
NGF/P75 in Cell Cycle and Tetraploidy

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Abstract

Neurotoxicity, neurodegeneration, and other disorders affecting neuron survival are often related to cell cycle reentry in neurons. Traditionally, cell cycle reentry of these postmitotic cells has been thought to be associated with apoptosis. Nevertheless, cell cycle reentry and DNA synthesis in neurons could also lead to tetraploidy which may explain long-lasting neurodegenerative processes. During development, a subpopulation of newborn neurons reactivates the cell cycle and becomes tetraploid in response to p75^{NTR} activation. These neurons enlarge their cell bodies and increase their dendritic trees, thus generating specific neuronal populations that innervate particular areas. Pathological states in the nervous system could also lead to p75^{NTR}-dependent neuronal tetraploidy.

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De novo tetraploid neurons might become structurally and functionally altered, thus leading to neurodegeneration at late stages of the disease. This chapter describes what is currently known about the interplay between p75^{NTR} and the cell cycle, stressing the role played by different p75^{NTR} interactors, including the MAGE and Bex1/NADE adaptor proteins and the transcription factors SC1, NRIF, and Sall2, in cell cycle regulation. The chapter also discusses on the effects of p75^{NTR}, as a cell cycle regulator, in neural stem cell proliferation, neurogenesis, and neuronal tetraploidization, as well as on the connection of p75^{NTR} in pathology, including its putative effects in neurodegeneration.

List of Abbreviations

A β	Amyloid- β
BDNF	Brain-derived neurotrophic factor
Bex-1	Brain-expressed X-linked 1
BrdU	Bromodeoxyuridine
cdk	Cyclin-dependent kinase
CMAGE	Chicken MAGE
CNS	Central nervous system
Dlxin-1	Dlx/Msx-interacting MAGE/Necdin family protein
E2F1	E2 promoter-binding factor-1
EGFP	Enhanced green fluorescent protein
ERK	Extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase
MAGE	Melanoma antigen
MAPK	Mitogen-activated protein kinase
NADE	p75 ^{NTR} -associated cell death executor
NGF	Nerve growth factor
NRAGE	Neurotrophin receptor-interacting MAGE homolog
NRIF	Neurotrophin receptor-interacting factor
NT3	Neurotrophin-3
p75 ^{ICD}	p75 ^{NTR} intracellular domain
p75 ^{NTR}	p75 neurotrophin receptor
PCNA	Proliferating cell nuclear marker
PNS	Peripheral nervous system
PR/SET	Positive regulatory/suppressor of variegation, enhancer of zeste, trithorax
Rb	Retinoblastoma
RGCs	Retinal ganglion cells
Sall2	Sal-like protein 2
SC1	Schwann cell factor 1
SGZ	Subgranular zone
SVZ	Subventricular zone
TNF	Tumor necrosis factor
Trk	Tropomyosin-related kinase

1 Introduction

Neurotrophins are best known as neuronal survival factors with multiple effects in the structure and function of the adult vertebrate nervous system (Huang and Reichardt 2001). The first neurotrophin to be described was NGF in the early 1950s (Levi-Montalcini and Hamburger 1951), being initially purified from sarcoma tumor cells (Cohen et al. 1954). Several decades later, other members of the neurotrophin family were characterized, including BDNF (Barde et al. 1982), NT3 (Hohn et al. 1990; Maisonpierre et al. 1990; Rosenthal et al. 1990; Jones and Reichardt 1990), and NT4/5 (Hallböök et al. 1991; Berkemeier et al. 1991). In the brain, mature neurotrophins coexist with their precursor pro-forms, namely, proNGF, proBDNF, and proNT3 (Fahnestock et al. 2001; Michalski and Fahnestock 2003). The neurotrophin pro-forms fulfill specific functions, usually opposed to that of the mature neurotrophins, including induction of cell death (Lee et al. 2001; Teng et al. 2005; Yano et al. 2009) and, in the case of proBDNF, synapse modulation (Lu 2003; Woo et al. 2005; Greenberg et al. 2009). These opposed effects can be explained due to the capacity of neurotrophins to interact with two different types of receptors, p75^{NTR} and the members of the Trk tyrosine kinase receptor family. p75^{NTR} was the first member of the TNF receptor family to be described (Chao et al. 1986; Johnson et al. 1986; Radeke et al. 1987). Unlike the Trk receptors, known to interact with specific neurotrophins (Kaplan et al. 1991; Klein et al. 1991a, b, 1992; Lamballe et al. 1991), p75^{NTR} can bind to, and be activated by, all neurotrophins (Rodríguez-Tébar et al. 1990, 1992). Although p75^{NTR} was initially thought to be a mere Trk co-receptor, it is now clear that this receptor is at the core of at least two other signaling networks, including the p75^{NTR}/sortilin platform that induces apoptosis in response to the pro-forms of the neurotrophins (Nykjaer et al. 2004; Teng et al. 2005; Yano et al. 2009) and the p75^{NTR}/NgR/LINGO-1 complex that regulates axonal outgrowth (Wang et al. 2002; Mi et al. 2004). In some cases, signaling through p75^{NTR} requires the release of its intracellular domain (p75^{ICD}), which can be translocated to the nucleus (Frade 2005; Parkhurst et al. 2010). This mechanism has been shown to lead to apoptosis (Majdan et al. 1997; Kenchappa et al. 2006, 2010; Podlesniy et al. 2006), and it can also participate in Trk signaling (Skeldal et al. 2011).

