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DNAJ proteins: more than just “co-chaperones”

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Chapter 8

General Discussion and Future Perspective

In order to be biologically active, every nascent polypeptide that emerges from the ribosomes should fold and acquire its native conformation. Non-native conformations lead to drastic events in the cell ranging from compromised biological activity of the protein (hence loss or gain of function) to its tendency to (self) aggregate or to inappropriately interact with cellular components. Maintenance of protein homeostasis is thus crucial to the cellular functioning. Heat shock proteins (HSP) act as molecular chaperones and form the first line of defense in chaperoning protein folding. In addition to their role in normal protein homeostasis, the transcriptional regulation of various HSPs (heat-stress induced ones) is increased upon proteotoxic stress through transcriptional factor heat shock factor-1 (HSF-1).

In parallel to the HSF-1 regulated heat shock response (HSR) in the cytosol, interconnected pathways in different cellular compartments also respond to acute proteotoxic stress, including the unfolded protein response (UPR) in the endoplasmic reticulum and the mitochondria. Each pathway not only induces the transcriptional up-regulation of genes that enhance (re)folding capacity, but also the expression of HSP members that assist in degradation of unfolded proteins through the proteasome and lysosome-mediated pathways, together protecting cells from stress. It is known that with aging, there is a decline in the functionality of chaperone networks and on the other hand, accumulation of damaged proteins occurs. Together, this has a cumulative effect on cellular protein homeostasis and leads to protein aggregation, corroborated by the many late-onset neurodegenerative diseases that are associated with aggregation of proteins, all implying that aged cells are more susceptible to proteotoxic stress.

The human genome encodes more than 100 different HSPs that are grouped into 7 different families plus several regulatory co-factors (1). In **Chapter 2**, we highlighted how different sets of HSPs are required under acute versus chronic stress. During *de novo* protein folding and for the refolding of acute stress-denatured-unfolded proteins, the functional cooperation of different HSPs is primarily aimed assisted (re)folding of folding competent proteins or (if not possible) their degradation by the proteasome. In chronic stress, the clients are mostly not or no longer folding competent, meaning that under such conditions, a HSP network that rather functions in specific targeting of the misfolded or even aggregated proteins for degradation is required. We show that different aggregation diseases have different HSP “barcodes” implying that protective effects of different HSPs clearly depends on the type of the misfolded protein that causes disease. Indeed, more and more results in the field indicate that at least two distinct networks of chaperones exist where one category can act as “foldases” and hence assist in refolding of heat-denatured substrates while the other act as “aggregation inhibitors” and suppress aggregate formation under disease conditions.

The existence of different ‘barcodes’ for the rescue of specific aggregation diseases suggests

that boosting HSF-1 activity is usually insufficient for long-term protection in most dominantly inherited proteinopathies as it likely may only compensate for certain consequences of the disease rather than that it directly acts on the aggregation-initiating protein.

Chaperoning polyglutamine (polyQ) diseases

Polyglutamine (polyQ) diseases arise when the polyglutamine tract in the disease-associated protein is expanded beyond a certain threshold. A proposed model for polyglutamine diseases based on the existing literature and the work carried out in this thesis is provided in Fig. 1. Mainly it can be divided into two parts:

- PolyQ peptide as a toxic species:* The expanded polyQ stretch containing corresponding disease-related proteins have a polyQ expansion length dependent propensity to aggregate (2). Several lines of evidence, however, show that the full length polyQ proteins themselves are not very aggregation prone, but that protease activation (e.g. by exocytotoxic events) followed by cleavage of the polyQ proteins into smaller fragments is required to initiate the aggregation process (3–8); Fig. 1 step I & II). In line, preventing fragmentation of polyQ huntingtin in a mouse model by introducing a caspase-6 resistant site adjacent to the polyQ expansion greatly delayed disease onset (4). Such cleavage leads to fragments containing the expansion that either directly or after further processing via the proteasome acts as aggregation seeds, which next also recruit full length proteins and other CAG repeat containing proteins such as transcription factors into the aggregates (6,9–11). Not only this; they also seed the aggregation of other proteins including the ones containing non-pathogenic polyQ stretches (12,13); **Chapter 4**, including transcription factors (TF) thus leading to a dysregulated transcription, as indeed seen in several CAG repeats disease (14–16), and hereby may contribute to disease pathology (Fig. 1, step VI). PolyQ aggregates can also recruit many other chaperones and proteasome components (6,17), but this predominantly seems to occur at later stages (18), suggesting that chaperone and/or proteasome depletion may not be a cause for disease initiation but rather be a late consequence of the disease and hence might play a role in disease progression.
- Aggregate intermediates as toxic species:* Aggregates formation is likely a multi-step process during which different intermediates may have different toxic potential. Although the discussion about what is more toxic (intermediate species or aggregates) is still ongoing, it is likely that different aggregate intermediates have different compromising effects on the normal cell functioning. In fact, it has been described that inclusion formation into amyloid fibers (step 2b) may be less toxic than earlier amorphous intermediates. These intermediates are more reactive and could e.g. impair the integrity

of cellular membranes, by altering ionic homeostasis, membrane potentials and energy stores and hence led to cell death (19). This can serve as a second component by which polyQ aggregates exert neurotoxicity (Fig. 1, step IV). In fact, disturbed mitochondrial Ca^{2+} signaling is reported in Huntington's disease (20,21).

