

Hereditary Disease Foundation / Jennifer Jones Simon Foundation

Huntington Structure and Function: An Enigma

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Overview

Solution structure of polyglutamine: Does it exist?

According to circular dichroism measurements reported by Ron Wetzel, neither short nor long polyQ peptides assume a stable structure in solution (where “short” and “long” here and elsewhere implies fewer or more Qs, respectively, than the roughly 40 necessary to make huntingtin pathogenic in humans). But antibodies from Paul Patterson’s laboratory bind long polyQ preferentially over short polyQ, according to peptide ELISA and Western blots of pathological and benign forms of huntingtin (Htt).

Participants questioned the biological relevance of the ELISA and Western observations when Patterson reported that the antibodies stain wild-type and Huntington’s disease mouse tissue sections at similar intensities. But dissimilarities in the subcellular pattern of staining suggest that the accessibility or conformation of the polyQ tract within Htt depends on context. Patterson proposed that structural differences between short and long polyQ exist *in vivo*, but cellular factors obscure it.

To account for antibody preference without presupposing that polyQ naturally assumes a stable structure, participants hypothesized that Patterson’s antibodies induce the structure to which they bind. It was proposed that Shiva Malek of Aurora Biosciences should test this hypothesis with a peptide assay that she is developing, with which she hopes to detect conformational changes in polyQ by fluorescence resonance energy transfer.

Participants suggested that the structure that antibodies hypothetically induce might be either (a) a fold that polyQ assumes within the context of the Htt protein, or (b) a conformation that Htt adopts in transition from soluble to aggregated form.

In light of hypothesis (a), participants suggested that polyQ might fold without antibodies in peptides that incorporated the immediate flanking sequences from Htt. They proposed engineering such peptides.

Interpretation (b) arose from consideration of Htt aggregation in thermodynamic terms, as described below.

Kinetic interpretations of aggregation and pathology

Ron Wetzel described peptide experiments showing that longer polyQs aggregate more rapidly than short ones--an observation that echoes the correlation between early disease onset and longer polyQ expansions in HD and other diseases.

An intermediate structure that does not form readily often limits, and in effect determines, the rate with which thermodynamic processes proceed. Because longer polyQ sequences appear to aggregate more rapidly, it was hypothesized that longer polyQ sequences more readily assume a rate-limiting transition structure.

Parsimony then led to the proposal that the accessible but unstable structure is the same that antibodies had been hypothesized to induce. According to both the transition-state and the induced-fit hypotheses, the structure to which

antibodies preferentially bind is induced more readily in longer polyQ sequences.

Because concentration strongly influences the rates of reactions, thermodynamic thinking led participants also to note the potentially critical importance of the concentration of soluble Htt in cells. Observing the analogy of aggregation to the cooperative processes of crystal nucleation and micelle formation, which depend exponentially on concentration, heightened this perspective. Participants were excited by the implication that a small diminution in the expression of Htt might delay and even prevent HD onset. They also considered the possibility that HD might specifically affect medium spiny neurons simply because they express more Htt than other cells. (No one was sufficiently familiar with expression studies to say, however, whether these cells indeed express Htt more highly than all others).

It was suggested that research should be aimed at understanding the cellular signals that control Htt expression through degradation and transcription, for example. It was suggested that such mechanisms might offer ways to pharmacologically lower the concentration of soluble Htt in cells.

Htt Structure

Participants said data were now too scant to model the structure of Htt or exon-1 with confidence, but proposed approaches to help constrain ideas. These included electron spin-labeling, antibody binding, proteolysis and mass spectrometry. Most of these approaches presuppose purified Htt protein, which no one has yet obtained. A concern that the peptides and fragments used in current studies may not be folding as they do in the intact protein added to participants'

desire to see purification succeed soon. It was proposed that crystallography of short polyQ peptides--free or bound to antibody--could enlighten speculation about the structures that would be accessible to longer polyQ sequences in solution and in aggregates.

Other ideas

Participants presented ideas and research aimed at screening for compounds that could prevent or treat HD, in particular through disruption of polyQ structure. Evidence that Htt interacts with chaperones in yeast, which Sylvia Krobitch presented, excited interest. Phylogenetic bioinformatics was suggested to discover which proteins are huntingtin's natural partners; tests of the toxicity of soluble Htt and aggregates were also proposed. Participants remarked that large-scale screening of compounds using multiple criteria remains the surest route to a therapy for HD.

Workshop Details

Introduction

Huntingtin structure tantalizes researchers because it could explain so much. It seems possible, for example, that the death or dysfunction of a single class of brain cells underlies all of the devastating symptoms of Huntington's disease. Furthermore, in people who die from Huntington's, huntingtin impacts these cells in a striking way, by aggregating into spherical deposits so big they dwarf the organelles.

Inheriting a single copy of certain *huntingtin* alleles ensures that a person will develop Huntington's. The pathological alleles that researchers have sequenced all differ in the same way from benign ones: At a specific location in the predicted protein sequence--lying within exon-1 of

the *huntingtin* gene--these alleles encode a succession of at least 36 to 39 glutamine residues, where benign alleles usually encode less than 29.

This observation makes the primary sequence of huntingtin--and its as-yet undeciphered structural consequences--look key to the cellular pathology of Huntington's disease (HD). The etiology of other diseases sharpens this perspective: Seven neurodegenerative disorders besides HD arise from expanded glutamine (Q) repeats in other proteins. All of the diseases depend on polyQ length and show roughly the same threshold of about 40 residues.

In HD itself, for which the most information exists, the data tie the polyQ tract to disease at every length; because longer polyQ tracts correlate with an onset of symptoms earlier in life. In fact, the data suggest that the 40Q threshold is an accident of human mortality, and that people with 28-residue tracts would develop HD if only they were to live, to say 150.

This thinking has brought many researchers to focus on huntingtin, polyQ, and the relationship between polyQ length and aggregation. Using partial Htt sequences in cultured cells and *in vitro*, researchers have been seeking to flesh out the relationship. Many expect that these efforts will identify a process to target with drugs and methods with which to assay candidate compounds for efficacy against HD.

This perspective framed most of the discussion at the December 10-11, 2000 workshop in Los Angeles.

