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DNA damage response, bioenergetics, and neurological disease: The challenge of maintaining brain health in an aging human population☆,☆☆,★

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1. Introduction

Genome Dynamics in Neuroscience (GDN) is a series of conferences organized and hosted by leading-edge scientists interested in genome dynamics, genome instability and neuroscience. The fourth conference in the series (GDN4, www.gdn4.com), held in late September 2012 in Oslo, Norway, focused on genome instability in aging and brain disease. GDN4 was an eye-opening and mind-expanding conference, which started with a "wake-up call" to neuroscientists, molecular geneticists, cancer biologists, and other researchers, many of whom have been diligently working to elucidate the molecular mechanisms of human aging and neurodegenerative disease. The wake-up call came in the form of the suggestion that the paradigm underlying modern biomolecular research is based on inaccurate assumptions, and that an improved paradigm, one with potential for much greater success in understanding aging and complex human diseases, must embrace the idea that bioenergetics and epigenetic phenomena are central determinants of human health and disease.

2. Energy, aging and disease

Douglas Wallace (University of Pennsylvania, USA) presented the opening keynote lecture at GDN4, in which he asked the audience to re-examine two assumptions that have largely defined the landscape of modern molecular biology and to consider new research directions based on a new paradigm (see Box 1, bullet points 1, 2 and 3, and Fig. 1). The first assumption is that the inheritance of phenotypic traits *strictly* follows the laws of

☆ GDN4 was organized into 10 sessions, including two sessions on DDR and Cell Fate, two sessions on Bioenergetics, two sessions on DNA repair, two sessions on Aging and Neurodegeneration, one session on Infection Biology, one session featuring young investigators and one panel/open discussion session focused on Future Challenges in Aging and Neuroscience, www.GDN4.com.

☆☆ The GDN4 Organizing Committee included Linda H. Bergersen, Vilhelm A. Bohr, Keith W. Caldecott, Arne Klungland, Peter McKinnon, Cynthia McMurray, Lene Juel Rasmussen, Yosef Shiloh, Tone Tønjum.

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Box 1. Key conclusions of GDN4

- A novel ''bioenergetics paradigm'' is emerging based on the critical role played by the mitochondrion in maintaining energy homeostasis in mammalian cells. This new paradigm contradicts and is incompatible with canonical paradigms that argue that defects in anatomical physiology or Mendelian genetics are sufficient to explain all human disease.
- Epigenomic variation modulates and has a large impact on genome structure/function, genome dynamics, neurological disease and ageing.
- Inter-specific, intra-specific, and epigenomic variability contributes to genome evolution and fitness (evolvability), and is relevant to human disease. Interspecific (chromosomal) genomic variation is long term and slow, while intraspecific genomic variation is short term and rapid, featuring changes in the mitochondrial genome. The epigenome also evolves rapidly, but epigenomic variation differs from inter- and intraspecific genetic variation in that it is DNA sequence-independent and reversible.
- ATM may play biologically important ''non-canonical'' roles including several that are kinase-independent and some that are cytoplasmic. ATM may be involved in the cellular response to several genotoxic stresses and synaptic vesicle formation. It may also be involved in synaptic vesicle formation.
- Stress response, inflammation and infectious agents contribute to the etiology of neurological disease.
- β-Amyloid-related pathology, including fibrillar β-amyloid plaques and intraneuronal tau-rich neurofibrillary tangles, is a shared symptom of normal aging and AD and non-AD dementia, but may not be a primary causal factor for AD disease pathology. Therefore, therapeutics that target β-amyloid accumulation or processing are likely to fail, and novel therapeutics based on systemic metabolic aspects of the disease are urgently needed.
- Large scale GWAS initiatives have validated as many as 10 loci that contribute to AD risk. However, our ability to discover AD susceptibility genes far exceeds our ability to understand the underlying biochemistry/pathophysiology of AD.
- ER stress, autophagy and proteasome degradation are key pathways the etiology of AD. Other relevant processes include inflammation, cholesterol metabolism, adaptive immunity, synaptic vesicle trafficking and exocytosis.
- Neuropathology in cells and tissue lacking sufficient DNA repair reflects defects in energy metabolism and high levels of oxidative stress, both of which are more toxic to brain neurons and glial cells than to many other cell types. As a consequence, these diseases often feature selective loss of Purkinje cells and/or general cerebellar atrophy, reflecting changes in astro-glial function.
- AD is the 'disease of the century,' and novel effective tools to predict impending symptoms of dementia are urgently needed.
- Age of the individual, per se, is the strongest known risk factor for sporadic cancer and many neurological diseases.