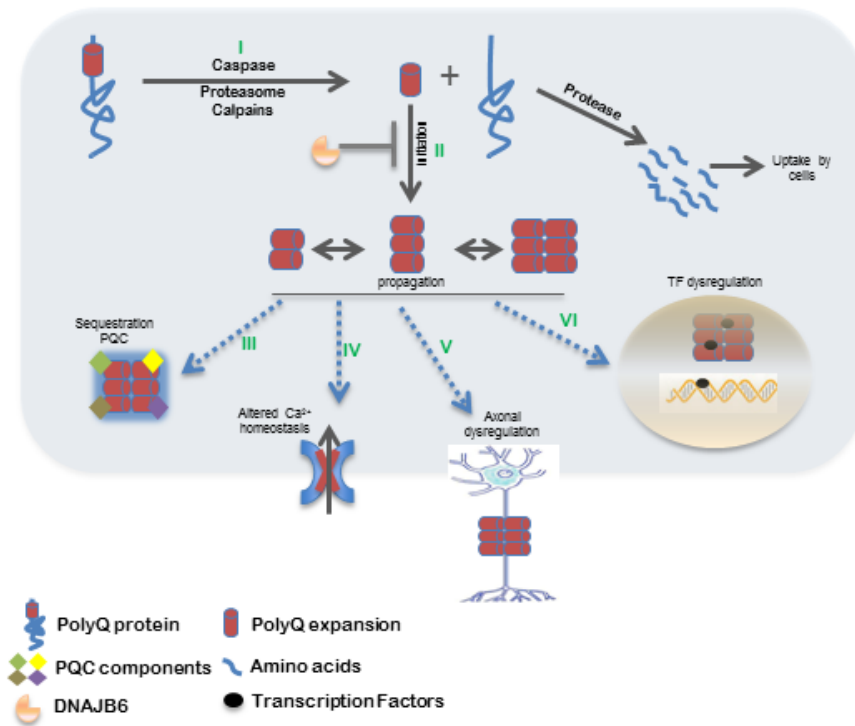


Figure 1: Model for polyglutamine aggregation leading to neurodegeneration: Cleavage of polyQ proteins via proteasome/proteases leads to release of aggregation prone polyQ domain and soluble chain (**Step I**). The soluble chain is further digested by proteases and amino acids are taken up by the cells. Aggregation of polyQ domain results in intermediate filaments and eventually amyloid fibrils (**Step II**). These have various dramatic effects on normal cellular functioning ranging from sequestration of other protein including PQC components like ubiquitin, HSPs etc (**Step III**), altered membrane potential which eventually disturbs Ca^{2+} homeostasis (**Step IV**), axonal transport dysregulation (**Step V**) and also entrapment of transcriptional factors leading to altered transcription of various other genes (**Step VI**). DNAJB6 act as a peptide chaperone and prevent "initiation" of aggregation (**Step II**) cascade by preventing further nucleation of other proteins.

Whereas formation of "inert" amyloid fibers, which could be assisted by chaperones, indeed may be cytoprotective in tissue culture cell systems (22), one must realize that loss of neuronal cells is actually a rather late event in the disease, long after neurodegenerative symptoms (neuronal dysfunction) are already seen (23). Moreover, such inclusions, albeit delaying toxicity, may directly impede on protein quality control as they may trap chaperones (18,24,25) (Fig. 1, step III) and as such interfere with the functions of these HSP in in

regular folding pathways. This can serve as a 3rd possible component contributing to the progression of neuronal dysfunction. Finally, a 4th pathway by which aggregates (both early intermediates and amyloid inclusion) may lead to neuronal dysfunction is by impairing axonal transport and thus synaptic functions (26–28)(Fig. 1, step V). This actually has actually been suggested to be a very early event in the disease (29,30) and at least for huntingtin this can be understood considering that the wild-type protein is actually thought to be involved in microtubular transport (31).

Whereas stimulating the HSR or overexpression of single components thereof, such as Hsp70, may interfere with some of the consequences after aggregate initiation like the conversion of early intermediates to fibrils (Fig. 1, propagation) or the rescue of TF from inclusions or could compensate for the loss of trapped chaperones (Fig. 1, step III, VI), our data in **Chapter 3** show that they are actually not very efficient in preventing aggregate initiation (step 2). This would be in agreement with the findings that Hsp70 overexpression delays disease phenotypes in flies, but without actually reducing protein aggregation (32). Yet, in mice amelioration of these consequences alone seems to be inefficient as Hsp70 transgenic mice showed no delay in disease onset and progression (33,34) and GA-treatment to activate the HSR only minimally delayed disease (35).

Thus, this polyQ peptide hypothesis may not only explain the success and failure of certain HSP to ameliorate aggregation and disease onset/progression, but also can explain why features of all the CAG repeat disease are so similar (including the effects of chaperones) irrespective of the fact that the polyQ expansion are found in such highly divergent genes encoding for proteins with entirely different functional and structural properties.

All this prompted us to screen for the potential of other members within the HSP families to reduce aggregate formation at an early stage. **Chapter 3** led to the identification of two members of the DNAJ superfamily, DNAJB6 and DNAJB8, that were extremely potent suppressors of aggregation initiated by polyQ containing proteins in cells and *in vivo* in a novel *Xenopus* model for HD. DNAJB6 further was found to be capable of preventing aggregation on Q-peptides *in vitro* (**Chapter 3**) and in cells (36), demonstrating it indeed acted very early on at the step of aggregate initiation thus preventing further nucleation. We next confirmed this in neuronal cells in which DNAJB6 interacted already with “soluble” polyQ fragments and protected against effects of polyQ proteins on neurite formation even before visible aggregates could be detected (**Chapter 5**). Most compelling, we demonstrate that the mere overexpression of DNAJB6 in neurons, delayed disease onset in the rapidly progressive and aggressive R6/2 model of HD (**Chapter 5**), a model in which ubiquitous expression of Hsp70 (33,34) overall activation of HSF-1 by GA (35) had not been effective. The

fact that the exclusive neuronal specific expression of DNAJB6 was protective furthermore strongly suggests that these diseases are initiated by neuronal cell degeneration and not by defects in astrocytes or glia cells as has recently been speculated (37,38) although the latter may have a modulating role in disease progression by preventing the possible cell-to-cell transmission of aggregates in these diseases.

