A. INTRODUCTION

The development of the brain from a unicellular stage to a multicellular complex organ has intrigued several neuroscientists. In order to understand the structure and function of the brain it was necessary to identify the highly specialized cellular components and changes in the development of such a complex organ from simplicity to a complicated array of phenomenon.

The earliest reference to brain was made by Sumerians in their attempt to depict euphoriant effects of poppy plants, some time around 4000 B.C. But the first available medical record of describing brain was in "The Edwin Smith Surgical Papyrus" dated around 1700 B.C. written by the Egyptian physician Imhotep (Swanson et al., 1999). Edwin Smith's papyrus, described for the first time the brain anatomy, the meninges of the brain, the bone marrow and the cerebrospinal fluid. Hippocrates and Plato believed brain as the seat of intelligence around 460-387 B.C. Ever since, several contributions have been made for a better understanding of the nervous system. Until the end of 19th century most biologists believed that there was a cytoplasmic continuity of cells in the nervous system.
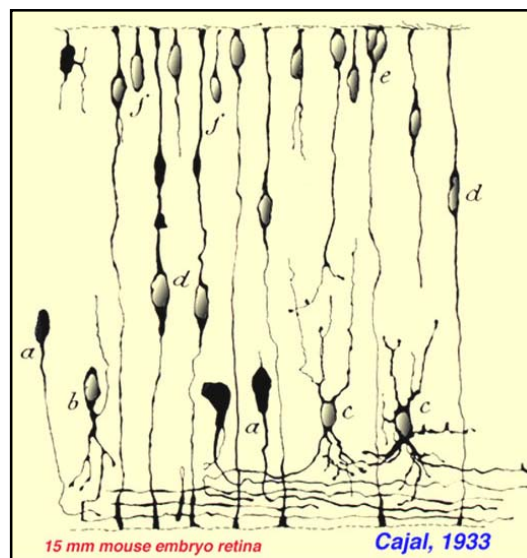

Fig (a): **Mouse retina stained by golgi staining**. Golgi stained embryonic mouse retina to show migration of cells followed by differentiation in the correct retina layers (From Cajal, 1933)

Camillo Golgi was one of the prominent scientists to suggest that nervous system was composed of not individual cells but a continuous syncytium. Golgi was the first to think about a link between neuroscience and psychiatry. In the early 20th century, Nobel laureate Santiago Ramón y Cajal considered as the founder of contemporary

neuroscience contradicted Camillo Golgi by showing that "neurons interact by way of contact or contiguity rather than by continuity". Cajal also conceptualized that "the dendrites and the cell bodies of neurons receive information, whereas the single axon transmits information to the adjacent neuron" (Swanson et al., 1999;Sanes et al., 2000;Kandel, 2000).

Since, the proposal of Santiago Ramón y Cajal on the theory of memory in 1894, stating that information is stored in the brain by anatomical changes in connections between neurons several developments have taken place in the understanding of neurons (Bailey et al., 2000). The terminology "Synapse" was defined as a focal point of communications between nerve cells by Charles Sherrington, the Nobel laureate. The formation of synapses by neurons makes them very unique from all other tissues of the body system (Sanes et al., 2000). The brain is composed of neurons which are supported by the glial cells. Neurons and glial cells work together to form the basis of the nervous system (Bloom and Swanson, 2003).

1.   *Molecular analysis of synaptogenesis*

Synapse formation is directed by a collaborative effort of an activity-independent guidance of growth cones by molecular cues (except in case of cortical pyramidal neurons or olfactory neurons, where the growth cones are guided by guidance cues modulated by neuronal activity) (Catalano and Shatz, 1998;Zheng, 2000;Ming et al., 2001) which specify initial neural connections and an activity-dependent process which gives shape to the initial connections (Misgeld et al., 2002;Munno and Syed, 2003). The early stages of synapse development depends on the genetic program of the cell and includes establishment of cell phenotype, target selection and distribution of initial synaptic connection (Roerig and Feller, 2000). The refinement takes place in a prolonged period during which several synapses are eliminated as well as added, requiring the action of multiple proteins that get regulated during the development; thus establishing adult patterns of connectivity and function (Katz and Shatz, 1996).

The earliest events in synaptic differentiation takes place in the absence of either pre- or post-synaptic activity, whereas the later refinement and restructuring depends on electrical activity (Zhang and Poo, 2001;Desai et al., 2002).