Disclaimers to the Paradigm

Among the first ideas that workshop participants expressed were caveats

regarding prevailing notions about HD pathology. Allan Tobin, Carl Leventhal, and others exchanged thoughts on the symptoms that people with HD show, attempting to tie them to the brain regions and cell types in which researchers see inclusions. But the evidence that they mustered was unsatisfying. Leventhal cited the effects of strokes and tumors in the striatum and in the basal ganglia, where the so-called spiny cells that are prone to inclusions reside. He said the effects indeed include upset of motor control, which is the chief capacity that HD hijacks and erodes. But he remarked that these lesions impact many cell types and the effects are diverse. They illustrate that motor feedback in the brain is distributed and complex, he said.

Tobin reminded participants that researchers know little about how pathological huntingtin impacts cells besides the spiny neurons, or even what functions the normal protein serves. Nevertheless, cells throughout the body express huntingtin (Htt), he said, and hence express the pathological huntingtin (Htt^{ex}, where "ex" stands for expanded polyQ). Given ignorance about Htt function and the largely circumstantial links between spiny cells and symptoms, Tobin said no one could say for sure that HD was just about spiny cells.

Ethan Signer voiced an additional caution regarding the role of inclusions. He cited experiments suggesting that inclusions were tangential to HD pathology--and that in fact they lessen it. In rat cells expressing a peptide corresponding to a particular Htt^{ex} exon-1, Signer said, researchers found that dissociating inclusions accelerated cell death rather than retarding it, as might have been expected.

But participants did not take this finding as a serious threat to the hypothesis that inclusions are pathological. Liberating the contents of inclusions after they form, many said, is not like preventing them. Such a release might swamp cells in proteins and fragments that they would never face at such high concentrations, if indeed at all.

Allan Tobin said later that the pathogenicity of inclusions nevertheless should be considered up in the air. As evidence challenging the prevailing view that they are pathogenic, he cited the effect of caspase inhibitors, which extend the lives of HD model mice, he said, yet apparently do not affect inclusions.

Ron Wetzel reminded participants that diseased medium spiny neurons contain not just inclusions, but also far smaller clumps of Htt. He suggested that aggregates as small as “two proteins stuck together” might be central pathological agents in HD. Wetzel said his lab had studied the aggregation of synthetic peptides and had found that small aggregates “recruit” protein more aggressively than do large ones. The aggregates might damage cells by roping in needed proteins, he said. In fact, Signer added that, inclusions contain about 30 to 40 proteins besides Htt. Sylvia Krobisch said she suspected small aggregates were toxic to the yeast in her studies (see below).

Valerie Daggett asked whether labs had injected exon-1 into cells to assess whether the peptides are toxic and in order to compare the soluble and aggregated forms. Signer said that he would like to see such tests done and doubted that they had been.

Wetzel went on to mention aggregates

in the nuclei of cells in mice and in culture, which contain fragments of Htt and several other proteins. He pointed out that at least one of these proteins is a transcription factor -- CREB-binding protein (CBP). Wetzel's statement *opened a can of worms*, because, by interfering with gene regulation, mobile Htt fragments might upset practically any metabolic program in practically any cell type. Signer said the likelihood of this prospect was more than remote because expression of nuclear-targeted exon-1 has the same effect on the P53 promoter as does P53 itself in cultured cells.

While the recruitment of transcription factors opened wide the question of what causes HD, it did not broaden the question infinitely. The transcription factors that Htt fragments recruit probably themselves contain polyQ tracts, several workshop participants said. Wetzel cited the innate capacity to aggregate that his lab sees in polyQ peptides. He also pointed out that CBP contains a polyQ tract.

Carl Johnson said that researchers should compile a comprehensive list of polyQ proteins from sequence data, which would be a short-list of proteins that Htt is liable to rope in through polyQ-polyQ binding. Signer said that researchers had compiled such a list before, and indeed most of the proteins were transcription factors. But he said that it was about time to update the list.

Wetzel said the seeming proliferation of routes by which Htt might perturb cells actually focuses rather than blurs HD pathology. The implication of polyQ tracts in most if not all of these proposed routes, he said, suggests that a common molecular phenomenon underlies many causes of dysfunction. So the burgeoning

complexity of HD pathology in fact provides even more reason to study the self-aggregation of polyQ and ways to disrupt it, he said.

The structure of aggregates and inclusions

Ralf Langen then precipitated discussion of inclusion structure. He asked whether inclusions bound molecules of Congo red or thioflavin--two dyes that bind the plaques associated with Alzheimer's disease, and which some researchers regard as diagnostic for beta-sheet structure, or beta-pleating.

Leventhal answered that researchers see no indications of beta-pleating within Htt inclusions [although one group actually has reported indications; c.f. *Neuroscience* 2000; 100(4):677-80]. But his answer prompted Wetzel to explain why he saw beta structure as possible or even likely.

Wetzel said first that beta-sheet is the structure that most proteins assume upon denaturation by heat--including the protein in Jello. So beta-sheet is a "default" state of proteins, he said. Next he argued that Congo red and thioflavin may not be faithful indicators of beta-structure. He said that his lab was using thioflavin to stain fibrils that formed from synthetic peptides, which were principally just a succession of Q residues. Wetzel said that changing a few atoms in the peptides did not alter their ability to form fibrils. But the change makes thioflavin appear not to stain them, he said, because the change upset its ability to fluoresce. So a failure to see staining does not exclude the possibility of beta structure within inclusions, Wetzel added, "We don't know how thioflavin works."

Nevertheless, a positive indication

from thioflavin is strongly suggestive, Wetzel said. So the fact that the dye does stain synthetic aggregates, he said, suggests beta-sheet. Measurements his lab has made using circular dichroism (CD) imply the same, he said.

Workshop participants asked Wetzel about the detailed structure of his fibrils. Wetzel replied that there was little he knew on the molecular scale. In fact, he said no disease-related aggregate had yielded to high-resolution studies.

Encouraged to speculate, Wetzel said a basis did exist to do so: Structural information about fibrils of the amyloid-forming protein transthyretin. But Daggett replied that this foundation was actually quite poor. She said the transthyretin structure that researchers discuss is itself just a model and X-ray data for the protein are ambiguous.

Don Crothers suggested that nascent methods for applying solid-state NMR to proteins should be able to help--though they have not yet yielded much information about prions or amyloid.

Langen said his lab, in fact, was studying amyloid fibrils with an NMR-related technique called electron paramagnetic resonance (EPR). He said that researchers in his lab monitor a radio signal from a so-called spin-label, which they attach chemically to a single site within the amyloid protein monomer. (The attachment follows mutagenesis to change the amino acid at that position to cysteine, he said, which has a unique chemistry and does not appear elsewhere in the protein). The label, or probe, is a small molecule with a stable, unpaired electron. Because the other electrons in the protein are paired (as in all proteins, under most conditions) the signal the lab

sees reflects conditions just at that one position.

