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The Role of Targeted Protein Degradation in Early Neural Development

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Abstract

As neural stem cells differentiate into neurons during neurogenesis, the proteome of the cells is restructured by de novo expression and selective removal of regulatory proteins. The control of neurogenesis at the level of gene regulation is well documented and the regulation of protein abundance through protein degradation via the Ubiquitin/26S proteasome pathway is a rapidly developing field. This review describes our current understanding of role of the proteasome pathway in neurogenesis. Collectively, the studies show that targeted protein degradation is an important regulatory mechanism in the generation of new neurons.

Keywords

ubiquitin ligase; F-box proteins; neurogenesis; neural induction

Introduction

In neurogenesis, neurons are produced from neural stem cells through successive steps characterized by dynamic changes in cellular protein profiles. Failure to properly regulate these changes in protein levels disrupts the balance of proliferating progenitors and differentiated neurons and can be detrimental to the development of the central nervous system (CNS) (Cremisi et al., 2003; Ohnuma and Harris, 2003; Salomoni and Calegari, 2010). With premature exit from the cell cycle, the size of the progenitor pool is depleted and with a delay in differentiation or cell cycle exit, the pool is expanded, changing the size of the brain and the neuronal subtype population (Donovan and Dyer, 2005; Fero et al., 1996; Goto et al., 2004; Guillemot, 2007; Spella et al., 2011). The focus in neural specification and differentiation has been regulation at the level of gene expression; however, regulation by targeted protein degradation is underappreciated yet is a burgeoning field in cell biology. For example, studies in cell cycle and cancer biology suggest that protein degradation is an essential strategy for regulating protein abundance in these highly dynamic processes (Lehman, 2009; Nakayama and Nakayama, 2006). This review summarizes the current understanding of targeted protein degradation in neural induction, patterning and neurogenesis and highlights potential areas for future research.

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Ubiquitination and ubiquitin ligases

The ubiquitin/26S proteasome pathway is one of the major ways in which proteins are selectively degraded (Hershko, 1983; Ciechanover et al. 1984). This pathway is a multi-step process, in which three enzymes catalyze the transfer of ubiquitin (Ub), a highly conserved 76 amino acid peptide present in all eukaryotes (Hochstrasser, 1996; Varshavsky, 1997), to a target protein (Fig. 1). The process of ubiquitination begins when the E1 Ub-activating enzyme activates and transfers Ub to a catalytic cysteine residue on the E2 Ub-conjugating enzyme, generating a Ub-E2 intermediate. Depending on the type of E3 ligase, the Ub is either transferred directly to the substrate or is held temporarily by the E3 prior to transfer (Hershko, 1983). In both cases, the E3 ligase facilitates transfer of Ub to the substrate. With multiple rounds of ubiquitination, a chain of at least 4 Ubs is attached to the substrate thereby targeting it for degradation in the 26S proteasome (reviewed in Pickart and Eddins, 2004; Wolf and Hilt, 2004). In contrast, proteins tagged with a single Ub are generally associated with endocytosis and protein sorting, and are either degraded in the lysosome, sub-localized within the cell or recycled by deubiquitination (Guterman and Glickman, 2004; Mosesson et al., 2009; Swanson et al., 2006; Todi and Paulson, 2011).

The E3 ligase proteins are responsible for target recognition, and thus are the most numerous and diverse of the three Ub addition enzymes (Smalle and Vierstra, 2004). They act as bridging factors that bring the E2-Ub complex and target protein together, via conserved binding domains for both. Based on their structure, E2 binding site, and ubiquitin transfer mechanisms, they are categorized into three major groups: RING (Really Interesting New Gene), HECT (Homologous to E6AP Carboxy Terminal), and U-Box (Fig. 2).

RING ubiquitin ligases

The RING ligase family of proteins is the largest and most diverse of the three E3 ligase families. They serve as scaffolds bringing E2 closer to the substrate and facilitating transfer of Ub directly from E2 to the substrate. Structurally, the RING E3 ligases feature a common RING finger domain subunit (Rbx1/Roc1/Hrt1) that binds E2 and a scaffold protein that links E2 to adaptor proteins required for substrate recruitment. Dependent on the components of the protein complex, the RING ubiquitin ligases are further categorized into Cullin-based (Fig. 2) or APC/C (Anaphase-promoting complex/cyclosome) Ub ligases (Pickart and Eddins, 2004). In this review, we will only discuss the Cullin-based Ub ligases, which play roles in neural patterning and neurogenesis.

