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Unraveling the role of ufmylation in the brain

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Ufmylation is an ubiquitin-like post-translational modification characterized by the covalent binding of mature UFM1 to target proteins. Although the consequences of ufmylation on target proteins are not fully understood, its importance is evident from the disorders resulting from its dysfunction. Numerous case reports have established a link between biallelic loss-of-function and/or hypomorphic variants in ufmylation-related genes and a spectrum of pediatric neurodevelopmental disorders. These include developmental and epileptic encephalopathy (DEE44), autosomal recessive cerebellar ataxia, congenital neuropathy, hypomyelinating leukodystrophy, and neurodevelopmental disorder with spasticity and poor growth, each presenting with varying severity (Zhou et al., 2024). Children affected by these disorders often exhibit a range of symptoms, including intellectual disability, seizures, microcephaly, abnormal electroencephalogram, impaired motor function, dystonia, and/or global developmental delay, which result in premature death in severe cases. Variants in ufmylation genes have been shown to disrupt UFM1 activation and/or transthiolation, thereby impairing the ufmylation pathway (Pan et al., 2023; Zhou et al., 2024). These findings underline the importance of maintaining balanced ufmylation activity for optimal brain development and function.

The ufmylation pathway encompasses an enzymatic cascade that facilitates maturation, activation, attachment, and deconjugation of UFM1 from target substrates. Specifically, UFSP1 and UFSP2 act as UFM1 proteases required for UFM1 maturation and deconjugation, while UBA5 (the E1 enzyme), UFC1 (the E2 enzyme), and UFL1 (the E3 enzyme) collectively catalyze the covalent attachment of UFM1 to lysine residues on target proteins (**Figure 1**). Additionally, DDRGK1 (also known as UFBP1) and CDK5RAP3 have been identified as ufmylation cofactors, functioning as scaffolding or subcellular localization proteins for the ufmylation machinery (Zhou et al., 2024). To date, several ufmylation targets have been identified, with RPL26 being the predominant target and implicated in selective autophagy of the endoplasmic reticulum (ER) and protein translation quality control in the ER. Furthermore, ufmylation has been associated with cellular processes including DNA damage response, autophagy, unfolded protein response, and membrane trafficking (Zhou et al., 2024). Despite widespread expression of the pathway components, the consequence of its disruption is particularly evident in the nervous system (**Figure 1**).

Ufmylation's role in the brain: Loss of Ufl1 in the central nervous system (CNS) leads to microcephaly and elevated apoptosis in the occipital region of the neopallium and death of mice within one day of birth (Muona et al., 2016). This study determined the requirement for ufmylation in neuronal development and survival. The ufmylation pathway components are found across the adult mouse brain, including the olfactory bulb, cortex, hippocampus, striatum, and cerebellum, as well as within the spinal cord. Notably, an abundance of ufmylated Rpl26 was identified in the hippocampus (Zhang et al., 2022), a site of continued neurogenesis during adulthood, and a region affected by epilepsy, a common feature of ufmylation deficiency disorders. MRI studies in affected children have revealed a spectrum of brain abnormalities, including cerebellar atrophy, thinning of the corpus callosum, white matter hyperintensities, delayed myelination, and reduced thalamic size (Muona et al., 2016), indicating a broad requirement for ufmylation throughout the brain.

Impaired ufmylation in the postnatal mouse through neuron-specific depletion of DDRGK1 or UFL1 results in abnormal behavior, such as increased aggression, and microcephaly by 3–4 months of age (Zhang et al., 2022). Both microcephaly and irritability are features observed in children with ufmylation deficiency disorders (Muona et al., 2016). Additionally, the loss of neurons in the CA1 region of the hippocampus and across the six cortical layers was identified in the mouse model. RNA-seq analysis of the cortex detected a downregulation of genes linked to synapse and dendrite development, while genes associated with glial function, microglial function, inflammatory responses, and ER stress were upregulated (Zhang et al., 2022). These findings underscore the critical role of ufmylation in neuronal function in the postnatal brain and suggest that inflammation and ER stress contribute to the pathophysiology associated with impaired ufmylation.