Langen said the probe yields information because spin signals look different when probes within a sample are less than 20 angstroms apart and because within this limit the signal varies with distance. From the one labeling site his lab has tested so far, Langen said he sees a pure signal, implying that a single inter-probe distance characterizes the entire sample. This result is very telling, he said. It implies that monomers assemble into fibrils regularly, such that every probe stands in the same orientation and at the same separation from its immediate neighbors, which are less than 20 angstroms away. This means that amyloid monomers cannot be globular within the fiber, Langen said. He concludes that their protein chains are unfurled and either stack or nest neatly.

Daggett remarked that the studies intrigued her very much. Similar measurements on aggregates of polyQ--or if possible exon-1--would be a big aid in understanding Htt aggregation.

Pathological and non-pathological solution structures: Do they even exist?

Daggett's suggestion led Wetzel to comment that spin-label attached at certain peptide positions might fluster aggregation--an idea that steered conversation toward the structure of polyQ peptides in solution.

But with a seemingly off-hand remark, Wetzel turned up a road-block that few participants seemed to have expected. Wetzel said that, according to CD measurements in his lab, polyQ lengths from 15 to 45 residues are all random coils--implying that pathological length or

not, polyQ has no stable structure in solution.

The revelation perplexed people. Most supposed that the soluble protein or peptides aggregate because their structure suits them to the geometrical and chemical constraints they encounter in the aggregates. Because the aggregation propensities of Htt, exon-1 and synthetic peptides all vary with polyQ length, people supposed that these lengths differed in structure. In particular, they supposed a difference arose across the dividing line of 40 residues relating to HD pathology. Yet Wetzel was saying that there was no structure to differ.

The stakes were more than academic because a stable solution structure was the premise of several assays for compounds to treat HD--including screens that workshop participants were developing. As Tobin remarked later, "We're in contract with Aurora on the premise that drugs can alter structure--if there's no structure, it's disturbing!"

The perplexity in fact was double, because Wetzel's results did not just defy expectations, they seemed to contradict other observations: this ability of antibodies to discriminate between pathological and non-pathological polyQ lengths both as peptides and within the complete Htt sequence. Participants hashed out which among the handful of monoclonal antibodies in use had this ability, and they concluded that all or most were from the lab of Paul Patterson.

Assays and other projects

The workshop did not immediately return to the conundrum of solution structures. Instead, Tobin asked Krobtsch to describe the assay she had been developing. The section below groups that

discussion with conversations surrounding other participants' work or ideas, although they did not all occur in succession or in exactly the order presented.

Silvia Krobitsch said she had transfected yeast with exon-1 DNA in tandem with DNA encoding the jellyfish Green Fluorescent Protein (GFP). She used variants encoding 25, 47, 72 or 103 residues of polyQ. The resulting exon-1/GFP fusions made the yeast that expressed it glow green, she said, and gave rise to green spots when and where proteins clumped. Krobitsch said the 25Q and 47Q proteins glowed diffusely, while the 72 and 103Q variants (corresponding to more pathological huntingtins) showed spots. The lengthier the polyQ, Krobitsch said, the spottier or clumpier the glow.

While these results only recapitulated what others have seen in mammalian cells, Krobitsch said, there could be a real advance for drug screening and hypothesis testing. Working with yeast is much easier than working with mammalian systems, she pointed out.

Krobitsch then described some testing she has already conducted using yeast. Her question was whether aggregation was aided and abetted or else undermined by chaperones, which catalyze the folding or unfolding of other proteins by buttressing them, as researchers believe. She took identical yeast strains that differ only in how strongly they expressed various chaperones, and she transfected them with the same exon-1/GFP DNAs. Krobitsch said that most of the chaperone differences had no impact on yeast fluorescence. But over-expression of either Hsp70 or Hsp104 diminished the spottiness of the 72Q and 103Q yeast. Furthermore, Hsp104 deficiency eliminated spots entirely. Yeast over-

expressing Sis1, meanwhile, showed two sizes of spots instead of one.

Krobitsch said that neither clumping nor the obstruction of clumping was toxic to yeast under her standard conditions. But she said she lately had discovered conditions under which pathological polyQ lengths, but not short ones, retarded growth. She said she does not know yet if the conditions impact clumping. But she said the findings lead her to believe that she shortly will have systems to screen for two different effects: Reduction of aggregation and diminution of toxicity.

Other workshop participants were interested in whether the dramatic impact of Hsp104 deficiency suggested a therapeutic strategy for use in people. But Krobitsch said this chaperone has no counterpart in humans, though people express members of the same chaperone family. She added that she suspects Htt has no homolog in yeast.

Participants suggested that Krobitsch should investigate where in yeast cells chaperones contact her fusion proteins--if they do at all. Other investigators might research the same question with regard to Htt in mammalian cells. The answers might reveal an unknown stage of processing or trafficking that Htt goes through--or show which of the familiar stages is an opportunity to encode a disinclination to aggregate. Silvia Cavagnero asked whether the particular chaperones that Krobitsch tested were believed to act during translation or afterwards. Krobitsch replied that evidence suggests both Hsp70 and Hsp104 act afterwards, and that Hsp104 directly disentangles protein aggregates.

Shiva Malek described three assays,

which she said she had brought to various stages of readiness. For the first, she had synthesized various lengths of polyQ and (in addition to a few other residues) attached complementary fluorophores at either end. She intended these for an *in vitro* detection scheme using FRET--or fluorescence resonance energy transfer. The assay pairs a “donor” and “acceptor” fluorophore of different colors. Illuminating a sample with wavelengths that only donors absorb causes acceptors to fluoresce, provided that the donors are close enough to acceptors to transfer energy quantum mechanically. Exactly how brightly acceptors fluoresce depends strongly on the distance between donors and acceptors, which makes the FRET peptides sensitive detectors for the disruption of polyQ structure, Malek said. The assay’s premise is that compounds that cause such a disruption are potential therapeutics. Malek will first test a common denaturant such as urea or heat to see whether there is indeed a solution structure to disrupt.

Crothers pointed out that a formula from polymer physics dictates how acceptor brightness will decline with increasing lengths of polyQ, if the peptides are structure-less, random chains. Examining whether peptides conform to this rule is another way to test for structure, Crothers said.