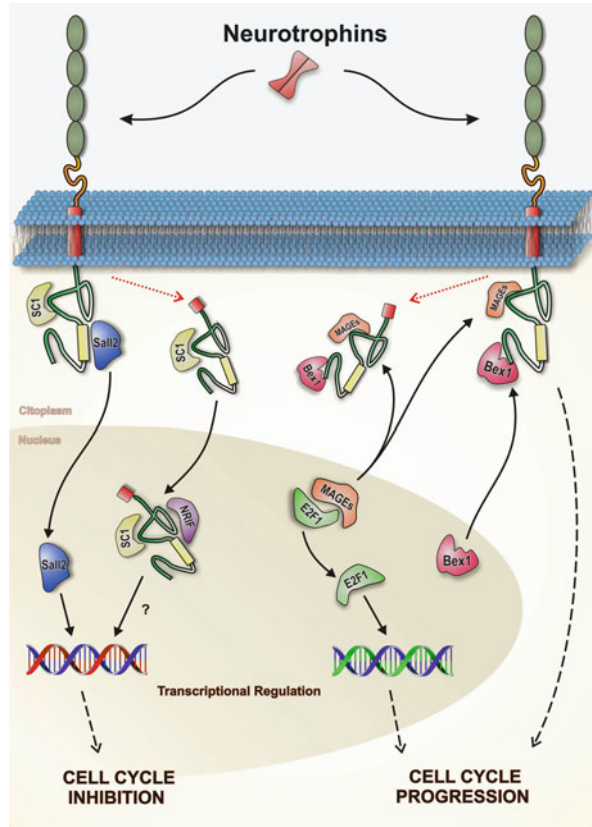
Mounting evidence indicates that neurotrophins can regulate cell cycle progression through both p75^{NTR} and the Trk receptors (López-Sánchez and Frade 2002). This chapter will firstly describe what is known about the interplay between p75^{NTR} and the cell cycle machinery. Then, the chapter will be focused on the role played by this receptor in the normal nervous system in terms of cell cycle regulation (proliferation of neural stem cells, embryonic and adult neurogenesis, and neuronal tetraploidization). Finally, a discussion on the possible involvement of p75^{NTR} as a cell cycle regulator in neurodegeneration will be presented.

2 Interplay Between p75^{NTR} and the Cell Cycle

p75^{NTR} is the founder member of the tumor necrosis factor receptor family. This receptor, characterized by the presence of one transmembrane domain and a type II death domain within its intracellular domain, is expressed by a variety of neuronal and non-neuronal cell types, frequently showing proliferative capacity. In this regard, p75^{NTR} was initially described in proliferating cells derived from pheochromocytoma, neuroblastoma, and melanoma tumors (Yankner and Shooter 1982). More recently, this receptor is often used as a marker of neural progenitor cells in the adult brain (Giuliani et al. 2004; Young et al. 2007; Bernabeu and Longo 2010) and as a tumor suppressor gene in a number of tumor cells (Krygiel and Djakiew 2001; Yuanlong et al. 2008; Jin et al. 2007; Khwaja and Djakiew 2003; Khwaja et al. 2006). p75^{NTR} has opposing effects on the cell cycle, inducing cell cycle arrest in some instances and cell cycle progression in other circumstances. For instance, p75^{NTR}-dependent cell cycle arrest, associated with reduced expression of cyclin D1, cyclin E, cdk2, E2F1, PCNA, and the cdk inhibitor p16^{INK4a}, decreased cdk2 activity, and hyper-phosphorylation of Rb protein, has been shown to occur in bladder tumor epithelial cells (Khwaja and Djakiew 2003). In human gastric cancer cells, p75^{NTR} expression suppresses proliferation by downregulation of cyclin A, cyclin D1, cyclin E, cdk2, and phospho-Rb and upregulation of the cell cycle inhibitors Rb and p27^{KIP1}. This antiproliferative activity in human gastric cancer cells is dependent on the presence of the death domain of p75^{NTR} (Jin et al. 2007). p75^{NTR} expression in hepatocellular carcinoma cells has also been shown to downregulate the expression of cyclin A, cyclin D1, cyclin E, cdk2, p-Rb, and PCNA and upregulate the expression of the cell cycle inhibitor Rb (Yuanlong et al. 2008). Examples of p75^{NTR}-dependent cell cycle progression are also available in the literature. For instance, p75^{NTR} has been shown to be expressed by human oral keratinocyte stem cells showing high in vitro proliferative capacity and clonal growth potential (Nakamura et al. 2007) as well as by human esophageal keratinocyte stem cells able to undergo self-renewal and self-amplification (Okumura et al. 2003). Moreover, p75^{NTR} can be detected in cycling cells from the subventricular zone of adult male rats (Giuliani et al. 2004), and the addition of NGF has been shown to revert p75^{NTR}-dependent cell cycle arrest in bladder tumor epithelial cells (Khwaja and Djakiew 2003).