Mendelian genetics, whereby the nuclear genome eclipses the mitochondrial genome in its relevance to fitness, survival and evolvability. The second assumption is that there is an anatomical basis for dysfunction at the level of the cell, organ, organism and species. To dispute the validity of the first assumption, Wallace presented recent studies from his laboratory, showing that heteroplasmic mice, carrying roughly equal proportions (1:1) of normal mtDNAs NZB and 129, display significant metabolic and cognitive impairment relative to homoplasmic isogenic animals (Sharpley et al., 2012). The phenotype of the heteroplasmic mice is characterized by reduced nocturnal activity, increased food intake, chronic anxiety, and inability to learn, while homoplasmic progeny of the dysfunctional parents are phenotypically normal. Interestingly, heteroplasmy for NZB and 129 drifts in a tissue-specific manner over successive generations, such that brain, heart, and muscle maintain a high level of heteroplasmy, while ovary, liver, and spleen selectively drift towards homoplasmy (Fig. 2). These data suggest strongly that admixture of two normal but different mtDNAs in mice that share the same nuclear genotype modulates phenotype, and can potentially lower overall fitness and survivability.

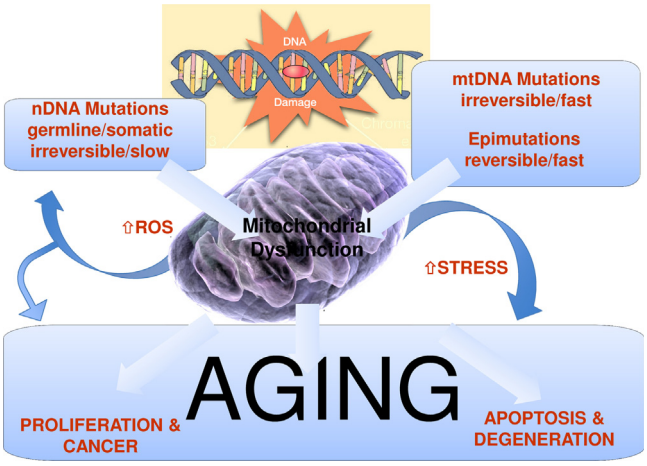

Fig. 1. Proposed conceptual framework: DNA dynamics, bioenergetics and complex human disease. Schematic diagram illustrating the proposed conceptual framework for understanding complex aging-related disease and cognitive dysfunction. See text for discussion.

Based on these and other data, Wallace argued that the mitochondrial genome critically influences fitness and survival at the level of the cell and the organism (Fig. 1). Wallace also presented evidence that mitochondrial dysfunction is tightly linked to many manifestations of human aging and to many human diseases that affect cognitive or neuromuscular function.

Considering these ideas and their relevance to complex human diseases, it becomes clear that tissue-specific pathology should not and cannot be understood simply as the consequence of tissue-specific dysfunction. Instead, as argued by Wallace and others at GDN4, in order to understand and effectively manage complex human diseases, including dementia, diabetes, and other syndromes characterized by progressive neurodegeneration, we should recognize and embrace the central importance of molecular bioenergetics and mitochondrial physiology. Such an approach acknowledges the fact that the mitochondrion and the mitochondrial genome play key roles in energy homeostasis (and thus viability) in mammalian biological systems.

In related work, Lene Juel Rasmussen (Center for Healthy Aging, Denmark) explored the relationships between mitochondrial dysfunction and genomic instability (Desler et al., 2007). For example, steady state dNTP concentrations decrease in human cells that lack mtDNA, promoting nucleotide misincorporation and an decrease in genomic instability. In *Saccharomyces cerevisiae*, replication fork blocking DNA lesions cause dNTP levels to increase during S-phase, promoting error-prone DNA translesion synthesis. However, this process is inhibited in yeast cells with dysfunctional mitochondria. These and other studies suggest crosstalk between mitochondrial and nuclear compartments.

