NEURODEGENERATION

EXPLORING COMMONALITIES ACROSS DISEASES

WORKSHOP SUMMARY

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Forum on Neuroscience and Nervous System Disorders

Board on Health Sciences Policy

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Willing is not enough; we must do."
—Goethe

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Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this workshop summary was overseen by **Joseph Coyle,** Harvard Medical School. Appointed by the Institute of Medicine, he was responsible for making certain that an independent examination of this workshop summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this workshop summary rests entirely with the rapporteurs and the institution.

Contents

1 INTRODUCTION **1**

2 RATIONALE FOR EXPLORING COMMONALITIES ACROSS NEURODEGENERATIVE DISEASES **9**

3 PROTEIN AGGREGATION **23**

4 TRANSMISSIBILITY **33**

5 MITOCHONDRIAL PATHOLOGY **45**

6 ERRORS IN RNA **57**

7 CLOSING REMARKS **65**

APPENDIXES

A References 69
B Statement of Task 77
C Workshop Agenda 79
D Registered Attendees 91

1

Introduction[1]

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD), are becoming increasingly prevalent in the United States due to an aging population (see, e.g., Alzheimer's Association, 2012; de Lau and Breteler, 2006; Hebert et al., 2003; Reitz et al., 2011; WHO, 2012).[2] Implications are grave for quality of life and health care costs (see, e.g., Alzheimer's Association, 2012; PDF, 2012).

Research on neurodegenerative diseases has expanded greatly over the past four decades (for example, see Young, 2009). Nevertheless, fundamental questions remain about the biology of these diseases, and further insights into the mechanisms of these diseases would help to inform the development of effective means to prevent and to efficiently treat them.

Traditionally, research and development efforts for neurodegenerative diseases have primarily considered individual diseases separately, and largely separate research communities and patient advocacy groups have emerged. Recent findings, however, have revealed certain commonalities in

[1] The planning committee's role was limited to planning the workshop, and the workshop summary has been prepared by the workshop rapporteurs as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants, and are not necessarily endorsed or verified by the Institute of Medicine and they should not be construed as reflecting any group consensus.

[2] Overviews of neurodegenerative diseases and other neurological disorders, as well as lists of relevant organizations and other resources, can be found on the website of the National Institute of Neurological Disorders and Stroke: http://www.ninds.nih.gov/disorders/disorder_index.htm (accessed October 29, 2013).

genetic and cellular mechanisms across neurodegenerative diseases. These findings suggest that it might be valuable—at least in some cases—to change the traditional way of studying these diseases by no longer seeing each as an independent entity, but rather as clinical variants of common cellular and molecular biological defects. This approach could help enhance basic scientific understanding of neurodegenerative disease, and could help with the development of biomarkers and new therapeutics.

In the spring of 2012, the Institute of Medicine's (IOM's) Forum on Neuroscience and Nervous System Disorders hosted a workshop to explore commonalities across neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, ALS, and FTD, and to identify potential opportunities for collaboration across the respective research and development communities. Participants came from academia; pharmaceutical and biotechnology industries; government agencies such as the National Institutes of Health and the U.S. Department of Veterans Affairs (VA); patient advocacy groups; and private foundations. Looking across the neurodegenerative diseases, workshop presentations and discussions aimed to do the following:

- Identify and discuss commonalities related to genetic and cellular mechanisms;
- Identify areas of fundamental science needed to facilitate therapeutics development; and
- Explore areas of potential collaboration among the respective research communities and sponsors.

CHARGE TO WORKSHOP PARTICIPANTS

In her opening remarks, Story Landis, director of the National Institute of Neurological Disorders and Stroke and co-chair of the workshop planning committee, remarked that people who work on Alzheimer's disease and ALS, for example, typically have their own meetings and have few opportunities to "sit down, roll up their sleeves, and begin to talk about common mechanisms." This type of conversation, she said, could be extremely interesting and informative for participants working on these different diseases and could help advance understanding of potentially promising therapies. John Trojanowski, co-director of the Center for Neurodegenerative Disease Research at the University of Pennsylvania and co-chair of the workshop planning committee, emphasized that the workshop format of shorter talks and extensive discussion periods was designed to encourage in-depth discussion among researchers specializing in different neurodegenerative diseases about the fundamental science and how this can drive therapeutic development.

Joel Kupersmith, chief research and development officer at the VA, which contributed funding for the workshop, charged workshop participants with identifying and discussing current research on commonalities across neurodegenerative diseases, research needs and opportunities, areas for collaboration among investigators and facilities, and infrastructure needed to advance research in this area. Kupersmith emphasized that the neurodegenerative diseases are a substantial part of the VA's research portfolio because the VA serves mostly an aging population. He also highlighted the potential for collaboration with VA investigators, who receive funding through the VA intramural research program.

ORGANIZATION OF THE WORKSHOP AND THIS SUMMARY

The workshop began with an examination of the rationale for examining commonalities across neurodegenerative diseases. Chapter 2 summarizes these presentations and discussions. Subsequent workshop presentations and discussions were organized around four topics: (1) protein aggregation and cellular mechanisms to prevent or eliminate it; (2) neurodegenerative disease transmission and immune therapy; (3) mitochondrial pathology in neurodegenerative disease; and (4) errors in RNA processing. These topics are summarized in Chapters 3 through 6, respectively. Given the time limits inherent in a 2-day workshop, it was not possible to exhaustively examine all possible cellular or genetic commonalities across neurodegenerative diseases. Planning committee members selected these four topics—from among various potential candidates—for discussion because of scientific interest in further exploring the mechanisms underlying these commonalities and/or the existence of promising therapeutics based on these mechanisms. Certain topics are well known to be shared mechanisms across many neurodegenerative diseases (e.g., protein aggregation), while others are in earlier stages of exploration (e.g., errors in RNA). Each chapter includes individual suggestions for future research priorities and other opportunities proposed by presenters during the workshop. The statement of task is in Appendix B, the workshop agenda is in Appendix C, and a list of registered participants is in Appendix D.

TOPICS HIGHLIGHTED DURING PRESENTATIONS AND DISCUSSIONS[3]

Several topics recurred across the 2 days of presentations and discussions. They are briefly summarized here, and discussed in much greater detail in subsequent chapters.

- **The need for a deeper understanding of cellular and molecular mechanisms, including those that may be common across neurodegenerative diseases:** The workshop was organized around exploring four mechanisms and pathophysiologies that appear to be common across multiple neurodegenerative diseases: (1) protein aggregation, (2) transmissibility, (3) mitochondrial pathology, and (4) errors in RNA. Some of these are better understood than others. For example, protein aggregation is a well-known commonality across many neurodegenerative diseases; mitochondrial dysfunction has been found across neurodegenerative diseases, although it is not known if it plays a causal role; and errors in RNA are at a much earlier stage of exploration. Regardless of how much attention a topic had previously received, there was a great deal of interest among many workshop participants in continuing to develop a deeper mechanistic understanding of the cell biology, both within a single disease and across diseases. Many participants suggested research questions about these mechanisms; examples are listed at the ends of the relevant chapters. Some participants noted the possibility of gaining a greater scientific understanding of these diseases through the examination of these commonalities; for example, perhaps understanding these commonalities better could shine light on why certain pathologies are found in multiple diseases with different clinical presentations.
- **Exploring commonalities across diseases may provide a promising approach:** Workshop presentations and discussions highlighted many reasons to pursue an approach of examining commonalities across neurodegenerative diseases. Participants discussed genetic and pathological overlaps across multiple neurodegenerative diseases, as well as genetic and cellular mechanisms that appear to be common across diseases, suggesting that cross-disease study could be appropriate and could help advance scientific understanding. A number of participants discussed various ways in which a cross-disease approach might help to advance therapeutics development,

[3] Rapporteurs' summary based on the presentations and discussions during the meeting and session chairs' summaries during the final session.

such as by leveraging findings from a disease that is better understood or easier to study to increase development of therapies for diseases that are less understood or harder to study. Participants also suggested a variety of pragmatic reasons to pursue this type of approach, such as sharing data and resources; combining areas of scientific and technical expertise; and tackling common challenges and barriers together.

- **A note of caution:** Several participants also emphasized, however, the importance of avoiding an "all or nothing" approach. They noted that it will be important to carefully examine the evidence and "tease out" when it makes sense to do cross-disease research and development and when the focus should remain on individual diseases. One participant raised the possibility that the diseases may have distinct initiating factors, but engage some common pathways at some point(s). There are also several examples in which promising approaches in one disease did not show similar promise for another disease, suggesting independent pathways in certain cases.
- **Therapeutic approaches based on common mechanisms:** Workshop discussions revealed significant interest in exploring ways to use the fundamental scientific understanding of these common mechanisms to drive therapeutic development. Presentations and discussions included some therapeutic approaches that are already at various stages of development, as well as ideas for promising new directions. Participants suggested various ways in which a cross-disease approach could help advance the development of therapeutics for neurodegenerative diseases. For example, one participant noted that identifying common threads across neurodegenerative diseases could help with target validation by at least showing that the result is based on multiple models rather than just one. Several participants noted that it might be helpful to start by testing new drugs in diseases that have features that make them easier to study (e.g., known genetic risk and onset estimate in Huntington's disease, shorter duration in Creutzfeldt-Jakob disease) before investing significantly in diseases that are harder to study (e.g., sporadic cases of Alzheimer's and Parkinson's diseases). Cross-disease therapeutic approaches could also enable cost sharing among disease-specific foundations and other entities, and could help reduce program risk by spreading it across multiple partners. One participant noted that a multiple-disease approach could be particularly beneficial for encouraging the development of therapeutics for rare diseases, if such a therapeutic also might be effective for a more common disease with a larger potential market.

- **Common challenges:** Participants discussed many challenges that are common across neurodegenerative disease research and development communities, and, in some cases, common to central nervous system (CNS) research and development in general. Examples included the lack of biomarkers, patient heterogeneity, lack of complete knowledge about the causes of these diseases, and the long latency before symptoms appear. Other challenges derived from problems with modeling neurodegenerative disease and impaired cognition in animals; and reliance on data from cell-free systems, cell cultures (often cells lines), and animal models, but rarely from human autopsy material.[4]
- **Opportunities:** Many participants highlighted opportunities to enhance the mechanistic understanding of these processes and diseases, and more generally to advance research and development. Some of these are specific to advancing research based on commonalities across neurodegenerative diseases. Others are topics frequently raised in the context of therapeutics development for CNS disorders. Example opportunities and strategies included

    - **Harmonizing measures:** Harmonizing genetic and pathology measures and developing pathological and clinical standards across diseases would provide a basis for further collaboration and research across neurodegenerative diseases, noted one participant.
    - **Identifying and validating biomarkers:** Throughout the sessions, many participants commented on the need to identify and validate both diagnostic and therapeutic biomarkers. Many presenters discussed the current state of the art in biomarkers being used in their research, and also discussed critical gaps and needs; these comments are summarized in Chapter 2 and included in subsequent chapters as applicable.
    - **Sharing resources, tools, and data:** In a variety of contexts, participants discussed the value of sharing resources, tools, and data among multiple investigators and/or academic and pharmaceutical entities. Examples of resources, tools, and data that could be shared included human genetics data to examine gene variants that may extend across disease populations,

[4] Challenges and opportunities related to the use of animal models in research and development for nervous system diseases were explored in greater depth in a March 2012 workshop also hosted by the Institute of Medicine Forum on Neuroscience and Nervous System Disorders. Titled *Improving the Utility and Translation of Animal Models for Nervous System Disorders*, a summary of the workshop is available online: http://www.nap.edu/catalog.php?record_id=13530 (IOM, 2013).

biomarker programs, induced pluripotent stem (iPS) cells, compound libraries, enzyme-linked immunosorbent assays, access to tissues, and access to analytic methods. Participants gave various examples in which pharmaceutical companies had already shared resources with academic investigators.

- **Collaborations and public–private partnerships:** Participants discussed a variety of potential collaborations and public–private partnerships that could help address some of the common challenges listed above. Several participants highlighted the Alzheimer's Disease Neuroimaging Initiative and the Parkinson's Progression Markers Initiative—large public–private consortiums that aim to validate biomarkers for their respective diseases—and suggested expanding this type of model to other diseases and arenas of investigation. Several participants also discussed the importance of collaborations between basic scientists and clinical researchers, and between academia and the pharmaceutical industry.
- **Funding:** Several participants suggested various funding mechanisms that could help support work that examines and leverages commonalities across diseases. Ideas included the development of dedicated programs and funding to identify commonalities; support specifically aimed at identifying targets and therapies that may benefit more than one disease; and support for collaborations among scientists interested in advancing this type of approach.

2

Rationale for Exploring Commonalities Across Neurodegenerative Diseases

Key Points Raised by Individual Speakers

- Ample rationale exists for integrated investigation of neurodegenerative disease.
- Neurodegenerative diseases show pathological overlaps. For example, many patients with a particular disease have more than one proteinopathy, and a single type of proteinopathy can be associated with multiple diseases.
- Neurodegenerative diseases have overlapping genetics. The same genotype can lead to disparate phenotypes, and the same phenotype may result from multiple genotypes.
- Formidable obstacles to research are present for all neurodegenerative diseases, including lack of biomarkers, long asymptomatic period before disease is manifest, lack of validated animal models, and costly clinical trials. These obstacles have contributed to a dearth of new therapies.
- Enhanced sharing of research findings and collaboration across disease-specific research communities could potentially help advance basic scientific knowledge about each disease and help facilitate therapeutics development, including therapeutics that may address more than one neurodegenerative disease.

Neurodegenerative diseases traditionally have been studied separately. The workshop was convened because of a growing recognition of potential commonalities across genetic and cellular mechanisms, which led to an interest in exploring these commonalities to (1) identify potential opportunities to better understand the basic science of neurodegenerative disease, and (2) develop new therapeutic approaches. However, the workshop began by asking the underlying question: Is there a rationale to justify studying neurodegenerative diseases together? Presentations and discussions examined common features across diseases, including pathological and genetic overlaps, common challenges, and practical considerations related to the infrastructure needed to study these diseases. These are examined in turn below.

COMMON FEATURES ACROSS DISEASES

Neurodegenerative diseases show pathological overlaps, noted some workshop presenters. For example, many patients with a particular disease have more than one proteinopathy, and a single type of proteinopathy can be associated with multiple diseases. Similarly, there are overlapping genetics across neurodegenerative diseases. The same genotype can lead to disparate phenotypes, and the same phenotype may result from multiple genotypes.

Pathological Overlaps

Neurodegenerative diseases are best known by their pathology. The pathological hallmarks of Alzheimer's disease, for example, are senile plaques made of amyloid-beta (Aβ) protein and neurofibrillary tangles made of tau protein. Parkinson's disease is best known by Lewy bodies made of the protein α-Synuclein. These two diseases and others are known as proteinopathies because they feature pathological protein accumulations thought to be responsible for neuron injury and death. Although each disease is associated with particular proteinopathies, Dennis Dickson of the Mayo Clinic in Jacksonville, Florida, pointed out their broad phenotypic spectrum, showing that each disease frequently reveals mixed proteinopathies that overlap with proteinopathies from other neurodegenerative diseases (see Table 2-1). Although Aβ plaques and tau tangles are paradigmatic of Alzheimer's disease, Lewy bodies typical of Parkinson's disease are found in more than 50 percent of Alzheimer's cases, and neuronal inclusions consisting of the protein TDP-43 are found in more than 40 percent. Similarly in dementia with Lewy bodies—a dementing disorder closely allied to Parkinson's disease, yet with some features of Alzheimer's—the paradigmatic α-Synuclein-rich Lewy bodies are accompanied by Aβ plaques in

TABLE 2-1 Pathological Hallmarks and Their Protein Components in Neurodegenerative Diseases

Neurodegenerative Disease	Pathology	Component Proteins
Alzheimer's disease	Senile plaques Neurofibrillary tangles Lewy bodies Neuronal inclusions	Aβ amyloid Tau α-Synuclein TDP-43
Parkinson's disease	Lewy bodies	α-Synuclein
Amyotrophic lateral sclerosis	Neuronal inclusions	TDP-43 FUS/TLS SOD1
Huntington's disease	Neuronal intranuclear inclusions	Huntingtin
Dementia with Lewy bodies	Lewy bodies Senile plaques Neurofibrillary tangles	α-Synuclein Aβ amyloid Tau
Frontotemporal diseases	Neuronal and glial inclusions	Tau TDP-43 FUS
Multiple system atrophy	Glial cytoplasmic inclusions	α-Synuclein
Prion diseases	Senile plaques	PrP protein

NOTES: FUS/TLS = fused in sarcoma/translocated in liposarcoma; PrP = prion protein; SOD1 = superoxide dismutase 1; TDP-43 = TAR DNA-binding protein 43.

60 percent of cases and tau tangles in 50 percent, he said. Furthermore, experimental evidence shows that some of these proteins from the same or different neurodegenerative diseases interact with one another, resulting in the acceleration of the disease process. The fact that there is such a high degree of mixed pathology and potential for interaction, in Dickson's view, provides rationale for studying these diseases together, and suggests that combination therapies are going to be crucial. A single therapy aimed at one proteinopathy may be found ineffective because the underlying disease has multiple proteinopathies.

Just as a single neurodegenerative disease can be associated with multiple proteinopathies, a single proteinopathy can also be associated with multiple diseases. This holds for the molecular defect four-repeat tau.[1] Four-repeat tauopathy is associated with at least three clinical presentations: (1) Progressive supranuclear palsy presents with an axial rigidity

[1] The 6 tau isoforms differ according to the number of repeats (3 or 4) of 18 amino acids at the C terminus.

and eye movement problems, in addition to atypical Parkinsonism; (2) corticobasal degeneration presents like a frontal lobe dementia, with focal cortical syndromes, including progressive apraxia or progressive aphasia; and (3) argyrophilic grain disease is an increasingly recognized disorder of the elderly that affects the medial temporal lobe and is associated with an amnesic cognitive impairment. Given the pathological heterogeneity, Dickson urged researchers, for therapeutic purposes, to search for "upstream targets, rather than downstream pathologies."

