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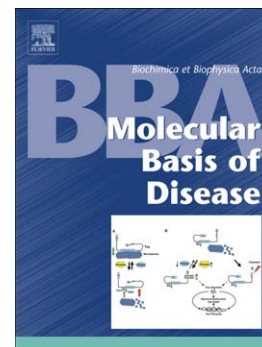
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Invited review

Mallory-Denk bodies: lessons from keratin-containing hepatic inclusion bodies

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Abstract

Inclusion bodies are characteristic morphological features of various neuronal, muscular and other human disorders. They share common molecular constituents such as p62, chaperones and proteasome subunits. The proteins within aggregates are misfolded with increased β -sheet structure, they are heavily phosphorylated, ubiquitinated and partially degraded. Furthermore, involvement of proteasomal system represents a common feature of virtually all inclusions. Multiple aggregates contain intermediate filament proteins as their major constituents. Among them, Mallory-Denk bodies (MDBs) are the best studied. MDBs represent hepatic inclusions observed in diverse chronic liver diseases such as alcoholic and non-alcoholic steatohepatitis, chronic cholestasis, metabolic disorders and hepatocellular neoplasms. MDBs are induced in mice fed griseofulvin or 3,5-diethoxycarbonyl-1,4-dihydrocollidine and resolve after discontinuation of toxin administration. The availability of a drug-induced model makes MDBs a unique tool for studying inclusion formation. Our review summarizes the recent advances gained from this model and shows how they relate to observations in other aggregates. The MDB formation-underlying mechanisms include protein misfolding, chaperone alterations, disproportional protein expression with keratin 8 > keratin 18 levels and subsequent keratin 8 crosslinking via transglutaminase. p62 presence is crucial for MDB formation. Proteasome inhibitors precipitate MDB formation, whereas stimulation of autophagy with rapamycin attenuates their formation.

Mallory-Denk bodies represent a unique tool to investigate aggregate pathogenesis.

Intracellular protein aggregates such as neurofibrillary tangles (Alzheimer's disease), Lewy bodies (Parkinson disease) or Mallory-Denk bodies (various chronic liver diseases) are morphological features of a variety of neurodegenerative, muscular and other diseases [1-3]. Inclusion bodies typically consist of misfolded and ubiquitinated structural proteins together with variable amounts of chaperones and the ubiquitin-binding protein sequestosome 1/p62 (p62; [4-6]). Based on the type of predominant structural protein, aggregates can be divided into subgroups such as synucleinopathies, tauopathies, polyglutamine- or intermediate filament-containing inclusions or TDP-43 (TAR-DNA binding protein 43) proteinopathies (Table 1). Aggregates share several features such as presence of highly insoluble, hyperphosphorylated and often crosslinked proteins which suggest common principles underlying their pathogenesis. Furthermore, aggregated proteins are often overexpressed or present at altered stoichiometric ratios.

While considerable efforts focused on elucidating the pathogenesis of tau- and synuclein-related disease processes, the formation and significance of intermediate filament (IF)-related aggregates is less well understood. The goal of our present review is to summarize the present knowledge about prototypic IF-related aggregates termed Mallory-Denk-bodies (MDBs; [7]), and to highlight how the understanding of hepatic inclusion body formation may enhance our perception of aggregates seen in neurodegenerative and other disorders. A special emphasis will be placed on ubiquitin-proteasome system (UPS), which represents one of the most consistent players in the development of aggregates.

IFs are components of the cytoskeleton and involved, in cooperation with actin microfilaments and the microtubules, in the maintenance of cell shape, locomotion, intracellular organization and transport [8,9]. IF proteins are encoded by ~ 70 different genes and comprise six subtypes which are, at least in part, expressed in a cell type (and differentiation)-dependent manner [10-12]. The family of IFs comprises *type I* (acidic) and *type II* (basic)

keratins, *type III* vimentin, desmin, glial fibrillary acidic protein, peripherin, and syncoilin, *type IV* neurofilaments, α -internexin, nestin and synemin, *type V* nuclear lamins, forming a meshwork at the inner layer of the nuclear membrane, and the "orphan" cytoskeletal proteins phakinin and filensin [10,12]. IFs are rather insoluble protein structures which are the major constituents of protein aggregates found in several hepatic, neurodegenerative and muscular disorders (Table 1; [1,10,13]). In addition, IFs represent regular components of structures called aggresomes, which arise when multiple small aggregates are deposited in a single, juxtanuclear location in a microtubule-dependent manner [14].

MDBs are the most prevalent and the best understood IF-related aggregates. They are hallmarks of steatohepatitis of the alcoholic (ASH) and non-alcoholic type (NASH; Figure 1). They are also seen in a variety of other chronic liver disorders including chronic cholestasis, particularly late stages of primary biliary cirrhosis (Figure 1c), Wilson disease and other copper-related disorders, various metabolic disturbances and hepatocellular neoplasms [7,15]. In idiopathic copper toxicosis and hepatocellular carcinoma (HCC), MDBs may concur with another type of cytoplasmic inclusions, termed intracellular hyaline bodies (IHBs), which share several components, except keratins, with MDBs (Figure 2; [7,16]). For example, about 16% of HCCs contain IHBs only and additional 16% contain both IHBs and MDBs, sometimes within the same cell suggesting a close relationship. Both types of inclusions share filamentous ultrastructure and IHBs have been designated as "globular" and MDBs as "reticular" hyaline [17]. In human diseases, "hybrid" inclusions were observed exhibiting a combination of morphologic and immunohistochemical features of both types of inclusions: IHB-like inclusions containing a small amount of (usually granular) keratin, and conversely, MDB-like aggregates with less densely arranged keratin granules (Figure 2). On the basis of *in vitro* transfection studies, the following scenarios leading to formation of MDBs, IHBs, and hybrids was proposed [18]: (i) classical MDBs arise if p62 and abnormal keratins accumulate at the same time in the cell in comparable amounts (Figure 3); (ii) hybrid inclusions are formed when p62