2.   *Activity-independent guidance for synaptogenesis*

Several molecules act as guidance cues aiding in fulfilling the basic criteria for an efficient synapse by positioning pre- and post-synaptic specializations apposing across the synaptic cleft (Yamagata et al., 2003). Proteins such as adhesion molecules hold the membranes of two apposing cells and hence have primary role in the synaptogenesis. Broadly, stating adhesion molecules play four distinct roles in synaptogenesis: (i) maintain the integrity and attribute stability to the synapses (ii) aid in recognition of the target region (iii) promote differentiation of pre- and post- synaptic specializations and (iv) probably are involved in regulation of synaptic structure and function (Serafini, 1999;Yamagata et al., 2003). Cadherins, immunoglobulin superfamily and integrins are major groups of adhesion molecules. Cadherins are calcium dependent adhesion proteins mediating homophilic interactions which are involved in recognition of synapse formation region (Serafini, 1999). Among all the 80 different types of cadherins found in the vertebrate system, N-cadherin has been well studied for its role in neurulation, regionalization of neuroectoderm, migration of neurons as well as in axonal growth (Itoh et al., 1997;Ranscht, 2000). Immunoglobulin superfamily (IgSF) members have a characteristic immunoglobulin domain (Rougon and Hobert, 2003). Neural cell adhesion molecule (NCAM), one of the members of the IgSF regulate the number of synapses and strength of synapses in an NMDA receptor dependent manner based on electrophysiological studies (Dityatev et al., 2000). SynCAMs, nectins and GPI anchored IgLONS such as opoid binding cell adhesion molecule, neurotractin are few other cell adhesion molecules having an immunoglobulin domain (Yamagata et al., 2003). Expression of SynCAM along with glutamate receptors in a heterologous cell coculture with hippocampal neurons induced synaptogenesis (Biederer et al., 2002). The nectin family forms homodimers with four of its isomers namely, 1, 2, 3 and 4. The nectin 1 and 3 isomers localize in the synapses formed between the mossy fiber and the pyramidal cells of adult mouse hippocampus. Inhibition of nectin 1 resulted in decrease of synapse size with a simultaneous increase in the synapse number. The authors speculated the increase could be a compensatory phenomenon for the smaller size of the synapses (Mizoguchi et al., 2002). Opoid binding cell adhesion molecule and neurotractin are observed in postsynaptic sites in cortex and hippocampus of adult mouse and are expressed in developmentally regulated manner reaching a peak at the adult stages (Miyata et al., 2003).

The mechanism leading to positioning of pre and post-synaptic specialization opposite to each other has been studied extensively at molecular level in neuromuscular junction (NMJ) synapses. These details are a generalized mechanism for CNS synaptogenesis (Sanes and Lichtman, 1999). In the NMJ synapse formation, there occurs a pre-patterning of the post-synaptic organization before the arrival of the nerve terminal. Acetylcholine receptors (AChRs) and neuregulin are involved in the pre-patterning in muscle fiber which seems to be prerequisite for the formation of subsequent synapses (Yang et al., 2001;Arber et al., 2002). Neuregulin has been implicated in the synthesis of AChRs synthesis (Sandrock, Jr. et al., 1997;Lin et al., 2001). With the arrival of the motor neuron to the pre-patterned post-synaptic terminal, Agrin is released and subsequent post-synaptic specializations take place (McMahan, 1990;Sanes and Lichtman, 2001). Agrin is synthesized both by the muscle as well as by the neurons but z-plus Agrin (neuron isoform) induces clustering of the AChRs at the site of neuron innervation in the presence of MuSK (muscle specific kinase) (DeChiara et al., 1996). In the absence of Agrin, there occurs a pre-patterned clustering of AChRs, which get dispersed on the neuron reaching the muscle, where as in the absence of MuSK, there does not occur any clustering of AChRs at all (Yang et al., 2001). Then follows anterograde and retrograde signalling pathways, which stabilize the synaptic connection formed with the formation of the active zone (Goda and Davis, 2003).

Succeeding the roles played by guidance cues, the activity-dependent processes take over the process of synaptogenesis by stabilizing the connections.