In Cullin-based ligases, the Cullin scaffold protein specifies the type of adaptor protein. Cullin1 binds to the adaptor Skp1, Cullin2 forms a complex with ElonginB/ElonginC, and Cullin3 does not utilize any adaptors and instead directly binds to BTB/POZ domains of target proteins (Kipreos, 2005; Sarikas et al., 2011). The best characterized Cullin-based ligases are the SCF (Skp1, Cullin1, and F-box) ligases, which are involved in numerous cellular and developmental processes from cell cycle progression (Nakayama and Nakayama, 2005), to stress response (Asada et al., 2008; Kuiken et al., 2012), to DNA repair (Galli et al., 2010; Jia and Sun, 2009; Kondo et al., 2004). SCF ligases function in such a wide variety of processes because of the large number of F-box proteins (Fig. 2a); *C. elegans* has 326, *Arabidopsis* has 694, and humans have more than 70 F-box proteins

(Gagne et al., 2002; Jin et al., 2004). The F-box protein features a conserved Skp1 binding domain at the N-terminus, termed the “F-box domain”, and a substrate recognition domain at the C-terminus. They are categorized into 3 groups based on their C-terminal domain: Fbxw (WD40 repeat domains), Fbxl (leucine-rich repeat domains), and Fbxo (other domains including Kelch, between-ring domain (IBR), F-box-associated domain (FBA), and more) (Jin et al., 2004).

HECT ubiquitin ligases

HECT-type ubiquitin ligases are single subunit enzymes (Fig. 2b) (Metzger et al., 2012) with intrinsic C-terminal catalytic activity that facilitates loading of Ub from E2 onto a conserved cysteine residue, and then transfers it to the target protein bound to its N-terminus (Kamadurai et al., 2009). The size of the HECT family is small in many organisms (e.g. approximately 30 HECT proteins in humans) (Metzger et al., 2012) and is divided into three subgroups based on their target interaction domain: the Nedd4 (Neural Precursor Cells Expressed Developmentally Down-regulated 4) group with tryptophan-tryptophan (WW) domains, the HERC (HECT and RCC1- regulator of chromosome condensation 1 domain) group with RCC1-like domains, and the Other group with other domains (Bernassola et al., 2008; Scheffner and Staub, 2007). Although the functions of many HECT E3 ligases are yet to be identified, an increasing number of studies indicate their importance in signal transduction (Chen and Corliss, 2004; Edwin et al., 2010; Inoue and Imamura, 2008), cancer (Bernassola et al., 2008) and other human diseases (Scheffner and Staub, 2007), and embryonic development (Sarkar and Zohn, 2012; Zohn et al., 2007).

U-Box ubiquitin ligases

The single subunit, U-box E3 ligases consists of a conserved U-box or RING-finger domain for E2 interaction and a divergent target binding domain (e.g. cyclophilin-like, tetratricopeptide, WD40, etc.) (Fig. 2c) (Hatakeyama and Nakayama, 2003). They have only recently been defined as a class of Ub ligases (Hatakeyama et al., 2001) and were initially thought to assist RING and HECT E3 ligase ubiquitination through transfer of an oligoubiquitin tail (Koegl et al., 1999). However, recent studies show that U-box proteins sufficiently achieve the polyubiquitination of target proteins in the absence of RING or HECT E3 ligases (Hatakeyama et al., 2001). The family is small in many eukaryotes with only seven U-box E3 ligases in humans (Marín, 2010). Despite this, they regulate many aspects of cellular life including a housekeeping function for degradation of unfolded or misfolded proteins (Hatakeyama and Nakayama, 2003), cell fate determination (Yamada et al., 2013), inflammatory response (Liu et al., 2011), and disease (Jang et al., 2011; Tetzlaff et al., 2008).

