A matter of perspective

The multifaceted role of UBE3A in Angelman syndrome development

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CHAPTER 7

General Discussion

GENERAL DISCUSSION

In 1993 Scheffner and colleagues identified UBE3A, also known as E6AP (E6 associated protein), as the ubiquitin-protein ligase that targets the tumor suppressor protein p53 for proteasomal degradation[1]. Four years after its discovery as an ubiquitin-protein ligase, it was recognized that mutations in the UBE3A gene were linked to a severe neurodevelopmental disorder: Angelman syndrome (AS)[2]. Soon thereafter a strenuous search began for UBE3A target proteins that could be responsible for one or more aspects of AS development. Two distinct categories of UBE3A targets have been identified over the years: (1) direct targets that do not require any additional protein to mediate their interaction with UBE3A, and (2) indirect targets, that are dependent on other proteins for the interaction with UBE3A. Most of the targets in the latter category require the HPV (Human Papilloma Virus) E6 oncoprotein for UBE3A binding/ubiquitination (e.g. p53, see also[3]). Since AS is a neurodevelopmental disorder, which is unrelated to HPV infection and cervical cancer, the search for UBE3A targets responsible for AS development focuses primarily on the direct ones. It should be noted, however, that there is recent evidence to suggest that UBE3A activity can be regulated by endogenous, non-viral related, proteins[4] and in **Chapter 4** we report that one of the newly identified UBE3A interactors might possess such a function.

The ubiquitin conjugating system is highly versatile (see **Chapter 1**) and is capable of linking various types of ubiquitin chains on the target proteins. The fate of the modified target protein is dependent on the length and type of ubiquitin chain that is linked to the target protein[5,6]. As limitless as these possibilities might look, for many years UBE3A has been considered an E3 ligase only capable of synthesizing K48-linked poly-ubiquitin chains[7,8] and responsible for proteasomal degradation of its targets. Even though this is true in particular circumstances[1,9-11], there is a growing list of targets that can be ubiquitinated by UBE3A but which are not degraded, suggesting a non-degradative function of UBE3A[12].

Two newly identified UBE3A interactors described in this thesis, RPH3A (**Chapter 3**) and NSUN2 (**Chapter 4**), are examples of proteins that are ubiquitinated by UBE3A, but that are not degraded. In particular, the ubiquitination pattern observed for RPH3A is non-canonical and very distinct from the ubiquitination pattern observed for UBE3A targets such as p53[1], RING1B[11] (and see **Chapter 3**) or RAD23[13]. RPH3A ubiquitination resembles more mono- or multi mono-ubiquitination that is usually associated with DNA repair, chromatin remodeling and protein signaling[14,15]. On the other hand, NSUN2 is clearly poly-ubiquitinated by UBE3A, but no degradation of the full-length NSUN2 could be observed in HEK293T cells. One possible explanation for this conundrum is that only efficient UBE3A interaction and

poly-ubiquitinated was observed for a fragment of NSUN2, NSUN2$^{232-544}$, while the full-length protein does not interact, and, consequently, is not ubiquitinated.

So, while RPH3A seems to be a direct target of UBE3A, the evidence for NSUN2 as a UBE3A target is inconclusive. What the biological consequences are of UBE3A-mediated ubiquitination of RPH3A remains to be investigated, but its unique localization at the synapse makes it a very interesting target for in depth analysis. Also the observation that a fragment of NSUN2 activates the ubiquitin protein ligase activity of UBE3A begs for further experiments.

PML (Promyelocytic Leukemia) has been previously described as an UBE3A target whose ubiquitination leads to proteasomal degradation[16]. This would be the perfect example of a canonical target of UBE3A, if it wasn't for the fact that PML is only ubiquitinated and degraded in a tissue specific manner (**Chapter 5**). Even though PML ubiquitination was observed in an *ex vivo* ubiquitination system, in mice UBE3A-dependent degradation was only seen in lung and kidney tissue, but not in brain. Our findings suggest not only that PML does not contribute to AS development, but also that UBE3A-dependent degradation of PML is tissue specific. These data imply that lung and kidney may in fact express a factor (protein), absent in brain, which ensures the successful degradation of PML following UBE3A ubiquitination.

Only by looking at the examples that were just described, it's clear that identifying "canonical" UBE3A targets (direct, poly-ubiquitinated and degraded by the 26S proteasome) is not a trivial task. To add complexity to this already complicated landscape, we describe in **Chapter 6** and **Appendix 1** a new function for a previously identified UBE3A interactor, PSDM4[17]. PSMD4 is an essential subunit of the proteasome involved in binding ubiquitinated substrates through its ubiquitin interaction motifs (UIMs). *In vitro*, PSMD4 serves as an "ubiquitous" target for any E3 ubiquitin protein ligase by binding to ubiquitin moieties on the E3 protein that are generated by self-ubiquitination activity of the E3[18]. For this reason, considering PSMD4 a canonical target of UBE3A is most likely incorrect. We now show that PSMD4 is much more than a simple UBE3A "interactor" and, in fact, functions as a chaperone to mediate UBE3A nuclear localization. Disruption of the UBE3A-PSMD4 interaction results in cytosolic mislocalization of UBE3A, a phenotype that is observed in an AS patients with a UBE3A missense mutation in the PSMD4 interaction domain. These observations underscore the essential role of UBE3A in the nucleus and are in line with the growing number of transcription factors that are, in fact, UBE3A interactors[12,19]. Further support for a role of UBE3A in the nucleus is its reported associated with euchromatin-rich

regions[20], which are transcriptionally active domains, where UBE3A could exert both its ligase-dependent[21] and -independent[22] functions.

By simply looking at the different chapters of this thesis, it's quite obvious that what started out as a simple quest to identify targets responsible for AS development, has turned into a exiting journey to understand UBE3A function itself.. On one hand we have characterized two novel "non-canonical" UBE3A targets, RPH3A and NSUN2, that shed some light on a possible role of UBE3A beyond its classical E3 ligase function in protein degradation. On the other hand, there is a well-known target of UBE3A, PSMD4, which has been given a new function. We should therefore broaden our horizon and look at these proteins with the new set of tools that this study has generated. This includes not only the search for additional factors that might mediate interaction of UBE3A with non-direct targets, but also the possibility that UBE3A-mediated target ubiquitination might serve purposes other than proteasomal degradation and, finally, the hypothesis that UBE3A's main role in the cell might be in the nucleus and not in the cytosol.

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