

Sirtuins as Targets for HD Treatment

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Abstract

The sirtuins are a class of protein deacetylases one of whom, SIRT1, has attracted the interest of researchers in various fields of medicine because it is linked to longer lifespan in several organisms. In January 2007, more than 20 scientists came together at a workshop titled “Sirtuins as Targets for HD Treatment” to discuss the therapeutic potential of these enzymes in Huntington’s disease (HD). Over two days, neurologists and scientists shared their knowledge of HD pathophysiology with leaders in the sirtuin field, who outlined the known effects of sirtuins on gene transcription, energy metabolism, and longevity. Both groups were encouraged by the overlap between their two fields, and intrigued by the possibility that SIRT1 activation may benefit HD not only as a consequence of slowing down aging and delaying the onset and progression of many diseases, but also because it may target many of the same cellular pathways that have specifically been implicated in HD.

Sharing both published and unpublished data from yeast, worms, flies, mice and humans, the group overwhelmingly agreed that the sirtuins are a set of targets worth pursuing in the context of HD. They also identified areas where discrepancies need to be reconciled and gaps in understanding need to be filled. One important concern to emerge from the workshop is how protective effects of SIRT1 activation will ultimately weigh against observations that SIRT1 may exacerbate HD pathology by deacetylating huntingtin and inhibiting its autophagic clearance. The potential of pharmacologic treatments like resveratrol and analogs were discussed, as were their limitations and contraindications. In all, participants were optimistic about the potential opportunity for HD therapy development and about the new lines of communication and collaborations the workshop helped facilitate.

Introduction

Sirtuins are a family of mammalian proteins named after the founding member in yeast, Sir2 (for silent information regulator 2). Sir2 is an enzyme with nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase activity (also known as class III histone deacetylase), and is part of a complex of transcriptional repressors/silencers that is evolutionarily conserved from prokaryotes to humans. The mammalian Sir2 family consists of seven homologues called *sirtuins*, or SIRT1-7. SIRT1 is the closest to Sir2 in terms of sequence and enzymatic activity, and also is the mammalian sirtuin most extensively studied to date. It was also the sirtuin most extensively discussed in this workshop.

Researchers in aging have been interested in Sir2/SIRT1 because of evidence that activity of Sir2/SIRT1 promotes longevity in several organisms. Age is a risk factor in many human diseases, and most certainly in neurodegenerative diseases, which have a late onset and progression of symptoms with time. The possibility then is that by activating SIRT1 and thereby extending lifespan and promoting healthier aging, one might effectively delay the onset and progression of age-related diseases like HD.

Huntington’s disease (HD) is a complex neurodegenerative disease, involving cognitive, psychiatric, metabolic and motor symptoms. So that the scientists who attended the workshop might have a better understanding of the disease, a man with HD and his wife generously shared their experiences with the participants. They had both watched his father suffer for more than ten years before receiving the correct HD diagnosis, so when this man’s first symptoms of HD appeared three years ago, they recognized quickly—and ominously—what was going on. Today,

his unwanted movements are well controlled by haloperidol, but his problems with memory and multitasking are so pronounced that he stopped working a few months ago. His wife expressed her fear, “watching him change slowly and knowing what the end result is going to be” And, of course, they worry for their three young children who are at risk of getting HD. The couple’s story helped the workshop participants appreciate the challenges of living with the disease, inspiring them to focus on finding new insights into the pathogenesis and new approaches to the treatment of HD.

Metabolic aspects of HD invite exploration of SIRT1

Many organisms, including humans, can live longer by restricting the number of calories consumed. Sir2/SIRT1 appears to mediate the life-lengthening effects of caloric restriction, suggesting Sir2/SIRT1 regulates the pace of aging in accordance with metabolism. This has led to much research into the mechanisms by which Sir2/SIRT1 regulates energy metabolism and how that may impact the aging process. For example, SIRT1 activity has been shown to influence pancreatic insulin secretion and glucose metabolism, as well as gluconeogenic/glycolytic pathways in the liver. At the cellular level, SIRT1 activity can influence the induction of genes for oxidative phosphorylation and mitochondrial biogenesis.

In addition to the idea that slowing down aging in general may delay the onset and progression of various diseases, there are many reasons to think that regulating aging in accordance with metabolism may be relevant to HD in specific ways. As noted by many HD researchers at the workshop, there is a long history of interest in energy metabolism in HD, fueled by a bevy of circumstantial clinical evidence such as pronounced weight loss, as well as experimental models which point to excitotoxicity, mitochondrial dysfunction, defects in calcium handling and dysregulation of ATP production.

Thus, there was much discussion during the first day of the workshop exploring the overlap between what is known about the metabolic aspects of HD and the role sirtuins play in regulating metabolism. Although any cure for HD will ultimately have to ameliorate neuronal dysfunction and pathology, participants wondered if more hints about disease pathogenesis can be gleaned from understanding the basic physiology of individuals with HD. Most were optimistic that once we understood in more detail how metabolism is altered in individuals with HD, SIRT1 activation might not only restore metabolism toward a healthier profile, but also potentially target the neurodegenerative process as well.

One important consequence of better characterizing metabolic abnormalities in individuals with HD is that the pathophysiologic processes can then be mechanistically studied—and therapeutic interventions tested—in HD mice models that have parallel changes. From the discussion, however, it became clear that obtaining definitive metabolism data from individuals with HD has been elusive for a number of reasons. Participants outlined what is known about energy and metabolism in individuals with HD and some of the HD mice models, and identified areas where more study is warranted. While there were straightforward suggestions such as conducting systematic screening of basic blood labs and hormone levels, much of the conversation circled around the most prominent metabolic observations in HD.

One striking feature of HD is that many people experience rapid and profound weight loss, even when consuming thousands of extra calories a day. Jang-Ho Cha of Massachusetts General Hospital (MGH) indicated that many HD neurologists generally consider rapid weight loss a bad sign, coinciding with a worsening of motor symptoms. In fact, some people at risk for HD even try to protect themselves by becoming overweight. However, there is no hard data

confirming a relationship between weight and severity of symptoms. Nancy Wexler suggested looking systematically at the data from the Venezuela families and from the larger data sets in the US to pursue these questions. She noted that it would be very straightforward to use the datasets to see if heavier individuals have a later age of onset for a given repeat length, for example.

Since SIRT1 can impact metabolism in many different types of tissues, participants were interested to know if the weight loss in people with HD is predominantly due to loss of fat or loss of muscle. Although individuals with HD have not been extensively studied in this way, Anne Young of MGH noted that HD is not a disease of frank muscle weakness, like ALS, so although individuals are very atrophic at the end stages, at the early stages they are still quite strong. A number of participants noted that a more precise understanding about weight loss in patients would facilitate mechanistic studies in HD mice models to determine whether they reproduce the human changes. Such an understanding might also lend insight into whether rescuing the weight loss phenotype might also improve other symptoms of HD.