amounts exceed the levels of aggregation-prone keratins, and (iii) IHBs result if only p62 is overexpressed without sufficient amounts of abnormal keratins. It is currently unknown, whether IHBs represent “pure” p62 aggregates or whether they contain additional, yet unidentified proteins.

MDBs can be easily reproduced in a mouse model by chronic griseofulvin or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding [7,19]. MDB formation in mice and men is reversible in that they become smaller and less numerous after discontinuation of the causative agent [7,20]. However, both “recovered” humans and animals remain highly predisposed to MDB re-formation [7,15]. Moreover, the MDB re-appearance can be induced by a variety of rather non-specific stress inducing agents as it has been shown in mice recovered on standard diet for one month, which are therefore termed as “primed mice” [7].

Despite the existence of several cell culture-based models [7,18], mouse studies were pivotal in elucidating the pathogenesis of MDB formation [7,21]. In the mouse model of MDB formation aggregates are induced by feeding a toxic substance. This is reminiscent of Parkinson disease models, where intravenously or subcutaneously administered drugs lead to generation of proteinaceous deposits [22]. In contrast, most animal models rely on genetic methods such as over-expression of wild-type protein or introduction of mutant proteins [13,23,24]. This approach is well suited to mimic genetically simple human diseases (such as diseases caused by expanded polyglutamine repeats), however the most common human inclusion bodies are found in genetically complex disorders such as Parkinson or Alzheimer’s disease [24]. The availability of a drug-induced model of human inclusion body formation represents an interesting alternative to study the pathogenesis and biological significance of such aggregates. Furthermore, a non-genetic model allows a convenient testing of factors potentially involved in inclusion formation, since there is no need for generating multiple transgenic animals. Hepatic aggregates are also ideally suited for pharmaceutical trials due to the ease of drug delivery and tissue availability. The goal of our review is to summarize the recent advances gained from the MDB

mouse model and to show how they can enhance our understanding of aggregates seen in multiple other diseases. As a part of the theme edition, our review will put a special emphasis on UPS.

Inclusion bodies share common molecular constituents.

While earlier studies suggested that inclusion bodies consist of randomly trapped proteins, it is now believed that they represent highly organized structures of proteins involved in their pathogenesis [25]. As a reflection of common underlying mechanisms, aggregates share several common constituents such as chaperones, ubiquitin, UBB(+1), p62, Valosin-containing protein, NEDD8 or proteasome subunits. Chaperones are among the most prominent components of inclusion bodies which likely try to refold damaged proteins or to wrap them up to limit toxic interactions with other cellular components [5,6]. Although chaperones are rather constant features of many aggregates, the chaperone composition differs among deposits. For example, MDBs contain significant amounts of heat shock proteins (Hsp) 70 and 90, whereas Hsp27 and α B crystalline, which are the major chaperones seen in Rosenthal fibers, are not obligatory constituents of MDBs [4,13,26]. In Lewy bodies, crystallins and Hsp90, but not Hsp70 belong to the main components [27]. Although the precise reason for such heterogeneity is unknown, the different structural components of inclusion bodies might be responsible. For example, keratins (i.e. the major constituents of MDBs, Figure 3a,b,d) associate with Hsp70 [28], whereas glial fibrillary acidic protein (GFAP), which is part of Rosenthal fibers, preferentially binds Hsp27 and α B crystallin [29].

Valosin-containing protein (VCP/p97) has been observed in multiple aggregates including MDBs [30,31]. VCP/p97 belongs to the AAA class of ATPases and seems to be involved both in protein aggregate formation and clearance [31,32]. In the case of MDBs, reduction of VCP/p97 increases inclusion body formation, whereas overexpression did not have an obvious effect [30]. In a cell culture model of polyglutamine diseases, VCP/p97 decrease delays aggre-

gate clearance [31]. In humans, a mutation in VCP/p97 causes inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia [33].

Several shared inclusion body constituents are involved in protein degradation. Most aggregates consist of highly ubiquitinated proteins, which likely reflect the cellular attempt to target misfolded proteins for proteasomal degradation. However, dependent on the particular lysine involved, protein ubiquitination may also facilitate autophagic clearance through interaction with p62 [34]. Ubiquitin may also be involved in coating of the damaged proteins, which should preclude further growth of the aggregate and prevent potentially toxic interactions [35]. MDBs as well as inclusions observed in tauopathies and polyglutamine diseases, but not synucleinopathies harbour a frameshifted form of ubiquitin termed UBB(+1) [36-38,39]. UBB(+1) arises as a consequence of dinucleotide deletion during or after transcription and is produced at similar levels in a variety of conditions [40]. It is efficiently degraded by proteasome at lower levels [39], but its accumulation can cause proteasome inhibition both in cell culture and *in vivo* [41,42]. Therefore, elevated UBB(+1) levels may both signalize impaired proteasome function and accelerate disease progression through additional proteasome impairment [40].

