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Review

UBE3A Isoform Biology in Health and Disease

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Abstract

Ubiquitin protein ligase E3A (UBE3A) is expressed in neurons throughout the brain and is critical for normal neuronal morphology and synaptic function. Altered UBE3A expression is implicated in several neurological disorders, most notably Angelman syndrome (AS), a rare genetic, non-degenerative, neurodevelopmental disorder that results from disruption of the maternal allele of *UBE3A* due to deletion, mutation, uniparental disomy, or imprinting center defect and leads to loss of neuronal UBE3A protein expression. Multiple isoforms of UBE3A are generated from the human gene sequence, and closely conserved mouse homologs show high sequence identity. Recent findings from the past few years using animal and cellular models, as well as insights from clinical studies in individuals with AS, have been instrumental for identifying isoform-dependent differences in expression, subcellular localization, structure, and contributions to both normal neuronal function and AS-related phenotypes. In this review, the current knowledge on the isoform-specific roles of UBE3A in normal physiology and disease pathology are summarized. Importantly, increased understanding of isoform-specific research questions will be informative for guiding future development of therapeutic strategies.

Keywords: UBE3A, isoforms, Angelman syndrome, expression, subcellular localization, function, neuron, translational science, human, mouse, gene therapy, development.

Introduction

Ubiquitin protein ligase E3A (UBE3A, also known as E6-AP) is essential for normal neuronal function and synaptic plasticity. Multiple isoforms of UBE3A exist, and prevailing evidence indicates that they each play distinct biological roles. Disruption to the normal expression and function of UBE3A gives rise to neurogenetic disorders, most notably Angelman syndrome (AS), which results from loss of expression in neurons. There is a significant unmet medical need for Angelman syndrome as no disease-modifying treatment is currently available and severe symptoms leave individuals unable to live independently. Greater understanding of the biology of UBE3A isoforms may provide new insights on therapeutic strategies for Angelman syndrome. This review considers isoform-dependent differences in expression, subcellular localization, structure, and functional roles of UBE3A in normal physiology and disease.

UBE3A Isoform Expression

The *UBE3A* gene is located in the 15q11–q13 region of chromosome 15. The sequence for *UBE3A* has a coding region of ~2.7 kb long containing 10 exons that encode 852-875 amino acids. *UBE3A* contains multiple functional domains: the E6 protein binding

