Fifth HFSP Awardees Meeting
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By Geoffrey Montgomery

Introduction

The Fifth Awardees Annual Meeting of the Human Frontier Science Program continued the annual meeting's tradition of opening and closing plenary lectures by a pair of renowned researchers from different disciplines within the life sciences. Held at the Natcher Conference Center of the National Institutes of Health, Bethesda, Maryland USA on June 5-8, 2005, the meeting featured an opening plenary talk by **Linda Buck**, a molecular biologist turned neuroscientist, who was awarded the 2004 Nobel prize in Medicine or Physiology for her pioneering studies of olfaction. The closing plenary lecture was given by **Steven Chu**, who won the 1997 Nobel Prize in Physics "for development of methods to cool and trap atoms with laser light" [1997 Nobel Citation], but has recently turned his lasers towards the illumination of the complex macromolecules of living systems. Indeed, the scientific breadth of the meeting's plenary speakers well reflected the interdisciplinary projects presented by the international network of HFSP investigators at the Bethesda meeting, described in 28 oral presentations and some 116 posters. As **Kathy Olsen** (Associate Director for Science, Office of Science and Technology Policy, Executive Office of the President, USA; now Deputy Director, National Science Foundation) said on the meeting's opening morning: "The challenges and opportunities in scientific research that we face in the 21st century are complex and are generally at the intersection of what we refer to as traditional disciplines…. During its evolution, HFSP has changed its emphasis to the current expanded focus on novel collaborations that bring together scientists from different disciplines, such as chemistry, physics, computer science and engineering, to focus on areas of complexity in life sciences."

Yet threading through the wide-ranging talks and posters at the Bethesda meeting--which included remarks by **Norka Ruiz Bravo** (Deputy Director for Extramural Research, NIH, USA) --were common themes as well. Here too the topics of the meeting's plenary lectures were beautifully representative. Steven Chu presented vanguard single-molecule studies of a 40-year-old mystery: how DNA's genetic code for proteins is recognized and read out by a cell's protein-translation machinery with such specificity and accuracy. Linda Buck spoke of another kind of "code" woven within a biological system of molecular recognition and chemical specificity: the olfactory "receptor code" that enables the nervous system to deconstruct the chemical attributes of a vast array of different odorant molecules and then reconstruct this "code" into the perception of smell in higher olfactory centers in the brain. This report highlights eight representative awardee presentations at the Bethesda meeting that carry forward central themes of the two plenary talks. What is the molecular nature of the processes that guide the development of neural pathways in the brain and give rise to the neural plasticity that underlyies learning, memory and the response of sensory systems to experience? What new tools will be required in the 21st century to understand the remarkable specificity of molecular recognition events in complex living systems and what advances in physics, chemistry and engineering can promote their development?

Unraveling the Sense of Smell: From Receptor Codes to Perceptual Reconstruction

Smell has been called the primal sense. The ability to detect and discriminate a wide array of odorants is essential to the way most organisms interact with their environment in order to survive and reproduce. "It is estimated that humans can sense as many as 10,000 to 100,000 chemicals as having a distinct odor," said **Linda Buck** (Fred Hutchison Cancer Research Center, Seattle, USA) in the opening plenary lecture. Each odorant is a volatile molecule with a different chemical structure; yet highly related structures can yield quite different perceptions. For instance, closely related aliphatic acid and alcohol molecules can elicit diametrically opposite sensations: octanoic acids are perceived unpleasantly as rancid, sour and repulsive, while octanols elicit pleasant smells of sweet, orange and rose. How can the mammalian nervous system make such fine discriminations among the vast universe of volatile molecules? What is the biological source of the olfactory system's chemical specificity?

In 1991, Buck and Richard Axel discovered a large family of genes encoding olfactory receptors. Mice, for which smell is the primary sense guiding behavior, have some 1,000 olfactory receptor (OR) genes; indeed, OR genes are the largest gene family in the entire mouse genome. In humans, however, smell has been displaced by vision and hearing as the primary sense guiding behavior, and this behavioral evolution is reflected in the human genome, which possesses only 350 intact OR genes, with another 300 non-functional OR pseudogenes. OR genes encode G-protein coupled receptors with hypervariable residues, consistent with the ability of OR proteins to interact with a multitude of odorants. "The discovery of odorant receptors explained how the olfactory system can detect a vast array of chemicals in the external world, "said Buck. "It also provided a set of tools to explore how the nervous system translates chemical structures into odor perceptions."

