Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Proteome-Scale Mapping of Perturbed Proteostasis in Living Cells

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Proteinopathies are degenerative diseases in which specific proteins adopt deleterious conformations, leading to the dysfunction and demise of distinct cell types. They comprise some of the most significant diseases of aging—from Alzheimer's disease to Parkinson's disease to type 2 diabetes—for which not a single disease-modifying or preventative strategy exists. Here, we survey approaches in tractable cellular and organismal models that bring us toward a more complete understanding of the molecular consequences of protein misfolding. These include proteome-scale profiling of genetic modifiers, as well as transcriptional and proteome changes. We describe assays that can capture protein interactomes in situ and distinct protein conformational states. A picture of cellular drivers and responders to proteotoxicity emerges from this work, distinguishing general alterations of proteostasis from cellular events that are deeply tied to the intrinsic function of the misfolding protein. These distinctions have consequences for the understanding and treatment of proteinopathies.

Proteinopathies are degenerative diseases in which specific proteins aggregate in distinct cell types. The most common and devastating diseases of the group include the neurodegenerative disorders, Alzheimer's disease (AD), and Parkinson's disease (PD). But beyond these, type 2 diabetes mellitus, inclusion body myopathy, and systemic amyloidoses are also proteinopathies. Outside of the rare condition, transthyretin amyloidosis (Maurer et al. 2018), no disease-modifying treatments exist for these diseases, and the public health significance cannot be overstated. They collectively cost the United States around $500 billion annually, and AD alone affects 4.5 million Americans.

Increasingly, it is becoming appreciated that a "one-size-fits-all" treatment strategy may not be feasible for proteinopathies. Substantial variability among individual genomes, cells, and protein conformations may lead to unique disease processes in individual patients (Jarosz and Khurana 2017). Broadly speaking, these diseases are thought to arise from an interplay between genetic factors and environmental "hits" that

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Editors: Richard I. Morimoto, F. Ulrich Hartl, and Jeffery W. Kelly
Additional Perspectives on Protein Homeostasis available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a034124
Cite this article as *Cold Spring Harb Perspect Biol* 2020;12:a034124

result in accumulation of misfolded proteins (Fig. 1A). In some informative cases, aggressive early-onset disease is driven deterministically by a dominant mutation or locus multiplication in the gene encoding the aggregating protein, tying that protein aggregation process causally to the disease. Once the protein-folding pathology is induced, there are toxic responses within the cell to the misfolding and mislocalization of proteins (Fig. 1B). A distinction can be made between responses that relate to the intrinsic function of the misfolded protein and general responses to misfolded proteins, the latter commonly referred to as disruption of the proteostasis machinery (Balch et al. 2008; Labbadia and Morimoto 2015). Finally, there may be toxic interactions among cells. For neurodegenerative diseases, these interactions may result in disruption of neuronal circuitry, abnormal interactions between neurons and glial or immune cells

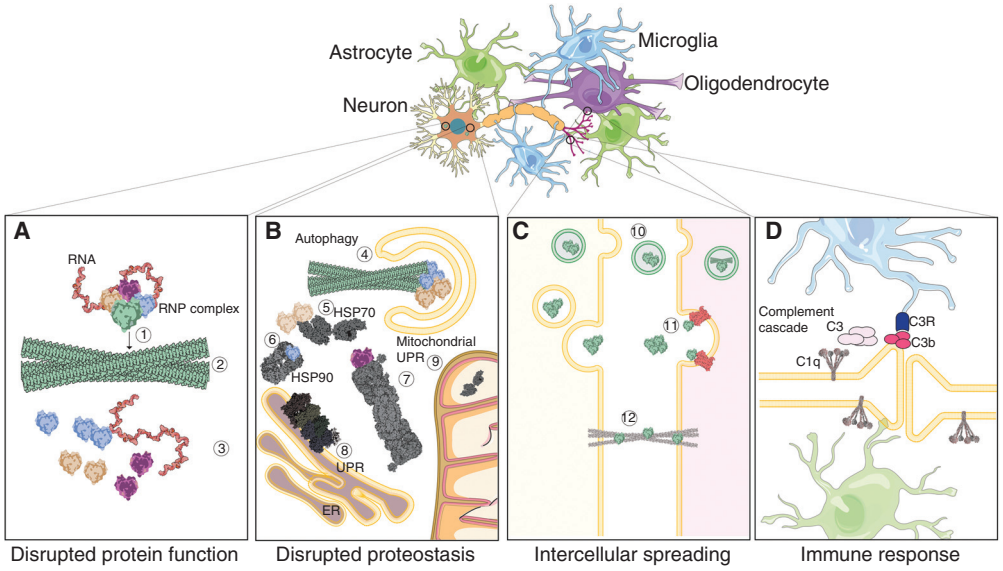

Figure 1. A summary of consequences of protein aggregation in proteinopathies. The brain is comprised of neurons and glial cells (astrocytes, oligodendrocytes, and microglia). Glio–neuronal interactions are critical for appropriate brain development and increasingly recognized as pivotal for neurodegeneration also. Here, we illustrate four consequences of protein misfolding: (*A*) Direct consequences of aggregation of a protein. In this example, one (green) protein component of a ribonucleoprotein (RNP) complex aggregates as a result of an environmental challenge, deterministic point mutations, or various genetic factors (*1*). This leads to formation of amyloid fibrils of the protein (*2*), thus relieving other protein/RNA members from the complex (*3*). Toxicity results from either loss-of-function of the RNP complex, or from toxic gain-of-function of misfolded or mislocalized RNP complex proteins. (*B*) Effects of protein aggregation on the cellular homeostasis machinery. Members of protein complexes require each other for correct folding and chaperoning. The first member of the complex forms an amyloid aggregate and is degraded in this illustration through engulfment by autophagic membranes (*4*). A second with exposed hydrophobic cores attracts Hsp70 for refolding (*5*). Another protein (blue) attracts Hsp90 to exposed hydrophilic surfaces (*6*). Another member (purple) is ubiquitinated and degraded by the proteasome (*7*). Sequestration of these chaperones or overwhelmed protein degradation pathways can lead to a vicious cycle that results in misfolding and abnormal accumulation of other proteins, eventually triggering an unfolded protein response (UPR) in the endomembrane system (*8*) or mitochondria (*9*). (*C*) Intercellular spread or self-templating of amyloid fibrils. Fibrils can be secreted (*10*) or taken up from the extracellular matrix by receptor-mediated endocytosis (*11*). Nanotubes between cells can also mediate transfer of fibrils from one cell to another (*12*). (*D*) Neuroinflammation and complement activation by neurons (via C1q expression) and glia results in C3b deposition at the surface of neurons. Microglia expressing C3 receptors can engulf such neurons, thus contributing to neurodegeneration. ER, endoplasmic reticulum.

(Fig. 1D; Liddelow et al. 2017; Salter and Stevens 2017). These may be driven by the pathologic templating and propagation of misfolded protein conformers in prion-like fashion between cells (Fig. 1C; Guo and Lee 2014; Jarosz and Khurana 2017).

Even within this elemental view of neurodegenerative disease pathogenesis, there are many unanswered questions. We discuss some of these in this review, including:

- What biological approaches can help us complete the genetic "map" of proteinopathies? Genetic factors have been pivotal in directing rational research in the proteinopathy field, but most of the heritability of these diseases remain unexplained.
- Are genetic factors that inform us about disease risk and initiation equally informative about mechanisms of disease progression? Or are important cellular and intercellular responses not captured by genetic analysis? Vulnerability factors involved in disease risk or initiation might be good preventative targets, but may not be good targets in late stages of the disease. On the other hand, if these diseases progress in an orderly fashion, distinct cell types may be in different disease states at any given time, and thus there may be a scope for preventative and reversal strategies simultaneously.
- How can we develop methods to systematically understand and target different toxic protein conformations? Much attention has been given to distinct biophysical forms, or conformers, of toxic proteins, and methods are sorely needed to tractably model these in the laboratory.

The answers to some of these questions may lie in methods that can capture systematically the molecular consequences of protein misfolding. A reductionist approach could plausibly use a variety of cellular assays to fully deconstruct cellular consequences in cellular models. In time, this could be achievable in different cell types and in more complex models to examine intercellular interactions. In this review, we describe distinct methods we and others have employed for such analysis. We focus on neurodegenerative proteinopathy. Some emphasis is placed on α-synuclein, the protein that misfolds in synucleinopathies including PD, because it has been extensively analyzed with these methods.