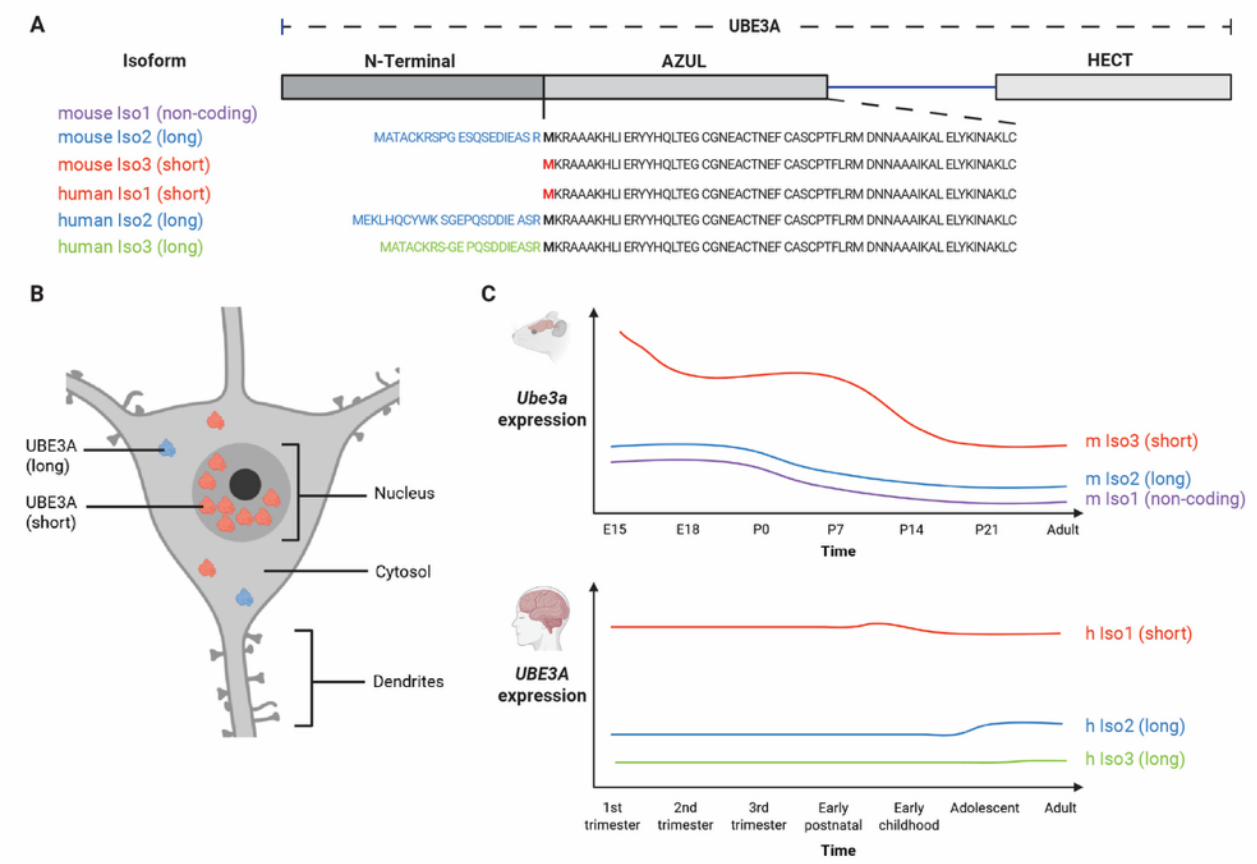

Figure 1.
(A) Schematic overview of the UBE3A amino acid sequence alignment for the mouse and human isoforms (adapted from Biagioni 2025 Fig 2 and Krzeski 2024 Fig 1).
(B) Subcellular localization and relative expression levels of UBE3A isoforms in the neuron.
(C) Expression pattern of *UBE3A* in brain in mice (adapted from Miao et al. 2013, Fig 2B) and humans (adapted from Judson et al. 2021 Fig 1C) throughout development.
Created with BioRender.

region, the amino-terminal zinc-binding domain of ubiquitin E3 ligase (AZUL), the HECT and RCC1-like domain 2 (HERC2), and the Homologous to E6-AP Carboxyl Terminus domain (HECT) (Huibregtse et al., 1993; Scheffner et al., 1994; Cooper et al., 2004; Kühnle et al., 2011; Avagliano Trezza et al., 2019). The loss of expression or function from the maternally inherited *UBE3A* gene causes Angelman syndrome, a rare neurogenetic, non-degenerative disorder with an incidence of 1:12,000-1:20,000) (Buiting et al., 1995; Fiumara et al., 2010; Mabb et al., 2011; Dagli et al., 2012). The most common causes for AS include maternal deletions within the chromosome 15q11-q13 region containing *UBE3A* (65-75%) or point mutations/variants within the *UBE3A* coding region (10-15%); less frequent causes include paternal uniparental disomy (UPD) (3-5%), an imprinting center defect (ICD) on the maternal allele (3-4%), and mosaicism (~1%) (Jana, 2012). Frequently, the genetic cause is a spontaneous *de novo* mutation involving the maternal *UBE3A* gene (Kishino et al., 1997; Matsuura et al., 1997). Inherited causes of AS are much rarer and due to a *UBE3A* point mutation or a subtype of ICD called submicroscopic deletion of the imprinting center, which results in bi-allelic neuronal silencing (Buiting et al., 1995; Van Buggenhout and Fryns, 2009; Dagli et al., 2012).

Early work identified 5 *UBE3A* mRNA transcripts generated by alternative splicing (Yamamoto et al., 1997) with more recent reports indicating there are ~50 mRNA transcripts (Yang and Huang, 2025). Three UBE3A protein isoforms (~100 kDa) have been identified in humans (isoform 1 [hUBE3A-iso1], isoform 2 [hUBE3A-iso2], and isoform 3 [hUBE3A-iso3]). These isoforms have complete sequence overlap with the exception of an extension at the N-terminal end in isoforms 2 and 3 due to translation from an independent start codon (Fig. 1A). hUBE3A-iso2 includes a 23-amino-acid N-terminal extension

and hUBE3A-iso3 includes a 20-amino-acid N-terminal extension. In line with the critical role of UBE3A in normal physiology and neurodevelopment, there are closely conserved murine homologs for the two human isoforms that make up the majority of expressed protein in neurons with 96% amino acid sequence identity to their respective orthologs (Zampeta et al., 2020). Extensive analysis to understand the different UBE3A isoforms has been completed using mouse-derived neuronal cell culture or using AS mouse models, such as the *Ube3a*$^{m-/p+}$ (Jiang et al., 1998) or *Ube3a*^{tm2Yelg} model (Wang et al., 2017); additional studies have used immortal cell lines, hESC-derived cells, AS donor hIPSC-derived neurons, and human post-mortem tissue.

