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Stress signaling caused by mitochondrial import malfunction can be terminated by SIFI: Importance of stress response silencing

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Protein aggregates, mitochondrial import stress and neurodegenerative disorders: A salient hallmark of several neurodegenerative diseases, including Parkinson's disease, is the abundance of protein aggregates (Goiran et al., 2022). This molecular event is believed to lead to activation of stress pathways ultimately resulting in cellular dysfunction (Eldeeb et al., 2022). Accordingly, many lines of research investigations focused on dampening the formation of protein aggregates or augmenting the clearance of protein aggregates as a potential therapeutic strategy to counteract the progression of neurodegenerative diseases, albeit with little success (Costa-Mattioli and Walter, 2020). Cell stress cues such as the accumulation of protein aggregates lead to the activation of stress response pathways that aid cells in responding to the damage. Despite the notion that the transient activation of these pathways helps cells cope with stressors, persistent activation can induce unwanted apoptosis of cells and reduce overall tissue strength as well as lead to an accumulation of aggregation-prone proteins (Hetz and Papa, 2018). Mutations in proteins involved in stress signaling termination can cause conditions like ataxia and early-onset dementia (Conroy et al., 2014). Therefore, it is crucial for stress response signaling to be turned off once conditions have improved. Nevertheless, the mechanisms by which cells silence these signals are still elusive.

Mitochondrial import stress is one type of stressor that has important implications for the development of human neurodegenerative disorders such as Parkinson's disease (Eldeeb et al., 2024b). Tellingly, mammalian cells have evolved sophisticated quality control mechanisms to sense and respond to dysfunctional mitochondrial import via specific stress response signals. Remarkably, transient induction of stress response signaling aids in attenuating cellular damage. Nonetheless, continued stress response signals can exacerbate the cellular system, eliciting cellular demise. The way in which cells turn off the stress response signals resulting from mitochondrial import arrest is not fully elucidated. Recent studies unveil that the stress signaling caused by mitochondrial import malfunction can be terminated by SIFI, an E3 ligase complex, yet inactivation of this complex can lead to an abundance of aggregation-prone proteins (Yau et al., 2017; Haakonsen et al., 2024). Furthermore, the discovery that SIFI mutant cells could be rescued when stress response silencing was induced, further underscores the importance of stress response silencing (Martinez Castillo and Evans, 2024).

UBR4 E3 ubiquitin ligase and regulation of mitochondrial import stress: Diverse stress response signals have been connected to protein-aggregation. How does the SIFI E3 ligase complex modulate the regulation of the abundance of aggregation-prone proteins? The authors previously identified UBR4, an E3 ligase that promotes the degradation of aggregate-prone proteins and is mutated in early-onset dementia. UBR4 is an E3 ubiquitin ligase first identified as part of the N-end rule degradation pathway (Eldeeb et al., 2018). To study and determine genetic interactions of UBR4, a whole genome CRISPR–Cas9 synthetic lethality screen was conducted after creating ΔUBR4 cells. The results showed that most interactions of UBR4 were with genes that controlled mitochondrial protein import or electron transport chain function. ΔUBR4 cells were more affected by inhibition of mitochondrial import or ETC factors than wild-type cells. Other significant interactions of UBR4 were with E3 ligase KCMF, calmodulin, the E2 enzyme UBE2A, and mitochondrial proteins. These results led to the conjecture that the E3 ligase SIFI is composed of UBR4, KCMF, and calmodulin, which are essential components for the complex's function (Haakonsen et al., 2024).

Does modulating UBR4 E3 ligase levels fine-tune mitochondrial protein import? Discovering the important relationships between UBR4 and mitochondrial import proteins led the authors to question if SIFI was involved in regulating mitochondrial import. To test this, they used flow cytometry-based assays to reveal that both the depletion of UBR4 genetic interactors and the introduction of chemical stressors that depleted ΔUBR4 cells inhibited mitochondrial import. However, deletion of UBR4 did not, *per se*, affect import. These results suggested that SIFI is not involved in regulating protein transport into mitochondria. When searching for SIFI substrates using protein stability reporters, it was discovered that SIFI targeted cleaved DAP3-binding cell death enhancer 1 (cDELE1) and eIF2α kinase heme-regulated inhibitor (HRI) which both have key roles in the cell stress response program. cDELE1 is involved in detecting mitochondrial import stress and HRI is a kinase involved in the stress response (Guo et al., 2020). Both cDELE1 and HRI proteins were stabilized when potassium channel modulatory factor 1 (KCMF1) was absent or when specific domains in UBR4 were deleted. These findings suggested that KCMF1 promotes the degradation of cDELE1 and UBR4. The authors also concluded that SIFI supports ubiquitylation of cDELE1 and HRI, which suggests that this is how SIFI regulates their activity. This underscores the importance of silencing the stress response because when SIFI is not present, like in ataxia and early-onset dementia, the cDELE1 and UBR4 are not degraded and continue to induce the stress response. As a result, this prolonged stress response can have detrimental effects on the cell (Haakonsen et al., 2024).

