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# Chapter 6

## DNAJ proteins and protein aggregation diseases

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**ABSTRACT**

Many neurodegenerative diseases are late onset diseases, associated with aggregation of proteins, implying that aged cells are more susceptible to proteotoxic 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. Several studies have revealed that availability of DNAJ proteins, the co-chaperones to the Hsp70 machine, could be a rate-limiting factor in handling diseased proteins within the cell. Further, in **Chapters 3 and 4**, we have identified DNAJB family members (DNAJB6 & DNAJB8) as the most potent suppressors of polyQ aggregation. In order to have a better understanding of role of DNAJ proteins in not only polyQ diseases but non-polyQ diseases as well, we reviewed the existing literature on it. In this Chapter, we highlight how DNAJ proteins can affect aggregation of disease-causing proteins, if and how this depends on their function as Hsp70 co-chaperones, and how much this depends on the type of protein causing the disease. Finally, we will discuss the five known degenerative diseases that are linked to mutations in individual DNAJ members and what mechanism may underlie these DNAJ chaperonopathies.

## INTRODUCTION

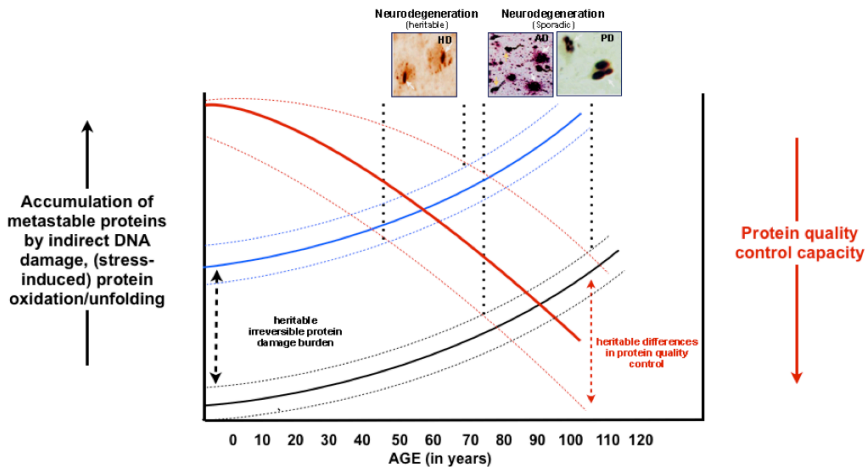
The crowded cellular environment renders any newly synthesized or partially (stress) unfolded polypeptide chain at risk of aggregation. All cells are thus equipped with a large set of molecular chaperones that can prevent unwanted protein-protein interactions and guide proteins to their native fold or direct them towards degradation (1-3). Molecular chaperones comprise several different families of unrelated proteins and many of them belong to the heat shock protein (HSP) family. The nomenclature of these proteins relates to their original discovery in the early 1960s, where temperature-induced puffs representative of the induction of heat shock genes were observed in the polytene chromosomes of *Drosophila melanogaster* larvae salivary glands (4), later established as corresponding heat shock proteins (5). This heat shock response (HSR) turned out not to be restricted to heat shock, but can be induced by many different forms of stress that caused direct or indirect protein unfolding or misfolding (6), which activates heat shock factor-1 (HSF-1) as the master regulator of the HSR. The conservation of HSPs and the HSR from bacteria to humans shows the importance of this response for the cell to survive environmental stress that impedes on cellular protein homeostasis (1). However, it became clear after the “human genome project” that each HSP family consists of many members, several of which are even not HS-inducible or regulated by HSF-1(7). This implies that even a higher degree of complexity and specificity exists in mammals to ensure protein homeostasis under many circumstances that requires specialized assistance.

Despite this elaborate network of HSPs, several pathological conditions and diseases are characterized by protein aggregation, indicating failure of the quality control system. This may either be because this system is overwhelmed under such conditions and/or not activated on time. The latter may be especially the case in slowly progressive protein aggregation diseases, where the HSR is not activated in a timely manner and only turned on in late(r) stages when cell death already has been initiated (8).

### *Protein aggregation diseases*

Protein aggregation forms the hallmark of many neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), Amyotrophic lateral sclerosis (ALS) or several polyglutamine diseases like Huntington’s disease (HD). All of these diseases, including those that are linked to heritable mutations, are late onset, implying that age-related factors contribute to the pathological process. On one hand such age-related factors may include a time-dependent increase in the burden of proteins requiring additional chaperoning (e.g. due to exocytotoxic stress events, protein oxidation, somatic DNA mutations leading to metastable proteins or infections) and on the other hand an age-dependent decline

in the functioning of the protein quality control system (Fig. 1). It has been observed that the HSR declines with age (9-11), which might be attributed to epigenetic alterations that affect the activation of HSF-1 (12). Interestingly, such a decline was much less prominent in centenarians (13, 14) supporting that this may, in part, underlie inter-individual differences in onset of aggregation diseases (Fig. 1). Likewise, differences between individuals in the proteostatic burden at birth, e.g. due to polymorphisms in genes, leading to altered 'foldability of proteins' (15) and/or the rate of decline in proteostatic burden (e.g. due to stress or infection) may be a factor in the actual age-of-onset of these protein aggregation diseases. This is most compelling for those cases in which genetic mutations predispose individuals for aggregation diseases, which in the case of neurodegeneration are typically characterized by a 3-4 decades earlier onset of disease than sporadic cases (Fig. 1).



