Second HFSP Annual Awardees Meeting
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By Geoffrey Montgomery

Introduction

At the conclusion of the HFSP Second Annual Awardees' Meeting, held June 9-12, 2002 in Ottawa, HFSPO President Masao Ito summarized how the qualities displayed by HFSP grantees and fellows embodied the central themes of the program: "Internationality, interdisciplinarity, and maximizing opportunities for young people."

In recent years, under the leadership of Secretary General Torsten Wiesel, President Ito, and its Board of Trustees, HFSP has redoubled its efforts to support truly frontier research in the life sciences at the international level. This frontier research revolves around the central challenge now faced by all life scientists: unraveling the immense and exquisite complexity of biological systems. Unlocking the enigmas of biological complexity will necessarily require a convergence of approaches from scientific disciplines outside biology—physics, chemistry, mathematics and computational science—as well as the development and creative deployment of new tools for scientific investigation. It also critically depends upon the support of the most talented young scientists across the world who are at the earliest stages of independent careers.

At the Ottawa meeting, held at the Marriot Hotel, awardees gave talks and poster presentations describing findings uncovered, and avenues of science opened up, during the periods of their HFSP grants and fellowships. In order to give some sense of the depth and range of awardee presentations at the Ottawa meeting, this report will highlight six representative projects which offered novel approaches to fundamental questions about the nature of complex living systems. How does the genome of a complex cell mobilize different genetic networks in response to different environmental signals? How do genetic networks pattern vertebrate embryos in developmental space and time? Can the complex protein signaling networks that govern cellular processes be unraveled and even re-designed? What new layers of genetic information might be lurking undiscovered in genomes, for instance in the so-called "deserts" or "empty spaces" between protein-coding genes? How does the visual system encode biologically relevant information about the structure of changing visual environments? How do infant brains develop from a "citizen of the world" state, capable of distinguishing between sounds from any human language, to a native speaker state?

These were some of the projects presented at the 2002 Ottawa meeting. The meeting also featured opening remarks by Arthur Carty (President, National Research Council Canada); a reception and dinner at the Canadian Museum of Civilization, with guest speakers Tom Brzustowski (President, NSERC) and Alan Bernstein, (President, Canadian Institutes of Health Research); and two outstanding plenary lectures by Roderick MacKinnon and Tim Hunt.

The molecular architecture of ion channels

In the first plenary lecture, Roderick MacKinnon (Rockefeller University, USA) concluded the first full day of the meeting with a guided molecular tour of potassium ion channel proteins (which underlie electric signaling by nerve cells and many other physiological processes), displaying a dazzling series of crystal structures for which he would receive a 2003 Nobel Prize in Chemistry. The second half of MacKinnon's talk was devoted to how a potassium channel opens in response to a gating signal, widening its entrance to a water-filled protein cavity in the cell membrane. The first half of the talk was devoted to the question that drove MacKinnon through two career changes (from medicine to neurophysiology, and then from neurophysiology to X-ray): how do potassium ions move from the water-filled protein cavity through a selectivity filter "tunnel" to the cell exterior with astonishing selectivity and speed?

MacKinnon showed the remarkable atomic-level correspondence between the cubical cages of 8 carbonyl oxygens lining the selectivity filter and the cubical cage of 8 oxygens in the water normally hydrating a K+ ion. Each protein cage matches the size of the water shell surrounding the K+ ion in its protein cavity, but not of a hydrated Na+ ion water shell. "And so when I look at this," said MacKinnon, regarding the marvelous evolutionary contrivance during his plenary lecture, "what I see is a protein that is really trying to mimic water—but for a potassium ion. "

The filter has evolved to mediate an energetically favorable transition by which the K+ ion sheds its shell of water only to become clothed with a cage of oxygens from the protein. Side-chains anchor the selectivity filter in the cell membrane, said MacKinnon, "and I think that provides the geometric constraints so that these cages are the right-size cages for a K+ ion to easily slip from the water into the protein." Moreover, as one ion slips into the tunnel of the selectivity filter, it electrostatically repels an ion already sitting in the tunnel, creating a "kind of bump-through mechanism that allows the ion to be pushed through the filter," and promoting high throughput.