Does SIFI-mediated function impact the activation and duration of the stress response signal? To address this question, the authors investigated the impact of modulation of SIFI on ATF4, a transcription factor that can be induced by HRI and plays a role in the integrated stress response (ISR) (Costa-Mattioli and Walter, 2020). To better understand how ATF4 is affected by SIFI, ΔUBR4 cells were treated with various stressors and ATF4 levels were evaluated. Results showed that ATF4 peaked at similar levels to wild-type cells treated with the same stressors but decreased much slower. This further supported the conclusion that SIFI works to turn off cell stress responses. CRep (encoded by Ppp1r15b) and GADD34 (encoded by the Ppp1r15a) are phosphatases that help SIFI by reversing the phosphorylation of eIF2α. eIF2α contributes to the stress response when it is phosphorylated by HRI. Experiments with cells that did not have GADD34 or CReP showed that SIFI did not affect the stability of these phosphatases and hence does not prevent ISR activation, but rather works primarily by restricting the duration of stress signals.

Targeting by SIFI complex–Helical degrons in HRI and DELE1: What are the degrons recognized by SIFI? The importance of the amino-terminal domain of HRI in engaging with SIFI complex was revealed when it was mutated or deleted. Absence of this motif inhibited ubiquitination and degradation by SIFI. Further testing of each alpha helix that comprises the domain showed that each helix can mediate recognition by SIFI. To study the importance of structural motifs in the cDELE1, a cleaved and mutant version of cDELE1 with deleted residues at a helix similar to HRI degrons was generated to bear a new amino terminus. These mutants exhibited metabolic stability and protection against degradation. Accordingly, it was concluded that SIFI can recognize multiple motifs in cDELE1. This underscores the notion that SIFI engages helical degrons in cDELE1 and HRI that regulates SIFI-dependent ubiquitination and subsequent degradation to turn off the stress response.

How does the SIFI complex recognize unimported mitochondrial proteins? Helical HRI and cDELE1 degrons are very similar to mitochondrial presequences that are involved in protein import which led to the question of whether SIFI can recognize unimported mitochondrial proteins prone to forming aggregates. Intriguingly, this seems feasible because upon mitochondrial import failure, these motifs can build up in the cytoplasm, making them potentially recognizable by SIFI. To test this conjecture, leveraging biochemical degradation and ubiquitination experiments, it was demonstrated that mitochondrial presequences were recognized by SIFI and import machinery through similar mechanisms. This underscores the notion that SIFI can recognize unimported mitochondrial proteins and does so by a molecular mechanism that is similar to mitochondrial import machinery.

Degradation and localization of proteins containing presequences were also investigated to see if they were associated. Testing this question involved either blocking import into the mitochondria or introducing mitochondrial stressors. Both cases resulted in the destabilization of the protein containing a pre-sequence. When UBR4 was deleted while mitochondrial import was blocked, this resulted in an accumulation of mitochondrial precursors with presequences. This supported the conjecture that, during mitochondrial import stress, SIFI can detect unimported proteins that have accumulated.

Do mitochondrial presequences and degrons supplement each other functionally? Since mitochondrial presequences and degrons involved in stress response have similarities, another question investigated was whether these presequences and degrons could supplement each other. To test this, the degrons in HRI were deleted and a presequence recognizable to import machinery was inserted as a replacement. The presequence insertion resulted in the restoration of the HRI degradation, which had been disrupted by the initial degron deletions. However, mutant presequences could not be identified by import machinery and were not successful in restoring HRI degradation. Additional tests integrated helical HRI and cDELE1 degrons to green-fluorescent protein, which allowed visualization of the degrons and showed successful mitochondrial localization. These results demonstrated that cDELE1 and HRI degrons and mitochondrial presequences, referred to as converging degrons, are crucial in both the localization and stability of proteins.

Since converging degrons function in both localization and protein stability, then mitochondrial presequences could potentially compete to interact with SIFI before DELE1 and HRI. This would allow for a delay in stress response silencing and provide more time to address the issues causing import malfunction. To test if this occurs, presequence peptides were tested for their ability to stop ubiquitylation of HRI by SIFI. Results showed that in a dose-dependent manner, presequences could inhibit HRI ubiquitylation and increase the concentration of precursors in the cytoplasm. This suggested that mitochondrial precursors can compete for access by redirecting SIFI from DELE1

and HRI, thus allowing continuation of the stress response signaling.