Multiple *Ube3a* isoforms are found in mice as a result of alternative splicing or polyadenylation, including mouse *Ube3a* isoform 3 (*mUbe3a*-iso3), the short coding isoform that is homologous to *hUBE3A*-iso1 (91% sequence identity). Mouse *Ube3a* isoform 2 (*mUbe3a*-iso2) is the long coding isoform (an additional 21 amino acid N-terminal extension) and homologous to *hUBE3A*-iso3 (92% sequence identity) (Fig. 1A).

For a more detailed diagram of the *UBE3A* exonic structure and comparisons across different isoform transcripts, see Figs.1 and 2 in (Yamamoto et al., 1997) and Fig. 4 in (Zampeta et al., 2020). There is no known mouse homolog for *hUBE3A*-iso2, which arose later in evolution (Zampeta et al., 2020). An alternative polyadenylation signal results in a different 3' UTR and a truncated, inactive non-coding mouse *Ube3a* isoform 1 transcript with a coding-independent role in brain development that does not have a known human homolog (Valluy et al., 2015).

UBE3A is biallelically expressed in most tissues. In mature neurons, however, it is solely expressed from the maternal allele due to genomic imprinting and silencing of the paternal allele through RNA transcription of a long non-coding RNA (>600 kb) called the *UBE3A* antisense transcript (*UBE3A-ATS*) that initiates in the 15q11-q13 region of the paternal allele (Huibregtse et al., 1991; Mishra et al., 2009; Greer et al., 2010; Sato and Stryker, 2010; Kaphzan et al., 2012; Margolis et al., 2015). Paternal *UBE3A* is expressed in all other cell types, including peripheral tissues and glial cells in the nervous system, with a reduction of 50% or more found in UBE3A expression in peripheral tissues and cultured glial cells (Dindot et al., 2008; Gustin et al., 2010; Grier et al., 2015). UBE3A is expressed broadly throughout the brain with the strongest expression in neurons, both glutamatergic and GABAergic (Gustin et al., 2010; Judson et al., 2014; Burette et al., 2017; Burette et al., 2018). UBE3A protein expression in the brains of WT mice was found to be slightly higher in some regions, including the cortex, olfactory bulbs, and thalamus, than in other regions evaluated, such as the hippocampus, striatum, hypothalamus, cerebellum, and midbrain (Gustin et al., 2010). For an in-depth review of the spatial and temporal expression of UBE3A, see (Biagioni et al., 2024).

At the neuronal level, work from Dindot et al. using a *Ube3-YFP* reporter mouse demonstrated that UBE3A protein expression can be found at high levels in the nuclei of neurons as well as in the cell body, dendrites, in the growth cones of developing neurites, and both pre- and postsynaptic compartments (Dindot et al., 2008). Subcellular fractionation confirmed a broad distribution of UBE3A as well, with the highest level detected in the soluble protein pool and lower levels found in the membrane, postsynaptic density, and insoluble pools (Gustin et al., 2010). Analysis using high-resolution immunogold electron microscopy and confocal co-localization confirms that expression is unevenly distributed, with the highest intensity/density of staining found in small puncta in the nuclei over euchromatin and in axon terminals and lower levels of labeling in the neuropil, dendrites, and spines (Burette et al., 2017). The highest concentration in surgically resected human temporal cortical tissue was in euchromatin-rich domains in the nucleus, the head and neck of dendritic spines, although not the postsynaptic density, and in axon terminals of neuropil (Burette et al., 2018). Glial cells (GFAP+ astrocytes) showed intense nuclear and diffuse cytosolic staining of UBE3A, which was reduced but not completely diminished in AS-derived astrocytes (Grier et al., 2015); however, the labeling in neurons was much higher than in glial cells (Burette et al., 2017).