Genetic Overlaps

Neurodegenerative diseases also have genetic overlaps (Bertram and Tanzi, 2005). Andrew Singleton of the National Institute on Aging (NIA) first focused on monogenic forms of neurodegenerative disease and their overlaps. The same genotype can lead to disparate phenotypes. For example, presenilin-1 (PS1) mutations, which account for the greatest fraction of early-onset familial Alzheimer's disease, are also found in the disease spastic paraparesis. Mutations in the gene leucine-rich repeat kinase 2 (LRRK2) are found in an autosomal dominant form of Parkinson's disease, yet also in several other diseases such as progressive supranuclear palsy, amyotrophy, and multiple system atrophy. Similarly, a recently identified mutation in the gene for *chromosome 9 open reading frame 72* (*C9orf72*) is the most common cause of familial amyotrophic lateral sclerosis (ALS). The mutation is also present in frontotemporal dementia (FTD) and clinically-diagnosed Alzheimer's disease. There are enough people with this particular mutation, noted Singleton, that researchers need to come together to begin to look for modifiers that explain phenotypic differences. A centralized DNA and data repository supported by some type of consortium, he said, would go a long way to advance the field.

There are also overlaps in risk loci, Singleton said. The microtubule-associated protein tau (MAPT) gene is shared by Parkinson's and progressive supranuclear palsy. The gene for α-Synuclein is shared by multisystem atrophy and Parkinson's disease. In his experience, Singleton said that the vast majority of risk loci associated with disease are not associated with protein coding changes. They are more likely to be associated with a change in basal expression of the protein, an effect on splicing, or an effect at a particular point in time. There are numerous ways, in short, that risk loci mediate their effects.

This sample of genetic overlaps speaks to the need to look across neurodegenerative disease, as opposed to focusing solely on one disease without regard to implications for others, said Singleton. For future research, he pressed for harmonization of methods for both pathology and genetics, such as the same genetic platforms and the same genetic readout. "I think

we are used to bringing data together, certainly within disease. The next challenge lies in bringing data together across diseases and bringing together groups that wouldn't necessarily work with each other." The bottom line, he said, is that each approach requires standardization and collaboration across disease communities.

Besides genetics and pathology, other overlaps occur in the mechanisms of disease pathogenesis. These overlaps were the prime focus of the workshop and are covered in ensuing chapters: protein aggregation, transmissibility within the central nervous system, mitochondrial dysfunction, and RNA processing errors.

COMMON CHALLENGES

Many speakers and workshop participants pointed to several well-recognized challenges to research posed by neurodegenerative disease. These challenges contribute to the low number and flat growth of new drug approvals for neurodegeneration (see Figure 2-1). Challenges cited by various workshop participants are described briefly below.

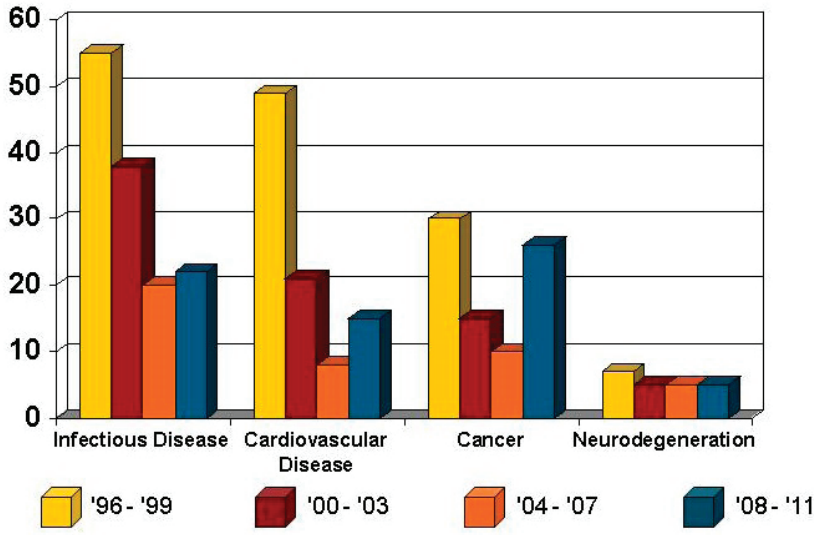

FIGURE 2-1 Drugs approved by the Food and Drug Administration from 1996 to 2011.
SOURCE: Data from Thompson Centerwatch; figure from Adrian Ivinson.

- Modeling neurodegenerative disease in animals is problematic because of species differences. Neurodegenerative disease often impairs cognition, but cognitive functioning is exceedingly difficult to model in animals.
- There is a lack of biomarkers with which to study neurodegenerative disease, especially its diagnosis. Without strong biomarkers, clinical trials are far more difficult to conduct because patients may be misdiagnosed.
- Patient heterogeneity in clinical trials often dilutes the capacity to find a medication efficacious.
- The causes of these disorders are not fully known, nor are the earliest times to intervene to modify the course of the disease.

Another daunting problem is the long preclinical period after the disease process is unleashed, but before appearance of frank symptoms, noted several participants. The disease process, as evidenced by pathological changes, can begin, in the case of Alzheimer's disease, two decades before symptoms become manifest (Alzheimer's Association, 2012). Once symptoms appear, they still may be too non-specific to warrant a definitive diagnosis. Yet by the time the disease is fully manifest, the global pathological damage to the brain is often so great that treating or slowing the disease course may be too late. Many argue that drug interventions are best in the asymptomatic stage while the brain is still resilient and capable of repair or compensatory changes. But administering drugs to asymptomatic individuals in clinical trials poses ethical and cost implications for the drug industry and for the Food and Drug Administration (FDA).

Drug trials, already expensive in neurological diseases, would likely be even more costly if studying asymptomatic people for whom the benefit may not accrue for years of taking drug therapy, according to several participants. Participants noted several potential barriers. For example, drug therapy could have to be administered for longer duration and the size of the trial would need to be larger. Another factor is that some patients with preclinical disease may never develop the disorder, which complicates analysis of efficacy. Furthermore, a number of costly failures loom large in deterring drug companies from making huge investments. Several large and expensive trials of antioxidants have not been successful (e.g., Galasko et al., 2012). The compounds tested had been successful in animal models, but the translation of the findings to humans was not effectively accomplished, most likely the result of species differences. Finally, pooling resources for clinical trials brings fears in drug companies of losing intellectual property, which deters investment. Amid the formidable array of challenges, as well as the economic downturn, several pharmaceutical companies have reduced their neurodegenerative disease divisions, according to press reports.

OPPORTUNITIES FOR COLLABORATION AND PARTNERSHIP

Given these challenges, Adrian Ivinson of Harvard Medical School pointed out that each of the disease communities has similar needs, such as defining more genetic risk factors for disease, elucidating genes and their contribution, defining phenotypes, understanding postgenomic modifications, identifying biomarkers, developing validated animal models, identifying and validating new drug targets, and translating results into effective clinical trials.

To meet the challenges confronting the field, Ivinson pressed for collaboration across the public and private sectors to develop clinical and pathological standards and to use them for preclinical and clinical research. He pointed out that resources—the tools, technologies, infrastructure personnel, and skill sets—are very similar, regardless of disease. In fact, he wagered that "a lab that was mostly focused on Alzheimer's disease today . . . could probably switch to Parkinson's disease tomorrow without changing much infrastructure." He urged collaborations in the preclinical phases when intellectual property considerations are not as salient as in clinical trials. He conceded that as research moves to large-scale clinical trials, there is greater difficulty in collaboration because of intellectual property. Still, he believes collaborations are the means to achieving not just greater efficiencies, and achieving them sooner, but also achieving better research outcomes. "I think for almost every stone you turn over, you will find an opportunity to collaborate," said Ivinson. He and other presenters acknowledged, however, that collaborative approaches to studying more than one disease have rarely been tried and may not be pertinent to all neurodegenerative diseases because of disease heterogeneity. Yet they were quick to add that the collaborative approach across diseases is worth trying where the data justify it.

The Alzheimer's Disease Neuroimaging Initiative is an 8-year initiative by coordinated teams of scientists in the public and private sectors to validate biomarkers for Alzheimer's disease (ADNI, 2010). It was showcased by several speakers as a good example of how to bring together public- and private-sector partners.

Story Landis, director of the National Institute of Neurological Disorders and Stroke (NINDS), mentioned two NINDS programs and one National Institutes of Health (NIH) Blueprint program that encourage collaboration. NINDS's NeuroNEXT (Network for Excellence in Neuroscience Clinical Trials) is a network for conducting Phase II clinical trials for neurological conditions through partnerships with academia, private foundations, and industry (NINDS, 2013). Landis noted that this network is unusual because it has a common institutional review board and prenegotiated agreements with all the sites, with the aim of going "from pro-

tocol to patient" in 2 months. Landis also mentioned the NINDS Cooperative Program in Translational Research (U01), which is a milestone-driven funding program with the goal of taking something from proof of concept in an animal system or a culture system to an investigational new drug, investigational device exemption, 510(k), or 510(k) de novo application to the FDA within the funding period (NINDS, 2012). Finally, Landis mentioned the Blueprint Neurotherapeutics Network, which "offers neuroscience researchers a 'virtual pharma' to develop promising hit compounds from chemical optimization through Phase I clinical testing" (NIH Blueprint, 2013).

EXAMPLE PROMISING TECHNOLOGIES

Several speakers described promising technologies to study the complex pathogenesis of neurodegenerative disease as well as to identify new treatments. These technologies are high-throughput screening, induced pluripotent stem cells (iPSCs), and yeast as a model system. This section does not exhaustively review current promising technologies, but rather summarizes certain examples that were discussed at the workshop.

High-Throughput Screening in Primary Neurons

Autophagy and other proteostasis mechanisms are constantly in flux. Obtaining a snapshot in time through ordinary microscopy cannot be used effectively to understand such dynamic cellular processes. A new method to study dynamic changes over time within single neurons has been devised by Steven Finkbeiner and colleagues (Sharma et al., 2012). Known as robotic microscopy (RM), the high-throughput and high-content automated imaging system can acquire images rapidly and automatically with single-cell resolution and enable high-throughput applications as the cell is followed longitudinally. The longest a cell has been followed thus far is 6 months. Automated analysis programs are used to obtain anatomic and physiological features of neurons and study how these change over time in a quantitative manner. Once cells are tagged with fluorescent biosensors, RM can be used to study events such as cell-wide protein misfolding, autophagy, and trafficking. New methods are being used to study early changes in protein aggregation and to determine the effects of neurodegenerative disease-causing proteins, said Finkbeiner. Combined with statistical methods, RM can determine the extent to which a variable can predict the fate of that same neuron at a later time. This capability is being used to uncover cause-and-effect mechanisms in neurodegenerative disease, such as whether changes are pathogenic, incidental, or beneficial (Arrasate and Finkbeiner, 2005).

Induced Pluripotent Stem Cells

Creating iPSCs from humans with neurodegenerative disease presents an opportunity for studying all facets of neurodegenerative disease, according to Finkbeiner. Currently, most cellular studies are conducted in cell lines derived from animal models, rather than from humans with disease. Many lines are not even primary neurons. To study human neurons, in particular, iPSCs can be developed from skin or blood cells from people with disease and reprogrammed with transcription factors or small molecules. With specific protocols, the cells then can be differentiated into neuron subtypes of interest (e.g., motor neurons). These differentiated iPSCs can be used to study pathological functioning, identify drug targets within the affected cells, and screen drugs to predict how they might fare in human clinical trials. In an iPSC line from a patient with ALS, the mutant nerve cells had higher levels of the TDP-43 protein and shorter survival than similar lines from control lines (Bilican et al., 2012). This validated the iPSC line as an accurate model because TDP-43 is well established as the protein that forms toxic aggregates in ALS. The downsides of iPSCs are their heterogeneity at baseline and the composition of the cultures after being differentiated; their high costs; the length of time to differentiate cells into brain cells of interest; and absence of protocols for making many brain cell subtypes, Finkbeiner observed.

In the discussion, one participant challenged the value of iPSCs for their immaturity and for their incapacity to model the effects of cell–cell interactions, which are highly important in neurodegenerative diseases. Another participant expressed concern about inherent variability across different iPSC lines. Finkbeiner responded that his laboratory now has approximately 50 iPSC lines and several clones from individual patients and has found that the variability is relatively small; their cell–cell interactions were recently studied in an ALS model (see Serio et al., 2013).

Yeast as a Model System

Yeast is a single-cell eukaryote with characteristics that are found in complex eukaryotic organisms. It has become a model for studying neurodegenerative disease because many of its fundamental cell pathways are relevant, such as mitochondrial gene function and autophagy, the process of self-degradation and clearance described in Chapter 3. Gregory Petsko of Weill Cornell Medical College described two crucial areas in which yeast can be useful for the study of neurodegenerative disease: mechanistic studies of pathophysiology and drug screening. These types of studies are relatively easy to model in yeast, thanks to their well-defined genome, fast growth, the fact that 80 percent of their proteins have some functional characteriza-

tion, and the ease of transfecting genes, especially human genes, into the yeast genome.

A recently published study by Petsko and colleagues highlights the utility of yeast for investigating pathophysiology of familial ALS (Ju et al., 2011). The research focused on the gene FUS/TLS, which, when mutated, is a cause of one subtype of familial ALS (Kwiatkowski et al., 2009; Vance et al., 2009). FUS/TLS is a nucleic acid binding protein that implements key functions related to RNA processing. When transfected and overexpressed in yeast, FUS/TLS mislocalizes to the cytoplasm rather than to the nucleus where it is normally found. In the cytoplasm FUS/TLS aggregates and subsequently kills the cell, thereby recapitulating the salient phenotype of ALS in motor neurons. The researchers determined that mislocalization into the cytoplasm occurs because of a defect in a region of the FUS/TLS protein that normally marks it for import into the nucleus. Petsko and colleagues then screened a library of 5,600 yeast genes to determine whether any of them could suppress FUS/TLS's toxicity. Yeast screens are fast and inexpensive, and produce clear-cut results. Emerging from the screen were five yeast genes that suppressed FUS/TLS's toxicity. The genes were all RNA-binding proteins. The investigators then identified one human homolog of the yeast genes and they found it, when cloned into yeast, to be effective in suppressing FUS/TLS's toxicity. The research implicates a possible insufficiency in RNA processing or RNA quality control in mediating toxicity of FUS/TLS. "It would be hard to find this sort of thing out so easily with this kind of time scale without using a model organism as facile as yeast," said Petsko.

RESEARCH NEEDS AND NEXT STEPS SUGGESTED BY INDIVIDUAL PARTICIPANTS

The workshop speakers identified many questions for future research and other opportunities for future action. Those related to the rationale for studying commonalities across neurodegenerative diseases are compiled here to provide a sense of the range of suggestions made (research suggestions that are specific to the topics covered in Chapters 3-6 are included at the ends of those chapters). The suggestions are identified with the speaker who made them and should not be construed as reflecting consensus from the workshop or endorsement by the Institute of Medicine.

Enhancing Collaborations

- Develop dedicated programs and funding to identify common threads. Support work that specifically aims to evaluate therapeutic targets or therapies shown to be beneficial in one disorder in models of other neurodegenerative diseases. (Finkbeiner)

- "Establish effective links between basic scientists and clinical investigators and between academia and the pharmaceutical industry to expedite the discovery and validation of potential drug targets and the development of novel therapeutics." (Mucke, citing the work of the Alzheimer's Association Expert Advisory Workgroup on the National Alzheimer's Project Act, of which he is a member [2012, p. 360])
- Create innovative programs to foster collaboration among consenting scientists. Consider allowing program officers to use supplemental support from NIH in a more flexible manner to encourage willing scientists to pursue such collaborations. (Finkbeiner)
- Harmonize pathology and genetic measures. Geneticists should work together to come up with a list of pathological and clinical standards across diseases. (Singleton)
- Catalogue ongoing efforts to produce neurodegenerative disease-related induced pluripotent stem cells. Identify who is creating them; how many exist; what their basic characteristics/homogeneity are; and how others get access. (Ivinson)
- NIA and/or NINDS should look toward funding shared/collaborative programs that support the development or enhancement of resources that could be made available to multiple investigators. These might include biomarker programs; mouse behavior (and other model systems) testing; iPS cells; shared compound libraries; and access to tissues and laser capture microdissection/nucleic acid capture and analysis. (Ivinson)
- Solicit a set of key questions that deal with the cell biology of neurodegenerative disease that can help focus the field on some of the critical basic science questions whose resolution would advance the field. (Finkbeiner)
- Share human genetics data, with the goal of data aggregation, to find truly causal gene variants that extend across populations or are population specific. (Mootha)
- Determine what accounts for selective vulnerability of neurons in neurodegenerative diseases. This question lies at the crossroads of many of the issues discussed, and involves molecular pathogenesis, genetics, cell biology, and systems biology. (Walker)
- Identify why aging is the most prevalent risk factor for neurodegenerative diseases. Specifically, determine whether a particular aspect of aging (e.g., a component of the proteostasis network) can be targeted to lower risk, or whether the diseases result from a general (and thus less tractable) decline in cellular integrity. (Walker)

Identifying Biomarkers and New Therapeutics

- Create a PPMI/ADNI[2]-like consortium funded by NIH and a public–private consortium of centers in academia and pharmaceutical companies to investigate mechanisms of neurodegenerative disease transmission and the potential efficacy of immune therapies as disease-modifying interventions. Unlike PPMI/ADNI, shared intellectual property (IP) could be generated and royalties derived could be distributed to all stakeholders who contribute funding and/or IP according to a predetermined formula. (Trojanowski)
- Identify new biomarkers in neurodegenerative disease. (Dunlop, Ivinson, Mehler, Mucke, Youle)
- Examine biomarkers longitudinally, which is highly valuable information for a clinical trial. (Rigo)
- Build a cohort of patients with C9orf72 mutations for a biomarker study in early or preclinical ALS/FTD. (Singleton)
- Create a catalogue of ongoing neurodegenerative disease biomarker collections and programs. This would enable researchers worldwide to locate the samples and patient records they need. (Ivinson)
- Screen novel therapies in the yeast system—extending to fission yeast—and apply to other simple well-characterized systems, such as nematodes, fruit flies, and zebrafish—thereby unifying the databases of these organisms. (Kowall)
- Complement biomarker discovery efforts with more innovative approaches to clinical trial design; for example, consider smaller trials in more homogeneous patient populations that include better biomarkers and more sophisticated neurocognitive instruments and better measures of brain function. (Mucke)
- Enforce rigorous design, analysis, and interpretation of clinical and preclinical trials. Standardization is a crucial feature wherever possible. (Games, Ivinson, Mucke)

Improving Animal Models[3]

- Generate experimental models that better simulate the multifactorial nature of neurodegenerative disease and use them to assess

[2] PPMI refers to the Parkinson's Progression Markers Initiative, which is a clinical study aiming to identify biomarkers of Parkinson's disease progression. The Alzheimer's Disease Neuroimaging Initiative (ADNI) has the same focus, but for Alzheimer's disease.