DNAJ proteins with unique SSF-SST region effective in polyQ anti-aggregation

A unique feature of only three DNAJBs (JB6, JB7, B8) proteins is the presence of a SSF-SST box in the C-terminus of the protein, which for DNAJB8, was demonstrated to be required for interaction with HDACs and for the formation of large oligomers (**Chapter 3**). Of these three, DNAJB7 did not prevent polyQ aggregation (Chapter 3). This might be because DNAJB7 lacks a conserved lysine at position 220 adjacent to the SSF-SST box, which was shown to be important for complete functioning of DNAJB6/8 chaperones in their anti-aggregation action on polyQ proteins (**Chapter 3**). The oligomeric structure of DNAJB6/8 and interactions with HDACs, in particular HDAC4, were found to be important for full DNAJB8 activity in cells, although HDACs are not needed for formation of oligomers as these spontaneously form *in vitro* (**Chapter 3**). Although we did not investigate whether DNAJB7 also forms large oligomers, we did not find any interaction of DNAJB7 with the three HDACs (preliminary: data not shown).

Together, these findings suggest that SSFT-SST box, responsible for both interaction with HDACs and HSPB oligomerization are unique features DNAJB6 and DNAJB8 required for their function as chaperone to prevent aggregation initiation by aggregation-prone unstructured peptides.

Interaction with Hsp70 to function as anti-aggregation chaperone: case of client specificity

Using *in vitro* approaches with purified proteins, it was further established that DNAJB6 alone, as a homo-oligomer, directly binds polyQ peptides and hereby greatly delays the “initiation step” of aggregate seeding, surprisingly without needing assistance of Hsp70 family members. In this way, DNAJB6 acts as a “holdase” for aggregation prone peptides to keep them in a state that is compatible for handling by the degradation machinery of the cell.

For this transfer, its interaction with HDAC and Hsp70s (client transfer to peptidase) may be required. The latter would also “recycle” DNAJB6 for preventing new seeding reactions. Within the time frame of our experiments, given the level of DNAJB6 overexpression and the efficient stoichiometry at which it can work (ten Q-peptides per DNAJB6 molecule), DNAJB6 recycling may not yet be required, which would explain the mere independence for its effects on Hsp70. Consistent with such an assumption, we found that the H/Q mutant of DNAJB6 that cannot interact with Hsp70 was as active as the wild-type DNAJB6 on expanded polyQ

protein (Q74-Htt), but slightly less active on the more aggregation-prone longer fragment (Q119-Htt).

Strikingly, this mere Hsp70 independence for suppression of polyQ aggregation was not found when Parkin C289G was tested as an aggregation-prone substrate. Here, the co-operation of DNAJ proteins (including that of DNAJB6 and DNAJB8) with Hsp70 was absolutely required for their anti-aggregation effects (**Chapter 7**). These data indeed confirm that DNAJB6 and DNAJB8 are 'normal' DNAJ proteins that do functionally cooperate with Hsp70. The data also reveal that, although several neurodegenerative diseases share protein aggregation as a common feature, the characteristics of the different aggregation-prone proteins impose a substantially different challenge on the cellular PQC system (**Chapter 2**). This idea is further supported by the results in this thesis where in fact several DNAJ members, which did not suppress aggregation of polyQ proteins, did protect against parkin C289G aggregation (**Chapter 7**).

For inhibition of parkin C289G aggregation, the SSF-SST deletion mutant was still effective. This implies that DNAJB6 and DNAJB8 can shift from more canonical, Hsp70 dependent dimers, effective in e.g. prevention of parkin C289G aggregation (**Chapter 7**), to non-canonical, largely Hsp70 independent oligomers that are effective as peptide chaperones that can prevent aggregation of polyQ (**Chapter 3**) and, as recently found, also of amyloid-beta (Månsson et al, *submitted*).

A question that remains to be solved concerns the clearance of the DNAJB6-bound aggregation prone peptides. Interestingly, our mass-spectrometry analysis revealed that in fact many peptides are bound to DNAJB6. So, does DNAJB6 act as "holdase" of peptides in a form competent for peptidases?

Intriguingly, DNAJB6 co-expression analysis associates DNAJB6 expression strongly with degradation processes (<http://thebiogrid.org/115360/summary/homo-sapiens/dnajb6.html>). Moreover, DNAJB6 has been shown to interact with keratin (39) and its role in keratin turnover is important to prevent toxic aggregation in chorionic trophoblast cells during chorioallantoic attachment in placental development (40). In fact, keratin network formation in trophoblast cells is a crucial event in placental development in mouse model (41) and DNAJB6 knockout mice showed defects in placental development (42), a process that requires large cellular remodelling and protein degradation. Although *in silico* studies show many other DNAJs are expressed during placental development (41), the lethality of DNAJB6 deficient mouse indicate that individual members of this gene family may have evolved with non-overlapping cellular and molecular functions.

Finally, the polydispersity of DNAJB6 is reminiscent of that found for many small HSP (sHsp) that also can form spontaneous oligomers with mostly dimers as building blocks (43). For sHsps, (de)oligomerization can be affected directly by e.g. temperature and pH, but it can also be affected by post-translational modifications, phosphorylation in particular (44,45). What regulates the DNAJB6 oligomeric dynamics is yet unknown and requires insight in its structure, which is not yet known. This will not only shed more light into this mechanism of DNAJB6 functioning, but also may help the development of strategies to drive DNAJB6 towards the formation of these large protective oligomers for therapy especially of patient with CAG repeat diseases.