It is crucial to understand if the harmful consequences of UBR4 deletion seen in conditions like ataxia and early-onset dementia are due to mitochondrial precursor aggregates or the persistent activation of stress response pathways. To test this, HRI and DELE1 were depleted in ΔUBR4 cells. The results demonstrated that ΔUBR4 cells with depleted HRI and DELE1 had reduced IRS, but mitochondrial import function was not impaired. Additionally, the HRI and DELE1 depletion restored ΔUBR4 cell proliferation. These findings suggested that persistent activation of stress response signaling is pivotal for the detrimental effects of UBR4 deletion rather than mitochondrial precursors prone to aggregation.

Further research was conducted on a small molecule that inactivates ISR called ISRIB. When tested with ΔUBR4 cells, ΔKCMF1 cells, UBR4-deficient stem cells, or neurons, ISRIB was successful in disrupting or halting ISR activation. To further understand the functional impact of ISRIB, ΔUBR4 cells were exposed to mitochondrial stressors or depleted of mitochondrial import factors. The import factors, TIMM8A and PMPCB used in these experiments are mutated in some neurodegenerative diseases making them important cellular components for study. Results of the experiment demonstrated that ISRIB was successful in rescuing the mutant UBR4 cells. These results, therefore, confirm the importance of studying ISRIB for neurodegenerative diseases caused by mitochondrial import stress because of its ability to inactivate the stress response (Haakonsen et al., 2024).

Concluding remarks and future directions: The results of this study underscore the remarkable role of stress response silencing and reveal that it is a highly orchestrated process controlled mainly by the E3 ligase SIFI (**Figure 1**). The molecular mechanism of stress response silencing involves identifying conserved sequences called converging degrons involved in both the degradation and localization of proteins. This work hypothesizes that the recognition by SIFI complex is due to exposure of these degrons from phosphorylation of HRI and cleavage of DELE1. Because of their similar degrons, mitochondrial precursors can compete with HRI (or cDELE1) for accessibility to SIFI complex allowing a delay in the silencing of a stress response until cellular conditions have improved. This suggests that degrons can silence a wide-range signal in response to a specific problem being fixed. Future research on elucidating the molecular mechanisms of stress response silencing to diverse mitochondrial stressors (including genetic and environmental) and other cellular stressors would be warranted (Eldeeb et al., 2024a; Narendra and Youle, 2024).

Although mutant UBR4 cells cannot properly degrade proteins prone to aggregation which allows them to accumulate in the cell, it was discovered that they could be saved if stress response signaling was silenced. This discovery suggests that compounds capable of inducing stress response silencing could be therapeutic for conditions that involve persistent or delayed inactivation of stress response signaling.

This work has important implications for uncovering potential novel therapeutic targets for neurodegenerative diseases caused by mutations in proteins that mediate the cellular stress response. These findings lay the foundation for investigating cellular components involved in the termination of cell signaling that is induced by stress. It is important to note that while the proposed treatment involving induced stress response signal silencing could help improve the survival of mutant cells, it does not address the problem of protein aggregates that accumulate in these cells. Further research addressing the link between stress response silencing and counteracting the accumulation of protein aggregates would be warranted. Overall, the exciting findings of this research show the potential for stress response silencing in tackling key aspects of protein aggregate diseases.

