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Angelman Syndrome: Insights into Genomic Imprinting and Neurodevelopmental Phenotypes

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Abstract

Angelman syndrome (AS) is a severe genetic disorder caused by mutations or deletions of the maternally inherited *UBE3A* gene. *UBE3A* encodes an E3 ubiquitin ligase that is expressed biallelically in most tissues but is maternally expressed in almost all neurons. In this review, we describe recent advances in understanding the expression and function of *UBE3A* in the brain and the etiology of AS. We highlight current AS model systems, epigenetic mechanisms of *UBE3A* regulation, and the identification of potential UBE3A substrates in the brain. In the process, we identify major gaps in our knowledge that, if bridged, could move us closer to identifying treatments for this debilitating neurodevelopmental disorder.

Keywords

Angelman syndrome; neurodevelopmental disorder; UBE3A; E6-AP; synaptic plasticity; critical period; autism; 15q11-q13; imprinting; epigenetic

Introduction to Angelman syndrome

Angelman Syndrome (AS) was originally described by Harry Angelman in 1965 and occurs in approximately one out of every 12,000 births [1, 2]. Patients with AS exhibit developmental delay, speech impairments, intellectual disability, epilepsy, abnormal EEGs (electroencephalograms), puppet-like ataxic movements, prognathism, tongue protrusion, paroxysms of laughter, abnormal sleep patterns, and hyperactivity [3]. Moreover, patients with AS often exhibit socialization and communication deficits that meet the diagnostic criteria for autism [2, 4], but it should be noted that autism diagnosis in AS can be confounded by co-occurring developmental delay [5].

In most cases, AS is caused by mutations or deletions of the maternally inherited *UBE3A* gene, which encodes a HECT (homologous to E6-associated protein C terminus) domain E3 ubiquitin ligase [6–8]. Because the paternal allele of *UBE3A* is epigenetically silenced (i.e., paternally imprinted) in most neurons but not other tissues (discussed below) [9–14], maternal inactivation of *UBE3A* causes a nearly complete loss of UBE3A protein selectively from the brain [14, 15]. AS typically (in ~75% of cases) arises from deletions (~6 Mb in size) within chromosome 15q11-q13, a region that contains *UBE3A*. In ~15% of cases, the maternal *UBE3A* allele alone is mutated [16], indicating that selective loss of brain UBE3A

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function can account for most AS phenotypes. A minority of AS cases arise from microdeletions that affect imprinting at the 15q11-q13 locus (~2–4%) or from paternal uniparental disomy (~7%), where two copies of an epigenetically silenced *UBE3A* allele are inherited [17, 18]. Interestingly, while 15q11–q13 deletions cause AS, the most genetically identifiable form of autism results from maternal duplication of the 15q11–q13 locus encompassing *UBE3A* [19–22].

Although pharmacological options to control mood and sleep disorders have been partially effective [23], in general, AS therapeutics have met with limited success. For example, frequent seizures in AS patients are especially difficult to treat. There are AS patients with unique seizure types and only a fraction of these individuals respond to pharmacological intervention [24]. As another example, Levodopa (L-Dopa), which is commonly used to treat Parkinsonian symptoms, is partially effective in treating late-onset movement disorders in a subset of AS patients [25]. Moreover, efforts to unsilence the intact paternal *UBE3A* allele with dietary supplements that increase DNA methylation have failed [26]. The paucity of therapeutic options highlights a need to learn more about AS pathogenesis, the mechanisms of imprinting, and downstream targets of UBE3A. Answers to these questions could lead to development of novel AS therapeutics.

Here, we review recent progress in understanding the mechanisms of *UBE3A* imprinting, how maternal *UBE3A* deficiency affects neurodevelopment, and how UBE3A protein regulates substrates and binding partners. In addition, we discuss avenues for future research, focusing on mouse models that are relevant to monitoring allelic expression of *UBE3A* and to the discovery of synaptic and cognitive phenotypes that result from a loss of UBE3A–substrate relationships.

Monitoring neuronal *UBE3A* imprinting

The genomic region spanning *UBE3A*, the *UBE3A antisense transcript (UBE3A–ATS)*, and the spliceosomal protein *SNRPN* (small nuclear ribonucleoprotein polypeptide N) [27] contains a large number of imprinted genes that are either paternally or maternally expressed in the human brain [27]. Mice possess a chromosomal region that is syntenic to human 15q11-q13 in which orthologous genes, including *UBE3A*, are also imprinted [28–30]. *UBE3A* is expressed from the maternal allele in most neurons, while the paternal allele is intact but epigenetically silenced (Figure 1a) [9–13]. Initial studies indicate that the *UBE3A* promoter region is unmethylated in mice and humans [17, 31], which may exclude differential methylation of the *UBE3A* promoter as a mechanism for maternal expression.