Degradation of key regulatory proteins in neurogenesis

Neurogenesis requires coordinated gene and protein regulation for controlled progression of neuron formation (Fig. 3). In this process, the neural stem cells either proliferate to maintain a pool of neural stem cells or exit the cell cycle to mature into neurons or glia. The neural stem cells are characterized by symmetrical cell division (Götz and Huttner, 2005) and the expression of neural stem cell maintenance genes such as the *SoxB1*s (Pevny and Plazcek,

2005), RE-1 Silencing Transcription Factor (*REST)* (Gao et al., 2011) and *Notch* (Zhou et al., 2010). To form neurons, neural stem cells undergo asymmetric cell divisions and a subset of proteins segregate differentially between the two daughter cells, one of which remains a stem cell while the other cell differentiates (Egger et al., 2010, 2007; Prehoda, 2009; Zhong and Chia, 2008). In the differentiating cell, the levels of REST (Ballas et al., 2005; Muraoka et al., 2008; Westbrook et al., 2008), Notch signaling (reviewed in Artavanis-Tsakonas et al., 1999; Hoeck et al., 2010; Mumm and Kopan, 2000), and SoxB1s (Bylund et al., 2003; Graham et al., 2003; Rogers et al., 2008; Savare et al., 2005) are down-regulated by repression of their gene expression and post-translational modifications. The decrease in Notch signaling is accomplished in part by degradation of the effector protein Hes1, which allows for the expression and stabilization of differentiation genes including the proneural gene, Neurogenin (Ngn) (Hatakeyama et al., 2004; Ishibashi et al., 1995; Kageyama et al., 2008; Yoshiura et al., 2007). Transient stabilization of Ngn allows for the activation and accumulation of its transcriptional targets important for differentiation and cell cycle exit (Ali et al., 2011; Hindley et al., 2012; Shimojo et al., 2011). As the interactions between proteins and signaling pathways restructure the proteome of the differentiating cell, the abundance of many proteins is regulated by targeted degradation by the ubiquitin-proteosome system.

Notch/Delta signaling pathway

Notch signaling plays a fundamental role in the maintenance of neural progenitors, neurogenesis, and gliogenesis and has been shown to be regulated extensively at the level of protein degradation (Shimojo et al., 2011; Zhao et al., 2009). The transmembrane receptor *Notch* interacts with membrane bound ligands, such as Delta, which promotes signaling in both cells. The dual signaling results in “lateral inhibition” where the Delta+ cell differentiates into a neuron and the surrounding Notch+ cells remain progenitors (Katsube and Sakamoto, 2005). Notch activation leads to proteolysis and the release of the intracellular domain (NICD), which translocates to the nucleus and interacts with the RBP-Jkappa family of proteins (also known as CSL - CBF1/Su(H)/Lag-1). When in a complex with NICD, RBP-Jkappa functions as an activator and turns on the expression of target genes. In the differentiating cell, Notch signaling is down-regulated by ubiquitination and degradation of NICD and the NICD effector, Hes1, allowing for transient up-regulation of Delta (Hirata et al., 2002; Matsumoto et al., 2011).

At least five E3 ubiquitin ligases: Suppressor of deltex/Itch (Cornell et al., 1999), SCF^{Fbxw7} (Oberg et al., 2001; Wu et al., 2001), Ligand of Numb-protein X (LNX) (Nie et al., 2002), Neuralized (Deblandre et al., 2001; Lai et al., 2001; Pavlopoulos et al., 2001; Yeh et al., 2001), and Mind bomb (MIB) (Chen and Corliss, 2004; Itoh et al., 2003) fine-tune Notch-Delta signaling. Studies show that these E3 ligases are key regulators of neural progenitor maintenance and neuronal differentiation. Suppressor of deltex/Itch (Chastagner et al., 2008; Qiu et al., 2000) and SCF^{Fbxw7} (Gupta-Rossi et al., 2001; Oberg et al., 2001; Wu et al., 2001) directly target NICD, whereas LNX degrades Numb, an antagonist of NICD (Nie et al., 2002). In the mouse brain, neuronal differentiation is blocked in the absence of SCF^{Fbxw7} due to stabilization of its targets Notch and c-Jun (Hoeck et al., 2010). SCF^{Fbxw7} is not only important for progression of neurogenesis but also for oligodendrocyte

development (Snyder et al., 2012). A missense mutation in the WD40 domain of zebrafish Fbxw7 increased Notch activity in spinal cord progenitors, leading to increased oligodendrocyte precursors at the expense of neurons (Snyder et al., 2012). Both studies suggest that SCF^{Fbxw7} regulates the amount of Notch activity to limit the number of oligodendrocyte progenitor cells derived from neural progenitors.

