

# Fourth HFSP Awardees Meeting Hakone, Japan May 15-18, 2004

By Geoffrey Montgomery

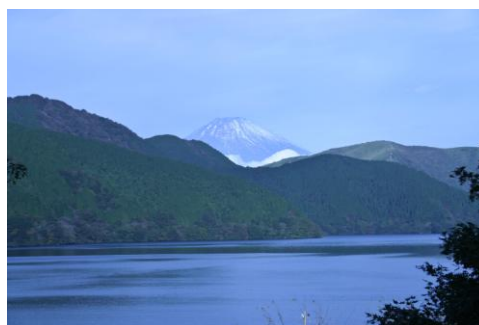
## *Introduction*



Among the opening speakers at the HFSP 15th Anniversary--Fourth Awardees Annual Meeting held in Hakone in May, 2004 was former Prime Minister Yasuhiro Nakasone, whose concept of a new international and interdisciplinary approach to the study of fundamental biological problems launched HFSP fifteen years before. "I think Mr. Nakasone is in a true sense a visionary," said Torsten Wiesel, Secretary General of HFSP. "To start an international program where scientists from different continents and different disciplines would collaborate, and in addition would carry out frontier research in the life sciences—that must have seemed like a dream that would be difficult to realize. But I think the dream has been realized...that the program has been a resounding success."

Of course, the success of ongoing investigations into fundamental biological problems is measured as much by the new questions and avenues of research opened up by new discoveries as by the discoveries themselves. This report aims to give a sense of a representative sample of the scientific studies presented at the Hakone meeting, and is not intended to be comprehensive. While the HFSP programs of scientific support take a unified, interdisciplinary approach to the complex mechanisms of living systems, rather than pigeonholing projects by traditional academic or disciplinary divisions, it is convenient for the purposes of this report to discuss the projects presented at Hakone according to a few broad domains of biological exploration.

The meeting was held at the Hakone Prince Hotel, whose beautiful lawns and paths stretched down to Lake Ashi and wove into paths through the forests of the surrounding national park. For those attendees staying in one of the traditional cabins located on a steep woody hill on the hotel grounds, it was like stepping inside a classical Japanese painting of human



habitations in harmony with nature. Mist, fog and light rain enveloped this lovely setting for much of the meeting, but the sound of the rain and silvery aura of the hotel's lamplights at night had their own charms, and only made several clear mornings and afternoons more dramatic, with many attendees going outside to look at the white-shouldered immensity of Mount Fuji looming across the lake.

The first part of the meeting was dedicated to the 15<sup>th</sup> anniversary of the meeting and featured presentations by former Prime Minister Nakasone and several leaders of Japan's major funding and scientific agencies. The scientific meeting itself consisted of talks and poster presentations by HFSP awardees, plenary

lectures by Christiane Nüsslein-Volhard and Nobutaka Hirokawa, and a special keynote lecture by Tadamitsu Kishimoto on “Challenges to Immune Disorders.”

*Guardians of the brain*

The opening plenary lecture was given by Christiane Nüsslein-Volhard, co-recipient of the 1995 Nobel Prize for Medicine or Physiology for her pioneering discovery of the network of genes that lay out the spatial organization of the early *Drosophila* embryo. Since then, Dr. Nüsslein-Volhard has been a leader in the scientific development of a relatively new model genetic system, the zebrafish, *Danio rerio*.



The zebrafish embryo is transparent, making it perhaps the best higher organism in which to study the processes of long-distance cell migration that characterize the development of many vertebrate organ systems and body structures. Dr. Nüsslein-Volhard told three stories of cell migration being studied in her laboratory: germ cell migration; the amoeboid-like migration of the macrophage-precursors to microglia, so-called “guardians of the brain” that serve as an innate immune system and provide the brain’s primary defense against infection, as well as interacting with glia and neurons in brain development and physiology; and lateral lines, which serve as the mechanosensory “ears” arrayed in specific locations along the fish’s body. Each lateral line is composed of three different cell types - sensory organ, nerve and glia - that must co-migrate across the embryo to these locations. Like the sound-sensing hair bundles in our inner ear, Nüsslein-Volhard explained, lateral lines are actually hair bundles which are in the skin and stick out, and sense changing rates of water flowing and vibrating around the body. Using an elegant combination of mutants, fluorescent protein expression, and cell ablation studies, Nüsslein-Volhard demonstrated how a migrating sensory primordium “tows the neurons to the point of destination,” which in turn guide the supporting glial cells’ migration. As in migrating germ cells, the sensory primordia apparently use a receptor called *Odysseus* to guide them on their embryonic journey (in *odysseus* mutants, these cells emulate Homer’s hero by wandering the Aegean sea of the embryo like lost windblown ships). “This coordinated migration ensures the correct innervation and connection of the sensory organ,” said Nüsslein-Volhard, “and we are now looking for other cases where such a mechanism might be used ...for proper axonal pathfinding.”

