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The Upper Hand of the Otu Amyloid Fibers: Increasing Enzymatic Activity and Prolonging Lifespan

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The formation of amyloid fibers is usually associated with aging and neurodegeneration. In this issue of *Molecular Cell*, Ji et al. (2019) demonstrate that the deubiquitinase Otu coalesces into amyloid-like fibers to enhance its activity and ensure its optimum biological function.

Many RNA binding proteins display low-complexity (LC) domains that are often intrinsically disordered (Franzmann and Alberti, 2018). LC domains are defined by poor amino-acid diversity, resulting in amino-acid repeats in so-called prion-like domains (Kato and McKnight, 2018), and they are often necessary and sufficient to promote liquid-liquid phase separation and droplet formation both *in vivo* and *in vitro* in an RNA-dependent fashion (Molliex et al., 2015; Murray et al., 2017; Patel et al., 2015). With time, some droplets can further polymerize into amyloid-like fibers that make them solid and irreversible (Molliex et al., 2015; Murray et al., 2017; Patel et al., 2015). Until now, the formation of amyloid-like fibers and aggregates is believed to be largely detrimental, often associated with aging or neurodegenerative diseases such as ALS and Alzheimer's disease.

Intriguingly, a number of RNA binding proteins have been shown to possess an enzymatic activity (Cano et al., 2010; Hentze et al., 2018). These proteins also harbor LC domains and are prone to self-assembly, raising the question as to whether these self-assembly properties are somehow linked to their enzymatic activity in addition to their binding to RNA. In this issue of *Molecular Cell*, Ji et al. (2019) show that this is the case for the *Drosophila* RNA binding protein Otu (ovarian tumor). Otu is a deubiquitinase whose activity has been shown to regulate germline stem cell differentiation (Ji et al., 2017). Otu also contains a LC domain in its C-terminal half composed of several proline-rich regions which appear important for Otu's RNA binding property, although its RNA binding domain has not been clearly mapped yet. Interestingly, the authors show that Otu protein self-assembles into amyloid-like fibers in a LC-dependent manner enhancing Otu enzymatic activity. They also show that both Otu self-assembly and enzymatic activity are critical *in vivo* to enhance *Drosophila* lifespan by preventing gut leaking. This shows that amyloid fiber formation can be beneficial at the organismal level.

The first part of the article by Ji et al. (2019) is dedicated to show that Otu protein phase separates into solid (not liquid) amyloid fibers, both *in vitro* from purified protein and *in vivo* upon overexpression in Hek293 cells. The formation of Otu fibers depends on RNAs and strictly depends on the presence of the LC domain. Importantly, the coalescence into amyloid fibers is directly correlated with Otu deubiquitinase activity that is dramatically increased. Without the LC domain, Otu deubiquitinase activity remains very low.

The role of Otu LC domain (and, by extension, the role of Otu amyloid-like fibers) is then tested *in vivo*. The shorter lifespan of *Drosophila* Otu mutants when compared to their control counterparts is used as a readout for Otu function. Indeed, this shorter lifespan is rescued to control level by expression of full-length Otu, but not by expression of Otu lacking its LC domain. Furthermore, the decreased longevity observed in Otu mutant is linked to gut leaking and the increased presence of antimicrobial peptides that result from an increased signaling through the immune deficiency (IMD) pathway. This pathway is indispensable for gut homeostasis, but when it signals excessively it leads to gut leaking. Using this biological readout, the authors confirm that the presence of Otu's LC domain is critical to rescue these defects. Taken together, these results suggest that Otu coalescence via its LC domain is linked to its full enzymatic activity, necessary for both gut homeostasis and normal lifespan.

Bam (Bag of Marble, a predicted RNA binding protein) is a known interactor of Otu. Bam/Otu complex formation positively controls the deubiquitinase activity of Otu that is a key factor in germline specification (Ji et al., 2017). The role of Bam is therefore investigated. Bam is first shown to also be a direct interactor of Otu in the gut. Accordingly, *bam* mutant exhibits the same decreased longevity, gut leaking phenotype, and activation of the immune response pathway as *otu* mutants. At the molecular level, Bam promotes Otu coalescence into amyloid-like fibers and enhances Otu deubiquitinase activity. Bam is itself recruited to the Otu containing granules, and as expected, Bam does not promote coalescence of Otu lacking its LC domain.

The last part of the article by Ji et al. (2019) investigates how Otu and Bam coalescence mediates their protecting effect in the gut, hence ensuring wild-type longevity. dTraf6 (*Drosophila* TNF-receptor-associated factor 6) is found to form a ternary complex with Otu and Bam. Interestingly, dTraf6 loss of function

reduces IMD signaling, and knockdown of dTraf6 in *bam* mutant suppresses the increased IMD signaling. This suggests that Otu/Bam somehow traps dTRaf6 and inhibits its function. However, unexpectedly for a deubiquitinase, Otu/Bam complex does not affect dTRaf6 stability but instead appears to restrict dTRAf6 activity.

Altogether, this manuscript reports that the deubiquitinase enzyme Otu and its binding partner Bam coalesce into amyloid-like fibers in an LC domain- and RNA-dependent manner. This coalescence activates Otu's enzymatic activity and provides the animal with a normal longevity through preventing inflammation in the gut through the release of anti-microbial peptides. This is an original and important finding that adds an exciting new dimension to amyloids in linking them to a positive role in regulating organ homeostasis and fly lifespan. The study also suggests a physiological functional role for amyloid-like coalescence.

However, several questions remain to be answered. The first is to ask how the coalescence of Otu enhances its enzymatic activity. Is Otu's active site revealed only when multiple Otu proteins come together? What is the oligomerization state necessary for Otu activity? Second, as Bam's sequence does not appear to contain any significant LC domain that would confer it with phase separation propensity, another opened question is how Bam increases the deubiquitinase activity of Otu while increasing its coalescence. This issue can be partly resolved by referring to the "driver/clients" model proposed by Banani (Banani et al., 2017), in which clients and interactors (here Bam) are recruited in self-assemblies driven by scaffolds (here Otu). We would have to argue that Bam binding to Otu slightly changes Otu conformation in such a way that it exposes more of its LC domain. Only structural analyses will shed light on this issue. Third, it also remains unclear why Otu binds RNA. Is it only to promote coalescence? Furthermore, what are the fates of these RNAs in Otu coalescence? RNAs are likely untranslated, as phase separation appears to be correlated with translation inhibition, at least *in vitro*. Finally, it will be interesting to investigate whether these findings can be expanded to other RNA binding proteins with enzymatic activities and whether LC domain-mediated coalescence regulates their enzymatic activity as well.

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