DNAJB6 a target for therapeutic intervention in polyglutamine diseases

The brain specific overexpression of DNAJB6 (so far) is without any deleterious phenotypic effects and the ectopic expression of DNAJB6 ameliorates HD phenotype in R6/2 mouse model without changing the general chaperone network unlike HSF-1 upregulation, indicating DNAJB6 does not evoke a stress response when overexpressed in neurons. As DNAJB6 is constitutively expressed in neurons, our data thus demonstrate that modulation of its levels or activity may be a realistic option for disease intervention. The promotor of DNAJB6 is, however, complex and no pathways have yet been established that strongly regulate its activity. However, we have generated promoter reported constructs to carry out unbiased compounds screens to identify DNAJB6 expression enhancing compounds. At the same time, better understanding of the pathways that regulate DNAJB6 will be important, not only for more dedicated screens towards activating certain receptors or transcription factors but also to understand and predict possible side effects of potential activators of DNAJB6 expression.

Instead of boosting DNAJB6 expression, one could also try to directly target DNAJB6 by modulating its functionality. This may e.g. be done through targetting HDAC activity as we showed these do affect DNAJB6 functionality. However, given the many targets that HDACs have, specificity may become a challenge here. A better approach might be to try to find compounds that directly modulate DNA oligomerization.

Disease initiation versus disease progression

Although DNAJB6 highly efficiently prevented “initiation” of aggregation for poly-Q peptides generated inside cells, apparently sufficient to delay disease onset in mice, we also found that it was completely unable to prevent aggregation initiated by polyQ oligomers added extracellularly (**Chapter 4**). Whereas these data are consistent with the proposed mode of action of DNAJB6 that it works on aggregate seeding and not elongation, the data would suggest that DNAJB6 may not be effective in the potential spreading of intracellular

aggregates in a prion-like manner as has been suggested for neurodegenerative diseases. Although our *in vitro* data confirm the earlier work by Ren et al., (12) that extracellular aggregate can enter cells and seed aggregation here, the role of prion like spreading in CAG repeat diseases is yet unclear. If true, however, this would imply that DNAJB6 (or similar poly Q aggregation inhibitors) would have to be activated as early as possible (in presymptomatic carriers) and that their activation in symptomatic patients may have limited effectivity. Such considerations should be tested in mouse models, once DNAJB6 activators have been discovered. Also, it should be considered if/how astrocytes and glia cells may play a role in preventing transmission of aggregates from neuron-to-neurons, for possible combination therapies with e.g. DNAJB6 activators.

CONCLUDING REMARKS

It is clear that different aggregation-prone proteins require different PQC handling. While some of them (e.g. polymorphism related problems) will require enhanced assistance for folding, others (e.g. disease-inducing mutants) may never reach the proper folded state and have to be properly disposed by the cell's protein degradation systems. The existence of different 'barcodes' for the rescue of specific aggregation diseases suggests that, although loss of protein homeostasis with aging might contribute to disease initiation, boosting HSF-1 activity alone may be insufficient for long-term protection in many dominantly inherited proteinopathies. While the promiscuous Hsp70 has been shown to be a rate limiting factor for protein refolding under acute stress, chaperones with some specificity and tighter holding capacities like the DNAJs and HSPB family members may be more effective in chronic diseases and could to steer cells how to better deal with specific disease causing substrates.

Further, a potential worry in all HSP overexpression or boosting studies is that it leads to network adaptations which would annihilate long-term effectiveness or that could lead to multiple side effects, including increasing carcinogenesis, as was demonstrated for the manipulation of HSF-1 activity (46). Although network adaptations are to be expected upon manipulation of the driving forces of chaperone machinery (e.g. Hsp90/HSPC or Hsp70/HSPA), such effects might be less likely for those components that only steer the specificity of these machines (e.g. HSPBs or DNAJs). Also, transgenic DNAJB6 mice used in **Chapter 5** did not show any deleterious effects with aging until the time followed (18 months of age). So, depending on the relative contribution of different folding and aggregation problems, different chaperones (HSP70, DNAJs, NEFs, other Hsp families) may be required to combat premature proteostasis collapse and neurodegeneration.

"In the end there is always that one last question left to answer"