It is known that R6/2 mice lose weight with progression of symptoms, but also have an increase in body fat at 8-9 weeks if fed either normal lab chow or a high fat and high sugar diet. Flint Beal of Weill Medical College of Cornell University noted work by Mark Mattson's lab showing that caloric restriction in N171-82Q mice delayed the onset of symptoms and increased life span, but also paradoxically *reduced* weight loss. While many participants questioned the advisability of having individuals with HD restrict calories, Leonard Guarente of Massachusetts Institute of Technology (MIT) noted that it is possible that multiple signaling pathways involving SIRT1 may be relevant, such that, for example, high fat diets and caloric restriction could both be protective for different reasons. He suggested that a lot could be learned by examining the various HD mice under several different types of diets.

Weight loss in HD cannot be attributed to inadequate supply of food or absorption of nutrients; rather increased utilization of energy is thought to be the culprit. Published studies indicate that individuals with HD do have increased energy expenditure, but it is still debated whether the increase in energy expenditure is solely due to increased movements or if there is also some underlying increase in metabolic rate even at baseline. Beal noted several studies with conflicting reports, and Young explained that the data obtained to date does not adequately answer the question because of the difficulty obtaining basal metabolic rates in people with continuous movements. She suggested answering this question by testing individuals who are at risk for HD in metabolic chambers to see if they have metabolic changes that precede the onset of movements.

To address energetic abnormalities specifically in brain tissue, Beal suggested looking at glucose utilization in the brain of people at risk for HD by PET scan. This is another area in which published data in people with HD has given conflicting results. Beal indicated that which region of the brain you are looking at, and when, are critical variables. He noted work from his laboratory measuring glucose utilization by deoxyglucose methods in two different HD mouse models (HdhQ111 knock-in mice and R6/2). They found that glucose utilization was increased early in these mice, but at later ages glucose utilization was significantly decreased. By proton NMR, Beal and colleagues found an increase in lactate production in the basal ganglia and the frontal, parietal and occipital cortex of individuals with HD. Increased lactate production indicates an energy deficit in these tissues, and specifically an increase in the rate of glycolysis, although is not known if this is an attempt to make up for decreased energy stores.

David Sinclair of Harvard Medical School asked if there was evidence of specific metabolic changes in tissues other than brain in HD, and Beal noted that he and colleagues also attempted to measure lactate in blood, but were unable to complete the study because of technical difficulties. They used phosphorous NMR, however, to observe that individuals with HD have a decreased ability to produce phosphocreatine in muscle, which is a sensitive marker of energy dysfunction. These results have been observed in both symptomatic individuals with HD and gene positive individuals who are at risk but do not have any symptoms of HD. Evidence of muscle fiber type conversion in human HD and the R6/2 mouse model has also been reported.

Another indicator that the metabolism of people with HD is altered is evidenced by their temperature regulation. According to Cha, HD patients generally feel warmer than others, and are more bothered by heat than by cold. “They walk around with no jacket in the winter and have the AC cranked all summer,” he noted.

Temperature dysregulation is also seen in HD mice. Beal cited a publication from Al La Spada’s lab showing that HD N171-82Q mice have difficulty maintaining core body temperature and develop hypothermia associated with impaired activation of adipose tissue, another process in which SIRT1 is implicated. Interestingly, Beal reported that when the ambient temperature in the HD N171-Q82 mice are raised is increased, the mice live longer. Extending this analysis to people with HD is complicated because temperature changes in mice involve metabolic changes in brown fat, which humans have very little of, noted Guarente. Nonetheless, Beal noted that the evidence of temperature dysregulation in several HD models prompted him to look for similar changes in people with HD.

Regulation of blood glucose is another area where HD symptomatology and SIRT1 may overlap. Studies indicate that 10% of people with HD are also diabetic, which is prevalence ten-fold higher than in the general population. In fact, Carl Johnson posed the hypothesis that diabetes is a non-fully penetrant phenotype in HD (i.e. with abnormal movements being fully penetrant). However, it is not clear if diabetes associated with HD is more likely to have a Type I or Type II etiology. For example, Beal hypothesized that in HD you start with a basic bioenergetic defect and then develop hyperinsulinemic diabetes from that, while Cha noted that what occurs might be a hypoinsulinemia that is due to mechanisms different than the autoimmune destruction of cells that occurs in Type I, giving a more partial phenotype. Beal has shown that the R6/2 mice get aggregates in pancreatic cells, and develop hypoinsulinemic diabetes. Beal has also found that transcription factors that regulate insulin secretion are also decreased.

Whether these changes also occur in humans is not known. Beal noted that he and Walter Koroshetz tried to conduct glucose tolerance tests in individuals with HD years ago but were unable to collect the samples they needed. Shin-ichiro Imai from Washington University School of Medicine suggested that in addition to specific metabolic tests such as glucose tolerance, a systematic screening of basic blood lab values and hormone levels from individuals with HD and mice models could be very informative.

Complicating all of these investigations, however, is increasing evidence pointing towards pathologic involvement of the hypothalamus and endocrine systems in HD, which would also have metabolic implications. Asa Peterson’s studies have shown that hypothalamic atrophy occurs early in HD, with specific loss of neurons expressing orexin and somatostatin, pathologies that may underlie sleep and circadian rhythm alterations in HD. Beal and Young also noted published reports of dramatic cell loss in other hypothalamic nuclei, such as the tubular

mammary and lateral tubular nuclei. Thus, alterations in the HPA axis complicates the analysis of metabolic changes in HD, and how these factors interact with SIRT1 based therapies remain to be seen.

Essentially all of the participants agreed that more extensive information about metabolic changes in HD is warranted. In addition to the ideas for experiments already outlined, Guarente emphasized that a lot could be learned by putting some of the HD mice models on different laboratory diets (such as normal chow, high fat, low fat, and caloric restriction), especially since much is known about SIRT1 in this context. Measurements suggested by participants included lifespan, weight, motor function, basal metabolic rate (in metabolic chambers), and body composition in terms of fat vs. muscle, or muscle-type switch.

Larry Marsh of the University of California, Irvine, among others, also stressed the importance of determining which of the symptoms, metabolic or otherwise, will be the most critical to target, noting that “living longer but still neurologically impaired is not necessarily better for the individual with HD.”

Introduction to Sir2/SIRT1 and its manipulation in HD animal models

Guarente provided an overview of Sir2/SIRT1 and the other mammalian sirtuins (see *Other Sirtuins SIRT2-7*). Sirtuins are a family of enzymes that can act as NAD-dependent deacetylases, removing acetyl groups from modified histones or other proteins and transferring it to the ADP-ribose moiety of NAD. They can also act as ADP-ribosyltransferases, transferring ADP ribose (ADPr) to proteins. SIRT1 has strong deacetylase activity and weak ADPr-transferase activity. In yeast, the longevity effects of Sir2 appear to be mediated by its deacetylation of a lysine residue of histone H4. The mechanism(s) by which SIRT1 extends lifespan in mammals and other organisms is not yet clear.