Two other E3 ligases, the HECT-type Ub ligase, Neuralized, and the RING-type Ub ligase, MIB, are enriched at the plasma membrane of the differentiating cell and target the Notch ligands Delta and Serrate for ubiquitination and internalization (Liu et al., 2012; Pitsouli and Delidakis, 2005; Yeh et al., 2001). Both ligands are monoubiquitinated, internalized by endocytosis and then degraded or transcytosed (Chen and Corliss, 2004; Daskalaki et al., 2011; Deblandre et al., 2001; Lai et al., 2001). A dominant negative form of Neuralized (NeurΔRING), which lacks the capacity to ubiquitinate leads to the accumulation of the ligands at the cell surface demonstrating the importance of ubiquitination in regulating their levels (Pavlopoulos et al., 2001). Thus, by controlling the availability of the Notch ligands at the cell surface, these Ub ligases modulate Notch signaling (Chitnis, 2006).

Not only do Neuralized and MIB modulate Notch signaling in trans, but they also do so in cis (Glittenberg et al., 2006). Cis-inhibition occurs when the receptor and the ligand interact at the same cell surface and counteract the trans-activation of Notch signaling by neighboring cells (del Alamo and Schweisguth, 2009). Neuralized and MIB play a critical role in suppression of cis-inhibition by promoting internalization of the ligands. In fact, ectopic expression of Neuralized in mutant cells with reduced levels of active Serrate (a Notch ligand) increased Notch activity, while NeurΔRING decreased Notch activity in cells lacking Delta (Glittenberg et al., 2006). This indicates that loss of Neuralized ubiquitination function increases cis-inhibition by preventing the clearance of Serrate from the cell surface (Glittenberg et al., 2006; Pitsouli and Delidakis, 2005).

As predicted by their ability to affect Notch signaling, Neuralized and MIB have essential roles during neurogenesis. For example, loss of MIB promotes premature neuronal differentiation in the zebrafish hindbrain (Bingham et al., 2003) and in *Xenopus* primary neurogenesis (Itoh et al., 2003), however, the cells that initiate the neurogenic fate fail to differentiate into mature neurons (Bingham et al., 2003). Although the initial increase in neurogenesis is likely due to suppression of Notch signaling, the decrease in terminal differentiation may be the result of depletion of neural progenitors in MIB mutants. Similarly, conditional inactivation of MIB in mouse forebrain and spinal cord promotes premature neurogenesis while depleting the pool of radial glial cells supporting a critical role for MIB in determination of the neuronal and radial glial identities (Kang et al., 2013; Yoon et al., 2008).

REST

Another key player in neurogenesis regulated by ubiquitin mediated protein degradation is REST. REST is proposed to regulate cell fate by preventing premature expression of neuronal genes in neural progenitors (Gao et al., 2011), and repressing expression of neuronal genes in other germ layers (Kok et al., 2012). To do this, REST binds to a 21 bp evolutionarily conserved neuronal restrictive silencing element and recruits co-repressors

and chromatin remodeling agents to change the topology of DNA to heterochromatin to silence gene expression (Ballas et al., 2005; Chen et al., 1998; Chong et al., 1995; Paquette et al., 2000; Schoch et al., 1996; Thiel et al., 1998). To accomplish these functions, REST is broadly expressed in all three germ layers during early development, but then restricted to the neural stem cells and progenitors of the brain (Armisén et al., 2002; Olguín et al., 2006; Palm et al., 1998). As neural stem cells differentiate into neurons, *REST* expression is down-regulated (Ballas et al., 2005), and the REST protein degraded (Westbrook et al., 2008). This degradation is regulated by the RING Ub ligase, SCF^{Fbxw1}/SCF^{TRCP} whose levels increase during the transition from stem cell to neuron and directly promotes REST ubiquitination (Westbrook et al., 2008). Knockdown of Fbxw1, the F-box protein of the Ub ligase complex that binds REST, results in decreased neuron formation indicating that the transition of stem cell to differentiated neuron is contingent upon a decrease in REST mediated by SCF^{Fbxw1}(Westbrook et al., 2008).

Degradation of key regulatory proteins in neural specification and patterning

BMP signaling pathway

Bone morphogenetic protein (BMP) signaling plays a number of roles in defining neural cell fate including specification of dorsal neuronal subtypes in the neural tube (Mehler et al., 1997) and initiation of neurogenesis in adult brains (Bond et al., 2012; Sabo et al., 2009). The BMP receptor and effectors of BMP signaling, the Smad proteins, are tightly regulated by ubiquitination and degradation (Inoue and Imamura, 2008).