Buck's laboratory found that individual neurons in the olfactory epithelium of the nose express only a single type of olfactory receptor. Moreover, neurons expressing a given OR gene appear to be randomly distributed within one of four non-overlapping zones in the olfactory epithelium. Working in collaboration with two researchers from the Life Electronics Research Center in Japan, Buck's group used calcium imaging in mice to visualize the responses of single olfactory sensory neurons to a series of different odorants. They then used reverse-transcriptase polymerase chain reaction techniques to isolate the OR gene expressed in each responsive cell. "These studies showed that each odor is detected by a combination of different receptors, and each odorant receptor recognizes multiple different odorants. However, different odorants are recognized by different combinations of ORs. So this indicated that the OR family is used in a combinatorial manner to encode odorant identities." Buck called the combination of different ORs activated by a specific odorant that odorant's receptor code. "And this combinatorial scheme could allow for the discrimination--not only the detection but the discrimination--of an almost unlimited number of different odorants."

Remarkably, axons from neurons in the nose that express the same OR gene converge on the same so-called glomeruli of the olfactory bulb, the next stage of the olfactory pathway. "This is very different from what we had seen in the nose," said Buck, where neurons expressing the same OR are randomly distributed within four broad zones. In addition, glomeruli responding to specific ORs had almost exactly the same location in different individuals. "This further indicated that olfactory information that is broadly organized into four zonal sets in the nose is transformed in the bulb into a highly organized and spatially stereotyped sensory map."

Buck reviewed her laboratory's exciting recent work at the next stage of olfactory processing, where so-called mitral cells from the olfactory bulb send axons that "dive in and branch" onto neurons in the olfactory cortex. Her laboratory used barley lectin coding sequences "knocked in" to specific OR genes in transgenic mice as a trans-

synaptic tracer to delineate the neural pathways taken by different OR-specific glomeruli, and found a quite different organization from earlier stages of the olfactory pathway. "While each neuron in the nose and in the bulb is dedicated to only one type of OR, each cortical neuron is likely to receive input from multiple different ORs," said Buck. "Given that each odorant is recognized by a combination of ORs, this scheme may allow the integration of multiple components of an odor's receptor code at the level of a single cortical neuron, and this of course could be important in the generation of different odor perceptions."

Development and Plasticity of Neural Pathways for Vision by a novel homeoprotein signaling mechanism

Experiments with transgenic mice indicate that the OR expressed by a neuron in the nasal epithelium plays an instructive role in guiding the targeting of axons from nose to olfactory bulb. Classic experiments from the 1960s by HFSP Secretary General Torsten Wiesel and his longtime colleague David Hubel showed that, in the visual system, axons carrying visual information from the two eyes become organized into so-called ocular dominance columns in the primary visual cortex. Moreover, the development of these ocular dominance columns undergoes a "critical period" of plasticity early in life during which visual experience—such as the deprivation of sight from one eye—can lead to dramatic anatomical restructuring. These classic studies established ocular dominance columns as the paradigmatic experimental system for studying how experience shapes the development of young mammalian brains. At the 2005 Bethesda meeting, HFSP Grant Team leader **Takao Hensch** (RIKEN Brain Science Institute, Wako, Japan) described remarkable new insights into the molecular nature of this paradigmatic developmental process

The project described by Hensch tied together three seemingly unconnected and rather puzzling findings on three different continents.

First, working in Paris, **Alain Prochiantz** (Ecole Normale Supérieure) fortuitously discovered a highly unexpected property of homeodomain transcription factor proteins, already famous for organizing embryonic bodies and brains into different areas whose development they then controlled. Beginning in 1991, Prochiantz's *in vitro* biochemical studies indicated that within their classical DNA-binding and transcription-regulating domains, homeoproteins possessed specific amino acid sequences that allowed them to exit a cell and enter neighboring cells. This suggested that in addition to acting as cell-autonomous transcription factors, homeoproteins may mediate a novel kind of intercellular signaling. However, no cell signaling function—no function of any kind—had been identified for these intercellular transport sequences.

Second, working in New York, **Ariel Ruiz I Altaba** (currently at the University of Geneva, Switzerland) found that the Otx-2 homeoprotein, which is expressed in the entire midbrain and forebrain in early mammalian embryos, by late embryogenesis and early post-natal development has its expression predominantly restricted to brain areas involved in the early stages of visual processing---retina, lateral geniculate nucleus and superior colliculus. What functions Otx-2 might have in these visual areas was unknown.

