DYRK1A-mediated Cyclin D1 Degradation in Neural Stem Cells Contributes to the Neurogenic Cortical Defects in Down Syndrome

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abstract

Alterations in cerebral cortex connectivity lead to intellectual disability and in Down syndrome, this is associated with a de cit in cortical neurons that arises durl16(h)23 TJ 0 -1.328 TD [(me)21(ch)21(an)15(is)25(ms)]

Sweet, 2009; Rubenstein, 2010; Sun and Hevner, 2014).

Around 80% of neocortical neurons are excitatory projection neurons that extend axons to distant intracortical targets and to subcortical regions, whilst the remainder are inhibitory interneurons involved in local circuits (DeFelipe et al., 2013; Greig et al., 2013). The distinct types of projection neurons are produced in the dors alon (pallium) of the embryo from multipotent neural known as radial glia (RG), and from more restricted p termediate progenitors (IPs). These neurons are gen outside pattern, rst generating the neurons that for to the ventricle (layer VI) and lastly those that form th layers (Layers II…III). Projection neurons within a la molecular characteristics and connectivity patterns, at they form (Greig et al., 2013; Molyneaux et al., 2

During neurogenesis, RG progenitors divide as ventricular zone (VZ) producing another RG cell an or an IP (Noctor et al., 2004; Haubensak et al., 2004). This progenitor moves to a more basal pro subventricular zone (SVZ), where it divides symme a pair of neurons directly or it does so after 1 to 3 ro amplifying divisions (

present at the onset of neurogenesis, as well as to the number of VZ (apical) and SVZ (basal) proliferative and neurogenic divisions (Huttner and Kosodo, 2005; Noctor et al., 2001, 2007).

Regulation of the cell cycle, and particularly of the G1 phase of the cell cycle, is important for the normal expansion of the neocortex in both rodents and primates (Dehay and Kennedy, 2007). G1 is a critical phase, integrating extracellular signals that induce either commitment to a further round of cell division, or withdrawal from the cell cycle and differentiation (Cunningham and Roussel, 2001; Dehay and Kennedy, 2007; Salomoni and Calegari, 2010; Zetterberg et al., 1995). Pioneering cumulative S-phase labelling experiments performed in the mouse embryo showed that as neurogenesis progresses the cell cycle of neocortical progenitors extends due to a progressive lengthening of the G1 phase (Takahashi et al., 1995). Moreover, there is evidence of a correlation between cell cycle length and neurogenesis, which has led to the formulation of the cell cycle length hypothesis (Gotz and Huttner, 2005). According to this hypothesis, the time that a progenitor spends in G1 determines the nal effect of a particular cell fate determinant, which could be equivalent (symmetric divisions) or distinct (asymmetric divisions) in the two daughter cells (Dehay and Kennedy, 2007; Gotz and Huttner, 2005; Salomoni and Calegari, 2010). Indeed, it was more recently shown that manipulating the duration of the G1 phase in neocortical apical progenitors alters the production of1pn3(i)-2Ny7(pa)dl2(t)-57(pa)dd p7(pa)dsarls (

DYRK1A have longer S phases than wild-type progenitors (Supplementary Fig. 4D). The increase in S phase duration calculated in this experiment (28% increase with respect to the wild-types) was similar to the increase calculated by assessing the accumulation of EdU (35%; Fig. 2E). However, we did nd signi cant differences between the Ts values calculated in the two experiments (Fig. 2E and Supplementary Fig. 4D). This

Therefore, to estimate RG-derived IP production at this developmental stage, we counted the $Tbr2^+$ cells that did not express the neuronal marker Tuj1 (arrows in Fig. 3D). The cell counts showed that Tg Dyrk1a embryos had more IPs than the wild-types (Fig. 3D). Together, these observations suggest that the de cit of early-born cortical neurons in the transgenic condition may result from an increased proportion of RG proliferative divisions at the expense of the neurogenic divisions (Fig. 3E). As progenitors undergoing proliferative divisions have longer S phases than the ones undergoing neurogenic divisions (Arai et al., 2011), the bias observed in the division mode of Tg Dyrk1a RG progenitors was consistent with the increased duration of the S phase in these progenitors (Fig. 2E and Supplementary Fig. 4).

