expression with peaks during the G1/S and G2/M phases (Sadasivam & DeCaprio, 2013). DYRK1A regulates protein levels of regulators of the G1/S phase transition in different ways. It controls the nuclear levels of Cyclin D proteins by phosphorylating a particular threonine residue in Cyclin D1/D3 (CCND1, CCND3), and this promotes Cyclin D nuclear export and degradation of the protein through the ubiquitin-proteasome pathway (J. Y. Chen, Lin, Tsai, & Meyer, 2013; Thompson et al., 2015). Knockdown of DYRK1A in fibroblast cells greatly increases Cyclin D1 levels and splits cells into two fates, with one subpopulation having an accelerated cell cycle with a significantly shortened G1 duration and the other entering an arrested state by costabilizing Cyclin D1 and the CDK inhibitor $p21^{Cip1}$ (J. Y. Chen et al., 2013). In addition, DYRK1A regulates $p21^{Cip1}$ at the transcriptional level through phosphorylation of p53 (J. Park et al., 2010).

2.4. Participation of DYRK1A in other cellular processes and signal transduction pathways

DYRK1A associated with the scaffold protein HAN11 (or DCAF7, WDR68) regulates glycogen synthase, a key enzyme in the regulation of glycogen synthesis by insulin (Skurat & Dietrich, 2004). DYRK1A is also a modifier of signaling mechanisms: it directly acts on receptor tyrosine kinase (RTK) signaling by phosphorylating Sprouty2. Both DYRK1A and Sprouty2 localize to growth cones of nerve terminals (Aranda et al., 2008). DYRK1A also interacts with SPRED1 and SPRED2, which inhibits the ability of DYRK1A to phosphorylate its substrates by reducing access to its kinase domain (Li et al., 2010).

DYRK1A contributes to the maintenance of normal circadian clock oscillation by phosphorylating Ser553 in cryptochrome 2 (CRY2), which serves as a priming step for glycogen synthase kinase 3 (GSK3)-mediated phosphorylation, thus leading to proteosomal degradation of CRY2 (Kurabayashi, Hirota, Sakai, Sanada, & Fukada, 2010).

DYRK1A interacts with proteins involved in protein processing and degradation: it phosphorylates presenilin, a component of the gamma secretase complex producing the Aβ peptide (Ryu et al., 2010), and it modifies neprilysin (NEP), which cleaves peptides on the amino side of hydrophobic residues and degrades the Aβ peptide (Kawakubo, Mori, Shirotani, Iwata, & Asai, 2017). Results of a yeast two-hybrid assay showed that DYRK1A is a novel binding partner of parkin (PRKN): DYRK1A phosphorylates parkin at Ser131, a modification that inhibits the E3 ubiquitin ligase activity of parkin (Im & Chung, 2015).

There is strong experimental evidence positioning DYRK1A within the endocytic network. DYRK1A phosphorylates synaptojanin1 (SYNJ1) and modifies its binding with amphiphysin and intersectin (Adayev, Chen-Hwang, Murakami, Wang, & Hwang, 2006; Adayev, Chen-Hwang, Murakami, Wegiel, & Hwang, 2006). In *Drosophila*, MNB-dependent phosphorylation of Synj1 is required for complex endocytic protein interactions and to enhance Synj1 activity *in vivo* (C. K. Chen et al., 2014). Through phosphorylation of Synj1 at Ser1029, MNB modulates the synaptic vesicle pool size (Geng, Wang, Lee, Chen, & Chang, 2016). DYRK1A was shown to phosphorylate Ser293 of amphiphysin in SY5Y cells (Murakami et al., 2006). It also phosphorylates Ser857 of dynamin1, and this phosphorylation is dependent on neuronal activity (Xie et al., 2012). DYRK1A can regulate neural development and synaptic plasticity through phosphorylation of Ser1048 in GluN2A, a subunit of the N-methyl-D-aspartate glutamate receptors (NMDARs) (Grau et al., 2014). Another target of DYRK1A is α-

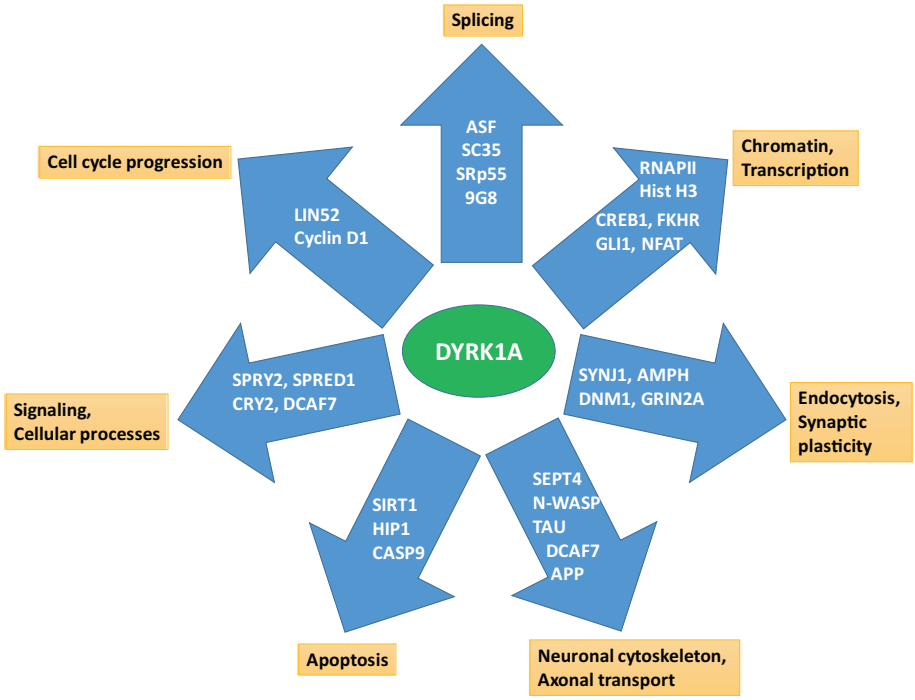


Fig. 2. Main functional roles of DYRK1A and proteins described as interactants or targets.

synuclein (Ser87), which is involved in the control of presynaptic signaling and membrane trafficking (E. J. Kim et al., 2006).

Cytoskeletal proteins involved in cell structure and trafficking, such as Septin4, a GTPase family member that serves as a scaffold for diverse molecules beneath the plasma membrane, are also DYRK1A targets (Sitz et al., 2008). In addition, DYRK1A influences the interplay between neuronal Wiskott–Aldrich syndrome protein (N-WASP) and growth factor receptor-bound protein 2 (GRB2), which control the actin skeleton and cytoskeletal architecture. DYRK1A phosphorylation of N-WASP inhibits Arp2/3-complex-mediated actin polymerization (Abekhoukh et al., 2013; Park et al., 2012a; Shin, Guedj, Delabar, & Lubec, 2007). Tau (MAPT), which promotes microtubule assembly and stability, is phosphorylated by DYRK1A (Frost et al., 2011; Yin et al., 2012) as well as two huntingtin-interacting proteins, HIP1 and HAP1a, associated with protein trafficking and the cytoskeleton, and these proteins also interact with DCAF7 (Kang et al., 2005; Z. Xiang, Haroutunian, Ho, Purohit, & Pasinetti, 2006). β-Amyloid precursor protein (APP) is phosphorylated by DYRK1A *in vitro*, and overexpression of DYRK1A increases the phosphorylation of APP on Thr668, which has been reported to modify APP conformation and could alter the role of APP in axonal transport and its processing by γ-secretases (Ryoo et al., 2008; Salehi et al., 2006). A summary of DYRK1A substrates/interactors is given in Table 1. The relevance of these interactions in brain development and function is discussed in parts 3, 4, and 5.

3. Neural numbers and organ size

The mammalian brain is formed of many neural types. During development, neurons arise from progenitors located in the proliferative regions within the anterior neural tube through a highly regulated process known as neurogenesis. Newborn neurons, which in most cases are produced prenatally, migrate and differentiate to form functional circuits during postnatal development. A proportion of differentiating neurons die by physiological apoptosis to balance the number of various neuron types with their corresponding target cells. Thus, alterations in neurogenesis and/or physiological cell death have an impact on the neuronal component of the brain circuits and, consequently, on its function. In this section, we summarize the current knowledge of the mechanisms by which DYRK1A/MNB kinases may regulate neural homeostasis and ultimately organ size. Owing to the relevance of the neocortex in cognition, the DYRK1A-mediated activities involved in neurogenesis and astrogliogenesis in this structure are explained in more detail (see schematic representation in Fig. 3).

3.1. Neurogenesis

The first evidence implicating DYRK1A/MNB in brain growth was published by Tejedor et al. in 1995. The authors showed that loss-of-function mutations in the *D. melanogaster mnb* gene caused a prominent size reduction in the optic lobes and central brain hemispheres in the adult fly; this reduction is due to the failure of *mnb* mutants to generate sufficient numbers of neurons during postembryonic neurogenesis. Recently, Shaikh et al. (2016) showed that *mnb* is weakly expressed in neuroblasts and that the expression of this gene is upregulated in newborn neurogenic precursors known as ganglion cells (Ceron, Gonzalez, & Tejedor, 2001). MNB in these precursors promotes the expression of the cyclin-dependent kinase inhibitor Dacapo, a homolog of vertebrate $p27^{Kip1}$, through a mechanism that involves the upregulation of the proneuronal transcription factor Asense and the homeodomain transcription factor Prospero. On the basis of these results and on the phenotype of *mnb* mutants, Shaikh et al. concluded that MNB is necessary for cell cycle exit and terminal differentiation in larval ganglion cells. In these mutants, many of these precursors continue to proliferate instead of exiting the cell cycle and they eventually die by apoptosis.

Drosophila MNB interacts with the adaptor protein Wings Apart (WAP, also known as Riquiqui), and this interaction promotes Yorkie-dependent tissue growth by stimulating phosphorylation-dependent inhibition of the Warts kinase (Degoutin et al., 2013). Yorkie (YKI; YAP/TAZ in mammals) is the major effector of the Hippo pathway, a universal governor of organ size and tissue homeostasis (Yu, Daniels, Wu, & Wolf, 2015). In addition, WAP interaction with MNB can control organ growth through the downregulation of the transcriptional repressor Capicua (CIC) (L. Yang et al., 2016), which is a key sensor of RTK signaling in *Drosophila* and mammals (Jimenez, Shvartsman, & Paroush, 2012). There is evidence that MNB, WAP, and CIC are implicated in a common pathway that controls brain growth (Tejedor et al., 1995; L.