[3] For additional discussion of opportunities related to animal models, see the summary of the March 2012 Institute of Medicine workshop on improving the utility and translation of animal models for nervous system disorders. The summary is available online: http://www.nap.edu/catalog.php?record_id=13530 (IOM, 2013).

combination treatments, which may be required to defeat neurodegenerative disease. (Mucke)

- Study the effect of aging in neurodegeneration by supporting the generation of conditional transgenic mouse models in which the different pathways that contribute to progression of disease could be manipulated in a temporal manner. (Cuervo)
- Generate animal models, free from intellectual property constraints, that are fully validated and available for minimal cost to academia and perhaps at a premium cost to industry. (Youle)

3

Protein Aggregation

Key Points Raised by Individual Speakers

- Protein aggregation is a common characteristic of many neurodegenerative diseases. The aggregates and/or oligomers appear to be toxic, causing injury or death to cells. In general, the greater the degree of aggregation, the greater is the severity of disease.
- Cells have specific organelles and other cellular components to clear protein aggregates, including proteasomes and lysosomes. Proteasomes are used to degrade smaller aggregates, whereas lysosomes are used for larger ones. The actions of proteasomes and lysosomes are controlled by a range of proteins, including ubiquitinating ligases, deubiquitinating enzymes, and chaperone proteins.
- Therapies that stimulate the cell's normal clearance mechanisms are likely to show promise for treating neurodegenerative disease, as are therapies that prevent protein aggregation in the first place.

Protein misfolding and other errors in protein generation occur frequently within cells, and the cell has evolved a range of mechanisms to ensure proper folding and to eliminate aggregated or otherwise damaged

proteins. A common characteristic of many neurodegenerative diseases is protein aggregation due to a failure of clearance mechanism(s).

The neurodegenerative disorders featured in the workshop share pathological accumulation in the brain of abnormal protein aggregates or inclusions that contain misfolded proteins. These diseases include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, dementia with Lewy bodies, frontotemporal diseases, and multiple system atrophy. The same protein can be found in more than one disease; mixed proteinopathies are highly prevalent (see Table 2-1). Proteins within the aggregate possess altered physical properties that are responsible for their misfolding. For example, the mutated huntingtin protein found in Huntington's disease contains excess repeats of the amino acid glutamine (Trottier et al., 1995). The alteration in structure leads to interaction with other proteins and subsequent aggregation that is dependent on age and length of the repeats (Voisine et al., 2010). In other cases, the protein itself may not necessarily be mutated, but it may be produced to excess by disease-related upregulation in protein expression. The sheer amount of additional protein being produced may tilt the balance toward misfolding, said Richard Morimoto of Northwestern University.

An abundance of misfolded proteins appears to be toxic to cells, leading to their injury and death. A disease's severity often correlates with the expression levels of the protein (Voisine et al., 2010; Williams et al., 2006). The toxic accumulation occurs in different parts of the brain and can be in the nucleus, cytoplasm, or extracellular space. Protein aggregation not only has been identified in humans with disease, but also has been replicated in biological model systems, such as in *C. elegans*, and with pure protein, according to Morimoto. Although not discussed in detail at the workshop, there is debate about whether certain forms of aggregates and/or components of aggregation mechanisms are neutral or even protective rather than toxic (see, for example, Selkoe, 2008; Spires-Jones et al., 2009; Williams and Paulson, 2008; Wolfe and Cyr, 2011).

This chapter summarizes workshop presentations about different mechanisms by which cells clear toxic protein aggregates and, for each, discusses potential therapeutics based on those mechanisms. Because protein aggregation is common across many neurodegenerative diseases, these therapeutic approaches might benefit more than one disease.

PROTEOSTASIS

Proteostasis, or protein homeostasis, is the collective term used to describe a variety of cellular processes designed to minimize damage from altered, misfolded, and otherwise damaged proteins. Morimoto stressed the importance of proteostasis for ensuring cellular health by proper folding

of proteins into native, soluble state instead of improper folding that leads to protein aggregation and cell toxicity. The so-called proteostasis network relies on chaperone proteins that guide protein folding, beginning with protein synthesis. Other chaperone proteins refold denatured proteins. The proteostasis network also relies on clearance mechanisms and detoxifying enzymes to degrade an excess of improperly folded proteins.

Aging, disease-associated mutations, polymorphisms, and energetic deficits place high demands on the proteostasis network, illustrated in Figure 3-1. Once the cell can no longer keep up with the heightened demand, the balance is shifted toward toxic accumulation of misfolded proteins. Morimoto pressed for better understanding of the upstream signaling events within the proteostasis network in order to prevent protein misfolding and aggregation. Preventing protein misfolding and aggregation is an appropriate therapeutic strategy, he said.

PROTEASOMES

One significant component of the proteostasis network is carried out by proteasomes, multisubunit complexes within the nucleus and cytoplasm that degrade soluble protein, according to Alfred Goldberg of Harvard Medical School. The vast majority of damaged proteins are normally degraded by proteasomes, and so too are smaller protein aggregates. The process begins with ubiquitin ligases that attach ubiquitin to damaged proteins or aggregates, marking them for proteasomal destruction. A chain of at least four ubiquitin molecules must be attached for the process to proceed. The bonding between the protein or small aggregate and ubiquitin leads the conjugate to attach to the opening of the proteasome, where the damaged protein is unwound and translocated through a small gate into the proteolytic core of the proteasome. There it is quickly digested into amino acids. If the binding and unfolding are not accomplished within seconds, deubiquitinating enzymes, such as USP14, inhibit proteasomal degradation by stripping away the ubiquitin tags. That releases the substrate from the proteasome and precludes degradation. Selectivity of the degradation process is conferred by a range of highly specific ubiquitin ligases that tack ubiquitin onto the damaged protein. There are at least 30 to 40 ubiquitin ligases of the E2 class and more than 650 of the E3 class. The combination of classes allows enormous opportunities for selectivity in the process of targeting proteins for elimination, Goldberg explained.

Another degradation pathway is via endosome engulfment and translocation to lysosomes, organelles that are larger than proteasomes. Goldberg described his work to identify the enzyme Nedd4, a membrane-associated ubiquitin ligase, which plays a major role in the clearance of α-Synuclein via the endosomal/lysosomal pathway. Nedd4 is found in neurons containing

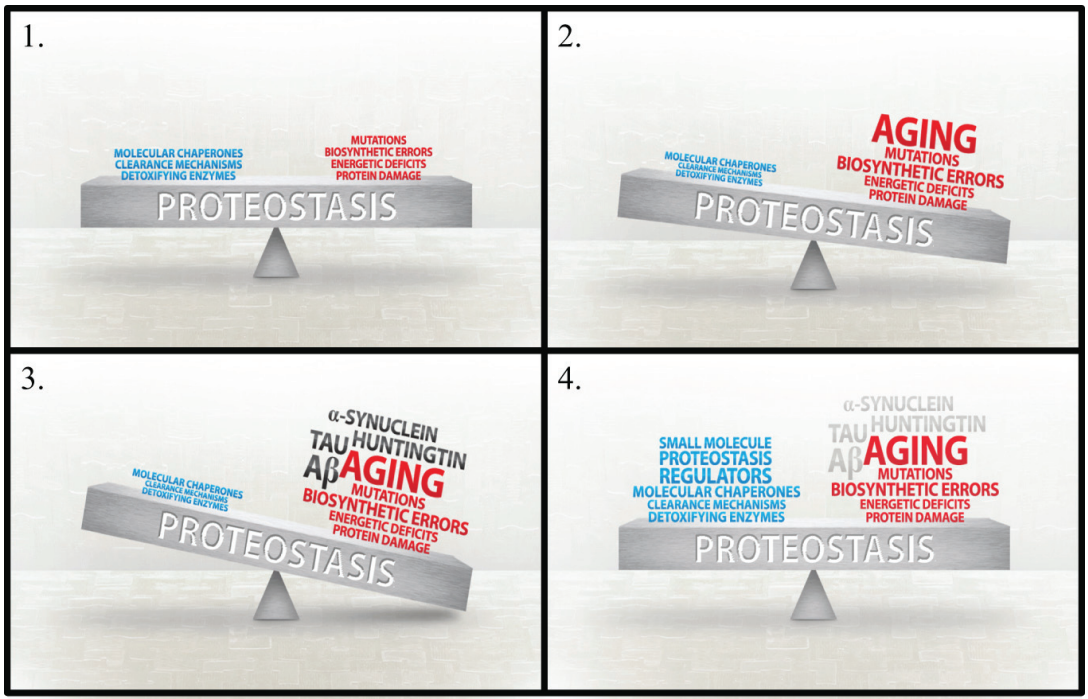

FIGURE 3-1 Proteostasis: balance between function and dysfunction. (1) In the optimal state, molecular chaperones, clearance mechanisms, and detoxifying enzymes keep in check mutations, biosynthetic errors, energy deficits, and protein damage. (2) Aging intensifies imbalance. (3) Disease-associated mutations further challenge the proteostasis network's ability to maintain balance. (4) Targeting upstream signaling processes may help restore the cellular environment by preventing misfolding and protein accumulation. SOURCE: Morimoto, 2012.

Lewy bodies. Its downregulation increases α-Synuclein content (Tofaris et al., 2011). (α-Synuclein can be degraded by other proteostasis mechanisms as well [Cuervo et al., 2004].) Finally, he pointed out that some protein aggregates tagged by ubiquitin and slated for destruction may be too large for the proteasome, in which case the complex forms a cork-like structure, clogging the proteasome and thereby leading to greater protein accumulation. How protein accumulation in the extracellular space is removed is unknown, Goldberg noted. Another area of insufficient knowledge is of proteasomal function specifically in neurons. Most of the research is done on other cell types, he acknowledged in response to questions.

In terms of therapeutic opportunities, Goldberg argued for stimulating proteasomal degradation by (1) identifying and tapping into the specific ubiquitin enzymes necessary for targeting and degrading proteins by various pathways; and (2) inhibiting deubiquitinating enzymes to prevent dissociation of protein-ubiquitin conjugate from the proteasome. Goldberg referred to the research identifying a small molecule that inhibits the specific deubiquitinating enzyme USP14 (Lee et al., 2010a). In this study, researchers found that the small molecule accelerated in vitro the degradation of neurodegenerative disease-related proteins tau and TDP-43 (Lee et al., 2010a). He observed that deubiquitinating enzymes are more amenable to drug targeting than are the ubiquitin ligases because they are cysteine proteases, which have a defined mechanism of action and highly specific targets.

AUTOPHAGY AND LYSOSOMES

Autophagy is a dynamic process of bulk degradation of cellular organelles and proteins; this includes proteins that are soluble as well as those that form into oligomers and aggregates. Autophagy clears them from the cell by lysosomes rather than by proteasomes, whose catalytic core is too narrow for bulk material to enter. The most common form of autophagy, known as macroautophagy, involves formation of an isolation membrane appearing around the bulk material to sequester it, then fusion of the edges of the membrane into a double-membrane structure known as an autophagosome. The autophagosome in turn fuses with lysosomes, which destroy the protein with their proteolytic enzymes. At least 35 autophagy-related genes essential for formation of autophagosomes have been identified (Yang and Klionsky, 2010).

Under normal conditions, autophagy occurs at a modest basal level. But under conditions of stress and nutrient depletion, autophagy is increased. Normal animals, whose autophagy in the central nervous system is blocked by knocking out essential autophagy genes (e.g., Atg5 or Atg7) needed to assemble the autophagosome membrane, proceed to develop neurodegen-

erative disease, as evidenced by behavioral deficits and loss of specific nerve cells (Hara et al., 2006; Komatsu et al., 2006).

A deficiency or outright failure of autophagy is thought to permit aggregation of misfolded proteins that lead to neurodegeneration. A key question is what causes the failure of autophagy in disease? The answer to this question could guide the creation of new therapies. There are several possible reasons for failure of autophagy. One is a failure by the autophagosome to recognize aggregated material. This was found to be the case in a study of Huntington's disease, according to Ana Maria Cuervo of Albert Einstein School of Medicine. In mouse models of Huntington's disease and cells from Huntington's patients, autophagosomes failed to efficiently trap protein deposits, organelles, and other cargo (Martinez-Vicente et al., 2010). The rest of the pathway was intact, for autophagosomes formed at a normal rate and fused appropriately with lysosomes. The failure was inefficient engulfment of cytosolic components. Cuervo speculated that the failure of recognition may stem from pathogenic proteins, such as the mutant huntingtin protein, which becomes attached to the inside of the membrane of the autophagosome, interfering with its capacity to recognize bulk cargo. Another cause of failure in autophagy might be that the aggregate itself induces damage to the pathway, rendering the autophagosomes unable to traffic within the cell. More specifically, Warren Hirst of Pfizer pointed out, the problem in one Alzheimer's case was the result of flawed fusion of the autophagosome with the lysosome. However, in another mouse model of Alzheimer's, Cuervo said, the failure occurred because the disease environment changed the pH, which is critical for lysosome's hydrolytic enzymes to work effectively (Lee et al., 2010b).

In the discussion, prompted by several questions, Cuervo said much remains to be known about autophagy and trafficking of autophagosomes in nerve cells. Lysosomes are less likely to be found in nerve cell processes as opposed to the cell body, so autophagosomes forming in the processes may need to traffic to the site of the lysosomes by retrograde transport up the microtubules, although this does not uniformly hold and ongoing studies support lysosomal presence in terminals. She stressed that movement of autophagosomes through retrograde transport is understudied.

One therapeutic opportunity is to enhance autophagy. This has been done successfully with the drug rapamycin, which induces autophagy. Rapamycin slowed the progression of Huntington's disease pathology in experimental models (Ravikumar et al., 2002). But the drug induces autophagy only weakly in physiologically relevant cells, such as cortical neurons. Steven Finkbeiner of the Gladstone Institutes and the University of California, San Francisco, described a small molecule (N^{10}-substituted phenoxazine) that induces autophagy specifically in neurons from the striatum, cortex, and hippocampus. He found that the compound was neuro-

protective in an animal model of Huntington's disease (Tsvetkov et al., 2010). These efforts will be difficult to translate into human research unless there are good biomarkers for measuring autophagy in a patient population, noted Finkbeiner.

Finally, therapeutic approaches can be designed to prevent protein aggregation altogether, thus obviating the need for therapies to induce autophagy. John Dunlop of AstraZeneca described a new drug, developed by Pfizer. Already approved in Europe, the drug tafamidis functions to stabilize the correctly folded tetramer form of the transthyretin (TTR) protein. This protein is destabilized in the genetic disease Transthyretin Familial Amyloid Polyneuropathy (TTR-FAP), a rare, progressive, and fatal neurodegenerative disease. In patients with TTR-FAP, the protein dissociates and forms amyloid fibrils, which, in turn, cause failure of the autonomic nervous system and/or the peripheral nervous system, among other bodily sites. The new medication, said Dunlop, counters the pessimistic view that protein–protein interactions are not likely to lend themselves to drug development. It also stands as testimony to the possibilities of drugs designed to combat protein aggregation, he said.

ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE

Recent findings show a strong correlation between the aggregation of misfolded proteins and the engagement of a stress response of the endoplasmic reticulum (ER), said Claudio Hetz, a professor at the University of Chile. Table 3-1 shows the neurodegenerative diseases for which evidence for ER stress has been documented in cellular/animal models and in postmortem human studies (for a review of this evidence, see Matus et al., 2011). The ER organelle is an "essential compartment for the maturation and processing of proteins" (Matus et al., 2011, p. 239). Hetz noted that ER stress triggers an adaptive response known as the unfolded protein response (UPR), which controls hundreds of genes related to protein quality control and folding. However, if these mechanisms of adaptation are insufficient to recover homeostasis of the ER, irreversible or chronic ER stress can also trigger cell death.

Hetz emphasized that the contribution of the UPR pathway to neurodegenerative diseases is not fully understood, including the circumstances under which it appears to provide an adaptive response that increases survival of neurons, as well as the circumstances under which it represents a pathological mechanism that leads to neuronal dysfunction or cell death when the damage is too high. Hetz and his collaborators have been working to further understand the role of UPR in neurodegenerative disease by generating new mouse models that enable them to manipulate the UPR and

TABLE 3-1 Endoplasmic Reticulum Stress in Neurodegenerative Diseases

Disease	Cellular/Animal Models	Human Studies (Postmortem)
Alzheimer's disease	✓	✓
Parkinson's disease	✓	✓
Amyotrophic lateral sclerosis	✓	✓
Creutzfeldt-Jakob (prion)	✓	✓
Multiple sclerosis	✓	
Huntington	✓	✓
Spinocerebellar ataxia	✓	
Spinal cord injury	✓	
Ischemia	✓	
Lysosomal storage disorders	✓	✓

see the impact on models of ALS, Huntington's disease, and Parkinson's disease (see, e.g., Hetz et al., 2008, 2009; Vidal et al., 2012; Zuleta et al., 2012). Some recent findings from a different research group indicate that mild ER stress ("preconditioning") may even inhibit the death of neurons by promoting autophagy, suggesting that preconditioning could have potential value in developing therapies for neurodegenerative diseases (Fouillet et al., 2012). During the discussion period, a workshop participant raised what he termed the "Goldilocks effect": Because a mild level of stress may be helpful but a high level will be harmful, it will be challenging to develop optimal therapeutic regimes. Hetz clarified that he thinks that decreasing the stress levels will always be good, but that perhaps a mild stress will be sufficient to trigger an endogenous adaptive response.