Three HECT-type Ub ligases, Smurf1, Smurf2 (Smad Ubiquitin Regulatory Factors) and NEDD4-2 negatively regulate BMP signaling by degradation of BMP pathway components. Smurf1 and Smurf2 reduce responses to BMP and TGFβ by directly targeting the Receptor regulated Smads (R-Smads) and indirectly targeting other components of the pathway using scaffold or adaptor proteins [for review (Cao and Zhang, 2013)], whereas NEDD4-2 targets the TGFβ type I receptor (Kuratomi et al., 2005) and the R-Smad, Smad2, for ubiquitination and degradation. Studies in *Xenopus* demonstrate that Smurf1-mediated repression of BMP signaling in the neuroectoderm is essential for dorsal ventral patterning and neural fold formation (Alexandrova and Thomsen, 2006; Zhu et al., 1999). In the absence of Smurf1, BMP signaling activity is increased such that the extracellular BMP antagonist Chordin does not induce neural gene expression in ectodermal explants, and neural fold formation and neural differentiation are disrupted (Alexandrova and Thomsen, 2006). Concordant with these data, increased Smurf1 neuralizes ectodermal explants and decreases BMP signaling in embryos, leading to the formation of a double axis (Zhu et al., 1999). In addition to targeted protein degradation of the R-Smad effectors of the BMP pathway, direct BMP targets are also regulated by ubiquitination and degradation. For example, degradation of the BMP target Xom (also known as Vent-2, Vox and Bbr-1) (Ladher et al., 1996; Schmidt et al., 1996; Trindade et al., 1999) in the dorsal regions of the developing *Xenopus* embryos is mediated by SCF^{Fbxw1} ubiquitin ligase (Zhu and Kirschner, 2002) allowing for dorsal specific gene expression. Contrary to these ubiquitin ligases that inhibit BMP signaling, the

RING-type ubiquitin ligase, Arkadia, enhances BMP signaling by inducing ubiquitination and degradation of inhibitory Smad7 (Koinuma et al., 2003; Liu et al., 2006).

Wnt signaling pathway

The canonical Wnt (Wingless/Integrase-1) signaling pathway, which plays central roles in anterior-posterior patterning of the neural tube (Kiecker and Niehrs, 2001), proliferation of neural stem cells in the developing dorsal neural tube (Chenn and Walsh, 2003; Dickinson et al., 1994; Megason and McMahon, 2002) and cortex (Woodhead et al., 2006), and dorsal interneuron specification in the neural tube (Zechner et al., 2007), relies on stabilization and nuclear translocation of β-catenin for transcriptional activation. In the absence of Wnt, cytoplasmic β-catenin is associated with a destruction complex consisting of Axin, Adenomatous polyposis coli (APC) and glycogen synthase kinase3β (GSK-3β). It is phosphorylated by GSK-3β and CKIα leading to ubiquitination mediated by SCF^{Fbxw1} ubiquitin ligase and degradation in the proteasome (Kitagawa et al., 1999; Liu et al., 2002)

Shh signaling pathway

Sonic hedgehog (Shh) signaling originating from the notochord generates a ventral concentration gradient in the neural tube, which opposes the dorsal BMP gradient and is essential to specify ventral neuronal subtypes (for review: Briscoe, 2009; Ribes and Briscoe, 2009). Shh signaling is mediated by two primary transcription factors, Gli2 and Gli3 that primarily function as an activator and repressor, respectively. In the absence of Shh signaling, Gli3 undergoes proteolytic processing to form a C-terminally truncated repressor. The Gli3 processing requires cAMP-dependent phosphorylation followed by ubiquitination via SCF^{Fbxw1} binding and degradation in the proteasome (Wang and Li, 2006). Although the role of degradation in Gli3 processing is not well understood, it was suggested that SCF^{Fbxw1} is involved in the degradation of the C-terminal cleavage product (Wang and Li, 2006). Conversely, only a minor fraction of Gli2 is processed to form a transcriptional repressor, but the full length Gli2 is readily phosphorylated and degraded in the absence of Shh signaling (Pan et al., 2006). Gli2 abundance is regulated by phosphorylation via protein kinase A, casein kinase 1, and GSK-3β, followed by ubiquitination by SCF^{Fbxw1} and degradation in the proteasome (Pan et al., 2006). The cleavage and degradation of Gli2 and Gli3 prevents transcriptional activation in the absence of Shh, ensuring that Gli mediated target gene activation only occurs when Shh is present.

