Chapter 1

Introduction
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Programmed cell death or apoptosis of neurons is a normal physiological phenomena associated with the development of the nervous system. During development, an excess number of neurons are produced initially. This massive production of neurons is followed by apoptosis during a restricted developmental period, leading to the elimination of as much as half of the originally produced cells (Hamburger and Levi-Montalcini, 1949). In addition to the extensive naturally occurring cell death in the developing nervous system, neuronal apoptosis is a prominent feature in a number of acute and chronic neurological diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS) (Mattson, 2000).

Neuronal survival requires a positive survival signal. Neurons usually obtain limited amounts of this survival protein / neurotrophic factor / neurotrophin from the target tissues they innervate. Neurotrophins such as Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5) generally bind and activate the Trk receptors, which are cell-surface receptors with intrinsic tyrosine kinase activity (Huang and Reichardt, 2003). Ligand induced dimerization of these receptors results in the phosphorylation of several tyrosine residues within its own cytoplasmic tail. These phosphotyrosines inturn serve as docking sites for other molecules such as phospholipase Cγ, phosphoinositide 3-kinase (PI(3)K) and adaptor proteins such as Shc and activate signaling pathways such as the MAP kinase pathway. Activation of these and other signal transduction cascades is known to be important for neuronal survival.
Adult neurons are characteristically postmitotic. In the developing mammalian CNS, neuronal precursor cells proliferate and differentiate into neurons within designated germinal zones. Once the neuronal precursors exit the cell cycle and enter a postmitotic state, these neurons migrate out of their proliferative zones and remain in a terminally differentiated state for the rest of their lives. Growing evidence however, suggests that they retain the ability to initiate events that lead to cell cycle progression. The current hypothesis states that neurons maintain their highly specialized structure and function by continuously holding their cell cycle in check, and, relaxation of this vigil may lead to cell death. This hypothesis is supported by the information that mitotic markers appear in neurons at risk for death in a variety of neurodegenerative conditions in mice and in humans. Studies have also shown that experimentally driving the cell cycle in mature neurons leads to cell death rather than proliferation (Becker and Bonni, 2004). Several studies have demonstrated correlations between deregulation of the cell cycle machinery and neuronal death both during development (Herrup and Busser, 1995) and during neuronal degeneration in the adult brain (Mattson, 2000). Deregulation of Cyclin Dependent Kinase 5 – an atypical Cdk which is active primarily in neurons, is an important event associated with neuronal apoptosis in several neurodegenerative diseases (Dhavan and Tsai, 2001). Interestingly, Cdk5 activator p35 is the most distant known member of the vertebrate cyclins (Lew et al., 1992; Tsai et al., 1994).

One indication that a differentiated cell, which is in the G0 phase of the cell cycle, is entering the cell cycle is the appearance of G1 phase associated D type cyclins. Due to this reason, cyclin D is considered a potentially major player in cell cycle related events in neurons. Specifically cyclin D1 is implicated in neuronal cell cycle re-entry. Cdk4 and Cdk6 are the cognate cyclin dependent kinase partners of cyclin D1.
The active cyclin D1/Cdk4/6 complexe phosphorylates retinoblastoma protein. Upon phosphorylation retinoblastoma loses its ability to sequester E2F transcription factor. E2F release from the Rb-E2F complex results in the transactivation of E2F-target genes, many of which are required for cell cycle progression e.g. cyclins, cdks and enzymes involved in DNA replication. However, the aberrant activation of the cell cycle machinery rarely leads to neuronal proliferation, instead apoptosis is induced (Liu and Greene, 2001).

Since neuronal differentiation is accompanied by cell cycle arrest, the fate of these cell cycle proteins has been thoroughly investigated during the process. Levels of most cyclins and Cdks, apart from cyclin D1, are lowered during the differentiation process (Yan and Ziff, 1995), thus, re-engagement of the cell cycle machinery may lead to “de-differentiation” of neurons and ultimately apoptosis.

Cyclin D1 has been directly implicated in neuronal apoptosis because cyclin D1 over-expression induces apoptosis of PC12 cells (Katayama et al., 2001). Also, inhibitors of active cyclin D1/Cdk4/6 complexes and administration of G1/S phase blockers rescue insult induced apoptosis in neuronal models (Farinelli and Greene, 1996; Ferrari and Greene, 1994; Park et al., 1997b). Over-expression of dominant negative Cdk4/6 also prevents death (Park et al., 1997a).

Despite several studies which highlight the role of cell cycle proteins like cyclin D1 in neuronal apoptosis, the mechanism by which levels of these proteins are upregulated and cause apoptosis is largely unknown. As these proteins are common players in neuronal differentiation and apoptosis, attempts to understand their regulation during neuronal differentiation may provide clues to understanding their deregulation during neuronal apoptosis. The major focus of this work was to
decipher the mechanism of regulation of cyclin D1 during neuronal differentiation and apoptosis. To this end, the following issues were addressed:

a. Mechanism of transcriptional regulation of cyclin D1.

b. Identification of signalling pathways that may control cyclin D1 levels during neuronal differentiation and apoptosis.

c. Dissection of the role of cyclin D1 in neuronal apoptosis.