The presence of UBE3A protein in both the nucleus and cytosol reflects isoform-dependent subcellular localization (Fig. 1B). In both mouse and human tissue and cells, the short isoform (mUBE3A-iso3 and hUBE3A-iso1) is predominantly found in the nucleus

and expressed at approximately 4-fold greater levels than the long isoform(s), comprising around 75-80% of total UBE3A protein levels (Miao et al., 2013; Avagliano Trezza et al., 2019; Sirois et al., 2020; Zampeta et al., 2020; Judson et al., 2021). The longer isoform(s) (mUBE3A-iso2, hUBE3A-iso2, and hUBE3A-iso3) contribute ~20% to total UBE3A protein levels and mostly localize to the cytoplasm (Miao et al., 2013; Avagliano Trezza et al., 2019; Sirois et al., 2020; Zampeta et al., 2020; Judson et al., 2021; van Esbroeck et al., 2025). Of the two long human UBE3A isoforms, hUBE3A-iso3, which has a conserved sequence similar to mUBE3A-iso2, is much more highly expressed (~15-19%) than the long hUBE3A-iso2 (~1-2%) (Sirois et al., 2020; Judson et al., 2021). The relative abundance of the different isoforms at the protein level is consistent across development (Avagliano Trezza et al., 2019). Subcellular fractionation analysis of hESC-derived neurons indicates that, while the nucleus is highly enriched for hUBE3A-iso1, this isoform is present in all cellular compartments that were assayed and may be evenly distributed. Overall, the higher relative expression of isoform 1 derives from a combination of dense expression in the much smaller volume of the nucleus and sparse expression throughout the higher volume of the cytoplasm (Sirois et al., 2020).

The level of *Ube3a* expression and subcellular localization shift during neuronal maturation. Expression is present from the paternal allele during very early development in AS mouse neurons (Grier et al., 2015) and is detectable in human-derived cell culture (Leung et al., 2009); this likely explains why early development during the first few months of life is closer to neurotypical development. The amount of UBE3A protein expression present in cortical tissue of an AS mouse is much lower than WT levels, but distinctly present at birth and the first few days of postnatal development. This is due to expression from the not-yet-silent paternal allele during maturation, which then drops sharply during the first week and into adulthood (Sato and Stryker, 2010; Judson et al., 2014). Subcellular localization also changes during this early stage of development, as UBE3A protein expression shifts from primarily cytosolic and synaptic with more limited nuclear expression at P6 in both AS and WT mice to primarily nuclear-localized expression with less expression in the cytosol (Sato and Stryker, 2010; Judson et al., 2014).

Analysis of mRNA transcripts from the three different mouse isoforms (*mUbe3a*-iso1, -iso2, and -iso3) during development (embryonic day 15 to adulthood) showed that non-coding *mUbe3a*-iso1 and long *mUbe3a*-iso2 are expressed at much lower levels than the short *mUbe3a*-iso3, which was nearly 4-fold higher at E15 and ~2- to 10-fold higher than the murine isoforms 2 and 3 at P14, a ratio that persisted to adulthood (Fig. 1C) (Miao et al., 2013). Transcript levels from all three isoforms decreased during late embryonic and early postnatal development, although transcript levels were stable from P14 to adulthood (Miao et al., 2013). RNA-seq data from publicly available datasets confirmed this developmental trajectory of relative isoform expression in tissues collected throughout the brain (forebrain, hindbrain, and midbrain) with ~3- to 4-fold higher RNA transcripts for the short *mUbe3a*-iso3 than long *mUbe3a*-iso2 during embryonic development to birth, which shifted to ~1.6 :1 short *mUbe3a*-iso3 to long *mUbe3a*-iso2 across all tissues collected from adult mice (Judson et al., 2021).

Analysis of publicly available human RNA-seq data showed a consistent ratio of around 4:1 *hUBE3A*-iso1 (short) to *hUBE3A*-iso3 (long) with iso2 (long) expression at near zero during pre- and post-natal development and into adulthood in different regions of the brain (Fig. 1C) (Zampeta et al., 2020; Judson et al., 2021). In addition to maintenance of this isoform expression ratio across development, the *hUBE3A* RNA isoform expression ratios are maintained across different brain structures (pre-frontal and sensory cortices; deep brain structures, hippocampus and thalamus; and cerebellum) (Judson et al., 2021), which suggests that an expression ratio with a relative abundance of *hUBE3A*-iso1 transcripts may be important for normal neuronal function. Overall, there is a remarkable conservation of sequence, ratio of endogenous expression of isoforms, and subcellular localization across species for UBE3A.

UBE3A Function

E3 proteins, including UBE3A, are critical for substrate recognition as a component of the ubiquitination process for protein degradation (Yamamoto et al., 1997). UBE3A was first identified

for its role in degradation of the tumor suppressor protein p53 via the ubiquitination proteosome system through binding the viral E6 protein (Huibregtse et al., 1991, 1993). Identified targets of E6-AP include p53, p27, Arc, and ephexin5, proteins that are crucial for regulation of the nervous system at the cellular and synaptic level - disruption of these interactions can lead to neurologic deficits (Huibregtse et al., 1991; Mishra et al., 2009; Greer et al., 2010; Margolis et al., 2010; Sato and Stryker, 2010). A more extensive understanding of UBE3A's putative molecular functions in health and disease, interaction partners, and target substrates can be gained from recent reviews (Lopez et al., 2018; Khatri and Man, 2019; Biagioni et al., 2024; Krzeski et al., 2024; Yang and Huang, 2025).