For a second FRET approach, Malek said she was engineering fluorescent molecules in cells, from which she will purify them for use *in vitro*. The cells will express DNA constructs that fuse exon-1 to “YFP,” a yellow form of GFP that is to be the donor in this assay. Malek said she has made a set of constructs, which each encode 25, 47, 72 or 103 residues of polyQ. A rhodamine moiety attached to an antibody will serve as the acceptor.

Malek said she is considering antibodies for polyproline as well as polyQ, since successive prolines in exon-1 flank the polyQ at both ends. With a decrease in rhodamine fluorescence, the assay would detect compounds that dislodged the antibodies, implying a structural change in polyQ.

The third approach pairs YFP with CFP (“C” for cyan) as bookends for exon-1. The color of CFP suits it as a donor for YFP, Malek said; but whether any exon-1 variants will draw the proteins close enough to transfer energy, she did not yet know. If they do, compounds that alter exon-1 structure within a sample should change the brightness of fluorescence originating from YFP, she said.

Finally, Malek described a fourth screen that would assay expression rather than structure. She said she plans to engineer a line of PC12 cells that will express polyQ-GFP fusion proteins, which should light up the cells. The screen’s premise is that a drug that diminishes expression of a polyQ protein is a candidate therapy for HD. Cells grown in the presence of a such a compound should glow less brightly than controls. Malek said she is using a particularly bright variant of GFP.

Johnson said such a screen required good controls, and he questioned whether any selective type of regulation existed, which a screen like this could turn up. Malek suggested RNA binding proteins, which Johnson acknowledged as a possibility, though one he considered unlikely.

Ron Wetzel described his lab’s studies of aggregation with synthetic polyQ peptides and two assays his group has designed around them.

Wetzel said that members of his lab monitor aggregation over time in one of three ways: Fluorescence of thioflavin (which binds to aggregates), light scattering (which increases as insoluble aggregates cloud the solution) and CD (which works, Wetzel said, because the signal from the aggregates has a characteristic beta-sheet component that grows as they accumulate). Wetzel said the three produce superimposable graphs. In each, the preponderance of aggregated peptide rises slowly to a peak rate and then levels off. The longer the polyQ, the shorter the lag before the rate peaks.

For assaying, Wetzel described what he called a microtiter sandwich ELISA, a system that uses 96-well plates and so facilitates the screening of many compounds or conditions in parallel. Lab members prepare wells by depositing about 50 nanograms of pre-aggregated peptide in a thin layer that sticks to the surfaces of the wells, Wetzel said. They then add a solution of modified polyQ molecules, which have a biotin moiety attached. The molecules stick to the aggregate layer, superimposing a biotin layer. The completion of the sandwich comes with the addition of the biotin-binding protein, streptavidin, which has been chemically coupled to a fluorescent ion, europium. Only the streptavidin that sticks remains in the well following a final rinse. More biotin means more streptavidin remains behind, which means brighter fluorescence from the well.

One way to use the sandwich system, Wetzel said, would be to incubate a test compound before adding streptavidin. To the extent that the compound interferes with aggregation, fewer biotins will adhere or remain stuck to the well afterwards, and the well will fluoresce less brightly

than controls. He said that even without robots a small lab might screen 10,000 drugs this way in 1-2 months in principle.

Participants expressed concern about the concentrations that Wetzel said his lab was using to make peptides aggregate. Proteins that associate in cells do so *in vitro* at lower concentrations, they argued. If Wetzel needs high concentrations to see aggregation, participants wondered, could the phenomenon he is watching relate to what occurs in cells? Wetzel replied that Htt^{ex} aggregates *in vivo* over decades, whereas his lab designs experiments to last a day or an hour. Watching biology in fast forward demands higher-than-biological concentrations, Wetzel argued.

Nevertheless, the high concentrations do handicap drug screening somewhat, he said. They limit the sensitivity with which the screen can discriminate the affinities of different aggregation inhibitors, even when the affinities aren't so high.

Cavagnero asked whether peptides above and below 40 residues in length showed different trends in aggregation.

By way of reply, Wetzel drew a graph showing the rise of Thioflavin-T fluorescence (as a proxy for aggregation) in solutions of 20 micromolar peptide over two days. He labeled five curves Q15, Q20, Q25, Q35 and Q45, corresponding to the number of Q residues in different peptides. The Q15 curve did not rise above the baseline, whereas the curves representing longer peptides did--and plateaued higher the longer the peptide they corresponded to. Wetzel said the separation between the different curves' plateaus showed an interesting trend. He pointed out that Q20 did not rise much higher than Q15, and that Q50 did not rise much higher than

Q45, but he showed that the spacing between Q20, Q25, Q30 and Q35 was much larger. This implied that length differences affect the propensity to aggregate most strongly in polyQ peptides between 20 and 40 residues long--or thereabouts. But Wetzel said he didn't know how to relate this trend to cells.

Cavagnero then asked Wetzel if he had plotted the rate at which different peptides aggregate as a function of length, and whether this gives a straight line. If it does, it would mirror the linear correlation between HD onset and the polyQ length encoded by pathological alleles. Wetzel replied that he hadn't made such a graph. But he added that because the aggregation curves were not mathematically simple, such an analysis would require some assumptions, or a theory, which he said he didn't have yet.

Nancy Wexler observed that compounds that disrupt the polyQ structure of Htt^{ex} would cause an unknown overall change in the protein and, furthermore, would impact its unknown function in unforeseeable ways. This meant that investigators were designing screens for compounds that, by design, might be unusable. Expressing a *polyQ-less* Htt in cells or mice might be one way to exclude this possibility in advance, she suggested.

Ethan Signer summarized 14 other assays that were going on in various labs and companies under and outside HDF auspices. He described some standard libraries of compounds that researchers buy and screen, and named which ones the different assays are sifting through or plan to. Many of the assays are screening the same libraries, according to Signer.

Adding Wetzel and Malek's screens to make a total of 16, Signer pointed out that

if each screen were to advance 100 prospective therapies, scientists would have 1600 compounds to put through trials. "I don't think we can put 1600 in mice," he said. "I think we need a way to get that number down."

Spier recommended that the HDF begin immediately to raise a horde of HD mice to test the coming compounds. He said cheap expertise and infrastructure in eastern Europe might make this possible.

On the other hand, a suggested route to whittle the number of candidates down was to "cross-assay" each one; i.e. to put each screen's picks through one other screen or more, and then to exclude those that don't show promise according to each successive screen.