The mechanisms used by p75^{NTR} to regulate cell cycle progression are beginning to be elucidated. In this regard, p75^{NTR} is known to activate different members of the MAPK family able to regulate the cell cycle (Ambrosino and Nebreda 2001; Zhang and Liu 2002), including JNK, p38^{MAPK}, and ERK (Casaccia-Bonnel et al. 1996; Susen et al. 1999; Costantini et al. 2005; Jiang et al. 2007). Moreover, the opposing effects of p75^{NTR} on cell cycle regulation can be explained by the different molecules with which this receptor interacts (see Fig. 1). p75^{NTR} can modulate the cell cycle through the interaction of its intracellular domain with a number of proteins known to regulate the cell cycle, some of which are transcription factors. In some instances, p75^{NTR} has been shown to sequester these molecules at the plasma membrane.

Fig. 1 Scheme of the signaling pathways used by p75^{NTR} to regulate the cell cycle. *Dotted arrows* represent the release of p75^{ICD} as a consequence of regulated γ -secretase-dependent cleavage of p75^{NTR}. *Solid arrows* describe the dynamics of the different proteins involved in the p75^{NTR} signaling. *Dashed arrows* represent the final biological output of the corresponding pathways. The *question mark* represents the putative transcriptional activity of p75^{ICD} complexed with NRIF and/or SC1. See main text for further details



In addition, p75^{NTR} is susceptible to undergo γ -secretase-dependent cleavage and release its intracellular domain (p75^{ICD}) which may subsequently translocate to the nucleus (Kanning et al. 2003; Frade 2005). The interaction of p75^{ICD} with transcription factors led to the suggestion that it can regulate gene expression when located in the nucleus. This notion has recently been confirmed as p75^{ICD} has been shown to translocate to the nucleus and activate a green fluorescent protein reporter (Parkhurst et al. 2010). Furthermore, NGF can induce the interaction of p75^{ICD} with the *cyclin E* promoter, thus inhibiting its activity in HeLa cells (Parkhurst et al. 2010), a result consistent with the observation that NGF-dependent nuclear translocation of p75^{ICD} in schwannoma cells (Frade 2005) correlates with cell cycle repression in these cells (Morillo and Frade 2008). The expression of p75^{ICD} in p75^{NTR}-negative PC12 cells downregulates cyclin D2 expression as well (Fritz et al. 2006). p75^{NTR} can regulate the cell cycle by means of recruitment of adaptor proteins such as different MAGE proteins or Bex1, known to be involved in the cell cycle regulation, and also by regulating the expression of cell cycle-regulating genes through transcription factors such as SC1, NRIF, and Sall2, all of them able to interact with p75^{ICD}.

2.1 MAGE Proteins

The MAGE protein family comprises more than 60 adaptor proteins in mammals, containing a conserved MAGE homology domain. Originally, MAGE proteins were discovered because peptides derived from MAGE gene products are presented on the cell surface of human melanoma cells by the major histocompatibility complex (Barker and Salehi 2002). MAGE proteins can be divided into two major groups: type I and type II MAGE proteins. The former are only expressed in tumors, male germinal cells, and placenta. In contrast, the latter group, defined by a phylogenetically distinct MAGE homology domain, comprises around ten different proteins expressed in both differentiating and adult cells (Barker and Salehi 2002). Most of the MAGE proteins are encoded by genes containing a single exon, suggesting that the whole MAGE protein family evolved from a retrotransposition event that occurred in an ancestral gene orthologue of the gene encoding NRAGE/Dlxin-1/MAGE-D1, the only MAGE gene containing exons in its sequence, followed by extensive gene duplication. This hypothesis is consistent with the existence of a single MAGE gene in all nonmammalian species including unicellular, plants, fungi, invertebrates, and nonmammalian vertebrates (López-Sánchez et al. 2007).