It is well established that dysregulated UBE3A activity results in disease states such as Angelman syndrome (lack of maternal *UBE3A* expression) or Dup15q syndrome (extra maternal copy of *UBE3A*); the clinical hallmarks and genetic causes of AS are described in more detail in these reviews (Williams et al., 2010; Mabb et al., 2011; Tan et al., 2011; Dagli et al., 2012; Buiting et al., 2016). However, increasing evidence suggests that UBE3A exhibits isoform-specific biology with respect to molecular structure, subcellular localization, and interaction partners that may play distinct roles in health and disease states. Results from isogenic hESC-derived neuronal cultures support the finding that loss of the short, nuclear isoform (hUBE3A-iso1) alone is sufficient to cause AS-related phenotypes as the hUBE3A-iso1 knockout (KO) cell line showed a partial AS electrophysiological phenotype, with no changes in the hUBE3A-iso2/3 KO neurons (Sirois et al., 2020). The observed phenotype was less abnormal than in AS patient iPSC-derived neurons, suggesting that the presence of hUBE3A-iso2 and/or hUBE3A-iso3 can partially compensate in the absence of hUBE3A-iso1 (Sirois et al., 2020). Clinical data supports the criticality of the short, nuclear UBE3A isoform as a missense mutation has been identified in patients that interrupts the start codon of *hUBE3A*-iso1 (short nuclear) but not the *hUBE3A* isoforms 2 and 3 (long cytosolic), and results in AS, albeit with less severe symptoms (Sadhwani et al., 2018).

The p.Met1Thr start codon pathogenic variant in *hUBE3A*-iso1 was identified to result in improved communication, gait, and more advanced overall developmental skills than other AS individuals despite putative disruption of the more critical short nuclear isoform (Sadhwani et al., 2018). This mutation shifts localization of the next most abundant hUBE3A-iso3 from the cytosol to the nucleus. This site is sensitive to substitution, and p.Met21Ala or p.Met21Thr shifts hUBE3A isoform 3 localization from the cytosol to the nucleus in murine neuronal and U2-OS cell cultures, although the equivalent site in mUBE3A-Iso2 is much less sensitive to substitution (Zampeta et al., 2020; van Esbroeck et al., 2025). One potential explanation for the milder phenotype is that nuclear expression of hUBE3A-iso3 can functionally compensate for the loss of hUBE3A-iso1. However, it is unknown whether this shift in subcellular localization impacts catalytic activity, and differences in N-terminal extension and isoform structure may limit binding with substrates that typically interact with isoform 1.
Bossuyt et al. screened 31 UBE3A missense mutations in neuronal cultures for disruptions in subcellular localization, catalytic activity, and stability and found the majority of the variants tested (55%) caused mislocalization with partial or complete loss of nuclear UBE3A and appearance of UBE3A signal in the cytosol (Bossuyt et al., 2021). All mislocalized variants also showed reduced UBE3A protein levels, likely due to incorrect folding, instability, and quicker degradation, which contrasted with the general characteristics of variants that maintained nuclear localization and typically resulted in reduced catalytic activity and increased amounts of UBE3A (Bossuyt et al., 2021) (see Fig. 5 in Bossuyt et al., 2021 for schematic overview of the location and classification of UBE3A missense mutations assessed). A limited number of variants have been identified that showed correct nuclear localization, normal ubiquitination, and normal or increased UBE3A levels in neuronal culture that have also been identified in individuals with milder neurodevelopmental delays that did not meet the criteria for AS (pAsn340del; p.Asp563Gly) or may be benign variants (p.Leu273Arg; p. Arg626Pro) (Bai et al., 2014; Geerts-Haages et al., 2020).