Third, in Japan, Hensch had identified a specific population of inhibitory interneurons in the visual cortex, called PV-basket cells, as playing a major role in mediating ocular dominance column plasticity during the critical period of early development. Using transgenic mice, Hensch's lab found that when the ability of these cells to produce the inhibitory neurotransmitter GABA was eliminated, the critical period fails to begin.

Moreover, as Hensch said in his talk at Bethesda, "we can rescue these mice by restoring GABAergic transmission, with benzodiazepines injected directly into the visual cortex." Indeed, this treatment "will trigger the critical period not only at the usual time, but, interestingly, even in adult mice. This led to prediction that the natural critical period in all of our brains is determined by the maturation of a late developing population of GABAergic cells."

The three researchers' HFSP-supported project began with the discovery of a tantalizing puzzle. Antibodies for the Otx-2 homeoprotein studied by Altaba showed, remarkably, that Otx-2 protein was localized to the same population of PV-basket cells identified by Hensch. Yet try as they might, the team could find no Otx-2 messenger RNA in the cortex. "And so this left us with a conundrum," said Hensch. "The Otx-2 protein is there at this late time in PV cells, yet it's not translated there. So we turned to this hypothesis, proposed by Alain Prochiantz several years ago from in vitro studies, that homeodomain-containing proteins can be transported, secreted and internalized thanks to various specific domains on their homeodomain. And so it was possible that the Otx-2 was arriving into the visual cortex from lower centers and in that way getting into PV-positive cells."

Indeed, the team performed both loss-of-function and gain-of-function experiments with Otx-2 which led to changes in the critical period that paralleled the changes elicited by the experimental manipulation of GABA signaling by PV interneurons. "Although we talk about the critical period for cortical plasticity," said Hensch, "the driving force may actually come from outside the cortex." Factors from the retina or lateral geniculate nucleus "may establish the cortical milieu for plasticity specifically through its actions on this inhibitory cell type."

In addition, Hensch described recent experiments, carried out by Prochiantz in collaboration with Christine Holt, showing that another homeoprotein (Engrailed-2) plays an instructive role in patterning connections between the retina and the optic tectum of frogs by passing from cell to cell and regulating protein translation in retinal axons. (**Nature** 438: 94-98 2005).

Unraveling the "RNA World" of Synapses

Long-Term Fellow **Gerhard Schratt** (Harvard Medical School, USA) described a fascinating convergence between two vanguard areas of biological investigation: the local translation of specific mRNAs within neurons at synapses and growing axons; and the control of mRNA translation by the novel class of regulatory molecules known as microRNAs. Schratt used gene expression "chips" to identify a select group of 48 mRNAs that are newly translated into protein upon the exposure of neurons to the growth factor BDNF (brain-derived neurotrophic factor). Many of these mRNAs were found to encode proteins that function at synapses (**J. Neurosci** 24:7366-77 (2004)). In a second screen, Schratt found that a candidate microRNA called miR-134 is localized to neuronal dendrites. Schratt then combined the findings of his two screens by searching the 3' untranslated regions of his 48 mRNAs for possible target sequences recognized by miR-134. He identified one potential target mRNA, Limk1, as being of particular interest, as it is known to regulate dendritic spine development. By studying the BDN--miR-13--Limk1 pathway in hippocampal neurons, Schratt was able to conclude that miR-134 negatively regulates the size of dendritic spines—the major sites of synaptic transmission—by inhibiting the local translation of LimK1. This represents the first case in which the regulatory role of a specific microRNA functioning at neural synapses has been identified, leading Schratt and laboratory head Michael Greenberg to propose that other cases of such microRNA regulation will be found in a variety of neural contexts, and to "speculate that miRNAs act locally at individual

synapses, thereby contributing to synapse-specific modifications that occur during synaptic plasticity" (**Nature** 439:283-289 2006).

Neurogenesis and Memory Formation

During the past decade a sea-change has occurred in the international community of neuroscientists with the recognition that new neurons continue to be generated in adult mammalian brains. An especially intriguing brain region displaying adult neurogenesis is the hippocampus, known to be essential for the formation of long-term memories in humans and other mammals. Yet while it has been shown that newborn neurons in the adult hippocampus survive and exhibit electrophysiological properties similar to pre-existing cells in the network, it has not yet been demonstrated whether these newborn neurons contribute to memory-formation in behaviorally relevant contexts.