**Figure 1. Hypothetical model for interindividual differences in the age-related decline in protein homeostasis impeding on the onset of familial and sporadic cases of neurodegeneration:** With aging, the cellular burden of protein damage increases (bold black line) due to molecular misreading, translation errors, protein oxidations, and somatic DNA lesions, leading to metastable proteins. Concurrently, the protein quality control machinery of the cell declines with age (bold red line). Acute stresses (viral infections, fever etc) may add to the total burden in protein damage at any age (not indicated). When the protein damage burden exceeds the (remaining) capacity of the protein quality control system, aggregation diseases are initiated. Interindividual differences in metastable proteins (e.g. due to polymorphisms) determine the burden at birth (dotted lines) and will be extremely high in cases where people carry mutations in the various familial forms of protein aggregation diseases (solid blue lines), leading to a 2-3 decades earlier onset of degeneration as compared to sporadic cases. Interindividual differences in protein quality control capacity at birth or the rate of its decline with ageing (dotted red lines) may further contribute to the interindividual differences in the onset of both sporadic and familial aggregation diseases. Different types of protein damage may require different chaperones: e.g. Hsp70 for assisted folding of metastable proteins or refolding of stress-unfolded proteins versus DNAJB proteins for lowering the burden of aggregation-inducing clients (see main text).

However, the types of protein aggregation risks as mentioned above are very different from each other. 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. In fact, depending on the type of mutated protein, different handling may be required. Given that cells are equipped with over 100 different HSP, it is conceivable that specific members within these HSP families may be required for each of these individual protein homeostasis issues.

#### *Diversity of HSPs*

As stated above, the human genome encodes for over 100 different HSP. Depending on their molecular mass, these HSPs have been classified into different families namely HSPH (Hsp110), HSPC (Hsp90), HSPA (Hsp70), DNAJ (Hsp40), HSPB (small Hsp) and the human chaperonin families HSPD/E (Hsp60/Hsp10) and CCT (TRiC) (16). The number of genes encoding the HSP family varies largely for each organism, e.g. the number of DNAJ proteins varies from 6 in *E. coli* to 49 in *Homo sapiens* (17, 18). The need of functional diversity for client specificity and/or processing, compartmentalization or developmental purposes can very well explain this expansion in HSP members with evolution (16, 19). All these different families of HSPs collaborate in an intricate manner in order to maintain protein quality control within the cell.

Central to this network is the so-called HSPA machine, minimally consisting of HSPA, nucleotide exchange factors (NEFs) and DNAJ proteins (18)(20-22). The Hsp70 machine works via binding and releasing the client proteins to/from HSP70 in an ATP dependent manner (18, 23, 24). Client specificity and processing fate of the machine is predominantly regulated by DNAJs and NEFs (18). While for refolding (stress unfolded proteins) reactions Hsp70s are thought to be rate-limiting (25, 26), evidence is emerging that for dealing with disease-related misfolded proteins the expression levels of DNAJs (see below), and to some extent NEFs, (18, 27-29) are (more) critical. In this review, we will specifically focus on the human DNAJ proteins as being the putative rate-limiting factor in the cell's ability to deal with (mutant) proteins that are causative of protein aggregation diseases. In addition, we will review how mutations in DNAJ members themselves cause protein aggregation disease.

#### *Diversity of DNAJ proteins*

Members of the DNAJ family were named after the founding DNAJ member in *E. coli*. Originally, the family also had been named the Hsp40 family, after one of the closest *E. coli* DNAJ orthologs, and most heat-inducible member of the family (30) (now annotated as DNAJB1: see (16)). Since many members of this family are not heat-inducible (7) and have molecular weights other than 40 kDa (18, 31), the Hsp40 nomenclature has gradually been abandoned.

Within the human genome, approximately 49 different J-proteins have been identified and

are suggested to be the main drivers of the specificity of the cellular Hsp70 machines (18). All DNAJ members contain the family-specific J-domain, by which they can bind to and stimulate the ATPase activity of the HSPA member in the Hsp70 machinery. The highly conserved His, Pro and Asp motif (HPD) within this J-domain is crucial for interaction of the DNAJ protein with the Hsp70 members (18, 31), which is why HPD mutants have often been used to determine whether actions of the DNAJ proteins are dependent or independent from its function within the Hsp70 machinery.

The members in the DNAJ family are, besides their J-domain, highly diverse and have been classified into three categories: class I, II and III (also referred as class A, B and C) (17, 31, 32). Category I is based on the domains present in the original *E.coli* DNAJ *i.e.* consists of a N-terminal J-domain, a glycine and phenylalanine rich region, a zinc – finger motif that is rich in cysteine followed by a C-terminal region. Class II is similar to Class I except that it lacks the Zinc finger motif (the cysteine rich region) and Class III is categorized as containing the J-domain located anywhere within the protein. However, this classification does not provide much insight into the functionality of the DNAJ proteins (18).