REFERENCES

1. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, et al. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones*. 2009 Jan;14(1):105–11.
2. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. *Annu Rev Neurosci*. 2000;23:217–47.
3. Ellerby LM, Hackam AS, Propp SS, Ellerby HM, Rabizadeh S, Cashman NR, et al. Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. *J Neurochem*. 1999 Jan;72(1):185–95.
4. Graham RK, Deng Y, Slow EJ, Haigh B, Bissada N, Lu G, et al. Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. *Cell*. 2006 Jun 16;125(6):1179–91.
5. Haacke A, Broadley SA, Boteva R, Tzvetkov N, Hartl FU, Breuer P. Proteolytic cleavage of polyglutamine-expanded ataxin-3 is critical for aggregation and sequestration of non-expanded ataxin-3. *Hum Mol Genet*. 2006 Feb 15;15(4):555–68.
6. Raspe M, Gillis J, Krol H, Krom S, Bosch K, van Veen H, et al. Mimicking proteasomal release of polyglutamine peptides initiates aggregation and toxicity. *J Cell Sci*. 2009 Sep 15;122(Pt 18):3262–71.
7. Koch P, Breuer P, Peitz M, Jungverdorben J, Kesavan J, Poppe D, et al. Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. *Nature*. 2011 Dec 22;480(7378):543–6.
8. Hübener J, Weber JJ, Richter C, Honold L, Weiss A, Murad F, et al. Calpain-mediated ataxin-3 cleavage in the molecular pathogenesis of spinocerebellar ataxia type 3 (SCA3). *Hum Mol Genet*. 2013 Feb 1;22(3):508–18.
9. Suhr ST, Senut MC, Whitelegge JP, Faull KF, Cuizon DB, Gage FH. Identities of sequestered proteins in aggregates from cells with induced polyglutamine expression. *J Cell Biol*. 2001 Apr 16;153(2):283–94.
10. Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, et al. Accumulation of mutant huntingtin fragments in aggregate-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell*. 2001 May;12(5):1393–407.
11. Venkatraman P, Wetzel R, Tanaka M, Nukina N, Goldberg AL. Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol Cell*. 2004 Apr 9;14(1):95–104.
12. Ren P-H, Lauckner JE, Kachirskaja I, Heuser JE, Melki R, Kopito RR. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat Cell Biol*. 2009 Feb;11(2):219–25.
13. Yang W, Dunlap JR, Andrews RB, Wetzel R. Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. *Hum Mol Genet*. 2002 Nov 1;11(23):2905–17.
14. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, et al. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc Natl Acad Sci U S A*. 2000 Jun 6;97(12):6763–8.
15. Valor LM, Guiretti D, Lopez-Atalaya JP, Barco A. Genomic landscape of transcriptional and epigenetic dysregulation in early onset polyglutamine disease. *J Neurosci Off J Soc Neurosci*. 2013 Jun 19;33(25):10471–82.
16. Yamanaka T, Nukina N. Transcription factor sequestration by polyglutamine proteins. *Methods Mol Biol Clifton NJ*. 2010;648:215–29.
17. Park S-H, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, et al. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell*. 2013 Jul 3;154(1):134–45.
18. Seidel K, Meister M, Dugbartey GJ, Zijlstra MP, Vinet J, Brunt ERP, et al. Cellular protein quality control and the evolution of aggregates in spinocerebellar ataxia type 3 (SCA3). *Neuropathol Appl Neurobiol*. 2012 Oct;38(6):548–58.
19. Lashuel HA. Membrane permeabilization: a common mechanism in protein-misfolding diseases. *Sci Aging Knowl Environ SAGE KE*. 2005 Sep 21;2005(38):pe28.
20. Panov AV, Gutekunst C-A, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci*. 2002 Aug;5(8):731–6.
21. Tang T-S, Slow E, Lupu V, Stavrovskaya IG, Sugimori M, Llinás R, et al. Disturbed Ca²⁺

- signaling and apoptosis of medium spiny neurons in Huntington's disease. *Proc Natl Acad Sci U S A*. 2005 Feb 15;102(7):2602–7.
22. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature*. 2004 Oct 14;431(7010):805–10.
 23. Yamamoto A, Lucas JJ, Hen R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell*. 2000 Mar 31;101(1):57–66.
 24. Cummings CJ, Mancini MA, Antalfy B, DeFranco DB, Orr HT, Zoghbi HY. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet*. 1998 Jun;19(2):148–54.
 25. Seidel K, Siswanto S, Brunt ERP, den Dunnen W, Korf H-W, Rüb U. Brain pathology of spinocerebellar ataxias. *Acta Neuropathol (Berl)*. 2012 Jul;124(1):1–21.
 26. Li H, Li SH, Yu ZX, Shelbourne P, Li XJ. Huntingtin aggregate-associated axonal degeneration is an early pathological event in Huntington's disease mice. *J Neurosci Off J Soc Neurosci*. 2001 Nov 1;21(21):8473–81.
 27. Chang DTW, Rintoul GL, Pandipati S, Reynolds IJ. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. *Neurobiol Dis*. 2006 May;22(2):388–400.
 28. Szebenyi G, Morfini GA, Babcock A, Gould M, Selkoe K, Stenoi DL, et al. Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron*. 2003 Sep 25;40(1):41–52.
 29. Lee W-CM, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a *Drosophila* model of Huntington's disease. *Proc Natl Acad Sci U S A*. 2004 Mar 2;101(9):3224–9.
 30. Gunawardena S, Her L-S, Bruschi RG, Laymon RA, Niesman IR, Gordesky-Gold B, et al. Disruption of Axonal Transport by Loss of Huntingtin or Expression of Pathogenic PolyQ Proteins in *Drosophila*. *Neuron*. 2003 Sep;40(1):25–40.
 31. Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell*. 2004 Jul 9;118(1):127–38.
 32. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet*. 1999 Dec;23(4):425–8.
 33. Hansson O, Nylandsted J, Castilho RF, Leist M, Jäättelä M, Brundin P. Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. *Brain Res*. 2003 Apr 25;970(1-2):47–57.
 34. Hay DG, Sathasivam K, Tobaben S, Stahl B, Marber M, Mestrlil R, et al. Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum Mol Genet*. 2004 Jul 1;13(13):1389–405.
 35. Labbadia J, Cunliffe H, Weiss A, Katsyuba E, Sathasivam K, Seredenina T, et al. Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *J Clin Invest*. 2011 Aug;121(8):3306–19.
 36. Gillis J, Schipper-Krom S, Juenemann K, Gruber A, Coolen S, van den Nieuwendijk R, et al. The DNAJB6 and DNAJB8 protein chaperones prevent intracellular aggregation of polyglutamine peptides. *J Biol Chem*. 2013 Jun 14;288(24):17225–37.
 37. Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Hausteiner MD, et al. Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nat Neurosci*. 2014 May;17(5):694–703.
 38. Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, et al. In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum Mol Genet*. 2010 Aug 1;19(15):3053–67.
 39. Izawa I, Nishizawa M, Ohtakara K, Ohtsuka K, Inada H, Inagaki M. Identification of Mrj, a DnaJ/Hsp40 family protein, as a keratin 8/18 filament regulatory protein. *J Biol Chem*. 2000 Nov 3;275(44):34521–7.
 40. Watson ED, Geary-Joo C, Hughes M, Cross JC. The Mrj co-chaperone mediates keratin turnover and prevents the formation of toxic inclusion bodies in trophoblast cells of the placenta. *Dev Camb Engl*. 2007 May;134(9):1809–17.
 41. Watson ED. 2005 Trophoblast Research