Signer himself suggested which screen to use to make the final cut. This was an assay of explanted striatal neurons, on which he said one company was basing its drug search. The obvious advantage of such a screen is that it tests compounds on the intended target cells, Signer said. But he said it has a big disadvantage: Because explanted cells never last long, the cells are beating a path to the grave as soon as they hit the culture dish, so the biggest effect a compound can have is to slow them down a bit. This makes the assay less sensitive than most others, he said. Tobin cited another potential disadvantage: One couldn't be sure one was really looking at the cells most relevant to rescuing medium spiny neurons, which might not be the neurons themselves but synaptic partners.

Other participants suggested winnowing candidates by taking into account how they spread through the body, and to rule out those unlikely to reach the spiny neurons. This

information was liable to be available for many of the compounds in the libraries that Signer had mentioned, they said. Wetzel suggested ruling out candidates that do not cross the blood-brain barrier.

Silvia Cavagnero said she is working to develop an assay that will monitor proteins as they come off ribosomes during translation *in vitro*. She is currently studying a protein that has nothing to do with Htt, but which she chose because it is small, crystallizable and because researchers have determined its structure at high resolution: Cold shock protein A, or CSPA. Cavagnero is testing whether hydrogen-deuterium exchange combined with mass spectrometry (MS) will enable her to monitor CSPA translation and folding. Once she has a monitoring system, she wants to fuse polyQ strands to CSPA and to assess the effect.

Todd Yeates discusses transthyretin

Todd Yeates described current ideas about the structure of transthyretin monomers and fibers and about fiber assembly *in vitro*. He said transthyretin forms soluble tetramers, which coalesce into fibers at low pH, and which perhaps only partially disassemble to do so. Evidence for incomplete disassembly comes in part from spin-label studies of the sort that Langen described, which Yeates said his lab had conducted through a collaboration with Wayne Hubbell at UCLA. Because probes on different monomers appear to be separated by the same distance in tetramers and fibers, an aspect of tetramer organization appears to persist in the fiber. Yeates said the persistent feature was probably a dimer- or half a tetramer. These may be the basic building blocks of fibers, he said.

In response to a question from Tobin,

Daggett described experiments from a paper by Jeff Kelley of Scripps, which described a way to inhibit the assembly of transthyretin fibers. She said the experiments mixed tetramers with the thyroid hormone thyroxin, to which the tetramers bind *in vivo* as substrate. *In vitro* they blocked fibers from forming, presumably by lending stability to the tetramers. Daggett said this was a promising starting point for rational drug design.

“Is there anything rational we can do with huntingtin?” Tobin asked. “If we knew more!” Daggett replied.

Melanie Bennet and others discuss purification and folding

Bennet said she is working toward X-ray diffraction studies to determine the structure of Htt in pieces or in toto. In parallel, she plans to analyze Htt structure by proteolysis and MS of the whole protein. She said she is still considering what peptides to attempt to crystallize or to co-crystallize with other proteins (e.g. antibodies), and said she has no crystals yet. Right now she is occupied with the task of purifying protein in amounts sufficient to work with, she said.

Several strategies she has tried have failed. She said that insect cell lines yielded only between 50 and 100 micrograms of full-length Htt, or not much. Peptides corresponding to the first 400 residues of Htt expressed well enough, but frustrated purification by clumping. The first 500 residues behaved similarly in bacteria, which did, however, express a purifiable exon-1 fusion protein.

Signer remarked that bacteria were liable to corrupt the Htt sequence and produce a heterogeneous mix of proteins, which he said he thought would be bad

for crystallography. In bacteria, the CAG codons for Q tend to mutate or “drift,” he said. Bennet replied that this sounded like an excellent point.

Mark Maffit said that he too had tried and failed to purify full-length Htt using the same insect cells. He said he had expressed Htt fused to GST (an enzyme that purifies well) and to a secretion signal sequence. The cells only actually secreted a small amount of protein, he said, but a lot more protein collected in inclusions and caused blebs or bulges in the cell. In other words, most of it was an inaccessible form. He worked with the meager amounts in the extracellular solution, but at each successive step of the purification he lost most of what he put in, he said. This quickly frittered away all he had and led him to table the project.

Signer suggested that the insect cell line might be causing Maffitt and Bennet’s problems. Because it’s non-mammalian, the clumping and misdirection of Htt and Htt fragments might reflect incomplete or inappropriate processing of the protein--which so far seems to have no close homologs in insects, participants pointed out. The appropriate processing might be anything, he said, because nobody knows what kinds of modifications Htt undergoes.

Bennet replied that she doubted Htt needs glycosylation--despite sites in the sequence that call for it--because the protein is not normally secreted. But Signer said there was no telling.

Johnson asked, given the problems that recombinant Htt and cell lines were posing, if anyone had thought to purify Htt from animals. “You’ve got an antibody,” he said. By this he meant that the avenue of affinity purification was

available. When it was suggested that animals probably expressed an impracticably small amount of Htt, Johnson said choline transferase refutes that idea. He said chicken brains contain just a few micrograms of it, yet biochemists had extracted it. They marched through a lot of chickens and then they did crystallography, he said.

Connotations of “the good old days” hung heavily in the air, and Crothers replied that the skills that once purified choline transferase may since have disappeared. Remarking that the food industry had figured critically in traditional biochemistry, Maffitt said the proximity of Oscar Meyer might yet come in handy to his lab, should he take up Htt again.

Participants proposed a few other ways around the purification problem. Signer suggested *in vitro* translation, which is routine for some proteins. Another person suggested expressing Htt in transgenic cow milk.

Workshop participants considered whether exon-1 fragments should be expected to fold. People pointed out that the peptide represents a sector of the gene, but not necessarily a natural domain of the protein. So the minimal folding unit that included the polyQ tract might be either larger or smaller than exon-1. Bennet said that identifying domains was the goal of the MS and proteolysis she wished to do. But she said she still lacked the protein to do it.

When the subject of Wetzel’s peptides reemerged in this context, participants hypothesized that in the context of Htt, polyQ indeed does fold, but that it relies on flanking or perhaps distant sequences to stabilize it. Wetzel’s polyQ peptides enjoy no such stabilization, however,

because they contain just polyQ and two residues at either end that, do not correspond to exon-1.

Daggett said that the right context could very well produce peptides that fold, and she encouraged Wetzel and Bennet to pursue many variations. She said studies of chymotrypsin inhibitor peptide showed how finicky and counterintuitive folding is. The peptide contains seemingly self-stabilizing elements: An alpha helix and strands of beta sheet. Yet snipping off even one amino acid from the peptide flusters folding completely, she said. So finding folds takes trial and error.