Analysis of the molecular signaling networks underlying these cellular migrations in vertebrates is still in its relatively early stages, and will benefit from the recently sequenced zebrafish genome.

*Deciphering  
Transcriptional and  
Signaling Networks*



A sense of post-genomic possibilities being realized was given in the opening awardee’s talk of the meeting, by Career Development Award recipient Jan Lohmann, who heads a new laboratory at the Max Planck Institute for Developmental Biology in Tübingen. Lohmann described his laboratory’s ongoing decipherment of the transcriptional network underlying the balance between stem cell proliferation and differentiation in *Arabidopsis thaliana*, the leading genetic system in plant biology.

In plants, all adult structures are generated from root and shoot meristem centers, making them a superb system for studying basic aspects of stem cell biology, with

important potential applications to agriculture and ecology. As an HFSP long-term fellow, Lohmann helped unveil the basic genetic pathway by which flowers differentiate from stem cells in shoot meristem. Three key genes, all encoding transcription factors, were identified by mutant studies. One gene, *Wuschel*, is critical for stem cell maintenance; when a stem cell center also expresses a second gene, *Leafy*, a shoot meristem is induced to express a third gene, *Agamous*. “*Agamous* has a dual role,” Lohmann explained, “being a patterning gene necessary to specify floral organs [by activating a set of flower differentiation genes], but also necessary to switch off stem cell maintenance,” by repressing the *Wuschel* gene. Together, these genes and transcription factors comprise three critical nodes in a most elegant feedback network; but how are these nodes linked? Flower development is critically dependent on such environmental factors as light and temperature: how do these environment inputs enter this transcriptional network, and what is the nature of the molecular circuitry connecting it?



By using DNA microarrays in a combination of mutant and wild-type plants and conducting a comparative analysis, Lohmann was able, for instance, to find four closely related genes encoding hormone signaling components as potential target genes of *Wuschel*. “We’re very excited about these genes,” Lohmann said, “because they have been implicated in the negative feedback regulation of [a plant hormone] that has been known for more than fifty years to be involved somehow in stem cell regulation—so this makes quite a bit of sense.” These studies have recently been published in *Nature* (438: 1172-1175 (2005)).

A poster by Career Development Awardee Ana Caño-Delgado from Barcelona told another side of plant stem cell differentiation: how so-called procambrial cells give rise to the plant’s vascular system, the water-conducting xylem, and the photo-assimilate (products of the photosynthetic cycle)-conducting phloem. “Basically a plant takes water through the roots,” said Caño-Delgado, “which is transported through the xylem to the aerial parts of the plants where photosynthesis occurs, and after photosynthesis the photo-assimilates go to all the organs in the plant through the phloem. So my interest is how the phloem and xylem tissues are differentiated in an ordered pattern from the activity of the procambrial cells. This is completely unknown. There are many studies in physiology that have suggested an important role [in this process] for hormones such as auxin, but my recent data suggest that steroid hormones also have a role in the differentiation of the procambrial cells into xylem and phloem tissues.”