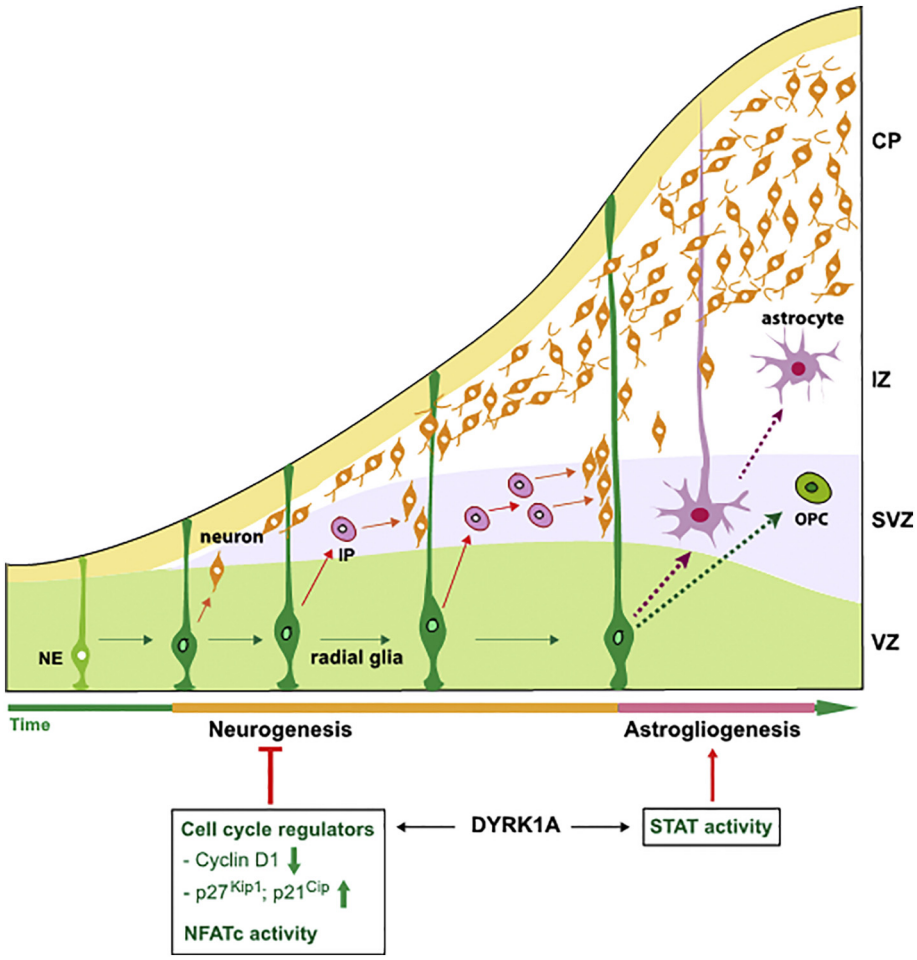


Fig. 3. Scheme illustrating the DYRK1A-mediated functions/activities involved in neural cell differentiation in the developing neocortex. Neurons and glial cells are produced sequentially from pluripotent radial glial progenitors (apical progenitors) or from progenitors with a more restricted fate (basal progenitors (IPs) and OPCs). During neurogenesis, DYRK1A regulates the neurogenic potential of radial glial progenitors regulating the expression of cell cycle regulator proteins (Cyclin D1, $p27^{Kip1}$, and $p21^{Cip}$) involved in the G1 to S phase transition. DYRK1A phosphorylation of NFATc transcription factors may regulate neuronal differentiation. Moreover, DYRK1A regulation of the pro-astrogliogenic factor STAT may have a role in astrocyte production. CP: cortical plate; IP; intermediate progenitor; IZ: intermediate zone; NE: neuroepithelial cell; OPC: oligodendrocyte precursor; SVZ: subventricular zone; VZ: ventricular zone.

Yang et al., 2016). Collectively, the studies performed in the fly indicate that in addition to the function of MNB in cell cycle regulation, other MNB-mediated activities may contribute to the brain size defects observed in *mnb* mutant flies.

The microcephaly displayed by human (see part 6), mouse (Fotaki et al., 2002; Raveau et al., 2018), and zebrafish (Kim et al., 2017a) models with loss-of-function mutations in *DYRK1A* indicates that the function of DYRK1A/MNB in brain growth is conserved across evolution. Morphometric studies performed in adult mutant mice with one (*Dyrk1a*$^{+/-}$ mice) or three functional copies of *Dyrk1a* (mBACTgDyrk1a mice) revealed that the effect of DYRK1A on brain growth is dosage dependent and region specific. In these two *Dyrk1a* mutants, neuron numbers in the adult neocortex inversely correlate with *Dyrk1a* gene dosage, whereas the opposite correlation has been observed in other brain regions (Guedj et al., 2012). This suggests that different DYRK1A-mediated activities are likely contributing to the cell number defects in mBACTgDyrk1a and *Dyrk1a*$^{+/-}$ mice.

There is evidence that DYRK1A, like MNB, inhibits cell cycle progression in neural progenitors by controlling the expression of cell cycle regulatory proteins. In SH-SYSY neuroblastoma cells, the overexpression of DYRK1A induces cell cycle exit and neuronal differentiation, an effect that correlates with increased phosphorylation of Cyclin D1 on Thr286, which promotes degradation of the protein, and of $p27^{Kip1}$ in Ser10, an event that stabilizes the protein (Soppa et al., 2014). Forced overexpression of DYRK1A in the chick spinal cord induces cell cycle exit and upregulates the expression of $p27^{Kip1}$ at the transcriptional level (Hammerle et al., 2011). In immortalized rat embryonic hippocampal progenitor cells, DYRK1A phosphorylates the transcription factor p53 at Ser15, thus leading to increased expression of several p53 target genes, including $p21^{CIP1}$, and attenuation of proliferation. Impaired proliferation and increased levels of phosphorylated p53 have also been observed in cortical neural precursors derived from a transgenic mouse line that expresses human *DYRK1A* (J. Park et al., 2010).

Regulation of cell cycle, particularly G1 to S phase progression, is crucial for the generation of appropriate number of neurons in the developing neocortex (Caviness, Takahashi, & Nowakowski, 1995; Dehay & Kennedy, 2007). These neurons are generated from pluripotent apical progenitors (also named as radial glial progenitors) in the ventricular zone (VZ) surrounding the ventricles or from neurogenic basal progenitors in the subventricular zone (SVZ) (Franco & Muller, 2013). In the mouse embryo, DYRK1A is expressed before the onset of neurogenesis, and the expression is maintained throughout corticogenesis (Franco & Muller, 2013; Hammerle et al., 2008; Kurabayashi & Sanada, 2013; Najas et al., 2015). The effect of an acute overexpression of DYRK1A on mouse cortical neurogenesis has been assessed by transfecting DYRK1A expression plasmids in apical progenitors through *in utero* or *ex vivo* electroporation techniques. In 2010, Yabut et al. showed that the overexpression of DYRK1A during mid-corticogenesis inhibits proliferation, thus leading to advanced differentiation (advanced production of basal progenitors and newborn neurons). Moreover, they

provided the first evidence that DYRK1A overexpression reduces nuclear Cyclin D1 protein levels. In similar electroporation experiments, Hammerle et al. showed that overexpression of DYRK1A promotes cell cycle exit, but in these experiments, differentiation was abrogated (Hammerle et al., 2011). To explain the failure of the progenitors that express DYRK1A under a heterologous promoter to differentiate and based on the dynamic expression of DYRK1A in precursor and differentiating neurons (Hammerle et al., 2008), the authors hypothesized that *DYRK1A* transcripts must be downregulated in the progenitors that exit the cell cycle to allow these cells to differentiate. Nonetheless, differences in the levels of the DYRK1A protein in the electroporated progenitors may explain the apparent discrepancy between these two studies. Indeed, it has been noted that *in vivo* electroporation of DYRK1A expression plasmids at concentrations that produce only a moderate increase in the DYRK1A protein does not appear to alter cortical neurogenesis (Kurabayashi & Sanada, 2013). Interestingly, Kurabayashi and Sanada showed that moderate overexpression of DYRK1A and RCAN1 delays neuron production, thus leading to an alteration in the fate of the neuronal progeny. Moreover, the authors demonstrated that this delay in neurogenesis resulted from the cooperative action of DYRK1A and RCAN1 on NFAT transcriptional activity. Thus, on the basis of these various *in vivo* overexpression studies, it is likely that DYRK1A regulates cortical neurogenesis by impinging on multiple pathways (e.g., cell cycle progression and/or NFAT activity).

The effect of DYRK1A on cortical neurogenesis has also been studied using the mBACTgDyrk1a mouse model, which overexpresses DYRK1A in a space- and time-regulated manner (Guedj et al., 2012; (Najas et al., 2015). In this model, neuronal production is delayed, thus leading to a deficit of neocortical neurons that extends until postnatal stages. This delay is caused by an augmented production of basal progenitors at the expense of neurons, which correlates with a lengthened G1 phase of the cell cycle in apical progenitors at the onset of neurogenesis. The levels of $p27^{Kip1}$ and $p21^{Cip1}$ in these progenitors are normal, but there is a significant deficit of nuclear CyclinD1. Conversely, *Dyrk1a*$^{+/-}$ apical progenitors have excess nuclear Cyclin D1 and produce more neurons at the expense of basal progenitors (Najas et al., 2015). These data indicate that variations in the amount of the DYRK1A protein change the division mode of apical radial glial progenitors through a mechanism that likely involves DYRK1A-mediated phosphorylation of Cyclin D1.

Active neurogenesis in the adult brain is restricted to the SVZ of the lateral ventricle and to the subgranular zone (SGZ) of the hippocampus (Alvarez-Buylla & Lim, 2004). There is evidence that epidermal growth factor receptor (EGFR) distribution varies between the daughters of adult SVZ neural stem cells (NSCs) and that inheritance of unequal receptor amounts determines the stem cell potential of this progenitor type (Andreu-Agullo, Morante-Redolat, Delgado, & Farinas, 2009). In these NSCs, DYRK1A distributes either symmetrically or asymmetrically during mitosis, and EGFR level and distribution in NSC daughter cells depend on the amount of inherited DYRK1A. Mechanistically, this is explained by the inhibitory effect of DYRK1A on endocytosis-mediated degradation of EGFR signaling. In the loss-of function *Dyrk1a*$^{+/-}$ mouse model, EGF-dependent cell fate decisions and long-term persistence of NSCs in the niche are disturbed (Ferron et al., 2010). Thus, normal levels of DYRK1A are required to sustain neurogenesis in the adult SVZ. Evidence obtained in DS mouse models (see part 7) indicates that DYRK1A also plays a role in neurogenesis of the adult hippocampus, but the underlying mechanism has not yet been described.

3.2. Astrogliogenesis

After the neurogenic period, the apical progenitors of the developing neocortex acquire the capacity to produce glial cells. In 2015, Kurabayashi et al. showed that the overexpression of DYRK1A upregulates the activity of the astrogliogenic transcription factor STAT in wild-type progenitors as well as the levels of Ser727 phosphorylation in STAT3, a modification that enhances STAT3 activity. They provide evidence that STAT3 phosphorylation by DYRK1A contributes to the overproduction of astrocytes in the neocortex of a trisomic DS mouse model with three copies of *Dyrk1a*. This pro-astrogliogenic role of DYRK1A is in apparent contradiction with the altered number of astrocytes observed in the adult hippocampus of the mBACTgDyrk1a and *Dyrk1a*$^{+/-}$ mouse models, which is decreased in the gain-of-function model and increased in the loss-of-function model (Fotaki et al., 2002; Guedj et al., 2012). Further investigations are needed to understand the possible role of DYRK1A in astroglial homeostasis.

3.3. Developmental apoptosis

The retina, a part of the CNS, has been extensively used as a model system to study neurogenesis in vertebrates (Cepko, 2014). The effect of *Dyrk1a* dosage imbalance in retinal neurogenesis has been assessed in the loss-of-function *Dyrk1a*$^{+/-}$ and gain-of-function tgYAC152f7 mice. In both models, retinal neurons are produced at normal rates and at the correct time. However, retinal thickness and cellularity at postnatal developmental stages are altered: decreased in the loss-of-function model and increased in the gain-of-function model. These alterations affect only the internal layers of the retina (internal nuclear layer and ganglion cell layer) and result from aberrant activity of the intrinsic apoptotic pathway in retinal differentiating neurons through a process involving CASP9 phosphorylation by DYRK1A (Laguna et al., 2008). Consistent with the antiapoptotic role of DYRK1A in retinal differentiating cells, the retinas of mBACTgDyrk1a mice are thicker and have more neurons in their internal layers than those of wild-type mice (Laguna et al., 2013).

Studies performed in the ventral brain mesencephalon of the *Dyrk1a*$^{+/-}$ mouse model showed that there is a deficit of dopaminergic neurons in this brain region like in the retina and that this deficit is not due to an altered neurogenesis but rather due to an increased CASP9-mediated cell death (Barallobre et al., 2014; Fotaki et al., 2002; Guedj et al., 2012). In the *dyrk1aa* zebrafish knockout model, brain size reduction seems to be caused by an increased neuronal cell death during development (Kim et al., 2017b). Collectively, these studies indicate that DYRK1A regulation of programmed cell death may contribute to the brain size defects associated with *DYRK1A* haploinsufficiency.