3. Imaging the brain during dysfunctional social interactions

Karl Deisseroth (Stanford University, USA), who presented the second keynote lecture, introduced the 'toolbox' of optogenetics, a technology that owes its existence in large part to groundbreaking studies performed in Deisseroth's laboratory in 2004 and 2005. When University of Oslo Rector Ole Petter Ottersen (University of

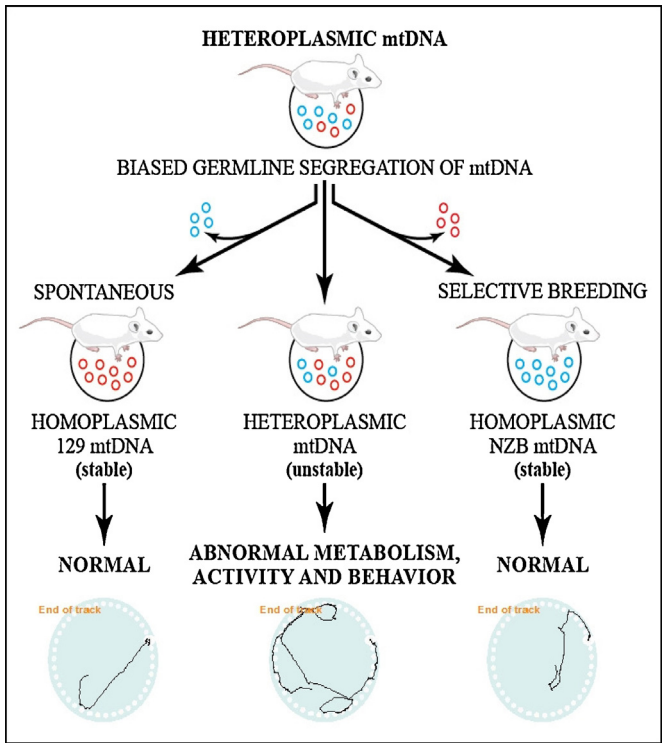

Fig. 2. Mouse model reveals phenotypic consequences of mitochondrial heteroplasmy. Isogenic C57BL/6J mice carrying a 1:1 admixture (heteroplasmy) of NZB and 129S6 mtDNAs were interbred over several generations and mitochondrial genotype of progeny was analyzed. The proportion of NZB mtDNA shifted, eventually producing NZB and 129 homoplasmic progeny. The NZB-129 heteroplasmic mice, but neither homoplasmic strain, demonstrated reduced nocturnal activity, increased food intake, altered respiratory exchange ratio, exacerbated response to stress, chronic anxiety and cognitive deficit.
Adapted with permission from Douglas Wallace.

Oslo, Norway) introduced Deisseroth, he touted optogenetics as one of the most important technical innovations of the last thirty years, suggesting that optogenetics, a technology that combines and leverages recent advances in optical science and molecular genetics, has the potential to explain how an individual neuron in the context of a specific neural circuit performs a specific function (and generates a specific behavioral outcome) in the intact brain of a living animal. Deisseroth summarized the characteristics of optogenetic systems, including functional control of vertebrate neurons with a single opsin gene that does not require cofactors, excellent safety and tolerability profile, millisecond sensitivity, and ability to deliver sustained and defined spike trains. Recent improvements include faster and more potent opsins, bistable (step-function) opsin mutants that can be switched into and out of stable excitable states with a single flash of light, generalizable methods for targeting opsins, and a fiberoptic/laser diode device that allows optogenetic control of any brain region in freely moving mammals. Recent studies in Deisseroth's lab have used optogenetic methods to investigate aspects of Parkinsonism, anxiety, social dysfunction, and other neuropsychiatric diseases (Tye and Deisseroth, 2012). During the last portion of his talk, Deisseroth described a novel rat model developed in his laboratory, which is being used to explore the possible role of excitation/inhibition imbalance in depression, autism and human psychiatric disorders characterized by dysfunctional social interactions. (Similar experiments were previously performed in Deisseroth's lab using a mouse model (Yizhar et al., 2011).) One experiment using the new rat model identified elevated gamma-wave oscillation as a consequence of altered excitation/inhibition balance in neurons in the medial prefrontal cortex. These and other data are consistent with the hypothesis that elevated gamma wave oscillation is a clinical correlate of social behavior disruption in rodents and humans. Ultimately, these or similar experiments may identify neural circuits that are promising therapeutic targets for treating depression and other neuropsychiatric diseases.