MAPPING PROTEOTOXICITY: GENETIC DETERMINANTS AND CELLULAR RESPONSES

To study proteinopathies, we can, on the one hand, systematically catalog the genetic requirements for proteotoxicity. This approach aims to identify the cellular setup—through forcing the absence or elevated expression of a specific gene—that exacerbates ("enhances") or ameliorates ("suppresses") toxicity associated with a misfolding protein. Most often in model systems, the proteotoxicity is induced by overexpression of a protein that is prone to misfolding, in accordance with the customary autosomal dominant and toxic gain-of-function mechanisms in proteinopathies. In a genome-wide screen, gene knockouts/deletions or gene overexpression for approximately every coding sequence are queried for their effect on a proteotoxic phenotype. However, genetic screens are typically not designed to report on cellular responses to protein misfolding. This is because the gene deletion or overexpression generally occurs before, or concomitant with, expression of the toxic protein. Rather, cellular response can be investigated by systematically measuring the changes that occur at the transcript and protein level (transcriptomics, proteomics) as a consequence of proteotoxicity.

To gain a global view of the cellular effects of protein misfolding, data sets from multiple "omics" approaches (e.g., genetic screens, transcriptomics, metabolomics, proteome-scale protein–protein interactions, ChIP-seq) can be integrated through bioinformatic methods (Fig. 2A; Tuncbag et al. 2016). Such in silico approaches can point to cellular pathway(s) most affected by a particular proteotoxic protein, and unveil relevant genes that might not have been recovered in individual screens, thereby increasing our understanding of the cytotoxic mechanism. As noted above, some of the genetic requirements or molecular consequences may

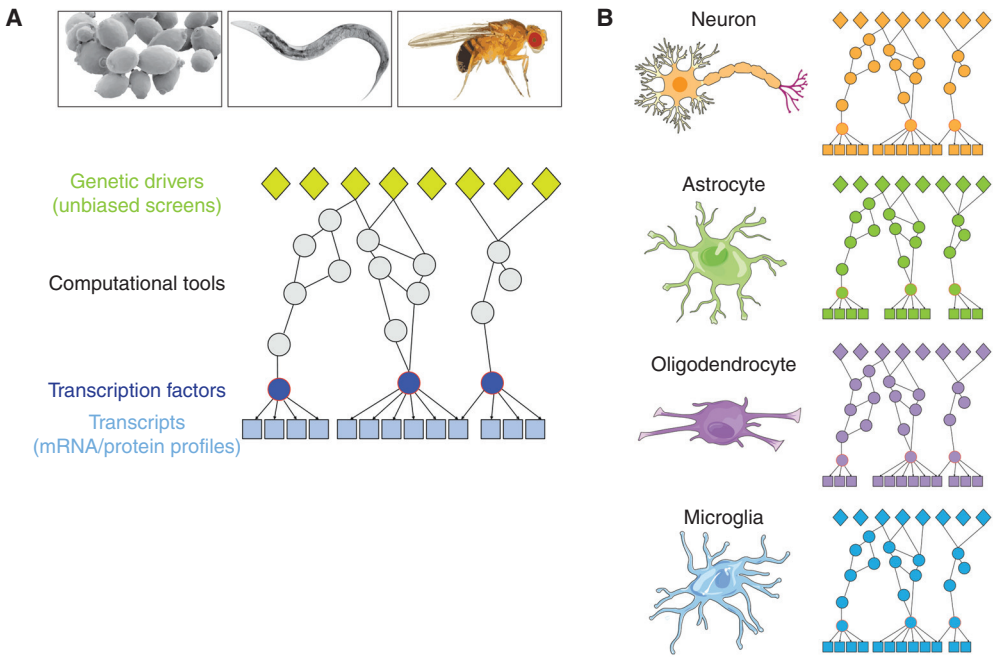

Figure 2. Building a global view of cellular response to proteotoxicity. (*A*) Bioinformatic methods can be applied to bridge data sets from genome-wide genetic screens and transcription profiles, thereby integrating genetic drivers and cellular response to proteotoxicity, and augmenting the list of molecular candidates implicated in the cytotoxic mechanism. Network-building algorithms can identify "hidden nodes" that are not recovered in individual screens. Such approaches are currently feasible in model organisms where multiple "omics" data sets are already available, but can one day be applied to human disease-relevant cell types (e.g., human neurons) as genome-wide genetic and molecular data sets become available. (*B*) Different cell types (e.g., neurons, astrocytes, oligodendrocytes, microglia) likely exhibit different molecular network profiles due to differential gene expression, so within proteotoxicity models, it will be important to obtain cell-specific data and build cell-type-specific networks. mRNA, messenger RNA.

reflect a general proteostatic response within a cell, whereas others relate to the intrinsic function of the protein that misfolds.

Genetic Screens

In proteinopathies, the principal aggregating protein (or proteins) are distinct in each disease, for example, α-synuclein in PD, β-amyloid and tau in AD, huntingtin in Huntington's disease (HD), TDP-43 in amyotrophic lateral sclerosis (ALS), prion protein (PrP) in prion diseases (e.g., Creutzfeldt–Jakob disease), and islet amyloid polypeptide (IAPP) in type 2 diabetes. Many of these proteinopathies have been modeled by transgenic overexpression in genetically tractable model systems, including baker's yeast cells (Table 1; for review, see Khurana and Lindquist 2010). The tractability of yeast cells is offset by lack of specialized neuronal features, and tractable metazoan organisms, including *Caenorhabditis elegans* and *Drosophila melanogaster*, have helped fill in that significant gap. Genome-wide and candidate genetic screens in these model organisms have been facilitated by high-throughput technologies such as knockout or overexpression libraries for every protein-coding gene (*Saccharomyces cerevisiae*), RNA interference (*C. elegans*, *D. melanogaster*), and transgenic stocks with P-element insertion mutations (*D. melanogaster*). Published data sets of genetic modifiers from high- and low-throughput screens against nine neurodegenerative proteotoxicities in yeast, flies, and worms have been

Table 1. Modeling human proteinopathies in genetically tractable model organisms

Misfolding protein	Proteinopathy	Model organism	References
α-Synuclein	Parkinson's disease	*Saccharomyces cerevisiae*	Outeiro and Lindquist 2003; Willingham et al. 2003; Dixon et al. 2005; Zabrocki et al. 2005; Sharma et al. 2006
		Drosophila melanogaster	Feany and Bender 2000; Auluck et al. 2002
		Caenorhabditis elegans	Lakso et al. 2003; Cao et al. 2005; Kuwahara et al. 2006; Hamamichi et al. 2008; van Ham et al. 2008
β-Amyloid	Alzheimer's disease	*S. cerevisiae*	Bagriantsev and Liebman 2006; Caine et al. 2007; von der Haar et al. 2007; Treusch et al. 2011; D'Angelo et al. 2013
		D. melanogaster	Finelli et al. 2004; Iijima et al. 2004; Crowther et al. 2005; Iijima et al. 2008
		C. elegans	Link 1995; Link et al. 2003
tau	Alzheimer's disease, FTDP-17	*S. cerevisiae*	Vandebroek et al. 2005
		D. melanogaster	Williams et al. 2000; Wittmann et al. 2001; Jackson et al. 2002; Blard et al. 2007
		C. elegans	Kraemer et al. 2003; Miyasaka et al. 2005; Brandt et al. 2009
huntingtin	Huntington's disease	*S. cerevisiae*	Krobitsch and Lindquist 2000; Muchowski et al. 2000; Duennwald et al. 2006a,b; Solans et al. 2006; Kayatekin et al. 2014
		D. melanogaster	Jackson et al. 1998
		C. elegans	Faber et al. 1999; Parker et al. 2001
Polyglutamine (polyQ)	Polyglutamine-expansion disorders (e.g., spinocerebellar ataxia type 1, type 3)	*S. cerevisiae*	Fernandez-Funez et al. 2000; Meriin et al. 2002, 2003
		D. melanogaster	Warrick et al. 1998; Kazemi-Esfarjani and Benzer 2000; Marsh et al. 2000
		C. elegans	Satyal et al. 2000; Brignull et al. 2006
TDP-43	Amyotrophic lateral sclerosis	*S. cerevisiae*	Johnson et al. 2008; Elden et al. 2010; Kim et al. 2014
		D. melanogaster	Kim et al. 2014
FUS	Amyotrophic lateral sclerosis	*S. cerevisiae*	Fushimi et al. 2011; Ju et al. 2011; Kryndushkin et al. 2011; Sun et al. 2011
SOD1	Amyotrophic lateral sclerosis	*S. cerevisiae*	Rabizadeh et al. 1995; Ticozzi et al. 2011
		D. melanogaster	Watson et al. 2008; Bahadorani et al. 2013; Gallart-Palau et al. 2016; Şahin et al. 2017
		C. elegans	Oeda et al. 2001; Gidalevitz et al. 2009; Wang et al. 2009
Islet amyloid polypeptide (IAPP)	Type 2 diabetes	*S. cerevisiae*	Kayatekin et al. 2018

cataloged and annotated in NeuroGeM (Na et al. 2013). Here, we distill four key insights from screens in proteinopathy models.