The functional contributions of different UBE3A isoforms to AS-related phenotypes have been dissected using *Ube3a* isoform-specific KO mice (Avagliano Trezza et al., 2019), isogenic hESCs

(Sirois et al., 2020), and isoform-specific hiPSC-derived neurons (Zampeta et al., 2020). Loss of the short, nuclear, and more abundant *mUbe3a*-iso3 resulted in a greater reduction of UBE3A protein expression and behavioral deficits similar to AS mice, including decreased rotarod performance, decreased nest-building material used, decreased marble burying, and increased time floating during forced-swim test, while the *mUbe3a*-iso2 KO performed similar to WT mice (Avagliano Trezza et al., 2019). Loss of *mUbe3A*-iso3 in the KO also resulted in synaptic changes indicative of disruption to the excitation-to-inhibition balance, suggesting that loss of the short, nuclear *Ube3a* isoform results in increased seizure susceptibility and that its expression is critical for maintaining stable electrical activity (Avagliano Trezza et al., 2019).

Expression of the non-coding mouse *Ube3a*-iso1 transcript is regulated by neuronal activity and enriched in the dendritic compartment. The *mUbe3a*-iso1 mRNA transcripts play a role in regulating the complexity of dendrites and spine morphology, even in the absence of protein expression, by pruning dendritic complexity and promoting spine maturation in opposition to *mUbe3a*-iso2/-iso3, which increased dendritic complexity (Valluy et al., 2015).

Downregulation of *Ube3a* in pyramidal neurons revealed an important role for the long cytosolic isoform (*mUbe3a*-iso2) in neural development, as rescue of inhibited apical dendrite outgrowth and impaired dendrite polarity was only found with expression of *mUbe3a*-iso2 (homolog of *hUBE3A*-iso3), while coexpression with isoform 1 or 3 failed to achieve rescue (Miao et al., 2013). However, it is clear from other studies that the dosage of mUBE3A-iso2 protein levels must be within a limited physiological range as neuronal overexpression of the cytosolic isoform *mUbe3a*-iso2 in mice caused anxiety-like behaviors, learning impairments, and reduced seizure threshold in addition to neuroanatomical pathology including large reductions in forebrain, hippocampus, striatum, amygdala, and cortical volume in line with phenotypes associated with Dup15q syndrome (Copping et al., 2017). One caveat is that the Tetracycline-Off system used to overexpress *mUbe3a*-iso2 targeted excitatory neurons due to the CamKIIa promotor used, and it is possible that imbalanced UBE3A expression among excitatory and inhibitory forebrain neurons contributed to an exacerbation of seizure phenotypes, as demonstrated in conditional *Ube3a* deletion experiments that showed selective loss of *Ube3a* from GABAergic neurons increased seizure susceptibility and EEG activity characteristic of AS (Judson et al., 2016).

Numerous efforts have been led to characterize the interacting partners of UBE3A. Krzeski et al. outlined potential UBE3A substrates and their subcellular localization in neurons (nucleus, cytoplasm, plasma membrane, dendrites, synapses, or mitochondria) (Krzeski et al., 2024). Martínez-Noël et al. used a proteomic approach using wildtype, dominant negative, and catalytically inactive versions of all the UBE3A isoforms to identify substrate interactions and characterize isoform-specific differences using T-Rex 293 or SH-SY5Y cell lines or a yeast two-hybrid screen (Martínez-Noël et al., 2012; Martínez-Noël et al., 2018). Although isoform 1 more efficiently pulled down proteasome subunits, there was a great extent of overlap across all three isoforms with the most unique potential interactions identified for isoform 2, suggesting there is redundancy across the isoforms to bind as part of protein complexes (Martínez-Noël et al., 2012; Martínez-Noël et al., 2018). It is also feasible that the relative abundance in a physiological setting would impact the availability of different isoforms and likelihood for direct or indirect substrate recognition/binding affinity. These findings of high overlap for potential substrate interactions across all isoforms with a limited number of unique interaction partners identified for individual isoforms may be due to differences in structural and/or biophysical properties.

Analytical testing to understand the structure, conformational dynamics, and interactions of the three hUBE3A isoforms demonstrated that some structural features, such as the AZUL domain structure and association with Rpn10, were consistent across all isoforms (Bregnard et al., 2025). However, sequence-dependent differences have also been identified. The two long isoforms (hUBE3A-iso2 and -iso3) have an extended N-terminal helix and are able to dimerize, a feature which may be responsible for oligomerization-dependent activation of the UBE3A, whereas only hUBE3A-iso1 and -iso3 contain a dynamic Zn-coordination site, which may contribute to an isoform-specific sensitivity to oxidative stress (Bregnard et al.,

2025), and may facilitate hUBE3A-iso3 compensation in the absence of hUBE3A-iso1. The unique combination of biophysical characteristics for each isoform and differences in N-terminal flexibility, conformational dynamics, and multimerization may result in differences in stability/turnover, subcellular localization, and interactomes (Bregnard et al., 2025).

