# Regulation of protein homeostasis in acute and chronic stress

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*DOI:*
10.33612/diss.96277662

# Appendices

Samenvatting in het Nederlands
Summary in English
Acknowledgements
Curriculum Vitae
List of Publications

## Samenvatting in het Nederlands

Het handhaven van een gezond proteoom (d.w.z. alle eiwitten in de cel) is cruciaal voor de cellulaire functie en het overleven van de cel. De eiwitbalans, of eiwithomeostase, in cellen staat voortdurend onder spanning door een verscheidenheid aan intrinsieke en extrinsieke stressfactoren. De systemen die de eiwitkwaliteit reguleren moeten zich daarom continu kunnen aanpassen. Als reactie op acute vormen van stress, zoals veranderingen in de omgeving en intrinsieke veranderingen in cellen, zoals tijdens differentiatie, kunnen cellen de eiwithomeostase opnieuw in balans brengen door induceerbare eiwitkwaliteitscontrole-routes te activeren. Om de basale eiwitcontrole te handhaven, wat relevant is in meer chronische vormen van stress zoals de expressie van ziekteverwekkende mutante eiwitten, zijn andere regulerende factoren of routes belangrijker. Dit is vooral relevant tijdens veroudering wanneer de acute stressreacties minder goed werken. Het doel van dit proefschrift was om de rol van eiwitkwaliteitscontrole in acute en chronische stress te bestuderen.

In hoofdstuk 1 hebben we de routes en componenten besproken die eiwithomeostase in eukaryoten reguleren. Hierbij is gefocust op het netwerk van heat shock proteïnen (HSP's). Onder fysiologische situaties is eiwithomeostase afhankelijk van de correcte vouwing, translocatie, assemblage en demontage van eiwitcomplexen en tijdige afbraak van eiwitten. Wanneer deze balans wordt verstoord, zijn de “heat shock response” (HSR) en de “unfolded protein response” (UPR) cruciale routes die worden geactiveerd om de eiwithomeostase opnieuw in evenwicht te brengen. Tegelijkertijd wordt de eiwitsynthese op verschillende niveaus geremd (minder aanmaak van ribosomale componenten en directe remming van translatie) om de belasting van het eiwitkwaliteitssysteem te verlagen. In tegenstelling tot acute stress, activeren chronische vormen van stress de HSR of de UPR vaak niet, zoals bijvoorbeeld bij de expressie van mutante eiwitten die zogenaamde eiwitaggregatieziekten veroorzaken (bijvoorbeeld de ziekte van Huntington). Dit gebeurt althans niet voordat de meeste schade al is aangericht. In deze gevallen zal intrinsieke weerstand tegen dergelijke mutante eiwitten vooral afhankelijk zijn van metabole routes die de basale niveaus van de componenten van de eiwitkwaliteitscontrole reguleren. Deze routes omvatten, onder meer, de insuline/IGF1- en insuline /IGF2-signaleringsroutes en reguleren de belangrijkste transcriptiefactoren die betrokken zijn bij de expressie van netwerken voor de eiwitkwaliteitscontrole.

Hoofdstuk 2 gaat in op de problemen die gepaard gaan met oververhitting (acute stress) zoals die zich voordoen in sommige kippenfokkerijen in China. Er werd gevonden dat voorbehandeling met aspirine, hetgeen de HSR activeert, de door hitte veroorzaakte schade in myocardiale cellen aanzienlijk kan verminderen. Belangrijk is dat het beschermende effect van aspirine afwezig was als tegelijkertijd de inductie van HSP’s wordt geremd. Dit is een sterke aanwijzing dat het inderdaad de opregulatie van deze HSP’s is, die de cardiomyocyten tegen oververhitting beschermt.