Yet limited information is available on the actual structure of DNAJ proteins. For some of the canonical members (the DNAJA and some DNAJB members) it is thought that the glycine/phenylalanine rich region acts as a spacer between the N-terminal J-domain and the rest of the molecule and makes it flexible. The C-terminal is thought to fold itself into  $\beta$ -sheet like structures that are essential for substrate binding and for dimerization of the molecule (31). The canonical DNAJ members dimerize in a V-shape manner, where each monomer binds to a part of unfolded substrate. Hereby it maintains and holds its extended conformation in the middle of both monomers. While the DNAJA members are structurally the closest to the DNAJ protein in *E. coli*, the best studied family member is DNAJB1, the heat-inducible Hsp40 discovered originally by Ohtsuka (30, 33). This protein is the most highly expressed member in most of the mammalian cells and tissues, in particular after heat shock (7). DNAJB1 assists HSPA1A/HSPA8 (Hsp70/Hsc70) in recovery from heat damage as evident from experiments on Luciferase refolding after heat shock both *in vitro* and in living mammalian cells (34, 35). A close relative of DNAJB1, DNAJB2, also recognizes heat unfolded luciferase but seems to be more involved in the targeting of luciferase and other (heat) unfolded proteins towards proteasomal degradation (36) a function that seems to depend on two ubiquitin binding motifs (UIM) in its C-terminus. This illustrates that the various DNAJ family members may not only serve to confer substrate specificity to the Hsp70 machines, but that DNAJ proteins may also play a role in the determination of the client's fate.

In this review, we will summarize the existing literature regarding the role of human DNAJ family members in aggregation diseases. In addition, the focus will be to illustrate if DNAJ

needs Hsp70 machinery or if it has evolved over time to combat proteotoxic stress independent of the J-domain, and hence of Hsp70 members.

#### *DNAJ proteins and polyQ diseases*

Cummings *et al.* were one of the first to test the role of chaperones in polyglutamine diseases. Affected neurons from spinocerebellar ataxia type-1 (SCA-1) patients and purkinje cells from ataxin-1 transgenic mice were shown to contain DNAJ family members, in particular DNAJA1. Furthermore, they showed that overexpression of DNAJA1 in a cellular model of SCA-1 prevented ataxin-1 aggregation. J-domain mutants were unable to suppress aggregation of ataxin-1, implying that DNAJA1 acted in the context of the Hsp70 machinery. Since HSP 70 was not found to be upregulated in affected neurons of the SCA-1 patients nor in transgenic animals, they suggested that DNAJA1 might associate with ataxin-1 aggregates independently from Hsp70 members, but it may not be capable of suppressing aggregate formation on its own (37). Genetic screening also revealed the involvement of DNAJA1 in suppressing toxicity in flies expressing ataxin-1 (38). Nuclear inclusions in these flies were more compact and smaller.

Not only DNAJA1, but also several other DNAJ members were also found to suppress aggregation of (other) proteins with expanded polyglutamine stretches. For example, de-repressed mutants of HSF1 induced DNAJB1 and Hsp70 and showed reduction in ataxin-1 mediated aggregates in a non-neuronal cellular model (39). In line, Muchowski *et al.* found that a combination of DNAJB1 with Hsc70 suppressed the assembly of huntingtin into detergent insoluble amyloid-like fibrils *in vitro* (40). Furthermore, in cell models, several DNAJA and DNAJB and at least two DNAJC family members (DNAJC7 and DNAJC29) were found to affect aggregation of polyglutamine-containing Ataxin-3, huntingtin fragments and the androgen receptor (41-49) (**Table I**). Therefore, there appeared to be little specificity with regard to which DNAJA or DNAJB is required for aggregation suppression. An exception to this rule, however, was found with an *in vivo* SCA-3 *Drosophila* eye degeneration model, where DNAJB1, but not DNAJA1, was found to be protective (43). However, except for our recent study, (48) a direct comparison between the various members within one model system has not been executed.

In general, in the studies where this question was addressed, the effects of most DNAJAs and DNAJBs were found to be J-domain dependent as evident from J-domain mutants that lacked aggregation suppressive activity (**Table I**). In a study by Bailey *et al.*, using polyQ androgen receptor (AR), it was demonstrated that DNAJB1, combined with Hsp70, enhances the proteasomal degradation rate of the polyglutamine protein (41). As DNAJB1 has no domains that directly links it to the proteasome and is generally found to enhance protein



refolding (when overexpressed together with Hsp70s) (7, 35, 50), a model may be deduced that the DNAJB1/Hsp70 combination keeps the polyglutamine protein in a non-aggregated, soluble form such that it can (in a stochastic manner) be degraded by the proteasome; see also (18). This is further supported by the fact that mutant huntingtin fragments with an expanded polyglutamine repeat generally form spherical and annular oligomeric structures, while in the presence of elevated DNAJB1 and Hsp70 levels these occlusions of polyQ epitopes were prevented (51). In addition, DNAJ proteins (with Hsp70s) may prevent interaction of other proteins with the polyglutamine aggregates. For example, this was suggested for interactions between the mutant huntingtin fragments and transcription factors that contain a polyQ stretch, such as TBP/CBP, that were prevented by DNAJB1 and Hsp70; hereby, one aspect of the toxic gain-of-function of the polyQ aggregates, i.e. interference with transcriptional regulation may be reduced (52). A similar model may apply to several of the other DNAJA and some DNAJB members.