In the conclusion of his 2003 Nobel lecture, MacKinnon noted that "electrophysiological studies have uncovered a multitude of connections between cellular biochemical pathways and ion channel function"; an "interconnectedness [that]…is beginning to reveal itself as complex and fascinating." At the Ottawa HFSP Awardees meeting, two HFSP Long-Term Fellows presented novel approaches to the challenge of understanding—and even re-designing—the links that connect protein-protein pathways and genomes into the complex networks controlling life processes.

Understanding and re-designing protein-protein interactions

Tanja Kortemme (HFSP Long-Term Fellow, University of Washington, HHMI, USA; currently Assistant Professor, UCSF) likes to summarize a central goal of her scientific investigations with an Italian proverb: "Tell me with whom you go and I'll tell you what you are." The subject of the path-breaking project Kortemme pursued as an HFSP fellow are the molecular interfaces used by proteins to recognize and bind to each other, thus creating the macromolecular complexes and networks underlying cellular regulation and complexity. The Italian proverb, says Kortemme, symbolizes the biological fact "that to truly understand the function of a protein within the context of a living organism, you need to understand the network of interactions it is making."

Working in David Baker's laboratory in Seattle, Kortemme undertook two pioneering and complementary approaches to the structural biology of protein-protein

interfaces. First, she has developed an all-atom computational model for predicting which amino-acid side-chains present at an interface are critical for molecular recognition and binding [Kortemme and Baker *PNAS* 99:14116-14121 (2002)]. Second, in a collaborative project, she used her computational model to design an artificial protein-protein interface that unites two DNA-binding domains that never come face to face in nature. This lab-created chimeric protein provided the first proof of principle of Kortemme's computationally-based design method, and also helped set the stage for the development of a new class of gene-specific reagents for use in research and perhaps also for medical diagnosis and therapy. More recently, Kortemme extended this computational strategy to alter the specificity of an existing interface between two proteins [for review, see Kortemme and Baker *Current Opinion in Chemical Biology* 8: 91-97 (2004)]. Now working in her own lab at UCSF, Kortemme aims to use this computational design method to pursue the long-term goal enunciated in her HFSP fellowship application: to unravel and re-engineer the complex signaling networks operating within living cells, one of the most exciting areas in the new field of Synthetic Biology.

Systematically exploring the links between the genome and a key signaling Pathway

"I think the beauty of biology is the complexity," says HFSP long-term fellow Julia Zeitlinger, "and that the challenge today is how we deal with that complexity." Working on yeast in Richard Young's lab at the Whitehead Institute (MIT), Zeitlinger has been at the forefront of using DNA microarrays to study large-scale, complex molecular and transcriptional circuits. Zeitlinger helped develop a major new method of analyzing transcriptional networks called "genome-wide location analysis." [Ren, B. et al.. Science 290:2306-9 (2000)]. In this method, all the protein transcription factors bound to the genome are cross-linked to DNA, freezing the cells in a certain transcriptional state. The cells are opened, their DNA fragmented, and then specific antibodies to nearly all the cell's transcription factors are used to pull out each DNA fragment to which a transcription factor is bound. The sequences and genomic locations of these DNA targets are then identified by an intergenic microarray that contains not protein-coding gene sequences, but the DNA sequences between genes. Included in these intergenic regions are critical control sequences, called promoters, which regulate transcription through the integrative action of the transcription factors that the promoter binds. Deciphering the genetic regulatory code [Michelson, PNAS 99:546-8 (2002)] somehow embedded in promoters and other control sequences is a central challenge now faced in functional genomic studies.

During her HFSP fellowship, Zeitlinger deployed these tools to study how a single transcription factor elicits completely different behaviors in living yeast cells: mating, performed in response to pheromone signals; and filamentation, in which yeast cells grow out and colonize new areas in response to starvation signals. Zeitlinger showed how, in response to the two different signals governing mating and filamentation, the same transcription factor Ste12 will bind to two different sets of promoters and regulate the expression of two different sets of genes [Zeitlinger et al., Cell 113:395-404 (2003)] Thus Zeitlinger has taken the problem of specificity in complex signaling and transcriptional networks down to the level of DNA regulatory sequences in a genome. Zeitlinger is currently applying these methods to the far more complex networks and genome of the Drosophila fruitfly.

