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Chaperones, protein homeostasis & protein aggregation diseases

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2014

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Minoia, M. (2014). *Chaperones, protein homeostasis & protein aggregation diseases*. s.n.

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CHAPTER 1

Aim of the thesis

Proteins fold in an unfavorable cellular environment due to molecular crowding and relatively high temperatures. When combined, these two conditions favor aggregation over folding. Molecular chaperones evolved to shift the equilibrium towards folding by preventing aggregation [1].

Imbalances in protein homeostasis are observed in a high number of disease states, including neurodegenerative disorders [2]. The predominant feature of these disorders is manifested by the formation of intracellular and/or extracellular deposits of aggregated proteins. Examples include the formation of intracellular inclusions containing aggregated α -synuclein in Parkinson's disease or huntingtin in Huntington's disease, as well as the extracellular β -amyloid plaques in Alzheimer's disease [3]. The risk of getting any of these diseases increases dramatically with age. As a consequence of aging, or as a result of mutations, the delicate balance of the synthesis, folding, and degradation of proteins is perturbed, resulting in the slow accumulation of misfolded proteins that form aggregates.

When unfolded or misfolded proteins accumulate in the cells and the protein-folding capacity is overwhelmed, specific stress responses are activated. To relieve stress and re-establish homeostasis, the cells activate specific signal transduction pathways, which induce the transcriptional upregulation of genes that enhance protein-folding capacity and quality control. Heat Shock Proteins, as a central components of the cellular network of molecular chaperones and folding catalysts, are the first components to be upregulated upon proteotoxic stress [4]. Members of the various groups of HSPs were initially classified according to their molecular weight: HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), and HSPB (small HSP) as well as for the human chaperonin families HSPD/E (HSP60/HSP10) and CCT (TRiC). Recently, HSPs have been grouped into different groups based on the sequence homology [5]. The protective functions of HSPs during stress have been investigated since a long time [6]. More and more evidence are accumulating that HSPs also have the ability to restore protein homeostasis in protein misfolding diseases [2,7]. In this thesis we explore how individual HSP and their cofactors function in protecting cells from stress and how the chaperone systems cooperate as a network and function in conjunction with the protein transport and degradation machineries to ensure misfolding protein disposal.

In the introductory chapter (Ch2), a summary of the existing literature will be provide on the role that HSPs play in preventing aggregation or/and toxicity of different disease-associated proteins and how each specific disease may be "barcoded" by a set of different disease modifying HSP activities. Furthermore, this chapter will provide an overview of the known chaperonopathies, which are diseases that are actually caused by mutations in HSP themselves. This part illustrates the importance of HSP-mediated quality control in normal cellular function and highlighting the

hypersensitivity of tissues with lower regenerative capacities to (age-related) decline in protein homeostasis.

In Chapter 3, we will provide novel insights in how cells utilize chaperones to regulate protein degradation under conditions in which proteasomal activity declines (like in aging). Several studies, including recent findings from our lab, had revealed that the small HSPB member HSPB8 collaborates with the HSPA1A-BAG3 complex in clearing protein aggregates containing e.g. expanded polyQ proteins [8,9]. At the same time data appeared in the literature that BAG3 is upregulated under conditions of proteasomal impairment, and that under such conditions cells switch to autophagy to ensure proper clearance of clients (the proteasome-to-autophagy switch). How proteasomal clients are re-routed to the autophagosomal pathway has remained unclear. In chapter 3, we describe that BAG3 plays a role in this switch. BAG3 induces the sequestration of HSPA1A-bound proteasomal clients into cytoplasmic puncta which indeed leads to their re-routing to autophagosomes for degradation. This switch occurs through competitive inhibition with its family member BAG1, which normally directs HSPA1A-bound clients to the proteasome.