RESEARCH NEEDS AND NEXT STEPS SUGGESTED BY INDIVIDUAL PARTICIPANTS

The speakers at the workshop identified many questions for future research and other opportunities for future action. The research suggestions related to protein aggregation are compiled here to provide a sense of the range of suggestions made. The suggestions are identified with the speaker who made them and should not be construed as reflecting consensus from the workshop or endorsement by the Institute of Medicine.

- Develop better mechanistic understanding of protein aggregation and clearance mechanisms. (Hirst, Ommaya, Rigo)

- Use conformation-specific antibodies to recognize specific structures in aggregation intermediates. (Finkbeiner)
- Develop methods to measure protein homeostasis and quality control. (Cuervo)
- Develop and standardize an in vitro model of α-Synuclein aggregation. (Kowall)
- Study propagation of amyloidogenic proteins and ways to prevent or arrest it. Will plasmapheresis, intravenous immunoglobulin, or other manipulations halt or reverse propagation? (Kowall)
- Use imaging and other methods to measure proteostasis and related quality control mechanisms in humans. (Cuervo, Ommaya)
- Develop understanding of heterogeneity of protein aggregation in normal and disease populations. (Cuervo)
- Develop deeper understanding of proteostasis within neurons and within specific neuron subtypes. (Dunlop, Ommaya)
- Study whether people with neurodegenerative disease have genetic susceptibility to protein overexpression or misfolding. (Ranum)
- Develop therapies that stimulate proteasomal degradation and autophagy. (Cuervo, Goldberg)
- Evaluate therapeutic targets or therapies in proteostasis that are shown to be beneficial in one disorder in models of other neurodegenerative diseases. (Finkbeiner)
- Identify the mechanisms of movement of autophagosomes through retrograde transport. (Cuervo)

4

Transmissibility

Key Points Raised by Individual Speakers

- Misfolded proteins appear to act as seeds or templates to cause misfolding of the same proteins from their native, soluble state into oligomers. The oligomers eventually coalesce to form insoluble aggregates that are found in all major neurodegenerative diseases.
- The protein aggregates appear to be transmissible by some type of cell-to-cell spread in vivo along anatomically connected pathways. The aggregates might become toxic to the cells in the pathway and lead to disease.
- Transmissibility can be interrupted by administration of antibodies to pathogenic proteins, suggesting immunization as a treatment strategy. Immunization requires that the target protein be found extracellularly.

The progressive accumulation of protein aggregates is the pathological hallmark of many neurodegenerative diseases (see Table 2-1 and Chapter 3). The question is what initiates the process of protein aggregation and subsequently enables it to progress and ramify through distinct pathways of the nervous system. The prevailing model for transmission within the nervous system is known as protein seeding or corruptive protein templating (Jucker and Walker, 2011; Lee et al., 2011). The "seeds" are the small amounts of

misfolded protein that are self-propagating: When they come into direct contact with native protein they convert it to the misfolded form, a process that leads to formation of oligomers; as oligomers accumulate they eventually coalesce into insoluble aggregates consisting of amyloid fibrils, which bear their characteristic beta-pleated sheet conformation (see Figure 4-1). The seed is the transmissible agent. The model is borrowed from what is known about the formation of misfolded amyloid proteins known as "prions" that are responsible for aggregating and spreading transmissible spongiform encephalopathies. The amyloid aggregates in transmissible

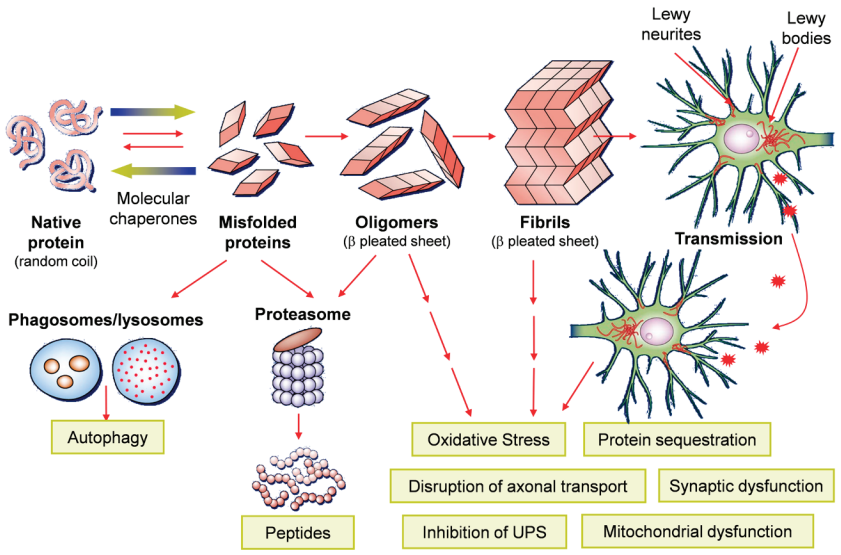

FIGURE 4-1 α-Synuclein mediated neurodegeneration. This illustrates hypothetical processes whereby normal α-Synuclein is converted into pathological α-Synuclein that fibrilizes and deposits into Lewy bodies/Lewy neurites of affected neurons in the brains of patients with Parkinson's disease (PD)/PD with dementia/dementia with Lewy body. Genetic abnormalities and poorly understood environmental factors may accelerate this process. Normal quality control systems (chaperones, ubiquitin proteosome and phagosome/lysosome systems) that prevent/reverse protein misfolding or eliminate misfolded proteins are overwhelmed. Remarkably, recent data suggest that the progression of PD and related disorders may be linked to the cell-to-cell spread of pathological species of α-Synuclein as illustrated in the upper right of the figure. The toxic consequences of pathological α-Synuclein are illustrated in the lower right of the figure.
NOTE: UPS = ubiquitin-proteasome system.
SOURCE: Trojanowksi, 2012. Adapted from Lee and Trojanowski, 2006 (adapted figure printed with permission from *Neuron*).

spongiform encephalopathies are structurally similar to those found in neurodegenerative disease, but their constituent amyloid proteins are different. In neurodegenerative disease the proteins are, most commonly, Aβ amyloid, tau, and α-Synuclein, whereas with prion diseases, the disease proteins are different pathological strains of prion proteins.

The term "transmissible," for the purpose of this workshop summary, is not synonymous with infectious. The term refers here to cell-to-cell spreading along pathways within the brain, as opposed to spreading or infectivity between individuals. There is no evidence as yet that pathogenic proteins in neurodegenerative diseases are infectious and thus spread between individuals. In contrast, prion diseases are not only transmissible across cells of the brain, but they also can be spread within and across species by direct contact of biological fluids, according to human epidemiology studies. The epidemiology shows that common neurodegenerative disorders do not exhibit infectivity, emphasized John Trojanowski of the University of Pennsylvania. This is a fundamental difference between the two types of diseases. Ignorance of this distinction can be highly injurious to people with neurodegenerative disease who may be needlessly stigmatized if they are erroneously deemed to have an infectious disease.

This chapter summarizes workshop presentations on the prion-like mechanisms by which protein aggregates might spread through the central nervous system. It then discusses the transmissibility of specific aggregated proteins in relation to certain neurodegenerative diseases, before examining the potential success of passive immunization to counter transmission within the brain.

PRION DISEASES AND THEIR TRANSMISSION

Both transmissible spongiform encephalopathies and neurodegenerative diseases are marked by insoluble protein aggregates made up of misfolded protein. Transmissible spongiform encephalopathies—also known as prion diseases—have been studied for decades because of their unique form of transmission by protein templating. The process by which prions propagate is similar to the process that underlies formation and generation of amyloid aggregates in neurodegenerative disease. Thus, prion diseases are important to study in order to understand how neurodegenerative disease might spread within the brain, according to Claudio Soto of the University of Texas Medical School at Houston.

The transmissible spongiform encephalopathies are a group of fatal neurodegenerative diseases. They include Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep. Although clinical manifestations differ among them, their pathological characteristics are similar, including extensive spongiform degeneration,

widespread neuronal loss, synaptic alterations, brain inflammation, and accumulation of protein aggregates that are toxic to cells (Diaz-Espinoza and Soto, 2010).

The propagation of prion disease, both within and across species, is by misfolded prion proteins, which are the "infectious" agents. The term "prion" was coined to denote a proteinaceous particle that is infectious. Evidence that the misfolded prions are the infectious agents has been accumulating for decades. The most definitive evidence, according to Soto, comes from two studies published during the past 7 years. The first showed that injection of highly purified prion protein, which had been amplified in vitro, caused disease in normal animals (Castilla et al., 2005). The second showed a similar result by injecting purified recombinant prion protein (Wang et al., 2010). Altogether, there have been fewer than 700 cases of prion transmission to humans, with most cases caused by ingestion of beef from cows with bovine spongiform encephalopathy (Jucker and Walker, 2011).

Soto reviewed the basic features of prion proteins and their infectivity. Prions are protease-resistant, amyloid-like β-pleated sheets of diverse sizes ranging from small oligomers to large aggregates. They can be acquired by different routes of exposure—including blood transfusion, intracerebral injection, oral ingestion, or intraocular and intranasal routes—but not through skin contact or inhalation. They are highly resistant to common sterilization procedures, including extremely high temperatures, ultraviolet (UV) radiation, treatment with detergents, and proteases. Soto described the seeding as an exponential process that begins slowly. There is a long lag phase during which monomers attempt to form stable oligomers. Once this occurs, there is a swift elongation phase during which oligomers act as seeds to form a far greater number of amyloid aggregates (Soto et al., 2006). In other words, the process begins slowly, but reaches a tipping point at which time there is a sudden acceleration in forming aggregates made up of amyloid fibrils. The process is accelerated by the addition of exogenous seeds. Misfolded prion proteins, oligomers, and aggregates are, in his view, "seeding competent," but the fine details of the seeding and transmission process are still elusive. Better understanding of the seeding process, in his view, will yield dividends for biomarker development and therapeutics for neurodegenerative disease.

Soto subsequently described his research on Aβ amyloid. His team has shown that Aβ amyloid can be transmitted to an animal by direct injection of human brain extracts from Alzheimer's disease cases. The formation and accumulation of aggregates increases progressively with time, and the aggregates are localized to brain areas distant from the injection site (Morales et al., 2011).

Several participants emphasized that members of the public need to

understand that Alzheimer's disease is not infectious in conventional ways, that is, through inhalation or skin contact. A recent study also found no evidence for human-to-human transmission of neurodegenerative disease–associated proteins in recipients of cadaveric human growth hormone (Irwin et al., 2013). One participant relayed his experience when a newspaper article implied that Alzheimer's disease was infectious. It engendered many calls to Alzheimer's organizations expressing confusion and fear. If scientific findings are not properly qualified, people may needlessly shun and stigmatize victims of neurodegenerative disease and their family caretakers—those already devastated by the disease.

TRANSMISSIBILITY OF SPECIFIC AGGREGATED PROTEINS IN SELECT NEURODEGENERATIVE DISEASES

This particular portion of the workshop focused on Aβ amyloid and tau transmission in Alzheimer's disease, and α-Synuclein transmission in Parkinson's disease. In describing the choice to focus on these specific topics, Trojanowski, chair of this session, noted, "We could have done more, but we as a group thought it was helpful to focus on areas that had made the most progress in cells and animal models."

Aβ Amyloid Transmission in Alzheimer's Disease

Lary Walker of Emory University described his research on the seeding process and the transmission of Aβ amyloid within the brain of Alzheimer's animal models. Several lines of evidence reveal that the seed for Aβ amyloid aggregates and plaques is indeed Aβ amyloid. The most salient evidence comes from studying the consequences of intracerebral injections of dilute Aβ amyloid-containing brain extracts from autopsied Alzheimer's cases into transgenic mice. The mice carry the human gene for Aβ precursor protein, from which Aβ amyloid is cleaved. One key experiment found that seeding is abolished or reduced by anti-Aβ amyloid antibodies, immunodepletion, or denaturation (Meyer-Luehmann et al., 2006). Other evidence shows that the seeded deposits are not from the injected brain extracts because there is a several months–long lag time in the formation of Aβ amyloid plaques. Finally, the seeded host must express human Aβ amyloid. Transgenic mice develop seeded Aβ plaques, whereas wild-type mice do not show any lesions after injection.

Walker's laboratory also established that seeding stimulates Aβ deposition elsewhere in the brain. His team showed that, after hippocampal injection of Alzheimer's disease extracts, Aβ plaques are found along axonally interconnected pathways, including the entorhinal cortex (Jucker and Walker, 2011). The opposite was the case when another team of

researchers injected the extract into the entorhinal cortex; it led to plaque formation in the hippocampus, relayed Walker. He speculated that the spreading occurred by axonal transport, but his group is only beginning to investigate the mechanisms of intracellular passage of seeds. The extract in these experiments is the supernatant of centrifuged homogenates from Alzheimer's disease brains or from Aβ precursor protein transgenic mouse brains. Small and soluble Aβ amyloid seeds from the dilute supernatant are potent inducers of plaques, noted Walker.

Tau Transmission in Alzheimer's Disease

Tau is a microtubule-associated protein that aggregates in its hyperphosphorylated form into neurofibrillary tangles that are a pathological signature of Alzheimer's disease. In this disease there is a characteristic progression of neurofibrillary tangles, starting in the entorhinal cortex in prodromal stages, proceeding to the limbic areas in early to moderate stages, and finally converging on the neocortex in late stages (see Figure 4-2).

In her presentation, Karen Duff of Columbia University focused on the prodromal period when the tangles are localized to the entorhinal cortex. She and her colleagues investigated whether tau could spread to anatomically appropriate pathways in the limbic system. They developed an animal model of a transgenic mouse expressing the human pathological tau gene

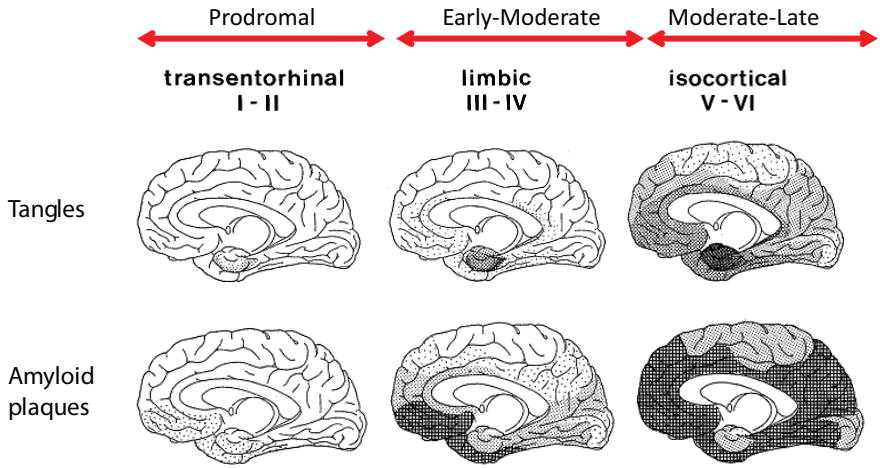

FIGURE 4-2 Plaque and tangle distribution at different stages of Alzheimer's disease progression (Braak staging).
SOURCE: Braak and Braak, 1991.

under the control of a promoter that is specific for the entorhinal cortex. This ensured that the animal only expressed tau pathology in the entorhinal cortex. Using this model, they found that in old animals, neurofibrillary tangles appeared in three monosynaptically connected nuclei to which entorhinal neurons project: the granule cells of the dentate gyrus, the subiculum, and the CA1 region of the hippocampus (Liu et al., 2012). Tangles were not evident in nuclei immediately neighboring the entorhinal cortex. They concluded that there was propagation of tau along anatomically connected neural pathways in older animals. In younger animals, neurofibrillary tangles remained restricted to the entorhinal cortex, without any evidence of spread, which indicated an age effect (Liu et al., 2012).

Duff's team also sought to discover the mechanism of tau spread. Both her team and another (de Calignon et al., 2012) proposed that tau is directly released into the extracellular space at the synapse as a result of degenerating axons. Once released it could be taken up by the postsynaptic neuron. Data from her in vitro work suggest that only small molecular-weight tau fibrils, not large aggregates, could be taken up by the postsynaptic neuron, she explained. Most importantly, tau's presence in the extracellular space and not in vesicles meant to Duff that tau could be targeted there by immune therapies. Finally, using a microchamber to separate different regions of the cell, her team found that, after administration, tau is taken up by neurons and transported both retrogradely and anterogradely. In the future, she would like to employ her transgenic mouse model to study interactions between Aβ amyloid and tau in the entorhinal cortex and she would like to understand the mechanisms of cell-to-cell spread as well as the functional impact of tau pathology.

α-Synuclein Transmission in Parkinson's and Other Neurodegenerative Diseases

α-Synuclein is a cytosolic protein thought to participate in the regulation of synaptic transmission and synaptic plasticity (Murphy et al., 2000). Amyloid fibrils of this protein constitute the major pathological signature of Parkinson's disease, dementia with Lewy bodies, multiple system atrophy (Lee et al., 2011), and a subset of Alzheimer's disease cases. Virginia M.-Y. Lee of the University of Pennsylvania described two new models for studying the seeding and transmission of α-Synuclein.