First, genetic screens in simple model organisms have uncovered disease-relevant biology. Homologs of known human disease risk factors have emerged as genetic modifiers (for reviews, see Khurana and Lindquist 2010; Kryndushkin and Shewmaker 2011; Tenreiro et al. 2017). For example, genetic screens in the yeast synuclein models recovered orthologs of multiple confirmed or putative risk factors for human parkinsonism, including *ATP13A2/PARK9*, *RAB7L1/PARK16*, *VPS35/PARK17*, *EIF4G1/PARK18*, and *SYNJ1/PARK20*, with validation of the findings in models from worm to rodent to induced pluripotent stem cell (iPSc)-derived neurons (Gitler et al. 2009; Dhungel et al. 2015; Khurana et al. 2017). Similarly, a genome-wide screen against β-amyloid (Aβ) toxicity in yeast recovered homologs of multiple risk factors of sporadic AD (including *YAP1802*, the yeast homolog of *PICALM*) as suppressors of toxicity, and these were also found to modify Aβ toxicity in worms and primary rodent neuronal models (Treusch et al. 2011). Remarkably, the identification of the yeast homolog of *ATXN2* as a modifier of TDP-43 toxicity in yeast led to the identification of this gene as a robust risk factor for ALS in humans (Elden et al. 2010) and a modulator of neurodegeneration in a mouse model of that disease (Becker et al. 2017).

Second, the same gene modifiers are not necessarily recovered in genetic screens of the same proteotoxicity in different model organisms, but often the same cellular pathways emerge. For example, genes in the vesicle trafficking pathway appear as modifiers of α-synuclein toxicity in both yeast and *C. elegans*, even if precisely the same genes do not emerge as modifiers in the different models. A case in point is the gene encoding the vacuolar assembly/sorting protein VPS41, which emerged as a modifier of α-synuclein pathology in an RNAi screen in *C. elegans* and was subsequently validated to be neuroprotective (Hamamichi et al. 2008; Ruan et al. 2010; Harrington et al. 2012). The homolog of *VPS41* did not emerge as a genetic modifier of α-synuclein in yeast genetic screens to date, although *YCK3*, which encodes the kinase that phosphorylates Vps41, as well as a host of other genes involved in endolysosomal trafficking were identified as modifiers (Khurana et al. 2017). The same genes may fail to be recovered in different screens of the same proteotoxicity due to technical limitations of the screens (i.e., if the screens have low genome coverage), or for biological reasons (e.g., redundant gene function, or differences in genetic requirement across strain backgrounds in the same species).

Third, there is little overlap in genetic modifiers against different proteotoxicities (e.g., α-synuclein, Aβ, TDP-43, tau, polyglutamine-expansion models), suggesting that each protein exerts different intrinsic genetic requirements and/or cellular responses despite a common protein misfolding pathology in human disease (Jarosz and Khurana 2017; Khurana et al. 2017). For example, genetic modifiers against α-synuclein toxicity in yeast are enriched in processes related to lipid metabolism and vesicle trafficking (Outeiro and Lindquist 2003), whereas modifiers against polyglutamine-expanded huntingtin are enriched for genes related to stress response, protein folding, and ubiquitin-dependent protein catabolism (Willingham et al. 2003). Along the same lines, kinases and phosphatases are the major class of genetic modifiers identified in a *D. melanogaster* model of tauopathy, but not of the polyglutamine disease model *SCA3* (Shulman and Feany 2003). These observations are consistent with hyperphosphorylated and aggregated tau as hallmark pathology in AD and frontotemporal dementia (FTD). Meta-analysis of published genetic screen data sets indicate that this trend holds true when comparing polyglutamine disease models (e.g., HD, SCA1, SCA3, SCA7) versus AD models (Aβ): polyglutamine disease models share many genetic modifiers that are not seen in AD models, consistent with a common underlying biology linking polyglutamine expansion (Na et al. 2013).

Fourth, despite the common molecular dysfunction (protein misfolding), genes in the proteostasis network do not appear as a major class of modifiers overlapping proteotoxicities (e.g., α-synuclein, Aβ, and TDP-43) (Jarosz and

Khurana 2017). Although proteostasis network components that are broadly protective against misfolded and aggregation-prone proteins do emerge as modifiers (Nollen et al. 2004; Silva et al. 2011), it is estimated that such genes account for only 3% of genetic modifiers across yeast, worm, and flies (Na et al. 2013). There are many possible explanations for their underrepresentation, including the setup strategy for genetic screens, the essential functions of certain genes in this pathway, and the redundancy of others. We return to this point again below.

Finally, the advent of CRISPR-Cas9 genome editing has extended the reach for high-throughput genetic screens against proteotoxicities (e.g., Kramer et al. 2018). For example, most genome-wide screens to date have been limited to looking at single genetic modifiers. In practice, combinatorial effects could be equally critical and valuable for functional genomics. This would be of particular interest for genes with redundant function, whereby knockdown of one gene is compensated by a second gene, masking the genetic modifier effect of the first. Recently, screening technology that combined randomized guide RNAs (gRNAs) with CRISPR-Cas9-based transactivation (so-called "CRISPRa" technology) was used for unbiased screening of transcriptional networks that modify α-synuclein toxicity in yeast (Chen et al. 2017). In this approach, the gRNA recruits a transactivating protein complex to the 5′ untranslated region (UTR) of a number of gene targets, enabling combinatorial effects of gene expression to be probed. Genes with altered expression in the presence of top gRNA hits from the screen were then shown to suppress α-synuclein toxicity in yeast and neuronal models. Most of the genes recovered in this way were not previously identified in single-gene overexpression or deletion screens (but tellingly had overlap in gene ontology [GO] categories). This study underscores the importance of orthogonal approaches to obtain a complete understanding of the cellular requirements and consequences of protein misfolding.

With CRISPR/Cas9-based approaches, whole-genome genetic screens previously conceivable only in tractable genetic organisms are now also within reach in human cells (Khurana et al. 2015). For example, Kramer and colleagues used the CRISPR-Cas9 system to conduct genome-wide knockout screens in human cell models of C9ORF72 dipeptide-repeat (DPR) protein toxicity associated with ALS and FTD (Kramer et al. 2018). Their cellular model of C9ORF72 DPR toxicity consisted of exposing K562 cells to proline-arginine and glycine-arginine synthetic polymers. Suppressors and enhancers of C9ORF72 DPR toxicity were validated in a secondary, pooled CRISPR-Cas9 screen in mouse primary neurons. Screen hits were enriched in endoplasmic reticulum (ER) stress, proteasome, nuclear transport, RNA processing, and chromatin modification pathways. One of the suppressors, *TMX2*, which encodes the ER-resident transmembrane thioredoxin protein, was further validated in human neurons. Knockdown of *TMX2* improved survival of motor neurons derived from a *C9orf72* ALS patient's iPScs (Kramer et al. 2018).