Silencing of the paternal *UBE3A* allele is predominantly thought to be caused by expression of a large (0.5–1.0 Mb) antisense RNA transcript (*UBE3A–ATS*) [10–12, 32]. As shown in mice, this RNA transcript is paternally expressed in neurons [13, 33], initiates near the differentially methylated Prader-Willi syndrome-imprinting center (PWS-IC), and runs through *Snurf (*Snrpn upstream reading frame*)/Snrpn* and *UBE3A* (Figure 1a) [10, 34]. Moreover, snoRNAs (small nucleolar RNAs) expressed from gene clusters located between *UBE3A* and *Snurf/Snrpn* [35] regulate neuronal nucleolar size [36], appear to be brain-specific, and are paternally expressed (Figure 1a) [11, 37]. DNA methylation and histone deacetylation at the maternal PWS-IC correlate with repression of the large transcript that includes the *UBE3A–ATS* [32, 38–41]. Changes in these methylation and acetylation patterns at the paternal PWS-IC locus are believed to permit production of the *UBE3A–ATS* from the paternal allele [11, 32, 38–41] (Figure 1a). In mice, high resolution SNP (single-nucleotide polymorphism) genotyping studies have demonstrated that only the 3’ end of *UBE3A* is imprinted, while the 5’ end is biallelically expressed (Figure 1b) [33]. This suggests a competition model involving *UBE3A/UBE3A–ATS* at the level of RNA-RNA

interactions, such that the *UBE3A–ATS* somehow interferes with the production of a portion of the *UBE3A* sense transcript in *cis*. However, other models of competitive interaction may also contribute to paternal-specific regulation (Figure 1c) [42].

An antisense mechanism of regulation is further supported by studies using inter-subspecific crosses of mice generating a 35 kb targeted deletion within the PWS-IC. Deletion of this region in mice leads to upregulation of the paternal *UBE3A* allele, suggesting that this region is required for paternal *UBE3A* silencing [32]. Replacing the mouse PWS-IC with the corresponding human region produces a surprising outcome that also supports an antisense mechanism of silencing. In this case, *Ube3a–ATS* is produced from paternal and maternal alleles, resulting in silencing of both paternal and maternal *UBE3A* [43]. Biallelic expression of the *Ube3a–ATS* could be due to reverse orientation selectivity of the human PWS-IC in mouse or overall species variations in PWS-IC regulation [43].

Recently, a knock-in mouse in which a *UBE3A–Yellow Fluorescent Protein* (YFP) fusion gene ($Ube3a^{YFP}$) is expressed from either the maternal or paternal allele, was used to monitor allelic contributions to *UBE3A* expression [44]. Paternal imprinting of *UBE3A* was found in neurons in the hippocampus, cortex, thalamus, olfactory bulb, and cerebellum. The UBE3A–YFP fusion protein was localized to the nucleus, with much lower levels of protein in axons and dendrites of the hippocampus [44]. Biallelic expression of *UBE3A–YFP* was observed in glial cells lining the lateral ventricles, confirming previous *in vitro* findings that paternal imprinting does not manifest in all cell types in the brain [13]. Such a model may be used to further characterize the distribution of paternal UBE3A expression in the brain during development. These studies will help to reveal the ontogeny of paternal *UBE3A* imprinting and, therefore, the earliest neurodevelopmental events that are susceptible to maternal *UBE3A* deficiency.

Recent developments in understanding the mechanisms of *UBE3A* imprinting in human patients have been obtained by studying induced pluripotent stem cell (iPSC) lines that were established from individuals with AS [31]. *UBE3A* imprinting is retained in neurons derived from both control iPSCs and AS patient iPSCs containing maternally-inherited deletions of 15q11-q13. In these stem cell-derived neurons, both maternal and paternal copies of the PWS-IC retain their differential methylation patterns. Moreover, the *UBE3A–ATS* transcript is expressed in iPSC-derived neurons, suggesting that the mechanisms of imprinting are retained in iPSCs and are conserved from human to mouse. Thus, programmed differentiation of human iPSCs could be useful for testing the mechanisms underlying human gene imprinting and may allow one to identify mechanisms that relax imprinting.

AS mouse models recapitulate many AS patient phenotypes

To date, three AS mouse models have been engineered with targeted mutations that mimic *de novo* chromosomal abnormalities underlying AS (Table 1). Because brain-specific paternal imprinting of *UBE3A* also occurs in mice, all three models are based on the maternal inheritance of a chromosomal deletion that includes *UBE3A*. Importantly, these models recapitulate the loss of UBE3A in neurons in the central nervous system (CNS) [45–47] and display several AS-relevant phenotypes [48–50]. Conversely, mice that inherit paternal *UBE3A* deletions express normal levels of UBE3A in CNS neurons [45–47] and are phenotypically normal [48, 50, 51].

The first mouse model of AS was generated by knocking out 3kb of sequence orthologous to exon 2 of human *UBE3A*. This mutation causes a frame shift and results in a null allele [48]. Mice that carry this mutation on the maternal *UBE3A* allele ($UBE3A^{m-/p+}$) display ataxia and epilepsy [48, 52, 53]. The ataxia presents as abnormalities in gait, motor coordination, motor learning [48, 51, 54], and reduced strength [54], which may model ataxia observed in

patients with AS. These motor deficits are generally ascribed to a loss of cerebellar UBE3A [52]; however, this has never been rigorously tested. Proprioceptive, spinal, and basal ganglia circuits regulate motor function [55–57] and are just as likely to contribute to gait disturbances when impaired. As a case in point, the extrapyramidal motor system may be dysfunctional, since ~25% of dopaminergic neurons in the substantia nigra are lost in *UBE3A*$^{m-/p+}$ mice, and deficits in dopamine-sensitive motor tasks are observed [51]. *UBE3A*$^{m-/p+}$ mice also exhibit audiogenic seizure susceptibility and have extended EEG polyspike and slow wave discharges that co-occur with episodes of behavioral immobility resembling absence epilepsy [48]. The penetrance and severity of seizures in *UBE3A*$^{m-/p+}$ mice are influenced by genetic background [48], suggesting that this phenotype is modified by other genes.