4. The disease of the 21st century!

Although hereditary diseases characterized by neurodegeneration are heavily studied, at least in part because they are linked to specific disease-causing mutations in well characterized human genes, many clinicians and basic science researchers feel there is as great or greater urgency to understand and learn to manage the more prevalent, more genetically complex human diseases, such as diabetes, obesity, heart disease, and Alzheimer's disease, because these diseases dramatically lower the quality of middle and late life in the rapidly aging human population worldwide. As expressed by George Martin (University of Washington, USA, Professor Emeritus), "Alzheimer's disease is the disease of the century!" Clearly, the combined social and economic cost of AD and AD-like dementias is very high and is expected to increase in the immediate future, as the average population age increases worldwide.

In this context, it was fitting that Gerald Schellenberg (University of Pennsylvania, USA), who presented the third keynote lecture, described the "state-of-the-science" of AD research. Schellenberg posed two challenging questions during his lecture: (1) Can we completely understand the genetic determinants of both early onset and late onset AD? (2) Does a common molecular mechanism underlie the clinical features and pathology of AD and other progressive neurological human disease syndromes, such as Pick's disease, progressive supranuclear palsy, Parkinson's disease and amyotrophic lateral sclerosis. With regard to the first question, standard genetic studies of familial early onset AD and large scale multi-center consortium-based genome-wide association studies (GWAS) of late onset sporadic AD have identified and validated at least 10 genetic determinants that influence risk of one or both forms of the disease (Naj et al., 2011). Schellenberg pointed out that effect size in GWAS does not predict clinical significance, but that a validated GWAS hit is usually highly reproducible and that GWAS studies have successfully identified potential drug targets for AD and other complex human diseases. Nevertheless, Schellenberg emphasized that "our ability to discover disease genes has far-outstripped our ability to understand the biochemistry and pathophysiology" of those disease-related genes and the proteins they encode. While emphasizing that AD is a complex disease that may reflect dysfunction in multiple pathways including cholesterol metabolism, inflammatory response, adaptive immunity, intracellular synaptic vesicle trafficking and/or exocytosis, Schellenberg also argued that AD might be best understood as a 'proteinopathy,' in which the pathology reflects ER stress and aberrant intracellular protein homeostasis following accumulation of aggregated and/or mis-folded proteins.

5. Probing the link between oxidative stress, DNA repair and aging

Several mouse models have been developed for studying whether and how defective DNA repair causes neurological degeneration or disease. For example, Cynthia McMurray used a mouse model for Huntington's disease (HD) to test the hypothesis that reactive oxygen species (ROS) directly promote disease-causing genetic instability in mice. The experiment was carried out using mice that develop HD-like symptoms and pathology in mid-life, due to the presence of a homozygous CAG-triplet repeat tract ($n = 150$) in the mouse homolog of the HD gene (Lin et al., 2001).

Remarkably, when these animals were dosed with the radical scavenging molecule XJB-5-131 attached to a mitochondrial membrane-targeting peptide derived from gramicidin S, HD clinical signs reversed and disease progression was prevented (Xun et al., 2012). These data strongly support the idea that ROS contribute to aging-related cellular damage in general, as well as contributing to HD-specific pathology, including neurodegeneration.

Because cognitive loss and neurodegeneration are common clinical signs of normal aging, human diseases that feature neurological symptoms in early or mid-life often mimic accelerated aging. The diseases that have been most informative and enlightening concerning mechanisms of normal human aging are relatively rare, affecting as few as a handful to as many as several hundred patients worldwide. These rare diseases include the segmental progerias, such as Werner syndrome, Bloom's syndrome and Cockayne syndrome. Bloom's and Werner syndrome, as well as several hereditary diseases associated with DNA repair deficiency are associated with dramatic increases in incidence of specific human cancers (i.e., xeroderma pigmentosum/skin cancer, hereditary non-polyposis colon cancer/colon cancer, ataxia telangiectasia (AT)/leukemia/lymphoma), while Cockayne syndrome does not feature pronounced cancer susceptibility.

Recent studies have focused on the potentially critical role of base excision repair (BER) in mitochondria and/or in neurons, based on the idea that oxidative stress and oxygen free radicals, combined with lower DNA repair capacity, contribute to normal aging. Such studies have exploited mouse models and sophisticated brain imaging and/or behavioral studies; for example, Vilhelm Bohr and his colleagues observed different memory capacity in mice predisposed to AD-like disease with or without deletion of one allele of DNA polymerase β (P. Sykora, unpublished data) and showed that deletion of BER glycosylase NEIL-1 in neurons interferes with short-term spatial memory and recovery from ischemic stroke (Canugovi et al., 2012). Magnar Bjørås also observed complex effects resulting from defects in NEIL-1 and/or NEIL-2 in the brains of mice exposed to ischemic injury.