New insights into the signaling pathways and networks mediating a host of basic biological processes were presented by HFSP Fellows and Grantees. For instance, Long-Term Fellow Katja Brückner (Harvard Medical School, USA) studies of *Drosophila* hematopoiesis extended known-parallels to the signaling pathways underlying blood cell development in mammals. Dr. Brückner showed that the *Drosophila* PDGF/VEGF receptor, known for its role in guiding cell migration in *Drosophila*, also controls cell survival in embryonic hemocytes through an anti-apoptotic mechanism. This work “establishes *Drosophila* as a model to study hematopoietic cell survival in development and disease.” Long-Term Fellow Pascale Dijkers (UC San Francisco, USA) developed a novel assay system, in which a fluorescent-tagged transcription factor translocates to *Drosophila* cultured cell nuclei in response to decreased oxygen levels, to study cellular responses to low levels of oxygen. These studies demonstrate that “nitric

oxide is indispensable for mediating signaling responses to hypoxia,” and have uncovered other potential components of the hypoxia signaling pathway, including a calcium-regulated phosphatase. Working in the green alga *Chlamydomonas reinhardtii*, Long-Term Fellow Angela Falciatore (University of Geneva, Switzerland) studies shed new light on the signaling pathways regulating chlorophyll synthesis. Chlorophyll is synthesized only in chloroplasts, yet most of the enzymes involved in this synthetic pathway are encoded by nuclear genes. How do these two cellular organelles communicate with each other in response to metabolic and environmental changes? Dr. Falciatore discovered that both light levels and signals from the chloroplast help regulate chlorophyll biosynthesis by regulating alternative splicing and expression levels of a single nuclear gene encoding two so-called FLP proteins. Additional experiments suggest that FLP regulatory proteins “may also have a protective role against photo-oxidative damage and act as stress proteins.”

The systematic elucidation of the basic transcriptional and signaling networks controlling development in several key model organisms has made possible the new field of “evo-devo” (evolutionary-developmental biology), by which biologists seek to unveil how variations on these well-established developmental themes underly the evolution of new morphologies. For instance, the conserved Hox cluster genes, originally discovered in *Drosophila*, control body-patterning along the anterior-posterior axis of all bilaterally-symmetric animals. Six conserved intercellular signaling pathways—the Notch, Wnt, EGF-Ras, Hedgehog, TGF-dpp, and insulin pathways—are deployed multiple times in different tissues within a single developing animal species, whether insect or mammal, and also determine cellular diversity between these species. How do these conserved gene and protein networks produce phenotypic diversity? And what does the evolutionary adaptability of these networks reveal about the molecular logic and architecture underlying biological complexity? Grant Team Principal Investigator Bern Schierwater (Tierärztliche Hochschule Hannover, Germany) presented his team’s intriguing explorations of Hox body-patterning genes in *Trichoplax adhaerens*, the only known species of the phylum Placozoa, which looks like a large, multi-cellular amoeba and is “by far the most simply organized metazoan—it lacks a head, foot, any kind of symmetry, and any kind of organ.” Dr. Schierwater’s team discovered that *Trichoplax* possesses only a single “Proto-Hox” gene, Trox-2, which is expressed at the periphery of the body in a mysterious inner layer of cells that may constitute a “hitherto unrecognized population of possibly multi-potential peripheral stem cells.” Inhibition of Trox-2 expression by RNA interference and antisense oligonucleotides completely stops the animal from growing and from reproducing by binary fission. These studies of this primitive, shapeless beast have opened a new perspective on the evolutionary origin of Hox-class genes and animal body plans., provided new evidence that these creatures are “living fossil” representatives of a basal animal phylum, and helped establish *Trichoplax* as an important new model system for research in development and evolution. Long-Term Fellow Yoshinori Tomoyasu (Kansas State University, USA) described his innovative studies, recently published in *Nature* (433: 643 – 647 (2005)), of the Hox-controlled genetic network accounting for how beetles got their strange forewings (used for body protection) and hind-wings (used for flying) as compared to *Drosophila* (which like all flies use their fore-wings for flying and vestigial hind-wings as flight balancing gyroscopes). Dr. Tomoyasu’s work has revealed that the Hox gene *Ultrabithorax*, which plays no role in the development of *Drosophila* wings, plays a directing role in the genetic network that produces beetle wings.

Grant Team Principal Investigator Marie-Ann Felix (Institute Jacques Monod, France) described her team’s extensive cellular, molecular and phylogenetic

investigations of vulval development in a host of nematode species. Two decades of intensive studies in *Caenorhabditis elegans* have demonstrated that the fates of the small cluster of cells making up the vulva are determined by multiple, converging intercellular signaling pathways. Dr. Felix's comparative studies are helping to reveal how these conserved signaling pathways can undergo adaptive change during evolution; how developmental redundancy in these pathways ensures robust cell fate outcomes despite stochastic developmental noise and environmental variations; and how the properties of this developmental system may bias the way in which the genetic variations underlying evolutionary change produce the phenotypes upon which natural selection acts.