Transcriptomics

A systematic assessment of 179 environmental and genetic perturbations in yeast (an organism where such comprehensive data are uniquely available) surprisingly revealed that transcriptional responses are largely distinct from genetic modulators when yeast cells were subjected to distinct stressors or environmental perturbations (Yeger-Lotem et al. 2009). Genetic hits were biased toward genes that encode regulatory proteins, whereas transcriptional profiling hits were biased toward genes involved in metabolic processes. The same study confirmed this exclusive nature of genetic and transcriptional data sets in a yeast model of α-synuclein toxicity. Genetic modifiers were dominated by genes encoding vesicle trafficking proteins. In contrast, messenger RNA (mRNA) profiling revealed up-regulated genes with oxidoreductase activities, and down-regulated genes relating to mitochondrial activity and ribosomal response to stress (Yeger-Lotem et al. 2009; Su et al. 2010). Notably, down-regulation of genes related to mitochondrial activity (along with dysregulation of lipid, and membrane transport) was also detect-

ed by genome-wide expression profiling in a *D. melanogaster* transgenic model of α-synuclein toxicity (Scherzer et al. 2003), and in expression analysis of postmortem substantia nigra tissue of PD patients (Hauser et al. 2005; Mandel et al. 2005; Duke et al. 2006; Zheng et al. 2010). Gene-expression analyses in individuals affected by PD also revealed perturbation of other cellular functions including chaperones, ubiquitin-proteasome protein degradation, vesicle trafficking, iron transport, synaptic transmission, oxidative stress, and dopamine metabolism (Hauser et al. 2005; Mandel et al. 2005; Duke et al. 2006; Zheng et al. 2010; Borrageiro et al. 2018). At least in the case of PD and α-synucleinopathy, these findings reveal a surprisingly conserved difference between transcriptional profiles (dominated by metabolic and mitochondrial genes) and genetic modifiers (dominated by vesicle trafficking genes) from model organisms to PD patients.

Proteomics

Proteomics is another important component to consider when capturing the macromolecular environment in the presence of a perturbation. Many studies have conducted quantitative proteome profiling in the presence of proteotoxicity in cellular and animal models, and patient brain samples (for review, see Kasap et al. 2017). Notably, proteomic analyses of PD research models concur with transcriptional profiling data in detecting dysregulation of proteins associated with mitochondrial function compared to controls. This holds true in *Drosophila* or mouse models expressing transgenic α-synuclein A53T or A30P mutations, or neurotoxin-induced neurodegeneration in rodents (e.g., MPTP, 6-OHDA) (Poon et al. 2005; Xun et al. 2007a,b; Diedrich et al. 2008; Lessner et al. 2010). Proteins in other molecular pathways are also significantly altered in models of PD, including calcium metabolism, actin cytoskeleton, membrane trafficking, and ribosomal components (Kasap et al. 2017).

Differences in protein levels between pathologic and normal conditions can provide useful insights for discovery of disease biomarkers or therapeutic targets. Proteomic and genetic analyses can inform druggable targets when used in a coordinated fashion. A recent study utilized this approach to identify the molecular mechanism through which calcineurin and FK506 contribute to α-synuclein toxicity in yeast and rat α-synuclein models (Van der Perren et al. 2015; Caraveo et al. 2017). Calcineurin is a calcium-calmodulin dependent phosphatase. A thorough genetic analysis revealed that increased activity of calcineurin is associated with α-synuclein toxicity in cellular models, and the calcineurin inhibitor tacrolimus/FK506 accordingly rescued this toxicity (Caraveo et al. 2014). Calcineurin is inhibited by tacrolimus/FK506 through the formation of a ternary complex with the FK506-binding protein FKBP12 (Liu et al. 1991). Mass spectrometry-based phosphoproteomics analysis in the yeast model demonstrated that FKBP12 contributes to α-synuclein toxicity by regulating calcineurin activity and promoting dephosphorylation of proteins involved in endocytosis, vesicle trafficking, actin reorganization, and ion channel regulation (Caraveo et al. 2017). Conversely, inhibiting the functional interaction between FKBP12 and calcineurin with low doses of tacrolimus restored phosphorylation of calcineurin- and FKBP12-dependent substrates involved in vesicular trafficking in an in vivo rat model of PD, resulting in restored dopamine transporter trafficking and dopamine secretion at presynaptic terminals. At the organismal level, tacrolimus improved behavioral deficits related to α-synuclein toxicity (Van der Perren et al. 2015; Caraveo et al. 2017). In this case, proteomic analysis provided critical insights into mechanisms through which genetic and pharmacologic targets relate to proteotoxicities.

ResponseNet: Integrating Genetic and Transcriptional Data

Because genetic modifiers and transcriptional responses are frequently distinct (see above), computational methods have been used to combine these two data sets to develop a coherent view of the cellular response to a proteotoxicity. Yeger-Lotem et al. (2009) developed an algorithm (ResponseNet) to bridge the data obtained from genome-wide screens and transcriptional

profiling, and thereby capture a unified cellular map of the proteins and genes responding to cellular perturbation. ResponseNet identifies molecular-interaction pathways connecting genetic screen hits and differentially expressed genes through known protein–protein interactions (Lan et al. 2011; Basha et al. 2013). This method can theoretically be extended to other types of "omics" data sets across different organisms and proteotoxicities (Fig. 2A). Furthermore, different cell types will likely exhibit different molecular network profiles, so within proteotoxicity models it will be important to generate cell-type-specific networks (Fig. 2B) and to use molecular interaction data that are cell-type and tissue-specific. Basha and colleagues have recently assembled such tissue-specific molecular interaction data in a database called DifferentialNet (Basha et al. 2018). Cell-type-specific networks may reveal differences in network connectivities that provide mechanistic insight into differential vulnerability to proteotoxicity across cell types, a unifying but poorly understood feature of proteinopathies.

MAPPING TO FIND MISSING HERITABILITY IN PROTEINOPATHIES

Despite decades of human genetic studies, the set of genes responsible for disease risk of many proteinopathies remains incomplete. Genes responsible for disease risk have traditionally been identified through family-based genetic linkage analysis and population-based genome-wide association studies (GWAS) (Cui et al. 2010; Flint 2013). Linkage analysis is generally better suited to detect rare variants with large effects, as in Mendelian disorders (e.g., HD). More recently, whole-exome sequencing has been favored to search for such variants. In contrast, GWAS are geared toward detecting common variants with smaller effects. GWAS have successfully uncovered causal genetic loci for a variety of complex genetic disorders (e.g., type 2 diabetes, schizophrenia) (Visscher et al. 2017). However, when the effect size and frequencies of the known causative loci are combined for a disease, they often explain only a small fraction of the heritability of the disease. Even in monogenic disorders like HD, our knowledge of genetic variants that modify disease onset or progression is far from complete (Arning 2016; Holmans et al. 2017). Put another way, a large proportion of genetic variation predisposing to proteinopathies or modulating their progression is unaccounted for.

What are the other genetic loci that are affecting disease susceptibility? This conundrum is referred to as the problem of "missing heritability" (Manolio et al. 2009). Many ideas have been put forth to explain it (Manolio et al. 2009; Eichler et al. 2010; Brookfield 2013). GWAS are limited in identifying single-nucleotide polymorphisms (SNPs) in linkage disequilibrium with the "true" gene locus conferring risk and may never pinpoint the causal gene (MacArthur et al. 2014; Schaid et al. 2018). Furthermore, traditional methods for evaluating common variants, such as replication of the association across populations, are not readily applicable for rare variants. Rare variants may be private to a family or small population and are thereby difficult to replicate in another population, leaving the status of the variant in doubt (Lupski et al. 2011; Casals and Bertranpetit 2012). In addition, for both classes of variant, combinatorial effects can be elusive.

As noted above, genetic screens against proteotoxicities in model organisms are enriched in homologs of known human genetic risk factors for neurodegenerative diseases. It is thus plausible that complete cellular dissection of proteotoxicity (through the different methods outlined in this review) could provide a candidate list of genes enriched in true human genetic modifiers. This kind of Bayesian approach—Bayesian because it begins with a list of genes a priori more likely to be involved with the disease process—may be synergistic with traditional human genetic analysis. Because tests to identify association between rare or common variants and complex genetic disease are statistically underpowered, one method to sidestep this limitation is by reducing the number of comparisons in genetic association studies through integration of information from available biological data sets in model organisms or cellular models. This idea is gaining traction and has recently

been tested in the PD field. For example, in a recent GWAS meta-analysis of PD cases and controls, candidate causal genes were assigned to novel PD-associated risk loci ($P < 5 \times 10^{-8}$) by using a "neurocentric" scoring-based strategy that takes into account multiple levels of experimental evidence, including expression quantitative trait locus (eQTL) and tissue-specific gene-expression data from central nervous system (CNS) cell types in mice and neurologically relevant phenotypic annotations in *Drosophila* (Chang et al. 2017).