Lee and her colleagues have developed the first neuronal model of spontaneous Parkinson's disease. It is a hippocampal neuron primary culture to which they add non-mutated, α-Synuclein preformed synthetic fibrils (Volpicelli-Daley et al., 2011). The cultured neurons are from wild-type, non-transgenic mice. The addition of the fibrils to the neuron cell culture recruits endogenous α-Synuclein to form pathologic, insoluble Lewy bodies

and Lewy neurites. α-Synuclein becomes hyperphosphorylated and ubiquitinated, but the purpose of these modifications is unclear to her. Lewy body–like pathology forms first in axons because axons bear the highest concentrations of endogenous α-Synuclein. The pathology is propagated both retrogradely and anterogradely throughout the entire neuron. The Parkinson's-like inclusions impair neuronal function and induce cell death. Lee said that this unique and easy-to-use primary neurons culture model will facilitate Parkinson's research and drug discovery. She offered to share the synthetic fibrils with other researchers.

The second model she used was of M83 transgenic mice that express the human gene for pathological α-Synuclein. Her team injected preformed fibrils and lysate from symptomatic animals into the stratum and/or cortex of asymptomatic transgenic mice. The recipient mice, which normally become symptomatic with Parkinson's-like motor impairment at 12 months of age, displayed motor impairment earlier. This finding is consistent with a previous study showing that addition of small quantities of preformed fibrils accelerates the kinetics of aggregate formation (Luk et al., 2007). At autopsy, her team found transmission of pathology throughout the entire mouse brain, including the olfactory bulb, the cerebellum, and the corpus callosum. Because pathology was evident in white-matter tracts, they believe this is the route for propagation of α-Synuclein throughout the brain. The seeded inclusions bore morphological and biochemical similarities to inclusions found in Parkinson's disease. The animals have a shorter lifespan, dying about 100 days sooner than did untreated animals. Finally, the incubation time from injection to death is consistent regardless of the age at which the injections took place. This transgenic model represents, in Lee's view, a new opportunity to study disease mechanisms and disease-modifying therapies. She is especially interested in determining how α-Synuclein is degraded, how endogenous seeds interact with the preformed fibrils, what enzymes are involved in phosphorylation and ubiquination, and how α-Synuclein inclusions affect cellular function prior to death.

IMMUNIZATION FOR NEURODEGENERATIVE DISEASE

This portion of the workshop dealt with research on two types of antibody immunizations, one targeted to the pathogenic protein α-Synuclein and the other to tau, to treat Parkinson's disease and Alzheimer's disease, respectively. Although the research is in the early stages, there have been successful treatments in animal models.

α-Synuclein Immunization for Parkinson's Disease

The rationale for antisynuclein immunization as a treatment strategy derives from the presence of α-Synuclein in the synapse, cerebrospinal fluid (CSF), and plasma membrane, where it would be accessible to antibody attack. Dora Games of Neotope Biosciences first reiterated the evidence supporting seeding and transmission of α-Synuclein in vivo and in vitro. She also noted that decreased expression of α-Synuclein reduces behavioral deficits and pathology, whereas addition of synthetic α-Synuclein fibrils exacerbates those outcome measures (Lim et al., 2010; Luk et al., 2012).

For the study of the efficacy of immunization, Games and her colleagues selected a transgenic mouse model expressing α-Synuclein because mice develop behavioral deficits and α-Synuclein aggregates throughout the temporal cortex and hippocampus similar to what has been described in Lewy body disease. After testing several antibodies, they selected one to the C-terminus region of α-Synuclein, and conducted in vivo and in vitro studies. Upon antibody administration, they found that the antibody crossed the blood/brain barrier and localized to lysosomes. Once in the brain, the antibody reduced behavioral deficits in learning and memory, and reduced the accumulation of α-Synuclein in axons and synapses (Masliah et al., 2011). The researchers used readout antibodies to label dystrophic neurites and somatic pathologies. Using two markers, they also found that immunization preserves synaptic integrity. The combined evidence led Games and colleagues to conclude that immunization is efficacious for synucleinopathies. In response to questions, she conceded that she does not know the mechanism by which antibodies are effective. In fact, she and her collaborators are unsure as to whether the antibodies work extracellularly and/or intracellularly. Her collaborator, Eliezar Masliah, does have evidence of antibodies penetrating the neuron cell membrane. In the ensuing discussion, Games added that much needs to be learned from ongoing clinical trials of antibodies in Alzheimer's disease. If the results, which are expected in several months, are negative, she urged investigators to evaluate why the failure occurred, whether it was the patient population, the timing of delivery, or other factors.

Games raised several issues regarding the immunization study, including whether transgenic models are relevant to human disease, and she expressed concern about the lack of common standards for readouts and assays, lack of incentive for replicating studies in academic settings, and the high cost of chemistry, reagents, and in vivo support. To reduce risk in clinical trials, she argued that diagnoses should be assessed earlier, progression must be assessed faster, target engagement must be verified in early phases of development, and diagnostic and treatment biomarkers must be validated.

Tau Immunization in Neurodegenerative Disease

Tau is the main component of neurofibrillary tangles, the pathological aggregate found in Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, and progressive supranuclear palsy. Because tau functions normally in the cytosol to stabilize microtubules and the tau tangles are intracellular, it has been widely assumed that only negligible concentrations of tau would be found in the extracellular space. That assumption has been discarded as a result of findings that reveal tau to be present in the CSF in young, healthy people, said Peter Davies of the Feinstein Institute for Medical Research. Further studies have shown tau in interstitial fluid (Yamada et al., 2011) and tau release from cultured neurons and transfected cells. The extracellular presence of tau justifies the use of antibody immunization, noted Davies.

At least four published studies show that antibodies against tau can reduce pathology and improve behavior of transgenic animals that express the mutant tau gene from humans (Asuni et al., 2007; Boimel et al., 2010; Boutajangout et al., 2010; Chai et al., 2011). Several of these studies were conducted in P301S transgenic mice. These mice are commonly used, said Davies, because they show many of the features of frontotemporal dementia and their tau pathology appears early. Using this mouse model, Davies and his colleagues revealed that antibodies directed at tau reduce pathology in the hippocampus and delay progression of disease (Chai et al., 2011). Davies reported being "very puzzled" by these findings because it is not clear to him how an extracellular reagent can exert these striking effects intracellularly. He reported spending months trying to determine if antibody penetrates intracellularly, but was unable to show it. There was discussion surrounding the question of whether antibodies worked extracellularly or intracellularly. In response to a question, Davies reported that, according to his in vitro studies, the degree of membrane depolarization does not affect the amount of anti-tau antibody levels in the culture medium.

He concluded his presentation by asking whether there is a pathogenic extracellular species of tau and what type of characteristics it has. He also raised questions about the predictability of the models to humans, especially because tauopathy, in the most common neurodegenerative diseases, is often accompanied by other proteinopathies (see Table 2-1). He urged testing in humans, and in a patient population with more pure tau pathologies, such as frontotemporal dementia or progressive supranuclear palsy.

RESEARCH NEEDS AND NEXT STEPS SUGGESTED BY INDIVIDUAL PARTICIPANTS

The speakers at the workshop identified many questions for future research and other opportunities for future action. The suggestions related to transmissibility and immunization are compiled here to provide a sense of the range of suggestions made. The suggestions are identified with the speaker who made them and should not be construed as reflecting consensus from the workshop or endorsement by the Institute of Medicine.

- Conduct epidemiology studies to directly study transmissibility of neurodegenerative disease and the rare likelihood of infectivity through surgical equipment and blood transfusions. (Trojanowski, Walker)
- Test the model of protein seeding and transmission by identifying the biophysical and cellular mechanisms by which seeds form in vivo, how they are cleared, and how they move within neurons and in the extracellular space once seeding begins to spread. (Cuervo, Walker)
- Study the proteolytic pathways by which seeded aggregates are degraded and study cellular response to seeding. (Lee)
- Determine whether transgenic animal-expressing mutant tau are relevant to humans with neurodegenerative disease. Determine whether a pathogenic extracellular species of tau exists and what type of characteristics it has. (Davies)
- Study interactions between aggregates of distinct proteins and focus on neural networks. (Duff)
- Determine if there is "cross-seeding" between misfolded proteins. (Walker)
- Before conducting clinical trials, establish target engagement, use validated diagnostic and treatment biomarkers, and use common standards for readouts and assays. (Games)
- Understand the mechanism(s) by which tau is released from neurons into extracellular fluids. (Davies)
- Develop therapies, such as immunization, that abolish transmission of misfolded amyloid proteins. (Davies, Duff, Games, Walker)
- For trials using anti-tau antibodies, test therapies in patients with pure tauopathy, such as frontotemporal dementia or progressive supranuclear palsy. (Davies)

5

Mitochondrial Pathology

Key Points Raised by Individual Speakers

- Because of their high energy requirements, neurons are especially vulnerable to injury and death from dysfunctional mitochondria.
- Pathological and physiological evidence reveals mitochondrial dysfunction in all major neurodegenerative diseases.
- Questions remain as to whether mitochondrial dysfunction is causal to neurodegenerative disease. Even if is not causal, mitochondrial dysfunction is still highly important and likely contributory to disease. Identifying therapies to improve mitochondrial function or to degrade dysfunctional mitochondria may make sense.
- Studying primary mitochondrial diseases can shed light on neurodegenerative diseases that show similar pathology. Because both types of diseases affect multiple pathways and organ systems, they require the approach of systems biology.
- Potential therapeutic approaches include medications that induce mitochondrial genesis, catalytic antioxidants to protect against reactive oxygen species, regulators of intracellular calcium, and regulators of redox potential across mitochondrial membrane. Maintenance of redox potential is crucial for mitochondrial integrity and control over oxidative phosphorylation.

Mitochondria are cellular organelles responsible for oxidative phosphorylation, the vital process of converting nutrients into adenosine triphosphate (ATP) molecules that provide the power for normal cell functions. Each neuron has at least hundreds of mitochondria. Because nerve cells are postmitotic, any mitochondrial damage that is sustained will accumulate with age and lead to dysfunction. Widespread damage to mitochondria causes cells to die because they can no longer produce enough energy. Indeed, mitochondria themselves unleash the enzymes responsible for cell death. The brain is especially vulnerable to mitochondrial dysfunction because its energy needs are higher than that of any other organ in the body. The brain accounts for only 2 percent of body weight yet consumes 20 percent of oxygen.

Mitochondrial functioning is determined by two separate genomes, one in the mitochondria, known as mitochondrial DNA (mtDNA), and the other in the nucleus. The mitochondrial genome encodes 13 proteins, all of which are vital to oxidative phosphorylation. The nuclear genome encodes approximately 1,500 genes involved in mitochondrial biology, including proteins necessary for replication of mtDNA, transcription, translation, and posttranslational modifications. There is only one copy of mtDNA, inherited from the mother, versus two copies of nuclear DNA, one from the mother and the other from the father. Mitochondria not only are responsible for oxidative phosphorylation, but they also play significant roles in metabolism and signaling, including fatty acid synthesis, ketone body metabolism, calcium homeostasis, and apoptosis. More specifically, mitochondria provide the majority of cellular energy in the form of ATP. They generate and regulate reactive oxygen species, they buffer calcium levels inside the cell, and they control apoptosis (Wallace, 2005, 2010).

Mitochondrial defects are found in pathological studies of all major neurodegenerative diseases, said Vamsi Mootha of Harvard Medical School. The range of mitochondrial defects includes fragmentation and other morphological changes, increased mutation rates in mtDNA, changes in permeability of mitochondrial membranes, changes in redox potential, accumulation of mutant proteins, and impaired oxidative phosphorylation (Reddy and Reddy, 2011). But whether these mitochondrial defects are causal in neurodegenerative disease is the fundamental question, Mootha said. The potential roles of mitochondria in neurodegenerative disease are, in his view, threefold: (1) they harbor primary lesions and thus serve as the primary source of disease pathology; (2) they function properly, but serve as mediators or amplifiers of disease; or (3) they are bystanders that do not contribute to pathology. Even if mitochondrial defects are not causal, they are likely contributory, noted Neil Kowall of Boston University, and thus any therapy that preserves, enhances, or corrects mitochondrial function is likely to be beneficial in forestalling cell death and disease progression.

This chapter summarizes workshop presentations that provide evidence of mitochondrial dysfunction in major neurodegenerative diseases. Because the evidence is unclear as to whether mitochondrial dysfunction is causal, it may be valuable to look at primary mitochondrial diseases and adopt a systems approach to research, several participants said.

MITOCHONDRIAL DYSFUNCTION AND NEURODEGENERATIVE DISEASES

As noted above, mitochondrial dysfunction is found in the major neurodegenerative diseases. This section outlines workshop presentations about mitochondrial dysfunction in Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, and Alzheimer's disease.

Parkinson's Disease

Parkinson's disease is characterized by a loss of dopamine-containing neurons in the brain region known as the substantia nigra. Pathological and other studies have convincingly shown that mitochondrial deficiency accumulates in this brain region upon aging, said Richard Youle of the National Institute of Neurological Disorders and Stroke. Youle's talk focused on the function of two proteins that are mutated in familial, early-onset Parkinson's disease: Parkin and PINK1 (PTEN-induced putative kinase 1).

The normal functions of Parkin and PINK1 have not been well understood until recently, Youle said. Evidence from multiple species is accumulating that these proteins normally work together to trigger clearance of damaged mitochondria, a process known as mitophagy. It stands to reason that, if mutated, they can fail to induce mitophagy, leaving dysfunctional mitochondria to accumulate within the cell and cause death. In this way, the failure of mitophagy is implicated in the etiology of early-onset Parkinson's disease (Narendra and Youle, 2011).

When mitochondria are under stress or damaged, remarked Youle, they accumulate PINK1. PINK1 is a mitochondrial protein ordinarily anchored to the mitochondrion's outer membrane at low concentrations. When the mitochondrial membrane loses its electrical potential—whether by DNA mutations, reactive oxygen species (ROS),[1] or other perturbations—PINK1 increases. Increasing concentrations of PINK1, in turn, serve to recruit Parkin, which is a ubiquitin ligase, from the cytosol. Parkin marks the damaged mitochondrion with ubiquitin, a process that triggers formation of an autophagosome (see also Chapter 3). The autophagosome engulfs

[1] ROS are produced as a byproduct of oxidative phosphorylation.

the damaged mitochondrion, then merges with a lysosome, which degrades it. The findings on PINK1/Parkin, which have been replicated in multiple laboratories, have established a novel pathway for mitochondrion quality control, said Youle. He noted that much of the earlier work in this area was done on cultured cell lines, because the compounds used to induce this pathway in cultured cell lines were too toxic to neurons. More recently, however, two groups have shown the pathway in neurons (Cai et al., 2012; Wang et al., 2011).

Youle relayed that his laboratory has started a drug screening program to identify compounds that stimulate the PINK1/Parkin pathway. While he acknowledged that people with early-onset Parkinson's may not benefit from stimulating the pathway because their PINK1 or PARKIN are mutated, people with sporadic Parkinson's disease may benefit, as might others with neurodegenerative disease whose mitochondria are dysfunctional.

Amyotrophic Lateral Sclerosis

ALS predominantly affects motor neurons, leading to progressive muscle wasting and paralysis. In animal models of ALS, mitochondrial abnormalities precede symptoms of disease (Manfredi and Xu, 2005). Electron microscopy has revealed structural abnormalities in mitochondria in spinal motor neurons and in the motor cortex of ALS patients. Neil Kowall of Boston University focused his presentation on SOD1 (Cu, Zn superoxide dismutase), the first identified gene responsible for causing ALS. The corresponding protein is mutated in about 20 percent of familial cases of ALS.[2] The most widely used animal model of ALS is a transgenic mouse carrying a mutant SOD1 gene. The mouse develops muscle wasting similar to that of ALS.

Mitochondria from motor neurons in this animal model exhibit smaller size, fewer number, defective membrane potential, and impaired fusion. Fusion of mitochondria is designed to distribute mtDNA to the mitochondrial population and preserve the capacity for oxidative phosphorylation. These morphological and physiological changes in mutSOD1 motor neurons are not seen in wild type SOD1 motor neurons (Magrane et al., 2012). mutSOD1 also alters the levels of at least 50 different mitochondrial proteins, including proteins involved in the electron transport chain and in fusion, suggesting a possible widespread effect of mutSOD1 (Karbowski and Neutzner, 2012).

mutSOD1 also inflicts mitochondrial damage, as assessed by an increase of cytochrome c in the cytosol. Because cytochrome c is an essential component of the electron transport chain, which is situated in the inner

[2] About 5 to 10 percent of ALS cases are familial.

membrane of the mitochondria, its release into the cytoplasm indicates disruption of mitochondria membranes. But this toxic effect only occurs in the presence of the protein Bcl-2, which can reverse its functional phenotype and become a toxic protein (Pedrini et al., 2010). The identification of Bcl-2 as a necessary contributor to SOD1 toxicity suggests that Bcl-2 could be used as a molecular target for drugs designed to inhibit its action (Pedrini et al., 2010). Bcl-2 may also be an important target not only in familial ALS, but possibly also sporadic ALS, said Kowall. That is because research has recently found that, in a subset of ALS patients with bulbar onset, wild-type SOD1 becomes hyperoxidized. In concert with Bcl-2, hyperoxidized wtSOD1 displays mitochondrial toxicity similar to that seen with mutSOD1. Thus, Bcl-2 represents a common link between familial and a subtype of sporadic ALS, and thus appears to be a good target for therapeutics that inhibit it.

Huntington's Disease

Huntington's disease is an autosomal dominant disease in which the mutated protein, mhuntingtin (mHTT), displays excess polyglutamine repeats. mHTT localizes to the outer mitochondrial membrane, where it exerts widespread and deleterious effects on mitochondria and selective loss of neurons in the striatum. Kowall said a great deal of evidence shows that mHTT reduces mitochondrial motility, alters mitochondrial morphology, causes calcium dysregulation, reduces oxidative phosphorylation, and depolarizes the mitochondrial membrane in lymphoblasts of Huntington's disease patients. The depolarization is increased with greater numbers of polyglutamine repeats. mHTT also alters the balance between mitochondrial fusion and fission (Lin and Beal, 2006; Reddy and Reddy, 2011).