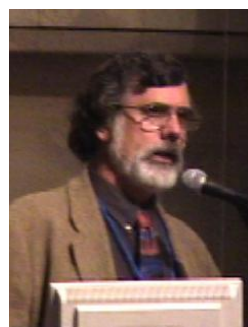
The evo-devo field emerged in the early 1990s in part through the kind of naturalist inclinations that drove Darwin aboard the *Beagle*; like the youthful Darwin himself, for instance, Dr. Tomoyasu confessed to a personal fascination with beetles. At the same time, such evolutionary studies are increasingly seen as fundamental to the problem of how complex gene and protein networks are structured: for the very architecture of these networks must somehow reflect the evolutionary processes that built them. Deciphering the combinatorial, modular logic of biological complexity and unraveling the adaptable processes of evolution are in many respects two sides of the same coin.

Perhaps the most fundamental revelation of the post-genomic era of molecular-network analysis is that genomes are full of "dark matter": microRNAs regulating gene expression that had remained largely



undetected for forty years, and which comprise a heretofore hidden layer of the genetic networks of cells and developing organisms. In Hakone, Long-Term Fellow Javier Palatnik (Tubingen, Germany) presented his pioneering studies of the JAW microRNA in *Arabidopsis*. JAW appears to be essential to leaf morphogenesis; a News and Views article accompanying the 2003 *Nature* paper published by Palatnik and his colleagues commented that studies of JAW "provide one of the most compelling cases that the newly discovered microRNAs have an important role in controlling development." In addition, initial studies of JAW's messenger RNA targets (made possible by genomic sequence analysis) provide hints that the layered complexity of the networks controlled by these novel regulatory molecules—in which families of microRNAs may bind to overlapping sets of target mRNA sequences—might rival the regulatory networks controlled by traditional transcription factors in their flexible evolutionary complexity.

*Cellular and Systems  
Neuroscience*

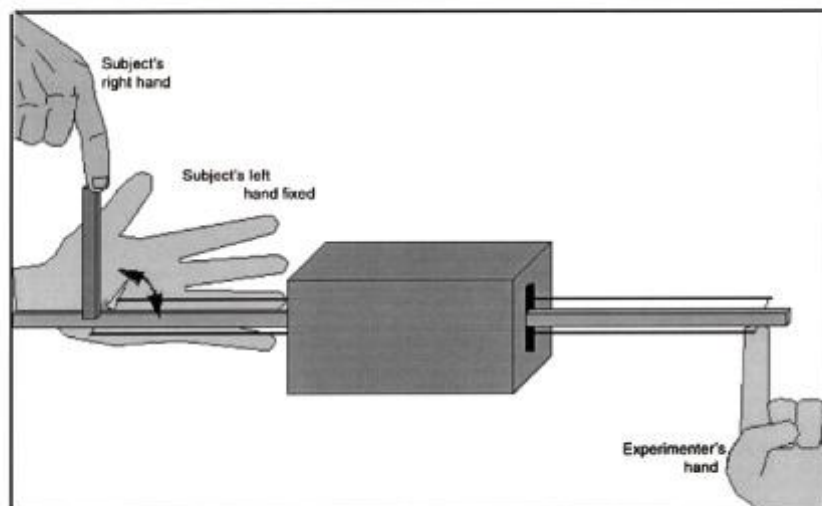


In a fascinating talk, Grant Team Investigator Bruce McNaughton (University of Arizona, USA) described recent advances in understanding how experiences lay down memory traces in the neural circuitry of the brain. McNaughton has been a pioneer in showing that when a novel experience (such a maze exploration by a rat) evokes specific patterns of neural activity in the hippocampus --a brain region known to be necessary for the formation of long-term episodic memories in humans and other mammals--aspects of this activity pattern are replayed during sleep or restful wakefulness in the period following this experience. McNaughton and his colleagues use multi-electrode recording and sophisticated