Linda H. Bergersen and her colleagues generated Neil-3 knockout mice (Magnar Bjørås) and transgenic mice that conditionally express a mutated form of uracil-N-glycosylase 1 (mutUNG1) in forebrain neurons (Arne Klungland), and used these mouse models to analyze the consequences of elevated frequency and increased persistence of hydantoin lesions or cell lethal abasic sites in mtDNA, respectively. Interestingly, mtDNA copy number and mitochondrial transcription are reduced in hippocampal neurons of mutUNG1 expressing mice, receptor density is lower in excitatory glutamatergic synapses in the dentate gyrus, and the size of the postsynaptic density in this brain region is abnormal. Bergersen and colleagues speculate that synaptic dysfunction in mutUNG 1expressing mice is causally related to mitochondrial loss and dysfunction (Lauritzen et al., 2011). Neil3$^{-/-}$ mice display learning and memory deficits and reduced anxiety-like behavior and neural stem/progenitor cells from aged Neil3$^{-/-}$ mice showed impaired proliferative capacity (Regnell et al., 2012). These data strongly support the idea that BER capacity influences complex neurological functions during early neurogenesis in mice, although the exact mechanisms are not yet fully understood and further confirmation is needed. Clearly, one of the major challenges for the future is to elucidate how genome dynamics modulate behavior in humans and other animals.

6. Defective DNA damage response and neurodegeneration

If protein homeostasis, ER stress and trafficking of damaged and/or aggregated protein play a key role in neurological disease, this is not to say that DNA damage, oxidative stress and altered cellular bioenergetics do not also play equally critical roles. Several sessions at GDN4 considered new insights into the cell-biological response to DNA damage (DDR) and genotoxic stress. A central conundrum in this field, central also to the overall focus of GDN4, is the molecular basis of the tissue-specific, brain region-specific, and cell type-specific dysfunction associated with nuclear-encoded defects in DNA repair or DDR (Shiloh and Ziv, 2013). In the first GDN4 session on DDR, Yosef Shiloh (Tel Aviv University, Israel) presented an overview of ataxia–telangiectasia (A–T) – genomic instability syndrome characterized by neurodegeneration, particularly a striking cerebellar atrophy. The responsible gene encodes the serine/threonine protein kinase, ataxia telangiectasia, mutated (ATM), most notable for its role in mobilizing the cellular response to DNA double-strand breaks (DSBs). Researchers studying A–T debate extensively the physiological and molecular basis of neurodegeneration associated with the disease, because its functional link to the DSB response is not obvious. Shiloh compared A–T to other genetic disorders characterized by cerebellar atrophy, including SCAN1, AOA1, AOA2 and A–T-like disease, emphasizing that the common feature of these DDR-related diseases is cerebellar degeneration, which is quite similar in most of them (Fig. 3). He suggested that the neurodegeneration in A–T might reflect a background role for ATM in the cellular response to several types of genotoxic stress; in particular, those that commonly occur in all body tissues and are by-products of normal metabolism. Importantly, post-mitotic brain neurons and glial cells exhibit higher oxidative stress than many other cell types, and ATM may play an important role in mitigating oxidative stress-induced damage in these cell types (Guo et al., 2010). However, the cerebellar atrophy characteristic of A–T and other DDR deficiencies is probably not caused by a specific neuronal defect; while these diseases can feature a striking loss of Purkinje cells, the structural and functional changes in the cerebellum may also reflect pervasive changes also in glial cells.

Consistent with the view that ATM plays a multi-dimensional and complex role in orchestrating the response to cellular stress, Peter McKinnon (St. Jude Children's Hospital, Memphis, USA) presented evidence that ATM directly regulates repair of Topoisomerase I-linked single-strand DNA breaks (McKinnon, 2011), and Karl Herrup (Hong Kong University, Hong Kong) reviewed the evidence that ATM regulates the transcriptional state of large chromatin regions, by indirectly regulating the balance between histone acetylation/deacetylation, thus modulating the histone