FGF signaling pathway

Fibroblast growth factor (FGF) signaling has a prominent role in anterior-posterior patterning of the neuroectoderm (Hongo et al., 1999; Kengaku and Okamoto, 1995; Kudoh et al., 2002; Lahti et al., 2012; Takahashi and Liu, 2006). The functional status of the FGF receptor, like other tyrosine kinase receptors, is affected by the availability of Sprouty proteins (Yu et al., 2011). Sprouty enhances FGF signaling by binding to and dissociating c-Cbl, a RING-type ubiquitin ligase, from the FGF receptor, thereby, allowing signaling to occur (Fong et al., 2003). However, when Sprouty is ubiquitinated and degraded by NEDD4 ubiquitin ligase, c-Cbl interacts with the receptor, resulting in ubiquitination and degradation of the FGF receptor (Edwin et al., 2010; Hall et al., 2003; Rubin et al., 2005).

Future directions

Still yet to be identified are the ubiquitin ligases that degrade key regulators of vertebrate neurogenesis including SoxB1 transcription factors and the proneural protein Ngn. This has been a challenge in part becasue many ubiquitin ligases are expressed at low levels, and the interaction between the ubiquitin ligases and their targets are often short-lived making global identification of targets difficult. Candidate proteins that degrade the key transcription factors in neurogenesis can be identified from studies in non-vertebrate models. For example, *Drosophila* basic helix-loop-helix proneural protein Achaete-Scute is targeted for degradation by the adaptor protein Phyl and the RING-finger ubiquitin ligase Sina (homolog of mammalian Siah) to regulate the timing of cell division in the sensory organ precursors (Chang et al., 2008). Studies show that ubiquitin ligases can target multiple proteins involved in the same developmental context such as Fbxl14/Ppa, which degrades multiple core regulators during neural crest specification (Lander et al., 2011). Therefore, the targets of Sina/Siah may be extended to other proneural proteins including Ngn.

Neurogenin2

The proneural, basic helix-loop-helix (bHLH) transcription factor Ngn2 promotes neuron and inhibits glia formation in the CNS (Kiefer et al., 2005; Ma et al., 1996; Thoma et al., 2012) and fine-tuning the level of Ngn2 is critical to controlled progression through neurogenesis. Levels of Ngn2 are low in neural progenitors and an increase in Ngn2 activity initiates the neurogenesis program (Hindley et al., 2012; Hirata et al., 2002; Vosper et al., 2009). To maintain the developmental potential of neural stem cells, Ngn levels are kept low and data indicate that this is due in part to a short protein half-life regulated by protein degradation (Hindley et al., 2012; Vosper et al., 2009).

The stability of Ngn2 is controlled by its phosphorylation state and the level of Ngn2 determines which target genes are activated. Highly phosphorylated Ngn2 is unstable with rapid degradation in the proteasome (Boix-Perales et al., 2007; Vosper et al., 2009, 2007) yet activates low threshold genes like *delta* (Hindley et al., 2012). Hypophosphorylated Ngn2 is required to induce high threshold genes such as *neuroD* (Ali et al., 2011; Hindley et al., 2012; Li et al., 2012). The phosphorylation state of Ngn2 is controlled by at least 3 kinases: GSK3β, and Cyclin A- and Cyclin B-dependent kinases (Cdk1 and Cdk2) (Ali et al., 2011; Li et al., 2012) but the proteins that bind and target Ngn for degradation are unknown.

