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CNS CELL SIGNALING: HOMEOSTASIS, DISEASE AND REPAIR

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1. ABSTRACT

"What mysterious forces precede the appearance of these processes…promote their growth and ramification…and finally establish those protoplasmic kisses…which seem to constitute the final ecstasy of an epic love story." ~Santiago Ramón y Cajal [1852-1934]

The science of neurobiology is now almost a century older than times when Spanish neuroanatomist and Nobel laureate Santiago Ramón y Cajal had wondered as above. Yet, these 'mysterious forces' have only been partially illuminated today and the posed question still remains worth pondering upon in contemporary times. What Cajal identified as 'forces' are basically key cellular signals that are transduced preceding growth and ramification. A precipitate of our knowledge today tells us that these 'forces' are mostly generated within and amongst members of the central nervous system (CNS). The present chapter is aimed at appreciating cellular signals and their transduction pathways which underlie the functional output of CNS during normal times, diseased conditions, and regeneration.

2. AN INTRODUCTORY ORIENTATION

Signal transduction forms the basis of cellular perception to an external signal. Generally, it refers to defined and regulated cascade of cellular events that identifies a certain signal at cell surface or in intracellular compartments (reception desk) followed by engagement of second messenger pathway(s) that finally enable the cell to respond to the signal.

2.1. General mechanism of cellular signal transduction

Ideally, there are four stages in any signal transduction pathway. The first stage involves binding of receptors by the ligand. These receptors could be intracellular (e.g. nuclear hormone

receptors), or may be exhibited on the plasma membrane. The second stage involves activation of receptors in response to ligand binding. Once activated, the receptor recruits several modulators (e.g. G-proteins) as the third step in the cascade. Finally, in the fourth step, second messengers (e.g. cAMP, ceramide) are activated which convey the signal downstream to effecter molecules (e.g. transcription factors, which translocated to the nucleus and induce activation of specific genes). Although most signal transduction pathways are structured around four-stage process, yet variations are also observed.

For a signal to be able to induce an appropriate response to the inducer, it must be specific, fast, and must be amplified along the way of transduction. Indeed, amplification is achieved when one receptor recruits several modulators, which in turn activate several second messengers (Figure 1).

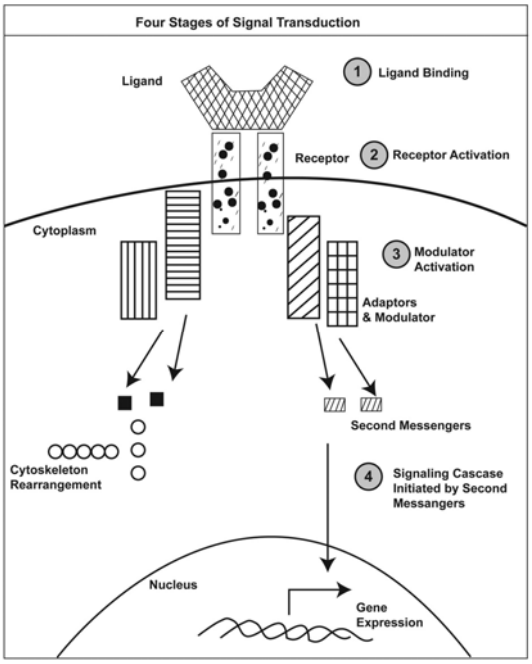

Figure 1: **Basic scheme of signaling**. Signal transduction pathways are usually composed of four stages as indicated. After ligand binding, receptor becomes activated and intracellular part of activated receptor recruits adapters and modulators that may produce second messengers. Second messengers may involve activation of enzymes like kinases and phosphatases, and/ or transcription factors. Finally, activation of transcription factors results in gene transcription, whereas signals not involving them mostly result in post-translational modification of existing proteins.

2.2. Signaling in CNS: a complex web of signaling in various cell types

Previous chapters of this book should have by now impressed the reader with the complexity of cellular types and function in CNS. The main cell types bathing in cerebro-spinal fluid are neurons, astrocytes, microglia, olidendroglia, and Schwann cells. Additionally, there are endothelial cells lining the blood-brain barrier (BBB). These cells, despite having distinguished functions of their own, are remarkably interconnected and demonstrate considerable amount of inter-cellular signaling between similar or dissimilar cells. Such crosstalk between different cell types forms the basis of several physiological outcomes like memory formation and axonal regeneration. Despite sharing several common signaling pathways, yet, sometimes same ligands induce strikingly opposite outcomes in different CNS cells. For example, few inducers of inflammatory response in glia cause degeneration of neurons. This suggests that there are cell-type-specific modulations of certain signaling pathways.