DISEASE	GENETIC DEFECT	Pathology* AT	CeD	ID	Ca
Normal	Normal	Ø	Ø	Ø	Ø
A-T	ATM	X	X	X	X
AOA1 (AOA2)	Aprataxin (Senataxin)	X	X	Ø	Ø
SCAN1	TDP1	X	X	Ø	Ø

* Clinical features: AT, ataxia; CeD, cerebellar degeneration; ID, immunodeficiency; Ca, cancer

Fig. 3. Shared features of human DNA repair-deficiency diseases. Disease name, genetic defect and presence/absence of cerebellar degeneration and cancer susceptibility are shown.

code and the folding/unfolding of transcribed chromatin domains (Li et al., 2012). These and other related data suggest that ATM plays kinase-dependent, kinase-independent, nuclear and non-nuclear roles in stressed cells, and that ATM is activated in response to many different stressors and multiple genomic lesions.

7. Understanding the astro-glial microenvironment: the next neuroscience frontier

In contrast to deep and experimentally based knowledge of the structure of the human brain, as well as extensive data on how neurons communicate with each other at synaptic junctions, there is a relative paucity of information on glial cells, how they interact with neurons, and how the astro-glial environment modulates brain activity. Nevertheless, as pointed out above, it is quite likely that brain aging and some brain diseases are directly linked to glial cell dysfunction. Before it is possible to test this hypothesis, it will be of the utmost importance to decipher glial–neuron interactions leading to thorough understanding of impact of the 'astro-glial microenvironment' on brain function.

8. Effects of ATP-dependent transport processes in neurodegenerative diseases

Jens Pahnke (University of Marburg, Germany) highlighted how ATP-binding cassette (ABC) transporters have been implicated in the regulation of Aβ levels and neurodegeneration in the brain. Functional abrogation of mitochondrial oxidative phosphorylation during aging is one important physiologic mechanism leading to ABC transporter dysfunction in the elderly. Altering the temporal aggregation profile of Aβ and pharmacological activation of ABC transporters could impede the neurodegenerative cascade during ageing that culminates in AD.

9. Mutagenesis and evolvability

For mutagenic changes distributed in genomic DNA strictly according to random chance, a very small fraction of events will have a beneficial effect (in a functional evolutionary sense), while the large majority of events are expected to be either inconsequential (i.e., a synonymous point mutation in a protein coding sequence) or have deleterious consequences. However, in contrast to the dogma that mutations are distributed randomly in a chromosome, Susan Rosenberg (Baylor College of Medicine, Texas, USA) discussed evidence that mutagenesis occurs in a non-random manner with respect to both time and space, and that this is equally true for prokaryotic and eukaryotic cells and for eukaryotic multicellular organisms. Referred to in other contexts as "adaptive" or "targeted" mutagenesis, Rosenberg referred to this as "stress-induced" mutagenesis, and demonstrated that in *Escherichia coli*, stress-induced mutagenesis requires expression of SOS (DNA damage response) genes and *RpoS*, which encodes a sigma factor that directs expression of downstream target genes; these genes in turn confer increased resistance to the stress-inducing factor(s) in the environment (Shee et al., 2011). In human cells, somatic hypermutation in antibody-expressing B cells might be viewed as a correlate of bacterial stress-induced mutagenesis. As discussed by Hans Krokan (Norwegian University of Science and Technology, Trondheim, Norway), somatic hypermutation is a process whereby cytosine residues in GC base pairs are enzymatically deaminated in variable regions of immunoglobulin genes in B-cells, and the resulting GU base pairs are subsequently repaired in an error-prone manner (Hagen et al., 2006). The end result of somatic hypermutation is increased antibody diversity, and a more robust adaptive immune response. In this scenario, somatic hypermutation is cell type-specific and targeted to specific regions of specific genes. Unfortunately, if any step in somatic hypermutation escapes tight regulation, B cell proliferation can increase; thus, dysregulation of somatic hypermutation is considered a risk factor for B-cell lymphoma. Nevertheless, Rosenberg argued that stress-induced mutagenesis in *E. coli*, and similar processes in eukaryotic species, increases the 'evolvability' of a cell, where evolvability is defined as the intrinsic ability of a cell to adapt through a genetic mechanism to a novel environment (or environments) (Rosenberg et al., 2012). Evolvability is distinct from Darwinian "fitness," which is defined as the relative ability of a population of cells or organisms to survive and reproduce in a single environmental context.