Because there are multiple effects downstream of Sir2/SIRT1 activity, it is challenging to determine which effect is most relevant in a particular model. This also prevents scientists from developing a general assay to measure SIRT1 activity, because they first must determine that a robust effect on one substrate (i.e. via Biomol enzyme assay or mass spectrometry) is relevant for another substrate or a specific outcome. Additionally, obtaining large amounts of acetylated substrates to work with in a purified system is very difficult (the analogous problem has complicated the study of phosphatases). Li Huei Tsai of MIT proposed screening a panel of acetylated peptides (which can be purchased commercially) to represent a number of different substrates *in vivo*. However, Imai noted that SIRT1 activity is NAD-dependent, and the regulation of the NAD concentrations in tissues is a critical factor that will be missing from any *in vitro* assay (see *NAD metabolism*). He suggested that expression profiling might be a more informative approach, perhaps choosing a panel of 10 genes to follow as reporters of SIRT1 activity.

Mammalian SIRT1 is ubiquitously expressed, with exceptionally high expression in the hippocampus and hypothalamus. In terms of subcellular compartmentalization, SIRT1 is thought to be primarily nuclear, although Imai noted evidence that SIRT1 can be shuttled between the nucleus and cytoplasm of neurons in the brain. In fact, he observed region-specific developmental changes in SIRT1 localization in the brain, with some regions having nuclear SIRT1 in young animals, but cytoplasmic SIRT1 in adults. How these phenomena relate to adult-onset diseases still needs to be investigated.

Currently, the tools researchers use to most commonly manipulate Sir2/SIRT1 in organisms include the following (all discussed in detail, below): 1) genetic over-expression of

Sir2/SIRT1, 2) null mutants or genetic Sir2/SIRT1 knock-outs, 3) treatment with resveratrol, which has been shown (among other things) to activate Sir2/SIRT1, and 4) manipulation of NAD concentration and/or treatment with nicotinamide.

Worms

Some of the first data indicating that SIRT1 activation is protective for degenerating neurons in HD come from *C. elegans*. Christian Neri (INSERM, Paris) summarized work from his laboratory showing that the worm homologue of Sir2/SIRT1, *sir2.1*, modifies neuronal polyglutamine toxicity. In the worm model, an exon 1-like 128Q huntingtin fragment expressed in a subset of sensory neurons causes neuronal dysfunction with visibly dystrophic axons. *Sir2.1* activation, either genetically or by resveratrol treatment, rescues these disease phenotypes, and *sir2.1* inactivation exacerbates them.

Neri further showed that the protective effects of *sir2.1* activation are dependent on *daf-16*, a member of the FOXO family of Forkhead transcription factors. Sir2/SIRT1 proteins deacetylate FOXO proteins which leads to inhibition of the expression of genes associated with cell death, and induction of genes that promote DNA repair and make cells resistant to oxidative stress. Interestingly, one of the human FOXO genes, FOXO3A, maps in a region of the human genome that has been shown to be a very strong modifier of age of onset of HD. This observation is currently being followed up by tracking SNPs in Venezuelan and French HD kindreds.

Discussing the *daf-16*/FOXO mechanism in more detail, the participants considered the subcellular localization of the signaling components. Responding to a question posed by Guarente, Neri noted that the DAF-16 protein is in the cytoplasm of neurons in the presence of either normal or mutant huntingtin. This raises the question of how cytoplasmic DAF-16 is protecting neurons by Sir2/SIRT1-dependent changes in transcription (i.e. in the nucleus). Noting recent evidence that Sir2/SIRT1 is shuttled between the nucleus and cytoplasm of cells, Guarente suggested that the translocation process may also be a therapeutically useful target. Along these lines, several investigators proposed also examining phosphorylation and other post-translational mechanisms that regulate the localization of Sir2/SIRT1.

Guarente also questioned whether the *sir2.1* over-expression and resveratrol treatment in *C. elegans* were indeed acting via the same mechanistic pathway. In worms, *sir2.1* activation can target not only *daf-16* but also *abu-11*, which is a gene involved in ER stress. Guarente noted that worm *sir2.1* activity normally represses *abu-11* expression, so if you knock out *sir2.1*, *abu-11* activity increases. However, in worms this effect is overridden by the effect of *sir2.1* on the *daf-16* pathway. Therefore, Guarente suggested knocking out *abu-11* in the HD worm model and testing whether resveratrol still increases lifespan. Although Neri had not yet tried this experiment, he stated that over-expression of *abu-11* alone in HD worms did rescue the phenotype.

Neri's group is conducting gene expression profiling in *C. elegans* to look for effects of altering the worm *sir2.1* or *daf-16* on the toxicity of mutant huntingtin as a shortcut to finding biomarkers and additional drug targets for HD. Sharing unpublished data, Neri reported that they had already screened several hundred genes controlled by *daf-16* in worms, and one third of these modify mutant huntingtin toxicity. They are currently characterizing those interactors which are conserved in humans to determine what mechanisms appear to be most relevant in disease.

Flies

Following up on the issue of whether SIRT1 activation and resveratrol treatment are equivalent, Larry Marsh and Leslie Thompson of the University of California, Irvine, outlined the results they have obtained in *Drosophila* models. In the HD fly model, N-terminal fragments of huntingtin are overexpressed, which leads to an easily quantified neurodegeneration of photoreceptors and early death of the flies. Thompson and Marsh's labs have previously shown that inhibiting Class I or Class II HDACs is protective in these models. Targeting the Class III HDACs, or sirtuins, however gave confusing results.

At the workshop, they reported that genetically increasing the fly orthologue of Sir2/SIRT1, called Sir2, allows the flies to live longer, but the photoreceptor neurodegeneration is exacerbated. If they genetically decrease Sir2, the photoreceptor phenotype is rescued and there is no effect on lifespan. Neuronal rescue also occurs with pharmacologic inhibition of Sir2 by sirtinol or nicotinamide. Thus, in flies there is a mismatch between neuron phenotype and general lifespan, and Sir2 inhibition, rather than activation appears to be better for neurons. Furthermore, the protection gained with inhibiting Sir2 activity was additive to the protection observed with inhibition of a Class I HDAC, RPD3 (using RPD3 and Sir2 double heterozygous knock-outs, or by pharmacologically inhibiting both RPDs and Sir2 with butyrate and nicotinamide, respectively).

However, when Marsh and Thompson treated their HD flies with resveratrol, they again observed unexpected results. Resveratrol treatment improves photoreceptor survival, paralleling the results with Sir2 knock-down in flies rather than Sir2 over-expression, which is opposite of what you would expect if resveratrol activates Sir2. These investigators were confused how treatment with a putative activator of Sir2 (resveratrol) and a putative inhibitor (nicotinamide), could both give the same protective results. The participants would come back to these issues after a more detailed discussion of nicotinamide (see *NAD metabolism*) to question whether nicotinamide given to flies will inhibit Sir2 or effectively increase NAD levels and activate Sir2. However, Guarente noted that Marsh and Thompson's data suggest that global Sir2 enhancement may not be an ideal goal, and that ultimately it might be best to regulate Sir2 up or down in only certain tissues, or even specific neurons. He also suggested that additional genes like *abu-11* might complicate the mechanisms, drawing a parallel to Neri's work in worms.