The mammalian MAGE II proteins Necdin, NRAGE/Dlxin-1/MAGE-D1, MAGE-H1, and MAGE-G1 as well as CMAGE are known to interact with p75^{NTR} through its intracellular domain (Salehi et al. 2000; Tcherpakov et al. 2002; Kuwako et al. 2004; López-Sánchez et al. 2007), and they have effects on cell cycle regulation. Therefore, these proteins are candidates for the effects of p75^{NTR} on the cell cycle. Necdin can bind to the transcription factor E2F1, thus blocking its capacity to trigger G1/S transition (Taniura et al. 1998; Kuwako et al. 2004). The activation of p75^{NTR} by NGF can recruit Necdin to the cell membrane, thus favoring E2F1-dependent cell cycle progression and subsequent death of differentiated neurons (Kuwako et al. 2004) (see Fig. 1). Alternatively, the presence of the p75^{ICD} fragment can displace both Necdin and CMAGE from their interaction with E2F1 in differentiating neurons, thus leading to cell E2F1-dependent cycle reentry and apoptosis (López-Sánchez et al. 2007) (see Fig. 1). NRAGE/Dlxin-1/MAGE-D1 can also block cell cycle progression (Salehi et al. 2000), likely by inducing the expression of the cell cycle-inhibiting protein p21^{Waf1} in a p53-dependent manner (Wen et al. 2004). NRAGE/Dlxin-1/MAGE-D1 associates with the plasma membrane when NGF is bound to p75^{NTR} (Salehi et al. 2000), as it occurs with Necdin (see above). Therefore, sequestering of MAGE proteins by p75^{NTR} seems to be a general mechanism for enhancement of cell cycle progression in different cellular systems.

2.2 Bex/NADE

p75^{NTR} is also known to interact through its death domain with the small adaptor-like proteins from the Bex/NADE family (Mukai et al. 2000; Vilar et al. 2006), including Bex1 and Bex3/NADE. Among them, Bex1 can constitutively

interact with p75^{NTR} (see Fig. 1) and favor cell cycle progression when overexpressed in differentiating PC12 cells (Vilar et al. 2006). This is consistent with the increase of Bex1 during S phase in PC12 cells, as it occurs with the presence of p75^{NTR} on the surface of these same cells (Urdiales et al. 1998). Upon NGF treatment, Bex1 translocates from the nucleus to the cytoplasm (see Fig. 1), suggesting that this mechanism is required for the effect of this protein on cell cycle regulation. Interestingly, in other cell systems Bex1/2 seems to act as a putative tumor suppressor gene in malignant glioma (Foltz et al. 2006), although in this case the participation of p75^{NTR} is currently unknown.

2.3 SC1

The p75^{NTR}-interacting protein SC1 is a member of the PR/SET domain-containing zinc finger protein family, which can be detected in both the nucleus and cytoplasm when overexpressed (Chittka and Chao 1999). SC1 is redistributed from the cytoplasm to the nucleus after NGF treatment in a p75^{NTR}-dependent manner, acting as a transcriptional repressor (see Fig. 1). Nuclear SC1 correlates with the loss of BrdU incorporation, likely due to its ability to repress cyclin E expression (Chittka et al. 2004). Interestingly, NGF has been shown to induce the association of endogenous p75^{ICD} with the cyclin E promoter (Parkhurst et al. 2010), suggesting that a complex formed by p75^{ICD} and SC1 can be formed in the promoter of different genes encoding cell cycle-regulating proteins (see Fig. 1).

2.4 Sall2

Sall2 is another transcription factor able to regulate cell cycle progression which can interact with p75^{NTR} through its intracellular domain (see Fig. 1). This protein is a tumor suppressor that becomes dissociated from its binding to p75^{NTR} upon interaction of the latter with NGF (Pincheira et al. 2009). Sall2 is known to activate p21^{Waf1} and promote growth arrest in neurons. This observation is consistent with the p75^{NTR}-dependent increase of p21^{Waf1} expression and decrease of Rb phosphorylation in breast cancer cells (Verbeke et al. 2010).

2.5 NRIF

The zinc finger proteins of the Krüppel family NRIF1 and NRIF2 are able to interact with the intracellular domain of p75^{NTR} through its juxtamembrane and death domains (Casademunt et al. 1999; Benzel et al. 2001) (see Fig. 1). These proteins have been suggested to be transcription factors based on structural characteristics and their ability to translocate to the nucleus (Gentry et al. 2004), and they prevent cell cycle progression when expressed in 293T cells. Interestingly, the release of p75^{ICD} correlates with the translocation of NRIF to the nucleus

(Kenchappa et al. 2006; Volosin et al. 2008), suggesting the complex NRIF/p75^{ICD} may participate in the regulation of the cell cycle, as it occurs with p75^{ICD} and SC1 (see above).