Uncovering a new layer of genetic networks mediated by regulatory RNA molecules

Gerhart Wagner (University of Uppsala, Sweden) described another pioneering investigation into the dark mysteries of the intergenic regions of genomes. Wagner's HFSP Grant team, which included bioinformatics and molecular genetics labs headed by Hanah Margalit and Shoshy Altuvia (Hebrew University—Hadassah Medical School, Jerusalem) , conducted a computationally-guided search of the so-called "gray holes" or "gene deserts" between protein-coding DNA sequences of the E. coli genome. The team was looking for a novel class of regulatory molecules: small antisense RNAs (miRNAs) that inhibit protein translation by binding to target mRNA sequences.

When the team began their search in 1999, said Wagner, "the reports of small RNAs

as bona fide regulators of gene expression was pretty anecdotal." Indeed, in 1999, no one had conducted a systematic search of the E. coli genome—the most intensively analyzed cellular genome in biology—for signs of such regulatory RNAs. Small regulatory RNAs were notoriously difficult to detect both experimentally and by DNA-sequence analysis. "We figured if these regulatory RNA genes exist, and people haven't found them, they're probably located in the empty spaces between genes," said Wagner.

Wagner and his colleagues developed a new computational strategy for identifying candidate regulatory RNA loci in these intergenic "desert" regions, searching for conserved bacterial sequences with predicted promoters and terminators spaced between 50-400 nucleotides apart. They then tested the expression of candidate sequences by Northern blot analysis under several cellular conditions, identifying 14 novel regulatory RNAs (Argaman et. al *Current Biology* 11: 941-950 (2001)). Shortly after they published their discovery, two other groups published their finding of a partially overlapping group of E. coli regulatory RNAs, the first wave in what another researcher would call a "tsunami" of small regulatory RNA discoveries in prokaryotes, plants and animals (Benfey *Nature* 425: 244-5 (2003); see also Wagner & Flardh *TIG* 18: 223-226 (2002)). Indeed, as Wagner said in Ottawa: "There's a whole world out there" of regulatory RNA molecules that conventional genetic and molecular analysis had failed to uncover previously. In bacteria, said Wagner, many researchers are working under the hypothesis "that small regulatory RNAs may be molecules that integrate cellular responses in bacteria," such as "the fine-tuning of cell responses to changing environments."

Oscillating proteins and complex genetic networks

In the second Plenary lecture, Tim Hunt (Cancer Research UK) described the strange 1982 experimental finding which opened a deep new path in understanding how cells divide, work that would lead to Hunt becoming a co-recipient of the 2001 Nobel Prize in Medicine or Physiology. Hunt was examining protein synthesis in newly-fertilized sea urchin eggs, which undergo rapid series of synchronous cell divisions. He added a labeled amino acid to track bands of newly-synthesized proteins on a standard auto-radiographic gel. Most of the labeled proteins got stronger and stronger as time proceeded post-fertilization. But one protein band behaved quite differently. "Here was this band that came up, it was the first one you could detect after fertilization—and then, just before the eggs divided, it disappeared. And the protein then proceeded to come back up again, and go away, and come back up again every time the eggs divided." It was a result, Hunt said, "that sort of knocked you between the eyes."

In a conversation with another scientist that night Hunt learned of a related experimental finding which led him to a startling conclusion concerning this mysterious protein which kept appearing and then vanishing. There was, said Hunt, "only one possible interpretation, bizarre though it seemed: this protein [that Hunt discovered that day] was being made, and then it was being destroyed. And that was unprecedented. Nobody had even thought about that before."

This oscillating protein became known as *cyclin*, and would be found to lie at the heart of the network of proteins that control cell division. A cyclin protein contains two domains, one governing its enzymatic activity, and the other governing its proteolytic destruction. Hunt showed a dramatic movie demonstrating that if the cyclin A protein's "destruction" domain is removed, cells enter mitosis but then get stuck—they never divide in two. Cyclin destruction is necessary in order to complete mitosis.