Ubiquitin-like protein NEDD8 has also been detected in numerous aggregates including MDBs [43,44]. Although its function is largely unknown, it can promote degradation of its substrates via adaptor proteins NUB1 and NUB1 ligand and this process is involved in clearance of Lewy body- like inclusions [45].

p62 is another constituent of a variety of aggregates [4,46]. It represents an ubiquitin-binding protein which may facilitate both proteasomal and autophagic degradation [47-49]. The potential involvement of proteasomal system in inclusion degradation is further highlighted by frequent co-localization of protein aggregates with proteasomal subunits [7,50,51].

Posttranslational modifications of proteins affect aggregate formation.

The similarities between inclusion bodies extend to protein modifications. Aggregated species are typically misfolded and highly insoluble. In addition, they are often hyperphosphorylated,

transamidated and partially degraded/proteolytically processed. Interestingly, these posttranslational modifications may have analogous impact on the aggregation process independent of the particular protein involved. For example, protein aggregates usually exhibit altered conformation with increased β -sheet formation as it has been shown for MDBs [52], inclusions seen in Alzheimer's, Parkinson, Huntington disease and many others disorders [5]. The uniformity of these structures is highlighted by antibodies cross-reacting with oligomeric species independent of their constituent proteins [53]. Furthermore, diverse aggregates can also be stained by agents with affinity for β -sheet protein conformation such as thioflavin or congo red [7,54]. Among them, congo red does not only stain aggregates, but also interferes with the process of protein misfolding and aggregation and shows some promise for treatment of inclusion-forming diseases [55].

Apart from phosphorylation (see below), oxidative modifications might be involved in protein precipitation. They cause conformational changes and exposure of hydrophobic domains, which would otherwise be hidden within the properly folded polypeptide. The exposed residues then interact with each other and other cellular components thereby resulting in protein aggregation [56]. For example, nitration and oxidation of α -synuclein generates crosslinked oligomers [57,58] and iron together with peroxide promotes NACP/ α -synuclein aggregation *in vitro* [59]. Several antioxidant compounds were also demonstrated to counteract inclusion formation in different settings. For example, S-adenosylmethionine and mito Q attenuated MDB formation in DDC-primed mice ([60]; Zatloukal et al., unpublished data), and antioxidant compounds show potent anti- α -synuclein-fibrillogenic properties *in vitro* [61]. However, such drugs exhibit a variety of different effects and the attenuation of aggregate formation may not be always related to decrease in oxidative stress. For example, lack of MDB formation after S-adenosylmethionine administration was explained as a result of interaction with epigenetic histone modifications rather than being a result of antioxidant properties [60].

The aggregated proteins are often hyperphosphorylated and protein phosphorylation may promote aggregate formation. To that end, MDBs consist of heavily hyperphosphorylated keratins [62] and ablation of keratin 8 S73 phosphorylation site in mice results in diminished MDB formation, whereas alteration of keratin 18 phosphorylation does not have an obvious effect ([63]; Harada et al., unpublished results). Furthermore, keratin 8 S73 is phosphorylated by p38 kinase and a p38 inhibitor prevents MDB formation in DDC-primed hepatocytes [64,65]. Similarly to keratins, α -synuclein is phosphorylated at serine residues with Ser 129 being the major phosphorylation site [66]. The extent of phosphorylation is considerably higher in insoluble synuclein when compared to the normal protein [66] and the abolition of the Ser 129 phosphorylation site results in decreased inclusion formation [67]. Tau is another established phosphoprotein and its abnormal phosphorylation promotes dissociation from microtubules, formation of neurofibrillary tangles and neurodegeneration [68]. GSK3 β , CDK5 and ERK2 are the most relevant kinases involved in this process and their specific inhibitors are currently evaluated as potential treatments in tau-related neurodegenerative diseases [68]. The beneficial effect of GSK3 β and CDK5 inhibitors may extend to inclusion body myositis, which contains hyperphosphorylated tau forming paired helical filaments [69]. Moreover, protein aggregation might be stimulated by stress-responsive kinase MEKK1 in polyglutamine disorders [70]. Finally, JNK kinase contributes to GFAP accumulation and expression of dominant negative JNK restores GFAP solubility in a cellular model of Alexander's disease [71].

Protein crosslinking by transglutaminases (TG) was observed in various inclusion bodies. It represents a largely irreversible protein modification, which results in protein oligomerization and was implicated in aggregate formation [72]. For example, keratin 8 within MDBs is crosslinked by TG2 and preventing this crosslinking might be responsible for greatly reduced MDB formation observed in TG2-knockout mice [73,74]. Enhanced protein crosslinking was detected in multiple other aggregate-forming disorders such as Inclusion Body Myositis [75], Alzheimer [76], Parkinson [77] or Huntington disease [78]. Crosslinking might be directly in-

volved in synuclein aggregation or aggregates seen in Huntington disease [77,79]. However, protein crosslinking does not always predispose to inclusion body formation as seen after crossing Huntington's disease (HD) R6/1 transgenic mice with TG2 knock-out mice, which even potentiated inclusion body formation [80].

Aggregated proteins are often partially degraded or result from regulated proteolytic processing. The former is true for keratins in MDBs [7,81], the latter for amyloid β in inclusion-body myositis and Alzheimer's disease [69,82] and proteins causing several polyglutamine disorders [83]. In the case of amyloid β , huntingtin and ataxin 3, partial proteolysis can promote aggregation [82,83]. Accordingly, inhibitors of responsible proteases may become interesting drug targets. For example, β -secretase inhibitors are currently under development and may prevent formation of amyloidogenic A β 42 fragment [84].