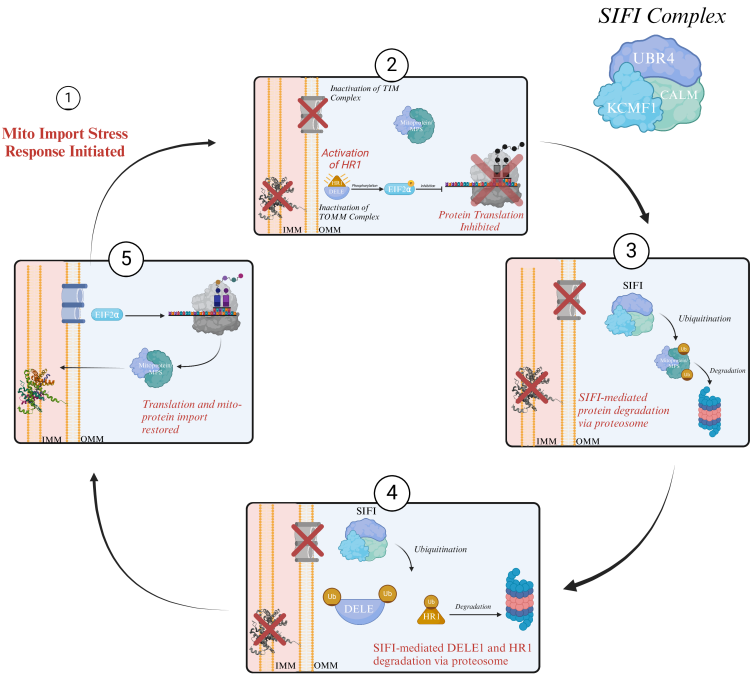

Figure 1 | **SIFI turns off the stress response signal through E3 ubiquitin ligase activity.**
Schematic depicting the reported roles of the SIFI complex (consisting of UBR4, KCMF1, and calmodulin [CALM]). (1) Mitochondrial import arrest initiates cell stress signals. (2) Halting mitochondrial protein import through the TOM/TIM complex, initiates the stress response where cDELE activates HRI to phosphorylate EIF2α ultimately resulting in inhibition of protein translation. (3) The SIFI complex mediated the clearance of unimported mitochondrial precursors from the cell through ubiquitin-proteasome degradation. (4) The SIFI complex engages the active HR1 and cDELE1 through the SIFI degron and mediates ubiquitin-dependent proteasomal degradation. This results in silencing the mitochondrial import stress response. (5) Silencing of the stress response restores EIF2α-mediated translation, resulting in mitochondrial protein expression and translocation through the TOMM/TIMM complex. Created with BioRender.com. DELE1: DAP3-binding cell death enhancer 1; HRI: eIF2α kinase heme-regulated inhibitor; Mito: mitochondria; TIMM: translocase of the inner mitochondrial membrane; TOMM: translocase of the outer mitochondrial membrane; Ub: ubiquitin; UBR4: ubiquitin protein ligase E3 component N-recognin 4.

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由线粒体转运功能异常引起的应激信号可以通过 SIFI 来终止：应激反应沉默的重要性
文章特色分析

一、文章重要性

1. 揭示新的细胞应激调控机制：

首次系统阐明 SIFI 复合体如何通过识别“converging degrons”（共轭降解信号）来终止由线粒体导入障碍引发的应激信号。

2. 为神经退行性疾病提供新视角：

指出持续应激信号（而非蛋白聚集本身）是导致细胞功能障碍的关键因素，这为治疗策略提供了新方向。

3. 提出潜在治疗靶点：

研究表明，通过药物（如 ISRIB）沉默应激反应可挽救因 UBRA 突变导致的细胞死亡，具有转化医学价值。

二、文章创新性特色

1. 提出“应激反应沉默”概念：

强调细胞不仅需要激活应激反应以应对损伤，更需要及时“关闭”该反应以避免持续损伤。

2. 发现 SIFI 复合体的多重功能：

- 降解未导入的线粒体前体蛋白；
- 降解应激信号通路中的关键激酶 HRI 和传感器 DELE1；
- 通过识别螺旋结构域（helical degrons）实现底物特异性识别。

3. 揭示“竞争性识别”机制：

线粒体前导序列与 HRI/DELE1 的降解信号结构相似，可竞争性结合 SIFI，从而延迟应激信号的关闭，为细胞修复争取时间。

4. 结合遗传学、生物化学与细胞生物学方法：

使用 CRISPR 筛选、蛋白质稳定性报告系统、泛素化实验等，多维度验证 SIFI 的功能。

三、对学科的启示

1. 重新审视神经退行性疾病的发病机制：

研究提示，应激信号持续激活可能比蛋白聚集本身更具破坏性，这挑战了传统以“清除蛋白聚集”为核心的治疗思路。

2. 推动“应激信号动力学”研究：

未来应更多关注应激信号的“开启-维持-关闭”全过程，而不仅仅是其激活机制。

3. 为药物开发提供新靶点：

SIFI 复合体及其调控通路（如 ISR）成为治疗线粒体相关神经退行性疾病的新靶标。

4. 拓展“降解信号”功能认知：

“Converging degrons”不仅控制蛋白稳定性，还参与蛋白定位，提示其在细胞信号转导中具有更广泛的功能。

总结

该文章通过揭示 SIFI 介导的应激反应沉默机制，不仅在基础细胞生物学层面提供了新的信号调控范式，也为理解神经退行性疾病的病理机制和开发新型治疗策略提供了重要理论依据和实验支持。其创新性在于将蛋白降解、线粒体质量控制与细胞应激信号的动态调控三者紧密结合，具有高度的学科交叉性和临床转化潜力。