If DYRK1A-induced degradation of Cyclin D1 is the mechanisms by which this kinase regulates G1 phase duration in RG progenitors and hence the fate of their daughter cells, lowering DYRK1A protein levels in these progenitors should also modify the proportion of neurons and IPs they produce. To test this prediction we did the same quanti cations in embryos heterozygous for a Dyrk1a null mutation ($Dyrk1a^{+/-}$ embryos) (Fotaki et al., 2002). The levels of DYRK1A protein in the telencephalon of E11.5 $Dyrk1a^{+/-}$ embryos were reduced (around 50%) with respect to the levels in the $Dyrk1a^{+/+}$ control littermates. Importantly, the levels of nuclear Cyclin D1 in $Dyrk1a^{+/-}$ dorsal RG progenitors were signi cantly increased (Supplementary Fig. 5B and C). As in the TgDyrk1a gain-of-function model, there were no differences between genotypes in the number of RG progenitors ($Pax6^+$ cells in the VZ; Fig. 4A). However, $Dyrk1a^{+/-}$ embryos had more Tbr1-expressing neurons than $Dyrk1a^{+/+}$ embryos (Fig. 4B). We could not detect any IP cells ($Tbr2^+$, $Tuj1^-$ cells) in E11.5 $Dyrk1a^{+/-}$ embryos (Fig. 4C), indicating that IP production is impaired in this Dyrk1a mutant. To con rm this, we counted the number of $Tbr2^+$ cells in $Dyrk1a^{+/+}$ and $Dyrk1a^{+/-}$ embryos two days later (at E13.5), an age where the germinal SVZ is well formed. The number of these cells was reduced in the VZ of $Dyrk1a^{+/-}$ embryos (Fig. 4D), showing that indeed mutant RG progenitors produce fewer IPs than the controls. Accordingly, Dyrk1a+/- mutants embryos at E13.5 and E16.5 (Fig. 5C), indicating that during neurogenesis the number of RG divisions was not altered in Dyrk1a transgenic embryos. Accordingly, there were no differences between genotypes in the numbers of RG progenitors during neurogenesis ($Pax6^{+}$ cells in a 100 μm-wide column were: wild-type, 127.11 ± 7.56; TgDyrk1a, 133.83 ± 6.23 at E13.5 and wild-type, 72.61 ± 3.98; TgDyrk1a, 80.53 ± 5.91 at E16.5). However, the number of $pH3^{+}$ cells in the SVZ (basal mitosis) was signi cantly increased in E13.5 TgDyrk1a embryos, which correlated with the increased number of IPs in the SVZ observed at this stage (Fig. 5C).

As neurogenesis proceeds, IPs become the main source of neocortical neurons (Kowalczyk et al., 2009; and Fig. 5A). To evaluate how the birthdate data shown in Supplementary Fig. 7C, suggests that Ctip2 neurons in the Dyrk1a embryos are produced earlier in development but at normal rates. In summary, our results show that a 1.5-fold increase in DYRK1A protein levels disturbs the number of neocortical neurons that are generated through development by direct and indirect neurogenesis.

As neurogenesis progresses and the length of the G1 phase increases, the levels of Cyclin D1 in dorsal VZ cells progressively decrease, and by the end of the neurogenic phase, by E18.5, Cyclin D1 immunolabelling in the ventricular proliferative region was very faint (Supplementary Fig. 8A). Similar to the situation at the onset of neurogenesis (Fig. 1B and C), the levels of nuclear Cyclin D1 inversely correlated to the levels of DYRK1A in E13.5 embryos (Supplementary Figs. 6 and 8B). In contrast and despite DYRK1A being still present in the VZ of E16.5 embryos (Supplementary Fig. 6), the levels of nuclear Cyclin D1 in Tg Dyrk1a VZ progenitors were normal (Supplementary Figs. 6A and 8C). Consistent with published data (Glickstein et al., 2009), we only detected a few cells expressing high Cyclin D1 levels in the SVZ. Therefore, our expression data and the phenotype of Tg Dyrk1a transgenic embryos indicate that the altered production of upper layer neurons observed in these embryos does not result from the regulatory action of DYRK1A on Cyclin D1 degradation.

The generation of macroglial cells in the mouse dorsal telencephalon begins around birth when VZ progenitors lose their capacity to generate neurons and become gliogenic (Kriegstein and Alvarez-Buylla, 2009). Immunolabelling for Cyclin D1 revealed the presence of a population of cells expressing high levels of the protein in the region above the SVZ, the intermediate zone, of E16.5 TgDyrk1a

Fig. 6. The TgDyrk1a model shows an advanced production of oligodendrocyte progenitors and decreased neuronal cellularity in speci c cortical layers. (A) Representative coronal sections from E16.5 wild-type (WT) and Tg Dyrk1a (Tg) brains immunostained for Olig2 and nuclei labelled with Hoescht. Yellow dashed rectangles indicate the region magni ed in the images on the right. Histogram shows the number of Olig2 $^{+}$ cells in a 300 µm-wide column of the cortical wall. (B) Representative coronal sections from P7 WT and Tg brains immunostained for Tbr1 (left), Ctip2 (middle) or Cux1 (right), and histograms showing the number of layer VI Tbr1 $^{+}$ neurons, layer V Ctip2 $^{+}$ neurons and layers II ..IV Cux1 $^{+}$ neurons in a 100 µ

the end of neurogenesis, and at P7, when radial migration has ended and projection neurons are in their final layer position (Miller, 1988). At these developmental stages, Tbr1 expression in the neocortex is almost restricted to layer VI neurons (Bulfone et al., 1995), which are mainly generated between E11.5 and E13.5 (Molyneaux et al., 2007). According to the neuron deficit observed in TgDyrk1a embryos during this period (Figs. 3B and 5D), the number of layer VI Tbr1^{+} neurons in

TgDyrk1a and Dyrk1a$^{+/-}$ mouse models, this defect leads to an altered number of projection neurons in the most internal layer of the neocortex.

The in vivo

We show here that IPs express DYRK1A and that at late corticogenesis the pool size of this type of progenitor is significantly diminished in TgDyrk1a embryos. Consistent with our Cyclin D1 expression data, it has been shown that cortical IPs do not express Cyclin D1 but instead express Cyclin D2 (Glickstein et al., 2009). Thus, the early exhaustion of IPs in TgDyrk1a embryos and the consequent deficit of late-born neurons

Authors' Contributions

MLA conceived the project. MJB, SN, PAL and SJC designed the experiments and analysed the data. SN, JA, PAL, ALA, and DO performed the experiments. JMD generated the mBACtgDyrk1a mouse model. SN, MJB and SJC interpreted the data. SN prepared the gures and MLA wrote the manuscript.

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