4. Synaptogenesis and neuronal functions

There are several spatiotemporal functional profiles for DYRK1A/MNB. DYRK1A is expressed during development at later stages (Marti et al., 2003; Song et al., 1996) and has been detected on the apical side of dendrites, in growing axons (Hammerle et al., 2003a; Hammerle et al., 2008), and in axonal growth cones (Vidaki et al., 2017). The expression of DYRK1A in differentiated neurons suggests a possible role in neurite formation (Gockler et al., 2009; Hammerle et al., 2008; Hammerle, Elizalde, et al., 2003b). This is supported by several studies that reported alterations in axon growth, dendritic arborization, or dendritic spines due to changes in DYRK1A dosage in gain-of-function or loss-of function models (transgenic mice, cell culture with siRNA, etc.). For instance, DYRK1A overexpression in primary mouse cortical neurons significantly reduced dendritic growth and complexity through disruption of the REST/NRSF-SWI/SNF chromatin remodeling complex (Lepagnol-Bestel et al., 2009). In addition, a genetic knockdown of *Dyrk1a* in cultured cortical neurons resulted in neurons with shorter and more branched neurites and fewer axons (Scales, Lin, Kraus, Goold, & Gordon-Weeks, 2009). This result is in line with that reported in a previous study that showed that pyramidal neurons in the *Dyrk1a* haploinsufficient mouse cortex have considerably reduced dendritic arborization and dendritic branching as well as fewer dendritic spines than those of wild-type animals (Benavides-Piccione et al., 2005;

Fotaki et al., 2002). Additionally, although specific knockdown of DYRK1A in COS-7 cells promoted filopodia formation (Park, Jung, Kim, Song, & Chung, 2012b), DYRK1A overexpression caused a reduction in dendritic spine formation in cultured hippocampal neurons. An additional study reported that cortical neurons from *Dyrk1a* overexpressing mice exhibited reduced dendritic spine density, dendritic filopodia length, and synapse formation and a dendritic spine phenotype with thinner and more immature spines (Martinez de Lagran et al., 2012). More recently, it was found that either knockdown or overexpression of *Dyrk1a* strongly represses dendritic spine formation in hippocampal neurons, thus indicating that *Dyrk1a* gene dosage is critical for proper dendritic spine development (Dang et al., 2018). However, previous studies suggested that the overexpression of DYRK1A increases the spine density of cortical pyramidal neurons (Altafaj et al., 2001; Thomazeau et al., 2014), an effect that can be prevented by a green tea extract enriched in the DYRK1A inhibitor EGCG. A recent work found that mutations in MNB/DYRK1A kinases perturb the overall neuronal outgrowth and maintenance of terminal branch length (Ori-McKenney et al., 2016). Finally, while DYRK1A overexpression reduces total neurite numbers and axon length in primary cultured mouse cortical neurons, a knockdown of *Dyrk1a* by RNA interference or missense mutations in *Dyrk1a* induced a significant decrease in neurite and axon length (Dang et al., 2018). All these findings highlight the impact of *Dyrk1a*/*Mnb* gene dosage on dendrite and axon development, and consequently synaptogenesis, an active process of synapse formation and maintenance that affects neuronal phenotypes and circuit structure.

The mechanisms underlying the function of DYRK1A in neurite formation, dendritogenesis, or synaptogenesis are largely unknown. Cytoskeletal modifications, regulation of transcription factors, and membrane trafficking have been suggested as potential stakeholders (Fig. 4).

4.1. Cytoskeletal arrangement

Axon growth and guidance is an important step in synapse formation. At the tip of outgrowing axons, motile growth cones sense guidance cues and translate this information into dynamic cytoskeletal reorganizations that orient growth in a specific direction (Sanes & Yamagata, 1999). Only one study has provided direct evidence that DYRK1A may control synaptogenesis through axon guidance. This study showed that DYRK1A and RTK, which are Sprouty antagonists, co-localize in the growth cone-like structures of cultured cortical neurons (Aranda et al., 2008). The authors proposed that DYRK1A phosphorylates and inhibits Sprouty, thereby promoting FGF signaling (Aranda et al., 2008), which in turn regulates axon guidance (Dabrowski, Terauchi, Strong, & Umemori, 2015; Shirasaki, Lewcock, Lettieri, & Pfaff, 2006). Two phases, synaptic assembly and synaptic formation, define synaptogenesis (Colon-Ramos, 2009). Cytoskeletal components, cytoplasmic scaffold assembly, and the trans-synaptic complex might collectively establish a checkpoint for the maturation of initial unstable assemblies into stable synapses. F-actin and its regulatory proteins are intriguing candidates for this task because they possess the combinatorial ability to interact with cell-surface proteins and with active zone proteins.

DYRK1A can regulate the cytoskeletal machinery (Colon-Ramos, 2009; K. Dowjat, Adayev, Kaczmarski, Wegiel, & Hwang, 2012; F. Liu et al., 2008; Martinez de Lagran et al., 2012; Park, Sung, et al., 2012a; Scales et al., 2009), thereby contributing to the development/establishment and maintenance of neurites and dendritic spines (F. Liu et al., 2008; Park, Sung, et al., 2012a) and ultimately neuronal function. As already mentioned, DYRK1A is both a nuclear and a cytoplasmic protein, with the latter existing in three pools: soluble, cytoskeletal associated, and membrane bound (Aranda et al., 2008; Kaczmarski et al., 2014; Marti et al., 2003). DYRK1A phosphorylates cytoskeletal proteins,

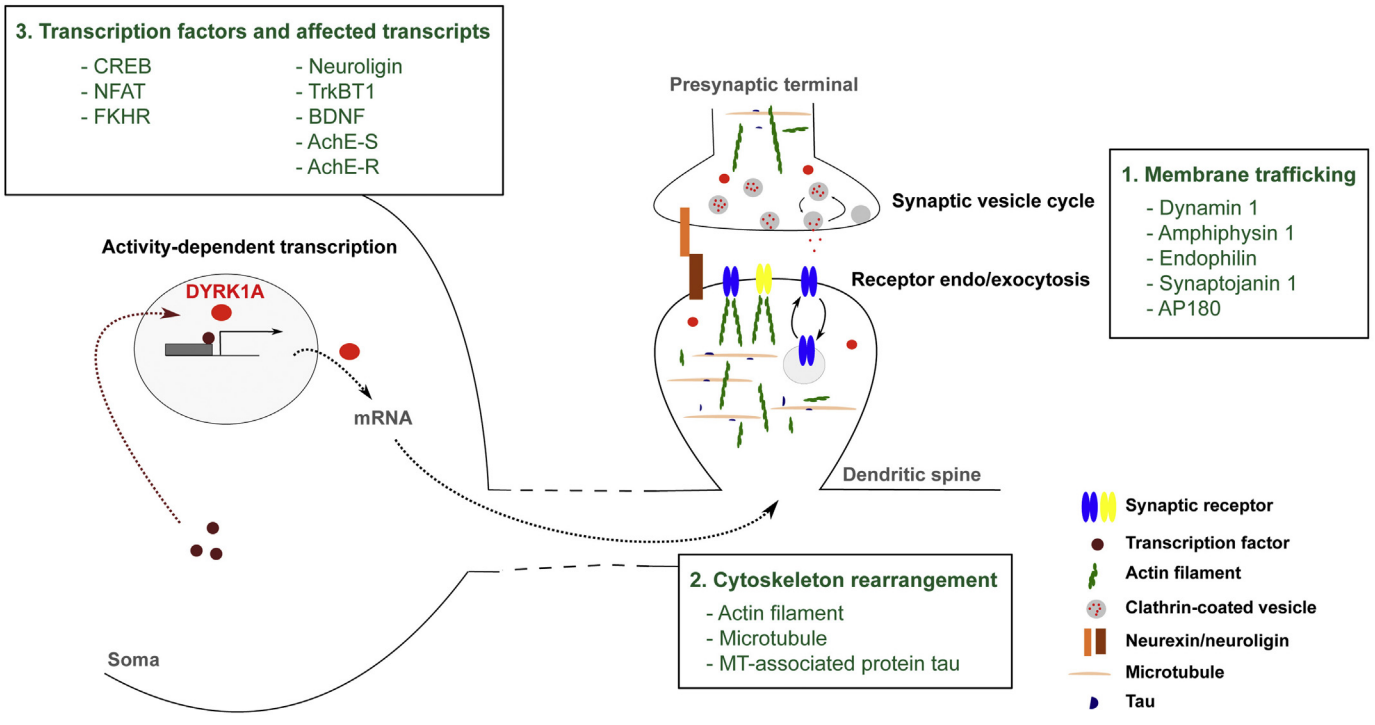


Fig. 4. Schematic model illustrating the putative involvements of DYRK1A at the developing and the mature synapse. DYRK1A regulates the exocytosis–endocytosis cycle of synaptic vesicles containing neurotransmitter in the presynaptic bouton and of receptors located at the postsynaptic membrane by phosphorylating core proteins such as dynamin 1, amphiphysin 1, synaptojanin 1, endophilin, or adaptator proteins such as AP180 (1). The presynaptic vesicle cluster and the postsynaptic compartment throughout the spine head are surrounded by a dense scaffold, the cytoskeleton, mainly composed of actin filaments and microtubules. DYRK1A is involved in cytoskeletal rearrangement by direct or indirect interactions with actin filaments, microtubules, or the microtubule-associated protein tau (2). DYRK1A can also impact the synapse formation and activity by regulating the transcription machinery, by its direct or indirect effects on transcription factors such as CREB, NFAT, or FKHR, or transcripts such as neuroligin, TrkBT1, BDNF, AchE-S, and AchE-R (3). AP180: clathrin adaptor protein; CREB: cAMP responsive element-binding protein; NFAT: nuclear factor of activated T cells; FKHR: forkhead transcription factor; TrkBT1: truncated Tropomyosin receptor kinase B receptor; BDNF: Brain-derived neurotrophic factor; AchE-S: acetylcholinesterase-S; AchE-R: acetylcholinesterase-R.

which then affect the outgrowth of microscopic fibers and actin assembly. Because of its enzymatic targets, DYRK1A is strongly implicated in the regulation of cytoskeletal protein assemblies such as actin (T. Liu, Sims, & Baum, 2009; Park, Sung, et al., 2012a), tubulin in the form of microtubules (Ori-McKenney et al., 2016; Scales et al., 2009), and the microtubule-associated protein (MAP) tau (F. Liu et al., 2008; Ryoo et al., 2008; Woods et al., 2001a).

4.2. Membrane trafficking

Synaptogenesis as well as spine morphology and function are also regulated by membrane trafficking pathways (Winkle & Gupton, 2016) in coordination with cytoskeletal dynamics (Hotulainen & Saarikangas, 2016; Newpher, Harris, Pringle, Hamilton, & Soderling, 2018; Spence & Soderling, 2015). DYRK1A is found in the presynaptic terminal of the neuromuscular junctions and in axons from the facial nucleus, thus suggesting a function for DYRK1A in these structures (Arque, Casanovas, & Dierssen, 2013; C. K. Chen et al., 2014). DYRK1A phosphorylates and thus regulates the protein–protein interactions of various factors involved in membrane trafficking, for example, the core endocytic proteins (Adayev, Chen-Hwang, Murakami, Wegiel, & Hwang, 2006b; Chen-Hwang, Chen, Elzinga, & Hwang, 2002; C. K. Chen et al., 2014; Geng et al., 2016; Murakami, Bolton, & Hwang, 2009; Murakami, Bolton, Kida, Xie, & Hwang, 2012; Murakami et al., 2006). DYRK1A-dependent regulation of clathrin-coated vesicle formation also contributes significantly to synaptic function. In both fibroblasts and neurons, overexpressed DYRK1A appears to inhibit endocytosis in transferrin internalization assays by causing defects in clathrin-mediated endocytosis (Y. Kim, Park, Song, & Chang, 2010). Moreover, DYRK1A phosphorylates dynamin, which is also critically linked to endocytosis and important for synaptogenesis (Fan, Funk, & Lou, 2016a; Schmid, McNiven, & De Camilli, 1998), and amphiphysin, which decreases the binding of amphiphysin to endophilin (Murakami et al., 2006). Endophilin is an endocytic protein, which like the clathrin heavy chain, can bind to DYRK1A (Murakami et al., 2009). DYRK1A inhibits the onset of clathrin-mediated endocytosis in neurons by phosphorylating dynamin1, amphiphysin1, and Synj1 and can promote the uncoating process of endocytosed clathrin-coated vesicles. Phosphorylation of the clathrin-coated vesicle adaptor proteins MAP1A, MAP2, AP180, and α- and β-adaptins by DYRK1A promotes the dissociation of these proteins from the clathrin-coated vesicle membranes (Murakami et al., 2012).