When comparing the various DNAJA and DNAJB members directly, we however found that some intriguing differences do exist between their efficacy and modes of action to combat polyQ aggregation (48). In fact, a subclass of DNAJB members was much more effective suppressors than the DNAJB1-like and DNAJA members. This included DNAJB2 previously identified by the *Cheetham group* as potent inhibitors of polyQ aggregation (47). DNAJB2 contains two ubiquitin-interaction motifs (UIMs) in its C-terminus and seems to be involved in directed targeting (as opposed to stochastic partitioning) of substrates to the proteasome (36). In fact, DNAJB2 does not support refolding of heat-denatured substrates as opposed to DNAJB1 (47), suggesting that it indeed plays a specific role in determining the fate (i.e. degradation) of the clients that it binds to. In this action, it still seems fully dependent on interaction with Hsp70 members. Such a model is also consistent with the findings of Gao *et al.* showing that DNAJB2 regulates the proteasomal degradation of ataxin-3 in a HSP70 dependent manner (56). Interestingly, DNAJB2 transgenic mice showed delayed disease onset and progression in a huntingtin mouse model (55) unlike Hsp70 transgenic mice that were non-resistant (59). This further supports the possibility that DNAJ proteins could be rate-limiting factors when it concerns polyQ-mediated protein aggregation and the consequential neuro-degeneration.

In our screen (48), we identified DNAJB6 and DNAJB8 as the most potent suppressors of polyQ aggregation (huntingtin, ataxin-3, AR). The functional activity of DNAJB6/8 in preventing polyQ mediated toxicity and aggregation was found to be largely independent of its J-domain and thus its interaction with Hsp70s. Rather, a unique serine rich region (SSF-SST) in the C-terminal of these proteins was found to be essential for aggregation prevention. DNAJB6 and DNAJB8 were found to be polydispersed oligomers (rather than

**Table I: Effects of DNAJ proteins on PolyQ diseases**

Family member	Disease	Aggregating protein	Model	Effect	HSP70-dependency	Reference
DNAJA1	HD	N-terminal huntingtin	Cellular; Mouse	Co-localization with aggregates	N.E	(44)
	SCA-1	Ataxin-1	Cellular	Aggregate suppression	Yes	(37)
	SCA-3	Ataxin-3	Cellular	Aggregate suppression	No	(42)
	SCA-3	Truncated Ataxin-3	Drosophila	Weak aggregate suppression	N.E	(43)
	SBMA	Androgen receptor	Cellular	Aggregate suppression	Unclear	(46)
	SBMA	Truncated androgen receptor	Cellular	Aggregate suppression	Yes	(41)
DNAJB1	HD	CAG-repeat	Cellular	No effect on Q-mediated toxicity	N.E	(42)
		Huntingtin exon 1	Cell free; Yeast	Aggregate suppression	Yes	(40)
	HD	N-terminal huntingtin	Cellular	Aggregate suppression	No	(44)
	HD	Huntingtin exon 1	Cell free; Cellular	Prevention of transcription factor sequestration	Yes	(52)
	HD	Huntingtin exon 1	Cell free	Prevention of spherical and annular oligomer formation of mutant huntingtin	Yes	(51)
	HD	Huntingtin exon 1	Cellular	Aggregate suppression	N.E	(53)
	HD	Huntingtin exon 1	Cellular	Aggregate suppression	Yes	(45)
	HD	Huntingtin exon 1	Cellular	Aggregate suppression	Yes	(45, 48)
	SCA-1	Human Ataxin-1	Drosophila	Aggregate suppression	N.E	(38)
	SCA-1	Ataxin-1	Cellular	Aggregate suppression	N.E	(39)
	SCA-3	Truncated Ataxin-3	Drosophila	Suppression of poly-Q mediated eye-degeneration; Aggregate suppression	Yes	(43)
	SCA-3	Ataxin-3	Cellular	Aggregate suppression	N.E	(48)
	SBMA	Truncated androgen receptor	Cellular	Aggregate suppression	Yes	(41)(41)
	SBMA	LBD of androgen receptor	Cellular	Aggregate suppression	Unclear	(47)
	SBMA	Androgen receptor	Cellular	No effect on aggregation	N.E	(48)
DNAJB2a	HD	CAG-repeat	Cellular	Aggregate suppression	N.E	(42)
		CAG-repeat	Drosophila	Suppression of acute poly-Q toxicity in the eye	N.E	(49)
	HD	CAG-repeat	Drosophila	Rescue of poly-Q mediated cell death	N.E	(54)
		Huntingtin exon 1	Cellular	Aggregate suppression	Yes	(36)
		Huntingtin exon 1	Mouse	Aggregate suppression; Improved neurological performance	N.E	(55)
		SCA-3	Ataxin-3	Cellular	Aggregate suppression	Yes
DNAJB2a/b	SBMA	Androgen receptor	Cellular	Aggregate suppression	Unclear	(47)
	HD	N-terminal huntingtin	Cellular I	Aggregate suppression and reduced toxicity	No	(57)
	HD	Huntingtin exon 1	Cellular; Xenopus	Aggregate suppression	No	(48)
DNAJB6a/b	SCA-3	Ataxin-3	Cellular	Aggregate suppression	N.E	(48)
DNAJB6b	SBMA	Androgen receptor	Cellular	Aggregate suppression	N.E	(48)
DNAJB6b		CAG-repeat	Drosophila	Suppression of acute poly-Q toxicity in eye; rescue of poly-Q mediated cell death	N.E	(54)
DNAJB8	HD	Huntingtin exon 1	Xenopus	Aggregate suppression	No	(48)
	SCA-3	Ataxin-3	Cellular	Aggregate suppression	N.E	(48)
	SBMA	Androgen receptor	Cellular	Aggregate suppression	N.E	(48)
DNAJC7		CAG-repeat	Drosophila	Suppression of acute poly-Q toxicity in the eye	N.E	(49)
DNAJC29	SCA-1	Ataxin-1	Cellular model	Knockdown of endogenous DNAJC29 causes cell death	N.E	(58)