Dysregulation of substrate binding significantly impacts neuronal processes and overall brain function as loss-of-function point mutations in the HECT domain can result in AS (Cooper et al., 2004; Yi et al., 2015). Work in neuronal cultures identified the direct interaction between PSMD4, a subunit of the 19S regulatory particle of the 26S proteasome, and the AZUL domain of UBE3A is required for nuclear targeting and facilitates binding the proteasome (Avagliano Trezza et al., 2019). This interaction is responsible for nuclear targeting, nuclear import, and retention in the nucleus; disruptions of the AZUL-PSMD4 binding through a missense mutation results in cytosolic expression and can result in AS presentation (Avagliano Trezza et al., 2019). While all isoforms contain this domain, the interaction between PSMD4 and the AZUL domain in the short nuclear isoform (mUBE3A-iso3) directly at the N-terminus leads to nuclear localization, and missense mutations in the AZUL domain (i.e., p.Cys21Tyr, p.Gly20Val) result in expression solely in the cytosol due to a lack of nuclear retention controlled by the zinc finger within the AZUL domain (Avagliano Trezza et al., 2019).

The prevailing data indicate that nuclear UBE3A plays a critical role in normal physiological function and that loss of the short nuclear isoform (hUBE3A-iso1 or mUBE3A-iso3) negatively impacts behavior and electrical activity in mouse models and cellular function in human-derived neurons. While cytosolic long UBE3A likely contributes to normal function, the presence of, or an increase in, long cytosolic UBE3A isoforms does not appear to be sufficient to compensate for the loss of nuclear UBE3A. It is currently unclear what the impact of selective loss of hUBE3A-iso2 or hUBE3A-iso3 would be in a developing human. Evidently identifying and characterizing the molecular and clinical features of isoform-specific UBE3A missense mutations - the second most common cause of AS cases (10-15%) - would inform our understanding of UBE3A biology (Malzac et al., 1998). Critically, it appears that replacement of nuclear UBE3A could improve clinical outcomes.

UBE3A Isoforms and Therapeutic Strategies

There are currently no approved disease-modifying treatments for AS; however, recent years have seen encouraging developments. A handful of investigational products have reached the clinical stage, including three antisense oligonucleotides (ASOs) that disrupt the paternal imprinting process to reinstate UBE3A expression from the normally silenced paternal allele and are at the Phase III clinical trial phase, and an AAV-*hUBE3A*-iso1-mediated gene replacement therapy is positioned to start enrollment for a Phase I/II trial. Phase 1 clinical trial data from the ASO Rugonerson (RO7248824) has provided proof-of-concept for safety, tolerability, and clinical improvements by increasing UBE3A expression in AS individuals (Hipp et al., 2025). For more in-depth reviews on current and future therapeutic development in AS, see (Markati et al., 2021) and (Copping et al., 2021).

The importance of different UBE3A isoforms in the rescue of AS clinical features can be informed by preclinical animal model studies that have used isoform-specific approaches to restore UBE3A expression to the brain. Achieving even limited amounts of UBE3A expression may enable clinically meaningful improvements as suggested by the milder clinical features seen in AS individuals with as few as 1% of cells expressing UBE3A due to mosaicism (Brockmann et al., 2002; Nazlican et al., 2004; Le Fevre et al., 2017). It will be important to understand whether manipulating the expression of any individual isoform affects the expression of the other isoforms for a given therapeutic strategy. The generation of conditional *Ube3a* overexpressing mice with an additional 1, 2, or 4 copies of *Ube3a* demonstrated similar ratios of short: long *mUbe3a* isoform expression, indicating that overexpression does not result in changes to the relative expression of either isoform (Punt et al., 2022). Additionally, removal of specific *hUBE3A* isoforms in hESC-derived neurons also does not appear to disrupt the production or localization of the remaining isoforms, suggesting

they are processed independently of each other (Sirois et al., 2020).

The first proof-of-concept study for treatment of AS with gene or protein replacement delivered AAV9-*mUbe3a*-iso3 with a ubiquitous promoter to adult AS mice via direct bilateral hippocampal injections (Jiang et al., 1998; Daily et al., 2011). This intervention successfully increased UBE3A expression to WT levels in the hippocampus and improved some AS-relevant phenotypes compared with AAV-GFP injected control mice, including associative learning and memory and supported partial recovery of hippocampal synaptic plasticity as assessed by long-term potentiation (LTP) (Daily et al., 2011). However, expression of the transgene was limited in distribution and was not present in other regions of the brain important for neurological function, such as the cerebellum, which likely explains the lack of rescue in motor deficits and overall partial rescue (Daily et al., 2011).