Protein overexpression or disproportional production accelerates inclusion formation.

Both increased protein expression and/or abnormal ratio between different protein species may predispose to formation of inclusion bodies and/or extracellular protein plaques. In support of the former hypothesis, individuals with trisomy 21, who have an extra copy of amyloid β gene, develop the characteristic Alzheimer disease pathology at early age [82]. Similarly, a triplication of α -synuclein locus causes early onset familial Parkinson disease [85]. In transgenic mice, Rosenthal fiber formation can be introduced by GFAP overexpression [13]. However, the ratio between different protein isoforms seems also to be important. For example, α -synuclein exhibits three different isoforms generated through alternative splicing and patients displaying dementia with Lewy bodies have up-regulated expression of α -synuclein 112 and down-regulated expression of α -synuclein 126 isoform [66]. Similarly, patients with sporadic Alzheimer disease exhibit a shift between different amyloid β peptides, rather than amyloid- β precursor protein overexpression [82]. Altered ratio between different neurofilament protein isoforms might be involved in aggregate formation in amyotrophic lateral sclerosis [86]. Improper stoichiometric balance between aggregate constituents rather than protein overexpres-

sion likely precipitates murine MDB formation. In support of that, K8 overexpressing or K18-null mice, who have increased K8/K18 ratio, produce inclusion bodies more rapidly, whereas mice overexpressing both keratins 8 and 18 do not [7,87].

Chaperones attenuate aggregate formation.

Since protein misfolding may result in inclusion formation [6], chaperones facilitating protein folding and repair or degradation of damaged proteins can protect from aggregation. Several studies support this hypothesis [70]. For example, overexpression of inducible Hsp70 or a concerted induction of several chaperones in HSF1 transgenes attenuated polyglutamine inclusion formation [88-90]. Accordingly, inclusion body formation would indicate either impaired or insufficient chaperone function. Indeed, MDB formation after chronic DDC intoxication is accompanied by decreased levels of inducible Hsp70 and GRP75, persistent chaperone modifications and compromised chaperone function [91]. This hypothesis may extend to other aggregate-forming disorders, since mouse models of Huntington disease and amyotrophic lateral sclerosis display reduced chaperone levels as well [92,93].

Proteasomal and autophagic degradation are involved in disposal of aggregation-prone proteins.

To avoid the accumulation of damaged proteins, cells developed two major degradative pathways, the UPS and autophagy. The putative role of the UPS in preventing inclusion body formations is based on three fundamental observations. The aggregated proteins are heavily ubiquitinated, i.e. marked for proteasomal degradation, several inclusion bodies (including MDBs) contain proteasome subunits and proteasome inhibition induces aggregate formation in different models [7,50,51,94-96]. Despite such compelling evidence, the relationship between UPS and inclusion formation is still not conclusively clarified and UPS seems to be only one of more players involved in complex aggregate pathogenesis. This is best evidenced in the case of Parkinson disease, where UPS participates in clearance of α -synuclein but other routes are also employed [97]. Support for the relevance of UPS in Parkinson disease also comes from genetic

studies, since mutations in ubiquitin ligase named parkin and deubiquitylating enzyme UCHL1 are found in patients with familiar Parkinson disease [98,99]. However, parkin represents a multifaceted enzyme and not all its functions are related to UPS degradation [99]. Similarly, injection of proteasome inhibitors into rodents were shown to cause parkinsonian features [96], but several labs failed to replicate these findings [99]. In the liver, proteasome inhibitors induced MDB formation in DDC-primed mice as well as transgenic animals overexpressing keratin 8 [95,100].

While the function of UPS to degrade damaged proteins is clearly established, it may not be able to provide sufficient protein degradation in certain conditions. For example, a recent study showed that an accumulation of lysine 48-linked polyubiquitin chains, i.e. chains marking proteins for UPS degradation, is a common feature in patients and animal models of Huntington disease [101]. Several reasons may account for the inability of UPS to degrade and recycle proteins. For example, cellular proteasomes might be depleted [102] or the proteasome activity decreased as it happens during aging and oxidative stress [103,104]. UPS might be unable to degrade larger oligomers possibly because such substrates do not pass through its narrow entry pore [105]. Some studies even claim that UPS becomes inhibited by aggregates, although this theory remains controversial and is not yet confirmed *in vivo* [50]. UPS was also shown to be inhibited by accumulation of frameshifted UBB(+1) [41,42]. Alternatively, some protein species might accumulate because they are difficult to digest [106,107]. The inability to degrade a subset of proteins was recently observed in MDB-forming mice, which show accumulation of ubiquitinated proteins in the insoluble, but not the soluble pool. The accumulation of insoluble ubiquitinated proteins coincides with formation of high molecular weight species, thereby suggesting that these large structures might be indigestible by the UPS [74].