statistical analysis of cross-correlated neural activity to explore “the relationship of this neural activity during behavior to the subsequent activity one sees when the animal rests. We want to examine whether or not patterns that occur during behavior and are presumably stored there [in the brain], reappear when the animal sleeps or rests after the experience, when its brain isn’t busy processing the outside world.” A major aim of the HFSP-supported work was to extend previous findings in the hippocampus to other brain regions, including subcortical brain regions known to be involved in integrating emotions, motivational signals and environmental input to direct behavior. The results McNaughton described were encouraging. “This gives us some indication that during its rest periods, the brain is in a state where it is retrieving, and rehearsing and perhaps re-wiring its memory system, and that this phenomenon is pervasive. It’s not limited to the hippocampus, but it affects wide-spread, although perhaps not all regions of the brain.” A major question for future work, said McNaughton, is analyzing the mechanism by which such neural activity pattern replay leads to memory consolidation in circuits distributed throughout the brain.

## *Why can't you tickle yourself?*



Blakemore *et al.*(1998) Nature Neurosci 1:635-640

In another intriguing presentation, 1999 Long-Term Fellow Kazuhiko Seki (formerly at University of Washington, USA and now at the National Institute for Physiological Sciences, Okazaki, Japan) opened his talk with a riddle: “Why can't you tickle yourself?” Seki described an experiment in which a lever draws a tickling implement over the palm of a subject’s left hand. If an experimenter pushes the lever, the subject feels a tickling sensation. However, if the subject pushes the lever with his or her own right hand, “this tickling sensation is much less.” Apparently some process of “sensory gating” is at work, whereby the brain sends inhibitory commands to suppress information coming from peripheral sensory circuits into the spinal cord: in other words, when desirable, the brain can exercise some degree of top-down inhibitory control over the information entering the peripheral nervous system. From the classical textbook presentation, one would expect such inhibition to be mediated by an inhibitory neuron feeding into a post-synaptic cell, where excitatory and inhibitory inputs are normally integrated. Yet, Seki explained, post-synaptic inhibition would offer quite a crude form of sensory gating: it would suppress all sensory inputs feeding into the post-synaptic cell in the spinal circuit. However, pre-synaptic inhibition mediated directly through the sensory axon feeding into this spinal circuit cell would be a much more specific

form of sensory gating, said Seki, “because you can suppress specifically this neural input without affecting other inputs.” While presynaptic inhibition was first described at the cellular level in 1957, Seki and his colleagues’ work has provided the first experimental demonstration of its behavioral relevance. Seki pioneered a system for studying presynaptic inhibition in monkeys trained to execute movements of their wrist. A *Nature Neuroscience News and View* commentary (6: 1243 (2003)) accompanying Seki’s paper remarked: “This is no small feat and is arguably one of the most challenging experimental preparations in neuroscience today.” The commentators added: “the techniques pioneered by Seki and colleagues to record neural activity in the spinal cord of awake, behaving non-human primates will continue to lead to important advances in understanding spinal function during volitional motor control.”

In other investigations of higher human brain function, Career Development Award recipient Katsuyuki Sakai, (Graduate School of Medicine; Tokyo, Japan) described functional magnetic resonance imaging (fMRI) studies on goal-directed behavior that he conducted as a Long-Term Fellow in London. Sakai’s findings indicate that the anterior prefrontal cortex “plays an important role in setting up future tasks by controlling the activity of different posterior brain regions according to the kind of task that the subjects are to perform. Given that the prefrontal cortex receives converging inputs from all parts of the brain, this region is unique in that it is capable of representing such highly abstract information and can be regarded as the key structure for goal-directed behaviors.” In Tokyo, Sakai plans to pursue the dynamic, goal-gated activity of these complex neural pathways using additional methods such as electroencephalography (EEG) that will provide higher temporal resolution.

Young Investigator Grant Team Principal Investigator Kuniyoshi L. Sakai (the “L.” was added, said Dr. Sakai, in order to inhibit neural confusion with the above Dr. Sakai who spoke before him) described his team’s pioneering and multi-faceted studies of the neural basis of syntactic comprehension in human language. The unique syntactic power of human language produces what Dr. Sakai called: “Discrete Infinity in the Brain: the ability to create infinitely many expressions from a finite store of memorized units (words).” But are these powers of syntactic arrangement and hierarchical nesting (recursion) dependent on general attention and memory processes in the brain? Or do they arise, as long proposed by Noam Chomsky, through brain processes specifically devoted to syntax, a “universal grammar” of human languages? Using an innovative combination of brain imaging and brain stimulation studies, Dr. Sakai and his team showed “for the first time that the human left prefrontal cortex is uniquely specialized in syntactic processing. Which means that activation [in this brain region] cannot be explained by general brain processes of memory, attention, learning.”