Mice

The closest mammalian homologue of yeast Sir2 is SIRT1. While the mechanisms of Sir2/SIRT1 activation are less understood in mammals, the promise that Sir2/SIRT1 activity may impact disease progression and symptoms has already prompted several groups to pursue studies in HD mice. Beal noted work done by Mark Mattson's group showing that calorie-restricted N171-82Q mice lived longer and even maintained their weight better. Sinclair shared unpublished data from his group together with Bob Ferrante, indicating that treatment of R6/2 mice with resveratrol extends lifespan by 12% with i.p. injection of resveratrol, and by 20% with 400-600 mg/kg resveratrol given in their food.

Both Sinclair's and Guarente's laboratories have created mice which over-express SIRT1, and are now breeding the SIRT1 mice with HD mice to see if genetic manipulation of SIRT1 impacts the disease process. Sinclair's mice are an inducible "floxed" SIRT1 transgenic, which can then be crossed with any CRE mouse to achieve the desired pattern of expression. They are currently crossing Nestin/Cre SIRT1 mice with R6/2 mice, but do not have any data to

report yet. The mice from Guarente's lab over-express SIRT1 in several tissues by knock-in of SIRT1 into the β -actin locus of the genome (with an IRES between β -actin and SIRT1). Guarente and Dena Cohen, a post-doctoral fellow in his lab at MIT, reported that SIRT1 expression is 3-fold higher in white fat of these mice, 2-fold higher in brown fat and brain, and normal in muscle and liver. Cha suggested that they also confirm over-expression levels of SIRT1 in the animals crossed with HD mice, since mutant huntingtin can change the expression of many genes.

Cohen reported early results that indicate that their SIRT1-over-expressing R6/2 males have a 25% longer lifespan. She has not been able to observe enough animals yet to determine if SIRT1 over-expressing R6/2 females have a more modest increase in lifespan, or none at all. Interestingly, Imai has made brain-specific SIRT1 transgenic mice, and he noted that they, too, have gender-specific behavioral differences, with females often performing better than males. Cohen has not completed the behavioral studies on SIRT1::R6/2 mice, but noted that because the SIRT1 mice themselves appear to perform better on rotorod tests than wild type mice, the analysis of the SIRT1::R6/2 mice might be complicated. Cohen and Guarente also noted that there is no rescue of weight loss in R6/2 in males or females with SIRT1 over-expression, but again, this analysis is complicated by the fact that SIRT1 transgenic mice are leaner than wildtype mice to start with. However, the data does suggest that weight loss is not what is limiting the lifespan of R6/2 mice.

All of the workshop participants were optimistic that the results from the SIRT1::R6/2 mice crosses will be informative. Cohen also eventually plans to give Coenzyme Q10 (CoQ) to the SIRT1::R6/2 mice to test for an additive effect. After a discussion of the potential Sirt2/SIRT1 signaling pathways that may be relevant in the context of HD (see below), it became clear that expanding the scope of the mice experiments was warranted, specifically to include both SIRT1 over-expressing mice and SIRT1 knock-out crosses not only with R6/2 mice but also with a full-length knock-in huntingtin mouse model.

Potential Sir2/SIRT1 signaling mechanisms in HD

In yeast, the longevity effects of Sir2 are mediated by its deacetylation of a particular lysine residue of histone H4 in the nucleus. Neri's work in *C. elegans* indicates that transcriptional regulation of oxidative stress and DNA repair elements are important. The mechanisms by which SIRT1 activation extends lifespan in mammals, however, are still not clear, as SIRT1 activity induces many different pathways. Which of these signaling pathways will be most relevant in the context of HD is an important question, as identifying the critical signaling mechanisms will also reveal new, and hopefully more selective, targets for sirtuin-based therapies.

Gene silencing and DNA repair

Johnson asked if there was evidence that SIRT1 silenced gene expression in mammals the way Sir2 does in yeast. Sinclair said that they have shown SIRT1 binds to repetitive sequences in DNA in mammalian cells. Then, when you heat shock or stress cells with H₂O₂, SIRT1 leaves the repeat sequences of chromatin and binds to other sites. Sinclair hypothesized that SIRT1 may move to regions with DNA breaks and be involved in DNA repair. Sinclair noted that the pattern of transcription in stressed cells is abnormal, and hypothesized that this might be a mechanism by which chromatin structure is disrupted and transcription is altered in disease. Cha wondered whether this mechanism is revealed by gene expression array results in

different HD models, where often there is an increase in expression of genes early and then downregulation of those same genes at later times. Cha wanted to know if the mechanisms Sinclair described happen in a gene-specific way, and Sinclair proposed investigating this with ChIP-on-ChIP assays to identify exactly where SIRT1 binds to DNA.

Thinking about DNA repair mechanisms, Beal noted that there is an increase in 8'-hydroxy-2'-deoxyguanosine (8-OH2'dG), a marker of oxidative damage to DNA, in peripheral blood of individuals with HD and in the cortex and striatum of postmortem human brain tissue. Beal noted the increase in 8-OH2'dG in plasma is 4-5 fold higher than controls, which is dramatically higher than they have observed in any other neurodegenerative disease (i.e. 8-OH2'dG is only 1.5 fold higher in Parkinson's disease, and barely detectable in Alzheimer's disease). Beal also found similar age-dependent increases in 8-OH2'dG in R6/2 mice, indicating this mechanism is reproduced in the animal model.

Robert Hughes of the Buck Institute for Age Research (California) noted that DNA damage and repair might be specifically relevant to somatic expansions in CAG repeats that can occur in mice and humans. In mice, somatic expansions have been shown to be dependent on DNA repair processes, indicating that somatic expansions at the HD locus stem from DNA damaging events. Thompson also noted that many DNA repair genes are altered in gene expression profiling of mouse and cell culture models.

PCG-1 α and mitochondria

PCG-1 α (for peroxisome proliferator activated receptor-gamma coactivator 1alpha) is a transcriptional coactivator protein that regulates gene expression by recruiting DNA-modifying enzymes to complexes containing classical transcription factors. PCG-1 α activity has been implicated in energy homeostasis, adaptive thermogenesis, β -oxidation of fatty acids and glucose metabolism. Of particular relevance to HD is the role of PCG-1 α in creating new mitochondria when they are needed, considering the evidence of mitochondrial abnormalities in the disease.