3. *Neuronal activity role in synaptogenesis*

Though several studies have been made, the specific manner in which activity modifies the synapse formation is still under speculation. Two general thoughts have emerged, one the Hebbian thought, which works at the level of single neurons and the next one is homeostatic modifications involving entire networks of neurons (Burrone et al., 2002). Donald Hebb proposed the Hebbian thought in the mid of 20th century, relating the role of neuronal activity to synaptic strengthening. His theory stated that a connection between a set of two neurons gets strengthened when the firing of the presynaptic and postsynaptic neurons are closely correlated in time (Katz and Shatz, 1996;Peled and Geva, 1999;Bailey et al., 2000). The patterned electrical activity during brain development plays an important role not only in organizing connections between the regions of the brain, but also in

maintaining the morphology of neurons and formation of local circuits (Turrigiano et al., 1994;Feller, 1999;Cline, 2001).

There are two kinds of electric activity, first one being spontaneous activity present during the embryonic stages; the next one is an experience driven activity appearing in the post-natal critical periods (Zhang and Poo, 2001). Spontaneous activity participates in the initial events like those of axonal outgrowth, pruning of synaptic connections, maturation of neuronal signalling and is known to be expressed through out the neuraxis by expressing calcium transients (Moody, 1998;O'Donovan et al., 1998). The experience driven electrical activities consisting of spike trains of varying frequencies are very critical; they are driven by the sensory experience and coincide with the presence of the critical periods of development (Katz and Shatz, 1996;Zhang and Poo, 2001;Desai et al., 2002). The sensory experience driven activity during the post natal critical period strengthens the connectivity by eliminating the weak and unstable synapses and consolidating the stable connections into mature forms (Katz and Shatz, 1996;Zhang and Poo, 2001). Neuromuscular junctions (NMJ) and visual system are the models which have given most of the insights to the underlying mechanisms of spontaneous as well as experience driven neuronal activity (Kavalali et al., 1999).

3.1 Neuromuscular junction

Extensive studies have been carried out on *Drosophila melanogaster* NMJ to investigate the role of neuronal activity on synapses. The NMJ synapses are glutamatergic in nature similar to excitatory synapses of the vertebrate brain (Davis and Bezprozvanny, 2001;Salinas, 2003). Receptor clustering in the post-synaptic density is formed in discrete fractions when the neuron reaches the muscle. The refinement of the discrete clusters into more concrete clusters takes place with the help of spontaneous neuronal activity (Saitoe et al., 1997). Spontaneous neuronal activity is sum of miniature endplate potential and action potentials.

Miniature endplate potential (mini) is generated on the spontaneous fusion of a synaptic vesicle with the presynaptic membrane, resulting in diffusion of neurotransmitter and simultaneous activation of postsynaptic receptors (Verstreken and Bellen, 2002). Action potential represents transient change of the membrane potential from a negative value to a

more positive by increasing the sodium permeability during depolarization, resulting in generation of an action potential (Purves et al., 1997;Matthews, 2001).

Saitoe and his colleagues, with their mutant fly experiments revealed that "minis" and not action potential generated fusions are responsible for clustering of glutamate receptors. Mutants of *Syntaxin-1A* or *Shibre* lacking both "minis" as well as action potential generated exocytosis could not cluster glutamate receptors. Mutations of *neuronal-synaptobrevin* or *cysteine string protein* in *Drosophila melanogaster* resulted in lack of action potential generated exocytosis only. Clustering of glutamate receptors did not get affected in these later mutants, suggesting "minis" a necessity for clustering of glutamate receptors (Broadie et al., 1995;Saitoe et al., 2001).
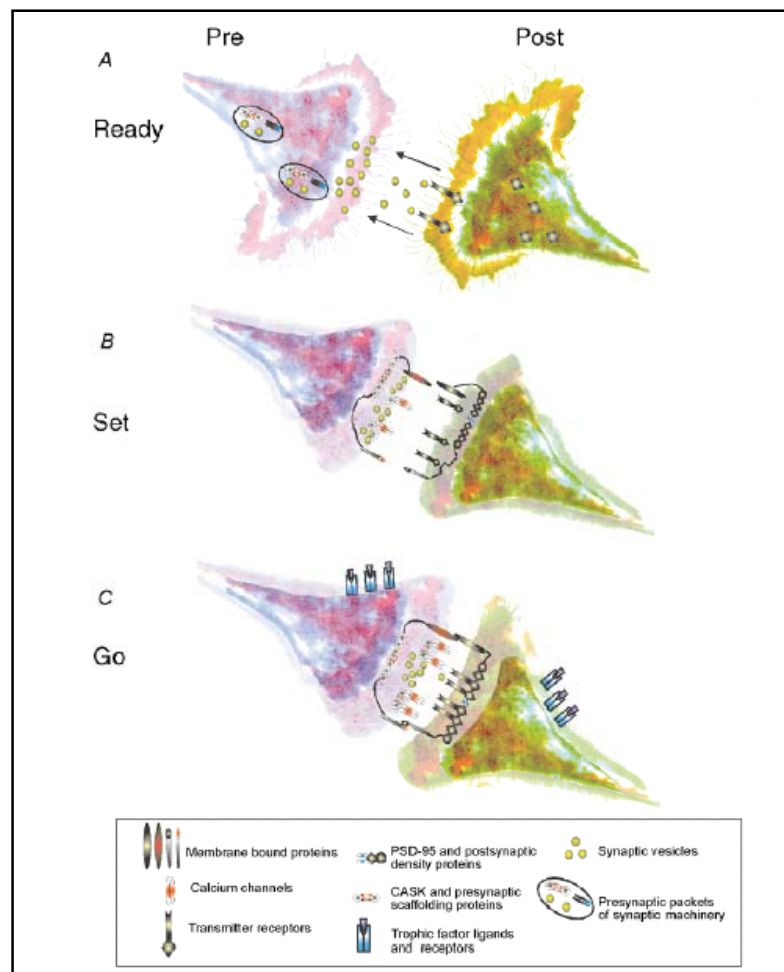