Several therapeutic strategies have recently emerged for Huntington's disease, Kowall noted. One avenue is to target a mitochondrial fission[3] protein to which mHTT binds, GTPase dynamin-related protein 1 (DRP1). The targeting of DRP1 is suggested by the finding that a dominant-negative $DRP1^{K38A}$ mutant, which reduces DRP1 activity, rescues mitochondria from the following adverse effects of mHTT: mitochondrial fragmentation, defects in anterograde and retrograde mitochondrial transport, and neuronal cell death. These findings were reported in cells from humans with Huntington's disease and from mice (Song et al., 2011). In other words, compounds that inhibit DRP1 might be useful as potential therapies.

Kowall described two more novel therapies. The first aims to detox-

[3] Mitochondrial fission is a quality control mechanism in which the mitochondrion divides into two, one healthy and the other containing the damaged portion of the mitochondria. The latter portion is degraded.

ify HTT. It involves intraventricular infusion of ganglioside GM1, which phosphorylates mutant HTT at specific serine amino acid residues. The approach not only curtailed the toxicity of HTT, but also restored normal motor function in symptomatic Huntington's disease mice (Di Pardo et al., 2012). The second therapy is with the already approved drug meclizine. This drug suppresses mitochondrial respiration and activates cellular survival pathways. In several models of Huntington's disease, meclizine was found to be neuroprotective (Gohil et al., 2011).

Alzheimer's Disease

Mitochondrial dysfunction precedes the pathological changes that are the hallmarks of Alzheimer's disease (Yao et al., 2009). Douglas Wallace of the Children's Hospital of Philadelphia proposed that the cause of Alzheimer's disease—and dementia more broadly—is from underlying dysfunction of the mitochondria. Beginning in 1993, Wallace's team found a mutation in one of the mitochondrial tRNA genes. The mutation correlated with 3 percent of late-onset Alzheimer's cases, 5 percent of Parkinson's, and 7 percent of the combined population. The finding was later corroborated by others. He asserted that subtle defects in tRNA will generate more global mitochondrial protein synthesis defects. Subsequently, his team began to study a mutation at the nucleotide position 414, which is adjacent to the control region promoter of mtDNA. The mutation previously had been shown to be increased with age in human fibroblasts (Michikawa et al., 1999). Wallace's team found the mutation in 65 percent of Alzheimer's brains and 57 percent of Down syndrome–dementia brains versus 0 percent of age-matched controls (Coskun et al., 2004).

Most recently, Wallace's team examined more globally the control region of mtDNA in tissue taken from the frontal cortex of the brain. The control region is responsible for regulating transcription of mitochondrial genes and helps copy mtDNA. They found the highest rate of somatic mutations in the Alzheimer's brain (Coskun et al., 2010). The mutation frequency was also elevated in Down syndrome–dementia cases relative to controls, but it was lower than that in Alzheimer's. Control tissue did show an age-related increase in mutation frequency, although the level was lower than that found in the other groups. The heightened rate of mutations was also found in serum and other tissues of Alzheimer's and Down syndrome cases, suggesting that the phenomenon is systemic. But, said Wallace, the brain is the most deeply affected tissue because of its disproportionately high energy demands. The study also found reduction in transcription of mtDNA and a reduction in the mtDNA copy number, implying a reduction in oxidative phosphorylation.

Turning to causation of neurodegenerative disease, Wallace expressed

the view that formation of Aβ plaques is not causal; rather, he hypothesized, Aβ protein is initially produced by cells as a compensatory means of *protecting* mitochondria. But as the protein continues to be produced, it begins to aggregate to form oligomers and larger aggregates that inhibit mitochondria, leading to cell injury and death. According to this model, the protein aggregates are contributory to the death of neurons in neurodegenerative disease, but not causal. Primary causation, according to his hypothesis, rests with dysfunctional mitochondria. He expressed the opinion that "bioenergetics is the common pathophysiological mechanism for all of these neurodegenerative diseases." He was then questioned in the discussion by several skeptical participants who did not agree with his causal attribution. In reply, Wallace described how his team had developed a way to introduce a cytochrome oxidase point mutation in mtDNA and found that the animal developed cardiomyopathy, myopathy, and pathological changes in hippocampal neurons, in retinal ganglion cells, and in the optic nerve. "This one particular point mutation—it has nothing to do with the nucleus—shows that energetics can affect all of those different functions," he asserted.

PROTEIN DEPOSITS AND TOXICITY TO MITOCHONDRIA

Multiple lines of evidence suggest that toxic proteins such as Aβ, apolipoprotein E (ApoE) fragments, and α-Synuclein can impair mitochondria, said Lennart Mucke of the Gladstone Institutes and the University of California at San Francisco. In this case, the damaged mitochondria would not be the primary cause of the disease, but rather would be secondary to the actions of aggregated proteins, which would be the primary cause. A significant amount of research shows that Aβ peptide accumulates in mitochondria, where they cause dysfunction and apoptosis (Manczak et al., 2006; Yao et al., 2009).

One possible mechanism by which protein deposits are toxic to neurons is by impairment of axonal transport of mitochondria. Mitochondria are generated largely in the cell body and need to be actively transported to the synapse, where energy need is high. Devoid of mitochondria, synaptic function can be impaired. Mucke and his team assessed the effects of Aβ and tau proteins on axonal transport of mitochondria (Vossel et al., 2010). They found that adding Aβ oligomers in culture quickly inhibited axonal transport of mitochondria in healthy neurons, a finding supported by earlier research. They also were interested in determining whether tau played a role. Reducing tau levels prevented Aβ oligomer-induced disruption of axon transport without affecting baseline axonal transport. The complete elimination of tau by gene knockdown also had the same effect. They concluded, "Aβ requires tau to impair axonal transport, and that tau

reduction protects against defects in Aβ-induced axonal transport" (Vossel et al., 2010, p. 198-a).

Another disease-related protein that impairs mitochondria is ApoE. The ApoE gene is the main susceptibility gene identified for late-onset Alzheimer's disease, and it is found on chromosome 19. Neurons produce ApoE when they are stressed by a host of factors, including aging, oxidative stress, trauma, and protein deposition. ApoE synthesis is thought to protect neurons from damage and to repair and remodel them. However, research has shown that the cleavage products of ApoE impair mitochondria (Brecht et al., 2004). ApoE e4, the allele associated with Alzheimer's disease, is most sensitive to being cleaved, whereas the other ApoE alleles are less so, said Mucke.

MITOCHONDRIAL DISEASES AND THEIR UTILITY FOR NEURODEGENERATIVE DISEASE

Given the uncertainty as to what roles mitochondrial dysfunction plays in neurodegerative disease, Mootha suggested the value of studying primary mitochondrial diseases, which refer to nearly 150 genetic diseases in which the lesion lies in a gene encoding a protein that is directly involved in mitochondrial biology. The diseases are heterogeneous, with dozens being the focus of study over many decades. Caused by genetic single-gene mutations or deletions, they follow Mendelian or a maternal pattern of inheritance.

Mitochondrial disease can shed light on neurodegenerative disease, said Mootha, in part because disease phenotypes are similar. For example, some mitochondrial disease phenotypes include ataxia, neuropathy, myopathy, deafness, and blindness. Indeed, several subsequent presentations focused on mitochondrial pathology in neurodegenerative disease, such as Parkinson's and ALS. Another reason why mitochondrial diseases carry import for neurodegenerative disease is that multiple organ systems are involved, just like neurodegenerative diseases, and their genetics are better characterized through an ambitious project known as the Mitocarta, which is an inventory of more than 1,000 mouse genes encoding proteins that localize to the mitochondria (Pagliarini et al., 2008). Finally, mitochondrial diseases are valuable, in his view, in providing "genetic extremes" that can help to determine whether or not a particular neurodegenerative disease may have mitochondrial defects as the root cause. Mootha advised looking for connections between mitochondrial and neurodegenerative diseases when there is at least some common ground, such as in pathogenesis, pathology, or biomarkers.

Even though mitochondria look similar upon microscopy, looks are deceiving. Mootha remarked on the enormous heterogeneity of mitochondria across different tissues. He reported that, after studying 14 different

tissues, research has found that mitochondria from 2 different tissues share only 75 percent of their proteins, whereas the remaining mitochondrial proteins are tissue specific. There is even physiological heterogeneity within an individual cell—mitochondria, for example, can possess different patterns of fuel usage. Given the diversity of phenotypes and genotypes, Mootha advocated for a systems approach to the study of mitochondrial function. Such an approach combines genomics, proteomics, metabolomics, biochemistry, and computer modeling to capture the dynamic range of complex interactions within cells and across tissues. Applying systems biology to neurodegenerative diseases would require identifying component parts, building wiring diagrams to connect these parts, identifying circuitry causal for the disease, and using the knowledge to develop therapies, he observed.

MITOCHONDRIA AND CELL DEATH

One commonality across neurodegenerative diseases is that they all feature a high degree of cell death. Here the focus is on mitochondria; mitochondria play a key role in regulating cell death, which occurs in specific brain regions across all neurodegenerative diseases. Cell death is of three types: (1) necrosis, which is the most chaotic form of death that involves cytoplasmic swelling, nuclear dissolution, and lysis; (2) apoptosis, an orderly form of death, reliant on ATP, that produces cell fragments that phagocytic cells are able to engulf and remove before the cell's contents disgorge onto surrounding cells and cause damage; and (3) autophagy, in which the cell degrades its cytoplasm and organelles via lysosomes (Martin et al., 2010). Mitochondria are the sites where antiapoptotic and proapoptotic proteins interact, and they regulate signals for cell death.

Lee Martin of Johns Hopkins University cautioned that cell death in humans versus animal models of neurodegenerative diseases may not be by similar mechanisms. He reported mouse–human species differences in the factors controlling the mitochondrial permeability transition (MPT), that is, an increase in permeability of mitochondrial membranes to small molecular weight molecules. MPT results from opening the mitochondrial permeability transition pore, a protein pore formed in mitochondrial membranes under certain pathological conditions. Induction of the permeability transition pore can lead to swelling of mitochondria and necrosis, and it also plays a major role in some types of apoptosis. Martin also noted species differences in signaling mechanisms of caspases, which are enzymes under the control of the mitochondria that are crucial to apoptosis, differences in caspace substrates, differences in mitochondrial fusion machinery, and in signaling mechanisms for DNA repair and metabolism, among others. Species differences in cell death confound the translation of findings from animal models into human clinical trials, he observed. He suggested modi-

fying the design of preclinical studies to rely less on mouse as models and more on human neural stem cell–derived neurons.

POTENTIAL BIOMARKERS AND THERAPIES

There are no established biomarkers or therapies for treating mitochondrial dysfunction in neurodegenerative disease. Wallace said his laboratory is working to develop them. One biomarker under development is near-infrared spectroscopy across the skin, using different infrared diodes that interrogate the redox potential of the respiratory chains. Wallace said his laboratory is also developing a biomarker using micro-organic breath analysis. They are hoping to get some surrogate variables that change in real time, and then go into a Phase I clinical trial and have at least a safety/efficacy indication.

Regarding therapies, this chapter has already mentioned a few in relation to specific neurodegenerative diseases. Focusing instead on *generic* therapies for mitochondrial dysfunction, Wallace said his first priority for therapy would be to stimulate formation of more mitochondria. Drugs to generate mitochondria are being tested in various animal models and cell culture systems. In particular, he noted that the drug bezafibrate has been found to increase mitochondrial biogenesis in cancer cells and ameliorate mitochondrial dysfunction (Wang and Moraes, 2011). It has not yet been tested in brain cells.

Other therapeutic options, Wallace explained, include (1) catalytic antioxidants to protect against ROS; (2) regulators of intracellular calcium; and (3) regulators of redox potential across mitochondrial membrane. Maintenance of redox potential is crucial for mitochondrial integrity and control over oxidative phosphorylation. One participant pointed out that antioxidant therapies have been uniformly ineffective in clinical trials, but Wallace responded that the doses may have not been high enough. Another participant advised targeting mitochondrial therapies in cases of threshold effects, that is, the point at which there is significant compromise of mitochondrial function. The participant also noted the possibility of mitochondrial therapies having secondary downsides.

RESEARCH NEEDS AND NEXT STEPS SUGGESTED BY INDIVIDUAL PARTICIPANTS

The workshop speakers identified many questions for future research and other opportunities for future action. The suggestions related to mitochondrial dysfunction are compiled here to provide a sense of the range of suggestions made. The suggestions are identified with the speaker who

made them and should not be construed as reflecting consensus from the workshop or endorsement by the Institute of Medicine.

- Develop deeper understanding of energy biology and interactions between bioenergetics and environmental influences. (Wallace)
- Identify biomarkers to follow mitochondrial functioning. (Lee, Mootha)
- Find biomarkers of mitochondrial decline. (Mootha)
- Identify new therapies that increase mitochondrial biogenesis. (Wallace)
- Identify therapies that interfere with mitochondrial contribution to pathogenesis of neurodegenerative disease, including therapies that increase mitophagy or stimulate activity of PINK1 and Parkin. (Mootha, Youle)
- Find therapies that detoxify mutant protein aggregates that interact with mitochondria. (Kowall)

6

Errors in RNA

Key Points Raised by Individual Speakers

- Various neuromuscular and neurodegenerative diseases—including certain types of amyotrophic lateral sclerosis (ALS), frontotemporal dementia, and Alzheimer's disease—feature toxic RNA or RNA-binding proteins.
- Non-coding RNAs (ncRNAs) are diverse classes of RNA molecules that are not translated into proteins. They are disproportionately expressed within the central nervous system, where they have roles in gene expression, development, neural network plasticity and connectivity, stress response, and brain aging. Base pair mutations to ncRNA can have widespread biological effects in light of ncRNA's unusually broad and interconnected gene and cellular regulatory roles, and they are implicated in neurodegenerative disease.
- Antisense oligonucleotides (ASOs) are being tested to treat RNA errors in human neurodegenerative disease. ASOs are designed to hybridize and then to block disease-related RNA sequences. ASOs have reached the point of being tested in a clinical trial of the neuromuscular disorder spinal muscular atrophy.

"Errors in RNA" is a generic term used here to refer to disease-related defects of three types: (1) defects in RNA itself; (2) defects in RNA-binding proteins that form ribonucleoprotein complexes with RNA; or (3) defects in proteins responsible for RNA assembly.[1] Defects of each type can hold deleterious effects that are broadly amplified because of RNA's regulatory roles in transcription, translation, epigenetic modification, and a host of other processes (Taft et al., 2010). Disease-causing RNAs can be in protein-coding mRNAs or in non-coding RNAs (ncRNAs), both of which can disrupt crucial cell functioning (Cooper et al., 2009).

In his opening remarks about the interest in examining RNA errors in human neurodegenerative diseases, Don Cleveland of the University of California at San Diego outlined a constellation of exciting recent discoveries that have identified toxic RNA-binding proteins in neurodegenerative disease. It has only been known for 6 years that dominant mutations in TDP-43 and FUS/TLS genes cause familial ALS and some rare cases of frontotemporal lobar degeneration. A wave of new research has established that the protein products of these genes are RNA-binding proteins that appear to be involved in multiple steps of RNA processing, such as alternative splicing, transcription, nucleocytoplasmic shuttling, or RNA transport (Lagier-Tourenne et al., 2010) (see Figure 6-1). Mutant TDP-43 protein-RNA alters nearly 1,000 splicing events (Polymenidou et al., 2011). Relatedly, spinal muscular atrophy is caused by a recessive loss of the RNA-binding protein SMN. Finally, the most prominent cause of inherited ALS is a GGGGCC hexanucleotide expansion in the C9orf72 gene.

This chapter of the workshop summary examines errors in RNA, including in RNA-binding proteins, their roles in neurological and neurodegenerative disease, and novel therapies to combat RNA errors. Unlike protein aggregation, which is well known as a pathological feature common to many neurodegenerative diseases, the recognition that there are errors in RNA in a series of neurodegenerative diseases is currently emerging, and these errors may not be regarded as primary pathogenic mechanisms in some conditions, such as Alzheimer's disease or Parkinson's disease.

RNA GAIN OF FUNCTION MECHANISMS IN NEUROGENERATIVE DISEASE: MICROSATELLITE EXPANSION DISORDERS

Microsatellite expansion disorders are ones in which mutations are repeating sequences of base pairs of DNA. Huntington's disease, for example, features a CAG expansion, leading to excess copies of the amino acid glutamine in the huntingtin protein. Laura Ranum of the University of Florida focused her presentation on the microsatellite expansion disorder

[1] Proteins that bind to RNA also bind to DNA.

