Third HFSP Awardees Meeting
Cambridge, UK
July 6-9, 2003

By Geoffrey Montgomery

Introduction

The Third HFSP Awardees' Annual Meeting, held in Robinson College, University of Cambridge on July 6-9, 2003, included an informal afternoon excursion to a local site as legendary in the modern history of biology as any laboratory: the Eagle Pub. It was here that Francis Crick and James Watson held many of their discussions concerning the quest to solve the molecular structure of DNA. And it was through the Eagle Pub's doors that Crick famously burst one February day in 1953, in the immediate aftermath of the discovery of the DNA double helix, declaring: "We have discovered the secret of life!"

The visit by HFSP awardees to the Eagle fifty years later provokes the question: where does the science of life stand now? Certainly all those who attended the meeting would agree with the hard-won wisdom that Francis Crick himself expressed in a conversation with this writer in 1998. "The general thing we have learned over those years," said Crick, "is that biological systems are a lot more complicated than you would have imagined…. I mean, I started out as a physicist, and I must in the back of my mind have thought that biology was simpler than it was. And we're lucky in the case of DNA, it *was* simpler than you might have expected, you see." Crick gave a high-spirited laugh. "But I hope one's learned one's lesson by now."

The Watson-Crick DNA double-helix offered a static model of the "secret of life" in which genetic information is inscribed in a one-dimensional sequence of nucleotide bases. The talks and posters of HFSP awardees fifty years later offered new interdisciplinary experimental approaches and theoretical models aimed at understanding the four-dimensional dynamic complexity of actual living systems.

How is DNA dynamically packaged and made available for use in the chromosomes of higher organisms? What specialized molecular environments enable the proteins encoded by DNA to fold into their mature three-dimensional form? How might the translation of these proteins be regulated in neurons to promote the synaptic plasticity underlying learning and memory? Do the molecular networks characterizing complex biological systems contain basic building blocks, network "motifs" analogous to the sequence motifs inscribed in one-dimensional DNA and protein sequences? Is it possible to study patterns of neural activity and axonal connections simultaneously in the most complex system in the known universe—the human brain?

These are some of the fundamental questions that were addressed by HFSP awardees. The meeting also featured opening and closing remarks by two leaders in UK life sciences: Julia Goodfellow (Biotechnology and Biological Sciences Research Council) and Sir George Radda (Medical Research Council). This report is not intended to be comprehensive: abstracts of all talks and posters are collected on an accompanying **link.** Rather, this report will highlight a few projects embodying some of the major themes of the frontier biological research discussed in Cambridge, including the studies of the two distinguished plenary speakers, both of whom have received support from HFSP.

Biological Energy

John Walker (MRC Dunn Human Nutrition Unit, UK) received the 1997 Nobel Prize in Chemistry for his structural studies of the key enzyme that catalyzes the synthesis of ATP, the energy currency of all cells. Until his recent move to the MRC Dunn Unit, all of Walker's work on ATP synthase was carried out at Cambridge's MRC Laboratory of Molecular Biology, the descendant of the Cavendish unit where the double helix was discovered and the first protein X-ray crystal structures solved by John Kendrew and Max Perutz. Walker's presentation beautifully illustrated the 21st-century challenge of understanding the dynamic details at the heart of living processes.

Whether derived from sunlight or respiration, Walker explained in his plenary lecture, the wellsprings of biological energy flow through a proton gradient or "proton motive force." The question addressed by Walker's studies is how the energy stored in this proton gradient in transferred into the chemical bond energy of the phosphate added to ADP (adenosine diphosphate) to make ATP. Walker's group showed that the protein subunits of the giant ATP synthase enzyme can be divided into four basic mechanical elements: a rotating ring or *rotor* embedded in the inner membrane of mitochondria is attached to a *stalk*; this stalk is implanted in a catalytic *head* fixed in place by a *stator* clamped to a membrane-anchored protein. Protons passing down an electrochemical gradient and through the enzyme cause the rotor to spin, which in turn causes the attached stalk to spin inside the fixed catalytic head. "So you are full of these little devices whirling around at 200 times a second," said Walker. Three of the six head sub-units have binding sites for ADP and phosphate, and Walker described experiments demonstrating how the rotary motion of the stalk is used to drive repeating six-step cycles of ATP synthesis and release inside the head. He also described evidence implying the existence of a "molecular spring" between the rotating ring of the rotor and its attached stalk. "The idea is: the ring is rotating and winding up the spring, and when the spring reaches a critical tension, click, it moves through 120 degrees," moving the stalk from one ATP-binding head subunit to the next. However, the nature of this molecular spring, and thus the roots of the rotation driving ATP synthesis inside the head, remains poorly defined. At the end of his 1997 Nobel address, Walker quoted a short poem by Robert Frost to describe this continuing molecular mystery, and this poem remains apt both for Walker's studies and many others described at the Cambridge meeting:

We dance round in a ring and suppose
The secret sits in the middle and knows

A Novel Model of Chromosome Dynamics

Twenty years after the Watson-Crick double helix discovery, two other workers at the Cambridge MRC Laboratory of Molecular Biology, Roger Kornberg and Aaron Klug, defined the basic packaging unit of DNA in the chromosomes of higher organisms, the nucleosome core particle made up of an octamer of histone proteins. In 1997, the high-resolution crystal structure of the nucleosome was solved in the ETH Zurich lab of Timothy Richmond. In 2000, HFSP Fellow **David Fitzgerald** traveled from the U.S. to Richmond's laboratory to explore the regulation of nucleosome arrangement on chromosomes by multi-protein machines called chromatin remodeling complexes.

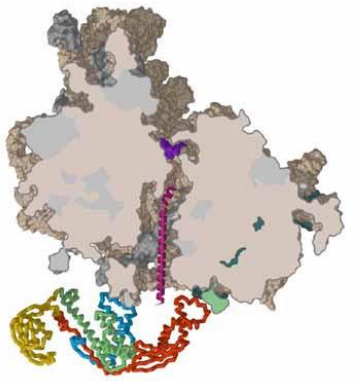
"Until the discovery of chromatin remodeling complexes," said Fitzgerald, "people generally considered [the arrangement of nucleosomes along DNA] to be relatively static" because of the tight-association between nucleosome histone proteins and the DNA wound around them. But in fact, chromosome structure is highly dynamic, said Fitzgerald. Chromosomes interact with remodeling complexes which "shift

nucleosomes along DNA to a nearby position, exposing gene regulatory sites or promoters, for instance." More dramatically, such complexes can "completely clear a large stretch of DNA which was previously occupied by numerous nucleosomes, which could expose the entire coding region of a small gene."

Fitzgerald helped develop a powerful new version of the baculovirus expression system, which enabled him to co-express all the protein components of the yeast ISWII chromatin remodeling complex. The baculovirus system also offers an important new tool for studying protein complexes in general [Berger, Fitzgerald & Richmond, *Nature Biotechnology* (2004) 22: 1583-7; see also accompanying commentary by P. Roy, "Baculovirus solves a complex problem" 22: 1527-8] . Like all chromatin remodeling complexes, ISWII is driven by hydrolysis of the ATP molecules created by the ATP synthase John Walker described. Fitzgerald reviewed his experiments that have led to a new mechanism linking ATP hydrolysis to the ISWII mediated-movement of nucleosomes along DNA. During an ATP hydrolysis cycle, the burning of successive ATP molecules "coincides with cycles of DNA binding, release and re-binding" by elements of the ISWII, while other "elements of the ISWII complex that bind to the nucleosome remain fixed," said Fitzgerald. "This binding and re-binding of DNA while keeping the binding site to the nucleosome fixed should result in a shearing strain," generating the force that is then dissipated by nucleosome movement along DNA. [Fitzgerald *et al* **EMBO J.** (2004) 3836-3843]. However, Fitzgerald said, the precise nature of this movement remains unknown.

The "naked" DNA modeled by Watson and Crick in the early 1950s gave way to the nucleosome-packaged DNA of the 1970s, the chromatin remodeling complexes of the 1990s, and the current efforts described by Fitzgerald to understand the dynamic regulation of chromatin organization in response to different cellular requirements. Similarly, the elegantly simple model of protein synthesis forged under the leadership of Francis Crick in the late 1950s has given way to a far more intricate picture. Two young HFSP awardees described novel strategies to peel back these added layers of complexity regarding the fundamental process of mRNA translation into protein.