PCG-1 α is a target of SIRT1, but there is some debate whether SIRT1 is an enhancer or repressor of PCG-1 α . Sinclair noted that treating mice with the SIRT1 activator resveratrol results in decreased acetylation of PCG-1 α , which makes PCG-1 α more active. Guarente added that SIRT1 over-expressing mice look similar to resveratrol-treated mice; they have decreased acetylation of PCG-1 α and increased mitochondria function. While many of the participants favored the idea that there is a positive relationship between SIRT1 and PCG-1 α , Guarente also noted that PCG-1 α is not more acetylated in SIRT1 knock-out mice, and Toren Finkel has observed repression of PCG-1 α by SIRT1 in PC12 cells.

Additional interest in PCG-1 α in the context of HD stems from work by Bruce Spiegelman's lab showing that PCG-1 α knock-out mice have a neurologic phenotype accompanied by striatal pathology. In general, PCG-1 α activity has been shown to not only upregulate mitochondria biogenesis, but also to upregulate reactive oxygen species (ROS) defense systems, in part via production of uncoupling proteins (UCPs). UCPs in the inner mitochondrial membrane allow passage of protons, which dissipates the mitochondrial voltage gradient. While this reduces the ability of the electron transport chain to generate ATP, it also prevents the gradient from getting so steep as to increase ROS production. When UCPs are impaired in brain tissue, as in Spiegelman's PCG-1 α knockout mice, the result is striking striatal pathology.

Workshop participants debated whether the potential therapeutic benefits of increased PCG-1 α activity in HD are more likely to be due to mitochondria biogenesis or anti-oxidative

mechanisms involving UCPs. Dimitri Krainc from MGH clarified that the evidence thus far favors antioxidants effects. He noted that Spiegelman's data shows that PCG-1 α is linked to UCP2 and UCP3 in striata, which is consistent with other reports that UCP2 is highly expressed in brain, and generally neuroprotective.

In terms of SIRT1 in this process, Krainc commented that if you piece together all the published reports, the link between SIRT1 and PCG-1 α in HD is compelling, but not yet definitive. He plans to use chromatin immunoprecipitation (ChIP) to see if SIRT1 is present at the PCG-1 α promoter together with huntingtin. He also said that from his experiments, it is not clear that deacetylation of PCG-1 α by SIRT1 in brain activates PCG-1 α . Imai added that there is no clear evidence that PCG-1 α is activated by SIRT1 in β -cells, either. He noted that SIRT1 effects on PCG-1 α may be dependent on the particular cell type, and suggested further that even for a particular cell type, the context dependency may be such that events in normal neurons may happen differently in diseased neurons.

Despite these unanswered questions, Beal emphasized that PCG-1 α could be an excellent therapeutic target. Bjoern Schwer of Children's Hospital in Boston is also very interested in PCG-1 α , and asked if there was a way to directly activate it in cells. Krainc indicated that Novartis made PCG-1 α activators for use in diabetes, and that he is now testing them in a neuronal context.

Autophagy and mutant huntingtin clearance

Dimitri Krainc's lab at MGH has also been looking at the acetylation and deacetylation of the huntingtin protein itself. Of the 15 different HDACs they over-expressed *in vitro*, only a few of them deacetylated huntingtin. SIRT1 was one of them.

Krainc identified the acetylation site on a caspase-6 (N558) fragment of huntingtin by mass spectrometry. He said that three different mass spectrometry experiments all gave the same result, that the acetylation site is a lysine near amino acid 400. An acetylation-specific huntingtin antibody made for this site indicates that this particular lysine is also acetylated in the full-length huntingtin protein, although Krainc noted that there are additional C-terminal lysines in the full-length protein that may also be acetylated.

Sharing striking unpublished data, Krainc said that acetylation promotes degradation of the caspase 6 fragment of mutant huntingtin by lysosomes. The same is also true for full-length huntingtin. Furthermore, he explained that in heterozygous knock in mice, only the mutant full-length huntingtin is acetylated; normal huntingtin is not. Furthermore, when he treated mice for 10 days with the HDAC inhibitors TSA and nicotinamide (in combination) to boost acetylation levels, he observed a selective decrease in mutant full-length huntingtin levels, with no change in normal huntingtin. This combination of HDAC inhibitors is also protective in primary neurons.

Interestingly, Thompson noted that data from her lab indicates that the exon 1 huntingtin fragment is also acetylated. The Thompson lab previously showed that phosphorylation of the N-terminus of huntingtin modulates its clearance, but she revealed at the workshop that they also have evidence that acetylation may affect clearance. Krainc did not detect acetylated lysine in the N-terminus when working with the larger fragment suggesting that acetylation and deacetylation mechanisms may be distinct in short vs. long fragments. Participants discussed how this may be related to aggregation of fragments and other differences observed between short vs. long fragment models.

Krainc also noted that SIRT1 in primary neurons grown in culture is nuclear, while fractionation studies indicate that acetylated huntingtin is predominately cytoplasmic. Therefore,

he thinks acetylation of huntingtin happens in the nucleus (i.e. CBP, one of the histone acetyltransferases that he found can acetylate huntingtin, is nuclear), but then acetylated huntingtin moves from the nucleus to the cytoplasm and gets enclosed by lysosomes. He further noted that there is little aggregation of mutant huntingtin in his model: he sees accumulation of acetylated mutant huntingtin in the cytoplasm only with lysosomal inhibition or if autophagy was blocked by specific gene mutation. If the critical lysine is mutated, huntingtin is neither acetylated nor degraded.

Krainc's speculated that acetylation may selectively target mutant huntingtin for degradation, leaving normal huntingtin untouched, which many think is exactly what a successful treatment for HD needs to achieve. Although his data indicates that acetylation/deacetylation of mutant huntingtin might be an excellent target for drug therapy, another important implication of his results is that activation of SIRT1, by promoting deacetylation of mutant huntingtin, would inhibit clearance of the disease protein. Since most believe that the presence of the mutant huntingtin protein—either in soluble or aggregated form—is problematic, Krainc's data suggest that SIRT1 activation might exacerbate the pathogenesis of HD.

On the other hand, it is not clear that SIRT1 inhibition would be beneficial. Guarente noted that, in addition to all of the other mechanisms that favor SIRT1 activation, it was reported at a recent Cold Spring Harbor meeting that by deacetylating and activating beclin, a protein involved in autophagy, SIRT1 activation also stimulated autophagy. Krainc did not specifically look at beclin, but others have shown that it is important for the autophagic clearance of mutant huntingtin. Thus, inhibiting SIRT1 could block autophagy and again, clearance of mutant huntingtin. From the discussion, it became apparent that competing mechanisms may be at hand, and the answer may lie in the balance between global autophagy and specific autophagy for individual substrates.

Krainc has induced autophagy globally via rapamycin, and found that it increases clearance of huntingtin along with a lot of other proteins. He added that it is unclear how acetylation in general affects autophagic clearance, but that activation of any histone acetyltransferase (HAT) alone does not induce autophagy. He reports that autophagy of mutant huntingtin is selectively increased by increasing activity of HATs or use of HDAC inhibitors. It remains to be seen how SIRT1 specific effects on huntingtin clearance vs. SIRT1 effects on autophagy in general will balance out.