Elucidating the segmentation clock

In his talk at the Ottawa meeting, Olivier Pourquie (IBDM Marseilles; currently Stowers Institute for Medical Research, USA) presented a slide representing the entry point into perhaps the most remarkable discovery in developmental biology in recent years. Like Tim Hunt's work on cyclin, it all began with the observation of a molecule undergoing bizarre oscillations—in this case the mRNA being expressed from a gene called *c-hairy1.* In 1997, Pourquie's lab found that *c-hairy1* is part of a developmental segmentation clock: a molecular oscillator driving the process by which vertebrate embryos form segmented structures called somites, which underlie the vertebrate body's metameric organization around a series of vertebrae, associated muscles, peripheral nerves and blood vessels.

With an HFSP grant, Pourquie organized an international network of four laboratories to study the nature of the segmentation clock. This has led to a series of new discoveries concerning the clock's molecular cogs and gears. By studying another cycling gene with whimsical name *lunatic fringe,* the network was able to connect to the clock to components of the Notch signaling pathway [Dale et al., *Nature* 421: 275-8 (2003)]. Moreover, Pourquie's group has defined key links between the segmentation clock and the famous Hox gene network that controls the morphological patterning of vertebrate bodies [for review, see Pourquié *Science* 301: 328-330 (2003)].

How do nervous systems represent the world? I. infant perception of language

"Perception works very strangely in adults," said HFSP Grant Awardee Patricia Kuhl (University of Washington, USA). "None of us perceive reality as it is. Instead, said Kuhl, the purpose of perception is to make our interactions with "the world work more quickly and easily." Two studies presented at the Ottawa meeting beautifully explored how the nervous system acts as a selective filter for "reality," remapping the physical input flowing into ears and eyes for adaptive purposes.

Kuhl's HFSP grant team conducted an international study of the development of infant language perception in Japan, Finland, France, Taiwan, Sweden and the United States. "If we want to begin studying language from birth, before a baby is bathed in language," said Kuhl, "you have to study the sound units of language, and not grammar. You can't understand how babies understand grammar" until they begin developing a working knowledge of their native language in their second year of life.

Using clever experimental protocols in which infants are trained to look towards a toy that will light up when a repeated language-sound like *ra-ra-ra* is followed by a different sound like *la*, Kuhl and her collaborators have found that infants 6-8 months old can discriminate sounds from any language in the world. Thus Japanese babies can hear the *ra-la* distinction that is so difficult for adult Japanese; and American babies can hear the Hindi phonological "place-contrast" sounds that adult Americans find nearly impossible to distinguish.

Together, all the world's languages contain some 800 different fundamental elements, 600 consonants and 200 vowels. But each language uses only about 40 basic sounds, a small subset of this larger phonological universe. "Babies start out by hearing all the distinctions of all languages," said Kuhl. "I call them citizens of the world". Then, between 10-12 months old, an infant's perception begins to organize itself around the small dictionary of sounds from the language to which it is exposed. "Experience works to narrow and allow them to generalize and categorize language sounds, so that they hear fewer distinctions over time." However, Kuhl's team found that simply exposing 9 month old U.S. babies to Mandarin Chinese for 12 half-hour sessions spaced over a month enabled the U.S. babies to match

Taiwanese baby performance in discriminating Chinese sounds. "Our hypothesis is that babies don't care whether [the sounds come from] English or Chinese." The language areas of the baby's brain "are using an algorithm that's taking statistics on the phonological input, and they're figuring out something about Chinese contrasts when listening to Chinese, and English contrasts when listening to English."

Kuhl's team is studying both monolingual and bilingual babies over the course of at least four years, enabling both a comparative and longitudinal analysis. "We're seeing a strong correlation between the ability to hear sound discriminations at six months and language performance at two years" in such measures as vocabulary and phrase perception and production. "Sound-perception is the front end of the language machine [developing in the baby's brain], it's the bottleneck. If you don't get through it, you don't acquire language." [for review, see Kuhl (2004) *Nature Reviews Neuroscience* 5: 831-43]

How do nervous systems represent the world? II, visual perception of changing environments

The brain's sole source of information about the visual world arrives through the action potentials transmitted by retinal ganglion cells, whose axons make up the optic nerve. Classical electrophysiological studies in the 1950s revealed key features of the coding rules that retinal ganglion cells employ. HFSP Fellow Toshiko Hosoya (Harvard University, USA; currently RIKEN Brain Science Institute, Japan) discovered, however, that these classical coding rules can be refined within seconds in response to scenes with a specific statistical structure, such as a scene dominated by vertically-oriented shapes. Hosoya's findings, conducted in Marcus Meister's Harvard laboratory and published as an article in *Nature* in 2005 (436: 71-77), are at the forefront of a new wave of investigations demonstrating "that the retina is more clever than was believed before," says Hosoya.