The first-discovered link between protein translation and folding



In his 1958 articulation of the "sequence hypothesis" by which the DNA nucleotide-base sequence was proposed to provide a code for the polypeptide sequence of proteins [Soc. Exp. Bio. 12:138-63], Crick wrote: "It is convenient at the moment to consider separately the synthesis of the polypeptide chain and its folding. It is of course possible that there is a special mechanism for folding up the chain, but the more likely hypothesis is that the *folding is simply a function of the order of the amino acids,* provided it takes place as the newly formed chain comes off the [RNA] template." However, it is now clear that in order to fold efficiently in real-world cells, most proteins require the assistance of "chaperones." HFSP Young Investigator **Elke Deuerling** (University of Heidelberg) described pioneering studies of Trigger Factor, the first direct link to be discovered between protein translation on ribosomes and chaperone-mediated protein folding. Moreover, Deuerling's collaborator, HFSP Young Investigator **Nenad Ban** (ETH Zurich) has recently solved the crystal structure of the "dragon-shaped" Trigger Factor chaperone protein hunched over a ribosome, yielding a rather spectacular new picture of the molecular cradle into which new proteins are born. In a conversation at the Cambridge meeting, Deuerling said that the HFSP Young Investigators Grant was "absolutely crucial" in starting her own laboratory at the University of Heidelberg, providing the critical seed funding for her research and allowing her to apply for and receive grant support from other sources. [go to Deuerling profile]

A new model system for studying local protein translation in neurons

The discovery of ribosomes and specific messenger RNAs localized to synapses has resulted in a recent sea-change in cellular neurobiology. Can local synaptic control of protein translation be the mechanism by which individual synapses regulate their protein composition and signaling strength, mediating the synaptic plasticity widely thought to underlie learning and memory? HFSP Fellow **Alejandra Gardiol** (University of Cambridge) described her bold effort to create a powerful new model-system in which to study local protein translation at synapses: the Drosophila neuromuscular junction. "I applied for this fellowship because HFSP was also looking along this line, encouraging you to try risky projects," Gardiol said. [go to Gardiol profile]

New tricks for classical model systems for studying vertebrate development and brain function

The observational science of embryology began more than two thousand years ago with Aristotle's staging of chick egg development, and for much of the twentieth century, said HFSP Grant Awardee **Esther Stoeckli** (University of Zurich), "the chicken embryo was one of the most important systems for studying vertebrate development, including the development of the nervous system." However, the lack of methods for genetic manipulation, especially for generating equivalents to the loss-of-function mutants that are the staple of research in mice and zebrafish, has hampered the usefulness of the chick in the post-genomic era. Stoeckli described her successful development of a combined *in ovo* electroporation/RNA interference method for silencing any gene of interest [Pekarik *et al. Nature Biotechnology* 21: 93-96 (2003)], bringing the chick to the forefront of vertebrate model systems amenable to functional genomics. Stoeckli's Zurich lab recently used this new *in ovo* RNAi technique, for instance, to discover a novel role for the Sonic hedgehog morphogen in guiding the growth of axons along the developing spinal cord. (Bourikas *et al. Nature Neuroscience* 8: 297-304 (2005)).

The mouse is the only mammalian system amenable to genetic studies through both forward genetic screens and reverse genetic manipulation. However, neurobiologists exploring vision, the best-characterized higher mammalian brain function, have until recently rarely studied the mouse. There has been a simple reason for this neglect. The dominant senses in the mouse are smell and hearing, not vision, and mouse cerebral cortex generally has been thought to lack the multiple specialized visual areas (visuotopic maps) characteristic of such vision-dominated mammals as cats, monkeys and humans. However, HFSP Grant Awardee **Andreas Burkhalter** described experimental evidence in the mouse for "at least eight visuotopic maps in addition to primary visual cortex," and the existence of the distinct ventral and dorsal streams of visual information processing characteristic of higher mammalian brains. This finding has caused much excitement among neurobiologists eager to use existing mutant mouse lines to study the development and function of mammalian visual cortex, employing genetic and molecular approaches impossible in traditional mammalian model systems. "That mouse visual cortex looks in some ways like a highly evolved system, with multiple areas, was really a revelation to me," said Burkhalter, opening up many new lines of study. [e.g., see Dong….Burkhalter *Vision Research* (2004) 44: 3389-3400].