4.3. Regulation of transcription factors

DYRK1A is localized in the nucleus in hippocampal neurons and has an NLS and a 13-histidine repeat for nuclear speckle targeting (Alvarez et al., 2003; Kentrup, Joost, Heimann, & Becker, 2000). This localization is consistent with the findings reported in several studies showing that DYRK1A regulates the splicing machinery at post-transcriptional and post-translational levels (Toiber et al., 2010; Wegiel et al., 2011b; Wegiel, Gong, & Hwang, 2011a). DYRK1A overexpression (in either fetal DS brains, engineered mouse models, or cultured cells) alters the phosphorylation and subcellular location of splicing machinery components and modifies key synaptic transcripts such as neuroligin 1 (Toiber et al., 2010). Such DYRK1A-regulated cell adhesion proteins have an important role in synaptogenesis and maintenance of adult synapse number (Kwon et al., 2012). The neurexin–neuroligin pair is an example of the synaptogenic membrane protein interactions needed for the establishment of functional trans-synaptic communication to guide synapse assembly. Moreover, overexpression of DYRK1A causes splicing aberrations resulting in changes in the expression levels of various mRNAs such as *TrkBT1*, *Bdnf*, *AchE-S*, and *AchE-R* (Toiber et al., 2010) involved in synaptogenesis.

Additionally, local mRNA translation is required for axon guidance and synaptogenesis (Dabrowski et al., 2015; Gao et al., 2012; Jung et al., 2011; Piper & Holt, 2004; Shirasaki et al., 2006), and *Dyrk1a* was found to be locally translated in axons in a Mena-dependent manner (Vidaki et al., 2017).

5. DYRK1A and neurotransmission

DYRK1A expression in mature brain neurons (Marti et al., 2003; Wegiel et al., 2004) and the possible role of two DYRK1A-regulated transcription factors, NFAT (Arron et al., 2006) and CREB (E. J. Yang et al., 2001), in synaptic function imply that DYRK1A may have a role in the maintenance of adult brain neural activity (Table 2).

5.1. Basal synaptic activity

Monitoring spontaneous neural activity revealed that DYRK1A might be involved in synaptic transmission. Cortical primary cultures with transgenic overexpression of DYRK1A exhibit significantly reduced numbers of spikes, thus suggesting a reduction in spontaneous activity (Martinez de Lagran et al., 2012). However, electrophysiological recordings revealed an increased miniature excitatory postsynaptic potential (mEPSP) amplitude but normal-evoked EPSP amplitudes at the *Drosophila* neuromuscular junction in a classical *Dyrk1a*/*mnb* fly mutant, which has a mutation next to the critical ATP-binding residue in the kinase-active site previously shown to influence MNB function and protein level (C. K. Chen et al., 2014). The authors observed no change in postsynaptic AMPAR in the *mnb* mutant, thus supporting the notion that the increased mEPSP amplitude was due to presynaptic changes. Indeed, they found that presynaptic overexpression of MNB in the *mnb* mutant protected the mEPSP phenotype. In addition, an increased synaptic vesicle size has been found in a variety of endocytic mutants (C. K. Chen et al., 2014). This is consistent with the role of DYRK1A in endocytosis as discussed above. Synaptic activity increases DYRK1A/MNB mobilization to endocytic zones and efficiently promotes synaptic vesicle recycling by regulating synaptojanin function, which directly has an impact on synaptic transmission. A similar increase in the amplitude of miniature excitatory postsynaptic currents (mEPSCs) correlated with increased spine density was observed in prefrontal cortex pyramidal neurons of mBACTgDyrk1a mice (Thomazeau et al., 2014).

5.2. Presynaptic changes: synaptic vesicle pool and dynamics

Changes in mEPSCs amplitude could formally occur through postsynaptic (receptor abundance or activity) or presynaptic (such as synaptic vesicle size) changes, but evidence discussed above favors a presynaptic mechanism. Consistent with this, DYRK1A/MNB is also located presynaptically (Arque et al., 2013; C. K. Chen et al., 2014) and is tightly coupled to the endocytic machinery, which can affect synaptic vesicle endocytosis and subsequently neurotransmitter release. Moreover, clathrin-mediated endocytosis is essential for the recycling of membrane after neurotransmitter release (Saheki & De Camilli, 2012).

In addition to regulating endocytosis, DYRK1A, through its interactors, could affect exocytosis, neurotransmitter release, or delivery of receptors at synaptic membranes. It was recently shown that the DYRK1A substrate dynamin 1 affects exocytosis in addition to its essential role in vesicle endocytosis (Anantharam et al., 2011; Chan, Doreian, & Smith, 2010; Gonzalez-Jamett et al., 2010), but the role of phosphorylation of dynamin 1 by DYRK1A in this process remains unclear (Park, Jung, et al., 2012b). It is known, however, that DYRK1A-mediated phosphorylation of MUNC18-1, a central regulator of neurotransmitter release, controls the interaction of MUNC18-1 with syntaxin 1, which stimulates SNARE-mediated exocytosis (with the formation of the SNARE complex required for membrane fusion during synaptic vesicle exocytosis) (Park, Jung, et al., 2012b). Finally, α-synuclein, a presynaptic protein involved in neurotransmitter release, is an additional substrate of DYRK1A (Burre, 2015) and has an important role in curvature stabilization, which is important for synaptic vesicle trafficking

Table 2
DYRK1A implication in synaptic activity.

Model	Approach	Phenotype	References
Basal synaptic activity			
Dyrk1a overexpression	cell culture, cortex	↓ spike number	Martinez de Lagran et al., 2012
Mnb overexpression	Drosophila NMJ	↑ mEPSP amplitude ↑ synaptic vesicle size	Chen et al., 2014
Dyrk1a overexpression	Mice, PFC, slice	↑ mEPSC amplitude ↑ dendritic spine density	Thomazeau et al., 2014
Dyrk1a overexpression	Mice, cerebellum, neuron culture	↑ NR2A-containing NMDAR expression ↑ decay NMDA-induced calcium transient	Altafaj et al., 2008
Dyrk1a overexpression	Mice,	E/I imbalance toward inhibition	Souchet et al., 2014
Ts65Dn crossed with Dyrk1a $^{+/-}$	Mice, HPC	Normalization of the density of GABAergic and glutamatergic synapse markers	Garcia-Cerro et al., 2014
Synaptic plasticity			
Dyrk1a overexpression	Mice, HPC, slice	↑ NMDAR-LTP ↓PPF-LTD Ø NMDAR-LTD	Ahn et al., 2006
Dyrk1a overexpression	Mice, PFC, slice	Ø NMDAR-LTP Ø eCB-LTD	Thomazeau et al., 2014
Ts65Dn + EGCG	Mice, HPC, slice	NMDAR-LTP protected by EGCG treatment	Xie et al., 2008
Ts65Dn + shRNA Dyrk1a viral injection	Mice, HPC, slice	NMDAR-LTP protected by shRNA Dyrk1a	Altafaj et al., 2013
Ts65Dn crossed with Dyrk1a $^{+/-}$	Mice, HPC, slice	NMDAR-LTP protected	Garcia-Cerro et al., 2014

NMJ: neuromuscular junction; PFC: prefrontal cortex; HPC: hippocampus; mEPSP: miniature excitatory postsynaptic potential; mEPSC: miniature excitatory postsynaptic current; NR2A: 2A subunit-containing N-methyl-D-aspartate receptor; NMDA: N-methyl-D-aspartate; E/I: excitation/inhibition; GABA: gamma-aminobutyric acid; NMDAR: N-methyl-D-aspartate receptor; PPF: paired-pulse facilitation; LTP: long-term potentiation; LTD: long-term depression; eCB: endocannabinoid; shRNA: short hairpin ribonucleic acid.

during both endocytosis and exocytosis (E. J. Kim et al., 2006; Lautenschlager, Kaminski, & Kaminski Schierle, 2017).

5.3. Postsynaptic changes

In addition to its effect on neurotransmitter release, DYRK1A can regulate synaptic receptor trafficking, including membrane delivery and internalization of glutamate receptors, AMPARs or NMDARs. NMDARs are involved in neural development, survival, synaptic plasticity, and memory processes. DYRK1A phosphorylates the NMDAR subunit GRIN2A on its C-terminal domain. This modifies the biophysical properties of the receptor in favor of channel opening, thereby resulting in a longer NMDAR-induced Ca^{2+} transient decay (Altafaj et al., 2008). This modification also decreases the internalization of NR2A-containing NMDAR, thus leading to an increase in the surface density of these receptors. The DYRK1A-dependent regulation of the amount and biophysical properties of synaptic membrane NMDARs indicates that DYRK1A participates in regulating Ca^{2+} signaling and ultimately contributes to synaptic transmission (Altafaj et al., 2008; Grau et al., 2014). DYRK1A might also contribute to the regulation of intracellular receptor trafficking by interacting with Sprouty, a protein associated with cytosolic vesicles involved in endocytic events controlling receptor trafficking (Aranda et al., 2008). Unlike NMDARs, NR1 and NR2AR receptors appear to be negatively regulated by DYRK1A. Mice overexpressing DYRK1A have lower levels of NR1 and NR2A receptors and CAMKII as well as a lower pCAMKII/CAMKII ratio (Souchet et al., 2014).

5.4. Excitation/Inhibition balance

DYRK1A seems to have an important role in controlling the excitatory/inhibitory balance, with DYRK1A overexpression increasing the number of inhibitory synapses and leading to enhanced inhibition. Increased expression of *Dyrk1a* in the mouse (mBACtgDyrk1a) induced molecular alterations in synaptic plasticity pathways and expression changes in GABAergic- and glutamatergic-related proteins leading to an excitation/inhibition imbalance related to GABA synthesis (Souchet et al., 2014). More precisely, this study showed that excess DYRK1A dosage induces the activation of the GABA pathway, thereby increasing the enzymes involved in decarboxylation of glutamate to produce GABA (GADs), a marker of GABAergic synapses, as well as vesicular transport of GABA (VGAT). By contrast, the protein involved in glutamate transport decreased, thereby resulting in elevated GABAergic neurotransmission and reduced glutamatergic transmission. Conversely, decreasing the level of DYRK1A induced a decrease in GABA production and an increase in glutamate transport-associated proteins (Souchet et al., 2014). Similar results were found in an additional study where DYRK1A overexpression enhanced the expression of GADs and reduced the expression of VGLUT, a marker of glutamatergic synapses, and these effects could be reversed by removing one copy of the *Dyrk1a* gene (Garcia-Cerro et al., 2014). It was recently reported that this DYRK1A-mediated increase in inhibition decreases the firing rate of cortical neurons. This perturbs the gamma range activity and thus affects the overall neuronal network (Ruiz-Mejias et al., 2016).

5.5. Monoamine modulation

Dyrk1a overexpression induces major deficits in serotoninergic, dopaminergic, and noradrenergic systems, thus revealing that DYRK1A modulates monoamine neurotransmission (London et al., 2018). Serotonin is related to GABA synthesis in the CNS, and evidence suggests interactions between glutamatergic transmission and the monoamine systems (Yuen et al., 2014). Thus, the dopamine and noradrenergic neurotransmitter systems are also important for neural and synaptic functions, especially in the prefrontal cortex known to be involved in intellectual disabilities (IDs) (Xing, Li, & Gao, 2016).

5.6. Alterations in synaptic plasticity

Long-term potentiation (LTP) and long-term depression (LTD) are examples of plasticity associated with either an increase or decrease, respectively, in synaptic strength following high-frequency stimulation of a chemical synapse. Only a few studies have reported that DYRK1A is involved in synaptic plasticity. One study found that overexpression of DYRK1A impairs the long-term synaptic plasticity of hippocampal

Schaeffer collateral-CA1 synapses without affecting normal basal synaptic transmission. Indeed, the authors found that although NMDAR-LTP is increased, a paired-pulse low-frequency stimulation-induced LTD (NMDAR and mGluR dependent) is reduced and classical low-frequency stimulation-induced NMDAR-LTD is abolished in hippocampi of mice overexpressing *Dyrk1a* (Ahn et al., 2006). Moreover, *Dyrk1a* overexpression induces functional alterations in the prefrontal cortex of mBACtgDyrk1a mice with dendritic alterations and anomalous NMDAR-LTP and endocannabinoid-dependent LTD (Thomazeau et al., 2014). Other studies used Ts65Dn mice, the most common rodent model for DS, to examine how normalization of the *Dyrk1a* gene dosage affects synaptic plasticity. Pharmacological inhibition of DYRK1A with EGCG corrects the LTP deficit observed in the hippocampus of the Ts65Dn mice (Xie, Ramakrishna, Wieraszko, & Hwang, 2008). Moreover, injecting the hippocampus with an adeno-associated virus carrying an shRNA sequence specific for *Dyrk1a* normalized DYRK1A expression in Ts65Dn mice and restored hippocampal LTP (Altafaj et al., 2013). Finally, crossing Ts65Dn mice with *Dyrk1a*$^{+/-}$ mice protected hippocampal LTP in offspring with normalized *Dyrk1a* copy number (Garcia-Cerro et al., 2014).