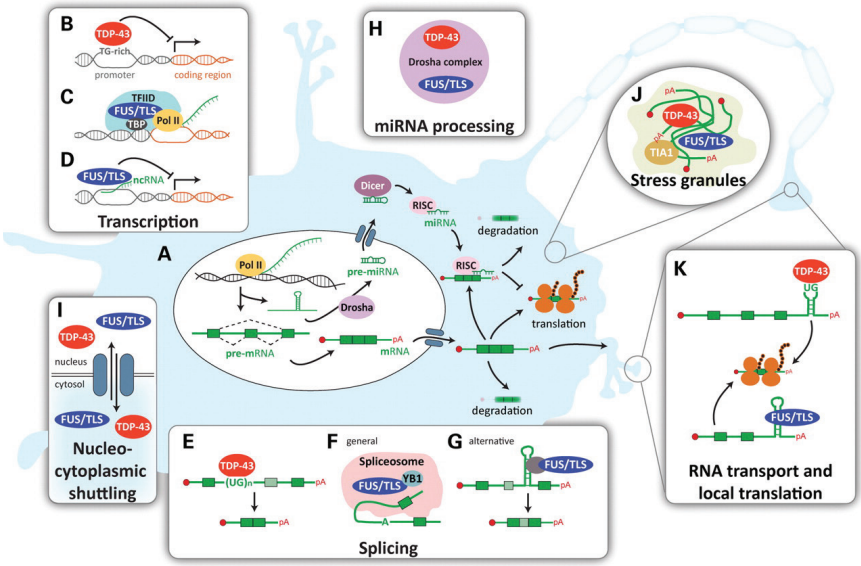

FIGURE 6-1 Proposed physiological roles of TDP-43 and FUS/TLS.
(**A**) Summary of major steps in RNA processing from transcription to translation or degradation. (**B**) TDP-43 binds single-stranded TG-rich elements in promoter regions, thereby blocking transcription of the downstream gene (shown for TAR DNA of HIV and mouse SP-10 gene). (**C**) FUS/TLS associates with TBP within the TFIID complex, suggesting that it participates in the general transcriptional machinery. (**D**) In response to DNA damage, FUS/TLS is recruited in the promoter region of cyclin D1 (*CCND1*) by sense and antisense non-coding RNAs (ncRNAs) and represses *CCND1* transcription. (**E**) TDP-43 binds a UG track in intronic regions preceding alternatively spliced exons and enhances their exclusion (shown for CFTR and apolipoprotein A-II). (**F**) FUS/TLS was identified as a part of the spliceosome and (**G**) was shown to promote exon inclusion in H-ras mRNA, through indirect binding to structural regulatory elements located on the downstream intron. (**H**) Both proteins were found in a complex with Drosha, suggesting that they may be involved in miRNA processing. (**I**) Both TDP-43 and FUS/TLS shuttle between the nucleus and the cytosol and (**J**) are incorporated in SGs, where they form complexes with mRNAs and other RNA-binding proteins. (**K**) TDP-43 and FUS/TLS are both involved in the transport of mRNAs to dendritic spines and/or the axonal terminal where they may facilitate local translation. Examples of such cargo transcripts are the low molecular weight NFL for TDP-43 and the actin-stabilizing protein Nd1-L for FUS/TLS.
NOTE: For additional details and citations associated with each of these proposed roles, see the source article.
SOURCE: Lagier-Tourenne et al., 2010.

myotonic dystrophy, a prominent example of RNA disruption, and she also focused on a new type of protein translation process that defies the canonical rules of molecular biology.

Myotonic dystrophy is a slowly progressive neuromuscular disease marked by wasting muscles, cataracts, heart conduction defects, endocrine defects, and myotonia, which is a slow relaxation of the muscles after voluntary contraction. Myotonic dystrophy comes in two different types: type 1, which is caused by a CTG expansion in the 3' untranslated region of the DMPK gene, and type 2, which is caused by a CCTG expansion in the intron in the ZNF9 gene (Ranum and Cooper, 2006). In both cases, the gene mutations encode an aberrant expansion containing RNAs that sequester proteins that normally regulate alternative splicing. As a consequence, these sequestered proteins fail to participate in their normal function of regulating alternative splicing. The associated splicing defects affect hundreds and thousands of genes, depending on the tissue. In transgenic animals with myotonic dystrophy, mis-splicing of a chloride channel causes myotonia, and mis-splicing of the insulin receptor is thought to lead to insulin resistance that is associated with the disease, noted Ranum.

In the process of studying myotonic dystrophy and the neurodegenerative disease spinocerebellar ataxia type 8, which features a CAG expansion (Koob et al., 1999), Ranum and colleagues uncovered an entirely new and surprising type of translation process for converting an RNA into protein: Repeat associated non-ATG (RAN) translation. According to the canon of molecular genetics, translation requires the initiating DNA sequence ATG—also known as the start codon. But her research showed that the ATG sequence was not required to translate CAG and CUG expansions. She and her colleagues showed that RAN translation takes place in vivo in myotonic dystrophy and spinocerebellar ataxia type 8 (Zu et al., 2011). They also showed that RAN translation is favored by hairpin turns and long repeats of DNA, as well as by cellular factors. She concluded her presentation by asking three key questions that need to be addressed in future research: (1) What is the role of RNA versus protein effects in neurodegenerative disease?, (2) How and why are RAN proteins expressed?, and (3) Is RAN translation a previously unrecognized pathogenic mechanism in neurodegenerative disease?

EMERGING ROLES OF NON-CODING RNA NETWORKS IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASE

ncRNAs are diverse classes of RNA molecules that are not translated into proteins. They exert complex regulatory and structural functions, including the biogenesis and function of nuclear organelles. According to various sequencing methods, there are likely to be hundreds of thousands

to millions of ncRNAs in the human genome. In fact humans have the highest number of non-coding sequences relative to other species in the animal kingdom (Mattick, 2007). A large fraction of ncRNAs is expressed within the central nervous system. There they participate in controlling gene expression, development, neural network plasticity and connectivity, stress response, and brain aging (Qureshi and Mehler, 2011). If an ncRNA has a mutated base or changes in expression, as appears to occur in certain neurodegenerative and other neurological diseases, it can potentially alter any or even all of these regulatory functions. Thus it is not surprising that ncRNAs have been implicated in neurodegenerative diseases, noted Mark Mehler of the Albert Einstein College of Medicine. In one example, a non-coding antisense RNA against β-secretase (BACE1-AS) may be a contributor to the pathogenesis of Alzheimer's disease by increasing BACE1 expression (Faghihi et al., 2008). BACE1 is an enzyme that cleaves amyloid precursor protein to generate fragments of the neurotoxic protein amyloid-β peptide (Aβ). The study showed elevated BACE1-AS in the postmortem brains of Alzheimer's cases.

ncRNAs bear four key features. First, they have low bioenergetic demand, meaning that to change their biophysics and conformational properties, they require about 20 percent of the energy demand of proteins. This is highly important, said Mehler, because every neurodegenerative disease is fundamentally a disease of bioenergetic failure. Second, ncRNAs are highly sensitive to intracellular and extracellular stimuli, conferring a link between genes and the environment. "ncRNAs are the most exquisite biosensors known," Mehler said. Third, ncRNAs are versatile: They uniquely interact with DNA, RNA, and proteins. Fourth, they exhibit diverse roles in epigenetic regulation, such as promoting DNA methylation, chromatin modification, and RNA editing. Epigenetics is changing the research landscape, considering that every cell in the brain has a unique epigenome that changes constantly because it is reflective of dynamic gene–environment interactions, Mehler added.

A final noteworthy feature of ncRNAs is their trafficking capacity. Not only do they shuttle intracellularly, but they are also transmissible by virtue of being secreted by cells within discrete classes of vesicles, including microvesicles and exosomes. Recent research has established that exosomes bearing ncRNAs, mRNAs, DNA, lipids, and protein products traffic not just between adjacent cells, but also through the bloodstream to other organs and potentially to the germ line. There is evidence, said Mehler, that mRNA in an exosome can be translated to protein in a recipient cell. It is even possible that ncRNAs account for some of the transmissibility of neurodegenerative disease-associated pathology. "The real take-home message is that this is a field where networks are going to be important," observed Mehler.

ANTISENSE OLIGONUCLEOTIDES AS THERAPIES FOR RNA-BINDING PROTEIN ERRORS

Antisense oligonucleotides (ASOs) are a class of emerging treatments for RNA errors in neuromuscular and neurodegenerative disease. ASOs are single strands of complementary base pairs that hybridize to highly specific target RNA sequences. Their role is first to hybridize and then to block the disease-related sequences from forming, or from functioning properly once they are formed. As a result of high specificity, ASOs possess the attractive feature that they minimize the likelihood of side effects. Frank Rigo of ISIS Pharmaceuticals described his approach to ASOs by pointing out that they are well suited for diseases with or without a known genetic cause and that are amenable to direct RNA therapeutic correction. The ASOs that ISIS develops work in one of two ways: by a Ribonuclease H method that cleaves and degrades the RNA once the ASO binds to the target sequence, or by a mechanism that modulates splicing. The latter approach is being used to treat spinal muscular atrophy (SMA) in a Phase I clinical trial, which is currently under way.

SMA is an autosomal recessive neuromuscular disorder marked by muscle atrophy and weakness. It is the leading genetic cause of death in infants and toddlers. It is caused by mutations in the SMN1 gene that lead to absence of the SMN protein, which is necessary for motor neuron survival. SMN1 protein is necessary for assembly of certain ribonucleoproteins. A very close copy of the gene, SMN2, is unable to compensate for the loss of SMN1 because exon 7 of the SMN2 gene fails to be transcribed, the result of which leads to an unstable SMN2 protein that is rapidly degraded. Rigo and colleagues successfully developed an ASO designed to prevent binding of certain splicing repressors in a manner that ensured inclusion of exon 7 in a transgenic mouse model that carries human SMN2 (Passini et al., 2011). Expression of the SMN2 protein is restored and can compensate for the lack of SMN1 expression. The treatment led to profound increase in survival of the SMA mouse model (Hua et al., 2011).

ASOs, noted Rigo, can be delivered systemically or centrally. The ASOs are endocytosed across the neuron cell membrane and make their way to the nucleus. He and his colleagues have shown in non-human primates that ASOs, delivered intrathecally, broadly distribute to central nervous system tissues, with the greatest concentrations in spinal cord gray matter and cortical regions. The lowest concentrations appear in subcortical regions. The ASOs have remarkably long half-lives of several months, a feature that makes them highly attractive considering that the route of administration is intrathecal. In a Phase I trial of an ASO targeted at the SOD1 mutation in ALS, the investigators have found good correspondence between human

pharmacokinetics as compared with that in non-human primates. The drug was well tolerated and no safety concerns were identified.

Rigo reported that ISIS has developed clinical experience with SMA and ALS, while myotonic dystrophy type I and familial dysautonomia are currently at the preclinical stage. They are also pursuing Huntington's disease by reducing the expression of the huntingtin protein with an ASO that operates by the ribonuclease H approach. They are also looking to reduce tau expression in Alzheimer's disease and frontotemporal dementia by both ribonuclease H and non-ribonuclease H approaches.

Despite progress, Rigo asserted that there are clear challenges to drug development for neurological disorders. Most diseases have relatively small patient populations, but they are still commercially attractive because of unmet medical need. He observed that ASO research and development comes with a great deal of risk, primarily because there are no established pharmacodynamic biomarkers for early drug efficacy: Measuring the target protein in cerebrospinal fluid and plasma is generally difficult. Uncertainty also surrounds regulatory and clinical development. To mitigate the risks, ISIS is working closely with the Food and Drug Administration (FDA). He said that his company has succeeded thus far by actively seeking regulatory input and fostering interactions with the FDA.

One of the most important challenges is to develop biomarkers, which will become highly important for gaining drug approval. Rigo said his company is trying to establish biomarkers for early indication of drug efficacy through expression analyses and proteomics. Without biomarkers it is more challenging to determine how to dose humans and how to follow pharmacodynamics over time.

When queried about regulatory problems in the discussion session, Rigo stressed the importance of meeting early with the FDA and being "engaged in an education process." He emphasized that there are no currently approved ASOs in neurodegenerative disease, which means that there is no clearly established path to drug approval. Story Landis of the National Institute of Neurological Disorders and Stroke suggested that he may wish to use her institute's Phase II clinical trials network. Landis asked: Once you have experience with one oligonucleotide, can this be applied to other oligonucleotides? Rigo responded: Yes, but a thorough safety evaluation for each oligonucleotide is needed.

RESEARCH NEEDS AND NEXT STEPS SUGGESTED BY INDIVIDUAL PARTICIPANTS

The speakers at the workshop identified many questions for future research and other opportunities for future action. The suggestions related to errors in RNA are compiled here to provide a sense of the range of sug-

gestions made. The suggestions are identified with the speaker who made them and should not be construed as reflecting consensus from the workshop or endorsement by the Institute of Medicine.

- Establish pharmacodynamic biomarkers for early drug efficacy readout because of the difficulty of measuring the target RNA-binding proteins in cerebrospinal fluid or plasma. (Rigo)
- Establish a clear clinical development path to drug approval because there are no currently approved disease-modifying drugs for many neurodegenerative diseases. (Rigo)
- Determine how and why RAN proteins are expressed. (Ranum)
- Identify the roles of RNA versus protein effects in disease. (Ranum)
- Discern whether RAN translation is a previously unrecognized pathogenic mechanism in neurodegenerative disease. (Ranum)
- Determine whether neural subtype-specific ncRNA networks contribute to selective regional cellular vulnerabilities. (Mehler)
- Identify the role of extracellular trafficking of ncRNAs and whether this novel process can lead to local and long-distance propagation of pathology. (Mehler)

7

Closing Remarks

Throughout the workshop, presentations and discussions focused heavily on deep mechanistic understanding of the cellular biology of neurodegenerative diseases. As illustrated in this summary, there remain many fundamental gaps in understanding about the causal mechanisms of neurodegenerative diseases and the roles of other cellular and molecular mechanisms in these diseases. Many participants noted the importance of further research within individual diseases. The observation of common mechanisms, pathologies, and genetics across different diseases suggests that it may be valuable—at least in cases where careful scrutiny and consideration of the evidence supports it—to move away from the tradition of studying individual diseases and instead consider whether they may be better understood as clinical variants of common cellular and molecular biological defects. Furthering understanding about the basic biology of the neurodegenerative diseases could help advance efforts to prevent and effectively treat them.

In the final session, the focus shifted toward broad trends and challenges in central nervous system (CNS) research and development. Participants suggested strategies for advancing research and development for neurodegenerative diseases. There was some debate about the extent to which there is hope and interest in therapeutics development for neurodegenerative diseases. Several participants expressed concern about large pharmaceutical companies' lack of interest in pursuing CNS development. John Dunlop of AstraZeneca, however, described this as a misconception and highlighted several areas in which large companies are working on CNS diseases. He noted that companies are not just focused on Alzheimer's disease, but also working on Parkinson's disease, Huntington's disease,

psychiatry, and chronic pain. Various participants mentioned the many biotech and medium-sized companies working on neurodegenerative diseases. Don Cleveland of the University of California, San Diego, described therapeutic approaches that target gene products known to be truly causative of disease, such as the treatments based on antisense oligonucleotides presented by Frank Rigo of ISIS Pharmaceuticals, as "at least one ray of real optimism" in therapies for neurodegenerative disease. Participants went on to discuss various ways to encourage therapeutics development for neurodegenerative diseases. For example, they noted that small and medium biotech companies may do innovative science and then partner with or be absorbed by larger companies. Several participants discussed how National Institutes of Health funding could help support Phase II clinical trials in small companies, as well as models of collaboration between academic investigators and companies.

In thinking about promising strategies going forward, several participants discussed the balance between going after a single receptor or single transcription factor versus embracing complexity. One participant noted that although the intrinsic complexity may be concerning to large pharmaceutical companies and a single focus may provide the appealing promise of clarity, the biological phenomena themselves may require a systems approach. Similarly, a number of participants highlighted the difficult balance between pursuing a diversified strategy and the reality of finite budgets. Lennart Mucke of the Gladstone Institutes and the University of California, San Francisco, noted that with personalized medicine, the days of pursuing one blockbuster drug are coming to an end. He mentioned the example of Herceptin and said, "As we understand patient population heterogeneity better, we will see that kind of personalized medicine approach expand. The pockets of investment will be smaller." He went on to say, "We need to diversify our strategy in drug development. We need to be prepared for the possibility that most sporadic neurodegenerative disorders have a multifactorial etiology and will require a multipronged therapeutic approach."

Some participants highlighted large collaborative efforts, such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Parkinson's Progression Markers Initiative (PPMI). John Trojanowski of the University of Pennsylvania called ADNI a great success story, encompassing public–private partnership, data made publicly available, and excellent use of funding in a strategic way during difficult financial times. He asked, "Why can't we do this again and again" for different diseases and biomarkers? Lucie Bruijn of the ALS Association encouraged this suggestion, and the group discussed differences in the levels of knowledge among the different diseases and how this might impact the appropriate timing for launching large collaborations.

While highlighting ADNI and PPMI and other large collaborative efforts, various participants also discussed the tension between encouraging large collaborations with agreed-upon strategic directions versus supporting investigator-initiated creative science. Story Landis, director of the National Institute of Neurological Disorders and Stroke, said that they have been sensitive to this issue and have been striving for "a reasonable balance." She noted that "there's huge tension between hypothesis-driven and discovery science, basic translational and large Phase III clinical trials, and keeping that balance in the current fiscal climate is not as simple as it would be if there were a different fiscal climate." Richard Hodes, director of the National Institute on Aging (NIA), also noted NIA's efforts—in the context of difficult financial circumstances—to achieve a balance of subject matter, mechanisms, and approaches. He noted that it is necessary to pursue both "large science" and smaller research projects funded through RO1 grants. Hodes also remarked on the importance of ongoing discovery science to inform the development of biomarkers.

During the last minutes of the workshop, a participant reemphasized the importance of the many open questions about cell biology that were raised over the course of 2 days of presentations and discussions. Lucie Bruijn of the ALS Association also emphasized the need for further careful exploration of the commonalities and differences among diseases, before launching large projects based on perceived commonalities across multiple diseases. In closing the workshop, Landis reminded the group of the provocative questions process that Harold Varmus implemented as director of the National Cancer Institute, and challenged participants to design a similar set of questions focusing on neurodegenerative diseases and basic cell biology of neurons.

Appendix A

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Appendix B

Statement of Task

An ad hoc planning committee will plan and conduct a public workshop that will explore commonalities across neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia, and identify potential opportunities for collaboration across the respective research and development communities. Participants will be invited from academia; pharmaceutical and biotechnology industries; government agencies such as the National Institutes of Health, the National Science Foundation, and the Department of Veterans Affairs; and patient advocacy groups. Looking across the neurodegenerative diseases, workshop presentations and discussions will

- Identify and discuss commonalities related to genetic and cellular mechanisms.
- Identify areas of fundamental science needed to facilitate therapeutics development.
- Explore areas of potential collaboration among the respective research communities and sponsors.

An individually authored workshop summary will be prepared based on the information gathered and the discussions held during the workshop in accordance with institutional policy and procedures.