Er zijn veel verschillende HSP’s in cellen en deze zijn verdeeld in verschillende families, elk bestaande uit een aantal verschillende leden. In hoofdstuk 3 hebben we ons gericht op de familie van de kleine HSP's (de zogenaamde HSPB-familie). Veel van de leden van deze familie waren betrokken bij de bescherming tegen acute vormen van

stress zoals hitteschok (bijvoorbeeld HSPB1). HSPB1 is echter niet, of slechts marginaal, effectief onder omstandigheden van chronische stress, zoals eerder genoemd bij de expressie van ziekteverwekkende mutante eiwitten. Een voorbeeld hiervan is polyglutamine (polyQ)-bevattende eiwitten die aggregatie-gevoelig zijn en verschillende ziekten kunnen veroorzaken, inclusief de ziekte van Huntington. Van een ander lid van dezelfde familie, HSPB7, was beschreven dat het juist een zeer krachtige werking had om polyQ-aggregatie tegen te gaan. Terwijl HSPB1 behoort tot de eiwitten die worden aangestuurd door de HSR, wordt HSPB7 niet geactiveerd door acute vormen van stress. HSPB7 wordt wel constitutief (zonder externe stress) sterk tot expressie gebracht in hartcellen. Zowel HSPB1 als HSPB7 bevatten echter beide het zogenaamde alpha-crystalline domain (ACD) dat de HSPB-familie kenmerkt. Waarom HSPB7 zoveel beter werkt dan HSPB1 bij het voorkomen van chronische stress was tot dusver onduidelijk. In hoofdstuk 3 laten we zien dat de anti-aggregatie activiteit van HSPB7 wordt bepaald door een stuk van het eiwit dat aan het zogenaamde N-terminale uiteinde (de "NTD") zit. De NTD van HSPB7 heeft geen duidelijke (secundaire) structuur, en is daardoor zeer flexibel, en is zowel noodzakelijk als voldoende om de ACD van HSPB-eiwitten te laten binden aan polyQ-bevattende eiwitten om zo hun aggregatie te onderdrukken. Ons onderzoek laat zien dat verschillende leden van de HSPB-familie verschillende soorten mis-gevouwen eiwitten kunnen herkennen. De ene (HSPB1) herkent meer generieke vormen van ontvouwing die plaatsvinden tijden acute vormen van stress zoals hitteschok (HSPB1) en kan daardoor onder deze conditie aggregatie onderdrukken, terwijl de andere (HSPB7) in staat is zich te binden aan amyloïde vormende eiwitten zoals polyQ eiwitten die chronische ziektes veroorzaken.

Hoe de expressie van niet-HSR-gereguleerde HSP's, zoals HSPB7, wordt gereguleerd is grotendeels onbekend. Het is echter aangetoond dat de insuline-groeifactor signaalroute (IGF-1 route) een belangrijke route is die eiwithomeostase onder fysiologische omstandigheden reguleert. Indien actief, remt de IGF-1 route HSF-1, de transcriptiefactor verantwoordelijk voor transcriptie van HSR-gereguleerde HSP genen. Daarnaast remt de IFG-route ook FOXO1, een andere belangrijke globale transcriptiefactor. In C. elegans is de verhoging van de activiteit van de homoloog van de humane FOXO1, DAF-16, voldoende om eiwitaggregatie te verminderen onder omstandigheden van chronische stress en wordt dit geassocieerd met verlenging van levensduur ("healthy ageing"). Hoe FOXO1 de eiwitkwaliteitscontrole bij zoogdieren regelt, is echter niet goed bekend. Op basis van gegevens verkregen met onderzoek naar DAF-16 in C. elegans, werd gesuggereerd dat deze werking van FOXO1 mogelijk gekoppeld was aan verhoogde expressie van HSPB eiwitten en (wellicht hierdoor) een verbeterde afbraak van eiwitaggregaten door autofagie. In hoofdstuk 4 vonden we dat FOXO1 inderdaad de aggregatie die geïnduceerd wordt door chronische stress (polyQ-eiwitexpressie) kan tegengaan en tegelijkertijd de expressie van HSPB eiwitten verhoogde. Tot onze verbazing bleek echter de verlaagde polyQ-aggregatie onafhankelijk te zijn van de opregulatie van HSPB eiwitten en ook onafhankelijk te zijn van eiwit afbraakroutes (autofagie en ook proteasomale afbraak). In plaats daarvan vonden we dat aanmaak (translatie) van polyQ-eiwitten was verminderd in cellen met meer FOXO1. Dit bleek geassocieerd met een sterkere interactie van zes RNA-bindende eiwitten (STAU1, IGF2BP3, DDX18, FUS, DDX41 en TAF15) met het polyQ mRNA waardoor specifiek minder polyQ eiwit kon worden aangemaakt.

