A proteasomal partner goes missing in Angelman syndrome

Loss-of-function mutations in the UBE3A ubiquitin ligase are associated with Angelman syndrome (AS), a severe neurologic disorder. A new study defines the role of mutations in an N-terminal “AZUL” domain as mediating direct binding to a proteasomal subunit and shows that this interaction is correlated with the ability of UBE3A to promote Wnt/β-catenin signaling. These results provide new insights into a central biomolecule in AS and suggest that defects in Wnt/β-catenin signaling may underlie some AS phenotypes.

Ubiquitylation is a post-translational modification that controls protein degradation by the proteasome. E3 ubiquitin ligases play a central role in this process by specifying which proteins are to be ubiquitylated. The ligase UBE3A (also known as E6AP)² was one of the first recognized ubiquitin ligases and the first belonging to the HECT domain family of E3s (Fig. 1). Its discovery stemmed from studies of the cancer-associated human papillomavirus E6 oncoproteins, which were found to bind directly to UBE3A, redirecting its substrate specificity toward proteins that it does not normally recognize, most notably, the p53 tumor suppressor (1). While this accounts, in large part, for the oncogenic properties of the HPV E6 proteins, a simple yet vexing question has been the identity of the normal substrates and functions of UBE3A. That is, which cellular proteins are ubiquitylated by UBE3A in normal cells? What biological pathways does UBE3A normally regulate? A new study by Kühnle et al. (2) provides insights into aspects of these questions in their report that the N-terminal domain of UBE3A binds the proteasome, with implications for development and disease.

The importance of untangling the normal functions of UBE3A came to the fore with the realization that Angelman syndrome (AS), a severe neurologic disease, is the result of loss of function mutations in UBE3A, with the effects being manifest primarily in the hippocampal and Purkinje neurons (3). Increases in UBE3A have in turn been linked to autism spectrum disorders, providing additional impetus to investigate this molecule. In AS, the simple hypothesis is that one or more of UBE3A’s neuronal substrates are not being ubiquitylated (and therefore not being degraded), leading to the severe phenotypes seen in these patients: intellectual disability, seizures, lack of speech, ataxia, and sleep and feeding disorders, and others. AS patients have a range of genetic disruptions to the UBE3A gene, including single amino acid substitutions or deletions; most of these fall within the C-terminal catalytic HECT domain and result in a loss of enzymatic ubiquitin-ligase activity (4), consistent with the hypothesis above. The starting point for Kühnle et al. (2) was the previous identification of a small number of AS patients harboring single-amino-acid substitutions (C21Y and G20V) near UBE3A’s N terminus. These mutations fall within an ~50-amino-acid zinc-binding domain known as the AZUL domain (amino-terminal zinc finger, UBE3A ligase). This domain is not required for and does not influence the catalytic ubiquitin ligase activity of UBE3A. The NMR solution structure of the isolated AZUL domain (5) further showed that it is unique to UBE3A proteins and apparent UBE3A orthologs. These observations suggested that the AZUL domain plays a fundamental yet uncharacterized role in UBE3A function.

To learn more about the AZUL domain, Kühnle et al. began by studying interactions between WT and mutant UBE3A proteins and the proteasomal subunit PSMD4 (also known as RPN10/S5α). A functional link between UBE3A and PSMD4 had been reported previously (6); however, it was not known if this was a direct interaction and, if so, what the point of contact might be. The authors found that the AZUL domain is necessary and sufficient to mediate a direct interaction with the 26S proteasome by binding to the PSMD4 subunit and that the AZUL domain AS mutations disrupt this interaction. PSMD4 is part of the 19S proteasome “lid” that sits atop the 20S core particle where proteolysis occurs. The 19S particle has several functions, including recognition of ubiquitylated chains, and PSMD4 functions as one of these ubiquitin receptors. So why would UBE3A bind to the proteasome? It’s not entirely clear, although this is not the first such example of a ligase being found at the proteasome. For example, association of the ligase Hul5/UBE3C with the proteasome allows for dynamic remodeling of ubiquitylated substrates at the proteasome (7). As for UBE3A, it’s possible that proteasome association is important for substrate delivery, that UBE3A ubiquitylates its substrates at the proteasome, or even that this interaction mediates UBE3A’s previously reported inhibition of the proteasome (8). Further investigation will clearly be important for understanding the precise biochemical function of UBE3A at the proteasome.
Kühnle et al. also analyzed UBE3A’s recently reported ability to mediate Wnt/β-catenin signaling in neurons (8). The authors confirmed that WT UBE3A was able to stimulate Wnt signaling, but found that AZUL AS mutant proteins or a protein lacking the AZUL domain did not. Importantly, the AZUL domain is not required for all functions of UBE3A. The authors showed that the AS mutants did not affect UBE3A’s function as a transcriptional regulator, while earlier studies showed that N-terminal deletions are still able to target p53 for HPV E6-dependent ubiquitylation (9), suggesting a complex interplay between the distinct domains, partners, and activities of UBE3A.

An important caveat to the study, acknowledged by the authors, is that the AZUL domain mutations also result in lower expression of the altered UBE3A protein. This complicates the issue, as it cannot be ruled out that the AS phenotype in these patients is at least partially due to lower levels of UBE3A; this would be consistent with the fact that the patients with the AZUL domain mutations have a less severe form of AS than those with complete loss of function alterations (10). More work will clearly be required to conclude that abrogated proteasome binding is a critical determinant in AS.

Despite this caveat, the work by Kühnle et al. (2) opens up new and important possibilities for understanding the role of UBE3A in neuronal function and AS (and potentially autism disorders, as well). At a basic level, these new insights may take us one step closer to identifying the substrates of UBE3A related to AS and the putative substrate(s) of UBE3A related to Wnt/β-catenin signaling. Furthermore, these results open the possibility that a defect in the Wnt/β-catenin signaling pathway, as a result of either loss of UBE3A catalytic activity or PSMD4/proteasome binding, may be a causative factor in AS. Further investigation will help us understand what it means when UBE3A abandons its post at the proteasome.

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Figure 1. Sites of Angelman syndrome single-amino-acid substitution and deletion mutations. A schematic of UBE3A is shown with the structures of the AZUL domain (PDB 2KR1) and the catalytic HECT domain in complex with the E2 enzyme UbcH7 (PDB 1C4Z).