While UPS represents the preferred way to dispose short-lived proteins under normal conditions, autophagy steps in when UPS is overwhelmed, inhibited or unable to digest certain protein species [108,109]. For example, UPS inhibition or the presence of certain aggregates aug-

ments autophagic degradation [110,111]. Autophagy may degrade substrates which are difficult to be degraded by UPS, such as proteins with expanded polyglutamine repeats [112,113]. Therefore, stimulating autophagy represents a promising target in aggregate-forming diseases, which can decrease the amount of inclusions and prevent cell death [108,113]. Similarly to polyglutamine diseases, autophagy is up-regulated during MDB formation in mice and further stimulation can prevent MDB formation *in vivo* [114]. A potential advantage of autophagic vs. UPS degradation is the fact that autophagy may also degrade smaller aggregates [97]. However, similar to the UPS, autophagic activity decreases during aging and may be impaired in certain disease conditions [109,115].

P62 promotes protein sequestration and facilitates proteins degradation.

P62 is one of the most abundant inclusion body constituents and was frequently implicated in aggregate formation [4,46, 116-120]. It represents on the one hand a linker molecule involved in NF κ B as well as other signalling pathways, and on the other hand, a ubiquitin-binding protein which interacts via its UBA-domain with species ubiquitinated via lysine 63. Although this modification is not typically associated with protein degradation, p62 has been shown to shuttle substrates to both proteasomal and autophagic machineries [47-49,121,122]. Under normal condition, the action of p62 seems to be protective, since it prevents accumulation of toxic, aggregation-prone proteins [49,123]. However, when autophagy is inhibited, it favours aggregate formation and is associated with increased oxidative stress, probably through stabilization of toxic protein intermediates [118].

Cell culture experiments showed that p62 is involved in MDB formation and that its ubiquitin binding site is a necessary prerequisite for this process [18]. For example, the combined transfection of p62, keratin 8 or 18 and ubiquitin resulted in aggregates with typical MDB ultra-structure; such inclusions, however, did not form when only K8, K18 and ubiquitin were transfected alone or in combination [18]. Furthermore, in DDC-primed hepatocytes, p62 inhi-

bition attenuated, whereas p62 overexpression enhanced MDB formation [124]. *In vivo* studies are needed to confirm the cell culture data.

Aggresomes and sequestosomes are terms describing the process of aggregate formation.

Inclusion body formation *in vivo* does not simply result from coalescence of misfolded proteins. For example, two aggregation-prone proteins expressed in the same cell do not necessarily co-aggregate together, but can form independent entities [125]. Cell culture experiments suggested that small protein aggregates form in the cell periphery first and are then transported via microtubule tracks to a central disposal place in the vicinity of the centrosome [14,126,127]. The structure resulting from this “aggregation of aggregates” is termed aggresome. It co-localizes with centrosome markers such as pericentrin or γ -tubulin and is surrounded by intermediate filaments displaying a cage- or ring-like arrangement [14,126-128]. In addition to sequestering potentially toxic proteins, aggresomes are thought to aid protein degradation by bringing them to a place rich in chaperones and proteasome subunits. Aggresome formation also likely facilitates autophagic degradation [129-131]. On the other hand, aggresome formation may lead to disorganization of microtubule arrays and disruption of the Golgi apparatus [132].

Among naturally occurring inclusions, Lewy bodies seem to resemble aggresomes [128], since they co-localize with centrosome markers and contain radiating intermediate filaments. The sequential process of aggresome formation is also replicated in cell culture and supported by the presence of small aggregates in neuronal processes (termed as Lewy neurites), which may represent the primary aggregates seen in aggresome formation [133,134]. On the other hand, neither mutant tau in neurofibrillary tangles nor mutant huntingtin seem to form aggresomes [5]. Similarly, MDBs do not co-localize with γ -tubulin, even though their formation in primary hepatocytes is inhibited by antimicrotubule agents [87,135].

The term "sequestosome" was proposed by Shin to designate a cytoplasmic storage compartment in the cell, not delimited by membranes, containing p62 in combination with polyubiq-

ubiquitinated proteins [136]. Similarly, to aggresomes, the author suggested that proteins stored in sequestosomes are upon proper signals released for proteasomal (or autophagic) degradation. This was later confirmed by several studies highlighting the importance of p62 for both proteasomal and autophagic degradation [48,49,137]. Since p62 is a common constituent of multiple human inclusion bodies and since these aggregates are usually ubiquitinated (Table 1; [4,46]), the term “sequestosome” is more suitable than “aggresome” to describe the aggregates seen in multiple human disorders [138]. Importantly, both terms are not exclusive in that some inclusions comply with both definitions [137].

Oligomers rather than macroscopic aggregates seem to be the toxic species.

One important aim of our review is to point out that the protein aggregation process follows the same rules independent of the tissue involved. Therefore, insights into MDB pathogenesis may enhance our understanding of aggregate formation in multiple other disorders and - vice versa -MDB research can greatly benefit from findings obtained when studying non-hepatic aggregates. As an example, findings from non-hepatic aggregates shaped our view of MDB formation process and will likely be helpful in deciphering the MDB significance in general. To that end, formation of large protein inclusions was long thought to be detrimental in multiple ways such as interfering with cellular transport processes (e.g., shown with microtubule-dependent transport in neurons), depriving the cell of vital components trapped in the aggregates or inhibiting the protein degradation machinery [104,139-143]. However, recent studies argue that large aggregates are biologically inert or even protective. For example, presence of MDBs per se does not seem to compromise hepatocyte viability in vivo and of transfected tissue culture cells in vitro [4,18]. In Parkinson and Huntington disease, there is little correlation between the presence of Lewy bodies/huntingtin aggregates and the extent of cell death [144,145]. Recently, Arrasate et al. [146] performed long-term monitoring of cells using an automated microscope and showed that the ones forming aggregates are less likely to die. Accordingly, a chemical compound, which promotes inclusion formation, lessened the toxic-

ity of aggregation-prone proteins in cellular models of Huntington and Parkinson disease [147].