Interspecific and intraspecific genomic variation is also highly relevant to understanding human aging and complex age-related diseases, including AD-like dementias and cancer (Figs. 1 and 4). Jan Vijg (Albert Einstein College of Medicine, New York, USA) reported on recent data from his laboratory and that of his colleague, Cristina Montagna, showing that the frequency of aneuploidy is very high in nuclei obtained from the cerebral cortex of the mouse brain, with chromosome-specific accumulation of aneuploidy in the aging mouse brain (Faggioli et al., 2012). They performed interphase FISH analyses with overlapping BAC probes, and quantified aneuploidy for up to 8 chromosomes in old and young mouse cortex, cerebellum and spleen. Remarkably, the estimated aneuploidy rate per chromosome was 1–2% in all three tissues of 4 month old mice. However, the increase in aneuploidy was only observed in cortex, not in cerebellum or spleen. After extrapolating from the 8 tested chromosomes to all chromosomes, in the aged cortex, on average, approximately half of all cells were aneuploid for at least one chromosome. They also showed that the age-related increase in aneuploidy only occurred in non-neuronal nuclei (i.e., in glial cells), possibly due to mitotic errors. Based on these and other data on the accumulation of various types of mutations in the human and mouse brain, Vijg argued that such high levels of age-dependent genomic instability in somatic tissue are relevant to human aging and aging-related neurodegeneration (Fig. 4). George Martin (University of Washington, USA, Professor Emeritus) largely concurred with this idea, stating that the primary mechanisms that generate intra-specific variation in healthspan

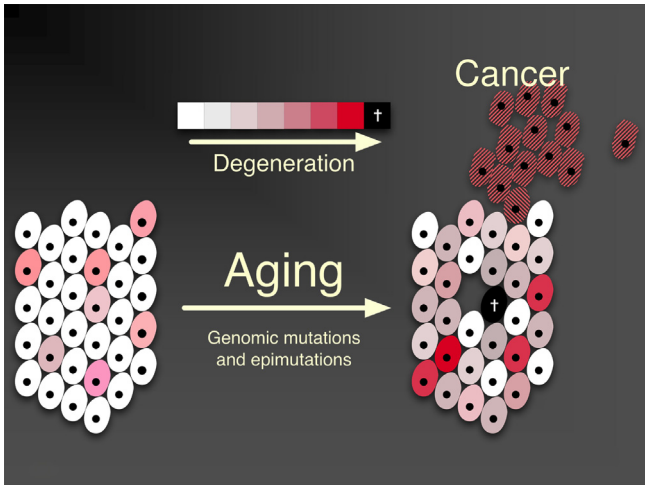

Fig. 4. Impact of mutations and epimutations on somatic phenotype. In a stochastic manner, mutant somatic cells acquire novel properties, leading to alternative outcomes: (1) clonal overgrowth of cells that acquire increased proliferative capacity (red); (2) degeneration of cell population through senescence or death (black); or (3) mutant cells with no selective advantage self-renew at the same rate as wild-type cells, independent of whether they are phenotypically distinct from wild-type soma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
Adapted with permission from Jan Vijg.

and lifespan include epigenetic drift, and nuclear and mitochondrial mutation in somatic tissues. Somatic and epigenomic mutations both occur over short time frames; however, epigenomic mutations are DNA sequence-independent and may sometimes be reversible. Interestingly, recent data indicate that epigenomic patterns can be transmitted across generations.

10. Symbiosis and versatility: the mitochondrion and the microbe

Human disease-causing bacterial infections, as exemplified by meningitis caused by *Neisseria meningitidis* or *Mycobacterium tuberculosis*, are an important and relevant area of neuroscience research today. *N. meningitidis* is a leading cause of meningitis, and *M. tuberculosis*-linked disease is the most frequently lethal bacterial disease worldwide, causing meningitis more frequently than any other extrapulmonary manifestation (Davidsen et al., 2007). The pathobiology of these CNS infections are highly informative: for example, it is critical to the infectious cycles of meningococci and mycobacteria that the bacteria are exposed to ROS and reactive nitrogen intermediates (RNI) generated by host granulocytes and macrophages, as a part of the host innate immune response. Because of their chemical reactivity, ROS and RNI cause severe damage to cellular macromolecules, including nucleic acids, nucleotide pools, proteins and lipids. The resulting genome instability represents both opportunity for adaptation, increased fitness and/or survival, as well as a liability, with associated risk of catastrophic mutagenesis, cell senescence or cell death. Interestingly, DNA repair profiles of the meningococci, mycobacteria and *E. coli* appear to be highly adapted to their unique environmental niches. Furthermore, meningococcal species actively promote uptake of 'foreign' DNA throughout the lifecycle, by a mechanism that requires biogenesis of a highly complex pilus. However, Tønjum and colleagues have shown that pilus-mediated DNA uptake by meningococci tends to have a conservative outcome, stabilizing the genome and preserving function, rather than promoting genetic variation. This represents a paradigm shift in the perception of the role of horizontal DNA transfer in meningococci and other bacterial species (Ambur et al., 2009; Frye et al., 2013).