Hughes commented that Krainc's data suggest that acetylation of mutant huntingtin is marking it for destruction, much like what is typically done by ubiquitination, and wanted to know if there is precedence. Guarente compared this to the nuclear LXR receptor, which gets deacetylated by SIRT1. When deacetylated, the receptor more active. However, without the acetyl group protecting the lysine, it gets ubiquitinated and broken down by the proteasome. So acetylated receptor is less active, but stable; deacetylated receptor is more active but less stable. Thus, he agrees a balance between competing events can be critically important.

Participants agreed these mechanisms should be studied in more detail in mouse models to order to clarify whether SIRT1 activity will improve or exacerbate HD pathophysiology. They suggested a series of collaborative experiments, crossing SIRT1 mice with full-length knock-in HD mice to biochemically examine how the acetylation and clearance of mutant huntingtin is affected. Crosses with both the SIRT1 knock-outs and SIRT1 over-expressors would be informative, because whether SIRT1 activity is good or bad could depend on the balance between SIRT1's effects on autophagy in general and SIRT1's specific effects on mutant

huntingtin. Furthermore, it would be extremely informative if an increase in mutant huntingtin levels via SIRT1 manipulation accelerates the phenotype in HD mice that express the full length huntingtin protein. Lastly, Thompson's observations of huntingtin exon 1 fragment acetylation can be followed up in SIRT1 crosses with R6/2 mice, which might also shed light on how similar or distinct acetylation mechanisms are in full-length vs. fragment huntingtin models.

While participants hypothesized whether the SIRT1 crosses with knock-in HD mice will look the same or opposite to the crosses with the R6/2 mice, they also wondered if survival, weight loss, and/or neuropathology might be also be dissociated. Imai suggested using his brain-specific SIRT1 knock-outs in some of these crosses to explore this possibility.

Novel signaling pathways

Class I and II deacetylases remove an acetyl group from a substrate and release it as free acetate. Class III deacetylases like Sir2/SIRT1, transfer the acetyl group to the ADP-ribose moiety of NAD generating O-acetyl-APD-ribose (OAADPr) and nicotinamide.

John Denu, of the University of Wisconsin Medical School, suggested that OAADPr may be a signaling molecule in its own right. For example, he noted that OAADPr has been shown to bind to macro-H2A, a histone isoform with a C-terminal domain that binds nucleotides that appears to play a role in gene targeting. Denu has also found that OAADPr targets a transmembrane ion channel, TRPM2 (for transient receptor potential melastatin like-2) that is activated by oxidative stress. TRPM2 is highly expressed in β -cells, glia cells, and neurons, where it can mediate cell death. Denu has found that ADPr and OAADPr activate the TRPM2 channel. Other reports suggest a mitochondrial origin of the ADPr and OAADPr that activate TRPM2 under oxidative stress, and Denu hypothesized that mitochondrial sirtuins may play a role in this process (see *Other Sirtuins SIRT2-7*).

Cha remarked that if OAADPr is an obligate by-product of Sir2/SIRT1 activity, it might also be utilized in developing an assay to measure Sir2/SIRT1 activity. Quantifying OAADPr production might circumvent the problem of measuring Sir2/SIRT1 activity with specific substrates. Denu is currently making non-hydrolyzable OAADPr derivatives for assay development and further studies.

Pharmacologically targeting Sir2/SIRT1

Resveratrol

Resveratrol, a compound that has received considerable attention in both the popular press and scientific publications because it is thought to contribute to the cardiovascular benefits attributed to red wine, has been reported to be an activator of Sir2/SIRT1 *in vitro*. Sinclair, who has worked extensively with resveratrol, suggested that it may prevent the N-terminal regulatory domain of SIRT1 from folding back onto the catalytic domain, thereby locking the enzyme in an active conformation. Resveratrol extends the lifespans of yeast, worms and flies, and current studies are examining the ability of resveratrol to impact aging in mammalian systems. Furthermore, in animal models of disease, resveratrol has been shown to inhibit tumors in a variety of cancer models, protect the heart and the brain against ischemic injury, and recently, to inhibit pathology in models of Alzheimer's.

Resveratrol is inexpensive and available either as the purified natural product or as a synthetic version. Sinclair noted that a significant problem is that resveratrol is easily degraded in fluorescent light, so special care is needed when doing experiments to maintain the active configuration. He stores resveratrol stocks under nitrogen, in the dark, in a -80 C freezer. Sinclair

recently reported several resveratrol analogs—some of which were more potent and stable—that he expects will be generally available in a couple of years. Neri said that colleagues at INSERM are also pursuing resveratrol analogs, and have ones that are more stable and less toxic.

One of the main questions concerning resveratrol is how much of its *in vivo* action reflects the modulation of Sir2/SIRT1 activity versus effects on other targets. Sinclair noted that there are reports in the literature of alternate targets of resveratrol but also that resveratrol does not appear to activate any of the other sirtuins. Also, his laboratory has mutated Sir2 in yeast so that it no longer binds resveratrol but still has enzymatic activity, and in those yeast resveratrol no longer has any effect. Guarente added that it would be interesting to knock-in that type of mutation in mice. Both Johnson and Beal also suggested doing a microarray analysis of the SIRT1 over-expressing mice and comparing them to resveratrol-treated mice. Sinclair said he also plans to do that, and compare them to the SIRT1 knock-out mice as well.

Another concern with the use of resveratrol in neurodegenerative disorders is the need for an active compound to cross the blood brain barrier (BBB). As noted by Johnson, for proof of concept studies, it might be best to bypass the BBB and deliver the compound directly into the brain. This is exactly what Sinclair and Tsai did in a collaborative study of resveratrol in a neurodegeneration model in which induction of p25 expression leads to axonopathy, neuronal cell death, and mice that exhibit impaired memory in fear startle tests. Intracranial delivery of resveratrol resulted in dramatic memory improvements, whereas delivery via the food was ineffective.

In Sinclair's collaboration with Bob Ferrante, resveratrol was mildly effective at improving symptoms and pathology in R6/2 mice when injected ip (100 mg/kg/day) or given in the chow (400-600mg). Sinclair reported that they observed 12-20% increase in lifespan, increased striatal volume, decrease in aggregates, improvement in rotorod, and even a decrease in weight loss.

Beal reported that he is also currently giving resveratrol to N171Q82 mice in their chow. Prompted by Johnson's concern about how much resveratrol is getting into the brain, Beal said he will examine mechanistic biomarkers in the brain, such as 8-OH²dG measures and lactate increases by proton NMR, which he has previously shown is attenuated by Coenzyme Q (CoQ). Beal and Johnson are also interested in eventually exploring whether the improvements observed with resveratrol and with CoQ are additive. Cohen and Guarente suggested giving CoQ to their R6/2::SIRT1 over-expressing double transgenic mice and looking for additive effects.