Fig (b) **Formation of synapses from recognition to connectivity**. A, as pre and postsynaptic growth cones approach each other, transmitter-receptor interactions, via the release of presynaptic vesicles, attract appropriate target growth cones by binding to and stimulating postsynaptic receptors. Various components of pre- and postsynaptic specializations, including presynaptic packets containing synaptic machinery and channels and postsynaptic proteins such as PSD-95 are mobile prior to contact. B, as extending growth cones contact, the growth cones smooth, and asymmetric interactions between membrane-

bound molecules such as neurexin/neuroligins, cadherins and integrins mark the synaptic site and stabilize pre and postsynaptic scaffolding proteins such as CASK and PSD-95. C, subsequent maturation of the synaptic contact and interactions between trophic ligands and receptors leads to clustering of calcium channels and synaptic vesicles at the pre-synaptic terminal and the transmitter receptors at the postsynaptic bouton. (From (Munno and Syed, 2003))

In vertebrates accumulation of AChRs in the postsynaptic terminal is an activity independent process, but their stabilization and maturation requires agrin. Maturation and stabilization of the post-synaptic terminal is an activity-dependent process (Sanes and Lichtman, 2001;Cohen-Cory, 2002). Pharmacological blockade of neuronal activity induced excessive motor axon sprouting and prevented normal synapse elimination process, most probably by modulating neurotrophins which regulate neuregulin expression and thereby AChR synthesis (Broadie and Bate, 1993).

Results from studies on NMJ point to the roles played by the spontaneous and sensory driven electric activity in refinement and maturation of neuronal connectivity.

3.2 Visual pathway

In the retina neuronal spontaneous activity consists of bursting action potentials occurring before eye opening that mediate the pruning of the lateral geniculate nucleus in the eye-specific layer. The precise connections between the retinal ganglion cells and their central target structures as well as formation of connections within the retina are facilitated by correlated firing of the ganglion cells and spatiotemporal patterning of the neuronal activity (Wong et al., 1995). Blocking of the spontaneous action potentials by tetrodotoxin prevents segregation of layers in the lateral geniculate nucleus (Penn et al., 1998).

The development of the ocular dominance columns in the mammalian neocortex has been quite speculative with respect to the role of neuronal activity (Crowley and Katz, 2002). Ocular dominance columns were initially described by Hubel and Wiesel in cats. Based on electrophysiological recordings they stated that the establishment of the ocular dominance columns are guided by innate mechanisms, and the competition based mechanisms are responsible for their later modification during the critical periods (HUBEL and Wiesel, 1963). LeVay and his colleagues observed by injecting tritiated amino acids (an anterograde transport tracer) in young cats, a continuous homogenous band of label in layer 4 of the primary visual cortex which might be a result of absence of segregation. Contradicting Hubel, Levay and his colleagues concluded that the precise organization of

the ocular dominance columns was not innately established but was a gradual process guided by the columnar rearrangement during critical period (LeVay et al., 1978). Experimental studies from monkeys also revealed similarity in the segregation of ocular dominance columns between the adults and the new born, suggesting the role of non-visually driven activity in segregation of the columns (Horton and Hocking, 1996). Recent findings form ferrets have also pointed out to the fact that ocular dominance columns are established by P37 and correspond to the beginning of the critical period (when neuronal circuits are susceptible to external sensory inputs) at P35 for their maintenance (Katz and Crowley, 2002).