**Abbreviations:**

N.E:	Not examined
HD:	Huntington's disease
SCA:	Spinocerebellar Ataxia
SBMA:	Spinal and Bulbar Muscular Atrophy
LBD:	Ligand binding domain

dimers, as found for all other DNAJs tested so far) for which this SSF-SST box was essential. Oligomerization (and thus the SSF-SST box) was essential for substrate binding and aggregation prevention. In addition, it was found to be essential for interaction with histone deacetylases (HDACs), of which HDAC4 seems important for DNAJB6/8 function through deacylation of 2 C-terminal lysines. The precise role of DNAJB6/8 deacetylation, however, still needs to be established but is not required for client binding.

All together, these data imply that most DNAJs which prevent polyQ aggregation do so through a classical mechanism, keeping the polyQ proteins competent for (proteasomal) degradation in an Hsp70 dependent manner (18). DNAJB2 is likely to be more potent than the other DNAJs as it actively targets the polyQ proteins to the proteasome. Finally, DNAJB6 and DNAJB8 work through an entirely different mechanism in a non-canonical, largely Hsp70 independent manner. Our recent unpublished work suggests that DNAJB6 may act at a very early stage in the aggregation process by preventing aggregate seeding (Mansson, Kakkar *et al.*, submitted; Gillis *et al.*, submitted).

#### *DNAJ proteins and protein aggregation diseases other than polyQ diseases*

Besides a protective role in polyQ diseases, some members of the DNAJ family have also been shown to play a role in other protein aggregation diseases. The first demonstration for a DNAJ involvement in a folding disease was by Meacham *et al.*, who found that upregulation of DNAJA1 or DNAJB1 in cells suppressed aggregation of the  $\Delta 508$  mutant of the cystic fibrosis transmembrane conductance regulator (CFTR), that is known to cause cystic fibrosis (60); (**Table II**). Although both DNAJA1 and DNAJB1 in the presence of Hsc70 were able to suppress aggregation of the mutant protein, the DNAJB1/Hsc70 combination was better in doing so. The data showed that DNAJB1 synergistically work with Hsc70 in early assembly of polypeptide chains in CFTR biogenesis, suggesting a mechanism in the context of the canonical mode of action of the Hsp70 machine. A recent report also suggests a role for DNAJs in tauopathy (AD); although no data on aggregation were reported, the rate of degradation of tau was accelerated by DNAJA1 in an Hsp70 dependent manner (61).

The aggregation of various mutants of  $\alpha$ -synuclein, synphillin, and PARK-1 that are all associated with PD could be alleviated by several DNAJ members, including DNAJA1, DNAJB1, DNAJB2 and DNAJB6 (62-64) (**Table II**). Similar to the inhibition of polyQ aggregation, the action of DNAJB2 was shown to be Hsp70 dependent. But, intriguingly, for suppression of PARK-1 aggregation, the presence of the UIMs in DNAJB2 was not essential, unlike the situation for polyQ (62). Also unlike polyQ aggregation, DNAJB6 was not more effective in preventing PARK-1 aggregation and its effects on restoring mitophagy than the DNAJB2 (62) or than DNAJA1 or DNAJB1 (unpublished observations). In fact, DNAJB6 was

now fully dependent on interaction with Hsp70 to exert its protection and it did not require its SSF-SST box that was crucial in preventing polyQ aggregation (unpublished observations). These data suggest that the aggregation processes for polyQ and PD-mutants are largely different and that for the latter stimulation of Hsp70 activity, unrelated to the type of DNAJ protein, may be sufficient for protection.