Speculation about structure

At the start of the last session of the final day of the workshop, Patterson walked up to the oversize notepad that stood on an easel at the head of the table, where he wrote out the sequence of exon-1: Seventeen letters and a string of “Q”s followed by another short string of residues. With the expertise in this room, Patterson said, he thought they could hypothesize what this peptide looks like and how expansion of the polyQ tract changes it. He asked, “Can’t we build models of this simple sequence?”

The number of conformations accessible to a peptide even as small as this was, *a priori*, gargantuan, Daggett explained. Only an understanding of the relative impact of intramolecular forces and solvent affects could narrow down the possibilities--and no general formulary exists for these factors, nor have experiments constrained possibilities for this particular peptide. So in the absence of homology to a known structure, Daggett said, modeling even this short a sequence would be an extremely speculative enterprise. On top of that,

there was the possibility that exon-1 does not even fold.

Still standing, Patterson cited a recent paper by Max Perutz, which proposed a structure in which strands of polyQ come together. The structure is an antiparallel beta sheet that further stabilizes itself with H-bonding between side chains above and below the plane of the sheet. What if it were supposed, Patterson asked, that the polyQ in exon-1 folded in the middle to form the Perutz structure internally? “Doesn’t that constrain possibilities?” he asked.

The consensus was no, or “not really,” and Patterson stood down. However, several people did find the scenario thought provoking and some speculation ensued. One thought that people had was that the act of bending the polyQ in half, at least on the written page, brings the flanking sequences in contact. This made it easier for people to conceive of flanking sequences as essential to stabilizing a polyQ structure in solution. Another thought was that the hairpin suggested a mechanism by which Htt might sequester an otherwise sticky and pernicious sequence. PolyQ expansion might cause it to unfurl or to bend asymmetrically, so that an unpartnered overhang of successive Qs results.

Is structure not the issue?

When the speculation paused, Yeates suggested an alternate way to think about aggregation; one which didn’t look to structure for explanations. He pointed out that lipids aggregate into micelles spontaneously at or above a critical concentration. Seemingly every shape of lipid aggregates, he said, but the different varieties do it at different concentrations.

Crothers then suggested two related

analogies. One was the conformational transition of peptides from random coil to helix in solution, which takes place only when peptides are long enough. The other analogy was crystal nucleation.

Crothers reminded workshop participants of the delay that precedes the precipitation of a crystal from solution. Absent a rough surface or a “seed” on which to build, he said, solute molecules may remain supersaturated in solution for years. Crothers said that the decades that precede the appearance of inclusions in humans (i.e. inferred from when symptoms appear) make Htt aggregation seem like just this sort of process. In fact, he said, under the broader rubric of “precipitation,” how could it not be?

Crothers said that the analogy to precipitation (which he said is probably more than just an analogy) has important implications regarding concentration. Because aggregates of just a few molecules are unstable, nucleation occurs only if they fall together simultaneously in large number--an unlikely event in which the concentration makes all the difference. In mathematical terms, he said, the likelihood depends on solute concentration raised to an exponent that is (in the context of reaction kinetics) very high - 10 or 12, for example--which corresponds to the number of molecules that need to come together. Crothers referred to this as high-order cooperativity.

If Htt aggregation is highly cooperative, Crothers said, one could expect that even a tiny diminution in expression--one bound to have little impact on the ability of Htt to carry out its normal cellular functions--could delay aggregation so long that it would never occur in a human life span. In other

words, if inclusions are central to pathology, one might prevent Huntington's disease this way.

Crothers suggested that researchers delve into the cellular mechanisms governing how much Htt they express, and he also suggested a short-cut: Targeting the Htt gene's promoter directly with drugs. He said people had engineered sequence-selective, synthetic DNA intercalators, which might serve this purpose. Later he cited Dervan at Caltech as the head of one such group.

Spier added that companies exist, which tailor zinc-finger proteins to DNA sequences a customer can specify. He also suggested the possibility of oligonucleotide therapy with antisense or ribozymes. [See J. Pharmacol Exp Ther 295:239 2000 for an anti-sense study]

Bennet pointed out that these sequence-based approaches would impact the healthy and pathological alleles alike; but then she said that this was not necessarily a problem.

Beyond suggesting a therapeutic approach, Crothers's perspective also suggested a possible insight into HD pathology: Why only spiny cells appear to show Htt inclusions. “Maybe striatal cells just have twenty percent more huntingtin,” Johnson said.

Signer said data exists that might either support or argue against that suggestion: Different tissues throughout the body do express Htt at different levels. He said he could not remember if the striatum expressed the most, but said he thought it was at least in the running.

A return to structure: Antibodies on trial

The potentially critical importance of concentration delayed a return to the issue of structure, but ultimately it led straight back to it. Crothers had advocated thinking of HD as a problem of kinetics--of which concentration is an aspect, but so is structure.

Crothers said the relation between polyQ length and the age at which HD symptoms appear connects the primary structure of Htt to kinetics. The natural next step, Crothers said, is to assume differences also in the three-dimensional structure of Htt.

Patterson said his lab had eight monoclonal antibodies that bound within or around the polyQ tract of Htt. Lab members both had blotted them to gels (i.e. Westerns) and stained tissue sections with them (i.e. *in situ*).

Several of the antibodies bound preferentially to Htt^{ex} on gels; on which the lab ran crude protein extracts from healthy versus HD mouse brains, and ran extracts from healthy versus HD human cells. Yet in comparisons of tissue sections between wild-type and HD model mice, there was no preferential binding, Patterson said, and with these words exposing the skeleton.

The data was in fact richer in detail, and had given him a lot to puzzle over, Patterson continued. One bag of pieces was that the antibodies bound only a subpopulation of Htt molecules *in situ*, and that different antibodies preferred molecules in different subcellular locales. Furthermore, the antibodies bound at different positions in and around exon-1's polyQ tract, which members of the lab had mapped by peptide array screening. Together these observations implied that the accessibility of the polyQ tract varied

across subcellular locales, and either conformational variation in Htt or binding by other proteins (or both) were responsible, Patterson said. He interpreted the preferences that antibodies show on gels to reflect real differences in the structure of pure Htt in solution. But these differences go away or are obscured in the cellular context, he said, because of the proteins or other factors that evidently impact Htt structure.

