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p27^{Kip1} Is a Microtubule-Associated Protein that Promotes Microtubule Polymerization during Neuron Migration

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SUMMARY

The migration of cortical interneurons is characterized by extensive morphological changes that result from successive cycles of nucleokinesis and neurite branching. Their molecular bases remain elusive, and the present work describes how p27^{Kip1} controls cell-cycle-unrelated signaling pathways to regulate these morphological remodelings. Live imaging reveals that interneurons lacking p27^{Kip1} show delayed tangential migration resulting from defects in both nucleokinesis and dynamic branching of the leading process. At the molecular level, p27^{Kip1} is a microtubule-associated protein that promotes polymerization of microtubules in extending neurites, thereby contributing to tangential migration. Furthermore, we show that p27^{Kip1} controls actomyosin contractions that drive both forward translocation of the nucleus and growth cone splitting. Thus, p27^{Kip1} cell-autonomously controls nucleokinesis and neurite branching by regulating both actin and microtubule cytoskeletons.

INTRODUCTION

The cortex contains neurons that are distributed within layers and are regionally organized into specialized areas that underlie sophisticated motor, cognitive, and perceptual abilities (Rash and Grove, 2006). Cortical lamination follows an "inside-out" sequence of neuronal placement and maturation that arises from the successive birth and orderly migration of two major classes of neurons, projection neurons and interneurons. In contrast to projection neurons that show rather simple bipolar morphology and directed migration along straight radial glia guides (Gupta et al., 2002), cortical interneurons extend multiple branches and move along various tangential paths that run across different substrates in the telencephalon (Anderson et al., 1997; Tamamaki et al., 1997), including progenitor cells, postmitotic neurons as well as radial glia fibers (Yokota et al., 2007). Indeed, migration of interneurons results from successive cycles of morphological changes that couple the saltatory progression of their cell body with the dynamic remodeling of their leading process. The nucleus alternates resting phases that correlate with the elongation of the leading process and movement phases associated with the splitting of growth cone-like structures (termed "growth cone" further in the text) that give rise to new branches. This stepwise behavior relies on cytoskeletal transformations that promote the rostral translocation of a cytoplasmic dilatation encompassing the centrosome and the Golgi apparatus into the extending leading process. This is followed by forward migration of the nucleus, and its perinuclear cytoplasm, a process named nucleokinesis (Luxton et al., 2010; Marin et al., 2010). Finally, interneurons undergo retraction of the trailing process and branching of the leading process (Bellion et al., 2005). Over the past few years, several studies have begun to define the molecular mechanisms underlying nucleokinesis in cell migration (Bellion et al., 2005; Gomes et al., 2005; Tsai and Gleeson, 2005). Key regulators were identified as proteins associated with actin or microtubule (MT) cytoskeletons. Among them are Cdk5, the MT-interacting proteins dynein, its cofactor Lis1, and Doublecortin that organize and control the MT cytoskeleton dynamics during cell migration (Kappeler et al., 2006; Kawauchi et al., 2006; Koizumi et al., 2006; Levy and Holzbaur, 2008; Rakić et al., 2009; Tanaka et al., 2004; Tsai et al., 2007). The polarity protein Par6α regulates centrosome positioning

and is required for the integrity of the MT cage surrounding the nucleus of migrating cerebellar neurons (Solecki et al., 2004). Importantly, this molecule couples regulation of both MT- and actin-based cytoskeletons during migration, by promoting the activity of myosin II (Solecki et al., 2009). In addition, small GTPases (Kholmanskikh et al., 2003; Kholmanskikh et al., 2006) and F-actin regulatory molecules (Bellenchi et al., 2007; Nagano et al., 2002) contribute to remodeling of the microfilament system during neuronal migration. In spite of these major advances, little is known about the molecular basis of tangential migration of cortical interneurons and there is currently no integrated view on how nucleokinesis is coupled to dynamic branching of the leading process to ensure an efficient migration of these neurons into the cerebral cortex.

In this study, we combined genetic and molecular approaches with real-time imaging to investigate the role of the Cip/Kip protein $p27^{Kip1}$ (termed hereafter p27) in cortical interneuron migration. First identified as cell cycle inhibitors mediating the growth inhibitory cues of upstream signaling pathways, the cyclin-CDK inhibitors of the Cip/Kip family have emerged as multifunctional proteins with roles extending beyond cell cycle regulation (Besson et al., 2008). In this study, we show that p27 is the predominant Cip/Kip protein expressed in cortical interneurons and time-lapse imaging revealed its major contribution to tangential migration. Indeed, p27 promotes interneuron migration by regulating nucleokinesis and leading process branching. At the molecular level, we found that p27 controls the activity of myosin II through the Rho kinase pathway to fine-tune nuclear translocation and splitting of the growth cone at the tip of the leading process. Furthermore, we identified p27 as a MT-associated protein (MAP), which harbors a prolin-rich domain required for MT polymerization in extending neurites. Thus, p27 promotes interneuron migration in the cerebral cortex by combining distinct cell cycle-unrelated activities.