Motor deficits and EEG abnormalities are similarly found in a second AS mouse model. In this model, the C-terminal sequence encoded by mouse *UBE3A* (corresponding to part of exon 15 and all of exon 16 of human *UBE3A*) was replaced with a β-galactosidase (lacZ) transcriptional reporter, resulting in a null allele [50]. Electrophysiological studies of these mice indicate that motor dysfunction may be related to abnormal cerebellar output caused by increased purkinje cell firing and rhythmicity [52]. Further EEG studies indicate sleep abnormalities, such as reduced rapid eye movement (REM) sleep [58], which has been reported in children with AS [59, 60]. Cognitive deficiency is another AS phenotype that has been extensively modeled in these mice [50]. Consistent with learning impairments in individuals with AS, these mice exhibit deficits in spatial learning and memory during Morris water maze task performance and deficits in contextual fear conditioning [50]. These learning impairments have also been observed in the original AS mouse model described above [48, 53].

AS mouse models based on targeted *UBE3A* disruption have reinforced the hypothesis that maternal *UBE3A* deficiency is the primary cause of AS. However, large maternal deletions of chromosome 15q11-q13 manifest in the majority of AS cases and are correlated with more severe clinical phenotypes [17, 23, 61], perhaps owing to the haploinsufficiency of neighboring genes such as *GABRB3* ($GABA_A$ receptor β3 subunit) and *ATP10A* (ATPase, class V, type 10A) (Figure 1). For, example, *Gabrb3* haploinsufficiency enhances seizure susceptibility in mice [62], and thus may explain why epilepsy is more severe in AS patients with large 15q11-q13 deletions than in individuals with *UBE3A*–specific insults [61]. Cre/*loxP* and *Hprt* (hypoxanthine-guanine phosphoribosyltransferase) minigene chromosomal engineering was recently employed to produce a mouse with a 1.6 Mb maternal deletion that disrupts the *Atp10a* and *Gabrb3* loci in addition to *UBE3A* [49]. This model may better represent large deletion classes of AS. Like mice with targeted *UBE3A* deletions, large deletion mice (*deletion*$^{m-/p+}$) exhibit AS-relevant deficits, including EEG abnormalities and motor and cognitive behavioral dysfunction [49] (Table 1). It will be informative to test *deletion*$^{m-/p+}$ mice for AS-relevant phenotypes that are not found in *Ube3a*$^{m-/p+}$ mice, including hyperactivity and increased social-seeking [63]. Comparative studies between *deletion*$^{m-/p+}$ and *UBE3A*$^{m-/p+}$ mice will help determine if the manifestation of these phenotypes requires the haploinsufficiency of other neighboring genes (e.g., *Gabrb3*), or if they are due to species-specific consequences of *UBE3A* loss. Future AS mouse models may involve even larger deletions of the entire region syntenic with human 15q11-q13 and approximate the ~6 Mb deletion found in many patients with AS. In the meantime, a current model with transgenic replacement, rather than a true deletion, of the entire AS homology region may prove useful in this regard [64, 65].

Changes in neuronal morphology in AS mouse models

To help understand the profound neurological deficits underlying AS, researchers have explored neuroanatomical correlates of abnormal connectivity and synaptic development in *UBE3A*$^{m-/p+}$ mice. These studies have almost exclusively focused on measuring dendritic spines at the single-cell level owing to the fact that *in vivo*, UBE3A is localized to postsynaptic compartments in addition to the nucleus [44]. Dendritic spine density (~15–20%) and length (~10–15%) are reduced in post-adolescent *UBE3A*$^{m-/p+}$ mice in cell populations that may be relevant to cognitive deficits (i.e., pyramidal neurons in CA1 of the hippocampus and in layer III-V of the cortex) and motor impairments (i.e., cerebellar purkinje neurons) observed in AS [44]. In pre-adolescent mice, spine density deficits of a similar magnitude are observed in the basal dendrites of layer II/III [45] and V [47] pyramidal neurons in primary visual cortex. Interestingly, spine density appears normal in the apical dendrites of these same layer V neurons, suggesting that *UBE3A* deficiency may potentially influence synaptic development in a compartment-specific manner. This finding contrasts with findings of reduced apical dendritic spine density in a previous study in which neurons were broadly sampled from throughout the cortex of older, post-adolescent *UBE3A*$^{m-/p+}$ mice [44]. Therefore, it is possible that region- as well as age-specific consequences of *UBE3A* deficiency contribute to synaptic abnormalities in AS. In general, dendritic spine phenotypes in *UBE3A*$^{m-/p+}$ mice are consistent with a role for UBE3A in regulating excitatory postsynaptic development and function, which is only beginning to be defined [66, 67].