**Table II: Effect of DNAJ proteins in Non-PolyQ diseases**

Family member	Disease	Aggregating protein (mutant)	Model	Effect	HSP70-dependency	Reference
DNAJA1	CF	CFTR ( $\Delta$ F508)	Cellular	Aggregate suppression	Yes	(60)
	PD	$\alpha$ -synuclein and synphilin-1	Cellular	Aggregate suppression	N.E	(63)
DNAJB1	OMD	PABPN1	Cellular	No effect	N/A	(65)
	PD	$\alpha$ -synuclein	Postmortem brain tissue	Present in lesions/inclusions	Unclear	(64)
	LBVAD DLBB NBIA1					
	PD	$\alpha$ -synuclein and synphilin-1	Cellular	Aggregate suppression	N.E	(63)
	ALS	SOD1	Cellular	Aggregate suppression; Neurite outgrowth; Reduced cell death	Yes	(66)
	OMD	PABPN1	Cellular	No effect	N/A	(65)
	CF	CFTR ( $\Delta$ F508)	Cellular	Aggregate suppression	Yes	(60)
	DM type 2	Human amylin	Cell free	Aggregate suppression	Yes	(67)
DNAJB2a	PD	Parkin (C289D)	Cellular	Aggregate suppression; Restoration of mitophagy	Yes	(62)
	RP	Rhodopsin	Cellular	Modulates rhodopsin processing and inclusion formation	No	(68)
		Rhodanese	In vitro	Aggregate suppression	N.E	(69)
	DNAJB2b; DNAJB6	Parkin (C289D)	Cellular	Aggregate suppression; Restoration of mitophagy	Yes	(62)
DNAJC5	CF	CFTR	Cellular	Promotes proteasomal degradation of CFTR	Yes	(70)

Abbreviations:

N.E:	Not examined
PD:	Parkinson's disease
DM:	Diabetes Mellitus
CF:	Cystic fibrosis
OMD:	Oculopharyngeal muscular dystrophy
RP:	Retinitis Pigmentosa
LBVAD:	Lewy Body variant Alzheimer's disease
DLB:	Dementia Lewy Bodies
ALS:	Amyotrophic Lateral Sclerosis
NBIA1:	Neurodegeneration Brain Iron Accumulation type 1

In analogy, for superoxide dismutase I (SOD-1) mutants, associated with familial amyotrophic lateral sclerosis (ALS), aggregation could also be inhibited by DNAJB1 in a HSP70 dependent manner (66) leading to improved neurite outgrowth and reduced cell death in a neuronal cell system. To the best of our knowledge, no information on DNAJB1 domain requirements or other DNAJ members has been reported for effects on ALS-related mutant protein aggregation. Although Hsp70 alone was also somewhat effective in preventing SOD-1 aggregation, again the combined effects of DNAJB1 and Hsc70 resulted in more than

additive effects, implying yet another example for canonical way of functioning of Hsp70 machine.

Single reports on aggregation of mutant Rhodopsin (68) and human amylin involved in Diabetes mellitus-2 (67) suggest that more aggregation diseases may be suppressed by DNAJ proteins, but more work on this is still required. One disease where the canonical members DNAJA1 and DNAJB1 were reported not to be effective is oculopharyngeal muscular dystrophy (OPMD), which is a poly alanine repeat disease (65), implying that canonical DNAJ-HSP70 machine actions are insufficient here. Given their different modes of action, it will be of interest to test (for example) DNAJB2 or DNAJB6 effects in OPMD.

So, for the non-polyQ diseases, a general picture emerges that also here DNAJ proteins could be effective, but clearly as part of the Hsp70 machinery. Whether any specificity exists in terms of type of DNAJ versus type of substrate remains to be investigated.

#### *DNAJ mutations as cause for disease*

The importance of the DNAJ proteins in regulating protein quality control is further underscored by recent data showing that mutations in 5 different DNAJ proteins actually are the cause of some neuropathies or myopathies (**Table III**).

The first report on a DNAJ chaperonopathy concerns mutations in DNAJC29 (also called saccin) that causes an early onset, autosomal recessive cerebellar ataxia with peripheral neuropathy referred to as ARSACS (71, 72). DNAC29 is an extremely large protein of 521 kDa with a J-domain present in the C-terminal part of the protein. Little is known about its function and how the various mutations, including mutations in the J-domain (80), lead to disease. However, DNAJC29 has been suggested to have chaperone-like activities as it can assist in luciferase refolding after heat shock (81). Also, siRNA-mediated DNACJ29 knockdown enhanced aggregation of polyQ mutant ataxin-1, which has lead to the suggestion that the large multi-domain may be able to recruit Hsp70 chaperone on mutant ataxia-causing proteins (58, 82). In addition, DNAJC29 was found to be involved in mitochondrial dynamics and loss of such a function of the mutants could be involved in causing ataxia: this would be consistent with finding in fibroblasts from ARSACS patients that show a hyperfused mitochondrial network (79). If and how a loss of either function of DNAJC29 is indeed related to its function as a DNAJ protein remains to be elucidated.