HFSP awardees also presented a number of novel approaches to the cellular and molecular study of neural processes. Long-Term Fellow Fekrije Selimi (The Rockefeller University, USA) presented a progress report on her highly ambitious efforts to unravel the molecular basis of synaptic specificity. She opened her talk with a beautiful Golgi-stain atlas of the mouse brain—a multi-tiered forest of twisting neural connections, dark glades of neurons interconnected by spidery arborizing axons—that laid out the challenge: neurons in the brain are of many specific types, interconnected in an exquisitely specific fashion. In the cerebellum, for instance, where Selimi has concentrated her studies, Purkinje cells receive precise synaptic input from four different kinds of neurons. “Moreover,” said Selimi, “all of those different connections are made on precise locations on the Purkinje cells.” For instance, one type of neuron connects to Purkinje dendritic

spines, a second type to its cell body. Yet during brain development, each neuron has many potential choices of a synaptic partner. “So what are the cues for such precise connections?” Is there “a different molecular code for each synapse of a specific type of neuron?”

In order to pursue this question, “we need to be able to purify a given synapse from the brain,” said Selimi, “and to do this, we had to develop a new experimental strategy.” Using knowledge of some of the receptors present at certain cerebellar synapses, Selimi generated different lines of transgenic mice with these receptors fused to Venus (a yellow fluorescent protein variant), which allowed biochemical purification of synaptosomes containing these receptors from the mouse brain. “The specific purification of a given synapse is possible!” was the heading on one of Selimi’s slides. The challenge now is to refine mass spectroscopic techniques for analyzing the molecular content of different purified synapses, as well as to find more synapse-specific markers” said Selimi.

A commentary to a 2003 *Nature Neuroscience* paper published by Long-Term Fellow Adi Mizrahi (Duke University, USA) asked “After development, how does [the brain] retain the flexibility to learn second by second, while preserving the fidelity of networks that store memories and direct behaviors over days and years?” . Mizrahi’s innovative imaging of specific neurons over time in mouse olfactory cortex has provided powerful new insights into this central question. Like the mammalian hippocampus, olfactory cortex is the site of adult neurogenesis—the creation of new neurons and synaptic connections from self-renewing neural stem cells—as well as learning-induced synaptic changes. Yet, what happens to the dendrites of existing neurons in this brain region as new cells are produced by neurogenesis, or when learning occurs? Pursuing this question required the ability to look at what happened to the dendrites of specific brain neurons over days and weeks. Mizrahi used existing lines of transgenic mice in which a small sub-population of olfactory cortex cells were labeled with fluorescent protein. Then he used two-photon imaging, “which allows you to put a detector very close to the source of [fluorescent] emission, and get super-high sensitivity compared to confocal imaging. So you can go very deep into brain tissue and it’s perfect for imaging living cells, because the damage [from laser-excitation of this fluorescence] is minimal.” Mizrahi also used a custom-built stereotactic device to place a living mouse’s head in the same spatial orientation for imaging studies over time. “So we can image the same cells a month apart, two months apart, at micrometer resolutions. You can teach the animal stuff, do whatever experiment that you want, and then go back to the same cells” and see how its dendritic structure may have changed. Future directions for this imaging method include the possibility of tracking gene and protein expression in living, individual neurons in the brain over long periods of time.

Remarkably, in his initial studies of olfactory cortex, Mizrahi found that while the dendritic structure of neurons could be altered by artificial pharmacological methods, “under natural challenges including neurogenesis, an odor enriched environment and olfactory-based learning, M/T cell dendrites [the synaptic connections being studied] remained highly stable. Thus, in a context of ongoing adult synaptogenesis, dendritic stability could serve as a structural scaffold to maintain the organization of local circuits.”