Dendritic arborization has yet to be studied in a detailed, quantitative fashion in *UBE3A*$^{m-/p+}$ mice. Three key lines of evidence support that these and other basic studies of neuronal morphology in this model are warranted. First, human postmortem findings indicate that the dendritic arborization of cortical pyramidal neurons is decreased in AS [68]. Second, gross cortical and cerebellar weight is reduced (by ~15%) in both juvenile and mature *UBE3A*$^{m-/p+}$ mice [48], strongly suggesting that morphological abnormalities in addition to decreased dendritic spine density are possible. Third, *UBE3A* expression is coincident with developmental processes that precede synaptogenesis, including neuronal migration and the establishment of polarity (i.e.,. axonogenesis, and dendritogenesis) [14]. These events collectively lay the cytoarchitectural foundation that supports possible alterations in synaptic connectivity, function, and plasticity in AS, which we discuss below.

Changes in synaptic plasticity in AS mouse models

Early investigations of altered synaptic plasticity in *UBE3A*$^{m-/p+}$ mice were inspired by findings of impaired contextual fear conditioning, which led to studies of whether long-term potentiation (LTP) of Schaffer collateral synapses in the CA1 hippocampal region was impaired. Standard high-frequency stimulation protocols evoke only a transient potentiation of these synapses in hippocampal slices from *UBE3A*$^{m-/p+}$ mice [48]. However, sustained LTP, similar to what was observed typically in wild-type mice, could be produced by increasing slice temperature and the number of high-frequency stimulations [69]. This suggests that the induction threshold for NMDA receptor- (NMDAR) dependent LTP is increased at CA1 hippocampal synapses in *UBE3A*$^{m-/p+}$ mice. NMDAR-independent LTP is also more difficult to sustain in *UBE3A*$^{m-/p+}$ slices [69], perhaps owing to deficient signaling downstream of Ca^{2+} influx. Accordingly, a defect in Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activation was found in the hippocampus of these mice [69]. This CaMKII activation defect correlates with chronic hyperphosphorylation at the threonine (Thr^{286}) autoactivation and Thr^{305} inhibitory sites of the CaMKII–α subunit. However, these changes may be age- and or region-specific, as no evidence of altered αCaMKII phosphorylation was found in the visual cortex of juvenile AS mice [47].

Mutation of αCaMKII Thr^{305} to alanine in AS mice abrogates inhibitory αCaMKII phosphorylation and, perhaps surprisingly, rescues both LTP and hippocampus-dependent learning deficits [53]. The mechanism of CaMKII hyperphosphorylation in AS mice remains elusive.

Deficits in neocortical synaptic development and plasticity in AS mice were first observed within the visual cortex [45]. In this region, typical developmental increases in the frequency of miniature excitatory postsynaptic currents (mEPSCs) are blunted in *UBE3A*$^{m-/p+}$ layer II/III pyramidal neurons. This finding corroborates measures of decreased dendritic spine density [44, 45, 47], but contrasts with a more recent study reporting similar decreases in mEPSC frequency without changes in synapse number in CA1 pyramidal neurons from acute hippocampal slices [66]. Decreases in AMPA/NMDA current ratios and synaptic AMPA receptor expression are also found in immature neurons cultured from *UBE3A*$^{m-/p+}$ hippocampi [66]. This indicates that, at least in the hippocampus at young ages, *UBE3A* deficiency may cause an increase in silent synapses (i.e., synapses lacking AMPA receptors) rather than a loss of synapses *per se*.

It is intriguing to speculate that decreased spine densities in *UBE3A*$^{m-/p+}$ mice might reflect the end-point of an experience-driven, activity-dependent process whereby synapses rendered silent at an early age are subsequently eliminated during later stages (Figure 2b). Experience-dependent activity clearly influences the emergence of synaptic deficits observed in *Ube3a*$^{m-/p+}$ mice. For example, dendritic spine deficits in pyramidal neurons of layer II/III visual cortex fail to develop if *Ube3a*$^{m-/p+}$ mice are deprived of visual experience [45]. Furthermore, LTP and LTD are not expressed at their normal induction thresholds at layer IV to II/III synapses in slices of visual cortex from juvenile *UBE3A*$^{m-/p+}$ mice, but late-onset visual deprivation rescues these synaptic plasticity deficits [45], Moreover, this rescue is not maintained if visual experience is restored [45]. Thus, experience is integral to the expression of plasticity deficits in the visual cortex of this AS mouse model.

Experience-dependent activity may also alter the expression of UBE3A itself. Increased rates of neuronal firing lead to increased levels of UBE3A through a process that requires the activity-regulated transcription factor Mef2 (myocyte enhancement factor-2) [66]. However, UBE3A levels in primary visual cortex are not altered in response to monocular deprivation [47], indicating that different paradigms of activity (e.g., neuronal firing patterns vs. rates of activity) may be required to influence changes in *UBE3A* expression. Indeed, treating cultured neurons with potassium chloride is well known to increase neuronal firing rates, while monocular deprivation does not substantially alter visual cortical firing rates, but only firing patterns [70]. Moreover, *UBE3A* may be subject to variable transcriptional regulation during development, including during critical periods. Finally, as UBE3A appears to target itself for proteasomal degradation [71–73], it may be that absolute measures of UBE3A alone cannot be relied upon to identify time-points and/or anatomical regions where *UBE3A* expression is altered. Detailed analysis of transcript:protein ratios may prove to be more informative in this regard. New technologies to reliably measure *UBE3A* expression and activity in the brain may illuminate temporal and regional UBE3A substrate specificity and, ultimately, the understanding of AS etiology.