RESULTS

$p27^{Kip1}$ Is Expressed by Migrating Cortical Interneurons and Their Progenitors

On the basis of recent findings demonstrating that p27 promotes the development of cortical projection neuron through cell-cycle-unrelated activities (Itoh et al., 2007; Kawauchi et al., 2006; Nguyen et al., 2006), we investigated its contribution to the differentiation and migration of cortical interneurons. p27 transcripts were broadly detected in periventricular regions of the telencephalon of embryonic day (E) 12.5 mouse embryos, including medial and caudal ganglionic eminences (MGE and CGE) where newborn interneurons and their progenitors reside (Figures 1A and 1B; Figure S1B available online). In contrast, the expression pattern of other Cip/Kip family members was restricted to specific telencephalic regions. $p21^{Cip1}$ mRNAs were detected in the presumptive hippocampus (Figure S1A), whereas $p57^{Kip2}$ transcripts were mostly observed in the developing septum and the subventricular zone (SVZ) of the MGE (Figures S1C and S1D). Immunolabeling of E12.5 embryo sections showed expression of p27 proteins in the SVZ and the mantle zone (MZ) of the MGE (Figures 1C and 1D), whereas the corresponding transcripts mostly accumulated in the ventricular zone (VZ) at the border of the SVZ (Figures 1B and 1D). To confirm that cortical interneurons and their progenitors express p27, we analyzed E14.5 embryos from heterozygous GAD67-GFP knockin mice (Tanaka et al., 2003). Immunolabeling with antibodies against GFP and βIII tubulin showed that all GFP-positive cells in the developing cortex were neurons (Figures S1E–S1I). In addition, p27 was detected in all GFP-expressing interneurons and their MGE progenitors (Figures 1E–1G″). It is worth noting that p27 also accumulated in the cytoplasm (Figures 1H–1I″) of cortical interneurons, thus arguing for cell cycle-unrelated activities during migration. Various markers of interneuron subtypes were coexpressed with p27, consistent with the observation for ubiquitous expression of p27 in cortical interneurons (Figures 1J–1M′).

$p27^{Kip1}$ Controls the Rate of Cortical Interneuron Migration Independently of Its Cell Cycle Regulatory Activity

The broad expression of p27 in MGE progenitors and its maintenance in cortical interneurons prompted us to test whether this protein was involved in functions beyond cell cycle regulation. Survival (Figures S2A–S2D), proliferation (Figures S2E–S2G), and cell cycle exit of MGE progenitors (Figures S2H–S2J) remained unchanged in p27 knockout ($p27^{-/-}$) embryonic brains (Fero et al., 1996). However, tangential migration of cortical interneurons was impaired (Figures S2K–S2R, S3A–S3D, and S3H–S3K), and this mostly in the intermediate and lower migratory streams (Figures S3J and S3K). Similar results were obtained after acute loss of p27 in interneurons from cultured E14 brain slices (Figures S3F–S3G′). In addition, bromodeoxyuridine (BrdU) birthdating experiments performed on p27CK embryos (a knockin mouse line in which point mutations in p27 disrupt its interaction with cyclins and CDKs; Besson et al., 2006) showed no defect in tangential migration of cortical interneurons (Figures S3H–S3K). These results strongly indicate that p27 cell-autonomously promotes tangential migration of cortical interneurons through mechanisms unrelated to its cell cycle regulatory activity.

We next performed real-time imaging using a conditional knockout mouse model that lacked expression of p27 and expressed GFP in cortical interneurons. This was achieved by breeding p27 lox ($p27^{f/f}$) (Chien et al., 2006) with Dlx5,6 Cre-GFP (termed hereafter DlxCre-GFP) (Stenman et al., 2003) mice. E12.5 DlxCre-GFP embryos showed robust expression of Cre recombinase and GFP in the ventral telencephalon, including the SVZ and MZ of the MGE where most cortical interneurons are generated. GFP expression was detected in postmitotic cells that expressed high levels of p27 and in few Ki67 positive cycling cells that showed lower expression of that protein. All GFP+ cells into the cortex of $p27^{+/+}$;DlxCre-GFP embryos expressed p27 and were interneurons. Cortical interneurons were consistently negative for p27 expression after Cre/loxP recombination compared to corresponding cells in controls (data not shown). Conditional removal of p27 in MGE occurred in SVZ progenitors without affecting cell proliferation (data not shown), possibly because $p57^{Kip2}$ is also expressed in MGE progenitors where it promotes cell cycle exit (Figure S1D; data not shown). The distribution of pioneer GFP+ interneurons was affected in several rostro-caudal regions of the cortex of E12.5 $p27^{f/f}$;DlxCre-GFP embryos compared to controls. Indeed, the migration front was delayed in all cortical regions (Figures 2A–2F) and the