Recently, it was determined that impaired ufmylation specifically affects a subset of neurons, enhancing our understanding of its impact on neuronal function. Single-cell RNAseq analysis of 3D cortical organoids derived from induced pluripotent stem cells derived from individuals carrying *UBA5* variants identified a reduction in both progenitor and mature inhibitory GABAergic interneurons and consistent with this the organoids exhibited an increased firing rate and reduced coordinated activity (Chen et al., 2024). GABAergic interneuron signaling is essential for maintaining excitation-inhibition balance and its disruption is linked to epilepsy, thus impaired GABAergic interneuron signaling may be an underlying mechanism for the clinical manifestations observed in affected children. Chen et al. (2024) also revealed that both cortical organoids and glioma cells expressing pathogenic *UBA5* variants exhibited elevated levels of p-PERK and p-eIF2α, indicative of activation of the unfolded protein response. Conversely, increasing the levels of the UBA5 variants reversed the unfolded protein response protein levels and normalized the firing rate (Chen et al., 2024). It will be interesting to see whether GABAergic interneurons in other brain regions are affected, as well as identifying additional neuronal populations that are dependent on ufmylation.

In 2023, it was demonstrated that *Uba5* is expressed in a subset of neurons and glia in *Drosophila* larval and adult brains, with a larger population of expressing cells in the adult brain (Pan et al., 2023). This challenges the previous belief that ufmylation occurs across all neuronal cells and suggests that ufmylation may play a more prominent role in maintaining neuronal integrity in the adult brain or it may be particularly critical in differentiated neuronal cells. Additionally, the authors demonstrated that while ubiquitous expression of *UBA5* was able to rescue the survival of *Uba5* mutant flies, *UBA5* expression in either neurons or glial cells alone, or in both cell types simultaneously, proved insufficient (Pan et al., 2023). This indicates that ufmylation activity both within and outside the CNS is required to ensure survival.

Ufmylation in aging and neurodegeneration: While impaired ufmylation is well-documented in association with neurodevelopmental disorders, it is becoming apparent that more subtle disruption of ufmylation may have an impact on aging and late-onset neurodegenerative diseases. Given the involvement of ufmylation in protein quality control mechanisms, essential for optimal protein synthesis, as well as its role in ER stress and autophagy, ufmylation is likely critical in supporting neurons during cellular stress conditions

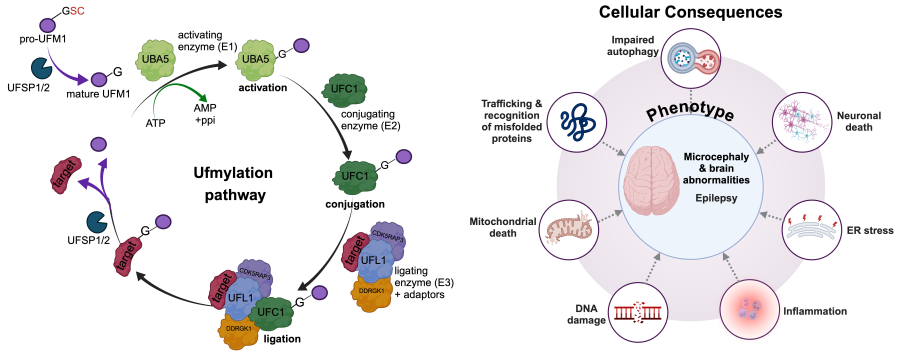

Figure 1 | **Consequences of impaired ufmylation in the brain.**
Left panel: Schematic depiction of the ufmylation pathway. Ufm1-specific proteases 1 and 2 (UFSP1, UFSP2) cleave pro-UFM1 to its mature form. UBA5 then activates UFM1 via thioesterification. UFM1 is transferred to UFC1, which, in concert with UFL1 and cofactors (e.g. DDRGK1 and CDK5RAP3), conjugates UFM1 to its substrates. Deufmylation of ufmylated proteins is catalyzed by UFSP1 or UFSP2. Right panel: Ufmylation plays a role in multiple cellular processes but how disruption of these processes leads to the phenotypes observed requires further investigation. Created with BioRender.com. AMP: Adenosine monophosphate; ATP: adenosine triphosphate; CDK5RAP3: CDK5 regulatory subunit-associated protein 3; CNS: central nervous system; DDRGK1: DDRGK domain-containing protein 1; ER: endoplasmic reticulum; PPi: inorganic pyrophosphate; UBA5: ubiquitin-like modifier activating enzyme 5; UFC1: ubiquitin-fold modifier conjugating enzyme 1; UFL1: UFM1-specific ligase 1; UFM1: ubiquitin-fold modifier 1; UFSP1: UFM1-specific peptidase 1; UFSP2: UFM1-specific peptidase 2.