TransposeNet: Transposing Molecular Networks across Species

If model organisms are utilized for functional genomics, a clear challenge arises in assigning cross-species homology. Assigning homology of genes and networks across species is a nontrivial exercise, particularly when sequences of homologous genes diverge over evolution. Ideally, a molecular network identified in one species could be "transposed" to another. A recent study achieved this for three genome-wide screens against α-synuclein toxicity in yeast. A computational approach (TransposeNet) was developed to transpose rich yeast molecular networks from yeast to human (Khurana et al. 2017).

Prior to network generation, TransposeNet comprised three steps (Fig. 3, steps 1 through 3). First, human homologs to yeast genes were assembled through consideration not just of sequence, but also of protein structure and known molecular interactions. This model of homology accounted better for sequence divergence across evolution, and demonstrably increased assignment of homologs across species. Only homologs expressed in brain were included. Second, molecular interaction data linking these human gene "nodes" were curated. However, human molecular interaction data (particularly genetic interactions) are sparse and result in fragmented, incomplete networks. To address this, an augmentation method was developed as a third component of TransposeNet: known molecular interaction data between yeast genes and proteins were transposed across species to augment the sparse human data sets. This enriched data set emerged as a critical component to generate network "coherence," namely, the appropriate interconnection of the majority of the original genetic hits into a single network on which genes/proteins of like function are linked together in close proximity.

Finally, having assembled the original genetic hits and known yeast/human molecular interaction data, the TransposeNet networks themselves were generated through an optimization framework based on the prize-collecting Steiner Forest algorithm (Fig. 3, step 4). The algorithm identifies the simplest way to connect the gene/protein "nodes" of the network through the most robust known protein–protein or genetic interactions. The algorithm also inserts genes ("predicted nodes") to solve the network problem in the most efficient way, thereby connecting genes not included in the original data set in the network. The resultant "humanized" networks were more complete and appropriately linked genes of related function together. Importantly, underscoring the strength of the approach, the resulting "humanized" network included human disease genes with no clear homologs in yeast as predicted nodes. The humanized network of α-synuclein toxicity modifiers thus revealed molecular connections between multiple different genetic forms of parkinsonism, and predicted pathologies (e.g., mRNA translation defect) that were validated in patient-derived neurons. Importantly the network approach could also infer connections to druggable targets for synucleinopathy (including calcineurin, described above, and another target Nedd4), even when those druggable targets were not recovered by the genetic screens from which the networks were generated. This type of computational approach may provide a blueprint for translating findings in model organisms for functional genomics and pharmacogenomics in human proteinopathies.

A "DRIVER-RESPONDER" ARCHITECTURE IN PROTEOTOXICITY

Distinguishing Causation and Progression in Neurodegeneration

Where abundant molecular interactome data are available, genetic and transcriptional data are

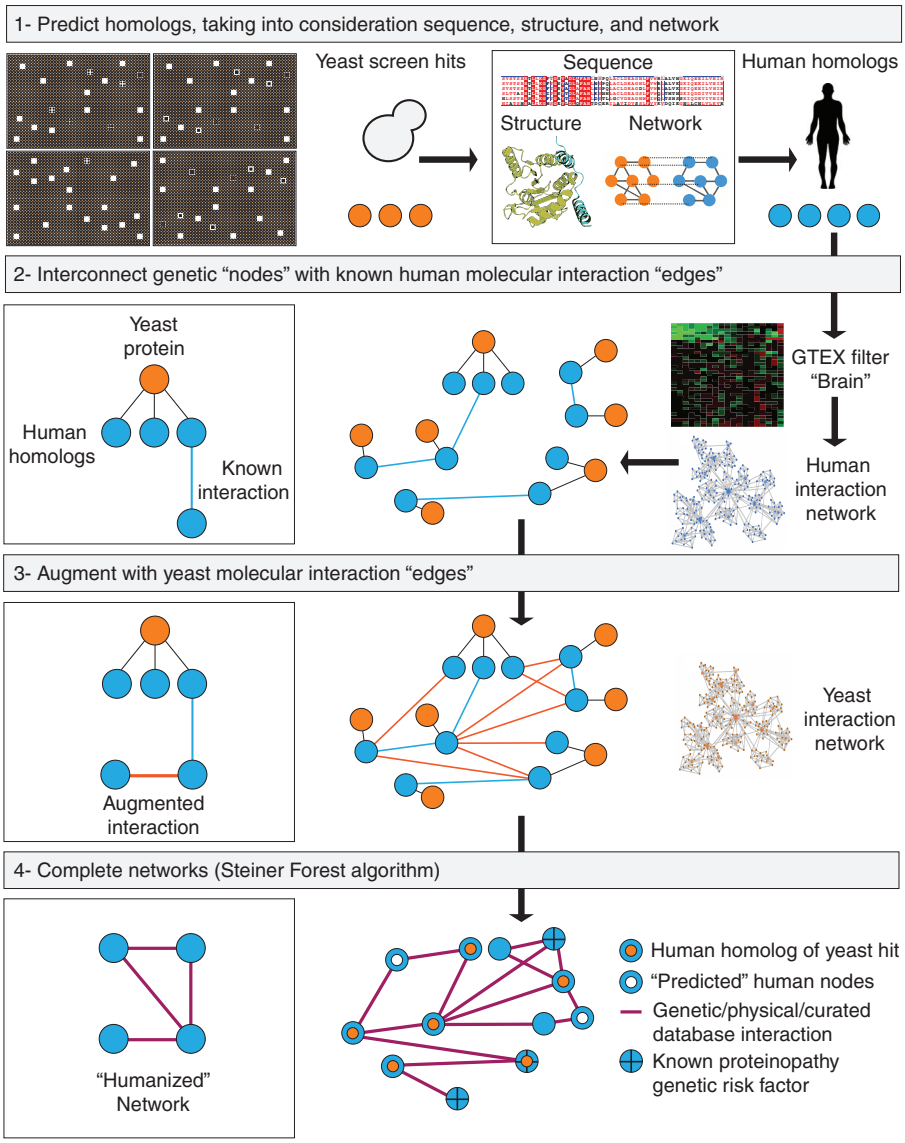

Figure 3. TransposeNet is a computational approach that may enable integration of cross-species molecular data to build a more coherent view of proteotoxicity. In this depiction of a recently published study (Khurana et al. 2017), modifiers recovered from genetic screens of proteotoxicity in yeast were "transposed" into the context of the human proteome. (*1*) Human homologs of yeast modifiers were generated through cross-species consideration of sequence, structure, and protein–protein interactions. (*2*,*3*) Genetic and physical interactions ("edges") between these human genes/protein "nodes" were curated not just from the relatively sparse existing human molecular interaction data sets (*2*) but also through augmentation of the much richer data set of homologous interactions in the yeast proteome (*3*). (*4*) A network was generated to connect the nodes through edges, employing a method known as the Steiner Forest prize-collecting algorithm. The advantage of this method is that it solves a "hairball" of interactions in the most efficient, robust way and introduces "predicted" nodes to do so. The "predicted" nodes can capture biology that was missed in the original screen. In the published example for α-synuclein toxicity, the method captured interactions between many genetic risk factors for Parkinson's disease through specific molecular pathways. It is presumed that the tool can be used to focus attention on specific human genes as potential genetic risk factors, aiding functional genomics efforts.

largely nonoverlapping, as noted above. Genetic screens to identify modifiers of proteotoxicities may be more geared toward identifying factors that either drive disease processes or vulnerability. In contrast, transcriptional profiling may be more germane for identifying responders to the toxic insult. It is worth remembering that genetic modifiers in simple yeast screens are often introduced into the yeast cell prior to the proteotoxic insult (or simultaneous with it), just as they would be in a human born with a gene variant. In contrast, transcriptional profiling assesses the response at various intervals after the toxic protein is overexpressed.

There is mounting evidence that drivers and responders to proteotoxicities may be different. For example, in HD, the archetypal polyglutamine-expansion disorder, it is well known that the longer the polyglutamine tract (or the CAG repeat that encodes it), the earlier the onset of the disease. However, a recent study found that disease duration is independent of this repeat length (Keum et al. 2016). Thus, factors other than the polyglutamine tract, whether genetic or otherwise, are more important in determining disease course and progression. Alternatively, the polyglutamine tract may also be important in driving progression, but in cells or circuits that are distinct from those involved in disease initiation.