The story did not go over smoothly, because it took a while for Patterson to express, while it took no time for participants to pounce at opportunities to discount his findings, which stood in consternating opposition to Wetzel's. They attacked from various angles, and some took several turns.

Tobin suggested that the complex staining actually might have nothing complex to say about Htt. He said it could mean that the antibodies also bound other polyQ proteins, such as TATA binding protein (TBP) and ataxins. As a control, Tobin proposed that Patterson spike his samples with an excess of one or the other of these proteins to show definitively that it does not bind them. Alternatively, Bennet said, Patterson could use an antibody for TBP on the usual samples his lab runs. He could show that TBP is indeed there, but the Htt antibodies don't see it.

Olivier Becker suggested that the length specificity on gels could reflect not structure but cooperativity: i.e. if longer polyQ molecules were bound doubly. Crothers asked Patterson if his antibodies were divalent, and he said most were. But he added that his lab had made a single-chain (and hence monovalent) antibody from DNA encoding one of the binding sites and said that it too showed the

length preference. That ruled out cooperativity, Crothers said.

Becker suggested another option: If two antibodies could fit together on one polyQ strand, they might huddle with an affinity that made the second antibody aboard stick more firmly than the first. Yet to create the appearance of antibody specificity, the affinity of the antibodies for each other would have to be of binding-site strength (i.e. pico or femtomolar affinity), making the scenario unlikely.

Signer took the similar staining in wild-type and HD model mice as exactly what one should not see from antibodies that prefer a pathological structure. “The pathology is in the mice, not on the gel,” Signer said.

Signer then encouraged participants to consider what might have happened to the true pathological structure of polyQ in the making of Patterson’s antibodies. The structure may have slipped past the cells that made them, he proposed. “I think we have to say that the immune system may not have been sensitive to it,” he said. The pathological conformation might be rare in the peptides that Paul’s lab injected, or easily broken by the antigen-presenting machinery on immune cells, he suggested.

Tobin too implied that he would question the biological relevance of the gel structures before he questioned the *in situ*. “The peptide could be a mess as a peptide and in the cell it could be constrained,” he said.

More or less in unison, about half the people in the room said, “Induced fit.” “Induced fit” appears in textbooks as a reply to the lock-and-key paradigm of

enzyme-substrate specificity, and asserts that the counterparts are not actually complementary except in union. According to one articulation of the paradigm, the key conforms to the lock to fit the tumblers after it is inserted. It occasionally assumes the complementary shape without the lock, but the lock prefers that shape (i.e. binds it with a more negative free energy) and so makes the key prefer it too.

In the specific context of polyQ, “induced fit” suggests that Patterson’s antibodies induce the structure they fit. It suggests also that that this structure is not absent but merely rare in the solution samples that Wetzel analyses by CD. On the other hand, the structure is less rare in populations of longer polyQ peptides, because these assume the shape with less resistance, the hypothesis implicitly suggests. The longer peptides would bind antibodies better for this reason.

Wetzel suggested that he might confirm induced fit by CD if there were some means besides antibodies to stabilize the inducible structure; for example, sucrose or cold. He might observe that for longer polyQ peptides less sucrose or less cold is necessary to produce a CD spectrum indicating structure, he said.

Becker had suggested earlier an experiment that would test induced fit more directly. Assuming that Malek’s FRET peptides would be unstructured like Wetzel’s, he suggested that she add Patterson’s antibodies to the solution. If they induced fit, the amount of transfer and therefore acceptor brightness should change. Malek said she had the antibodies and would try it as soon as possible.

A synthesis of ideas

The induced fit hypothesis led

Crothers to propose a synthesis of ideas, which offered to solve three problems at once: The dependence of HD onset and aggregation on polyQ length; the sluggishness of aggregation; and the relevance of Patterson's antibodies to pathology.

Crothers arrived at the synthesis by considering thermodynamic kinetics. Revising a view he had expressed earlier, he said that, actually, a difference in the three-dimensional structure of Htt^{ex} is not quite the most natural way to account for their greater-than-normal propensities to aggregate. "If you want to have a huge effect on a process," Crothers suggested, "look at its transition state." Yeates and Daggett voiced their agreement with Crothers.

Crothers proposed that long polyQ peptides and Htt^{ex} might differ from their short polyQ counterparts only in the frequency and ease with which they assume an "aggregation transition state;" i.e. the most unstable intermediate shape that the dissolved molecules must pass through as they morph into the form they take in the aggregates. Then he proposed that this transition structure might be the very one that Patterson's antibodies bind. The hypothesis fits the observation that the antibodies bind preferentially to the polyQ lengths that prefer to aggregate, he said, and it reduces to one the number of proposed alternate shapes for polyQ.

Crothers went on to say that, according to this scenario, even non-pathological Htt could assume the transition structure; it just did so more rarely. The wild-type protein aggregates too slowly to cause problems, as a result; yet at higher concentrations it would form inclusions and perhaps be as deadly as any of those with longer polyQ tracts.

There is indeed some experimental support for this idea, Signer remarked: Over-expression of wild-type Htt makes animals sick. But individuals inheriting two Htt^{ex} alleles instead of one--and who presumably express Htt^{ex} in higher concentrations--nevertheless show disease no sooner. Tobin replied that the diploid evidence was facing challenges, he believed. [And still other evidence for or against might come from k.i. mice expressing different amounts of the same Htt^{ex} or exon-1]

As an arbitrary candidate transition state, Crothers suggested the Perutz structure, although he said it might be anything. He proposed that researchers try to crystalize polyQ bound to antibodies to determine the structure by X-ray diffraction, or to try NMR. Bennet said she was considering antibody crystallization options already.

Don Crothers sketches a kinetic perspective

After Crothers had argued several points from what he called a "kinetic perspective," he went to the notepad to explain the perspective explicitly. He drew a chemical reaction diagram: Writing "gene," "protein" and "degradation" from left to right, and drawing arrows connecting them. He also drew an arrow from the word "protein" down to "aggregation."

Crothers said that the mechanisms naturally governing the abundance of Htt--whatever they are--aren't liable to register an accumulation of Htt that is locked inside aggregates (nor would the mechanisms recognize the presumably abnormally folded proteins on the surface of an inclusion, he said). Pointing to the

diagram, he said that, rather, such mechanisms might drive expression of the “gene” at full tilt to keep “protein” in steady supply, even while “aggregate” withdraws it from solution. Cells might also be expected to suppress movement through the “degradation” pathway, he said, since this would be another way to counter the diminution of protein.