3. SIGNALS MAINTAINING NORMAL CNS HEALTH AND FUNCTION

3.1. Major signaling pathways maintaining CNS homeostasis

Regulation and/or maintenance of axonal growth, dendritic pruning, synaptogenesis and synaptic refinement, and neuronal survival/death are essential for the proper functioning of the nervous system. These functions are carried out following the interaction of neurotrophins with their plasma membrane receptors, Trk receptor tyrosine kinases (Trks) and p75 neurotrophin receptor ($p75^{NTR}$) and increase in cytoplasmic Ca^{2+}. In the mammalian brain four neurotrophins have been identified: nerve growth factor (NGF); brain-derived neurotrophic factor (BDNF); neurotrophin 3 (NT3) and neurotrophin 4 (NT4, also referred to as NT4/5) (Zweifel et al., 2005; Lu et al., 2005). Trk family of receptor tyrosine kinases comprises of three different receptors, Trk A, Trk B and Trk C. $p75^{NTR}$ is a member of the tumor necrosis receptor super family (Huang and Reichardt 2003). In general, activation of Trk receptors stimulates neuronal survival, differentiation, neurite outgrowth, synaptic plasticity, and function. $p75^{NTR}$ acts as a facilitator of Trk-mediated neuronal survival as well as an inhibitor of cell growth and promoter of apoptosis (Nykjaer et al., 2005).

Neurotrophins are synthesized as proneurotrophin precursors of ~27-35 kDa. These precursors of neurotrophins are cleaved either within the cell by the serine protease Furin (trans-Golgi network) and pro-convertase or in the extracellular space by the plasmin and matrix metalloprotineases (MMP3 and MMP7), affording mature neurotrophins of about 13 kDa. While mature NGF preferentially binds to and activates Trk A (k_D ~ 1-10nM), BDNF and NT4 (NT4/5) exhibit high affinity for Trk B. On the other hand, NT3 binds to and activate Trk C. Mature neurotrophins have slightly lower and similar affinities for $p75^{NTR}$, while proneurotrophins exhibit high affinity for $p75^{NTR}$ (Barker 2004).

3.1.1. Trk receptor signaling:

The extracellular domain of Trk receptors is made up of three leucine-rich 24 residue motifs flanked on either side by a cysteine cluster (C1 is on the outer side and C2 is in the inner side), followed by two immunoglobulin (Ig)-like domains and a single transmembrane domain. The cytoplasmic domain of Trk receptors contains several tyrosine motifs (Huang and Reichardt 2003). The major ligand binding site on Trk receptors is located in the region proximal to the Ig-C2 domain. Binding of neurotrophins to Trk receptors triggers receptor dimerization, autophosphorylation of tyrosine residues and activation of several signaling pathways. There are ten conserve tyrosine residues in each Trk receptors. Phosphorylation of Y670, Y672, and Y675 potentiate tyrosine kinase activity by pairing these negatively charged residues with basic residues in their vicinity. Phosphorylation of additional residues creates docking sites for adaptor proteins including Ras-Raf-MEK-Erk-CREB, PI3-kinase-Akt, PLCγ-Ca^{2+}, NF-κB and atypical protein kinase pathways. In Trk A receptor, phosphotyrosine 490 creates a docking site for Shc, fibroblast growth factor receptor substrate 2 (FRS2) which then activates Ras and PI3 kinase. However, phosphorylation of 785 residue recruits PLCγ-1. Activation of these pathways leads to local control of axonal growth, neuronal survival and metabolism.

Neurotrophin-Trk receptor complexes are internalized and retrogradely transported from distal axons to the neuronal cell body where they signal to the soma to mediate target-dependent survival, growth and gene expression. The neurotrophin-Trk receptor complex is internalized by four mechanistically diverse and highly regulated pathways: macropinocytosis; clathrin-mediated endocytosis; caveolae-mediated endocytosis and Pincher-mediated endocytosis. The kinase activity of Trk is probably required for receptor internalization.