Likewise, as noted above, in the case of α-synuclein toxicity in cellular models or in patients, genetic hits are enriched in genes encoding proteins involved in vesicle trafficking, but transcriptional responses are enriched in genes encoding proteins localized to the mitochondria and genes involved in oxidative metabolism. Interestingly, an emerging literature is revealing mechanistic links between trafficking and mitochondria. For example, α-synuclein itself has been localized to mitochondria-associated ER membranes (Guardia-Laguarta et al. 2014), and binds to the mitochondria-associated tethering protein Vapb to disrupt mitochondrial function (Paillusson et al. 2017). Mutation of the Parkinson's gene *VPS35* (that genetically interacts with α-synuclein) leads to mitochondrial fragmentation. This has been attributed to abnormal interactions between mutant Vps35 and mitochondrial dynamin-like protein (DLP1) (Wang et al. 2016). The endocytic and actin cytoskeletal machinery are intimately related, and perturbations induced by α-synuclein in the actin cytoskeleton lead to mitochondrial dysfunction (Ordonez et al. 2018). Beyond mitochondrial dysfunction, it is conceivable that "responders" to different proteotoxic stressors include other components of the proteostasis network, for instance the unfolded protein response, chaperones, proteasomal and lysosomal degradation machineries, and so forth.

Another major thrust of current neurodegenerative disease research involves factors outside neurons, and even outside the nervous system, that may be pivotal in disease progression. These types of disease mechanisms may not be accessible to modeling within model organisms that lack specialized biology. For example, there is deepening interest, in the prion-like self-templating behavior of amyloids and their ability to spread trans-synaptically or between neurons and glial cells (Brettschneider et al. 2015; Jarosz and Khurana 2017). Increasing evidence suggests that, just as for bona fide prions like PrP, specific conformers of proteins prone to misfold may have tropism for certain cells and circuits (Falcon et al. 2018). This could explain how distinct disease processes may result from the misfolding of the same protein (Jarosz and Khurana 2017; Peng et al. 2018).

There has also been burgeoning interest in neuroimmune interactions, in particular the potential role of microglial dysfunction. This has been partly driven by the recovery of many genes enriched in microglia in human genetic studies of neurodegenerative disease (Salter and Stevens 2017) in conjunction with elegant cellular studies that distinguish normal from pathogenic interactions between microglia and neurons (Krasemann et al. 2017). Even more recently, there has been growing appreciation that viral infection may have a role to play in the initiation and progression of neurodegeneration (Eimer et al. 2018). It is plausible that neuroimmune mechanisms may be involved in neurodegenerative disease progression after an initiating proteotoxic stress. Alternatively, in late-onset "sporadic" forms of the disease it is conceivable that protein

misfolding is initiative through either defective neuroimmune mechanisms or perturbed clearance of toxic proteins from extracellular spaces.

This distinction between drivers and responders to proteotoxic stress may have significant implications for therapy. Genetic modifiers may alter vulnerability to protein misfolding and cytotoxicity, but they may miss critical proteostasis pathways that respond to proteotoxic stress. It is intriguing, for example, that for the most part the central proteostasis machineries (chaperones and so forth) are not commonly implicated in human genetic studies of neurodegenerative disease, and also in genetic modifier screens of proteotoxicity in model organisms. This is surprising because these pathways are known to be critical to the handling of misfolded proteins and deeply tied to pathways of aging. But this does not necessarily detract from the potential importance of these pathways. It could be, for example, that the core components of the chaperone machinery are essential for all of protein biogenesis and required to support embryonic development, and thus detrimental mutations would not be recovered. Or it could be that alterations in these pathways do not affect predisposition to disease but modulate severity or rate of progression. Conventional case-control human genetic studies would miss these factors, and yet they might be very important for therapeutics. Likewise, genetic screens in cellular models such as yeast cells could miss key genetic drivers of pathologies in aged organisms, and screening at different time points in organisms that can be aged (like the fly or worm) may be useful for identifying these factors. Finally, many of these factors may act in combination. As noted above, human genetic analysis of proteinopathies has far from exhausted a search for combinatorial effects of genes, and the majority of heritability factors remain as yet unrecovered.

Spatial Mapping to Identify Drivers

One intriguing feature that has emerged from the genetic architecture of neurodegenerative diseases is how little overlap there is between distinct diseases. With the possible exception of apolipoprotein E genotype, there is not a single genetic risk factor known to definitively predispose to different degenerative proteinopathies. It is possible that major defects in the proteostasis machinery that could predispose to misfolding and aggregation of multiple toxic proteins would be lethal, or that there is enough redundancy in the machinery to protect against the effect of such mutations.

Another possibility is that the genetic modifiers of a proteotoxicity relate in part to the intrinsic function of the protein that misfolds. Put another way, modulation of pathways intrinsically related to this function are major drivers of disease. Considerable evidence supports this possibility, particularly for proteins like huntingin and ataxin proteins (reviewed recently in Jarosz and Khurana 2017). Recently, this hypothesis was indirectly tested via mapping of interacting proteins. For example, Hughes and colleagues exploited protein interaction data of huntingtin protein (via yeast two-hybrid and affinity pull down-mass spectrometry) to identify candidate genetic modifiers of huntingtin in a *Drosophila* model of HD. They thereby connected genetic drivers to functional interactors of the misfolding protein (Kaltenbach et al. 2007). Proximity biotinylation labeling with an ascorbate peroxidase (APEX) tag (Han et al. 2018) enables this kind of analysis in living neurons, and was recently applied to α-synuclein (Chung et al. 2017). Genetic modifiers of α-synuclein toxicity in yeast cells ("genetic map" as described above) were cross-compared to proteins <10 nm radius from α-synuclein in neurons (Chung et al. 2017; Khurana et al. 2017). The spatial and genetic maps significantly overlapped, most clearly for vesicle trafficking and mRNA-binding proteins. Thus, the intrinsic location and protein interactions of α-synuclein are directly related to its mechanism of toxicity when it misfolds. This may turn out to be a general theme, explaining in part the exquisite specificity of protein-misfolding pathologies.

CAPTURING SPECIFIC PROTEIN CONFORMERS IN LIVING CELLS

Underlying the conceptual framework of drivers and responders to neurodegeneration, there is a

physical dimension at the level of biomolecules that needs to be understood. The methods we describe above delineate proteins and pathways mediating the cellular effects of toxic proteins. But at the molecular level, these cellular networks need to be resolved as specific protein–protein interactions, and as interactions between proteins in specific conformational states. To achieve this level of understanding, there are a growing number of approaches to assess alterations of protein structure and protein–protein interactions in living cells.

It is straightforward to grasp the disruption of a network if a mutation arises in an "executive domain" of a protein, such as a catalytic site, a well-folded protein-interaction domain or a posttranslational modification site. However, there are numerous Mendelian mutations that are mapped to intrinsically disordered regions (IDRs), or low-complexity domains (LCDs) (Castello et al. 2013). Nowhere is this clearer than the case of ALS, where mutations of TDP-43 and FUS proteins are concentrated in LCDs (Harrison and Shorter 2017). Although IDRs seem to have low information content, they had been well-recognized in yeast prions and polyglutamine-containing proteins as the primary source of prionogenic potential. For instance, conversion to a prion conformation in yeast depends on long stretches of asparagines and glutamines (N/Q) (Halfmann and Lindquist 2010), and computational algorithms trained on these LCD sequences enabled the discovery of novel prions (Alberti et al. 2009). But beyond sequence, the last decade has brought remarkable biophysical insights into the nature of intrinsically disordered proteins. These included seminal observations of the gel-like properties of nuclear pore complexes (Frey et al. 2006) and the liquid behaviors of P-granules of *C. elegans* oocytes (Brangwynne et al. 2009). A new understanding of the material properties of proteins has emerged in which phase separation contributes to cellular organization through the formation of membraneless organelles (MLOs). Moreover, this process is highly sensitive to mutation of such proteins (Shin et al. 2017; Boeynaems et al. 2018). Although the nomenclature for this phenomenon is as abundant as the newly discovered MLOs (prion-like aggregation, liquid–liquid phase separation, droplets, ribonucleoprotein bodies, phases, granules, etc.), the term "condensates" has been widely accepted in the field (Banani et al. 2017) as it is inclusive of all the physical states (liquid, gel, amyloid fibrils) of biological polymers.