DYRK1A could also indirectly affect synaptic plasticity. As mentioned previously, DYRK1A overexpression turns the excitation/inhibition balance toward inhibition. LTP deficits in the hippocampus have been associated with enhanced GABA-mediated inhibition (Fernandez et al., 2007; Yoshiike et al., 2008). An increase in the inhibitory function mediated by GABAergic synapses may interfere with processes required for learning and memory, as indicated by LTP deficits in the dentate gyrus (DG; Palop et al., 2007). Consistent with this, the $GABA_A$ receptor antagonist picrotoxin was found to prevent such LTP deficits observed in an animal model of AD (Kleschevnikov et al., 2004). In addition, DYRK1A could indirectly affect synaptic plasticity by phosphorylating and inactivating GSK3β (Song et al., 2015), a protein that has a pivotal role in synaptic plasticity (Bradley et al., 2012). Finally, by forming a multiprotein complex with Ras, Raf, and MEK1 (Kelly & Rahmani, 2005), DYRK1A might affect MAPK/ERK signaling, which is vital for synaptic plasticity (Impey, Obrietan, & Storm, 1999).

6. DYRK1A deficit, autism, and MRD7

Classical genotype–phenotype association studies of rare cases with partial monosomy 21 (Chettouh et al., 1995; Matsumoto et al., 1997) in combination with the physical map of the chromosomal region for DS (Osoegawa et al., 1996) narrowed the loci of monosomy 21-associated microcephaly and intrauterine growth retardation to a 1.2 Mb segment at 21.q22.2 that contains several genes including *DYRK1A* (Matsumoto et al., 1997). In 2008, Moller *et al.* identified two unrelated patients carrying a *de novo* balanced translocation that truncates *DYRK1A*. The overlapping phenotypic traits of these two patients, which include microcephaly, intrauterine growth retardation, and febrile seizures, indicated that *DYRK1A* haploinsufficiency alters brain development and function. Three years later, a study performed in a cohort of 3009 individuals with ID (intelligence quotient (IQ) below 70) identified one patient with a *de novo* microdeletion of 52 Kb affecting the last three exons of *DYRK1A* (van Bon et al., 2016). Depending on the clinical features presented by this patient and by previously reported patients with either chromosomal rearrangements that truncate *DYRK1A* (Moller et al., 2008) or with large deletions encompassing this gene (Fujita et al., 2010; Lyle et al., 2009; Matsumoto et al., 1997), van Bon *et al.* proposed that the heterozygous disruption of *DYRK1A* causes a distinctive clinical syndrome, characterized by the presence of mild to severe ID, microcephaly, intrauterine growth retardation, facial dimorphisms, impaired motor functions, and behavioral problems. The phenotype presented by haploinsufficient MNB/*Dyrk1a* flies and mice supported this hypothesis (Fotaki et al., 2002; Tejedor et al., 1995).

The sequencing of *DYRK1A* in a cohort of 105 patients with ID who exhibit two or more symptoms from the Angelman syndrome spectrum identified one patient with a frameshift *DYRK1A* variant affecting the N-terminal part of DYRK1A (Courcet et al., 2012). This patient presented epileptic seizures, speech delay, and other clinical manifestations compared to the patient described in the study by van Bon et al. (2011). On the basis of this and the phenotype of patients with microcephaly and large deletions encompassing more than one chromosome 21 gene (Oegema et al., 2010; Valetto et al., 2012; T. Yamamoto et al., 2011), Courcet *et al*, proposed that MRD7 (OMIM #614104) is caused by heterozygous disruption of the *DYRK1A* gene.

Three additional *de novo DYRK1A* pathogenic variants, one splice site variant and two frameshift variants predicted to delete parts of the DYRK1A protein that are important for its function (O'Roak et al., 2012), were identified by exome sequence analysis of a large number of families from the Simons Simplex Collection, which is a well-characterized cohort of patients with idiopathic autism spectrum disorder (ASD) (Fischbach & Lord, 2010). ASD is a complex and heterogeneous developmental disorder (DD) characterized by impairments in social interaction and communication and repetitive, restricted behaviors. ID and language delay are the most frequent comorbidities of the disorder (Lord and Bishop, 2015; Geschwind and State 2015). Interestingly, the three ASD probands carrying pathogenic *DYRK1A* variants show a complex phenotype that includes mild to severe ID, microcephaly, and impaired speech (van Bon et al., 2016). Since 2013, high-throughput (whole-exome or gene target) sequencing applied to cohorts of patients with DD/ID, with or without ASD, identified 42 *de novo* truncating variants (frameshift, splice–acceptor, splice–donor, and nonsense) and missense mutations (Bronicki et al., 2015; Dang et al., 2018; Ji et al., 2015; Luco et al., 2016; Redin et al., 2014; Ruaud et al., 2015; van Bon et al., 2016) in *DYRK1*A. Additional studies performed with larger cohorts of patients with DD, including the Deciphering Developmental Disorders Study (Deciphering Developmental Disorders, 2015), substantially increased the number of *DYRK1A* point mutations and small insertions and deletions (INDELs) associated with these disorders (De Rubeis, et al., 2014; Kosmicki et al., 2017; Stessman et al., 2017). These studies found that *DYRK1A* is one of the most frequent *de novo*-mutated genes in ASD (Iossifov et al., 2014; Kosmicki et al., 2017; Stessman et al., 2017; Yuen et al., 2014), thus accounting for 0.1–0.5% of the ASD population (van Bon et al. 2015) and for approximately 0.5% of syndromic ID (Evers et al., 2017).

DYRK1A-truncating variants are concentrated in the N-terminal region and the kinase domain of DYRK1A, whereas most missense mutations are within the kinase domain (Earl et al., 2017; Ji et al., 2015; Luco et al., 2016; van Bon et al., 2016) and are predicted to affect the enzymatic activity of the protein (Evers et al., 2017). Functional studies showed that missense *DYRK1A* variants might impair enzymatic function by affecting catalytic residues or by compromising the structural integrity of the DYRK1A kinase domain (Widowati, Ernst, Hausmann, Muller-Newen, & Becker, 2018).

The clinical phenotypes of patients with *DYRK1A de novo* pathogenic variants or chromosomal mutations involving this gene defined a distinct ID syndrome. Core symptoms of the *DYRK1A*-related syndrome (also known as *DYRK1A*-haploinsufficiency syndrome or MRD7) are microcephaly, intrauterine growth retardation, developmental delay, seizures, speech problems, ASD or ASD-related deficits (e.g. stereotypies and anxious behaviors), neonatal feeding problems, hypertonia, gait disturbances, and a characteristic dysmorphic facies (Bronicki et al., 2015; Earl et al., 2017; Ji et al., 2015; Luco et al., 2016; van Bon et al., 2016). Consistent with the effect of *Dyrk1a* haploinsufficiency on mouse brain development (Fotaki et al., 2002), several structural brain alterations including cerebral atrophy, hypoplasia of the corpus callosum, and a thin optic chiasm have been detected by magnetic resonance imaging (MRI) in some patients with mutations in *DYRK1A* (Ji et al., 2015; Kim, Lee, et al., 2017b). Clinical studies, especially brain imaging studies, in a larger group of patients, together with studies in cellular model systems and in animal models, like the *Dyrk1a*$^{+/-}$ mouse model that displays many of the neurological traits in *DYRK1A*-related

syndrome (Arque et al., 2008; Fotaki et al., 2002; Fotaki, Martinez De Lagran, Estivill, Arbones, & Dierssen, 2004), are necessary to understand the pathogenesis of the syndrome.

7. DYRK1A and Down syndrome

DS, most commonly resulting from a complete trisomy of HSA21, is associated with several alterations in brain development and function. The trisomy underlying DS has provided particular insight into how deregulated gene expression in the brain leads to altered brain function, specifically cognitive impairment.

7.1. Overexpression in the trisomic context

The *DYRK1A* gene is located on chromosome 21, thus suggesting that this gene may be overexpressed in cells from patients with DS. In a microarray study of mRNA from DS lymphoblastoid cells, 29% of expressed chromosome 21 transcripts were elevated in DS. Of these 29%, 22% were increased proportionally with gene dosage and 7% were amplified. In this cell type, *DYRK1A* was 1.4-fold overexpressed (Ait Yahya-Graison et al., 2007). In the brain of mice with three copies of the *Dyrk1a* gene, the *Dyrk1a* mRNA level was increased by 1.5-fold (hYACtgDyrk1a and mBACtgDyrk1a) (Guedj et al., 2009; Guedj et al., 2012) and the DYRK1A protein level was overexpressed by 1.6-fold (cortex), 1.9-fold (hippocampus), or 1.7-fold (cerebellum) fold in mBACtgDyrk1a mice. Similar overexpression levels were observed in models with partial trisomy (Ts65Dn and Dp(16)1Yey) (Souchet et al., 2014). DYRK1A expression in the brain of patients with DS-associated trisomy was also, on average, 1.5-fold elevated, and this overexpression was preserved across a wide range of ages (W. K. Dowjat et al., 2007).

7.2. Phenotypes in patients with DS

7.2.1. Genetics and analyses of partial duplications

DS occurs in 1 in every 750 live births and encompasses a constellation of features caused by partial or complete trisomy for chromosome 21 HSA. In particular, an altered copy number for segments of Hsa21 containing the *DYRK1A* gene can induce morphological defects and cognitive impairments (Delabar et al., 1993; Papoulidis et al., 2014; Rahmani et al., 1989; Ronan et al., 2007). However, the genotype–phenotype correlation approach is limited by the small number of partial trisomies and the heterogeneity of clinical phenotypic descriptions; another group of partial trisomies produced discrepant results (Korbel et al., 2009), which could not associate a specific set of genes with impairment of cognition. Linking phenotypes observed in trisomy 21 to the overexpression of *DYRK1A* can be done more efficiently by using two complementary strategies: i) analysis of mice models with different levels of DYRK1A and ii) assessing effects of treatments using DYRK1A inhibitors in mouse models and in patients with DS (see below).

Defects similar to those observed in DS have been reproduced in a number of different mouse models of DS (Ts1Rhr, Ts65Dn, Ts1Cje, Dp(16)1Yey) as well as in mice with altered copy numbers of *Dyrk1a* (hBACtgDyrk1a, hYACtgDyrk1a, mBACtgDyrk1a, *Dyrk1a*$^{+/-}$). Interestingly, a phenotype rescue experiment crossing Ts65Dn mice with mice monosomic for a 33-gene chromosomal segment containing *Dyrk1a* (Ms1Rhr) produced progeny with a normal learning phenotype, thus indicating that triplication of this 33-gene region produces the cognitive deficit (Belichenko et al., 2009). A complete phenotypic assessment of Ts1Rhr mice, trisomic for the 33-gene segment, showed that trisomy of this region is sufficient to produce significant alterations in behavioral tests such as the open-field, novel object recognition, and T-maze tasks. In Ts65Dn, Ts1Cje, and Ts1Rhr mice, LTP in the fascia dentata, a brain region critical to learning and memory, could be induced only after blocking GABA(A)-dependent inhibitory neurotransmission. In addition, widespread enlargement of dendritic spines and decreased density of spines in the fascia dentata were preserved (Haas et al., 2013). Thus, cognitive impairment in DS appears to derive from molecular and structural changes related to an altered copy number within this 33-gene region. Among the 33-genes in this region, *DYRK1A* is an attractive candidate for inducing brain structure alterations and cognitive impairment phenotypes.

7.2.2. Morphology

Brachycephaly and increased ventricle size have been reported in individuals with DS (Allanson, O'Hara, Farkas, & Nair, 1993; Pearlson et al., 1998; Schimmel, Hammerman, Bromiker, & Berger, 2006). Prenatal EGCG treatment and *Dyrk1a* dosage reduction modified craniofacial features in Ts65Dn mice and brain morphology in YACtgDyrk1a mice (Guedj et al., 2009; McElyea et al., 2016), thus suggesting that DYRK1A overexpression alters brain morphology.