Administration of an engineered construct including *hUBE3A*-iso1 in combination with a secretion signal and cell-penetrating peptide sequence to adult AS mice or AS rats either directly to the hippocampus or by ICV injection to target a broader distribution of isoform 1 protein expression outside of the AAV-mediated transduction resulted in improvements in associative memory, motor learning, and LTP (Nenninger et al., 2022). Delivery of the short nuclear isoform (hUBE3A-iso1 or mUBE3A-iso3) to neonatal and young adult immunodeficient AS mice using a lentivector-transduced hematopoietic stem cell system also showed promising improvements in AS-relevant behavioral phenotypes (Adhikari et al., 2021). A direct protein replacement strategy using recombinant human UBE3A-iso1 protein delivered bilaterally directly to the hippocampus in adult rats was also able to improve associative memory and LTP (Dodge et al., 2022). Together these findings support that expression of *hUBE3A*-iso1 is sufficient to improve functional and neurophysiological outcomes.

More recent work from Judson et al. used a dual-isoform neuronal-specific PHP.B-*hUBE3A* vector administered ICV to neonates with different strength Kozak sequences to bias expression towards short (*hUBE3A*-iso1) over long (*hUBE3A*-iso3) isoforms close to the short:long isoform ratio seen in wildtype mice (~3.5:1 short to long in WT mice, ~2.7:1 in AAV-dual isoform) and across human development (Judson et al., 2021). This strategy resulted in broad protein expression throughout the brain that significantly improved motor learning and mouse-specific behaviors in AS mice as well as decreased seizure susceptibility and kindling-associated hippocampal pathology (Judson et al., 2021). In the absence of a side-by-side comparison using the same capsid and promoter, it is currently unclear whether the combined dual-isoform strategy is more effective than *hUBE3A*-iso1 or *hUBE3A*-iso3 alone.

Long cytosolic isoforms (hUBE3A-iso2, -iso3) play a role in neural development and may be able to compensate for loss of isoform 1 to some degree, but a strategy relying solely on *hUBE3A*-iso3/*mUbe3a*-iso2 expression may be less effective and risk exacerbating phenotypes or precipitating deleterious outcomes when overexpressed too highly (Copping et al., 2017). It is unclear why overexpression of the long isoform is associated with a greater risk of adverse outcomes, but this could reflect isoform-specific molecular dynamics pertaining to turnover/stability, catalytic activity, and/or subcellular localization. Further studies manipulating the relative expression of each isoform could provide useful insights relating to disease pathology or highlight therapeutic strategies to avoid.

It is assumed that ASO treatment, by virtue of targeting the native *UBE3A-ATS*, is isoform-agnostic and results in a balanced expression ratio of all three isoforms similar to the endogenous ratio, although this is not yet known. It is also unknown what ratio of isoform expression is most beneficial to individuals with AS and whether ASOs could be engineered to achieve the optimal therapeutic isoform ratio. Furthermore, it is unclear whether an isoform-specific strategy might lead to better outcomes for a subset within the heterologous AS population, including missense mutations that may have variable isoform-specific effects. It will also be important to consider how the stage of neuronal development at the time of treatment may be impacted by isoform-specific changes that occur during development.

Earlier treatment is generally associated with better outcomes, such that very early *in utero* or post-natal timepoints have been proposed to achieve more

effective rescue of AS by restoring *UBE3A* expression during critical developmental windows and allowing for lower payloads of therapeutic agent (Schwab and MacKenzie, 2021; Clarke et al., 2024). Future studies should seek to better understand isoform-dependent functions and protein-protein interactions that could inform therapeutic strategies through identification or optimization of new small molecule drugs or putative biomarkers.

Conclusion

UBE3A exhibits isoform-dependent differences in expression levels, subcellular localization, structure, and substrate interactions that can change during development. Current data indicate that hUBE3A-iso1 is essential for neuronal physiology and behavioral function and support prioritizing therapeutic strategies relying on expression of this isoform, or that express hUBE3A-iso1 with hUBE3A-iso3 at close to the 4:1 endogenous ratio. As the field enters the next stage of therapeutic development, it will be necessary to obtain a deeper understanding of how the different UBE3A isoforms are expressed and function in normal neurons to guide future therapeutic strategies.

Conflict of Interest

H.B. is currently an employee of GEMMA Biotherapeutics.

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