Bacteria are also important low-complexity genetically amenable model organisms. For example, bacteria in the genus *Neisseria* express a broad-spectrum O-linked protein glycosylation system that provides a useful model for studying protein-associated glycans from a structural and functional perspective (Vik et al., 2012). Michael Koomey (University of Oslo, Norway) presented studies on the role of these important protein post-translational modifications (PTMs) in localizing proteins to a specific subcellular compartment (i.e., periplasmic space, plasma membrane). Glycosylation of pilin plays a role in intracellular pilus dynamics and in biogenesis of extracellular type IV pili. Together, these studies provide new perspectives on the functional correlates of bacterial protein glycosylation and glycoform diversification in eukaryotes, where they are critical, among other things, for brain cell cohesion. In fact, without O-linked glycosylation of membrane components, neurons and glial cells would not demonstrate normal adhesive properties, which would most certainly lead to neuropathology. Along these lines, a neisserial model system has been developed to explore aspects of bacterial respiration, and their relevance to mitochondrial bioenergetics in eukaryotic cells (Aspholm et al., 2010).

11. Future directions

A highlight of GDN4 was the panel discussion near the end of the conference, during which conference presenters and participants identified the most critical immediate challenges facing researchers studying DNA damage/repair, clinical and experimental neuroscience and aging-related neurodegenerative disease. Consensus points that emerged during the panel discussion ranged from understanding the neurological bases of human behavior to elucidating epigenetic mechanisms that regulate genome dynamics (Box 2). Conference participants also felt that there is an urgent need for new and better tools for predicting onset of AD and AD-like dementias.

Box 2. Critical challenges for understanding neurodegeneration and aging

- Identifying and characterizing determinants of cell fate in the astro-glial–neuronal microenvironmental niches **represents the next neuroscience frontier**.
- Identifying pre-clinical biomarkers and predicting clinical onset of AD, the disease of the century.
- Understanding the interplay between interspecific, intraspecific and epigenetic variation and the impact of each on genome evolvability and fitness.
- Understanding how the epigenome influences genome dynamics and human disease.
- Identifying metabolites and exosomes as useful biomarkers and developing effective diagnostic and therapeutic targets for neurodegenerative diseases.
- Understanding the direct link between neurological/synaptic function and behavior.
- Understanding how the microenvironment and the precise connectome of an individual neuron influences perception, cognition and behavior.
- Identifying rapidly evolving regions of the human and microbial genomes.
- Defining the role of non-coding RNA species in regulatory genome dynamics.
- Developing and exploiting novel technologies, such as NIMS and optogenetics, to overcome research bottlenecks in neuroscience.

As mentioned above, the increasing incidence and cost of managing AD and AD-like dementias is already a social/medical/economic problem of major significance. As this trend continues, it will become increasingly more difficult to promise or promote improved health span among the elderly, without unduly drawing upon economic, medical and social resources needed to educate and nurture the shrinking sub-65 year-old fraction of the human population. Because brain health, in all life phases, may be the key to the 'good life,' the challenge for the future is not only to characterize and understand neuroscience and DNA repair at the level of metabolites, molecules and cells, but also to understand human cognition and experience, using as many distinct but convergent technologies as possible. Bioenergetics and mitochondrial function are emerging as novel keys to this more 'organic' approach, at least in part because all biological systems harness and regulate chemical energy to create biological function.

The last imperative is to address the need for novel translational approaches that bridge the gap between basic and clinical science, because the ultimate goal is to increase quality of human life and minimize suffering from disability and neurological disease. The most imminent problem worldwide for neuroscientists may be to learn what measures conserve cognitive capacity among the elderly. With any luck, this knowledge might lead to tools for preventing or treating neurological disease in young and middle-aged individuals as well.

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