associated with aging and neurodegeneration. In 2023, a neuroprotective role for ufmylation in aging *Drosophila* neurons was identified (Li et al., 2023). This protective effect was mediated by Atg9, a transmembrane protein crucial for autophagosome formation and lysosome fusion, through its interaction with Ddrgk1. The study demonstrated that Atg9 overexpression in neurons lacking ufmylation alleviated age-related deficits associated with the motor function (e.g., climbing activity), neuronal apoptosis, and lifespan. Additionally, it restored normal levels of LC3-II and p62, key markers of autophagic flux. Atg9 was further shown to enhance c-Jun N-terminal kinase activity, a pathway implicated in aging and oxidative stress and reduced in Ddrgk1-deficient flies. Recently, Wang et al. (2024b) highlighted the biological relevance of ufmylation for autophagy by demonstrating that ufmylation of VCP/p97 is essential for Beclin-1 stability, enabling the assembly and activity of the PtdIns3k complex critical for the initiation of autophagy. Ufmylation of VCP/97 was also shown to promote phagophore and phagosome formation, enhance autophagosome-lysosome fusion, and restore LC3-II levels. Importantly, VCP/p97 variants (R155H and L198W) known to cause inclusion body myopathy with early-onset Paget disease and frontotemporal dementia 1 (IBMPFD1) and frontotemporal dementia and/or amyotrophic lateral sclerosis-6 (FTDALS6) impede ufmylation of VCP/p97 (Wang et al., 2024b), suggesting a potential link between ufmylation and the pathophysiology of these disorders. Altogether, these findings suggest that ufmylation may be critical for the regulation of autophagy dynamics in the nervous system.

A recent functional genomic analysis using CRISPRi in a human induced pluripotent stem cell 4R tauopathy model determined that reducing key components of the ufmylation pathway (UBA5, UFM1, UFBP1, UFC1, or UFL1) reduced the accumulation of Tau aggregates. Likewise, UBA5 knockdown in the hippocampus of a Tau-spreading mouse model decreased the spread of Tau aggregates (Parra Bravo et al., 2024). These findings indicate that ufmylation may facilitate the spread of Tau aggregates. Consistent with this, in the context of progressive supranuclear palsy and Alzheimer's disease, both marked by Tau aggregate accumulation, analyses of brain tissue revealed significantly reduced free UFM1 levels in neurons with Tau inclusions, suggestive of increased ufmylation (Parra Bravo et al., 2024).

Tau and α-synuclein, both of which are prone to misfolding and implicated in Parkinson's disease, were revealed to undergo ufmylation (Wang et al., 2024a). Ufmylation facilitates their secretion through misfolding-associated protein secretion, involving the ER-associated enzyme USP19. In the context of α-synuclein, the knockdown of ufmylation genes using siRNAs reduces α-synuclein secretion in HEK293T cells. Notably, impaired RPL26 ufmylation does not affect α-synuclein secretion, indicating that α-synuclein secretion via ufmylation is independent of ribosomal ufmylation. However, in Ufm1-deficient *Drosophila*, α-synuclein secretion is only partially reduced and not completely impeded indicating that parallel pathways contribute to its secretion. Additionally, Wang et al. (2024a) demonstrated via immunoprecipitation assays that ufmylation preferentially targets misfolded proteins, exemplified by enhanced ufmylation of FLAG-GFP-10 (truncated unfolded GFP form) compared to FLAG-GFP, which is exacerbated by the presence of USP19. The interaction of UFM1 with USP19 further supported their coordinated action in the misfolding-associated protein secretion pathway (Wang et al., 2024a). These findings underline a possible role for ufmylation in both protein trafficking and recognition of misfolded proteins in the nervous system.

Ufmylation in the neuromuscular system: While most affected individuals described to date primarily have phenotypes associated with the CNS, some case reports also describe significant involvement of the peripheral nervous system. Additionally, infants with severe motor peripheral neuropathy, without discernible signs of CNS involvement, have been identified (Cabrera-Serrano et al., 2020). Further insights into the dual involvement of CNS and peripheral nervous system were provided by demonstration that Uba5-deficient zebrafish exhibit peripheral nerve damage prior to detectable CNS or muscle changes. Moreover, whilst skeletal muscle integrity is not affected, mitochondria within the skeletal muscle become enlarged and show signs of degeneration at later stages (Serrano et al., 2023). This suggests that impaired skeletal muscle function, due to disruptions to mitochondrial function, may play a role in the disease presentation. However, UFC1 knockdown in human skeletal muscle organoids results in increased maximum force generation (Molendijk et al., 2022). UFC1 knockdown in mice, also leads to a subtle increase in muscle cross-sectional area, alongside upregulation of proteins associated with muscle contraction, such as fast-type Troponin T (TNNT3), the translation machinery, and signal recognition particle-mediated translocation to the ER (Molendijk et al., 2022). This suggests that ufmylation may play a regulatory role in the skeletal muscle through the modulation of protein homeostasis (Molendijk et al., 2022). However, it is also possible a compensatory mechanism is triggered by ufmylation deficiency to maintain muscle homeostasis.