7.2.3. Dendritic alterations

Several studies have reported abnormal cortical lamination patterns, altered dendritic arbors and spines, aberrant membrane electrophysiological properties, reduced synaptic density, and abnormal synaptic morphology in patients with DS (L. E. Becker, Armstrong, & Chan, 1986; Dierssen & Ramakers, 2006; Marin-Padilla, 1976; Takashima, Iida, Mito, & Arima, 1994). Alterations in the dendritic structure should have a major impact on the processing of afferent information by single neurons. At the level of the neuronal network, even modest alterations in dendritic structure and organization of many neurons, as seen in other MR, will lead to considerable changes in overall information processing.

7.2.4. Cognition

Trisomy 21 reduces IQ to between 20 and 80 (Anneren & Edman, 1993). In contrast to normally developing children and other cases of MR, there is a progressive IQ decline in DS beginning in the first year of life; therefore, the ratio of mental age to chronological age is not constant. By adulthood, IQ is usually in the moderate-to-severe level of impairment, thus suggestive of ID (IQ 25–55) with an upper limit on mental age of approximately 7–8 years, although a few individuals have IQs in the lower normal range (IQ 70–80) (Pennington, Moon, Edgin, Stedron, & Nadel, 2003).

A randomized, double-blinded, placebo-controlled, pilot study has shown preliminary results on the safety and clinical effects of EGCG, a DYRK1A inhibitor, in young adults with DS. A visual recognition task that measures visuoperceptual processing, the weakest component of visual memory in individuals with DS, was used. This measure has proven to be sensitive to hippocampal functioning, in particular of the perirhinal cortex, which is critically involved in object recognition memory and is reduced in size in DS. EGCG-treated individuals showed higher accuracy in visual memory recognition and spatial working memory, thus suggesting a positive effect of this compound on both the hippocampal and prefrontal system, in particular the ventromedial, ventrolateral, and dorsolateral cortices (De la Torre et al., 2014).

Following up on these promising results, a phase II study compared an "EGCG plus cognitive training" group and a "placebo plus cognitive training" group. This study showed that patients in the EGCG group performed better than those in the placebo group in some cognitive tests and in adaptive behavior after the 12 months of treatment. The EGCG and cognitive training group had better preservation of recognition memory tasks and improvement in executive function than the placebo and cognitive training groups.

In both trials, the main effect of EGCG was the improvement of immediate recognition memory. This measure is sensitive to hippocampus, in particular, perirhinal cortex activity, in addition to regions such as the ventromedial cortices. In DS, the altered function of the hippocampus and prefrontal cortex contributes to memory and executive functioning deficits and to distinct connectivity disturbances in frontal and anterior temporal structures. It was proposed that the efficacy of EGCG depends, at least partly, on the inhibition of DYRK1A kinase

activity. Total homocysteine plasma concentrations, used as a surrogate biomarker of DYRK1A kinase activity, were increased in the "EGCG and cognitive training" group to levels not observed in the "placebo and cognitive training" group and returned to baseline concentrations after discontinuation of treatment (de la Torre, et al., 2016). However, in this study, a reduction in total cholesterol and oxidized LDL concentrations was also noted. Thus, a lipid-lowering effect combined with a reduced lipid oxidation might contribute to the therapeutic effect. Other mechanisms of action of EGCG should not be disregarded, including epigenetic, protection from mitochondrial dysfunction, and antioxidant effects (Vacca & Valenti, 2015).

7.3. Phenotypes in murine models

7.3.1. Morphogenesis

Comparison of mice models with increased (hYACtgDyrk1a, hBACtgDyrk1a, and mBACtgDyrk1a) and decreased (*Dyrk1a*$^{+/-}$) gene copy number suggests that DYRK1A controls brain morphogenesis: global brain region-specific variations observed in gain-of-function models mirror their counterparts in the loss-of-function model (Ahn et al., 2006; Guedj et al., 2012; Sebrie et al., 2008). Increased ventricle size has been observed in coronal sections from BACtgDyrk1a and through *in vivo* MRI from YACtgDyrk1a mice (Guedj et al., 2012). This phenotype is also present in Ts65Dn, Ts1Cje, Ts2Cje mice, and additional trisomic mouse models that contain additional copies of orthologous regions of HSA21 (Ishihara et al., 2010). In the above described trisomy models, the dorsal and lateral portions of the third ventricle are more enlarged than the ventral portion.

7.3.2. Neurogenesis

As in the TgBACDyrk1a mouse, cortical neurogenesis in Ts65Dn and Ts1Cje trisomic embryos is impaired (Chakrabarti, Galdzicki, & Haydar, 2007). Moreover, Ts65Dn apical progenitors have longer cell cycles and less nuclear Cyclin D1 than the euploid littermates (Chakrabarti et al., 2007; Najas et al., 2015). Remarkably, the genetic normalization of *Dyrk1a* gene dosage in Ts65Dn embryos increases the amount of Cyclin D1 to normal levels and normalizes the production of early-born cortical neurons (Najas et al., 2015). The importance of DYRK1A overexpression in DS cortical neurogenesis is supported by the effect on the expansion of the cortical wall produced by the treatment of Ts1Cje embryos with a potent inhibitor of DYRK1A kinase activity (Nakano-Kobayashi et al., 2017). These results indicate that alterations in the formation of the neocortex in DS start early in development and that triplication of the *DYRK1A* gene contributes to the neurogenic cortical defects associated with this syndrome.

Ts65Dn mice also exhibit postnatal neurogenic defects, but treatment of Ts65Dn pups from day 3 to day 15 with the DYRK1A-inhibiting phytochemical EGCG restored neurogenesis; total hippocampal granule cell number; and levels of pre- and postsynaptic proteins in the DG, hippocampus, and neocortex of these animals (Stagni et al., 2016). Genetic normalization of *Dyrk1a* expression in Ts65Dn mice restored the proliferation and differentiation of hippocampal cells in the adult DG and the density of GABAergic and glutamatergic synapse markers in the molecular layer of the hippocampus (Garcia-Cerro et al., 2014). The increased dosage of *Dyrk1a* in the Ts1Cje mouse model of DS augments the propensity of progenitors to differentiate into astrocytes. This tendency is associated with enhanced astrogliogenesis in the developing neocortex, which is likely due to the potentiation of the DYRK1A-STAT pathway in progenitors (Kurabayashi, Nguyen, & Sanada, 2015). This work indicates that overexpression of DYRK1A likely contributes to the aberrant astrogliogenesis associated with DS.

7.3.3. Differentiation

The density of interneurons that control the level and type of inhibition is significantly increased in layers 3-6 of the somatosensory cortex of Ts65Dn mice (Perez-Cremades et al., 2010). The density of GABAergic synapse markers is also increased in the inner molecular layer of the hippocampus (Martinez-Cue et al., 2013). Similar density changes have been reported for the stratum radiatum of YACtgDyrk1a, BACtgDyrk1a, and Dp(16)1Yey (Souchet et al., 2014) mice, thus suggesting that this phenotype is strongly linked with *Dyrk1a* overexpression.

7.3.4. Learning and plasticity

A functional screen using yeast artificial chromosomes covering 2 Mb of human 21q22.2 identified a 180 kb fragment containing *DYRK1A* that associated with learning defects in the Morris water maze (MWM) (Smith et al., 1997). Additionally, animals overexpressing the full-length mouse *Dyrk1a* cDNA exhibit altered motor skill acquisition and impaired spatial learning and cognitive flexibility in the MWM (Altafaj et al., 2001). Transgenic mice containing one extra copy of the human *DYRK1A* gene display significant impairment in hippocampal-dependent memory tasks in the MWM. Interestingly, shifts in both long-term potentiation and long-term depression were observed in these animals (Ahn et al., 2006). In addition to alterations in LTP and LTD in the prefrontal cortex, the BACtgDyrk1a mouse also showed impairment in motor learning and novel object recognition task (Souchet et al., 2014; Thomazeau et al., 2014).

Mice with partial trisomy, which more closely mimics the alterations seen in DS, have also been used to study the role of *Dyrk1a* overexpression in specific phenotypes. In these animals, genetic or pharmacological correction of DYRK1A levels not only corrected the defects in neurogenesis but also the excitation–inhibition imbalance, density of GABAergic and glutamatergic markers, and learning impairment (Catuara-Solarz et al., 2016; De la Torre et al., 2014; Garcia-Cerro et al., 2014; Guedj et al., 2009; Nakano-Kobayashi et al., 2017; Souchet et al., 2015).

8. Neurodegenerative diseases

8.1. AD and DS with AD

The cytoplasm and the nuclei of scattered neurons of the neocortex, entorhinal cortex, and hippocampus of patients with AD, DS, and Pick's Disease have increased DYRK1A immunoreactivity. DYRK1A is found in sarkosyl-insoluble fractions, which are enriched in phosphorylated tau in AD brains, thus suggesting a possible association of DYRK1A with neurofibrillary tangle pathology (Ferrer et al., 2005). Compared to control levels, the *DYRK1A* mRNA level in the hippocampus is significantly elevated in patients with AD (Kimura et al., 2007). In the AD brain, overactivation of calpain is associated with truncation of the C-terminus of DYRK1A. Quantification of the different forms of DYRK1A suggests that AD is associated with a decrease in the full-length DYRK1A protein and an increase in the truncated form, which is still active (Jin et al., 2015). In parallel with proteomic studies, genetic studies have tried to establish a link between *DYRK1A* variants and AD. A study in a Japanese population reported a clear association between *DYRK1A* and AD risk, but the risk genotype (rs2835740) was not associated with increased expression of *DYRK1A*, thus suggesting that increased *DYRK1A* expression is induced by AD (Kimura et al., 2007). In a case–control Spanish cohort, a study examined genetic variations in *DYRK1A* by genotyping and haplotype tagging SNPs and detected no difference between the patient and control groups either overall or after stratification by the APOE epsilon4 allele (Vazquez-Higuera et al., 2009) (Fig. 5).

8.1.1. Tau

Cellular shape is mainly determined by microtubules. MAPs are responsible for brain microtubule stabilization and maintainance of the assembled tubulin polymers. Tau is one of the most widely studied MAPs. Tau was originally found to enhance microtubule stability and

polymerization but is currently known to have a number of other functions including controlling microtubule modifications, altering the stiffness and mechanical properties of the microtubule polymer, controlling the spacing of microtubules within axons, and regulating microtubule motor transport (Ramkumar, Jong, & Ori-McKenney, 2018). The DYRK1A protein can modify the functional properties of Tau by two different mechanisms that are altered in AD: i) *Tau* splicing and ii) Tau phosphorylation.

8.1.1.1. Tau splicing. Six different isoforms of *Tau* generated through alternative splicing are expressed in the adult human brain (Goedert, Spillantini, Jakes, Rutherford, & Crowther, 1989). Inclusion or exclusion of *Tau* exon 10 (E10), which encodes the second microtubule-binding repeat, gives rise to Tau isoforms with either four (4R) or three (3R) microtubule-binding repeats. Almost equal levels of 3R-tau and 4R-tau are expressed in the normal adult human brain. Mutations in the *Tau* gene associated with frontotemporal dementia FTDP-17 cause dysregulation of *Tau* E10 splicing. An altered 3R-to-4R ratio in the brain also occurs in corticobasal degeneration, Pick's disease, and progressive supranuclear palsy. DYRK1A plays a very significant role in multidimensional control of tau isoforms through various splicing factors. Of note, expression of 3R-tau can be modulated by peptide amyloid β (Aβ), and 3R-tau levels increase with the progression of AD. This increase might also result from aberrant DYRK1A levels. Alternative splicing is controlled by both exonic and intronic enhancers and silencers, which are controlled upstream by splice factors. DYRK1A phosphorylates the splicing factor ASF at different residues (see part 2) and inhibits the ability of ASF to promote *tau* E10 inclusion (Shi et al., 2008). DYRK1A phosphorylates ASF, thereby driving the splice factor into nuclear speckles. Moreover, phosphorylation of ASF by DYRK1A inhibits their association with nascent *tau* transcripts, thus increasing 3R-tau levels and causing an imbalance of the 3R-4R tau isoforms (Wegiel, Kaczmarski, et al., 2011). DYRK1A regulation of 9G8 activity through phosphorylation modulates *tau* E10 splicing (Ding et al., 2012). SC35 phosphorylation by DYRK1A suppresses the ability of SC35 to promote *Tau* E10 inclusion, whereas downregulation of DYRK1A promotes 4R-Tau expression (Qian et al., 2011). Phosphorylation by DYRK1A also inhibits SRp55 ability to promote *Tau* exon 10 inclusion. Upregulation of *DYRK1A*, as in DS, could lead to neurofibrillary degeneration by altering the alternative splicing of *tau* exon 10 and increasing the 3R-tau-to-4R-tau ratio (Yin et al., 2012). Treatment with EGCG from gestation to adulthood suppressed 3R-tau expression in mice (Yin et al., 2017).