Interestingly, mutations in BAG3 have been implicated in multiple muscular and cardiac diseases. In particular, a mutation in proline 209 (P209L) leads to a severe and extremely early onset myofibrillar myopathy with associated cardiopathy [10]. Based on the mechanistic insights obtained in Chapter 3, we characterized how the BAG3-P209L mutation affects BAG3 function. Our data suggest that this mutant has dominant negative effects on BAG3 wild-type function. Even though BAG3 P209L can bind to its partners and still can induce sequestration of HSPA1A-bound proteasomal clients into cytoplasmic puncta, the routing of cargo into autophagosomes seems impaired (Chapter 4).

In chapter 5, we next revisited earlier findings obtained in our lab that revealed certain members of the sHsp/HSPB family (HSPB6, HSPB8, HSPB9 and especially HSPB7) could protect against aggregation and toxicity associated with expression of polyQ proteins that cause neurodegenerative diseases like Huntington's diseases (HD) of several types of Spinocerebellar Ataxias (SCA 1, SCA 2, SCA3, SCA6 and SCA7)[11–14]. To test whether these some HSPB members might also be protective in another aggregation-disease, we chose as a substrate the RING domain mutant (C289G) in the E3 ligase PARK2/PARKIN, which is associated with autosomal recessive juvenile onset Parkinson's disease (PD). The PARK2 C289G mutation results in its aggregation. These aggregates are distinct in terms of their appearance, intracellular localization and biochemical characteristics from those formed by polyQ proteins. Interestingly, except for HSPB7 that is found to be effective in preventing both polyQ- and mutant PARK2- related aggregation, we now discovered other members (HSPB1, HSPB2, HSPB4) as the most potent suppressors of PARK2

C289G aggregation than previously identified as suppressors of polyQ aggregation. These data suggest that HSPB members may be less promiscuous in client recognition as previously anticipated. Moreover, we found that the mechanism of action of the four HSPB members in preventing mutant PARK2-associated aggregation was not dependent on the activity of cellular Hsp70s, which challenges the current view in which the ATP-independent HSPBs should always depend on ATP-regulated HSP for chaperone activities (Chapter 5).

Finally, we went back to polyQ diseases, aiming at developing an improved model to investigate the possible role of chaperones and protein quality control in disease attenuation. Current disease models for HD or SCA3 have mostly used non-physiological overexpression of mutant huntingtin or ataxin-3 fragments with enlarged CAG repeats. Using these models a number of important paradigms for the pathophysiological mechanisms of the disease have been provided [15–17]. For example, it has been shown that the disease is caused by a gain of toxicity mechanism, related to aggregation of the polyQ containing protein [17–20]. Moreover, these studies have identified a number of potential therapeutic targets. Since we and other have shown that boosting various components of the protein quality control systems can modify the disease progression in cellular and animal model systems [13,21,22]. Consistently, in both HD and SCA patients the CAG repeat length is inversely correlated with the age of onset (AO) of the diseases consistent with findings that the CAG repeat length is directly correlated with the aggregation proneness of respective polyQ proteins [23]. However, in each of these diseases large variations in AO between individuals that contain the same CAG repeat have been observed and, for example SCA3 up to 50% of the variation in AO between individuals must be due to other (genetic) factors than the CAG repeat length itself. To more conclusively test whether aggregation-modifying HSP derived from cell model studies may be determinants of the CAG-repeat length independent variation in AO, we urgently need a patient-representative neuronal models. The technology of induced pluripotent stem cell (iPSC) generation could provide such a solution as now it is possible to generate neurons from patient-derived cells. In chapter 6, we executed an exploratory/feasibility study by generating iPSC lines from 3 different SCA3 patients with comparable CAG repeat length but with largely different AO. We show that neurons can be developed from iPSCs from all patients and preliminarily characterized the neurons in terms of aggregation sensitivity and HSP expression (Chapter 6).

In the last chapter (Ch 7), I integrate the findings of this thesis and the literature to further illustrate the important role of the chaperones in protein quality control, especially when the cellular protein homeostasis is perturbed. Furthermore I explain how boosting specific HSP activities may be explored in the future to as means to alleviate protein aggregation diseases and reduce age-related decline in (neuronal) cell fragility.

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