Hughes suggested that resveratrol also be tested in a full-length knock-in HD model given Krainc's results showing that SIRT1 can deacetylate full-length mutant huntingtin, dampening its clearance by autophagy. Thus, by stimulating SIRT1, resveratrol treatment may increase accumulation, aggregation and cleavage of mutant full-length huntingtin, potentially exacerbating toxicity. He suggested examining whether resveratrol increased or accelerated various pathological markers in full-length knock-in HD mice, and emphasized that this knowledge is critical before moving into human trials in HD.

Participants debated in detail the prospect of resveratrol trials in humans. Sinclair said that resveratrol appears to be very safe. In addition to being clean in all the animals it has been tested in, it is currently in clinical trials outside the US for diabetes and MELAS syndrome. Sinclair remarked that he believes that it is safe enough that people at risk for HD could consider taking it. Nonetheless, Sinclair warned that his lab has tested many samples of resveratrol sold in pharmacies and health-food stores, and none of them have SIRT1 activating activity. There is not yet a source of resveratrol for human consumption.

In discussing the possibility of setting up a resveratrol trial for HD, Beal shared with Sinclair some of the insights he has gained from CoQ clinical trials. He emphasized that the safety of resveratrol will have to be proven first. Then it would be possible to carry out a small Phase II trial in 40-50 individuals with HD, with resveratrol classified as a nutritional supplement rather than as a drug. To demonstrate a biological effect, Beal suggested that if he repeats the glucose tolerance experiments and finds abnormalities in individuals with HD, they could take resveratrol for 3 weeks and be then measured again. Other metabolic endpoints might include MRI of muscle to measure its ability to regenerate phosphocreatine, or muscle biopsy to look for changes in Type I to Type II fibers. To address whether resveratrol crosses the BBB, he suggested measuring brain lactate levels. Beal noted that for CoQ, it took 6 weeks to 3 months for effects to become apparent, but results may be quicker with resveratrol, which shows effects after 2 weeks in mice. If these types of studies yielded suggestive results, he recommended extending the study to include quantitative MRI of the cortex, as Diana Rosas measured significant changes after one year.

NAD metabolism

As Class III deacetylases, the activity of sirtuins is dependent on NAD. Accordingly, Sir2/SIRT1 activity can be altered by modulating NAD synthesis and/or metabolism. Imai outlined for the participants how NAD is used by Sir2/SIRT1, and then recycled within different cells.

In the process of Sir2/SIRT1 removing the acetyl group from a protein and transferring it to the ADP-ribose of NAD, nicotinamide is generated. Nicotinamide is an inhibitor of Sir2/SIRT1, and cellular concentrations of nicotinamide may frequently be inhibitory in yeast, worms and flies, as these organisms have only one enzyme that converts nicotinamide into nicotinic acid (which is used to replenish NAD) and that enzyme is generally present at a low level.

NAD recycling in mammals uses different enzymes. In mammals, nicotinamide is broken down by Nampt (for nicotinamide phosphoribosyl transferase) into nicotinamide mononucleotide (NMN), which then gets converted to NAD. Nampt activity is high in most mammalian tissues, but as Imai noted, some tissues, including the brain and pancreatic β -cells, do not have Nampt. This prompted the participants to consider whether there are some conditions in mammalian neurons (i.e. DNA damaging conditions) in which nicotinamide might reach concentrations high enough to inhibit Sir2/SIRT1, and what the consequence of this might be.

Imai went on to explain that nicotinamide is small and membrane permeant, and is likely to leak out of cells and into bloodstream, where interestingly, an extracellular version of Nampt circulates. He hypothesized that for tissues like the brain and β -cells, which also lack Nampt, the nicotinamide produced by NAD utilization may be converted to NMN in the blood, and then the NMN or resynthesized NAD is transported back into the cells. If this is indeed the case, then rather than attempting to manipulate NAD recycling (and by extension, SIRT1 activity) inside neurons, it might be possible to gain therapeutic benefit by targeting this process in blood.

For example, Guarente suggested infusing NMN in mice and seeing if it boosts NAD levels in cells, including nerve cells in the brain. Imai said that he has obtained positive results with NMN in his β -cell specific SIRT1 over-expressing mice. With increased β -cell SIRT1 activity, these mice are initially resistant to challenge with free fatty acids, though they lose their resistance upon aging. When Imai injected aged β -cell SIRT1 over-expressing mice with NMN,

they again had improved glucose tolerance. Imai thinks that the β -cells of the aged mice may be depleted in NAD, and that this limits SIRT1 activity. Following this line of thinking, participants wondered if this could be true for neurons in the brain, and whether NMN can cross the blood brain barrier to also restore NAD in neurons, potentially protecting them against neurodegeneration.

This promising idea prompted participants to discuss niacin in neurodegenerative diseases. Imai noted that the term “niacin” can refer to either nicotinic acid or nicotinamide. In either case, if you ingest niacin as a dietary supplement, what gets absorbed and taken up into the blood is nicotinic acid, which is then converted into NMN. Guarente noted that niacin has been shown to be protective in cardiovascular disease and experimental autoimmune encephalomyelitis models of multiple sclerosis. Some participants were convinced it is worth investigating in HD models as well.

The discussion of NAD metabolism also shed new light on some of the research discussed at the workshop in which nicotinamide was used as a Sir2/SIRT1 inhibitor, since in some models nicotinamide might be rapidly recycled into NAD, and thus more likely to enhance Sir2/SIRT1 activity rather than inhibit it. Thus, Thompson wondered if you had drugs that could increase or decrease nicotinamide, which one would be better for individuals with HD? Thompson and Marsh found that niacin and nicotinamide were both protective in HD flies, but Beal did not see any benefit when he gave nicotinamide to R6/2 mice.

Participants agreed that more experiments in this area are worth doing. Imai said that he is very interested in feeding normal mice NAD metabolites and looking for changes in SIRT1 activity in different tissues, and would like to collaborate with others to examine this specifically in HD mice. Participants proposed testing if NMN protects R6/2 mice, as well as examining if R6/2::SIRT1 over-expressing double transgenic mice are protected even more if they are fed NMN. Imai noted that if NAD levels in the brain do decline with age, looking in a HD mice model with a longer lifespan may also be informative.

NAD metabolism may also impact the activity of sirtuins other than SIRT1 in these models, based on research from Sinclair’s lab (see *Other Sirtuins SIRT2-7*). Angela Hafner from Sinclair’s lab explained that Nampt is induced by DNA damage in cells treated with H₂O₂ and that Nampt translocates to the mitochondria, where NAD concentrations increase. The increase in mitochondrial NAD confers a neuroprotective effect against H₂O₂ that is not dependent on SIRT1, but rather on SIRT3 and SIRT4, which are both localized in mitochondria.