3 The Role of p75^{NTR} as a Cell Cycle Regulator in Nervous System Physiology

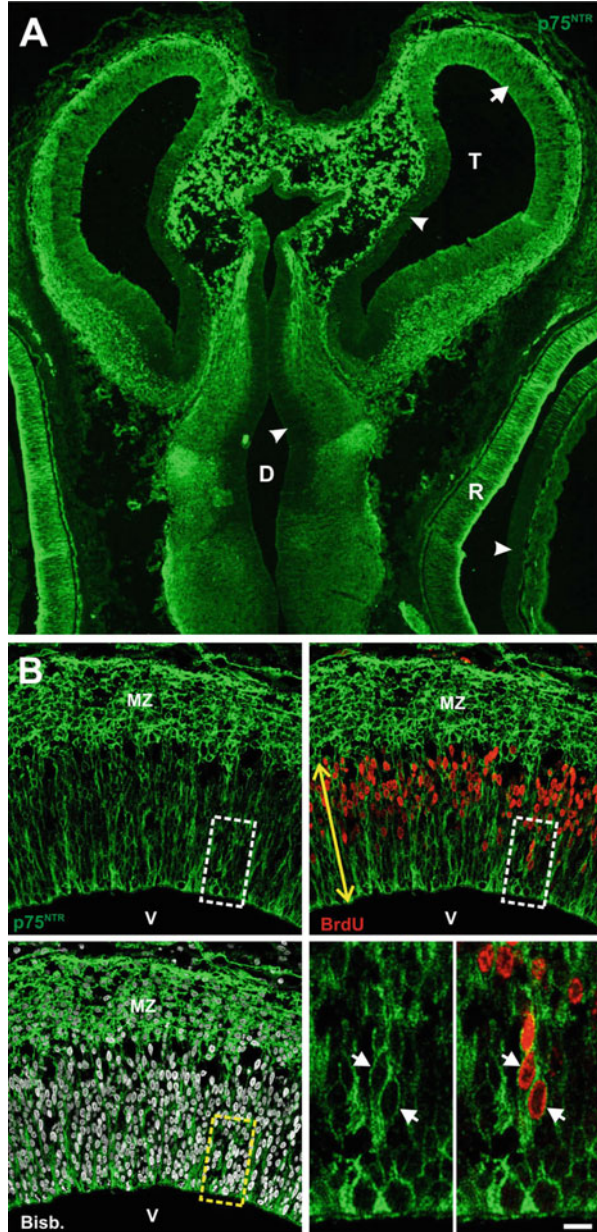
A critical feature of the nervous system is the requirement of neurons to be postmitotic. The avoidance of cell division in these cells is likely derived from their complex morphology, required for their function, which is incompatible with the reorganization of the cytoskeleton necessary for the mitotic process. Therefore, at certain point of neural development, proliferating neuronal precursors stop dividing and differentiate as neurons in a process referred to as neurogenesis. During development, p75^{NTR} can be detected in the proliferating epithelium of the retina (Frade and Barde 1999; Morillo et al. 2010; López-Sánchez et al. 2011), spinal cord (Frade and Barde 1999; López-Sánchez et al. 2011), and other structures of the CNS including the dorsal telencephalon in the chick (Fig. 2), suggesting that it can participate in the neurogenic process. Furthermore, p75^{NTR} is expressed by neural crest cells (Bannerman and Pleasure 1993), a proliferating cell population giving rise to the PNS, among other structures. Some evidence exists for the participation of p75^{NTR} in cell cycle withdrawal of precursor cells and neuronal differentiation during embryonic development (Hapner et al. 1998; Hosomi et al. 2003; Zhang et al. 2009). Much more evidence exists for the participation of p75^{NTR} in adult neurogenesis and neuronal tetraploidization during embryonic development. These two processes are further described below.

3.1 Adult Neurogenesis

For many decades it was assumed that the generation of functional neurons from precursors (i.e., neurogenesis) only occurs during embryonic and perinatal stages of development in mammals. This view has been ruled out, and nowadays it is widely accepted that neural stem cells can be present in the adult nervous system, being able to proliferate in vitro giving rise to neurospheres (Reynolds and Weiss 1992). Neurons are known to be produced during adulthood in two main brain regions, in the SVZ of the lateral ventricle (giving rise to the rostral migratory stream that colonizes the olfactory bulb) and the SGZ from the dentate gyrus of the hippocampus (producing dentate granule neurons).

Interestingly, p75^{NTR} is expressed by cycling cells in the SVZ of adult male rats (Giuliani et al. 2004). This p75^{NTR}-positive cell population integrates all of the neurosphere-producing SVZ precursors, which are known to enhance neuronal production in response to NGF or BDNF (Young et al. 2007). The importance of p75^{NTR} in SVZ adult neurogenesis is further supported by the observation that the neurogenic potential is reduced in neurospheres derived from p75^{NTR} knockout

