CELL-TYPE-SPECIFIC MODULATION OF PROTEIN HOMEOSTASIS IN HEALTH AND DISEASE

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Cells have highly conserved quality control machineries that detect, prevent, and resolve protein damage. The absence or malfunction of these machines can result in cellular dysfunction that is associated with the onset of protein misfolding diseases. When protein folding and clearance balance protein biosynthetic processes, protein homeostasis (proteostasis) is achieved, which ultimately prevents the accumulation of misfolded and aggregated proteins within cells. The health of the cell is therefore linked to the robustness of its proteostasis network.

Cell-type- and tissue-specific regulation of protein expression result in different functional and morphological characteristics, suggesting that proteostatic requirements may also vary between tissues. One possibility is that all cells have generic proteostatic machinery that can cover the folding requirements of a range of proteomes. Alternatively, the identity and concentration of chaperones and proteostatic machinery are highly cell-type specific and titrated to approximate the *in vivo* client protein needs to deal with highly specialized challenges in protein biogenesis. These two strategies are not mutually exclusive, as some components of the cellular proteostasis machinery may be similar in all cells, while others may be finely tuned to the cell's specific needs. Our challenge is to understand how the proteostasis network is established and maintained in healthy cells and to understand the course of events that lead to its collapse during aging and disease.

Using a set of well-characterized molecular tools with diverse metastable proteins and fluorescent reporters we assessed the protein homeostasis in a living organism. We first established assays to monitor the function and folding of these probes *in vivo* (Karady *et al.,* 2013). Different groups studying proteostasis now use these tools.

Employing these tools, we developed a strategy to delineate the chaperone network that function in body wall muscle of *C. elegans*. Specifically, we used ts *unc-45* mutations as a sensitized genetic background for candidate screening approaches, focusing on specific chaperone families such as HSP70, HSP90 and sHSP. Single amino acid substitutions in the UCS domain of *C. elegans* UNC-45 are responsible for temperature-dependent motility defects and myosin disorganization phenotypes, when animals are grown under restrictive conditions. In contrast, these *unc-45* mutants show no movement or myosin organization defects at the permissive temperature. Intriguingly, we identified the genes *CeHsp90 (daf-21)*, *CeHop (sti-1) CeAha1 (C01G10.8)* and *Cep23* (*ZC395.10*) that specifically caused a synthetic movement defect in combination with *unc-45(ts)* mutant animals but not wild-type worms. We went on to examine whether the *CeHop*-, *CeAha1*- and *Cep23*-associated synthetic phenotypes are caused by sarcomeric disorganization monitoring the subcellular arrangement of myosin heavy chain

A. While treatment of wild-type animals with *CeHop*, *CeAha1* and *Cep23* RNAi did not affect myofilament organization, depletion in *unc-45(e286)* mutant animals resulted in complete disruption of sarcomeric structures and MYO-3 mislocalization already at permissive conditions, comparable to *unc-45(ts)* single mutants grown at the restrictive temperature. Thus, our candidate approach identified novel cofactors supporting the regulatory function of the myosin assembly chaperone UNC-45, indicating that muscle function is governed by a fine-tuned proteostasis network (Frumkin et al., 2014). We therefore suggest that different co-chaperones form functional modules *in vivo* that are specialized for a set of substrates to specifically impact their folding. This view of functional modules within the proteostasis network is supported by the roles of co-chaperones in directing chaperone function and suggest that proteostatic machinery is at least in part cell-type specific and titrated to the cell needs.

To understand the underlining mechanism that leads to dysregulation of proteostasis in protein misfolding diseases, we carried out in depth characterization of proteoatsis capacity in different tissues and under physiological and stress conditions. We determine that proteostasis is remodeled at the transition to adulthood, resulting in a strong decline in folding capacity and change in expression of proteostasis machineries in different somatic tissues (Fig. 1). This change can be regulated by the reproductive system and specifically by germline stem cell arrest (Shemesh *et al.,* 2013; Feldman *et al.,* 2014; Shai *et al.,* 2014).

Our data, therefore, suggest that the transition to adulthood corresponds to a regulatory window during which time environmental conditions and germline competence are weighed to determine reproductive potential and the mode of proteostasis required. Our data therefore suggest that during development cellular proteostasis is regulated in a cell specific manner but that at the transition to adulthood cell-nonautonomous regulation of quality control systems results in a collapse of somatic proteostasis regardless of the tissue affected. Given that imbalance in proteostasis contributes prominently to age associated diseases, it is a priority to determine the regulatory signals at the transition to adulthood that result in proteostasic collapse to understand how the quality control systems in living multicellular organisms are remodeled.

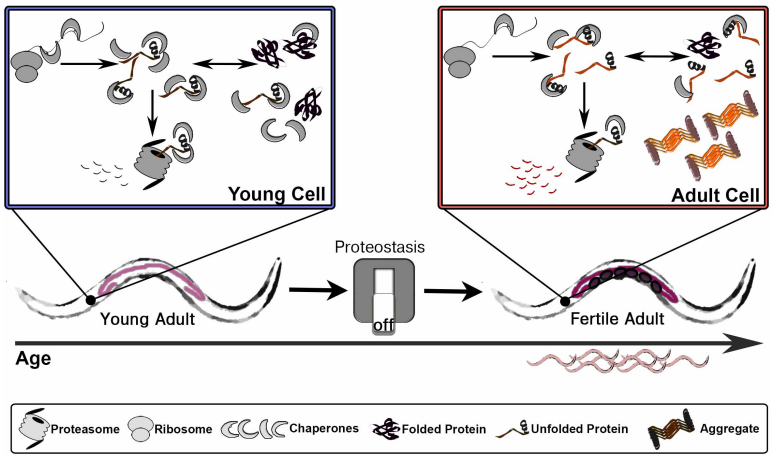

Fig 1. Proteostasis is remodeled upon transition to adulthood. Changes in expression and function of chaperones, the ubiquitin proteasome system and autophagy modulate cellular proteostasis capacity, resulting in accumulation of misfolded and aggregated proteins during adulthood. This change coincides with the onset of oocyte biomass production.