Long-Term Fellow **Victor Ramirez-Amaya** (University of Arizona, USA) described significant progress towards addressing this question. Ramirez-Amaya has employed a novel method of detecting hippocampal neurons activated when a mouse explores its spatial environment, by detecting the expression of an immediate early gene called *Arc,* and tracking the location of *Arc* mRNA within neurons. His studies indicate that "two or more waves of *Arc* expression may be required to stabilize behaviorally induced spatial representations [spatial memories] in hippocampal-neocortical circuits, and the reactivation of *Arc* may represent an anatomical signature of the synaptic activity that underlies [memory] consolidation." (**J. Neurosci** 25: 1761-68 (2005)) At the 2005 Awardees meeting, Ramirez-Amaya described preliminary studies showing that new-born hippocampal neurons exhibit similar patterns of *Arc* expression after spatial exploration of a maze, providing important evidence that newborn neurons can be integrated into neural networks responsible for the formation of long-term memories.

The Molecular Biology of Walking

Locomotion—walking step-by-step in rhythmic sequence with speeds ranging from a slow stroll to a quickstep march—is controlled by a circuit of neurons in the spinal cord. HFSP Grant Team Principal Investigator **Martyn Goulding** demonstrated how this relatively simple neural circuit, known as a "central pattern generator" (CPG), is serving as a powerful paradigm for understanding how more complex brain circuits integrate information. Goulding described the developmental studies in his and other laboratories which have led to increasingly precise knowledge of the "transcription factor codes that specify these neuronal cell types in the spinal cord. And now we are using these transcription factors as developmental tools to study how these circuits function."

Using the transcription factor "code" of specific CPG interneurons to pinpoint the expression of different transgenes in mice, Goulding's team combined several state-of-the-art methods both for genetically ablating and acutely silencing neural activity. "I think that these technologies are very important," said Goulding, "because they allow one to go into localized neural circuits and surgically remove one or two classes of cells and look at the functional consequences." Specifically, Goulding's team used diptheria-toxin-encoding transgenes to genetically destroy a specific class of inhibitory interneurons known as V1, whose function had previously been quite mysterious. The team then used *Drosophila* allatostatin receptor-encoding transgenes to silence these same cells conditionally upon experimental exposure to allatostatin. (When activated, the foreign allatostatin receptor suppresses an endogenous ion channel critical for neural activity.) Both genetic "surgeries" led to a rather unexpected and counterintuitive finding: silencing V1 inhibitory neurons does not prevent mice from walking slowly, but they can no longer walk rapidly in a normal rhythmic fashion. Apparently the V1 inhibitory neurons are required for the spinal cord CPG to generate

fast bursts of motor output and thereby control walking speed.

In a recent *Nature* paper describing this work (**440:** 215-219 (2006); HFSP long-term postdoctoral fellow Guillermo Lanza was one of the two lead authors of this paper), Goulding's team noted the broader implications of the ability to use neural transcription factor codes to perform genetic surgery on neural circuits. "This and similar genetic approaches now make it feasible to selectively probe the function of small populations of neurons, which should facilitate the mapping of neural circuits at higher resolution than was previously possible." Indeed, an accompany *News and Views* perspective on the Goulding team work remarked that "developmental biologists and physiologists have tended to approach the problem of circuit formation and function from very different angles, without much dialogue between them. …The work presented [in the Goulding team *Nature* paper] sets up a firm bridge across the river that has so far divided developmental biology and physiology."

Molecule Movies of Protein Translation

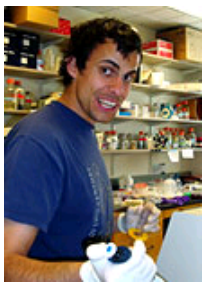
The beginning of the 21st century has yielded a series of beautiful, atomic-resolution X-ray structures of a long-sought crystallographic quarry: the bacterial ribosome, the large molecular machine that translates messenger RNA into polypeptide chains. Yet protein translation proceeds through a series of dynamic steps essential to accurate incorporation of amino acids into a growing polypetide chain, and X-ray structures by their nature can provide only static images. In his plenary lecture, physicist-turned-biologist **Steven Chu** (Lawrence Berkeley National Laboratory, USA) showed how "single-molecule studies of the ribosome allow one to see the dynamics between known static structures," yielding new insights into the 40-year-old mystery of how protein translation proceeds in living cells with a mere 1 in 10,000 amino acid error rate.