Fig. 2 p75^{NTR} expression in the chick brain at developmental day 6 (E6). (A) Horizontal section through the E6 chick brain, immunostained with the ChEX anti-p75^{NTR} antibody (kindly provided by L. F. Reichardt, University of California San Francisco). p75^{NTR} is expressed in specific regions of the neuroepithelium (*arrow*), while this protein cannot be detected in other areas of the neuroepithelium (*arrowheads*). *T* telencephalic derivatives, *D* diencephalic derivatives, *R* retina. (B) A high magnification from the telencephalic wall (dorsal hyperpallium) from E6 chick embryos treated with BrdU for 20 min. BrdU is incorporated by nuclei of progenitors undergoing S phase (*red*) which, due to the interkinetic nuclear movement, are mostly located far from the ventricle (*V*). Nevertheless, some nuclei from p75^{NTR}-expressing precursors incorporate BrdU when located close to the ventricle (*dashed box* and *arrows* in the magnification of the *dashed box* shown in the *bottom right panels*). Nuclei were labeled with bisbenzimidazole (*Bisb.*). Abbreviation: MZ mantle zone. Bar – 65 μ m (A), 25 μ m (B)



mice (Young et al. 2007). p75^{NTR} might also regulate proliferation of SVZ precursors in pathological states. For instance, p75^{NTR} activation with A β ₁₋₄₂, the pathological form of A β in Alzheimer’s disease, has been shown to favor proliferation and neuronal differentiation in neurosphere-forming adult progenitors,

an effect that is not observed in $p75^{\text{NTR}}$ knockout mice (Sotthibundhu et al. 2009). Moreover, an increase in the proliferation activity of $p75^{\text{NTR}}$ -positive SVZ precursors has been described in rats subjected to experimental allergic encephalomyelitis, a demyelinating disease widely used as an experimental model for human multiple sclerosis (Calzà et al. 1998). In the SGZ, $p75^{\text{NTR}}$ is expressed in dividing SGZ cells (Okano et al. 1996; Bernabeu and Longo 2010), and it seems to participate in adult hippocampal neurogenesis as neurogenic potential is reduced in the SGZ of $p75^{\text{NTR}}$ knockout mice (Bernabeu and Longo 2010; Colditz et al. 2010), maybe due to reduced survival of neuroblasts (Catts et al. 2008). Neurogenesis can also take place in different regions of the nervous system in response to injury. This is the case of the adult dorsal root ganglia, in which a subpopulation of glia may play a role in neurogenesis after peripheral nerve injury. These cells express $p75^{\text{NTR}}$, and in vitro they have been reported to form neurospheres (Li et al. 2007). Neurogenesis in the striatum in response to focal cerebral ischemia can be also potentiated by $p75^{\text{NTR}}$ since the proportion of neurons incorporating BrdU was increased in this paradigm (Zhu et al. 2011).

3.2 Neuronal Tetraploidy

The acquisition of multicellularity in evolution has allowed the emergence of the somatic lineage, in which genomic modifications may occur without transmission to the descendants. These genomic alterations, including somatic polyploidy, may participate in the differentiation of specific tissues. This is the case, for instance, of the liver and heart in vertebrates, which have both been shown to contain polyploid cells (Anatskaya and Vinogradov 2007).

Examples of somatic polyploidy in neurons have been described in some invertebrates (Coggeshall et al. 1970; Manfredi Romanini et al. 1973). In contrast, the presence of polyploid neurons in the vertebrate nervous system has been under controversy for decades (see Swartz and Bhatnagar 1981). It was not until the advent of modern techniques such as fluorescent in situ hybridization, flow cytometry, slide-based cytometry, and quantitative PCR analysis of nuclear DNA that the classical belief that all vertebrate neurons contain a diploid DNA amount (Swift 1953) was challenged. Recent analyses, for instance, have proved that 10 % of cortical neurons display a more than diploid DNA content in humans (Mosch et al. 2007). In this regard, tetraploid neurons have been shown to exist in different neural structures (López-Sánchez et al. 2011) including the normal vertebrate retina. In this latter tissue, tetraploid neurons constitute a subpopulation of RGCs that, in the chick, innervate lamina F in the stratum-griseum-et-fibrosum-superficiale of the tectal cortex (Morillo et al. 2010). In the retina, tetraploid RGCs are generated as they migrate to the ganglion cell layer, soon after they undergo their final mitosis and acquire neuronal markers (Morillo et al. 2010). During this stage differentiating RGCs express $p75^{\text{NTR}}$ and a subpopulation of these neurons incorporates BrdU, thus becoming tetraploid