Identification of brain substrates for UBE3A

UBE3A is a HECT E3 ubiquitin ligase that ubiquitinates protein substrates, leading to their degradation by the ubiquitin proteasome system (UPS) [74, 75]. Multiple mutations in *UBE3A* have been attributed to defective UBE3A stability or catalytic function [23, 76]. The ubiquitination and degradation of p53, the first identified substrate of UBE3A [77, 78],

require not only UBE3A, but a viral cofactor E6, hence the initial naming of UBE3A as an E6-associated protein (E6-AP) [77–80]. Notably, E6 is not required for UBE3A E3 ligase activity, and E6 serves only as a bridging factor to facilitate the interaction of UBE3A with certain substrates (such as p53). Although E6 is thought to be absent in the brain, increased p53 levels in CA1 pyramidal neurons and purkinje cells in AS mice have been reported [48], suggesting that E6-independent ubiquitination of p53 might occur. Loss of UBE3A has also been shown to increase p53 levels in cultured Neuro2A cells [81]. However, UBE3A regulation of p53 remains controversial, since p53 levels were found to be normal in one *UBE3A*–deficient mouse line [50]. Later work described a DNA-repair enzyme, HHR23A (human homologue A of Rad23) as the first E6-independent substrate for UBE3A in non-neuronal tissue [82]. To date, no endogenous E6-like cofactors for UBE3A have been described.

Drosophila express an ortholog of *UBE3A*, *Dube3a*, and can therefore be utilized as a model system to genetically identify UBE3A–dependent substrates. Human UBE3A was overexpressed in flies to examine potential decreases in protein content. The Rho-GEF (guanine nucleotide exchange factor) Pbl (pebble) / ECT2 (epithelial cell transforming sequence 2) was one of 20 proteins found to be differentially regulated when UBE3A was overexpressed [83]. Furthermore, overexpressing human UBE3A could partially rescue the rough eye phenotype in Pbl-overexpressing flies, suggesting that UBE3A attenuates Pbl levels and that Pbl may be a UBE3A–dependent substrate.

Additionally, UBE3A has been implicated in regulating the cyclin dependent kinase inhibitor p27, both in heterologous cells and in brain tissue [84]. UBE3A interacts with p27, and promotes its ubiquitination *in vitro* [84]. Consistent with this finding, loss of UBE3A results in decreased turnover of p27 in heterologous cells *in vitro* and leads to increases in p27 protein levels in cerebellar purkinje, cortical, and hippocampal neurons [84]. Moreover, a loss of UBE3A enhances p27 transcription in the cerebellum, suggesting that UBE3A not only regulates the degradation and turnover of p27, but also its transcription [84]. In the cortex, p27 promotes neuronal differentiation and migration in cortical projection neurons [85]. Therefore, potentially increased levels of p27 in *UBE3A*–deficient mice might result in the premature migration and differentiation of cortical neuronal progenitors and alter the laminar architecture of the cortex. However, such a possibility awaits further experimental testing.

The immediate early gene, Arc (activity-regulated cytoskeleton-associated protein), was also recently characterized as a substrate for UBE3A [66, 86]. Arc is brain-specific and is rapidly upregulated in response to increases in neuronal activity. Furthermore, Arc is known to promote the endocytosis of AMPA receptors [87, 88] and is required for learning, long-term memory, and homeostatic plasticity [86, 89–91]. UBE3A binds Arc *in vivo* and promotes its ubiquitination *in vitro* [66]. Interestingly, seizure or learning protocols induce abnormally elevated Arc expression in *UBE3A* knockout mice relative to wild-type controls [66], suggesting that UBE3A is required for Arc turnover in the brain during bouts of elevated synaptic activity. Changes in UBE3A levels correlate with surface expression of the AMPA receptor subunit GluA1 that are inversely correlated with Arc levels, suggesting that UBE3A regulates AMPAR endocytosis by controlling Arc protein levels (Figure 2) [66]. However, it remains unclear if Arc ubiquitination is defective or if Arc has an extended half-life in the AS brain. Intriguingly, basal levels of Arc increase during development [86]. It is tempting to speculate that if UBE3A ubiquitinates Arc during critical periods for experience-dependent plasticity, Arc levels in *UBE3A*–deficient neurons may increase to pathological levels over a time course coincident with the onset of AS phenotypes (Figure 2).