“The adult brain is clearly able to engage a range of plasticity responses,” wrote the commentators to the *Nature Neuroscience* paper in which Mizrahi and Lawrence Katz presented these conclusions. “Moreover, it is able to do this in a manner and to a degree that does not imperil network integrity and disrupt



ongoing learning and behavior; in the case of adult neurogenesis, such tight regulation of plasticity may even be a prerequisite for effective integration into established circuits. ...After all, change endures, but the [neural] slate is not wiped clean.”

*Structure and Dynamics of  
Complex Molecular  
Mechanisms*

Among the most exciting studies presented at the Hakone meeting were a number of novel chemical and physical approaches to the dynamic molecular complexity of living cells. Grant Team Principal Investigator Angus Lamond (University of Dundee, UK) described his team’s studies on the “dynamic nucleus.” Rather ironically, while the complete DNA content of nuclear genomes have been sequenced in a host of higher eukaryotes, the nucleus remains the most poorly characterized organelle in higher cells. Using the ribosome-synthesizing nucleolus as a model system, Lamond’s team has used an interdisciplinary combination of cell-biology, advanced light-microscopy with time-lapse image analysis, proteomics and mass spectrometry to launch “a concerted attack on the dynamic organization of the cell nucleus.” Lamond emphasized that while the nucleus is highly organized, characterized by sub-nuclear structures whose exact function is under intense investigation, “within this internal organization, proteins move around and exchange” back and forth between sub-nuclear structures and the surrounding nucleoplasm. His team concluded that, for instance, there is not a fixed set of proteins (known as the “proteome”) in the nucleolus: rather, it is dynamic, with proteins flowing in and out, presumably in response to different cellular demands and conditions.

Young Investigator Dominique Fourmy (ICSN-CNRS, France) described innovative studies of the molecular movements essential for ribosome-mediated translation of RNA, conducted with a team consisting of chemist Joseph Simpson (UC San Diego) and physicist Koen Visscher (University of Arizona). In one project, the structure of the RNA signal used by the HIV virus to promote –1 translational frame-shifting (a process essential to viral replication) was solved by NMR spectroscopy. By combining structural and biochemical approaches with physics-based “optical tweezers” techniques that allowed them to explore ribosome translocation along a single, stretched messenger RNA molecule, the team has observed backward, force-induced slippage of individual ribosomes, a process that may have important implications for understanding the molecular nature of frame-shifting in viral replication and other biological contexts.

Long-Term Fellow Jerome Boisbouvier (NIH; currently at Laboratoire de RMN, Grenoble, France) described the development of an important new method for accurately measuring long-range distances and angles in nucleic acids and proteins using cross-correlated NMR spectroscopy. The method allows the observations of dipolar couplings between protons separated by as much as 12 angstroms, and has been demonstrated to be effective with both DNA and RNA molecules. In addition to providing more accurate distance information for determining the geometry of local structures than previous methods, Boisbouvier notes, “this information will be particularly useful in the assembly of fragments of well-defined local structure, such as helical regions in complex nucleic acid structures, or elements of secondary structure in proteins.”

Long-Term Fellow Antoine Van Oijen (Harvard, USA) described his studies “watching single enzymes at work on DNA” by biophysical analysis of various components of viral DNA replication machinery, studies performed in close collaboration with a leading structural biologist and an enzymologist. “Single molecule biophysics has been around for the last 10 years, and there have been huge developments in this field,” said Van Oijen. “What I’d really like to do is to

develop these techniques beyond the point where people look only at single molecules..” Nearly all biological processes are carried out not by isolated proteins, but by protein complexes whose exact constituents often change as the process proceeds. The challenge now, said Van Oijen, is to begin to reconstruct this multi-protein dynamic complexity in *in vitro* systems amenable to biophysical analysis. techniques.” Van Oijen will be pursuing this challenge at his own lab at Harvard, where he has been offered a faculty position.