Appendix C

Workshop Agenda

Neurodegeneration: Opportunities for Collaboration Across Disease-Specific Research and Development Communities—A Workshop

April 30-May 1, 2012

Pew DC Conference Center
901 E Street, NW, Washington, DC

Background: Neurodegenerative diseases are becoming increasingly prevalent in the United States due to the aging population. Implications of these diseases are grave, both for individual and family quality of life and for health care costs. Recent findings have revealed potential commonalities and parallelisms in genetic and cellular mechanisms across neurodegenerative diseases. Enhanced sharing of research findings and collaboration across research communities could potentially help advance basic scientific knowledge about each disease and about neurodegeneration and neurodegenerative diseases in general. Furthermore, enhanced basic scientific understanding could facilitate therapeutics development for neurodegenerative disorders, including therapeutics that may address more than one neurodegenerative disease. This workshop will explore commonalities across neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia, and identify potential opportunities for collaboration across the respective research and development communities. Speakers and participants will be invited from academia; pharmaceutical and biotechnology industries; government agencies such as the National Institutes of Health, the National Science Foundation, and the Department of Veterans Affairs; and disease advocacy groups.

Meeting Objectives: The objectives of this workshop are to look across the neurodegenerative diseases—including Alzheimer's disease, Parkinson's disease, ALS, and frontotemporal dementia—and

- Identify and discuss commonalities related to genetic and cellular mechanisms.
- Identify areas of fundamental science needed to facilitate therapeutics development.
- Explore areas of potential collaboration among the respective research communities and sponsors.

DAY ONE: April 30, 2012

8:00 a.m. **Welcome and Opening Remarks**

Story Landis, *Workshop Co-Chair*
Director
National Institute of Neurological Disorders and Stroke

John Trojanowski, *Workshop Co-Chair*
Co-director, Center for Neurodegenerative Disease Research
University of Pennsylvania

8:10 a.m. **U.S. Department of Veterans Affairs and Neurodegeneration Research: Current Efforts and Future Goals**

Joel Kupersmith
Chief Research and Development Officer
U.S. Department of Veterans Affairs

SESSION 1: OVERVIEW OF COMMON FEATURES ACROSS NEURODEGENERATIVE DISEASES

Session Objectives: The objectives of this session are to provide a genetic, clinical, and pathological framework to the notion that commonalities exist across neurodegenerative diseases. While this meeting focuses on discrete diagnostic entities, it is likely that this section may use examples from entities that cross these boundaries. Specifically, this session will

- Provide an overview of the genetic complexity of different neurodegenerative diseases.
- Discuss common and distinguishing features of the genetics of different neurodegenerative diseases.
- Discuss the clinical heterogeneity of monogenic disorders.

- Describe and discuss how pathology is likely to inform us about etiologic overlap between entities and provide illustrative examples of this overlap.
- Discuss the rationale for looking across neurodegenerative diseases to advance scientific understanding and explore innovative approaches to therapeutics development.

8:20 a.m. **Genetic Overlap and Complexity of Phenotypical Expression**
ANDREW SINGLETON, *Session Chair*
Senior Investigator, Laboratory of Neurogenetics
National Institute on Aging

8:30 a.m. **Pathological Overlap**
DENNIS W. DICKSON
Professor of Laboratory Medicine and Pathology
Mayo Clinic

8:40 a.m. **Translational Route Challenges: Is Combining Diseases Informative or a Distraction?**
ADRIAN J. IVINSON
Director, Harvard NeuroDiscovery Center
Harvard Medical School

8:50 a.m. Discussion Among Speakers and Attendees

SESSION 2: PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASES

Session Objectives: The objectives of this session are to look at protein aggregation across the neurodegenerative diseases—including Alzheimer's disease, Parkinson's disease, Huntington's disease, ALS, and frontotemporal dementia—and

- Highlight commonalities related to protein aggregation across these diseases, for example, autophagy.
- Discuss promising opportunities for collaboration among the respective research communities.
- Identify areas of fundamental research about protein aggregation that would facilitate biomarker and therapeutics development.

- Identify the next steps that research sponsors, investigators, and others should take to facilitate collaborative research and drug development in this area, including frameworks for partnerships and collaboration.

9:10 a.m. **Overview of Status of the Field and Session Objectives**
JOHN DUNLOP, *Session Co-Chair*
Vice President, Discovery
Neuroscience Innovative Medicine Unit
AstraZeneca

LUCIE BRUIJN, *Session Co-Chair*
Chief Scientist
ALS Association

9:20 a.m. **Proteostasis Challenges in Neurodegenerative Diseases**
RICK MORIMOTO
Professor of Molecular Biosciences
Northwestern University

9:30 a.m. Discussion Among Speakers and Attendees

9:45 a.m. **The Selective Degradation of Misfolded Proteins and Protection Against Neurodegenerative Diseases**
ALFRED GOLDBERG
Professor of Cell Biology
Harvard Medical School

9:55 a.m. Discussion

10:10 a.m. BREAK

10:25 a.m. **Autophagy in Neurodegenerative Disease**
ANA MARIA CUERVO
Professor, Department of Developmental and Molecular Biology
Albert Einstein College of Medicine

10:35 a.m. Discussion

10:50 a.m. **Protein Aggregation in ALS and Huntington's Disease**
CLAUDIO HETZ
Professor, University of Chile
Adjunct Professor, Harvard School of Public Health

11:00 a.m. Discussion

11:15 a.m. **Development of Assay Systems in Observing Aggregates and Development of Small Molecules**
STEVEN FINKBEINER
Director, Taube-Koret Center, Gladstone Institute for Neurodegenerative Disease Professor, University of California, San Francisco

11:25 a.m. Discussion

11:40 a.m. **Drug Discovery Efforts**
WARREN HIRST
Associate Research Fellow, Neurodegeneration & Neurologic Diseases
Pfizer

11:50 a.m. Discussion

12:30 p.m. LUNCH

SESSION 3: MITOCHONDRIAL PATHOLOGY AND NEURODEGENERATIVE DISEASE

Session Objectives: The objectives of this session are to look at mitochondrial pathobiology across the neurodegenerative diseases—including Alzheimer's disease and other dementias, Parkinson's disease, and ALS—and to

- Highlight differences and commonalities related to mitochondrial dysfunction and pathology across the diseases.
- Discuss opportunities for the development of mitochondria-related biomarkers and therapeutic interventions.
- Identify next steps that research sponsors, investigators, and others should take to facilitate collaborative research and drug development in this area, including frameworks for partnerships and collaboration.

1:30 p.m. **Overview of Status of the Field and Session Objectives**
LENNART MUCKE, *Session Chair*
Director and Senior Investigator, Gladstone Institute of Neurological Disease
Professor of Neurology and Neuroscience
University of California, San Francisco

1:40 p.m. **Systems Biology and Disease**
VAMSI K. MOOTHA
Professor
Department of Systems Biology, Harvard Medical School
Department of Medicine, Massachusetts General Hospital

1:50 p.m. Discussion Among Speakers and Attendees

2:05 p.m. **Neuronal Cell Death in Human Neurological Disorders and Their Animal/Cell Models**
LEE MARTIN
Professor of Pathology, Neuroscience
Johns Hopkins University

2:15 p.m. Discussion

2:30 p.m. **Parkinson's Disease**
RICHARD J. YOULE
Senior Investigator
National Institute of Neurological Disorders and Stroke

2:40 p.m. Discussion

2:55 p.m. BREAK

3:10 p.m. **ALS and Huntington's Disease**
NEIL KOWALL
Professor of Neurology and Pathology, Boston University
Chief of Neurology, VA Boston Healthcare System

3:20 p.m. Discussion

3:35 p.m. **Alzheimer's Disease**
DOUGLAS C. WALLACE
Director, Center for Mitochondrial and Epigenomic Medicine
Michael and Charles Barnett Chair of Pediatric Mitochondrial Medicine and Metabolic Disease
The Children's Hospital of Philadelphia

3:45 p.m. Discussion

4:45 p.m. **Wrap-Up: Highlights and Key Themes of Day One**
STORY LANDIS, *Workshop Co-Chair*
JOHN TROJANOWSKI, *Workshop Co-Chair*

5:00 p.m. ADJOURN DAY ONE

DAY TWO: May 1, 2012

8:00 a.m. **Welcome and Objectives of Day Two**
STORY LANDIS, *Workshop Co-Chair*
Director
National Institute of Neurological Disorders and Stroke

JOHN TROJANOWSKI, *Workshop Co-Chair*
Co-director, Center for Neurodegenerative Disease Research
University of Pennsylvania

SESSION 4: NEURODEGENERATIVE DISEASE TRANSMISSION AND IMMUNE THERAPY

Session Objectives: The objectives of this session are to

- Provide an overview of the latest concepts on transmission of neurodegenerative diseases, including evidence that suggests that disease progression may occur through the cell-to-cell spread of pathological disease proteins.
- Explore how targeting transmissible species of α-Synuclein as well as tau and Abeta using immune therapy may be used to treat Parkinson's disease and Alzheimer's disease, respectively.

- Identify the next steps that research sponsors, investigators, and others should take to facilitate collaborative research and drug development in this area, including frameworks for partnerships and collaboration.

8:15 a.m. **Overview of Status of the Field and Session Objectives**
JOHN TROJANOWKSI, *Session Chair*
Co-director, Center for Neurodegenerative Disease Research
University of Pennsylvania

8:25 a.m. **Transmission of Prions and Alzheimer's Disease Abeta Amyloid**
CLAUDIO SOTO
Professor of Neurology
Director, Center for Alzheimer's Disease and Related Brain Disorders
The University of Texas Medical School at Houston

8:35 a.m. Discussion Among Speakers and Attendees

8:50 a.m. **Transmission of Alzheimer's Disease Abeta Amyloid**
LARY C. WALKER
Research Professor of Neuroscience
Emory University

9:00 a.m. Discussion

9:15 a.m. **Transmission of Alzheimer's Disease Tau Amyloid**
KAREN DUFF
Professor, Department of Pathology
Columbia University

9:25 a.m. Discussion

9:40 a.m. BREAK

10:00 a.m. **Transmission of Parkinson's Disease α-Synuclein Amyloid**
VIRGINIA M.-Y. LEE
The John H. Ware 3rd Professor in Alzheimer's Research
Department of Pathology and Laboratory Medicine
Director, Center for Neurodegenerative Disease Research
University of Pennsylvania School of Medicine

10:10 a.m. Discussion

10:25 a.m. **α-Synuclein Immunization for Parkinson's Disease**
DORA GAMES
Head of Pharmacology
Neotope Biosciences

10:35 a.m. Discussion

10:50 a.m. **Tau Immunization for Alzheimer's Disease and Related Tauopathies**
PETER DAVIES
Head
Litwin-Zucker Center for the Study of Alzheimer's Disease and Memory Disorders
The Feinstein Institute for Medical Research

11:00 a.m. Discussion

11:45 a.m. LUNCH

SESSION 5: ERRORS IN RNA

Session Objectives: The objectives of this session are to

- Discuss how errors in RNA-binding proteins are causes of neurodegenerative diseases, including ALS, frontotemporal dementia, and spinal muscular atrophy, as well as triplet nucleotide expansion as a risk factor in disease (e.g., ataxin and ALS).

- Discuss disease mechanisms for diseases with toxic RNAs, including myotonic dystrophy and other triplet nucleotide repeats where there are toxic RNAs or aberrant translation of the expansions.
- Explore potential biomarkers and therapies for RNA-binding protein errors in SMA, TDP-43, FUS, and C9orf72.
- Discuss yeast models to identify therapeutics and the emerging roles of non-coding RNA networks in the pathogenesis of neurodegenerative diseases.
- Identify the next steps that research sponsors, investigators, and others should take to facilitate collaborative research and drug development in this area, including frameworks for partnerships and collaboration.

12:45 p.m. **Overview of Status of the Field and Session Objectives**
DON CLEVELAND, *Session Chair*
Professor and Chair, Department of Cellular and Molecular Medicine
Head, Laboratory for Cell Biology
Ludwig Institute for Cancer Research
University of California, San Diego

12:55 p.m. **Overview of RNA Gain-of-Function Mechanisms in Neurodegenerative Disease**
LAURA RANUM
Professor of Molecular Genetics and Microbiology
University of Florida

1:05 p.m. Discussion

1:20 p.m. **Overview of Therapies for RNA-Binding Protein Errors**
FRANK RIGO
Assistant Director, Core Antisense Research
ISIS Pharmaceuticals, Inc.

1:30 p.m. Discussion

1:45 p.m. **Yeast Models to Identify Therapeutics**
GREGORY A. PETSKO
Gyula and Katica Tauber Professor of Biochemistry & Chemistry
Brandeis University

1:55 p.m. Discussion

2:10 p.m. **The Emerging Roles of Non-Coding RNA Networks in the Pathogenesis of Neurodegenerative Diseases**
MARK F. MEHLER
Alpern Professor of Neurology, Neuroscience and Psychiatry and Behavioral Sciences
University Chair, The Saul R. Korey Department of Neurology
Albert Einstein College of Medicine

2:20 p.m. Discussion

2:45 p.m. BREAK

SESSION 6: FUTURE DIRECTIONS AND NEXT STEPS

Session Objectives: A panel will synthesize and discuss key highlights from the workshop presentations and discussions, including

- Identify key promising areas for future cross-disease research and collaboration.
- Discuss opportunities for partnerships—public–private and across disease-specific communities—to advance neurodegeneration research and therapeutics development.
- Discuss challenges to advancing research and therapeutics development for the neurodegenerative diseases and potential mechanisms to address these challenges.

3:00 p.m. Panel Discussion (Session Chairs from Previous Sessions):
STORY LANDIS, *Session Co-Chair*
Director
National Institute of Neurological Disorders and Stroke

JOHN TROJANOWSKI, *Session Co-Chair*
Co-director, Center for Neurodegenerative Disease Research
University of Pennsylvania

ANDREW SINGLETON
Senior Investigator, Laboratory of Neurogenetics
National Institute on Aging

John Dunlop
Vice President, Discovery
Neuroscience Innovative Medicine Unit
AstraZeneca

Lucie Bruijn
Chief Scientist
ALS Association

Lennart Mucke
Director and Senior Investigator, Gladstone Institute of Neurological Disease
Professor of Neurology and Neuroscience
University of California, San Francisco

Don Cleveland
Professor and Chair, Department of Cellular and Molecular Medicine
Head, Laboratory for Cell Biology
Ludwig Institute for Cancer Research
University of California, San Diego

3:30 p.m. Discussion Among Speakers and Attendees

4:30 p.m. ADJOURN

Appendix D

Registered Attendees

Thomas Berger
Veterans Health Council

Neil Buckholtz
National Institute on Aging

Shailesh Chavan
Biotest Pharmaceuticals

Jiu-Chiuan (J. C.) Chen
University of Southern California

Wen Chen
National Institute on Aging

Roderick Corriveau
National Institute of Neurological Disorders and Stroke

Maria Dennard
U.S. Department of Housing and Urban Development

Cerise Elliott
National Institute on Aging

Danielle Evers
Office of Science and Technology Policy, The White House

Rona Fields
Associates in Community Psychology

Sam Gandy
Mount Sinai Hospital

Hugo Geerts
In Silico Biosciences

Barry Greenberg
University Health Network–Toronto

Mazen Hamadeh
York University

Mark Hegarty
Cassidy & Associates

Richard Hodes
National Institute on Aging

Andreas Jeromin
Banyan Biomarkers, Inc.

Cynthia Joyce
SMA Foundation

Bill Kaemmerer
Medtronic, Inc.

John Kehne
Translational Neuropharmacology Consulting, LLC

Judith Kelleher-Andersson
Neuronascent, Inc.

Madeline Kelly
GlaxoSmithKline

Zaven Khachaturian
PAD2020–The Campaign to Prevent Alzheimer's Disease by 2020

Walter Koroshetz
National Institutes of Health

Michael Krams
Janssen Pharmaceuticals

Alan Leshner
American Association for the Advancement of Science

Mack Mackiewicz
National Institute on Aging

Kathleen Maguire-Zeiss
Georgetown University Medical Center

Bronwen Martin
National Institute on Aging

Stuart Maudsley
National Institutes of Health

Greg Miller
Science

Poojashree Mishra
National Institute of Mental Health and Neuro Sciences, India

Richard Morris
National Institute of Allergy and Infectious Diseases

Rajendrani Mukhopadhyay
American Society for Biochemistry and Molecular Biology

Eric Nelson
National Institute of Neurological Disorders and Stroke

Alexander Ommaya
U.S. Department of Veterans Affairs

Misha Pavel
National Science Foundation

Steven Perrin
ALS Therapy Development Institute

Suzana Petanceska
National Institute on Aging

Creighton Phelps
National Institute on Aging

Philip Posner
Oak Ridge Institute for Science and Education/Oak Ridge Associated Universities

Ronald Przygodzki
U.S. Department of Veterans Affairs

Lorenzo Refolo
National Institute on Aging

John Reppas
Neurotechnology Industry Organization

Caroline Rodgers

Philip Rubin
Executive Office of the President of the United States

Sethu Sankaranarayanan
Bristol-Myers Squibb

Heather Severson
StudioGraphilia

Beth-Anne Sieber
National Institute of Neurological Disorders and Stroke

Nina Silverberg
National Institute on Aging

Judy Siuciak
Foundation for the National Institutes of Health Biomarkers Consortium

D. Stephen Snyder
National Institute on Aging

Michael Steinmetz
National Eye Institute

Cheryl Stroud
North Carolina One Health Collaborative

Rebecca Swain-Eng
American Academy of Neurology

Anna Taylor
National Institute of Neurological Disorders and Stroke

William Thies
Alzheimer's Association

Molly Wagster
National Institute on Aging

Richard Weidman
Vietnam Veterans of America

Bradley Wise
National Institute on Aging

Lauren Wolf
Chemical & Engineering News/ American Chemical Society

Alice Wyrwicz
Northshore University HealthSystem