Ephexin-5, a RhoA guanine nucleotide exchange factor (also known as Vsm-RhoGEF), is another possible substrate for UBE3A [67, 92]. Ephexin-5 is highly expressed in the CNS, where it interacts with the ephrin B2 receptor (EphB2) [67]. Ephexin-5 constitutively activates RhoA, which leads to suppression of excitatory synapse number during development [67]. *Ephexin-5* null mice have increases in excitatory synapse number with correlative increases in dendritic spine density [67]. The degradation of Ephexin-5 is stimulated by Ephrin B binding to EphB2 and mediated by UBE3A [67]. Ephexin-5 degradation by UBE3A thus relieves the suppression of excitatory synapse development. *UBE3A*$^{m-/p+}$ mice have elevated levels of Ephexin-5 protein and decreased levels of ubiquitinated Ephexin-5 [67], suggesting that UBE3A facilitates Ephexin-5 degradation. Hence, the decreases in dendritic spine density identified in *UBE3A*$^{m-/p+}$ mice [44, 45, 47] may reflect defects in the degradation of Ephexin-5 by UBE3A (Figure 2). Interestingly, both Ephexin-5 and Arc share a conserved UBE3A–binding domain sequence [66, 67], indicating that a bioinformatics approach may be able to identify additional UBE3A substrates (Table 2).

An additional factor adding to the potential complexity of this system is that three UBE3A isoforms, resulting from differential splicing, have been reported [93]. Whether these three isoforms have different functions in the brain has yet to be elucidated. However, a possible complication in the identification and verification of UBE3A substrates stems from the recent observation that isoform 2 of UBE3A is a binding partner for cytosolic and synaptically isolated 26S proteasomes in the brain [94]. Depression of synaptic activity in cultured hippocampal neurons, via a chemical LTD protocol, leads to the dissociation of UBE3A from the proteasome and a subsequent reduction in UPS activity [94]. This suggests that UBE3A may regulate overall proteasome activity following changes in synaptic plasticity [94]. UBE3A isoforms 1 and 2 were also found to interact with purified proteasome subunits in non-neuronal tissues [95–98]. Clearly, the role of UBE3A in modulating proteasomal function and its ubiquitination of substrates will require further analysis.

Conclusions and future directions

Although research is beginning to unveil the connections between *UBE3A* function and AS, there are still fundamental questions remaining to be answered (Box 1). For instance, does the *UBE3A* antisense mechanism fully account for why *UBE3A* is epigenetically silenced in the brain but not other tissues? If yes, can expression of the functionally intact, but epigenetically silenced, paternal *UBE3A* allele be upregulated by pharmacological means or by genetically manipulating *UBE3A–ATS* transcription? Of equal importance, is upregulation of UBE3A in the adult brain capable of rescuing neurodevelopmental and/or cognitive deficits observed in AS? Or, does UBE3A need to be upregulated during critical developmental periods? Answering these questions might provide clues to developing therapeutic strategies for AS.

Another important question relates to how widespread *UBE3A* imprinting is in the nervous system. Protein expression mapping in wild-type mice indicates variability in UBE3A levels throughout the brain, within and among various neuron populations [46]. This variability could be due to differential transcriptional/post-transcriptional regulation of maternal *UBE3A* expression or relaxed paternal imprinting, possibly caused by variable overrun of the maternal *UBE3A* transcript from the large *Ube3a–ATS*. In fact, there is evidence that paternal imprinting is relaxed in visual cortex neurons prior to the critical period [47]. Detailed expression mapping of endogenous *UBE3A* expression in wild-type versus AS mice, or maternal versus paternal *UBE3A -YFP* expression, will be required to reveal allelic contributions to the heterogeneity of *UBE3A* expression in the nervous system.

It is clear that interest in *UBE3A* for its role in AS has accelerated a new area of research and has led to important insights into the role of UBE3A in neurons [99]. It is equally clear that there are a number of critical gaps in our knowledge concerning the mechanisms of *UBE3A* imprinting, the substrates of UBE3A, and the role that UBE3A plays in synaptic and circuit function. By addressing these critical gaps, the field will move closer to identifying potential therapeutic targets at which novel AS drugs could be directed.

Box 1: Outstanding Questions

- Why is *UBE3A* imprinted specifically in neurons?
- Does the *UBE3A* antisense mechanism fully account for neuron-specific epigenetic silencing of *UBE3A*?
- What are the parameters for competition between sense and antisense transcripts at the paternal *UBE3A* locus?
- Why do neurons with imprinted *UBE3A* expression show higher levels of UBE3A than biallelically expressing cells [44]?
- How widespread is *UBE3A* imprinting in the nervous system? Does it occur in the peripheral nervous system or is it restricted to the central nervous system?
- Can the study of neurons in which imprinting is relaxed inform us about the mechanisms of *UBE3A* silencing?
- Can differential UBE3A levels within and among neural circuits tell us anything about circuit vulnerability and the manifestation of characteristic AS phenotypes?
- Why is neuronal UBE3A localization primarily nuclear while its major known substrates are cytoplasmic?
- Is UBE3A function in the nucleus required for regulation of synaptic plasticity?
- 10.) How is the ubiquitin ligase activity of UBE3A regulated?
- 11.) Are there brain-specific E6-like factors that dictate substrate specificity for UBE3A?
- 12.) Are there additional UBE3A substrates, and do they differ according to subcellular compartment, cell type, brain region, and developmental period?