In the Hakone meeting’s final plenary lecture, Nobutaka Hirokawa (Tokyo University) gave a *tour de force* presentation of the many dynamic faces of a single family of proteins, the kinesin molecular motors. He began by showing a movie of kinesin motors zipping along microtubules in living cells, with dynein molecular motors moving in the opposite direction. “It’s like a freeway,” said Hirokawa, “traffic is mixed, it seems very dangerous!” Over thirty different forms of kinesin are used by mammalian cells to transport organelles and other cargo. In neurons, for instance, kinesin is used to transport mitochondria and precursors of synaptic vesicles from the cell body to the synapse. Hirokawa’s laboratory has been the world-leader in studying how the many different classes of kinesin perform these precise transport duties. Long-term memory formation in the hippocampus, for instance, depends on the simultaneous activation of both AMPA and NMDA receptors on the same post-synaptic cell. Hirokawa showed that within the same neuron, one class of kinesin [KIF-5] transports AMPA-receptor containing vesicles to synaptic membranes, while a second class of kinesin motors [KIF-17] transports NMDA-receptor vesicles. He then showed a movie comparing the water-maze learning skills of a normal mouse compared to one transgenically-engineered to overexpress the NMDA-transporting KIF-17 kinesin protein. Remarkably, the learning and memory skills of the transgenic mouse were dramatically enhanced. “Very smart,” said Hirokawa. “So what happens in this guy’s brain?”

By looking at biochemical signaling pathways known to be activated by the NMDA-receptor mediated learning, Hirokawa showed how the increase in this specific kinesin protein had triggered a positive feedback loop of molecular pathways underlying learning and memory.



While humans have dozens of different kinesin motors for carrying different cargos along microtubules in different cell types, the human genome encodes only a single form of the dynein motor that runs along microtubules in the opposite direction. Dynein is much bigger than kinesin, and much more complicated in terms of the proteins with which it complexes. Moreover, while kinesin possesses only a single ATP-binding site for burning cellular fuel to power its molecular walks along microtubules, dynein possesses four additional ATP-binding sites. In a stunningly elegant set of experiments presented in the last awardees’ talk of the Hakone meeting, Long-Term Fellow Roop Mallik (UC Irvine, USA) described how these extra ATP-binding sites may underly an unprecedented property of dynein motors that he discovered: dynein’s ability to act as a self-regulating gear which adjusts its “step-sizes” along microtubules in response to changing cargo-loads. The overexpression of one form of the kinesin motor may make mice smart, but Mallik seems to have discovered how biological evolution has worked to weave “intelligent” machine

design into a single protein.

“We use gears all the time in our daily life,” said Mallik in an conversation after his talk. “And the idea that Nature has ‘thought’ of that a long, long time ago, has implemented a gear at the level of a few nanometers—that’s very exciting to me.” Mallik plans to use the third year of his fellowship upon his return to his native India, where he will continue his studies of molecular machines using such advanced biophysical methods as laser-based optical traps. “Trying to understand complexity in biological systems, that is my main goal,” he comments. “In vivo, inside the cell, we know that there are multiple motors that are working together and may be coordinating and collaborating with each other—but we don’t know how that’s happening. My feeling is that we need to take things to the next level of complexity”; to analyze a few motors acting simultaneously on a single cargo perhaps along with a regulatory factor such as dynactin, a giant protein complex that is crucial to dynein function in vivo but whose role remains quite mysterious. Indeed, Mallik and his lab supervisor Steven Gross recently wrote a review article speculating about how some of the unique features of dynein may enable it to prevent the molecular motor “traffic jams” alluded to by Hirokawa. Mallik thinks that the next generation of experiments, which he has already begun, should be “aimed at a level which is in between single molecule studies and very complex systems—complex but not too complex. That is the next step which I want to attempt to take.”

As Young Investigator Derek Toomre (Yale, USA) said in an informal conversation over a traditional Japanese breakfast: “Bringing together fields that presently don’t connect is what the HFSP is all about, and this meeting is really a nice example of these kind of interactions and cross-talk. You get to meet all these talented people working in areas outside your own, people from many different countries.” Toomre felt that the Hakone meeting mirrored the international and multi-disciplinary interactions among members of his own grant team, which is studying the dynamic regulation of intracellular membrane traffic. This HFSP grant awarded in 2003 has brought together three scientists from the U.S., France and Switzerland with expertise in cell and molecular biology as well as computer vision and computer modeling. “The multidisciplinary aspect, this is the critical element,” said Toomre. Synergistically combining methods from different fields is “enabling us to begin to do things we would never have envisioned” a few years ago, to study a complex biological process with “much more rigor and to develop a new [experimental] tool kit not only for us but for the larger community.” This is indeed the spirit of innovation through interdisciplinary collaboration that the HFSP aims to foster.