Remming met de CRISPRi techniek van twee van deze eiwitten, STAU1 en DDX18, in cellen met meer FOXO1, leidde tot verlies van effectiviteit van FOXO1 bij het remmen van de polyQ-aggregatie. Deze gegevens tonen aan dat de FOXO1 geactiveerde transcriptionele route niet alleen eiwitkwaliteitscontrolenetwerken verbetert, maar ook de mRNA-surveillance waardoor de synthese van mutante polyQ-eiwitten wordt verminderd.

Naast IGF-1 brengen zoogdiercellen een andere IGF tot expressie: IGF-2. Deze IGF-2 route is nog niet eerder bestudeerd in de context van proteotoxische stress. Onlangs heeft de groep van Claudio Hetz aangetoond dat de ER-gerelateerde transcriptiefactor XBP1 (een hoofdcomponent van de UPR) wordt gereguleerd door IGF-2. Verder laten ze zien dat deze route ook de accumulatie van polyQ-aggregaten vermindert en het begin van symptomen in Huntington-muismodellen vertraagde. Hoe de door IGF-2 gereguleerde route deze bescherming biedt, en of de mechanismen vergelijkbaar zijn met of overlappen met effecten gerelateerd aan IGF-1, was onbekend. In hoofdstuk 5 laten we zien dat IGF-2, in tegenstelling tot FOXO1 in de IGF1-route, de snelheid van synthese van mutant polyQ huntingtin niet beïnvloedt. IGF-2 had echter ook geen effect op de degradatie van polyQ-huntingtin via de autofagie en proteasomale routes. Wat werd gevonden, is dat IGF2-signalering de secretie van polyQ-huntingtin via exosomen stimuleren kon. Bovendien liet analyse van menselijk hersenweefsel en bloedmonsters van Huntington patiënten een verlaging van het IGF2-niveau zien. Tezamen suggereren deze data dat IGF-2 de accumulatie van abnormale eiwitaggregaten buffert en wellicht gedereguleerd is in de ziekte van Huntington.

Onze studies (samengevat in hoofdstuk 6) ondersteunen het idee dat acute stress en chronische stress grotendeels verschillen en verschillende netwerken van eiwitkwaliteitscontrole vereisen voor optimale bescherming. Zelfs binnen één chaperonne familie, zoals geïllustreerd voor de HSPB familie, zijn verschillende leden niet alleen verschillend gereguleerd, maar beschermen ze ook tegen verschillende vormen van stress. Onze gegevens over de IGF-routes laten verder zien hoe het eiwitkwaliteitscontrolesysteem de cellulaire eiwithomeostase bij chronische stress op meerdere manieren reguleert. Dat gebeurt niet alleen door de chaperonne (vouw) capaciteiten en degradatiecapaciteiten te verbeteren, maar ook door de eiwitsynthese te reguleren en zelfs door aggregaten extracellulair te dumpen. Deze meerdere lagen van eiwitkwaliteitscontrole onderstrepen het belang van het handhaven van eiwithomeostase voor de fitness van cellen en organismen.

## Summary in English

Maintaining a healthy proteome is crucial for cellular function and survival. In cells, protein homeostasis is constantly challenged by a variety of intrinsic and extrinsic stress factors that require adaptations of protein quality surveillance pathways. In response to acute forms of stress, such as environmental changes as well as intrinsic changes in cells like during differentiation, cells can rebalance protein homeostasis by activating highly inducible protein quality control pathways. To maintain basal protein control, relevant in more chronic forms of stress such as the expression of disease-causing mutant proteins, other regulatory factors or pathways may become more important especially under conditions of aging where the acute stress responses have declined. The aim of this thesis was to study the role of protein quality control pathways in acute and chronic stress.