Because regulatory mechanisms are liable to be part of the problem, Crothers said, they could be part of a solution. If researchers could flesh these mechanisms out, he said, they might discover how to convince cells to permit protein abundance plummet, and so rob aggregates of their supply. “I think we need to understand the regulation of this gene,” Crothers said.

Many workshop participants remarked later that Crothers’s ideas about concentration, gene regulation and kinetics in general were among the most important to emerge in the two days of talks.

In response to Crothers’s suggestion about where to direct research, Tobin replied that researchers had actually already paid quite a bit of attention to Htt degradation. In fact, he said, the experiments that Krobitch described--in which chaperone over-expression diminished aggregation--might turn out to be just the kind of regulatory effect that he suggested pursuing. In support of this idea, Tobin said that mutations in suppressor proteins that down-regulate the expression of the chaperones Hsp40 and Hsp70 in flies lead to an increased degradation rate of the polyQ protein associated with spinocerebellar ataxia 3.

Signer said he knew the paper that Tobin was referring to, but that he had

not interpreted the results the same way: He supposed that the increased chaperone activity had sped the folding and expression of proteins, which accumulated because degradation was normal. He said his first guess for the chaperones was that they figured in assembly and folding. But chaperones feed proteasomes, Tobin replied, so that HSP40 and HSP70 may be feeders of the “cell garbage disposals.” This interpretation satisfied him more, he said.

Tobin, asked whether the one-way arrow leading from “protein” to “aggregates” in Crothers’s diagram instead should be two-ways--which it should be if aggregation is reversible. If so, the effect of down-regulating protein concentration might be to reverse and not just to arrest or retard disease.

Wetzel replied that peptide aggregation is reversible, according to what his lab sees. Aggregates of long peptides dissociate too slowly to observe, he said, but aggregates of “Q15” eventually diminish in size. Others cited the study that Signer had mentioned, in which researchers had dissociated cell inclusions in culture.

An alert to potential misinterpretations

Earlier in the workshop, before he discussed his perspective around the diagram, Crothers had voiced similar ideas in more ambiguous terms, which seemed to lead some workshop participants to misunderstandings.

Citing Crothers’s perspective as the source, several remarked that inclusions implied an abnormally high abundance of soluble Htt--such that this high level might be a key factor in spiny cell dysfunction. But Crothers’s scenario implies that soluble Htt stays steady, or

that the cell tries to keep it so.

Other participants remarked that feedback mechanisms malfunction, or that feedback runs out of control in the face of aggregation. But Crothers proposed that feedback mechanisms might operate normally, and could respond to drugs to command them differently.

Closing Remarks

Spier said he is pursuing a project that did not mesh squarely with the themes workshop participants had been discussing. As background, Spier cited a report that antibodies against amyloid beta, the protein that aggregates in the brains of people with Alzheimer's, had promoted recovery when injected into so-called Athena mice, which model the disease. The study assessed the physical and mental competency of the mice using the "Morris water maze," which tests whether mice can memorize the location of a submerged platform in a pool of water, too cloudy to see through and too deep to stand in, unless a mouse finds the platform.

Spier said he wished to try the same tack in an HD model mouse; the R6/2. He said he might go by either of two routes: Injecting antibodies such as Patterson's, or inducing mice to raise their own antibodies, by inoculating mice with aggregated polyQ. As workshop participants immediately realized, a key difference between Alzheimer's and HD makes it impossible for Spier to truly recapitulate the strategy of the Athena study: In HD model mice, antibodies would not be able to reach the inclusions, which are intracellular, unlike amyloid.

While Spier conceded the point, he said the experiment would nevertheless test an important hypothesis. No one

knows whether a transmissible agent spreads dysfunction between cells in HD, Spier said. He hypothesized that full-length or fragmentary Htt becomes liberated with the rupture of dead cells, and that it acts as such an agent.

Patterson commented that he thinks the experiment is important to do.

As the workshop came to a close, Tobin asked participants to say what important ideas they would take away. Spier said that the workshop discussions had convinced him more than ever that HD is a prime target for gene therapy. He said he hoped the HDF would pursue this option vigorously. He suggested that HD researchers stay apprised of techniques as they become practical, safe and pass ethical scrutiny, and that they have sequences and ideas ready to go when the techniques have been proven so. He suggested that HDF hold a workshop on this theme.

Johnson remarked in closing that researchers would do well to try bioinformatics strategies to identify Huntington's natural partners; in particular to narrow down the 117 candidates that have been suggested by the yeast 2-hybrid screen (an assay that tests just the ability of two protein fragments to stick together). He suggested considering whether homologs of the candidate partners appear along with Htt homologs in the genomes of distantly related species--an approach to which is called phylogenetic profiling. He also said that researchers needed to do more homework on which cells express Htt when and how much. Signer expressed a similar thought: "The issue of concentration is now glaringly obvious," he said.

Daggett used her closing remarks to moderate what she called her cynical earlier comments about modeling Htt with computers and short peptides. She had come to be cynical, she said, after participating in modeling prions. When other researchers solved some real structures experimentally, and models proved wrong, she looked back on the theoretical work as a tragic waste of talent and funding. However, one can't predict when the empirical structure will arrive, Daggett said. With people dying, researchers have to do the best they can--and that includes modeling.

Isabella Karle said that researchers could stand to learn a lot more about the orientations in which the so-called R-groups of individual Q residues prefer to drape and to bond with other moieties. She said evaluating a model such as Perutz's was challenging given current knowledge. But Karle said that crystallography of very short polyQ peptides (e.g. 5 or 6 residues) could reveal a lot, and she advocated pursuit of this goal. In response, Yeates hinted that the lab of David Eisenberg at UCLA had preliminary results along these lines.

Bennet said in summary that the screening of large libraries of compounds using the strategies that people had described inspired her with the most optimism. She predicted that this research would prove to be the fastest route to HD treatments, and said they deserved to be the funding priority. Others expressed agreement. As far as basic science, Bennet said she hoped research would address how the structure of exon-1 and the polyQ tract depends on the rest of Htt. She said the antibodies from Patterson's lab could be immediately useful tools in this regard, and she hoped crystallographic data would follow not too far behind, she said.

More than any other aspect of the workshop, people cited that the visit by a woman with HD and her husband had made the strongest impression on them.

Many workshop participants said they had never met anyone with Huntington's disease before. The encounter had given an added impetus to everything that followed. Avron Spier best conveyed this feeling when he said, "She reminded me of why I am doing science"