This led to the current understanding that small oligomers/protofibrils rather than huge aggregates are the toxic species. This is best evidenced in Alzheimer's disease. For example, injection of A β into brain of rats resulted in toxic effects, which could be alleviated by co-injection of an oligomer-specific antibody [148]. Accordingly, both pathogenic α -synuclein mutations and the A β 42 form protofibrils more rapidly than non-pathogenic proteins [149,150]. In addition, dopamine can build adducts with α -synuclein, thereby stabilizing their oligomeric structure [151]. The latter observation may explain the selective dopaminergic neurotoxicity of α -synuclein in Parkinson disease [151]. The toxicity of oligomeric species may be due to formation of pore-like structures, which can be formed by A β , α -synuclein as well as polyglutamine peptides [152,153]. In addition, non-fibrillar α -synuclein inhibits proteasomal activity and oligomeric species might be generating reactive oxygen species through interactions with redox-active metal ions [154,155].

Concluding remarks.

Our review summarizes the recent advances in our understanding of MDB pathogenesis and shows how they relate to observations made in other aggregates. MDBs are hepatocellular inclusions while the most widely studied aggregates are typically found in neurodegenerative disorders. Despite the inherent differences between the tissues involved, the inclusions share common molecular constituents, analogue posttranslational modifications, alterations in protein expression and the involvement of protein repair and degradation machineries. Therefore, aggregates seen in different human disorders should be considered an extended family, which has a common foundation, but contains members with distinct personalities. Furthermore, MDBs represent an interesting tool to test the effect of pharmaceutical interventions on aggregate formation. This might be especially useful to evaluate the role of UPS in inclusion pathogenesis, as for example the proteasome inhibitor bortezomib is already FDA-approved

for treatment of multiple myeloma.

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Table 1. Examples of intracellular protein aggregates (inclusions) and corresponding human diseases

Subgroup*	Inclusion body	Major protein constituent	Ubiquitin	P 62	Cell type	Disease
IF-related inclusions	Mallory-Denk body	K8/K18	+	+	Hepatocyte	ASH, NASH, etc
	Desmin bodies	Desmin	+	+	Myocytes	Desminopathies
	NF inclusions	NF	+/-	N.D.	Neurons	NIFID
	Rosenthal fibers	GFAP, vimentin	+	+	Astrocyte	Alexander's disease etc
Tauopathies	Neurofibrillary tangles	Tau	+	+	Neurons	Alzheimer's disease
	Neurofibrillary tangles	Tau	+	+	Neurons	PSP
	Pick body	Tau	+	+	Neurons	Pick's disease
Synucleinopathies	Lewy bodies	α -Synuclein	+/-	+	Neurons	Parkinson's disease
	GCI	α -Synuclein	+/-	+	Oligodendrocytes	Multiple system atrophy
	Lewy bodies	α -Synuclein	+/-	+	Neurons	Dementia with Lewy bodies
Polyglutamine Diseases		Huntingtin	+	+	Neurons	Huntington's disease
		Atrophin-1	+	N.D.	Neurons	DRPLA
		Ataxin-1	+	N.D.	Neurons	Spinocerebellar ataxia 1
TDP-43 proteinopathies		TDP-43	+	+/-	Neurons, oligodendrocytes	FTLD-U
		TDP-43	+	+	Neurons	ALS
Others	-	APP, Tau, others	+	N.D.	Myocytes	Inclusion body myositis
	IHB	p 62	+/-	+	Tumor cells	HCC
	IHB	p 62	+/-	+	Hepatocytes	ICT
	Bunina bodies	SOD 1	+	+	Neurons	Familial ALS

* The classification into groups is based on the predominant aggregate constituents. For example, Lewy bodies belong to synucleinopathies even though they contain minor quantities of neurofilaments, i.e. members of IF protein family [1].

ALS, amyotrophic lateral sclerosis; APP, Amyloid- β precursor protein; ASH, alcoholic steatohepatitis; DRPLA; Dentatorubral pallidoluysian atrophy; FTL-D-U, Frontotemporal lobar degeneration with ubiquitin positive, tau-/ α -synuclein negative inclusions; GCI, glial cytoplasmic inclusions; IF, intermediate filament; K, keratin; NASH, non-alcoholic steatohepatitis; N.D., not determined; NF, neurofilament; NIFID, neuronal filament inclusion disease; PSP, Progressive supranuclear palsy; IHB, intracellular hyaline bodies; HCC, hepatocellular carcinoma; ICT, idiopathic copper toxicosis; SOD 1, superoxide dismutase 1; TDP-43, TAR-DNA binding protein 43

Table 2. Selected murine models of human aggregate forming diseases

Inclusion body	Animal model	Reference
NFT	wt/mt Tau alone/with other proteins	[24]
Lewy bodies	wt/mt α -synuclein	[24]
Lewy bodies	Administration of rotenone, MPTP, MPP ⁺ etc.	[22]
GCI	wt α -synuclein	[24]
MDBs	Feeding with DDC/griseofulvin	[7]
Htt aggregates	Truncated/full-length form of mt htt	[23]
Rosenthal fibers	wt/mt GFAP	[13]

GCI, Glial cytoplasmic inclusions; GFAP, Glial fibrillary acidic protein; htt, huntingtin;