SoxB1 proteins

The SoxB1 group transcription factors, Sox1, Sox2 and Sox3, are important for the maintenance of neural progenitors (Elkouris et al., 2011; Holmberg et al., 2008; Thiel, 2013, Rogers et al. 2009). SoxB1 proteins have a prominent role during induction of the neuroectoderm (Rogers et al., 2009, 2008), and, in vertebrates, they continue to be expressed in proliferating neural stem cells and progenitors (Pevny and Rao, 2003). As cells differentiate and exit the cell cycle, *sox2* and *sox3* expression is down-regulated and their decrease promotes cell cycle exit and neuronal differentiation (Graham et al., 2003). Conversely, constitutive expression of *sox2* or *sox3* maintains the proliferative capacity and

inhibits neuronal differentiation of progenitors (Archer et al., 2011; Graham et al., 2003). Numerous studies of Sox2 regulation indicate that a major mechanism for decreasing SoxB1 levels and promoting neurogenesis is at the transcriptional level (Iwafuchi-Doi et al., 2011; Mariani et al., 2012; Miyagi et al., 2006; Saigou et al., 2010; Takemoto et al., 2006; Zappone et al., 2000). Little is reported on the roles of post-translational modification and targeted protein degradation. However, in many vertebrates, both Sox2 and Sox3 have consensus PEST degradation motifs as estimated by the epestfind software (http://emboss.bioinformatics.nl/cgi-bin/emboss/epestfind), indicating that they may have short half-lives, a characteristic of proteins targeted for degradation. In fact, the preliminary data from our research group suggests that the half-life of Sox3 in *Xenopus* is short and approximately 2.5 hours (unpublished data). In addition, the fact that the SUMO conjugated Sox2 fails to bind DNA suggests an essential role for post-translational processing as a means of regulation of function (Tsuruzoe et al., 2006).

Conclusion

In closing, review of the literature cumulatively show that targeted protein degradation and ubiquitin E3 ligases provide a specific mechanism to eliminate key transcription factors and signaling pathways components important for neurogenesis and the development of the nervous system. This regulation at the protein level allows for a rapid change in the proteome of the cells and the timely and ordered progression of neurogenesis, adding another layer of complexity to this already complex process. As the precise role of ubiquitin E3 ligases and the corresponding targets are identified, we will gain further knowledge of the regulation of the present checkpoints in neurogenesis as well as identifying the novel regulatory relationships that are essential for deciphering the details of making new neurons.

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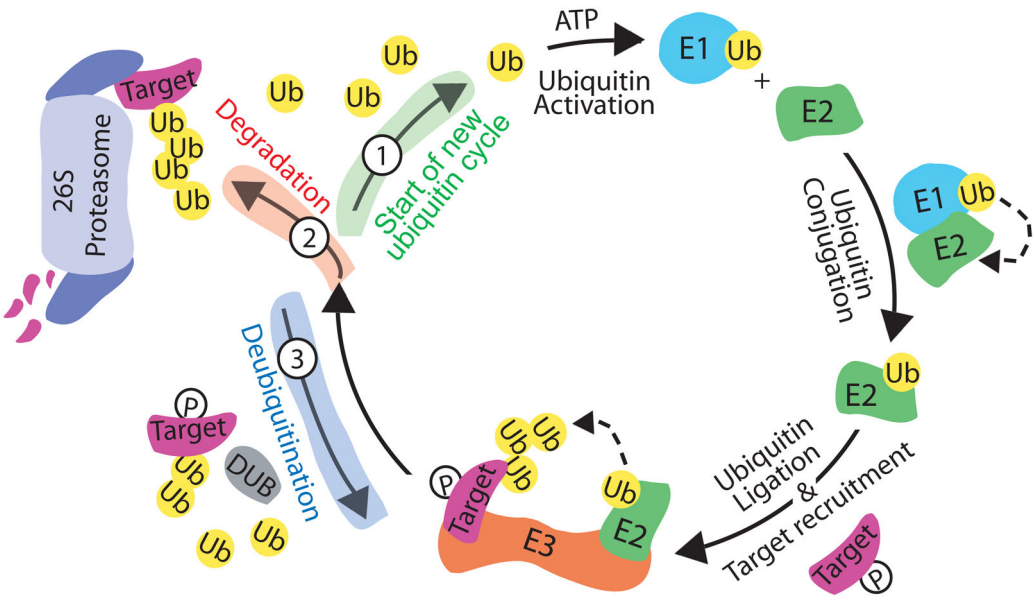

Figure 1.