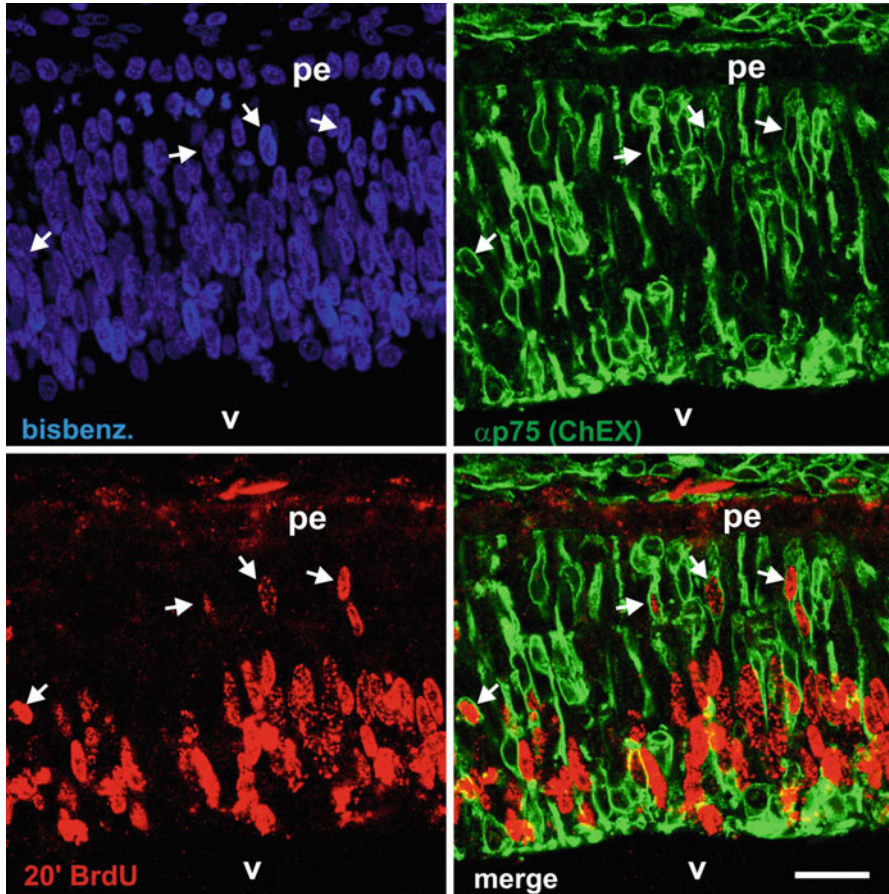


Fig. 3 Colocalization of $p75^{NTR}$ and BrdU in apically located cells from the E6 chick retina. Embryos were treated for 15 min with 40 μ l of a solution containing 10 μ g/ml BrdU and then fixed. Sagittal sections through the E6 chick retina were obtained, and they were subsequently immunostained with the ChEX anti- $p75^{NTR}$ antibody (green) and an anti-BrdU antibody (red) as previously described (López-Sánchez et al. 2011). $p75^{NTR}$ is expressed by BrdU-positive cells with nuclei located at the apical portion of the neuroepithelium (arrows). Nuclei were labeled with bisbenzimidazole (Bisb.). Abbreviations: *pe* pigment epithelium, *v* vitreous body. Bar, 18 μ m

throughout an endoreduplicative mechanism (Morillo et al. 2010; see also Fig. 3). Our work has demonstrated that DNA duplication in differentiating RGCs is triggered by NGF through $p75^{NTR}$, since blocking antibodies against these molecules were able to prevent cell cycle reentry (Morillo et al. 2010). BrdU incorporation in neural progenitor cells expressing $p75^{NTR}$ can also be observed in other neural structures of the chick embryo such as the spinal cord (López-Sánchez et al. 2011) or the telencephalon (Fig. 2b). Although further analyses are still needed to demonstrate that these $p75^{NTR}$ -positive cells are equivalent to those neurons

becoming tetraploid in the retina (Morillo et al. 2010), these results suggest that the participation of p75^{NTR} in neuronal tetraploidization is a general feature of the developing vertebrate nervous system.

Somatic polyploidy can be generated by different mechanisms usually linked to alterations of the mitotic cycle, including endoreduplication (i.e., a modified version of the cell cycle characterized by S phase without mitosis) and endomitosis (i.e., a mitotic cycle in the absence of anaphase/cytokinesis thus resulting in genomic DNA duplication) (Edgar and Orr-Weaver 2001; Ullah et al. 2009). Tetraploid RGCs are originated in the developing retina through an endoreduplicative cycle since they remain in a G2-like state after replicating their nuclear DNA (Morillo et al. 2010). Evidence exists that the neurotrophin BDNF, through its receptor TrkB, fulfills a critical role in preventing G2/M transition in retinal tetraploid neurons. Indeed, blockade of endogenous BDNF resulted in an increase of differentiating RGCs undergoing mitosis (Morillo et al. 2010). Therefore, neurotrophins, including NGF and BDNF, seem to play a critical role in neuronal tetraploidization in vertebrates. Tetraploid neurons that undergo mitosis finally die (Frade 2000; Morillo et al. 2010; López-Sánchez et al. 2011), and the regulation of the G2/M transition in these cells is likely crucial for removal of specific neuronal types during development (Frade et al. 1997), thus adjusting the ratio between tetraploid and diploid neurons in specific areas. Importantly, this process may be involved in neuronal death associated to neurodegenerative diseases (see below).

Polyploidy is usually linked to enlarged cell size (Edgar and Orr-Weaver 2001; Ullah et al. 2009), a concept that can be extrapolated to the vertebrate nervous system. For instance, neuronal hypertrophy has been shown to occur in tetraploid strains of *Xenopus laevis*, whose neurons contain significantly enlarged cell somas and dendrites (Szaro and Tompkins 1987). As expected, our studies have revealed that tetraploid RGCs show enlarged cell somas and dendritic arbors (Morillo et al. 2010), suggesting that somatic tetraploidy is associated with morphological and functional diversity in the nervous system.