In Chapter 1, we reviewed the pathways and components that regulate protein homeostasis in eukaryotes with a focus on the network of heat shock proteins (HSPs). Under physiological situations, protein homeostasis relies on appropriate folding, translocation, assembly and disassembly, and timely degradation of proteins. When this balance is disturbed, the heat shock response (HSR) and the unfolded protein response (UPR) are crucial pathways that are activated to rebalance homeostasis. In parallel, protein synthesis is attenuated at various levels (ribosomal biogenesis, translation) to reduce the burden on the protein quality system. Unlike acute stress, chronic forms of stress such as the expression of mutant proteins that cause so-called protein aggregation diseases (e.g. Huntington's disease) often do not activate the HSR or the UPR, at least not until most damage has been done. Here, intrinsic resistance to such mutant proteins will much rely on metabolic pathways that regulate the basal levels of PQC components. These include the insulin/IGF1 and insulin/IGF2 signaling pathways negatively regulate key transcription factors involved in protein quality control networks

Chapter 2 addresses the problems associated with acute heat stress as it occurs in the chicken breeding industry. We found that aspirin pre-treatment, that activates the HSR, can reduce heat stress-induced injury in myocardial cells in vitro and in vivo. Importantly, the protective effect of aspirin was suppressed when the induction of heat shock proteins was inhibited, strongly suggesting that it is indeed the upregulation of these HSPs that protect the cardiomyocytes against heat shock.

There are many different heat shock proteins in cells and these are divided in different families, each consisting of a number of different members. In Chapter 3, we focused on the family of small HSPs (the so-called HSPB family). Many of these members had been implicated in protection against acute forms of stress (e.g. HSPB1). HSPB1, however was not or only marginally effective under conditions of chronic stress, such as the expression of polyglutamine containing proteins that cause disease like Huntington's disease. Strikingly, another member of the same family, HSPB7, had been described as a very potent member to prevent polyQ aggregation. Whereas HSPB1 belongs to the proteins driven by the HSR, HSPB7 is not activated by acute forms of stress, but constitutively and highly expressed in cardiac cells. Yet both HSPB1 and HSPB7 share the so-called Alpha-Crystallin Domain (ACD) that characterizes the HSPB family. Why HSPB7 works so much better than HSPB1 in preventing chronic stress had remained unclear. In chapter 3, we show that this activity of HSPB7 is driven by its N-terminal region (NTD). This NTD of HSPB7 is highly disordered (i.e. has no clear secondary structure) and is both necessary and sufficient to allow the ACD of HSPB proteins to bind to and suppress the aggregation of polyQ containing proteins. This shows that different members of the HSPB family may recognize different types of protein misfolds, one that is more generic and required to suppress against acute forms of unfolding stresses like heat shock (HSPB1) and one that is capable of binding to amyloidogenic proteins that may cause chronic disease (HSPB7).

How the expression of non-HSR-regulated HSP like HSPB7 is controlled has remained largely unknown. However, the insulin/insulin-like growth factor (IGF) signaling pathway has been shown to be an important pathway that regulates protein homeostasis under physiological conditions. When active, the insulin/IGF signaling pathway negatively regulates HSF1 (for basal transcription of HSR regulated HSP) and FOXO1 (a main global transcription factor, associated with protein homeostasis and longevity). In C. elegans, the elevation of the activity of DAF-16 (homolog of FOXO1 in C. elegans) is sufficient to reduce protein aggregation under conditions of chronic stress and is associated with life span extensions. However, how FOXO1 regulates protein quality control in mammals is not well known. Like for DAF-16 in C. elegans, FOXO1 was suggested to be linked to elevated expression of small HSP and improved clearance of protein aggregates by autophagy. In Chapter 4, we found that FOXO1 indeed elevated small HSP transcription, upregulated autophagy, and reduced aggregation induced by chronic stress (polyQ protein expression). However, reduced polyQ expression was found to be independent of the upregulation of small HSPs and independent of any degradation pathway (autophagy nor proteasomal degradation). Instead, we found that translation rates of polyQ huntingtin were reduced upon FOXO1 overexpression. This was associated with an enhanced interaction of six RNA binding proteins (STAU1, IGF2BP3, DDX18, FUS, DDX41 and TAF15) with polyQ mRNA. Moreover, inhibiting STAU1 and DDX18 by CRISPi suppressed the action of FOXO1 in reducing polyQ aggregation. These data show that the FOXO1 activated transcriptional pathway not only up-regulated PQC networks, but also enhances mRNA surveillance to reduce the synthesis of mutant polyQ proteins.