**Table III: Mutations in DNAJ proteins resulting in chaperonopathies**

Family member	Mutation	Inheritance	Associated Disease	Characteristics	Reference
DNAJC29 (saccin)	c.3484 G>T, p.E 1162X; and c.11,707C>T, p.R 3903X in SACS gene	Recessive	Spastic ataxia of Charlevoix-Saguenay (ARSACS)	Early-onset cerebellar ataxia with spasticity and peripheral neuropathy; siRNA results in impaired ataxin-1 aggregation prevention	(58, 71-73)
DNAJC19 (Tim18 or Pam18)	IVS3-1 G-C on chromosome 3q26.33	Recessive	Dilated cardiomyopathy with ataxia (DCMA)	Mitochondrial protein import defects, (74) severe, early onset cardiomyopathy, growth failure and cerebellar ataxia, testicular dysgenesis	(74)
DNAJB2 (HSJ1)	Splice mutation in HSJ1 gene at chromosomal location 2q34-q36.1	Recessive	Distal Hereditary Motor Neuropathy (dHMN)	Neuromuscular disorder, progressive muscle weakness and atrophy at the distal part of the limbs, lower motor neuron degeneration.	(75)
DNAJC5 (CSPa)	c.346_348 delCTC, c.344T>G leads to p.Leu116del and p.Leu115Arg	Dominant	Autosomal dominant adult-onset neuronal ceroid lipofuscinosis (ANCL) or Kufs disease	Progressive neurodegeneration and early death. Lysosomal accumulation of auto-fluorescent lipopigment in neuronal cells.	(76, 77)
DNAJB6	Phe93Leu, Phe89Ile or Pro96Arg in G/F domain	Dominant	Limb-Girdle Muscular Dystrophy type 1D (LGMD1D)	Postnatal onset of progressive weakness & muscle atrophy; impaired prevention of polyQ aggregation in muscle tissue	(78, 79)

The second recessive DNAJ-related chaperonopathy is due to mutations in the specialized DNAJ protein DNAJC19, also referred to as Tim18 or Pam18 (18). These mutations affect the DNAJC19 splicing and cause an autosomal recessive cardiomyopathy (DCMA) (74). Work on the DNAC19 ortholog in yeast already had demonstrated that DNAJC19 is involved in mitochondrial import (83, 84) and consistent with this, the DCMA patients carrying two affected alleles with DNAJC19 mutations display defective mitochondrial import and develop a very early onset of both cardiomyopathy and cerebellar ataxia (74).

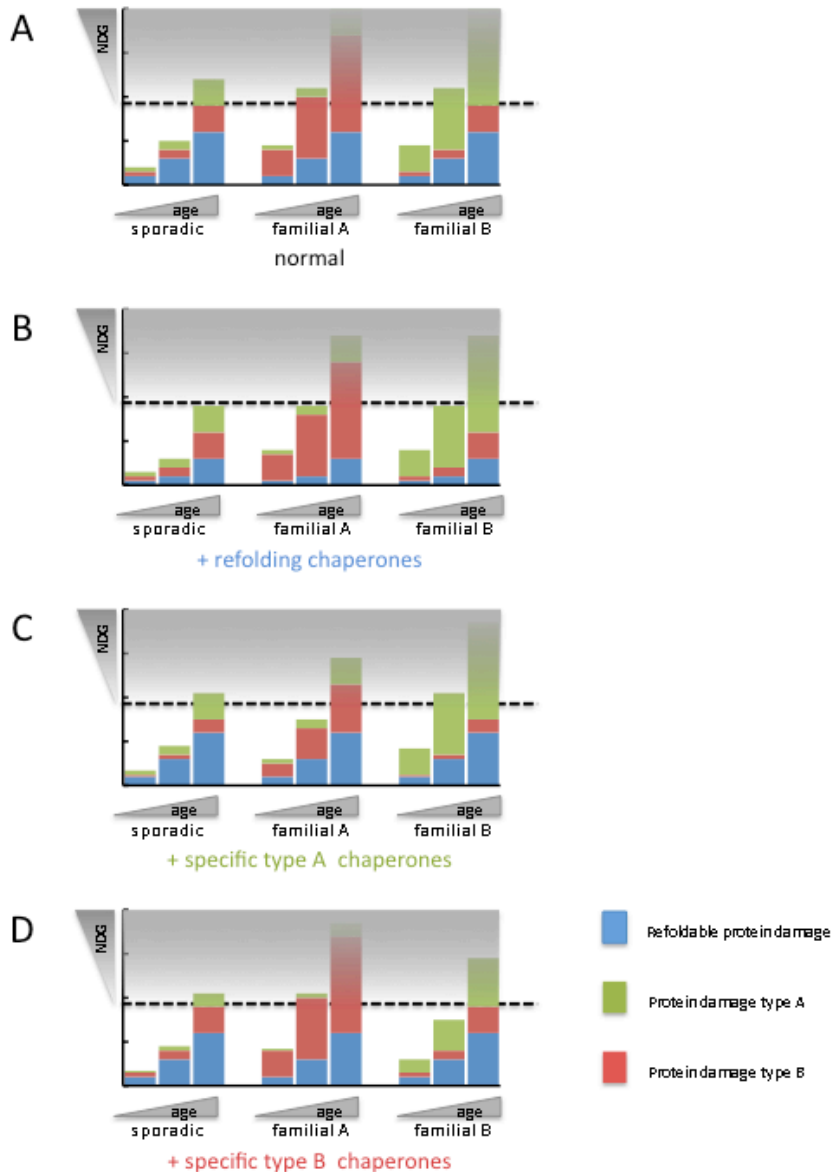
The third recessive disease that is associated with DNAJ mutations concerns a rare distal hereditary motor neuropathy (dHMN) in which DNAJB2 is mutated (75). dHMN is characterized by muscle weakness of the extremities and many other candidate genes have already been reported to cause dHMN, including some dominant variants associated with mutations in the small HSPs, namely HSPB1(85), HSPB3 (86) and HSPB8 (87). The recessive form of the disease associated with DNAJB2 involves splice mutations in DNAJB2 that lead to reduced levels of the long, ER associated isoform (DNAJB2b) and to undetectable levels of the shorter cytosolic form (DNAJB2a) (75); the latter isoform was reported to suppress many of the above-mentioned aggregation diseases, including many polyQ diseases and PD (Table I/II), implying that the loss of this chaperone function may be extremely disrupting for motor neuronal-muscular maintenance.