MDBs, Mallory-Denk bodies; mt, mutant; NFT, neurofibrillary tangles; wt, wild type

FIGURE LEGENDS:

Figure 1. Histological and ultrastructural features of alcoholic and non-alcoholic steatohepatitis. (a,b) Alcoholic steatohepatitis is characterized by steatosis, ballooning of hepatocytes, MDB formation (arrows in a,b), neutrophil-granulocytic inflammation as well as pericellular fibrosis. (c) At the ultrastructural level, MDBs consist of irregularly arranged filamentous rods (type II according to Yokoo, upper panel) and sometimes contain an electron-dense granular core highlighted by asterisk (type III according to Yokoo, lower panel). (d-f) Ballooning degeneration of hepatocytes, often associated with pericellular fibrosis (arrowheads in f), and MDB appearance is also seen in non-alcoholic steatohepatitis (arrows in d,e). (g,h) In idiopathic copper toxicosis, ballooned MDB-containing hepatocytes are often surrounded and even penetrated by neutrophils (arrow in g). Furthermore, intracellular hyaline bodies (IHBs) may be present even within the same cell and in close association with MDBs (arrows in h); a,d,e,g,h, hematoxylin and eosin staining; b,f, chromotrope aniline blue staining.

Figure 2. IHBs and MDBs are observed in hepatocellular carcinoma and Idiopathic copper toxicosis. Samples from trabecular hepatocellular carcinoma (a,b) and Idiopathic copper toxicosis (c-g) were visualized with chromotrope aniline blue staining (a) or immunohistochemically labelled with antibodies to keratins (c,d), p62 (b,f,g) and ubiquitin (e). MDBs as well as IHBs react with ubiquitin (e) and p62 (b,f, g) antibodies (IHBs are indicated by arrows in e-g, appear also as red inclusions in a). Keratin antibodies strongly decorate MDBs (arrowheads in c, d), whereas IHBs are typically keratin-negative (arrows in c, d). However, some IHBs contain occasional keratin granules suggesting that IHB material (p62) provides a matrix for incorporation of keratins. Note that hepatocytes within the presented Idiopathic copper toxicosis sample contain both MDBs and IHBs, sometimes in close proximity, whereas the presented trabecular hepatocellular carcinoma harbours only IHBs.

Figure 3. MDBs regularly stain with antibodies to ubiquitin, keratins and p62. Liver sections from a cirrhotic patient with alcoholic steatohepatitis (a,b), a patient with primary biliary cirrhosis (c) and non-alcoholic steatohepatitis (d-f) were stained with antibodies to keratins 8 and 18 (a,b,d), ubiquitin (e) and p62 (c,f). MDBs are strongly labelled by all three antibodies (e; arrows in a, arrowheads in d). Note that ballooned hepatocytes containing MDBs show greatly diminished or even absent cytoplasmic keratin immunostaining, whereas normal-sized hepatocytes retain their keratin cytoskeleton (arrows in a, arrowheads in d).