Using an experimental combination of optical microscopy, fast video cameras, Fluorescence Resonance Energy Transfer (FRET) and a variety of chemical tricks, Chu's laboratory has, in effect, made stop-action "movies" of the initial steps of protein translation: the steps by which a transfer RNA (tRNA) with a specific anti-codon RNA base-sequence (attached to its corresponding amino acid) enters the ribosome's "Decoding Site" and is recognized and bound by a matching codon sequence on a messenger RNA. In particular, two different fluorescent tags are attached to two different tRNAs—a green-sensitive fluorescent tag bound to a tRNA in the central "P" or peptidyl transfer site; and a red-emitting tag attached to the adjacent "A" site where initial decoding occurs. By exciting at green wavelengths and observing the intensity of red light emitted by the neighboring tRNA, one gets a direct correlate of the molecular distance between the two tRNAs expressed as different ratio FRET states. Moreover, one can use different antibiotics binding to different parts of the ribosome and its molecular partners to stall translation at different stages corresponding to these different FRET states, allowing Chu's laboratory to connect its dynamic FRET movies to ribosome crystal structure data. By comparing matching and non-matching tRNA within the ribosome, these crystal structures have shown that the ribosome recognizes the geometry of correct codon--anticodon base pairs in a sequence independent manner, causing the ribosome to wrap more tightly around the tRNA in the A site.

Chu's FRET studies have now shown that this wrapping process "causes the tRNA to move into a position so it is more likely to make stabilizing contacts with the ribosome," which in turn somehow triggers the activation of a GTPase center located 90 angstroms away. GTP hydrolysis of the elongation factor EF-Tu is an irreversible step separating this initial codon--anticodon selection process from a subsequent "proofreading" step which Chu's laboratory is currently analyzing by attaching another fluorescent tag to a mutant version of EF-Tu.

"The biology of the ribosome is beginning to be reducible to chemistry and physics," Chu concluded. "We can ask quantitative questions about biological mechanisms—and we can answer these questions." Indeed, at the end of his talk, Chu sketched his laboratory's ongoing single-molecule dynamic studies of other complex processes such as gene transcription and the signaling pathways underlying nerve growth. "So this is really exciting—and other groups are doing similar kinds of work—because it opens up the ability to see [complex events] at essentially single-molecule resolution in living cells in real-time."

Direct Observation of Proteins at Atomic Resolution in Living Eukaryotic Cells



Steven Chu's plenary lecture highlighted progress in the ability to observe dynamic changes between known structural states of macromolecules involved in core biological processes. On a related note, HFSP Long-Term Fellow **Philipp Selenko** (Harvard Medical School, USA) reported significant advances towards a breakthrough in *in vivo* structural biology: the ability to observe structural changes in proteins as they carry out complex processes inside living eukaryotic cells.

After undergraduate training as a physicist in his native Vienna, Selenko studied nuclear magnetic resonance (NMR) spectroscopy as a graduate student, a line of investigation he continued as a post-doc in Gerhard Wagner's laboratory at Harvard Medical School. By fortunate coincidence, working in the same building were biologists who studied the African clawed frog *Xenopus laevis*, a model system for many different areas of cell and developmental biology. Selenko attended many *Xenopus* talks and became accustomed to descriptions of how biologists micro-injected compounds of all kinds into *Xenopus* oocytes (unfertilized eggs) to probe cellular behavior. Then one day during a beer hour shared with *Xenopus* biologists, his creativity perhaps stimulated by a pint or two, Selenko had what he calls a "crazy idea." NMR spectroscopy depends on substituting NMR-active atomic isotopes, such as Nitrogen-15, into biomolecules. "And I thought: Why not inject a [N-15] labeled protein [into a *Xenopus* oocyte] and just look at it by NMR, and see what you get?" Structural biology by X-ray crystallography and high-resolution electron microscopy depend on pure samples in a crystalline or vitrified state removed from living context; but, Selenko reasoned, high-resolution, liquid state NMR might enable him to "see" protein structures at atomic resolution within their natural cellular environment. Selenko wrote a proposal for a 2004 HFSP fellowship describing "a couple of experiments that were just ideas at that stage," but whose feasibility he has subsequently demonstrated.