8.1.1.2. Tau phosphorylation. The accumulation of paired-helical filaments (PHF) is the most characteristic neuropathological lesion of AD, including AD in DS. Abnormally phosphorylated Tau is a component of PHF (Bancher et al., 1991; Grundke-Iqbal et al., 1986; Hanger et al., 1991). DYRK1A phosphorylates human tau at Thr212 *in vitro*, a residue that is phosphorylated in fetal tau and hyperphosphorylated in filamentous tau from AD brain. Phosphorylation of Thr212 primes tau for phosphorylation by GSK3 at Ser208 in vitro (Woods, Cohen, et al., 2001a). In a screen of 572 kinases in an AD model, Azrosa et al. found increased levels of *Dyrk1a* mRNA in the brain of Tg-PS1/APP mice and revealed that DYRK1A is involved in tau phosphorylation pathways (Azorsa et al., 2010). Consistent with this, an elevated *DYRK1A* mRNA level was associated with tau phosphorylation at Thr212 (Kimura et al., 2007). DYRK1A also phosphorylates other tau residues. These sites are phosphorylated in adult DS brains but not in age-matched controls. Increased expression of DYRK1A in Ts65Dn mice is also associated with increased phosphorylation of tau (F. Liu et al., 2008). In cell cultures, DYRK1A inhibition by harmine reduced Tau phosphorylation at multiple AD-related sites (Frost et al., 2011). Finally, DYRK1A inhibition reduced β-amyloid and tau pathology in mouse models of AD (Branca et al., 2017; Naert et al., 2015).

8.1.2. APP processing

DYRK1A phosphorylates APP on threonine 668 *in vitro* and in mammalian cells. The amounts of phospho-APP and Aβ are increased in the brains of transgenic mice overexpressing human DYRK1A, and the amounts of phospho-APP as well as those of APP are elevated in human DS brains (Ryoo et al., 2008).

APP function may also be indirectly affected by changes in Tau, which, as discussed above, can be modulated by DYRK1A. Tau overexpression and/or mutations impair axonal transport. A study that used

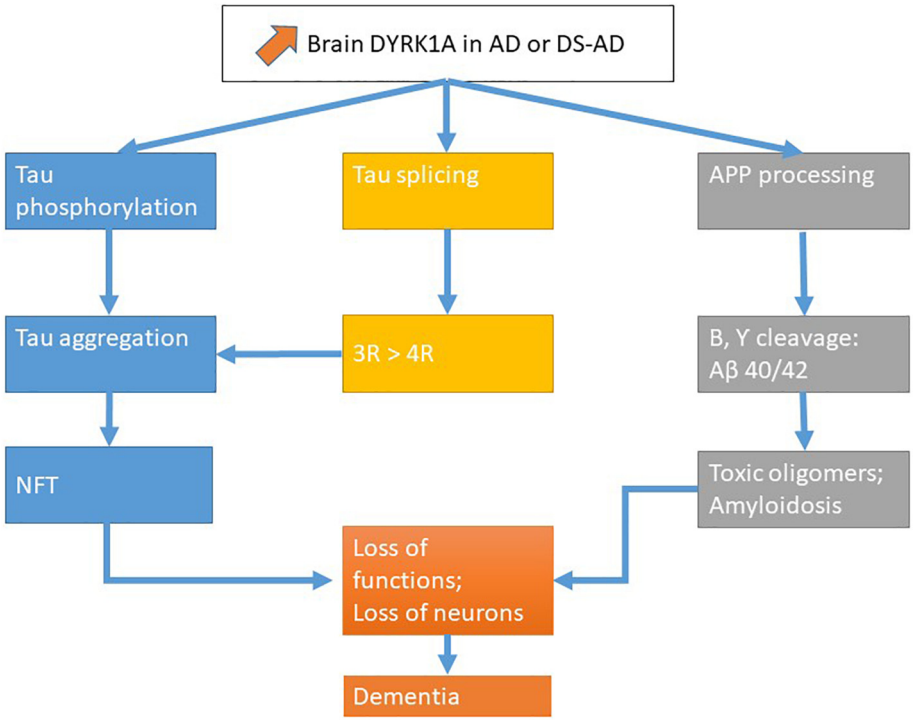


Fig. 5. Consequences of increased DYRK1A level on β-amyloidosis and neurofibrillary degeneration.

a trans-splicing strategy to modulate Tau exon 10 inclusion/exclusion in differentiated human-derived neurons found that 3R-tau favored anterograde movement of APP vesicles, thus suggesting that an alteration of the 3R-to-4R ratio may alter APP metabolism (Goldstein, 2012; Lacovich et al., 2017).

DYRK1A might also affect the degradation of Aβ. Gene expression of the major Aβ-degrading enzyme NEP in sporadic AD brains is decreased beginning at the early stages of disease development, and there is an inverse relationship between *MME* (*NEP*) gene expression and Aβ accumulation, thus indicating that the downregulation of NEP is at least one cause of sporadic AD. NEP is downregulated in fibroblasts of patients with DS compared to fibroblasts from healthy controls. Treatment with the DYRK1A inhibitor harmine and knockdown of the *DYRK1A* gene upregulate NEP in fibroblasts (Kawakubo et al., 2017). Recently, EGCG was shown to induce extracellular degradation of amyloid β-protein by increasing NEP secretion from astrocytes through the activation of ERK and PI3K pathways (N. Yamamoto et al., 2017).

Normalizing *Dyrk1A* gene dosage in aged Ts65Dn mice protected them from senescent cell density in the cingulate cortex, hippocampus, and septum; prevented cholinergic neuron degeneration; and reduced APP expression in the hippocampus, Aβ load in the cortex and hippocampus, the expression of phosphorylated Tau in Ser202 in the hippocampus and cerebellum, and the levels of total tau in the cortex, hippocampus, and cerebellum (Garcia-Cerro, Rueda, Vidal, Lantigua, & Martinez-Cue, 2017). Proposed consequences of increased DYRK1A level on β amyloidosis and neurofibrillary degeneration are schematized in Fig. 5.

8.1.3. Peripheral DYRK1A

A search for novel peripheral biomarkers in plasma from two different cohorts consisting of patients with AD and control patients revealed that the DYRK1A level is decreased in AD and also in AD with mild cognitive impairment. This decrease is also present in lymphoblastoid cell lines derived from patients with AD. Combining the assessment of DYRK1A with BDNF and homocysteine, two DYRK1A-related markers, provides high sensitivity, specificity, and accuracy for detecting AD. Further investigations are needed to identify the cause of these variations (Janel et al., 2014; Janel et al., 2017) and to classify plasma DYRK1A as a diagnostic or prognostic marker.

8.2. α-Synuclein dementias

Synuclein is a soluble, natively unfolded protein that is highly enriched in the presynaptic terminals of neurons in the CNS. Dementia with Lewy bodies (LB) is characterized by α-synuclein (SNCA) accumulation and degeneration of dopaminergic and cholinergic pathways, and Ser129 in SNCA is selectively and extensively phosphorylated in synucleinopathic lesions (Fujiwara et al., 2002). In a *Drosophila* model of PD, Ser129 phosphorylation is crucial for mediating α-synuclein neurotoxicity and inclusion formation (L. Chen & Feany, 2005). DYRK1A phosphorylates α-synuclein in transformed and primary neuronal cells on Ser87, which leads to α-synuclein aggregation and a decrease in cell viability (E. J. Kim et al., 2006). Furthermore, phosphorylation of α-synuclein at Ser129 promoted fibril formation *in vitro*. *In vitro* kinase assays of anti-DYRK1A immunocomplexes demonstrated that DYRK1A can phosphorylate α-synuclein at Ser87. It is not yet clear whether DYRK1A can also phosphorylate Ser129. It was proposed that α-synuclein neurotoxicity in PD and related synucleinopathies may result from an imbalance between the detrimental, oligomer-promoting effect of Ser129 phosphorylation and the neuroprotective action of Tyr125 and Ser87 phosphorylations that inhibit toxic oligomer formation (L. Chen et al., 2009). A pilot study identified the DYRK1A rs8126696 polymorphism as a risk factor for developing an α-synuclein-associated dementia (Jones, Aarsland, Londos, & Ballard, 2012). Collectively, the above data indicate that DYRK1A in pathological brain regions may contribute to aggregations of SNCA peptides, which are a major component of amyloid plaques in the brains of patients with AD (Irwin, Lee, & Trojanowski, 2013).

8.3. Parkinson's disease

PD is a long-term degenerative disorder of the CNS that mainly affects the motor system. The motor symptoms of PD are the result of reduced dopamine production in the basal ganglia of the brain. Cognitive and behavioral problems such as dementia and depression often arise as the disorder develops.

The *DYRK1A* rs8126696 T allele was associated with early onset in a cohort 297 Chinese patients with PD (Fan et al., 2016b). An additional study in the Chinese Han population identified the TT genotype derived from SNP rs8126696 of the *DYRK1A* gene as a possible risk factor for developing sporadic PD, especially for men (Cen et al., 2016). Increased DYRK1A expression in the BACtgDyrk1a mouse model is associated with increased survival of mesencephalic dopaminergic neurons (mDA) of animals injected with 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), a toxin that activates caspase-9-dependent apoptosis in mDA neurons (Barallobre et al., 2014).

DYRK1A may influence the behavior of parkin, the protein product of the first gene known to cause autosomal recessive familial PD. Multiple kinases phosphorylate parkin at several distinct sites and regulate its ubiquitin E3 ligase activity. DYRK1A directly phosphorylates parkin at Ser131 *in vitro*, which inhibits the E3 ubiquitin ligase activity of parkin and, consequently, its neuroprotective function in dopaminergic SH-SY5Y cells exposed to 6-hydroxydopamine (Im & Chung, 2015). In PD, α-synuclein aggregates often contain and sequester Septin4 (Sept4), a polymerizing scaffold protein. In a yeast two-hybrid screen, Septin4 was identified as a DYRK1A-binding partner, which colocalizes with DYRK1A in mouse neurons. DYRK1A phosphorylation of septin4 is inhibited by harmine (Sitz et al., 2008), thus suggesting a role for DYRK1A in health of dopaminergic neurons.

9. Inhibitors

9.1. DYRK1A inhibitors and their bioavailability

The elevated activity of DYRK1A in several human diseases makes DYRK1A an attractive potential target for therapeutic drugs, and much effort has been applied toward developing DYRK1A inhibitors. The chemical compounds shown to inhibit DYRK1A in living cells are listed in Table 3. In this chapter, we focus on chemicals applicable to disease models and discuss issues relating to their future application.

As mentioned before, prenatal exposure to green tea polyphenols protects from brain defects induced by overexpression of DYRK1A (Guedj et al., 2009), particularly EGCG, which is the most abundant catechin in green tea and a known DYRK1A inhibitor (Bain et al., 2007; Bain, McLauchlan, Elliott, & Cohen, 2003). Of note, EGCG is a noncompetitive inhibitor, thus suggesting that regions outside the DYRK1A active site are important for its enzymatic activity. However, EGCG has profound effects on other signaling pathways. EGCG is an antioxidant/metal chelator and a known inhibitor of proteasomes, matrix metalloproteinase, dihydrofolate reductase, DNA methyltransferase, topoisomerase II, and telomerase (Khan, Afaq, Saleem, Ahmad, & Mukhtar, 2006; Mandel, Amit, Weinreb, Reznichenko, & Youdim, 2008), and it is difficult to attribute its pharmacological effect solely to the inhibition of DYRK1A.