4 p75^{NTR} in Neurodegeneration: The Tetraploid Connection

Cell cycle reentry in neurons represents a critical feature of both acute neuronal damage and chronic neurodegenerative diseases (Wang et al. 2009). In this regard, cell cycle regulators have been proposed as therapeutic targets for stroke (Osuga et al. 2000), excitotoxicity (Verdaguer et al. 2004), trauma (Di Giovanni et al. 2005), and Alzheimer's disease (Woods et al. 2007).

In cases of acute injury to the CNS such as kainic acid excitotoxicity and trauma, cell cycle reentry is usually stopped at the G1/S phase and it leads to rapid apoptosis (Kuan et al. 2004; Byrnes et al. 2007). In contrast, chronic neurodegenerative diseases are normally associated with DNA synthesis in particular neuronal populations, which can be arrested at the G2/M transition and survive for some time before dying by apoptosis. This is the case, for instance, of neurons subjected to ischemia-hypoxia (Byrnes et al. 2007; Burns et al. 2007).

These neurons incorporate BrdU and remain alive for days, before they die. Examples of cell cycle reentry and hyperploidy have also been shown to occur in Alzheimer's disease (Yang et al. 2001; Mosch et al. 2007; Arendt et al. 2010). Furthermore, increased expression in neurons of cell cycle regulators involved in G1/S transition has been described in patients suffering amyotrophic lateral sclerosis (Ranganathan and Bowser 2003, 2010; Ferraiuolo et al. 2007), Parkinson's disease (Höglinger et al. 2007), Huntington's disease (Pelegrí et al. 2008; Fernandez-Fernandez et al. 2011), and different tauopathies (Stone et al. 2011). Furthermore, the Parkin2 gene, known to be a frequent cause of early-onset Parkinson's disease, is a tumor suppressor gene whose inactivation results in an increase in cyclin E levels (Veeriah et al. 2010). Neuronal cell cycle reentry in pathological states has often been interpreted as de novo adult neurogenesis (Fallon et al. 2000; Arvidsson et al. 2002; Curtis et al. 2003; Becker et al. 2007). Nevertheless, this conclusion has been proved to be erroneous in some cases. For instance, the use of conditional transgenic mice expressing EGFP under the control of the nestin promoter allowed Burns et al. (2007) to demonstrate that ischemia-hypoxia-dependent BrdU incorporation mostly occurs in differentiated neurons.

In the adult nervous system, p75^{NTR} is reexpressed in various neuropathological conditions, including Alzheimer's disease (Hu et al. 2002), amyotrophic lateral sclerosis (Lowry et al. 2001), Parkinson's disease (Chen et al. 2008), and Huntington's disease (Zuccato et al. 2008), likely participating in the etiology of neurodegeneration (Dechant and Barde 2002). p75^{NTR} is therefore a candidate for the induction of cell cycle reentry leading to tetraploidy in the affected neurons (Frade and López-Sánchez 2010). Since developmentally regulated tetraploidy in neurons correlates with cell body and dendritic tree enlargement (see above), it is conceivable that a similar effect may occur in adult neurons undergoing de novo tetraploidization, thus explaining, at least partially, the pathological signs of the disease. Structural alterations in neurons becoming tetraploid may correlate with functional changes, including alterations of metabolic activity, higher rate of membrane biosynthesis, changes in neuronal circuits, and alterations in electrical signal propagation (see Frade and López-Sánchez 2010). Furthermore, genome duplication may result in changes of gene expression in particular loci, as those observed in liver and heart cells (Anatskaya and Vinogradov 2007) and tetraploid mouse embryos (Kawaguchi et al. 2009). It is likely that all these alterations associated with tetraploidy in neurons could functionally compromise the affected neurons. Further insights on the molecular mechanisms triggering tetraploidy in neurons will probably be crucial for the design of appropriate therapeutic tools for patients that suffer from neurodegenerative diseases.

5 Conclusion

p75^{NTR} is a pleiotropic receptor that fulfills several critical functions in the nervous system, including the regulation of the cell cycle in both embryonic and adult nervous system. Indeed, p75^{NTR} seems to be involved in stem cell

maintenance, neurogenesis, and neuronal tetraploidization. The molecular mechanisms used by p75^{NTR} to regulate the cell cycle are far from being fully understood, but they are likely based on its capacity to interact with a number of cell cycle regulators, as well as its ability to translocate its intracellular domain to the nucleus to act as a transcriptional regulator. The capacity of p75^{NTR} to regulate the cell cycle has opened new research avenues not only focused on the normal development of the vertebrate nervous system but also related with neuropathological conditions, since deregulation of this receptor in the adult brain could participate in neurodegenerative diseases. It is likely that future research in this field will yield hints of possible therapeutic approaches for these devastating diseases.

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