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Glossary

Allele	One of two or more forms of a given DNA sequence of a gene
Genomic Imprinting	A genetic process whereby genes are differentially expressed depending on their parent-of-origin inheritance

Context-dependent fear conditioning	A behavioral paradigm where animals learn to fear a neutral stimulus when paired with a noxious or painful stimulus. Brain regions involved include the amygdala and, when cued by spatial context, the hippocampus
Epigenetic:	Heritable and reversible modifications to nucleotides or chromatin that can alter gene expression without a change to DNA sequence
Ocular Dominance Plasticity	A form of experience-dependent plasticity that occurs following monocular visual deprivation whereby synaptic connections between the deprived eye and the cortex are weakened or eliminated while connections between the nondeprived eye and the cortex are strengthened. This form of plasticity is most robust during critical periods of postnatal development
Prader-Willi syndrome:	An imprinting disorder that typically results from deletions within the paternal copy of chromosome 15q11-q13. Note that deletions within the maternal copy of this chromosomal region typically result in Angelman syndrome
Prognathism	Jaw malformation due to abnormal extension or bulging of the lower jaw

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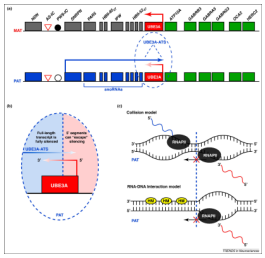

Figure 1.

Possible mechanisms for *UBE3A* imprinting in the brain. **a**. A map of the maternal (MAT) and paternal (PAT) human chromosome region 15q11-q13 containing *UBE3A*, adapted from Lalande and Calciano [27]. Maternally expressed genes are depicted in red and paternally expressed genes are depicted in blue. Non-imprinted genes are represented in green. *Top*: Methylation at the maternal PWS imprinting center (PWS-IC, black circle) globally represses expression of surrounding genes (gray boxes), including the *UBE3A* antisense (*UBE3A–ATS*) transcript. However, the maternal copy of *UBE3A* is expressed (red arrow). *Bottom:* On the paternal chromosome, the PWS-IC contains a cluster of CpG sites that are unmethylated (open circle), permitting paternal gene expression (blue boxes), including the *UBE3A–ATS* transcript (blue arrow). The *UBE3A–ATS* (0.5–1.0 Mb in length) overlaps the paternal *UBE3A* locus, resulting in transcriptional silencing of *UBE3A* (red arrow fading to white). Open triangles represent the AS imprinting center (AS-IC). Neighboring genes upstream of *UBE3A* include: *NDN* (necdin) and genes encoding snoRNAs [*SNRPN PAR5* (Prader-Willi Angelman Syndrome region 5), *HBII-85*$_{27}$ *HBII-52*$_{47}$ and *IPW* (Imprinted in Prader-Willi syndrome)]. Neighboring genes downstream of *UBE3A* include: *ATP10A*, the $GABA_A$ receptor β3, α5 and γ2 subunits (*GABRB3, GABRA5, GABRG3*), *OCA2* (Oculocutaneous albinism II) and *HERC2* (Hect domain and RDL 2). **b**. Zoomed in region from **a** depicting a cutoff (dashed vertical blue line) beyond which the silencing of *UBE3A* transcription by the *UBE3A–ATS* is incomplete [33]. Left of the line, the *UBE3A–ATS* transcript (dark blue shading) competes with the sense transcript (light red shading), resulting in silencing of full-length *UBE3A* sense transcripts. In contrast, to the right of the line, truncated paternal 5’ segments of the *UBE3A* sense transcripts (red) are produced [33]. **c**. Hypothetical mechanisms of *UBE3A–ATS*/sense competition at the paternal allele. *Top*: Collision model [42]. If transcription can only occur in one direction at a single time, RNA polymerases (RNAPII) transcribing the *UBE3A* sense strand (red) are competed off of their templates by oncoming complexes engaged in transcription of the *UBE3A–ATS* strand (blue). *Bottom:* RNA-DNA interaction model [42]. Production of the *UBE3A–ATS* induces histone modifications (HM) that modify chromatin architecture along the *UBE3A* locus. Transcriptional elongation of *UBE3A* is prematurely aborted at these regions, yielding truncated *UBE3A* sense transcripts (red). Note that similar models of RNA regulation have been described for genomic imprinting at other loci, such as *Xist*, a non-coding RNA (ncRNA) that contributes to X chromosome inactivation and *Air*, a paternally expressed ncRNA that leads to silencing of paternal insulin-like growth factor 2 receptor (*Igf2r)* [100].

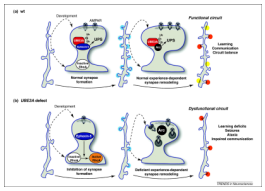

Figure 2.

Schematic model illustrating the potential contribution of UBE3A to neuronal morphology and developing neural circuits. **a**. During synaptogenesis, UBE3A ubiquitinates and promotes the degradation of the RhoA-GEF Ephexin-5 by the UPS [67] leading to inactivation of RhoA and facilitates formation of dendritic spines (highlighted in blue). UBE3A also ubiquitinates and promotes the degradation of Arc [66], an immediate early gene that facilitates the experience-dependent remodeling of pre-existing synapses by mediating AMPA receptor (AMPAR) endocytosis [87, 88]. This remodeling allows functional neural circuits to arise during development. Red circles, synapse elimination; yellow circles, growth of new spines. **b***UBE3A* deficiency results in the accumulation of Ephexin-5 and Arc, as observed in the *Ube3a*$^{m-/p+}$ mice [66, 67]. Increased Ephexin-5 levels lead to an enhancement in active RhoA levels, which results in deficits in excitatory synapse formation [67]. Inappropriately high accumulation of Arc leads to excessive endocytosis of GluA1-containing AMPARs from glutamatergic synaptic sites [66], hence reducing excitatory synaptic transmission. This also increases the number of silent (AMPAR-lacking) synapses, which may subsequently be eliminated during experience-dependent synapse remodeling. The resulting synaptic and circuit dysfunction may underlie various AS phenotypes, including learning deficits, ataxia, seizures and impaired social/communication skills.