Harmine is a β-carboline alkaloid that inhibits the kinase activity of DYRK1A and interferes with neurite formation (Gockler et al., 2009). Harmine has psychotropic effects owing to its inhibitory activity on monoamine oxidase A (MAO-A); therefore, groups have applied structure–activity relationship (SAR) analysis of harmine derivatives to design DYRK1A inhibitors without MAO-A inhibitory activity (Drung et al., 2014; Ruben et al., 2015). Meridianin is a natural alkaloid from the marine tunicate (Giraud et al., 2011; Yadav et al., 2015) shown to inhibit DYRK1A. Additionally, Leucettine L41 was extracted and

Table 3
DYRK1A inhibitors.

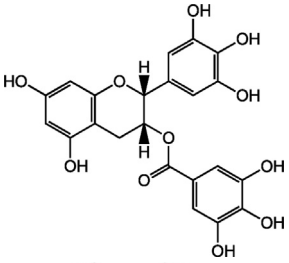
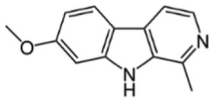
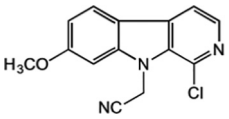
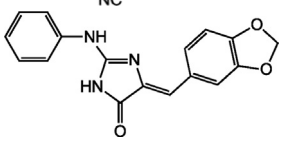
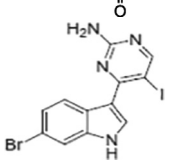
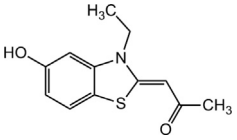
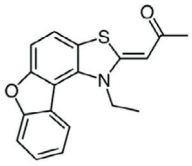
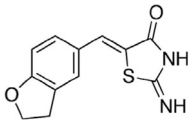
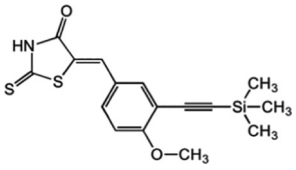
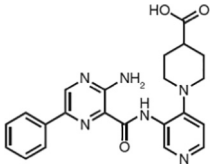
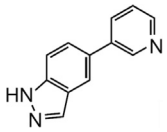
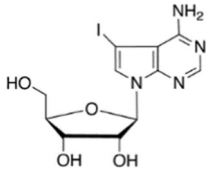
	Structure	Specificity (other targets except for Clks)	Bioavailability and disease models tested	References
From natural products and their derivatives				
EGCG		Proteasomes matrix metalloproteinase dihydrofolate reductase DNA methyltransferase topoisomerase II telomerase COMT Vimentin	Yes, but low DS Cancer	(Bain et al., 2003; Bain et al., 2007)
Harmine (β-carboline)		MAO-A	Yes (plant alkaloid)	(Gockler et al., 2009)
AnnH75 (β-carboline)		Haspin	ND	(Ruben et al., 2015)
Leucettine L41		GSK3α/β	Yes AD model	(Debdab et al., 2011; Naert et al., 2015; Tahtouh et al., 2012)
Meridianin (31)		CDK5	ND	(Giraud et al., 2011; Yadav et al., 2015)
INDY type (TG003 derivatives)				
INDY		Pim1, CDK2, CK1, GSK3α/β[a]	No INDY tested in *Xenopus* embryos with DYRK1A-overexpression	(Ogawa et al., 2010)
BINDY		Pims, CHK2, Pims, CDK2, FLT3[b]	ND	(Masaki et al., 2015)
Targeting DYRK1A protein stability				
CaNDY		Haspin, CK2, CDK9[b]	ND	(Sonamoto et al., 2015)
FINDY		Gsk3β, MARK4, Pims, PLK3[a]	ND	(Kii et al., 2016)
From cell-based functional assay				
GNF7156		GSK3β	ND Diabetes	(Shen et al., 2015)
ALGERNON		CDK2, CDK5, Haspin, HGK, ROCKs[b]	Yes DS	(Nakano-Kobayashi et al., 2017)

Table 3 (*continued*)

Structure	Specificity (other targets except for Clks)	Bioavailability and disease models tested	References
			
From the drug repurposing			
5-IT 	GSK3β	Yes Diabetes	(Dirice et al., 2016)

[a] Determined by kinase panel assay at 10 μM (INDYand FINDY).
[b] 1 μM (BINDY, CaNDY, and ALGERNON) concentrations of compounds.

optimized from marine sponges, thus showing potent inhibitory activity against DYRK1A in cell culture and in brain slices (Debdab et al., 2011; Tahtouh et al., 2012). L41 treatment rescued memory impairment in Aβ-injected mice (Naert et al., 2015), thus indicating its bioavailability.

Based on the structural similarity of the ATP-binding pocket of DYRKs and CLKs, INDY (inhibitor of DYRKs) was developed by altering the CLK inhibitor TG003 to enhance its specificity for DYRKs (Ogawa et al., 2010). INDY has potent inhibitory effects on DYRK1A in cultured cells and prevented the abnormal development of *Xenopus laevis* embryos overexpressing *Dyrk1a*. On the basis of the crystal structure of the DYRK1A/INDY complex, the novel inhibitor BINDY was designed and synthesized by replacing the phenol group of INDY with dibenzofuran to produce the BINDY derivative. Treatment of 3T3-L1 preadipocytes with BINDY-hampered adipogenesis was by suppressing gene expression of the critical transcription factors PPARγ and C/EBPα (Masaki et al., 2015). GMF7156 (along with GMF4877) was identified by screening of the pancreatic β-cell proliferation-promoter for insulin secretion in diabetes (Shen et al., 2015). Two new inhibitors, FINDY and CaNDY, targeting DYRK1A protein stability/maturation have been developed. These inhibitors suppress intramolecular autophosphorylation of Ser97 in DYRK1A and antagonize the interaction of DYRK1A and the kinase-specific co-chaperone CDC37, thus leading to the degradation of DYRK1A in living cells (Kii et al., 2016; Sonamoto et al., 2015). DANDY was also obtained from the search of the common structure supposedly inhibiting DYRK1A (Gourdain et al., 2013). Recently, its effectiveness in animals has been reported in a DS model with 2 weeks of administration (Neumann et al., 2018).

Additional inhibitors have been identified by screening for promoter activity of specific target genes. GMF7156 (along with GMF4877) was identified by screening of the pancreatic β-cell proliferation-promoter for insulin secretion in diabetes (Shen et al., 2015). ALGERNON was first identified from the screening of proliferation-enhancer in NSCs, thereby aiming to correct the aberrant neurogenesis observed in individuals with DS/DS murine models (Nakano-Kobayashi et al., 2017). ALGERNON is water soluble, distributes to the brain tissue after oral administration, and exhibits good pharmacokinetics. ALGERNON enhances the proliferation of NSCs in the DG of the mouse hippocampus. ALGERNON treatment of pregnant dams prevented morphological brain abnormalities, including the thinned cortical plate, typically observed in Ts1Cje embryonic mice. Remarkably, when compared to untreated trisomic offspring, ALGERNON-treated mice exhibited normal cognitive behavior.

Finally, a DYRK1A inhibitor was identified by a drug repurposing strategy. 5-Iodotubercidin (5-IT), an annotated adenosine kinase inhibitor previously reported to increase proliferation of islets, was found to be a potent and selective inhibitor of DYRK1A and proven to be effective in diabetes models (Dirice et al., 2016).

9.2. Future of DYRK1A inhibitors: possible applications and safety

Considering the involvement of DYRK1A in psychiatric/developmental diseases such as DS and AD, clinicians and patients have long anticipated the development of practical DYRK1A inhibitors. Of note, a clinical trial of EGCG in patients with DS has been conducted as discussed in section 7 (de la Torre, et al., 2016). EGCG supplementation for 12 months significantly improved visual recognition memory and adaptive behavior after cognitive training compared to those parameters in untreated controls.

ALGERNON is a more specific inhibitor of DYRK1A than EGCG and, as discussed above, has been used prenatally to prevent cognitive deficits in the Ts1Cje mouse model (Nakano-Kobayashi et al., 2017), thus suggesting the possibility of a prenatal therapy for DS. Prenatal treatment is not realistic at present, but ALGERNON also stimulates adult neurogenesis meaning that ALGERNON has therapeutic potential not only for the treatment of DS but also for a wide range of disorders involving progressive or permanent neuronal loss, including neurodegenerative diseases and traumatic brain injury. ALGERNON may also be applicable for treating the consequences of Zika virus infection. Zika is associated with an increased rate of microcephaly, and DYRK1A is reported to be upregulated in Zika-infected human neural progenitor cells that exhibit dysregulated cell cycle progression and attenuated growth (Tang et al., 2016).

Before DYRK1A inhibitors can be applied for therapeutic purposes, we need to consider that these inhibitors might be double-edged swords. DYRK1A has crucial roles in brain development, synaptic maturation, and functions (as discussed in previous chapters), and excess inhibition of DYRK1A could lead to deleterious side effects. Truncating mutations that reduce *DYRK1A* gene dose cause microcephaly and autism in humans, and loss-of-function mutations in *DYRK1A Drosophila* and mice orthologs produce defects in neurogenesis (Courcet et al., 2012; O'Roak et al., 2012). Of note, WT offspring of pregnant mice treated with ALGERNON trended toward impaired learning behaviors (Nakano-Kobayashi et al., 2017), which is reminiscent of MDR7 patients with known *DYRK1A* disruptions (Bronicki et al., 2015; Moller et al., 2008; Oegema et al., 2010; Ruaud et al., 2015; van Bon et al., 2016). However, there is information indicating the safety of ALGERNON; prenatal administration of ALGERNON in mice had no effect on the pup weight or other physical or behavioral factors, namely, including rectal temperature; righting reflex; whisker twitch; ear twitch; reaching behavior; wire hang performance; grip strength; reaction to key jangling

(Nakano-Kobayashi et al., 2017); and the pup's performance in several behavioral tests, including the open field test, light/dark transition test, elevated plus maze test, Crawly's social interaction test, tail suspension test, and forced swim test (Nakano-Kobayashi et al., unpublished data). Effective biomarkers that reflect DYRK1A activity are needed to establish the clinical therapeutic potential of these DYRK1A inhibitors.

10. Conclusion

The large number of substrates phosphorylated by DYRK1A and the wide range of interacting partners indicate that DYRK1A is capable of controlling a variety of molecular processes. These processes underlie several physiological functions at different stages of life: during neurogenesis at early development, in neuronal plasticity during brain functioning, and during aging. Analysis of chromosomal rearrangements and mutations have revealed that a deficit in the *DYRK1A* gene dosage is a likely cause of MRD7, a form of autism, and an excess in gene dosage is a likely cause of the cognitive alterations present in DS. These observations indicate that DYRK1A is a critical gene for synaptopathies. The role of DYRK1A in synaptic plasticity has been confirmed in models designed to reproduce alterations of DYRK1A levels in *Drosophila*, mice, and zebrafish. Many reports from humans and animal models also indicate a role for DYRK1A during neurodegenerative processes: with abnormal truncation events or abnormal peripheral levels of DYRK1A in patients with AD or with altered phosphorylation of DYRK1A targets involved in α-synuclein dementias or in PD.

The molecular pathways controlling the DYRK1A level and the overall molecular and regulatory controls exerted by DYRK1A on cognitive processes remain to be completely established. Evidence for crosstalk between DYRK1A-related pathways and for regulatory loops is still emerging.

The role of DYRK1A in developmental and neurodegenerative diseases makes this protein kinase an attractive drug target to not only normalize prenatal development and improve cognition in youths and adults with DS but also treat neurodegenerative diseases. However, considering the complexity of DYRK1A targets, adverse effects could be a key issue in clinical trials. Therapeutic risks may arise from i) off-target drug effects, as it is known that other serine threonine kinases can be targeted by DYRK1A inhibitors or ii) excessive inhibition, as it has been shown that severe developmental anomalies can arise when DYRK1A levels fall below normal. In the case of AD, inhibition in the periphery might be associated with adverse effects, for example, inducing an increase in homocysteine. Therefore, the proper objective is to achieve a balanced level of DYRK1A, and this will depend on the controlled use of existing or novel inhibitors; conversely, in the case of MRD7 or other conditions with decreased DYRK1A levels, therapeutic perspectives will rely on the discovery of activators.

Conflict of interest

The authors do not have any conflict of interest to declare.

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