Condensates are widely thought to organize the cellular milieu in a spatiotemporal manner. Classical cellular compartments are now recognized as MLOs; P-bodies/stress granules (Decker and Parker 2012), Cajal bodies (Nizami et al. 2010), nucleolus (Feric et al. 2016), and Balbiani bodies (Boke et al. 2016). Some of these granules exist constitutively, such as P-bodies, although their numbers increase upon mRNA decay-related stress. In contrast, stress granules quickly form de novo upon translation-related stress (Protter and Parker 2016). Although these physiologic protein accumulations are carefully controlled by the cell, their inherent metastability gives them a dangerous edge. Point mutations in the IDRs (Harrison and Shorter 2017), malfunctioning of the chaperone machinery (Mateju et al. 2017), aging (Alberti and Carra 2018), ATP depletion (Patel et al. 2017), pH changes (Munder et al. 2016), or cellular crowding (Delarue et al. 2018) can drive these metastable proteins into pathological aggregates. Cells can cope with such aggregates to a certain extent through degradation (autophagy/proteasomal degradation) (Balchin et al. 2016), or sequestration (JUNQ/INQ or IPODs) (Miller et al. 2015). However, beyond a point, the cell succumbs and, as noted above, proteotoxicity can result and may be amplified when some conformations are templated and spread within or between cells (Jarosz and Khurana 2017). Abnormal protein conformation may thus be critical to both the genesis and progression of proteinopathies. It is, therefore, imperative to integrate this conformational information into our understanding of cellular pathologies in proteinopathies.

Technologies to Measure and Control Aggregation Complexes and States in Living Cells

Traditional biochemical methods for assaying protein aggregation may entail lysis of the cell

but many tools have been developed for in vivo detection of condensation. Within intact cells, our knowledge has mostly arisen from skillful usage of fluorescent microscopy of tagged candidate proteins. In vivo dynamics of these condensates are measured by FRAP (fluorescence recovery after photobleaching) complemented by in vitro droplet formation assays with purified components (Brangwynne et al. 2009; Li et al. 2012; Caudron and Barral 2013; Zeng et al. 2016). Complementation of split fluorescent proteins have also been extensively utilized to track α-synuclein aggregations in vivo (Lázaro et al. 2014). Frequently, these condensates are a complex mixture of proteins and RNA. APEX methods (noted above) can be used to identify the heterogeneous composition of granules before or after condensation events. Stress-dependent composition of stress granules, for example, have been identified through proximity labeling of G3BP1-APEX (Markmiller et al. 2018). In some instances, solid cores of condensates are strong enough for extraction and proteomic analysis (Jain et al. 2016). Hubstenberger et al. (2017) have developed a fluorescence-activated particle-sorting method to check for the composition of P-bodies before and after stress conditions by labeling LSM14.

Amyloids can be investigated by methods developed in the prion field. Semidenaturing agarose gel electrophoresis (SDD-AGE) or filter trap assays have been extensively used to screen hundreds of wild yeast strains for [PSI^+] prion (Halfmann et al. 2012), or to search for amyloidogenic propensities of Q/N rich proteins in the yeast proteome (Alberti et al. 2010). However, a major challenge is to examine these entities in vivo with minimal intrusion. Recently, Newby and colleagues developed a highly sensitive, dynamic in vivo protein aggregation tracking method called yeast transcriptional reporting of aggregating protein (yTRAP). yTRAP makes use of a synthetic zinc finger (ZnF) fold and an activator moiety attached to a protein under investigation (Newby et al. 2017). ZnF recognizes a highly specific cognate DNA sequence upstream of a fluorescent reporter. This simple logic allows channeling the aggregation state of a protein to a fluorescent output (Fig. 4A). The modular nature of yTRAP permits tracking of multiple prion aggregations simultaneously and its high sensitivity, combined with flow cytometry, allows differentiating different strains of the same prion, a crucial distinction especially for the neurodegeneration field.

Importantly, many condensates contain RNA molecules, and low-complexity regions are highly represented in RNA-binding proteins (RBPs) (Castello et al. 2013). The unexpected finding that numerous RBPs contained IDRs and could be precipitated in biotinylated isoxazole from cell lysates opened up intense research into the biophysical properties of RNP granules (Han et al. 2012; Kato et al. 2012). Certain RBPs with IDR regions, especially components of stress granules, can solidify in vivo, a process known as maturation or hardening, causing loss of their protein/RNA clients, thus altering cellular "ribostasis" (Ramaswami et al. 2013). Hardened stress granules have been implicated in ALS pathology (Li et al. 2013). To find aggregation-prone RBPs, Newby et al. created a library of yTRAP strains for RBPs in the yeast proteome. This allowed simultaneous measurement of RBP aggregation states upon a chemical or environmental stress, such as chaperone inhibition or overexpression. Moreover, the library permitted tracking of coaggregation events in a time-resolved manner (Newby et al. 2017). It will be crucial to extend these libraries to other protein families, such as transcription factors, and transfer the methodology to mammalian systems.

Although amyloids are extremely stable, their nucleation is rare due to kinetic barriers. An important question in amyloidogenesis is how initial protofilaments form and self-template. To track these rare nucleation events in vivo, the Halfmann laboratory developed DamFRET (Khan et al. 2018). This method relies on a photoconvertible mEos3.1 fluorescent tag. Semiconversion of mEOS1 allows a single tag to represent a protein of interest as FRET pairs. Therefore, an increasing FRET signal occurs when the tagged protein becomes more tightly aggregated (Fig. 4B). With this single tag and flow cytometry, the aggregation of any protein can be measured as a function of its concentra-

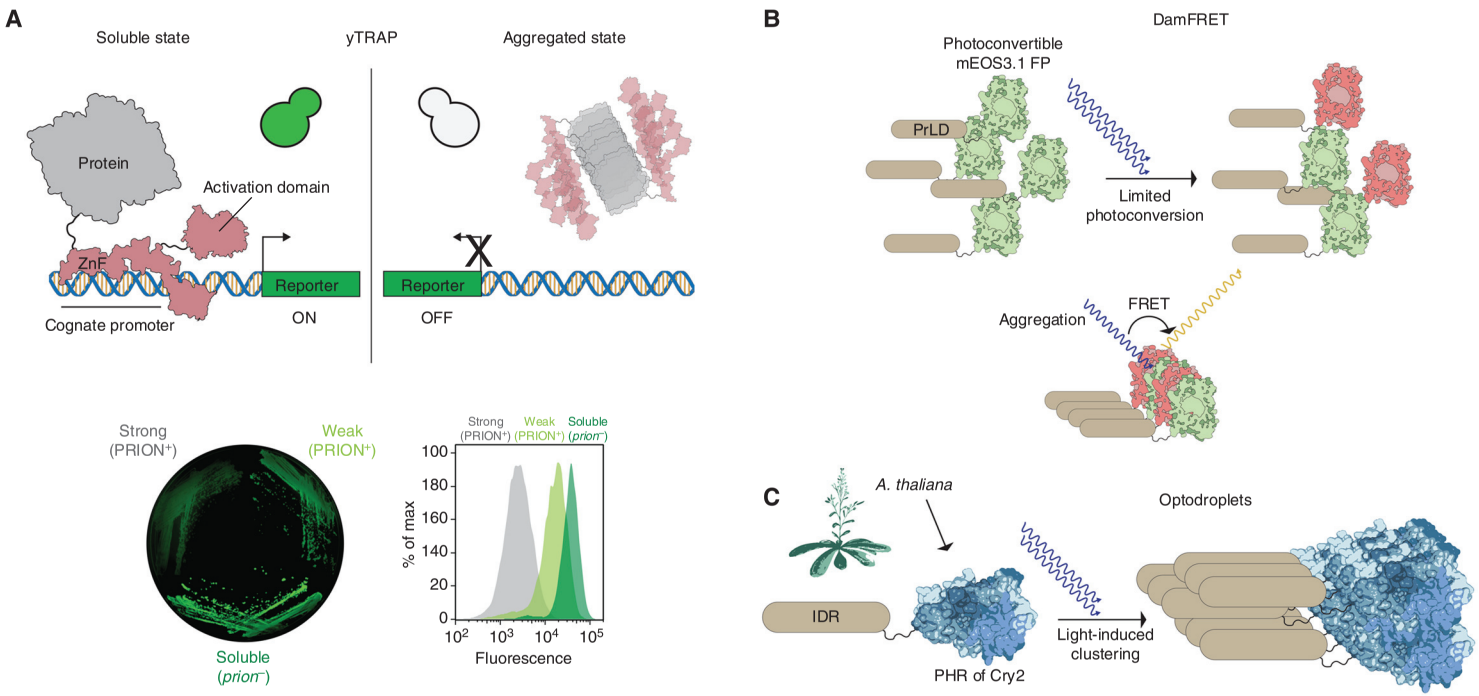

Figure 4. New technologies to measure and control protein condensation in vivo. (*A*) Yeast transcriptional reporting of aggregating protein (yTRAP) relies on tagging a protein of interest with a synthetic zinc finger (ZnF), which binds specifically to its cognate promoter. This yTRAP module contains an activation domain of VP16. In the soluble state, proteins diffuse freely and activate the reporter (in this case, mNeonGreen but could be easily interchanged or another reporter added for dual sensing of proteins). Upon aggregation, the signal is lost. The fluorescence can be measured sensitively with a CCD camera or flow cytometry. Three different strains of a yeast prion can be separated with this method. (*B*) DamFRET (distributed amphifluoric FRET). A protein contacting a prion-like domain (PrLD) is attached to mEOS3.1. Upon conversion of a subpopulation of mEOS1, two fluorophores can act as FRET pairs, if they are in close proximity. Therefore, the FRET signal acts as a proxy for concentration-dependent nucleation of prion-like proteins. Optodroplets rely on the photolyase homology region (PHR) domain of Cry2 protein of *Arabidopsis thaliana*. This domain self-associates upon exposure to blue light. The intrinsically disordered regions (IDRs) can be induced in vivo for supersaturation.