Table 1

AS mouse models and associated phenotypes[a]

AS mouse	Genetic alterations	Molecular Phenotype	Cell Morphology Changes	Plasticity Deficits	Seizure Susceptibility[d]/ abnormal EEG	Behavioral Deficits	Refs
Ube3a$^{m-/p+}$	Deletion of maternal sequence orthologous to exon 2 of human *UBE3A*[b]	Loss of UBE3A protein in a majority of neurons in the brain	Reduced dendritic spine density in cortex (layers II/III,V), cerebellar Purkinje cells, and hippocampal CA1 pyramidal cells	Impaired induction of hippocampal LTP (Schaffer collateral synapses) Impaired induction of visual cortex LTD and LTP (layer IV to II/III synapses) Impaired ocular dominance plasticity	$Occasional spontaneous tonic-clonic seizuresf, Increased audiogenic seizure susceptibility[f,g] Electrographic seizures and spike-wave discharges associated with behavioral inactivity[g]	*Motor:* Subtle gait abnormalities, impaired motor coordination, impaired motor learning, reduced grip strength *Cognitive:*Impaired contextual fear learning, impaired spatial learning *Communicative*: N/A *Social:* Normal social-seeking behavior	[44, 45, 47, 48, 53, 63, 69]
Ube3a$^{m-/p+}$	Deletion of maternal sequence orthologous to exons 15 and 16 of human *UBE3A*[c], replacement with IRES[a]-*lacZ*	Loss of UBE3A protein in a majority of neurons in the brain	N/A	N/A	Electrographic seizures and spike-wave discharges associated with behavioral inactivity Decreased REM sleep[e], possibly abnormal sleep consolidation	*Motor:* Impaired motor coordination, impaired motor learning *Cognitive:* Impaired contextual fear learning, impaired spatial learning *Communicative:* N/A *Social:* N/A	[50, 52, 58]
Deletion$^{m-/p+}$	1.6 Mb maternal deletion disrupting *UBE3A Atp10a*, and *Gabrb3* loci	Loss of UBE3A protein in a majority of all neurons in the brain; haploinsufficient for *Gabrb3* and *Atp10a*	N/A	N/A	Frequent spontaneous tonic-clonic seizures[e] Electrographic seizures and spike-wave discharges associated with behavioral inactivity and subtle myoclonia[e]	*Motor:* No obvious gait abnormalities or limb weakness, impaired motor coordination, impaired motor learning *Cognitive:* Impaired contextual fear learning, impaired spatial learning *Communicative:* Increased ultrasonic vocalizations in newborn pups	[49]

AS mouse	Genetic alterations	Molecular Phenotype	Cell Morphology Changes	Plasticity Deficits	Seizure Susceptibility[d]/ abnormal EEG	Behavioral Deficits	Refs
						Social: N/A	

[a] Abbreviations: N/A (Not Analyzed), IRES (internal ribosome entry site)

[b] Corresponding to exon sequence of GenBank accession No. X98022

[c] Corresponding to exon sequence of GenBank accession No. AF009341

[d] Background strain-dependent phenotype

[e] C57/BL6J;

[f] 129/SvEv;

[g] 129/Sv-C57BL/6;

Table 2

List of potential UBE3A substrates in the brain[a]

Protein	Neuronal function of substrate	Detection of UBE3A interaction[b]	Substrate Localization	Brain regions with increased expression in AS model mice[c]	Refs
p53	Involved in neuronal apoptosis, differentiation, axon outgrowth [101]	Co-IP *In-vitro*[d]	Cytoplasmic Nuclear	Purkinje cells, Hippocampus (CA region), Neuro2A cells	[48, 102]
p27	Promotes neuronal migration and differentiation in cerebral cortex [85]	Co-IP[d]	Nuclear	Purkinje neurons, CA2 Hippocampus, Cortical Neurons	[84]
Pbl/Ect2	Contributes to neuronal outgrowth and neuronal size [103]	Co-IP[d]	Cytoplasmic Perinuclear	Purkinje neurons	[83]
α-synuclein	Found in juxtanuclear aggregates in neurodegenerative disorders.	N/A	Cytoplasmic Nuclear Vesicular	Neuro2A cells	[104]
Arc	Controls surface AMPAR levels	Co-IP[d] *In-vitro*	Cytoplasmic Nuclear Vesicular	Hippocampal lysates	[66]
Ephexin5	Controls excitatory synapse number and dendritic spine density	Co-IP[d,e]	Synaptic	Brain lysates, Hippocampal cultures	[67]

[a] This table is limited to UBE3A substrates that have been best described in the brain.

[b] Abbreviations:N/A,Not Analyzed; Co-IP, Co-immunoprecipitation.

[c] Refers to use of Jiang et al., model [48]

[d] Cell lysates

^{e}Brain lysates