In addition to IGF1, mammalian cells express another IGF, IGF2, which is not well studied yet in the context of proteotoxic stress. Recently, the group of Claudio Hetz demonstrated that the ER-related transcription factor XBP1 (a main component of the UPR) is regulated by IGF2 and this pathway decreases the accumulation of polyQ aggregates and delayed the onset of symptoms in Huntington mouse models. How the IGF2-regulated pathway provides this protection and whether or not the mechanisms are similar to or overlapping with effects related to IGF1 was unknown. In chapter 5, we show that IGF2 treatment, unlike FOXO1 in the IGF1 pathway, does not affect the rate of synthesis of mutant polyQ huntingtin. However, IGF2 also did not enhance clearance of polyQ huntingtin via autophagy and proteasome. Rather, IGF2 signaling enhanced the secretion of polyQ huntingtin through exosomes, possibly involving changes in actin dynamics. Analysis of human postmortem brain tissue and blood samples from HD patients showed a reduction of IGF2 level, suggesting IGF2 as a relevant factor deregulated in HD, operating as a disease modifier that buffers the accumulation of abnormal protein aggregates.

Our studies (summarized in Chapter 6) support the idea that acute stress and chronic stress are largely different and require different protein quality control networks for optimal protection. Even within one chaperone family, as was illustrated for the small HSP, different members are not only differentially regulated but also protect against different forms of stress. Our data on insulin/IGF pathways further uncovered how the protein quality control system regulates protein homeostasis in chronic stress in multiple manners, not only by improving chaperone (folding) capacities and degradative capacities, but also by regulating folding demand and even by damage dumping extracellularly. These multiple layers of protein quality control underscore the importance of maintaining protein homeostasis for cellular and organismal fitness.

## Acknowledgements

*One sees clearly only with the heart. Anything essential is invisible to the eyes. It's the time you spent on your rose that makes your rose so important.—<Le Petit Prince>.*

Two years and nine months ago, with one hundred more fellows we brought forth upon this continent, a new era, conceived in dreams and dedicated to the proposition that challenges can make us stronger. Now I have waded through the shore from my Yin to my Yang to my Yangtze, showing that the dream or any dream so conceived and so dedicated indeed can long endure. Here I acknowledge all people who walked me along to here, when I stand next to you, I never stood alone (<*Gettysburg Address*>, < *The Warrior Song*>).

I had three supervisors. My Chinese supervisor, Prof. Endong Bao, is the first beacon in my career. Once, though my eyes could see I still was a blind man, though my mind could think I still was a mad man (<*Carry on Wayward Son*>). Bao showed me the way from young and foolish to science. My Dutch supervisor, Prof. Harrie Kampinga, is my life-long mentor, not only in science but also in life. His guidance is the wind at my back and the rains falling soft upon my fields. Harrie is such a great leader, do as he says, I can always see road rise up to meet me (<*Irish Blessing*>). I can imagine that whenever in my career and life I will be confused or hesitating about something, I will ask myself: "what would Harrie do". Dr. Steven Bergink was my dedicated daily supervisor. Steven is an excellent scientist. He is smart and can think fast. He fulfilled his duty to everyone and kept the group running well.

Thanks go to the reading committee, Prof. Verbeek, Prof. van Eden, and Prof. Reggiori for assessing my thesis. I also would like to thank Prof. Claudio Hetz and his group as allowing me to be a contributor to IGF2 work initiated in their lab (chapter 5 of this thesis).

People come and people leave. Standing behind me as paranymphs are Els Kuiper and Wouter Huiting. They are the only two "surviving" PhDs, witnessing my entire stay here at the UMCG; together we worked in the lab, explored in the science. Thank you, guys, for your company. Thanks for the company and colleagueship also go to Jan Vonk, Eduardo de Mattos, Niels Alberts, Gabriel Furtado, Abhinav Pandey. Thanks also to the master students Felix Salles, Hanna Terpstra, and Danara Vonk who contributed to some of my experimental chapters. Although we never met, thanks also to Jing Yang, Michel Vos, and Christos Papagiannopoulos who provided the groundwork for some of my projects.