It has been known for a half century that retinal ganglion cells do not signal a pixel-by-pixel description of the raw image intensity of a scene, but rather local contrasts in space and time. The neurons do not respond well to static stimuli, and generally fire action potentials only when there is a local difference in light intensity within the neuron's "center-surround" receptive field--the small region of space the cell surveys. "The retina transmits deviations from the average statistical structure of a scene," Hosoya said in an interview, providing the basic signals that enable the brain to perceive the edges and motion of objects, for instance. "Our question was: What if the statistical structure of a scene changes.... For instance, if you go into the woods, the [tree-filled] scene is vertically very similar, but horizontally not similar."

Hosoya performed multi-electrode recording in isolated salamander and rabbit retinas exposed to simplified, computer-generated versions of such highly-structured scenes, measuring how a ganglion cell's receptive field might change its normal circular, center-surround shape. Remarkably, he found that ganglion cells exposed to a scene dominated by vertical bars, for instance, change their receptive field within seconds so as to become less sensitive to vertical stimuli and more sensitive to horizontal stimuli. In Hosoya's forest analogy, it seems that the retina adapts its coding rules to filter out the unchanging vertical structure of the trees so as to be better able to detect some horizontal shape that might be predator or prey. "Thus," conclude Hosoya and Meister, "pattern adaptation is not merely a scheme for efficient recoding but rather serves to strip from the visual stream predictable and therefore less newsworthy signals."

Hosoya and Meister have proposed an elegant new model to account for the dynamic retinal coding-rule changes they observe, involving plastic changes in the inhibitory synapses whose inputs generate the "surround" of a ganglion neuron's center-surround receptive field. In experimental support of this model, these

dynamic changes are eliminated by pharmacologically blocking the inhibitory neurotransmitters used by the amacrine cells that form these synapses.

Conclusion

Originally trained as a physicist at Tokyo University, Toshiko Hosoya is an excellent example of HFSP's emphasis upon the principles of (in President Ito's words) "internationality, interdisciplinarity and opportunities for young people." In late 2003, Hosoya returned to the United States from Japan to head his own research unit at the RIKEN Brain Science Institute, where he will continue his studies of the vertebrate retina. As a young physics undergraduate, Hosoya began reading and thinking intensively about how the inner logic of neural networks might best be investigated. As a graduate student, he trained as a Drosophila molecular geneticist because "I thought that someday by molecularly [identifying different] neurons and modifying their functions, once could develop a very powerful tool to analyze neural network functions." Hosoya's studies of a gene governing the developmental choice between neurons and glia were highly successful, and he obtained a large grant to continue them. But he felt he needed to learn new theoretical and experimental approaches to neural network function, and concluded that Meister's Harvard lab was the best place in the world for him to pursue this goal. His HFSP long-term fellowship enabled him to go to Meister's lab and change scientific fields for a second time. Now, with his own group at RIKEN, Hosoya plans to combine the sophisticated electrophysiological analysis he learned as a post-doctoral fellow with the genetic analysis and manipulation of neural circuits that he contemplated as an undergraduate and graduate student.

"From the beginning of my scientific career, I wanted to understand how neural networks process information," said Hosoya. "I feel that the research I started through the HFSP [long-term fellowship], is what I've always wanted to do."

Indeed, the interwoven themes of interdisciplinarity, internationality, and opportunities for young scientists were everywhere on display at the Ottawa meeting, in conversation at the poster sessions, coffee breaks and meals. Tanja Kortemme said these intense and collegial discussions across the gamut of the life sciences made this "the best meeting I've ever attended in my scientific career--it was a fantastic atmosphere. And you really got the feeling that you were part of some larger community of international scientists that HFSP is creating."