Award Lecture: Defects in the keratin cytoskeleton disrupt normal murine placental development and trophoblast cell function. *Placenta*. 2007 Apr;28 Suppl A:S111–115.

42. Hunter PJ, Swanson BJ, Haendel MA, Lyons GE, Cross JC. Mrj encodes a DnaJ-related co-chaperone that is essential for murine placental development. *Dev Camb Engl*. 1999 Mar;126(6):1247–58.

43. Haslbeck M, Franzmann T, Weinfurter D, Buchner J. Some like it hot: the structure and function of small heat-shock proteins. *Nat Struct Mol Biol*. 2005 Oct;12(10):842–6.

44. Parcellier A, Schmitt E, Brunet M, Hammann A, Solary E, Garrido C. Small heat shock proteins HSP27 and alphaB-crystallin: cytoprotective and oncogenic functions. *Antioxid Redox Signal*. 2005 Apr;7(3-4):404–13.

45. Garrido C, Paul C, Seigneuric R, Kampinga HH. The small heat shock proteins family: the long forgotten chaperones. *Int J Biochem Cell Biol*. 2012 Oct;44(10):1588–92.

46. Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, et al. HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell*. 2012 Aug 3;150(3):549–62.

Appendix

SAMENVATTING

Om een biologisch functie te kunnen vervullen zijn moet iedere uit de ribosomen ontstane polypeptide 'vouwen' om een vorm te verkrijgen waarin deze het beste kan functioneren. Als echter onverhoopt een onbedoelde vorm ontstaat heeft dit tot gevolg dat er grote problemen in de cel ontstaan. Deze problemen variëren van aangetaste biologische activiteit van het eiwit (verlies van functie of dominante negatieve effecten) tot het verwerven van toxische functies via de neiging tot (zelf) aggregatie van het eiwit. Het behouden van de eiwithomeostase is derhalve cruciaal voor het functioneren van de cel. Hiertoe beschikt het menselijke genoom over meer dan 100 verschillende *Heat Shock Proteins* (HSP) die zijn gegroepeerd in 7 verschillende families en verschillende regulerende co-factoren. HSPs functioneren als moleculaire chaperones en vormen de eerste verdedigingslinie bij het begeleiden van het vouwen van eiwitten. Aanvullend op hun rol bij het behouden van de homeostase van eiwitten onder normale groeiomstandigheden, worden de niveaus van de verschillende HSPs verhoogd door activatie van de transcriptiefactor Heat Shock factor 1 (HSF-1) als er externe omstandigheden zijn (zoals temperatuursverhoging) die eiwitten doen ontvouwen om zodoende de eiwithomeostase te herstellen.

Het is gebleken dat tijdens veroudering de werkzaamheid van chaperone netwerken afneemt. Daarnaast neemt het aantal beschadigde eiwitten tijdens de veroudering toe. Gezamenlijk zorgt dit voor een gestage afname in de eiwithomeostase en leidt dit tot de aggregatie van eiwitten, wat bijvoorbeeld te zien is in vele neurodegeneratieve ziekten die op latere leeftijd ontstaan en die worden gekenmerkt door de aanwezigheid van eiwitaggregaten in de neuronen.

In **hoofdstuk 2** hebben wij een algemeen overzicht gegeven van het functioneren van verschillende HSPs in de cel tijdens enerzijds algemene acute proteotoxische stress en anderzijds chronische stress die wordt veroorzaakt door verkeerd gevouwen eiwitten waarvan bekend is dat ze leiden tot verschillende (erfelijke vormen van) neurodegeneratieve ziekten. Tijdens het *de novo* vouwen van eiwitten en tijdens het hervouwen van gedatureerde ongevouwen eiwitten tijdens acute stress, blijkt dat de functionele samenwerking van verschillende HSPs primair gericht is op het (opnieuw) vouwen van eiwitten die daartoe in staat zijn en (indien dit niet mogelijk is) op de afbraak van deze eiwitten door het proteasoom. Tijdens chronische stress zijn de eiwitten echter meestal niet of niet langer in staat om te vouwen, waardoor onder deze specifieke omstandigheden een ander HSP netwerk noodzakelijk is dat voornamelijk gericht is op het afbreken van verkeerd gevouwen of geaggregeerde eiwitten. De betaamde gegevens uit de literatuur suggereren verder dat de verschillende neurodegeneratieve ziekten en de verschillende eiwitten waardoor ze worden veroorzaakt ieder verschillende HSP 'barcodes' hebben. Dit geeft aan dat deze

ziekten weliswaar allen door het gemeenschappelijke probleem van eiwitaggregatie worden gekenschetst, maar dat elke afzonderlijke ziekte een eigen, specifieke oorzaak kent en dat derhalve ook verschillende HSPs nodig zijn voor beteugelen van zowel het ontstaan als het verloop van elk van deze specifieke ziektes.