The Ubiquitin/26S proteasome pathway targets proteins for degradation. Ubiquitin is conjugated to E1 activating enzyme with ATP hydrolysis. Ubiquitin is then transferred to E2 conjugating and E3 ubiquitin ligases. E3 ubiquitin ligase facilitates the recognition and recruitment of the target protein to be degraded and the transfer of ubiquitin to the target. The pathway cycles at least four times to polyubiquitinate the substrate (1), which is then recognized and degraded in the 26S proteasome (2). Alternatively, deubiquitinating enzymes (DUBs) can reverse ubiquitination by cleaving off ubiquitin chain and prevent degradation (3).

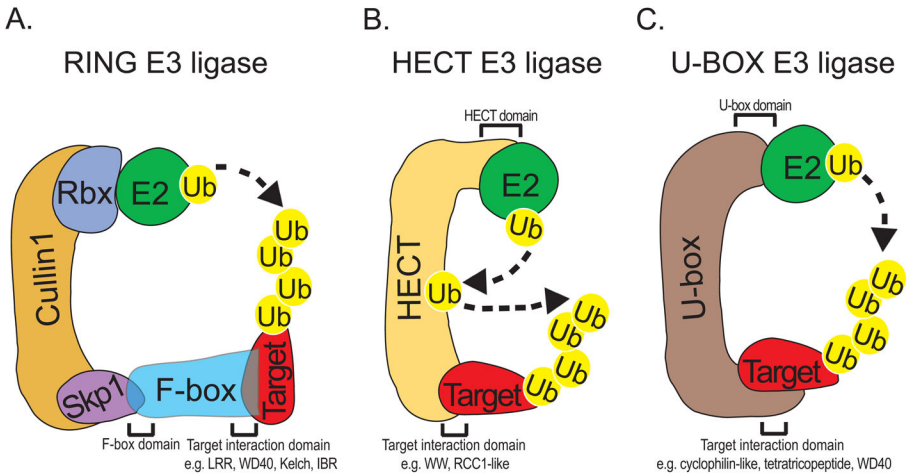

Figure 2.

Classes of Ubiquitin E3 ligases. **a.** RING E3 ligases form multi-subunit protein complexes that generally include RING finger protein Rbx and a Cullin scaffold. Cullin1-based SCF ubiquitin ligase is shown. Target proteins are recruited to the complex by F-box proteins, which bind to the adaptor protein Skp1 via the conserved F-box domain. Ubiquitin-loaded E2 binds to the complex via Rbx and transfers ubiquitin to the target proteins. **b.** HECT E3 ligases are monomeric proteins that interact with E2 via a conserved N-terminus HECT domain and with target proteins via divergent C-terminus domains. HECT proteins have intrinsic ligase activity and act as ubiquitin acceptors from E2 before transferring Ub to target proteins. **c.** U-box E3 ligases bind to E2 via a conserved U-box/RING domain and to target proteins via divergent domains including WW, cyclophilin-like, and tetratricopeptide. They do not have intrinsic ligase activity and their ubiquitin transfer system is similar to RING E3 ligases.

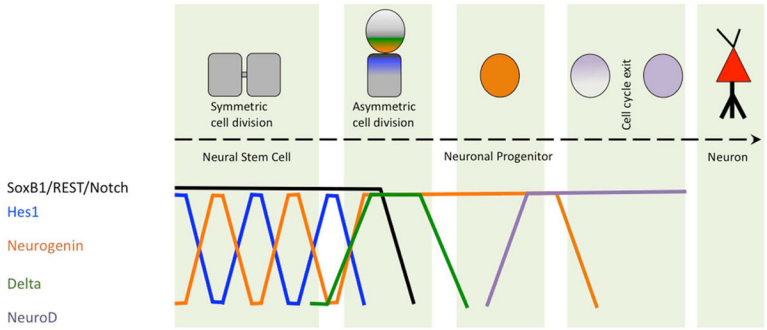

Figure 3.

Protein abundance is dynamic during neurogenesis. Neural stem cells are characterized by slow, symmetric cell division. As they progress through neurogenesis and proneural proteins are expressed, the plane of divisions is changed and they divide asymmetrically generating a neural stem daughter cell and neuronal progenitor daughter cell. The cell (orange) that accumulates gene products required for neuronal differentiation exits the cell cycle and differentiates into a neuron. During neurogenesis, the complementary oscillation of Hes1 and Ngn is lost. The factors that maintain neural progenitors (SoxB1, Notch, REST) are degraded. Delta and Ngn are stabilized transiently to allow time for Ngn to induce target genes such as *neuroD* and the regulators of cell cycle exit.