Conclusion: While the core ufmylation components exhibit widespread expression, and the requirement for ufmylation has been clearly shown in multiple cell types and pathways, the nervous system is particularly vulnerable to its disruption. It is unclear whether neuronal damage stems from perturbations in neuron-specific pathways, disruption of ubiquitous cellular processes, or a combination of both. In addition to its established role in severe early-onset neurodevelopmental disorders, it is becoming increasingly clear that ufmylation has a role in later-onset disorders and modulation of the ufmylation pathway may provide an approach to slow disease progression. The paucity of identified ufmylation targets hinders the exploration of these questions and we, and others, are working to identify ufmylation substrates and associated pathways that impact neuronal homeostasis.

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揭示 ufmylation 在大脑中的作用
文章特色分析

一、文章重要性
1. 阐明 ufmylation 在神经发育与疾病中的核心作用
- 文章指出，ufmylation 通路的功能丧失与多种儿童神经发育障碍密切相关，如发育性和癫痫性脑病、小脑共济失调、先天性神经病变等，严重影响患儿生存质量甚至导致早夭。
- 通过动物模型和人类干细胞研究，揭示了 ufmylation 在神经元存活、突触发育、兴奋-抑制平衡等方面的关键作用。
2. 连接神经发育疾病与神经退行性疾病
- 文章不仅聚焦于早期神经发育障碍，还探讨了 ufmylation 在衰老和神经退行性疾病（如阿尔茨海默病、帕金森病、FTD 等）中的潜在作用，扩展了该修饰的病理生理意义。
3. 为治疗干预提供潜在靶点
- 研究表明，调控 ufmylation 通路可逆转部分病理表型（如 UPR 激活、神经元放电异常），提示其作为治疗神经发育和神经退行性疾病的新靶点。

二、文章创新性特色
1. 多层面整合最新研究成果
- 文章整合了从果蝇、斑马鱼、小鼠到人类类器官模型的多物种研究数据，系统阐述了 ufmylation 在神经系统中的表达、功能及其失调后果。
2. 揭示神经元特异性与细胞类型依赖性
- 最新研究发现 ufmylation 并非在所有神经元中普遍存在，而是特定于某些神经元亚群（如 GABA 能中间神经元）和胶质细胞，挑战了既往认知，提示其功能具有细胞类型特异性。
3. 拓展 ufmylation 在蛋白质质量控制与分泌中的作用
- 文章指出 ufmylation 不仅参与内质网应激、自噬等经典通路，还促进错误折叠蛋白（如 α-突触核蛋白、Tau）的分泌，这为理解神经退行性疾病中蛋白质聚集的传播机制提供了新视角。
4. 强调神经肌肉系统的参与
- 除了中枢神经系统，文章还探讨了 ufmylation 在外周神经和骨骼肌功能中的作用，提示其在全身性蛋白质稳态调控中的广泛影响。

三、对学科的启示
1. 推动 ufmylation 从“未知修饰”向“关键调控机制”转变
- 文章系统总结了 ufmylation 在神经系统中的多重功能，将其从一个相对冷门的类泛素化修饰提升为神经发育与疾病中不可或缺的调控通路。
2. 促进跨疾病机制研究
- ufmylation 同时涉及神经发育疾病和神经退行性疾病，提示某些分子通路可能在不同生命阶段具有不同的病理意义，鼓励研究者从发育与衰老的双重视角审视疾病机制。
3. 呼吁更多底物鉴定与机制探索
- 文章指出目前已知的 ufmylation 底物有限（如 RPL26、VCP/p97 等），鉴定更多底物及其功能将是未来理解其神经特异性作用的关键。
4. 为精准治疗提供新思路
- 由于 ufmylation 通路成分明确、可调控性强，其作为治疗靶点具有较高潜力，尤其是在逆转 UPR、自噬障碍和蛋白质聚集等方面。

总结
这篇文章在系统性、前瞻性和跨疾病关联性方面具有显著优势，不仅总结了 ufmylation 在神经系统中的已知功能，还指出了未来研究方向，为神经科学、发育生物学和神经退行性疾病研究提供了重要的理论框架和实验依据。