Vervolgens hebben wij polyglutamine (PolyQ, CAG) ziektes als een model gebruikt om te kunnen identificeren welke leden van verschillende HSP ziekten het meest effectief blijken in het onderdrukken van door PolyQ-gemedieerde aggregatie. PolyQ ziekten worden veroorzaakt door een verlenging van CAG herhalingen in genen, zoals huntington, verschillende vormen van ataxia's of de androgene receptor, wat respectievelijk resulteert in de ziekte van Huntington, diverse vormen van Spinocerebellaire Ataxia (SCA's) of spinale en bulbaire spier atrofie (SBMA). De gecodeerde eiwitten bevatten abnormaal lange polyglutamine ketens. Terwijl de volledige lengte polyQ eiwitten volledig goed zijn gevouwen en relatief ongevoelig zijn voor aggregatie, kunnen stress condities die zogenaamde proteases activeren ertoe leiden dat de polyQ eiwitten worden gefragmenteerd in kleinere stukjes. Die kleinere fragmenten met daarin de polyQ keten zijn moeilijk verder af te breken en aggregeren heel makkelijk waarbij dan (in een soort sneeuwbal effect) ook de intacte polyQ eiwitten mee samenklonteren. Ook andere eiwitten met normale polyQ keten (waaronder enkele belangrijke transcriptie factoren) en veel leden van de HSP familie kunnen hierin meeklonteren.

Het ontstaan van eiwitaggregatie is dus een meerstaps proces. Er is nog veel discussie over de vraag hoe en wat hierin toxisch is. Het is echter waarschijnlijk dat verschillende vormen van aggregaties verschillende negatieve effecten hebben op het normale functioneren van de cel, variërend van fysieke schade aan celmembranen, depletie van essentiële transcriptiefactoren, depletie van HSPs en dus van de kwaliteitscontrole van eiwitten, tot aan de fysieke blokkade van transport in axonen nodig voor het genereren van actiepotentialen.

Wij hebben gevonden dat twee leden van de DNAJ chaperone familie (DNAJB6 en DNAJB8) enorm sterke onderdrukkers van polyQ aggregatie zijn. (**Hoofdstuk 3**). De anti-aggregatie activiteit blijkt afhankelijk van zogenaamde *SSF-SST box* in the C-terminus van, die verantwoordelijk is voor de formatie van grote oligomeren. Door gebruik te maken van *in vitro* benaderingen met gezuiverde eiwitten, hebben we vastgesteld dat DNAJB6 zelfstandig als een homo-oligomeer aan polyQ peptiden bindt en daardoor de 'initiatie stap' in de aggregatie vertraagd, verrassend genoeg zonder de hulp van HSP70 familieleden, waarmee DNAJs normaliter samenwerken.

Dit gegeven motiveerde ons om een DNAJB6 transgene muis te genereren met extra hoeveelheden DNAJB6 in neuronen. Het kruisen van deze muis met een model voor de

ziekte van Huntington, de zogenaamde R6/2 muis, toonde aan dat DNAJB6 het ontstaan van huntingtine aggregatie sterk kon vertragen en de levensduur van de R6/2 muis met 23% kon verlengen (**Hoofdstuk 5**). Deze verlenging van de levensduur met behulp van een enkele HSP is de grootste ooit gemeten voor dit ziekte model en laat zien dat DNAJB6 een veelbelovend doelwit is in de bestrijding van polyQ ziekten.

De resultaten van recent onderzoek geven aanwijzingen dat ook neurogliale cellen en astrocyten aangetast kunnen zijn in polyQ ziekten en bijdragen aan of zelfs het initiëren van de ziekte bepalen. Omdat DNAJB6 alleen tot expressie komt in neuronen, betekenen de gevonden data beschreven in **Hoofdstuk 5** echter dat de aggregatie van eiwitten in neuronen (en dus niet gliale cellen/astrocyten) waarschijnlijk de initiatie en meest cruciale stap in het uitbreken van de ziekte is. Dit wordt verder ondersteund door de data in **Hoofdstuk 4**, waarin wij laten zien dat DNAJB6 niet om kan gaan met aggregatie die is geïnitieerd door polyQ peptiden van buiten de cel, terwijl deze volgens sommigen een rol zouden spelen bij de cel tot cel verspreiding van de ziekte, zoals dat het geval is bij prion ziekten (ziekte van Creutzfeld-Jacob of gekke-koeien ziekte). Gezien het feit dat DNAJB6 dus wel de ziekte kan vertragen in muizen, betekenen deze dat in hoofdstuk 4 dat “prion-propagatie” ofwel geen grote rol speelt in polyQ ziekten ofwel dat het geen rol speelt bij de ziekte initiatie, maar alleen in de mate van progressie van de ziekte, waarin dan vervolgens gliacellen en astrocyten mogelijk ook een rol zouden kunnen spelen.

Zoals onze analyse van de literatuur beschreven in **Hoofdstuk 2** al aangaf, zijn er voor verschillende voor aggregatie gevoelige eiwitten verschillende eiwit kwaliteitscontrolemechanismen nodig. Om dit verder experimenteel te toetsen hebben wij in **Hoofdstuk 7** de effecten van HSPs onderzocht op hun mogelijkheid tot het vertragen van de aggregatie van een gemuteerd Parkin eiwit (PARK C289G) dat verantwoordelijk is voor een erfelijke vorm van de ziekte van Parkinson. Dit onderzoek is op een soortgelijke wijze uitgevoerd als het onderzoek naar polyQ aggregatie in hoofdstuk 3. Er werden opvallende verschillen gevonden tussen twee substraat studies waaronder:

In tegenstelling tot PolyQ aggregatie kunnen alle geteste DNAJ familieleden (inclusief DNAJB6) op evengoed de aggregatie van PARK C289G verminderen;

In tegenstelling tot PolyQ aggregatie, hangt het succes van het onderdrukken van aggregatie van PARK C289G door DNAJB6 (en de andere geteste DNAJAs) wel af van functionele interacties met met Hsp 70.