tion. Kinetic barriers for aggregation can certainly be overcome by overexpression of the protein under investigation; however, its supersaturation may occur in the cell because of overcrowding, change of cellular milieu, failure of protein control mechanisms, or aging as discussed above. Therefore, it is highly desirable to have precise spatiotemporal control of nucleation events in vivo by means other than overexpression. Optodroplets, developed for this purpose, are chimeric fusions of proteins under investigation and a blue light–inducible oligomerization domain of Cry2 from *Arabidopsis thaliana* (Fig. 4C). Using this technique, Shin and colleagues determined the in vivo dynamics of triggered condensations and their solidifications in time (Shin et al. 2017).

Collectively, these powerful and complementary approaches set the stage for detailed molecular dissection of protein aggregation states and multiprotein complexes.

CONCLUDING REMARKS

Here, we surveyed some methods that are enabling proteome-scale dissection of proteotoxic mechanisms at the cellular and organismal level. As noted above, the emerging biological data sets promise to, in turn, help us focus on specific gene variants emerging from larger-scale genetic studies for which pure human genetic analysis will most likely become an exercise in diminishing returns.

Driven by the classical tools of molecular biology, the preponderance of knowledge to date has (not surprisingly) been at the gene, transcript, and protein level. But chemical genetic profiling (Piotrowski et al. 2017), metabolomic profiling (Evers et al. 2017), and survey of chromatin and epigenetic factors (De Jager et al. 2018) are revealing novel mechanisms. For instance, recent elegant work has connected genomic instability, chromatin relaxation, and the aberrant activation of transposable genetic elements in tauopathy, providing entirely new perspectives on how age-related cellular changes might amplify the toxic effects of misfolded proteins (Guo et al. 2018; Sun et al. 2018). Computational approaches are also being employed to make better biological sense of the "hairballs" of large-scale interaction networks and to interrelate different large-scale data sets (Khurana et al. 2017; Kedaigle and Fraenkel 2018).

For all of their utility, the data sets described in this review have largely emerged from model systems that have not captured human cellular biology or the specific protein conformations that give rise to disease. There is a growing appreciation that specificity of proteinopathies is driven by distinct conformational states of proteins. These may be unique among diseases (Woerman et al. 2018), differ between individual patients, and be critically dependent upon cellular milieu (Peng et al. 2018). These unique conformers have not been modeled in cellular or organism models, nor have they been profiled in a human cellular context. But this will now change as technologies that capture both human host cell and toxic protein strain are available.

At the level of capturing human cell biology, somatic-cell reprogramming and the advent of iPSc is enabling the generation of patient-specific cells and distinct cell types from these patients. Cells can be arrayed in cocultures in specific orientations, aided by microfluidics or 3D laser printing. Stunning organoid technologies enable 3D patient-specific tissues to be generated too. These technologies are becoming available for the cells and tissues most affected in proteinopathies, from CNS (Lancaster and Knoblich 2014) to pancreatic islets (Zhou and Melton 2018) to muscle (Pourquié et al. 2018). At the level of capturing disease- and patient-specific conformer, biochemical approaches that capture and amplify these from brain (Peng et al. 2018) and spinal fluid (Shahnawaz et al. 2017) are being developed. Elusive cell types, such as microglia, can now be generated from iPSc and incorporated into 3D organoids (Muffat et al. 2016).

The amalgamation of these distinct technologies will provide an unprecedented view of proteotoxicity, amenable to the many methods outlined in this review. But a new wave of technologies will render it possible to profile 2D and 3D models, or patient-derived postmortem human tissue, in a spatially specific way (Fig. 5). Single-cell sequencing technologies are poised to reveal somatic mosaicism at the level of genome (Evrony et al. 2012) and transcriptome

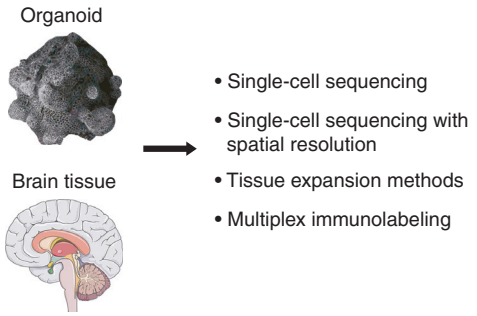

Figure 5. Emerging sequencing and imaging technologies. An unprecedented view of 3D postmortem tissue and stem-cell-derived organoid models is emerging through breakthrough techniques. These include single-cell DNA sequencing (Lodato et al. 2015) and RNA sequencing (Ecker et al. 2017). Our visualization of gene expression will be increasingly spatially resolved with more widespread use of techniques that can visualize hundreds or thousands of messenger RNA (mRNA) transcripts in tissue, including fluorescent in situ sequencing (FISSEQ) (Lee 2017) and highly multiplexed error-robust FISH (MERFISH) (Moffitt et al. 2016). Visualization will be enhanced by tissue-expansion methods (Chen et al. 2015; Ku et al. 2016; Wang et al. 2018) and methods like SWITCH that combine delipidation of tissue with multiplex antibody analysis (Murray et al. 2015).

(Ecker et al. 2017). Next-generation barcoding and sequencing have now also been applied to achieve transcriptional profiling of spatially resolved intact tissue, with methods such as fluorescence in situ sequencing (FISSEQ) (Lee 2017) and highly multiplexed error-robust FISH (MERFISH) (Moffitt et al. 2016) that can now be applied to 2D and 3D models of proteinopathy or human tissue samples. Stunning developments in microscopy will enhance our capacity to profile and understand proteinopathies in 3D, for example, the ability to expand tissue with expansion microscopy (Chen et al. 2015; Ku et al. 2016) or to combine clearing of lipids with multiplex antibody analysis (Murray et al. 2015).

In a time of aging populations, the personal and societal consequences of proteinopathies, from AD to PD to diabetes, will become even more devastating. It is heartening that our understanding of these diseases, from factors that drive them to those that mediate progression, is commensurately increasing. Unbiased methodology at the level of cell, tissue, and organism promises to enrich our understanding of underlying biological mechanisms. We predict that sorely needed preventative and therapeutic options for patients will follow closely behind.

ACKNOWLEDGMENTS

We apologize to colleagues whose work could not be cited owing to space limitations. We thank Dr. Mel Feany for critical reading of the manuscript. Work in our laboratory is supported by the New York Stem Cell Foundation, the National Ataxia Foundation, the Dr B.R. and Dr C.R. Shetty Initiative for Parkinsonian Disorders, the Barbara Bloom Ranson Fund for Multiple System Atrophy (MSA) Research and the Brigham Research Institute of Brigham and Women's Hospital. In addition, V.K. is supported by a National Ataxia Foundation Young Investigator Award and the 2018 Bishop Dr. Karl Golser Award. I.L. is supported by awards from the American Parkinson Disease Association (APDA) and PhRMA Foundation, and E.H. by the Human Frontier Science Program (HFSP, LT000717/2015-L). V.K. is a Robertson Investigator of the New York Stem Cell Foundation. Some of the graphics in this chapter were adapted from Servier Medical Art templates available at https://smart.servier.com. Servier Medical Art is licensed under a Creative Commons Attribution 3.0 Unported License.

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