Potential complications of SIRT1 activation

Most of the evidence from the literature indicates that activation of SIRT1 has beneficial effects on lifespan, metabolism, and healthy functioning of cells. Thus, most disease therapies are directed at increasing the activity of SIRT1. However, two important pieces of evidence revealed at the workshop suggest that that SIRT1 activation may not be beneficial in the context of HD: Marsh and Thompson’s *Drosophila* data showing that Sir2 inhibition rescues the neuronal phenotype in the HD fly model (and Sir2 activation exacerbates it), and Krainc’s data indicating that SIRT1 deacetylation of mutant huntingtin inhibits its autophagic clearance. Important follow-up experiments are needed to determine if the upregulation of SIRT1 increases full-length mutant huntingtin toxicity in HD knock-in mice, or whether this is outweighed by the other benefits conferred by SIRT1 activation.

Imai and Guarente also pointed out that although caloric restriction and treatment of resveratrol have been shown to lengthen lifespan and promote healthy aging via SIRT1-

dependent mechanisms, it is still debated whether increases in SIRT1 activity due to caloric restriction or resveratrol occurs in all tissues, or whether the benefits come from increases in SIRT1 activity in only select tissues.

Johnson asked whether there are additional toxicities to consider when upregulating SIRT1 in all tissues. Both Imai and Guarente noted that global upregulation of SIRT1 could theoretically lead to diabetes if it increased PCG-1 α in the liver and increased gluconeogenesis. Neri added that FOXO1 activity can also lead to atrophy of muscle, although it is not clear if this is all due to SIRT1, or if SIRT1/FOXO1 effects in muscle can be overridden by PCG-1 α . These issues will have to be resolved before targeting SIRT1 activation in any disease, and examined specifically in the context of HD. Imai suggested that comparing results from HD mice crossed with global SIRT1 over-expressing mice vs. brain specific SIRT1 over-expressing mice will inform these concerns.

Similarly, it is also unclear how different tissues will respond to NMN or other potential therapies aimed at regulating NAD. NMN may be more selective for tissues like the brain with limited NAD biosynthesis, making it a good therapeutic approach. However, NMN may be problematic if it also boosts SIRT1 activity in tissues like the liver and alters insulin levels, for example.

Lastly, Johnson proposed that if some complications are associated with chronic upregulation of SIRT1, this might be overcome with intermittent therapy, such as episodic treatment of NMN or resveratrol.

The other sirtuins (SIRT2-7)

In mammals there are seven sirtuins, SIRT1-7. While SIRT1 is the sirtuin most extensively studied and the only one activated by resveratrol, some of the other sirtuins may also prove to be useful targets in HD, particularly those which appear to have critical roles in mitochondria function, oxidative stress and DNA repair.

Guarente summarized what is known about the different sirtuins at this time. He noted that SIRT2 is very abundant in the brain, though probably more abundant in glial rather than neuronal cells. Young found this interesting, as there is significant gliosis in HD. She cited a study by Jean-Paul Vonsattel that reported increased striatal oligodendrocytes in people at risk for HD prior to neuronal loss. SIRT3 is also highly expressed in brain, as well as other metabolically active tissues, like muscle and the heart. SIRT4 and SIRT5 are expressed ubiquitously, SIRT6 is highly expressed in cancer cells, and SIRT7 is only expressed in dividing cells.

In terms of subcellular compartmentalization, SIRT2 is most often found in the cytoplasm, whereas SIRT3, SIRT4, and SIRT5 are localized in mitochondria. SIRT6 and SIRT7 are primarily nuclear. In terms of enzymatic activity, SIRT2, SIRT3 and SIRT7 are only known to have deacetylase activity, whereas SIRT4 and SIRT6 may only have ADPr transferase activity. The nature of the activity of SIRT5 is not yet determined.

Linda Kaltenbach of Duke University has studied effects of all of the sirtuins in an acute rat brain slice model biolistically transfected with constructs encoding an exon 1 huntingtin fragment with 73 glutamines. Using fluorescent reporters, the morphology of transfected neurons is followed for five days, during which time there is an increase in inclusions, neurite retraction and eventually cell death. Kaltenbach observed no effect with RNAi inhibition of SIRT1, over-expression of cDNA encoding SIRT1, or treatment with resveratrol. Interestingly, she found that

over-expression of cDNA encoding SIRT2 is protective, and early results suggest that RNAi suppression of SIRT3 may also be protective.

Sinclair's lab is also looking more broadly at the ability of sirtuins to protect neurons from a number of different insults by over-expressing them in neuronal cell lines. Hafner reported that over-expression of SIRT1 or SIRT3 was protective against A β toxicity, but SIRT2, SIRT4 or SIRT5 over-expression was not. She also noted that SIRT3 over-expression protects differentiated neuroblastoma cell lines and cerebellar granule neurons against oxidative stress by H₂O₂, whereas SIRT3 siRNA sensitizes the cells. The protection observed is dependent on SIRT3 deacetylase activity.

Guarente and Schwer were particularly interested in this SIRT3 data, and wondered if the protection involved stimulation of the TCA cycle via acetylation of acetylCoA synthase by SIRT3. They noted that SIRT3 deficient mice (generated in Fred Alt's lab at Harvard Medical School) seem to be normal, and that, surprisingly, so do SIRT2::SIRT3::SIRT4::SIRT5 quadruple knock-out mice. Several investigators hypothesized that these mice might appear normal at baseline but might show a phenotype in response to stressors. The activation of compensation mechanisms was also discussed.

Lastly, David Houseman of MIT noted that the portion of chromosome 11 where SIRT3 is encoded is often duplicated in persons with Beckwith Wiedemann Syndrome (BWS). He wondered if individuals with BWS have higher SIRT3 activity, and if they can inform us in new ways as to the role of SIRT3 activity in humans.

Concluding thoughts

In summary, the workshop participants agreed that SIRT1 appears to be a promising therapeutic target in HD. They were encouraged by results indicating that resveratrol treatment and genetic over-expression of SIRT1 may be beneficial in R6/2 mice, and optimistic that by investigating how SIRT1's deacetylation of huntingtin affects its clearance in HD mice, additional valuable insight will be provided. Collaborations were established to pursue a number of studies in both fragment and full-length huntingtin models: caloric restriction and special diets, genetic crosses with both global and regional-specific SIRT1 knock-out and over-expressing mice, and treatments with resveratrol, NMN, niacin, and CoQ in various combinations.

Interest in SIRT1 as a therapeutic target in other diseases is clearly helpful, as clinical trials are already underway to assess the safety of resveratrol, and a number of companies are in the process of making drugs with improved bioavailability and stability. If studies in animal models of HD support the hypothesis that SIRT1 activation is worth pursuing in people with HD, the means to do so may soon follow. Furthermore, workshop participants stressed that researching the mechanisms by which SIRT1 improves metabolism and lifespan could lead to the discovery of novel pathways containing new HD targets and, though we know much less about the other sirtuins, they too may provide therapeutic opportunities. Despite the challenges ahead, scientists left the workshop inspired to continue pursuing SIRT1 as a therapeutic target and optimistic that additional work will lead to treatments that make a difference for people with HD.