Special thanks also go to the special force of the lab, the technicians: Jeanette Brunsting, Maria van Waarde-Verhagen, Bart Kanon, Marianne van der Zwaag, Mirjam Baanstra, and Hette Faber (who sadly passed away). You people know every corner of every freezer and each slot in all boxes; you are the magical force keeps the earth rotating, the stars twinkling and, by the way, the lab running. Thanks also to Klaas Sjollema (UMIC) for his help with the microscopy and to Frank Perton (Department of Endocrinology) for his assistance with radioactive experiments.

I also want to thank the Chinese Scholarship Council (the CSC) and the RUG/UMCG. CSC for sponsoring most of my cost to study here, providing me with this great opportunity, to cross the ocean to work, think and live in a western country. The RUG/UMCG for accepting me here, allowing me to experience the dynamics of science in multiple cultures. I appreciate this great country of the Netherlands where the value of each is fully exerted: a tiny country so great.

Thanks to my parents who raised me up and shared their fortitude with me. Thanks also go to my friends: Y. H. Hu, P. Shen, Bela, Winne, Marina and Anna for the great time we spent together. Thanks to M. Wu my nice roommate and Q. Jiang who shared a wonderful trip with me. Peter and Gus, my

skydiving coaches: thanks for taking me up to the sky. Sjouke, my scuba diving instructor: thanks for show me the world under water. Thanks a lot to other supervisors, technicians, colleagues and students work or used to work in the department, you are great.

People come and people leave. It is my turn to say goodbye. But the courage of self-challenging, the spirit of science and the spark of the great of the people, by the people, for the people shall not perish from the earth (<*Gettysburg Address*>).

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## Curriculum Vitae

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### *Education:*

2008/9-2012/6 (BA) Jinling Institute of Technology. Majored in Veterinary Medicine.

2012/9-2014/6 (MA) Nanjing Agricultural University. Majored in Basic Veterinary Medicine.

2014/6-2016/10 (Ph. D) Nanjing Agricultural University. Majored in Basic Veterinary Medicine.

2016/10-2019/4 (Ph. D) University of Groningen/University Medical Center of Groningen. Majored in Biomedical Sciences of Cell & Systems (Cell Biology)

## List of Publications

1. **Wu D**, Xu J, Song E, Tang S, Zhang X, et al. (2015) Acetyl salicylic acid protected against heat stress damage in chicken myocardial cells and may associate with induced Hsp27 expression. Cell Stress Chaperones 20: 687-696.

2. **Wu D**, Zhang M, Lu Y, Tang S, Kemper N, et al. (2016) Aspirin-induced heat stress resistance in chicken myocardial cells can be suppressed by BAPTA-AM in vitro. Cell Stress Chaperones.

3. **Wu D**, Zhang M, Xu J, Song E, Lv Y, et al. (2016) In vitro evaluation of aspirin-induced HspB1 against heat stress damage in chicken myocardial cells. Cell Stress Chaperones 21: 405-413.

4. Zhang XH, Zhu HS, Qian Z, Tang S, **Wu D**, et al. (2016) The association of Hsp90 expression induced by aspirin with anti-stress damage in chicken myocardial cells. J Vet Sci 17: 35-44.

5. Zhang X, Qian Z, Zhu H, Tang S, **Wu D**, et al. (2016) HSP90 gene expression induced by aspirin is associated with damage remission in a chicken myocardial cell culture exposed to heat stress. Br Poult Sci 57: 462-473.

6. **Wu D**, Vonk J, Salles F, Vonk D, et al. The N-terminus of HSPB7 drives its activity to suppress polyQ aggregation. J Biol Chem. (DIO:10.1074/jbc.RA118.007117).

7. Yang J, **Wu D**, Furtado G, Papagiannopoulos C, et al. FOXO1 prevents polyglutamine aggregation by inhibiting protein synthesis in a STAU1 and DDX18 dependent manner. (Manuscript in preparation).

8. García-Huerta P, Troncoso-Escudero P, **Wu D**, Henríquez D, et al. Insulin-like growth factor 2 (IGF2) protects against Huntington´s disease through the extracellular disposal of protein aggregates. (Manuscript in preparation).