Next to these three recessive DNAJ chaperonopathies, two dominantly inherited diseases were found to be associated with DNAJC5 and DNAJB6 respectively. The mutations in DNAJC5 lead to an autosomal dominant adult onset neuronal ceroid lipofuscinosis (ANCL), also known as Kuf's disease, and is one of the nine types of these progressive neurodegenerative genetic disorders (76, 77) that are characterized by presynaptic dysfunction and lysosomal accumulation of proteolysis resistant proteins in neurons. DNAJC5 (or CSP $\alpha$ ) already has been extensively studied and is an evolutionary well-conserved, unique protein that is localized to synaptic vesicles where it forms a trimeric complex with Hsc70 and small glutamine-rich tetratricopeptide protein (SGT) (88, 89). Loss of CSP $\alpha$  functionality impairs SNAP25 function leading to deficits in SNARE complex assembly (89-91). CSP $\alpha$  also was found to regulate the polymerization of dynamin and thus may affect exo- and endocytic coupling (92). The dysfunction of the mutant DNAJC5/Hsc70/SGT chaperone complex might affect the folding quality of many client proteins and make them vulnerable to aggregation and degradation (93).

Finally, mutations in glycine-phenylalanine rich domain of DNAJB6 have been found to cause an autosomal dominant myopathy known as limb-grid muscular dystrophy type 1D (LGMD1D) (78, 79). The mutations results in abnormal protein accumulation and autophagy pathology in muscle tissue. The mutants were suggested to have lost their function. When over-expressed in muscle cells, the mutants were slightly impaired in their ability to prevent polyQ protein aggregation (78). This would suggests that DNAJB6 haplo-insufficiency is sufficient to cause disease and further underscores how important precise regulation of DNAJB6 levels might be for normal cell functioning. Interestingly, our preliminary data suggest that this minor "loss of function" of DNAJB6 is not seen in non-muscle cell types, implying that additional cell type related substrates or modulators may be involved that could explain the tissue specificity of the disease.

### **CONCLUDING REMARKS/PERSPECTIVES**

Early in life, the cell's protein quality control system is able to combat folding problems, but with aging these pathways start to fail, resulting in accumulation of damaged and misfolded proteins causing protein aggregation diseases (Fig. 1). While Hsp70 has been shown to be a rate limiting factor for protein refolding which could e.g. include handling of metastable proteins as they accumulate with age, it has little impact on preventing aggregation of disease-associated proteins. As shown in this review, the DNAJ proteins may seem more rate determining in the latter case. It is also clear that the type of disease-associated protein is extremely relevant for what type of DNAJ and protein quality control is required for prevention. So, depending on the relative contribution of different folding and aggregation problems, different chaperones (HSP70, DNAJs, NEFs, other Hsp families) may be required



**Figure 2: Hypothetical model for client and chaperone dependent contributions to aged related degeneration in different individuals.** Age dependent increases in protein burden consists of different types of clients that are either refoldable (blue boxes) or are non-refoldable clients of different types (green and red boxes), such as typically elevated at birth already in familial diseases (here exemplified as A and B). The higher burden at birth leads to an earlier onset of neurodegeneration (NDG) in such diseases (Panel A: see also fig. 1). Depending on the type of chaperones used for overexpression (Panels B-D) and the fractional contribution of each of the types of protein burden in each of the different individuals, the chaperones may or may not be able to significantly reduce the onset of NDG, sometimes even without directly acting on the disease-associated protein itself (e.g. as in the case of the refolding enhancing chaperones: Panel B).



to combat premature proteostasis collapse and neurodegeneration (see Fig. 2 for such a hypothetical model).

So far, only limited studies have systematically compared the effects of different components of the chaperone network on the various diseases and aging in general. Given that the human chaperonome contains over 100 different HSPs, there is still much out there to hope for as putative disease-specific targets for therapy. One such strategy that we are currently pursuing is the boosting of DNAJB6 activity in polyglutamine diseases. In addition, we started to compare all DNAJA and DNAJB family members for their ability to prevent the aggregation associated with other aggregation-causing disease associated proteins. In Chapter 8 we report on such a screen for suppressors of aggregation of the parkin C289G mutant that is associated with heritable juvenile parkinsonism.

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