Selenko first focused his attention on a N-15 labeled model protein domain called GB1, which is made by streptococcal bacteria and which is completely inert in *Xenopus* oocytes and other eukaryotic cells. A microinjection machine automatically injected labeled GB1 into *Xenopus* oocytes, and a NMR spectroscope scanned these samples 16 times over about 45 minutes. "It was late at night, and I came back [to the spectroscope after 45 minutes] and began processing my data on the computer screen, and I was just amazed." The NMR spectra of the GB1 inside Xenopus oocytes was perfectly superimposible on the NMR spectra of pure GB1 samples, just as would be expected for an inert protein. Every point on the spectra could be assigned to a specific amino acid residue in the GB1 protein sequence, and "the pattern of points is like a fingerprint of the three-dimensional conformation of that protein."

When Selenko's pilot studies of GB1 were published in *Proceedings of the National Academy of Sciences* [103: 11904-9 (2006)] an accompanying commentary by Charlton and Pielak noted that it marked "an important advance in endeavors [to gain atomic-level knowledge of molecules in living cells] by providing the first high-resolution glimpse into how the cytoplasm of a higher eukaryotic cell can affect the properties of a

folded protein." Selenko has also conducted preliminary studies of larger and more complex proteins involved in such processes as programmed cell death, indicating that his novel approach for *in vivo* protein structural analysis "can provide atomic-resolution data for a wide variety of complex biological processes."

In his poster at the 2005 Awardees' Meeting, Selenko also reported preliminary observations of conformational changes in a key protein involved in programmed cell death, indicating that his novel approach for structural analysis of proteins in living cells "can provide atomic-resolution data for a wide variety of complex biological processes."

Elucidating the Role of Aqueous Environments in Biological Function with a Nano-Mechanical Probe

Young Investigator Grant Team Principal Investigator **Suzi Jarvis** (Trinity College, Ireland) reported significant progress towards the development of Frequency Modulation-Atomic Force Microscopy (FM-AFM) especially designed to probe the aqueous environment of biological materials. Jarvis's team represents an unusual collaboration involving three scientists from outside the life sciences. Jarvis is a physicist and **Tjerk Oosterkamp** (University of Leiden, The Netherlands) an applied physicist who "consider ourselves tool-builders or instrument-makers," while **Rachel McKendry** is a chemist who has succeeded in "functionalizing" the carbon nanotube tips used in their FM-AFM experiments by attaching a model receptor molecule (biotin) with special cross-linkers.

"What forces would a virus feel as it approaches the surface of a cell it infects?" asked Jarvis. "These are the kind of question we are interested in." Most of a cell's mass is made of water, and the subtle interplay between hydrophilic and hydrophobic interactions is central to nearly all biological processes, including protein folding, protein-protein and protein-nucleic acid interactions, and the assembly of macromolecular machines and cell membranes. Yet hydration forces "are probably the least understood of all fundamental intermolecular forces and the subject of much scientific debate," noted Jarvis.

In their first proof-of-principle experiments, the team showed that their modified version of FM-AFM could measure the mechanical properties of water structure in several situations, including model versions of compliant lipid bilayers. "This was something that was not clear would be possible at the start of the project," said Jarvis, "because there was a lot of speculation that the thermal motion of these lipid bilayers would smear out the very forces we were trying to see. But we found that as we improved our measurement system, it has been possible to access these forces." The team, which will continue to collaborate beyond the HFSP grant period, has also studied unbinding forces associated with single receptor--ligand (biotin--avidin) interactions, "highlighting the potential use of FM-AFM to study a range of biological systems, including living cells and/or single biomolecule interactions." (Higgins *et al.*, **Nanotechnology**, 16:285-89 2005).

The Organization of Rare Molecular Events in the Nucleus and the Source of Genetic "Noise"

Long-Term Fellow **Attila Becskei,** working in the MIT physics laboratory of Alexander van Oudenaarden (Cambridge, USA), described his design of an artificial genetic circuit for amplifying fluctuations in gene expression in yeast cells, and the surprising findings this "noise-amplifier" revealed. Studies have shown that cells which are genetically identical and living in the same environment can have greatly varying concentrations of different proteins; moreover, these fluctuations are

increased by positive feedback reaction networks. What is the ultimate source of this intrinsic intracellular "noise" in gene expression? Is this noise a necessary consequence of molecular reactions in a cell with small numbers of particular mRNA species, as Poisson's law of small numbers might suggest?