Hence, combining the findings suggest activity blockade rather than preventing segregation of the dominance columns, desegregates the ocular dominance columns previously present. The role of spontaneous activity cannot be ruled out in the initial formation of the ocular dominance columns, though there are no supportive experimental evidences

4.   *Activity-dependent gene and protein regulation*

Neuronal activity modulates the calcium currents (Li et al., 1996). The most critical factor of neuronal activity is a change in post-synaptic (sometime pre-synaptic) membrane potential, which is usually a depolarization (Peled and Geva, 1999). Calcium transients arise either from membrane depolarization or release of calcium from internal pools (Zhang and Poo, 2001). Calcium influx occurring through N-methyl-D-aspartate receptors, AMPA receptors and voltage gated calcium channels are necessary to maintain the topographic organization by affecting the gene expression, protein synthesis, synaptic plasticity, neuronal development and survival (Ghosh and Greenberg, 1995;Corriveau, 1999;Debski and Cline, 2002).

Calcium by acting either directly through its cytoplasmic effectors or by acting as a second messenger regulates gene or protein expression (Ghosh and Greenberg, 1995;Nedivi, 1999;Zhang and Poo, 2001). The synaptic plasticity that results from the calcium influx has been observed in two forms, one is short-term plasticity (STP) and the other long-term synaptic plasticity (LTP) (Bailey et al., 1996;Corriveau, 1999). The long-term and short-term synaptic plasticity occurs at the level of transcription or translation and post-translational level respectively (Sheng et al., 1990).The STP is mostly regulated by post-

translational modification without the necessity of any protein synthesis (Kandel, 1997). The STP and LTP are the simple cellular basis of our learning and memory (West et al., 2001;Tartaglia et al., 2001;Pittenger and Kandel, 2003;Bailey et al., 2004).

4.1 Gene regulation

The remodelling of synapses by repeated neuronal activity affects gene expression, what correlates with neurotransmitter mediated signalling resulting in long-term synapse remodelling (Naeve et al., 1997). The long-term synaptic plasticity depends on protein synthesis i.e., transcription and translation.

Calcium moves into the cell and to the nucleus where it activates a calcium inducible gene called CREB (cAMP response element binding protein) and other complimented transcription factors most likely by binding to calmodulin and activating CaM (calmodulin) kinases (Sheng et al., 1991;Stevens, 1994;Ghosh and Greenberg, 1995;West et al., 2001). This is one of the earliest steps involved in the cascade of transcription regulation. The families of genes which are regulated by neuronal activity are known as the "activity-dependent regulated genes". These genes are classified in two groups based on their kinetics i.e., the rapidly induced genes are the immediate-early genes (IEGs) and the slowly induced ones are the delayed response genes (DRGs).

Genes of transcription factors (*c-Fos, c-Jun, zif268, Arg 3.1*), cytoskeleton associated proteins (Arc), for neuronal migration and outgrowth (GAP-43, Narp) extracellular proteases and molecules such as brain-derived neurotrophic factor (BDNF), dynorphin, serum inducible kinase, major histocompatibility complex I and *cpg15* are some that are regulated by neuronal activity (Ghosh and Greenberg, 1995;Naeve et al., 1997;Nedivi, 1999;Waltereit et al., 2001). For example, *cpg15* over expression in the optic tectum of *Xenopus laevis* tadpole resulted in faster growth of the dendritic branches and formation of mature arbors. Electrophysiological studies on *cpg15* over expressing tectal cells, revealed faster synaptic maturation resulting in higher incorporation of AMPA receptors (Nedivi et al., 1998;Cantallops et al., 2000). Serum inducible kinase (SNK) belonging to the polo family of kinases is targeted to spines and degrades spine associated Rap GTPase (PSD protein) involved in spine morphology. Role of SNK is speculated to have negative feedback mechanism on synaptic efficiency upon elevated neuronal activity (Pak and Sheng, 2003). The expression of Narp (neuronal activity-regulated pentraxin), a member