De data van deze experimenten laten inderdaad zien dat niet alle aggregatie ziekten gelijk zijn en dat er verschillende manieren zijn waarop aggregatie kunnen worden geïnitieerd en dat voor remming van elk van deze, verschillende HSPs nodig zijn. Bovendien laat de

experimenten in hoofdstuk 7 zien dat DNAJB6 een 'normaal' DNAJ is dat (ook) normaal met Hsp70 kan saemnwerken net als alle DNAJs, ondanks het feit dat DNAJB6 ook HSP70-onafhankelijke acties heeft zoals het vertragen van polyQ aggregatie.

Samengevat kan worden geconcludeerd dat DNAJB6 een veelbelovende kandidaat is voor het vertragen van polyQ ziekten en tevens de potentie heeft (hoewel het mechanisch gezien om een andere route gaat) om Parkinsonisme te vertragen. Echter, een potentieel probleem voor langdurige stimulatie van de activiteit van HSP is dat deze benaderingen kunnen leiden tot een verandering, cq aanpassingen van het chaperone netwerk van de cel. Die kunnen vervolgens leiden tot het verlies van de effectiviteit op de lange termijn of tot het ontstaan van ongewenste neveneffecten. Dergelijke veranderingen van Chaperone netwerken zijn aangetoond na manipulatie van de "motoren" van de chaperone machinerie (bijvoorbeeld bij HSP90/HSPC) of na overstimulatie van bijvoorbeeld HSP70 of HSF-1; die laatste twee manipulaties worden zelfs geassocieerd met verhoogde kans op het ontstaan van kanker. Echter, zulke effecten zijn wellicht minder snel bij die onderdelen die de specificiteiten van deze 'machines' besturen, zoals bijvoorbeeld de DNAJ eiwitten. Overeenkomstig met deze veronderstelling laten de resultaten met transgene DNAJB6 muizen in **Hoofdstuk 5** ook geen grote aanpassingen van deze netwerken zien en lijken deze muizen vooralsnog (na 18 maanden) gezond en vertonen geen verhoging in het ontstaan van tumoren. Derhalve zou DNAJB6 een veelbelovend aangrijpingspunt voor therapeutische interventies kunnen zijn.

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LIST OF PUBLICATIONS

- **Kakkar V**, Meister-Broekema M, Minoia M, Carra S, Kampinga HH: Barcoding heat shock proteins to human diseases: looking beyond the heat shock response. *Dis Model Mech.* 2014 Apr;7(4):421–34.
- Vonk WI, **Kakkar V**, Bartuzi P, Jaarsma D, Berger R, Hofker MH, Klomp LW, Wijmenga C, Kampinga HH, van de Sluis B: The Copper Metabolism MURR1 domain protein 1 (COMMD1) modulates the aggregation of misfolded protein species in a client-specific manner. *PLoS One.* 2014 Apr 1;9(4):e92408.
- Månsson C, **Kakkar V**, Monsellier E, Sourigues Y, Härmark J, Kampinga HH, Melki R, Emanuelsson C: DNAJB6 is a peptide-binding chaperone which can suppress amyloid fibrillation of polyglutamine peptides at substoichiometric molar ratios. *Cell stress & chaperones*, 2013 [1355-8145].
- **Kakkar V**, Prins LC, Kampinga HH: DNAJ proteins and protein aggregation diseases (Review). *Current topics in medicinal chemistry*, 2013 [1568-0266].
- Hageman J, Rujano MA, van Waarde MA, **Kakkar V**, Dirks RP, Govorukhina N, Oosterveld-Hut HM, Lubsen NH, Kampinga HH: A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. *Mol. Cell (2010) Feb 12;37(3):355-69.*
- **Kakkar V**, Kuiper EFE, Kampinga HH: Role of DNAJ chaperones in anti-aggregation activity on mutant Parkin (*under review*).
- **Kakkar V**, Bergink S, van der Zwaag M, Kloosterhuis NJ, Bates GP, van Deursen J, van de Sluis B, Kampinga HH: Ectopic expression of DNAJB6 ameliorates Huntington's disease phenotype in R6/2 mouse model (*submitted*).
- **Kakkar V**, van Waarde M, Melki R, Kampinga HH: DNAJB6 fails to prevent the nucleation of intracellular polyQ proteins mediated by extracellular purified polyQ-peptide fragments (*manuscript in preparation*).

EPILOGUE

The stability of the cellular proteome is absolutely crucial for the health and lifespan of an organism. Disruption in protein homeostasis with age accelerates the accumulation of misfolded and aggregated protein junk within the cell. Protein aggregation is tightly associated with neuronal degeneration and diseased conditions. Given the multidimensional functioning and the capacity of molecular chaperones along with protein degradation machinery suggests protein quality control to be a necessary and efficient process to maintain proteome integrity. Nevertheless the late onset of various neurodegenerative diseases is indicative of the fact that these protein quality control mechanisms become overwhelmed with age and hence contribute to the disease conditions. Also, members of different chaperone families have been evolved to differently regulate substrate recognition or clearance. Understanding the functioning of molecular chaperones with regard to different substrates and hence different target areas can enable the understanding of the disease itself. This can serve as the most promising therapeutic intervention in many neurodegenerative diseases.