Figure 1

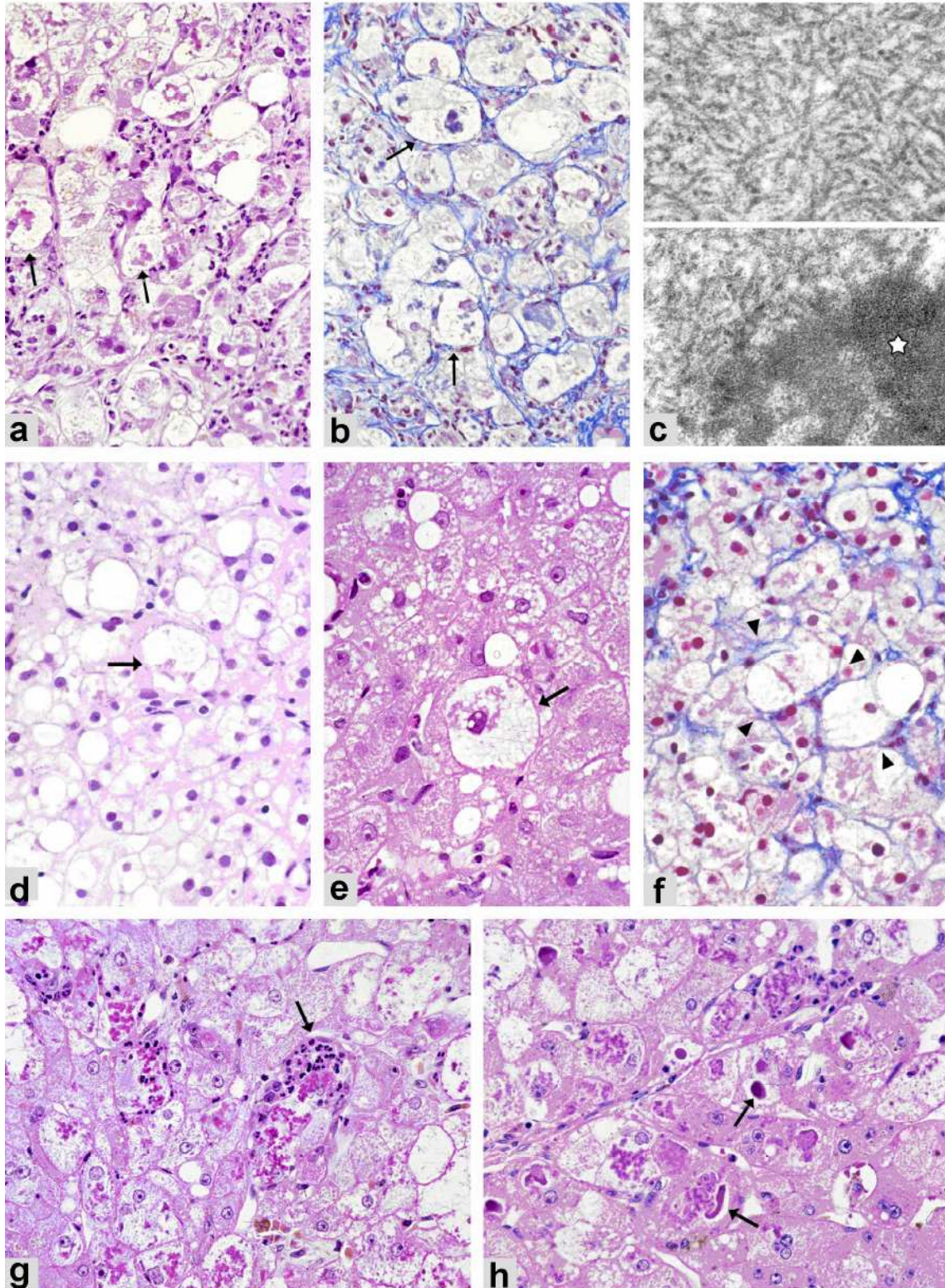


Figure 2

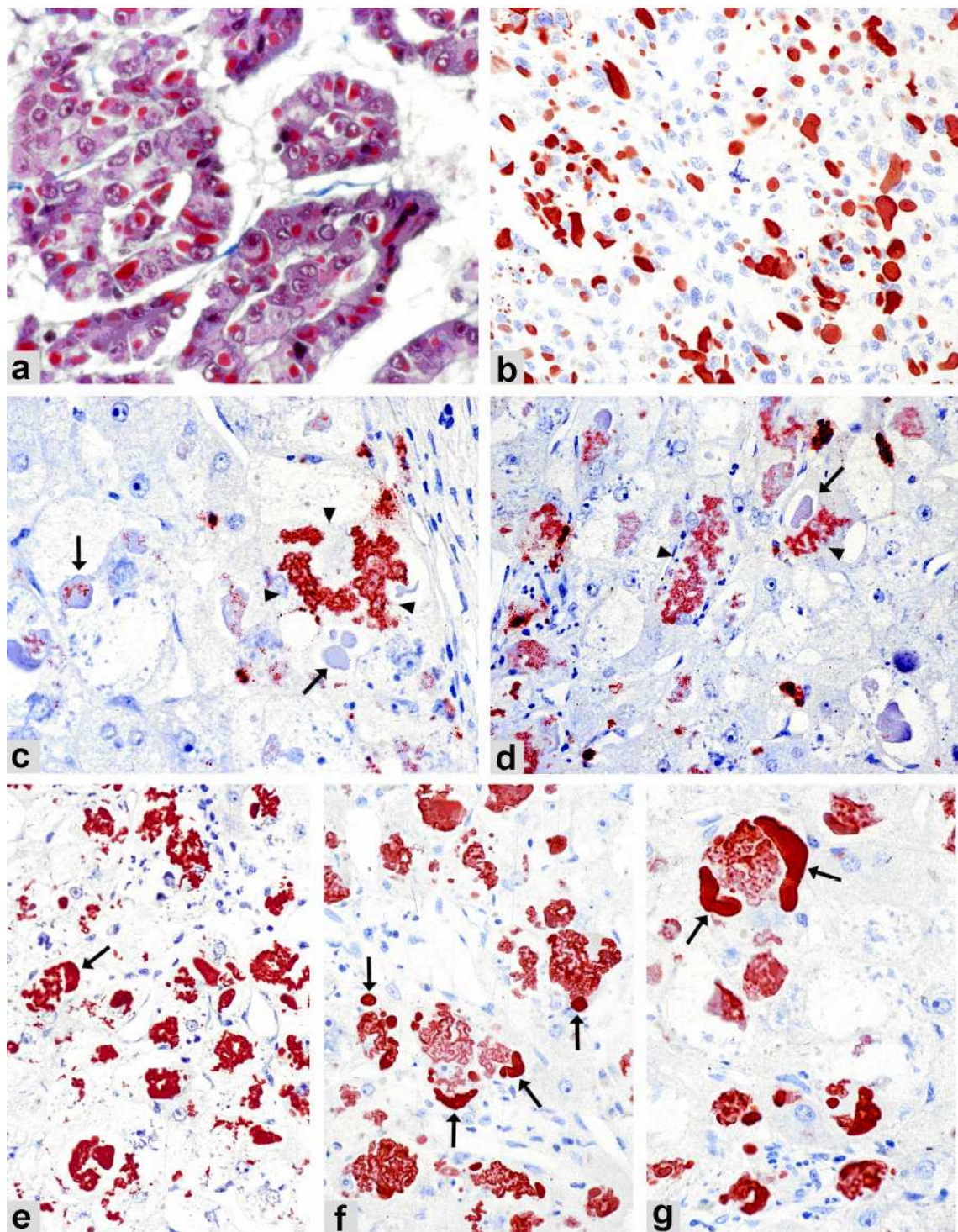


Figure 3