of the pentraxin family of proteins, is up regulated by seizures and co clusters with AMPA receptors when expressed either in the same or contacting cells (O'Brien et al., 1999;O'Brien et al., 2002). Narp when co-expressed with other members of the pentraxin family like NP1, forms complexes resulting in higher synaptogenic activity (Xu et al., 2003). Major histocompatibility complex I (MHC I) gene expression gets decreased on blockage of neuronal activity during the period when spontaneous retinal activity refines the visual system to form mature and segregated pattern of connections (Huh et al., 2000). MHC I is expressed in a pattern close to the pattern of activity-dependent plasticity observed in early postnatal retina, lateral geniculate nucleus and adult cerebellum and hippocampus (Boulanger et al., 2001). In the adult hippocampus MHC is required for long term potentiation as well as long term depression, forms of activity dependent synaptic plasticity (Boulanger and Shatz, 2004). Synaptic activity and action potential generation induce the secretion of BDNF that modulates synaptic strength and neuronal connectivity (Poo, 2001).

Expression profiling of genes in embryonic culture of rat cortical neurons after KCl (50 mM) mediated depolarization revealed 469 genes that were activity-dependent regulated. The genes were functionally classified into 19 functional categories (Li et al., 2004) with the set of transcription factors leading the group.

4.2   Protein regulation

Neuronal activity induces elevation of calcium thereby regulating a wide range of protein expression (Li et al., 1996). Neuronal activity is also known to modulate the protein expression for stabilizing synaptic connections at the translational level. There are various mechanisms for the protein regulation finally resulting in clustering of receptors, expression of receptors on cell surface, change in the subunit composition of receptors, change in protein turnover and change in the expression of protein both in structural and functional aspect (Vallano et al., 1996;Lissin et al., 1998;Quinlan et al., 1999;Ehlers, 2003).

Short-term synaptic changes are mediated by regulation of proteins at the post-translational level (Goggi et al., 2003). This is most likely due to the action of cAMP dependent protein kinases (Nguyen and Kandel, 1996;Kandel, 1997). For short-term changes post translational modifications occur on existing proteins (Peled and Geva,

1999). The various post-translational modifications which are responsible for short-term synaptic plasticity are phosphorylation, dephosphorylation, myristoylation, palmitoylation, ubiquitination, sumoyilation and proteolysis (Farazi et al., 2001;Ehlers, 2003;Schwartz and Hochstrasser, 2003). Phosphorylation or dephosphorylations are the most common post-translational modifications which occur by the addition or removal of phosphate groups to serine, threonine and tyrosine residues in proteins via the activation of protein kinases or phosphatases (Ron, 2004). Myristoylation is a lipid modification of the protein at its N-terminus, attributing secondary functions to protein such as anchoring protein to the membranes and consequently regulating signal transduction and translocation. It is an enzymatic process involving myristoylCoA:protein N-myristoyltransferase (NMT), which recognizes the characteristics of the substrate protein and attaches a lipid moiety to the N-terminal glycine (Boutin, 1997). Palmitoylation is a post-translational modification involving in addition of 16 carbon fatty acid, palmitate to a cysteine residue by making a thioester bond. This is unique in being a dynamic process, which is reversible as compared to myristoylation which is an irreversible phenomenon (Bizzozero, 1997). Palmitoylation has been implicated to aid in the process of protein sorting, axonal growth, G-protein signalling, ion channel clustering, presynaptic signalling and postsynaptic plasticity (El-Husseini and Bredt, 2002). Ubiquitination is known as "kiss of death" process for the proteins. The protein gets inactivated by attaching ubiquitin, which is a small molecule that acts as a signalling molecule for the protein transport machinery to transport to the proteasome for degradation (Ehlers, 2003;DiAntonio and Hicke, 2004). Sumoylation, is biochemically analogous but functionally very distinct to ubiquitination. It involves the covalent attachment of a SUMO (small ubiquitin-related modifier) to substrate proteins. This sumoylation results in conferring crucial functions including protein localization, stability, nucleocytoplasmic signalling and transport (Muller et al., 2001). Proteolytic processing or ectodomain shedding is another kind of post-translational modification involving enzymatic cleavage of a protein, leading to a releasable fragment and a membrane attached stump (Loeb and Fischbach, 1995;Han and Fischbach, 1999;Shirakabe et al., 2001). Proteolytic processing plays a critical role in protein regulation by removing protein when they are not needed, as well as transform dormant proteins to its active form or vice versa (Ehrman and Clausen, 2004).