Indeed, said Becskei, "we know that in yeast, 75% of yeast mRNAs are expressed at a level of less than 1 copy per cell, particularly mRNAs of transcription factors." However, by studying yeast transcription factors involved in cell cycle regulation, Becksei showed that the physical position of genes along the chromosome can be a more important determinant of gene expression noise than low numbers of RNA transcripts. In higher organisms, said Becskei, "I think these observations might be especially important in the field of immunology, where there are a number of differentiation steps involving stochastic patterns" of gene expression and positive feedback. "If the unit of fluctuation [in gene expression] is not the low number of mRNA molecules but rather the individual random instances of gene activation, which in turn is determined by how these genes are organized along the chromosome and in the nucleus, then gene expression is still stochastic." Moreover, this stochastic gene expression can be used to promote different cellular programs of of differentiation, said Becskei, "but there is still some coordination in these fluctuations because genes that are co-localizing in the same part of the nucleus have coordinated levels of gene expression." The spatial organization of genomes may have evolved in part to coordinate and thus take advantage of rare, stochastic molecular events. When Becskei's studies were published in *Nature Genetics* (37: 937—44 (2005)) an accompanying commentary concluded: "The results of Becskei *et al.* point in some unexpected directions, and more surprises are probably in store."

New Chemical Approaches to Deciphering the Glycosaminoglycan Code

Glycosaminoglycans are linear polysaccharide chains that are often attached to proteoglycan proteins at cell surfaces or the extracellular matrix. Though they are involved in a remarkably diverse range of biological and disease processes, ranging from axon pathfinding in developing brains to tumor growth and metastasis, the molecular mechanisms of glycosaminoglycan functions are poorly understood. There is increasing evidence that sulfation motifs arranged in specific three-dimensional patterns along the linear polysaccharide backbone form a "sulfation code" that somehow specifies how a particular glycosaminoglycan functions. However, the decipherment of the glycosaminoglycan "sulfation code" has been greatly hindered by their complex chemical structure, the inability to purify specific glycosaminoglycans from heterogeneous natural sources, and the fact that genetic deletion of sulfotransferase genes results in global changes to many different glycosaminoglycans simultaneously.

In a *tour de force* demonstration of the power of synthetic chemistry coupled to cell biology and developmental neurobiology, an interdisciplinary HFSP Young Investigator Grant Team led by **Linda Hsieh-Wilson** (Caltech, USA) has for the first time studied the function of a specific chondroitin sulfate (CS) glycosaminoglycan in a biological system. Hsieh-Wilson's team applied new advances in oligosacharide synthesis to construct libraries of CS glycosaminoglycans with different sulfation patterns. They were able to recapitulate the biological activity of a CS polysaccharide in a small molecule—a CS-E tetrasaccharide with a specific sulfation pattern, showing this small molecule can induce the growth of hippocampal and other neurons, including dorsal root ganglion sensory neurons of the spinal cord. "That's exciting," said Hsieh-Wilson, "because a tetrasaccharide is within the reach of synthetic chemistry. We can make the natural sequence, we can make non-natural analogues and tools—such as affinity agents and imaging agents—and use chemistry to

understand the structural determinants and mechanisms of these sophisticated biopolymers."

Hsieh-Wilson's team devised experiments to show that the position of sulfate groups on this tetrasaccharide was critical for its ability to promote neural growth. Altering the sulfation pattern, but not the overall charge, of their synthetic sugars abolished biological activity. "This is significant, because it's the first direct demonstration that the sulfation of chrondroiton sulfates directs it activity," said Hsieh-Wilson. "Previous studies using heterogeneous polysaccharides couldn't answer this question, because you always wondered whether the activity you were seeing was due to other sulfation patterns in the heterogeneous mixture. Using synthetic chemistry, we can relate the biological activity to a specific sulfation pattern such as CS-E."

This work has led to the hypothesis that different CS sulfation patterns recruit specific growth factors to the cell surface of neurons, triggering signaling cascades that underlie neuronal growth and regeneration. Moreover, the power of synthetic chemistry has allowed Hsieh-Wilson's team to begin testing this hypothesis by constructing carbohydrate microarrays for identifying specific CS-protein interactions. Their initial studies have pointed to two specific growth factors, NGF and midkine, as strong candidates for mediating the neural growth processes in their system.

"Over the past 50 years, DNA has been shown to have to have rich information content," said Hsieh-Wilson, citing the genetic code for proteins, as well as cis-regulatory sequences controlling transcription, epigenetic modifications of chromatin, and a host of other functions. "As we continue to study glycosaminoglycans, I think we'll continue to find common themes in the way that these biopolymers use molecular structure to encode information. For instance, a protein may bind to a low-sulfated region and then slide along the chain looking for the right activating sequence. What I find exciting is that we now have a chemical approach, and a set of tools, that are allowing us to unlock the structure and function of this important class of biopolymers."

Building New Bridges between Worlds of Science

Hsieh-Wilson has concentrated her attention on the specific role of CS glycosaminoglycans in promoting the growth of types of neurons that are important in cases of neurodegenerative disease and spinal cord injury. "We're now using our synthetic compounds to see whether we can regenerate neurons *in vivo*." On a related note, Hsieh-Wilson was greatly intrigued to learn that her pioneering "neurochemical" project intersected with another of the major discoveries described at the Bethesda Awardees' Meeting: Takao Hensch's talk on the role of secreted homeodomain proteins in regulating critical period plasticity in the visual system. Hensch showed evidence suggesting a role for chondroitin polysaccharides in specifically attracting Otx-2 to the PV interneurons which appear to regulate this cortical plasticity. Indeed, speaking before he knew of Hsieh-Wilson's breakthrough in synthetic polysaccharide chemistry, Hensch told the audience how the application of chondroitinase enzyme removes a net of sugars surrounding PV cells, abolishing their uptake of Otx-2; and how the same treatment has been used to reawaken the critical period of postnatal plasticity in adult rodent visual cortex. Hensch said that "there's a possibility here, which was not predicted from *in vitro* studies, that *in vivo*, there is a code, or some kind of mechanism, which specifically directs Otx-2 to PV cells," a chemical "code" which may depend on the specific chondroitin sugars surrounding these cells. In conversations later in the Bethesda Awardees' meeting, both Hensch and Hsieh-Wilson remarked on the extraordinary convergence between their widely disparate research interests, and noted how unlikely they were to have experienced this cross-pollination between disciplines at the specialized meetings they normally attend.

Indeed, in separate conversations, Hsieh-Wilson and the Hensch team both reflected on the unique properties of the HFSP grant-program that were crucial to their pioneering projects. Gesturing towards Hensch, team member Ariel Ruiz I Altaba remarked that normally "our two worlds don't talk to each other"—the worlds of embryology and those who study postnatal brain development. "Alain [Prochiantz, the third member of the team] is actually the person who saw the common interest. There was a common interest but not a common language in the beginning. Certainly Takao and I, we didn't do anything alike [until this collaboration]. But the observation my lab made in 1998 about Otx-2 being expressed in the visual pathway made sense to Alain, because Alain had been working on homeodomain proteins and had a hypothesis to test [that homeodomain proteins could be mediating a novel form of intercellular signaling]. And it made sense to Takao to test [this idea], because it could be one molecule that could be interesting in terms of the sorting out, molecularly, what's happening in a system that he's been working on for a long time." Yet despite this convergence of interests and hypotheses, said Ruiz I Altaba, "I think its fair to say that HFSP was the only source of funding for this kind of project."

For her part, Linda Hsieh-Wilson spoke of the "challenge of finding support for interdisciplinary studies, in our case trying to combine synthetic chemistry with cell biology." Her HFSP grant was her laboratory's main source of support for critical early work on the glycosaminoglycan "sulfate code." Moreover, Hsieh-Wilson emphasized the importance of HFSP's policy of not requiring preliminary data in order to support a well-argued and truly original research proposal that might open deep new inroads into the challenge of biological complexity. When her laboratory's work on the sulfation code commenced, "we did not have enough data to apply for an NIH R01 grant, but HFSP was willing to support us with a grant based on an idea." In May 2005, the month before the Bethesda Awardees Meeting, the Howard Hughes Medical Institute announced that Hsieh-Wilson had been appointed an HHMI Investigator at Caltech, affirming the fruitful development of Hsieh-Wilson's HFSP-funded "idea," and providing generous and flexible support for its future growth across the boundaries dividing traditional scientific disciplines.