Synaptic specificity: guideposts, intermediate targets, and the molecular biology of connectivity

"One of the findings to emerge in modern neurobiology," said **Cornelia Bargmann** [UCSF, now Rockefeller University, USA] in the second plenary lecture of the meeting, "is that there are far more cell types than previously thought in virtually every region of the brain. For instance, there are thirty-five different classes of amacrine cells in the retina, and the synapses found within and between these classes are highly reproducible." How is this intricate specificity of synaptic connections achieved? In particular, how does a given developing neuron recognize its proper synaptic partner?

"There are ten trillion synapses in a mammalian brain," said Bargmann, but only about "seven thousand synapses in the entire nervous system of C. elegans," the worm studied in Bargmann's laboratory. Indeed, C. elegans is the only animal whose entire neural wiring diagram is known, work done by John White at the LMB in Cambridge, where Sydney Brenner established the worm as a model biological system. Bargmann described elegant studies of the development of one synaptic circuit mediating egg-laying. First, Kang Shen in her laboratory found that the site of a synapse in this circuit is determined not simply by the interaction between the presynaptic and postsynaptic cell. "There are other cells, guidepost cells, that contact the developing nerve processes, align them with each other, and instruct the synapses where to form." Bargmann noted evidence in vertebrates pointing to the existence of similar guidepost cells.

Secondly, Bargmann described Shen's systematic hunt for synaptogenesis mutants that disrupted this circuit. These mutants led to the molecular identification of the SYG-2 signal sent by the guidepost neuron, and the SYG-1 receptor on the pre-synaptic neuron, proteins belonging to the immunoglobulin superfamily. Moreover, the C. elegans SYG-2 guidepost signal is homologous to a gene which is mutated in a human congenital kidney syndrome, which also sometimes results in ataxia, indicating that at least one of the three known human genes of the SYG-2 class may also be involved in neural connectivity.

The relative simplicity of the C. elegans makes it a formidable model system for studying the basic processes by which its neuronal networks become wired. Two pioneering approaches to the challenge of unraveling complex biological networks are described below and in the accompanying awardee profiles.

A new method for imaging neural function and connectivity in living human brains

HFSP Young Investigator **Dae-Shik Kim** (University of Minnesota; currently at Boston University Medical School) developed a novel method for studying neural activity and anatomical connectivity simultaneously in living human brains, by combining fMRI functional imaging and diffusion tensor MRI. In an accompanying short profile, Kim describes how the seed funding for this "risky project", which now has many promising implications for both basic and clinical research, was made possible by the HFSP philosophy of supporting well-formulated, high-stakes projects even in the absence of preliminary results. [go to Kim profile]

Discovering the fundamental building block of complex biological networks

HFSP Grant Awardee **Uri Alon** described his pioneering work to define the basic building blocks, or "motifs," of complex biological networks, beginning with the well-characterized gene transcription network of E. coli. Alon, a young physicist-turned-biologist, has become a leader among a new wave of scientists trained in the physical sciences, who are applying powerful new computational and quantitative approaches to the emerging science of "systems biology." (Similarly, in the golden age of molecular biology in the 1950s and 1960s, physicists such as Crick, Max Delbruck, Seymour Benzer, and Walter Gilbert played a seminal role in introducing a new mindset into the emerging science of molecular biology.) Alon and his colleagues' work--in which computational analysis, theory and

experiment are closely interwoven--has identified network motifs recurring within complex biological networks, each with their own architecture and information-processing function. Remarkably, some of these circuit elements are reminiscent of the modular components used by human engineers to build complex systems. This "raises a fundamental challenge," Alon has written: "understanding the laws of nature that unite evolved and designed systems." [go to Alon profile]

Building bridges across national and disciplinary boundaries

In her plenary lecture at the conclusion of the Cambridge meeting, Cornelia Bargmann summarized the enthusiasm and stimulating international and interdisciplinary atmosphere of the Cambridge meeting, expressed in lectures, poster sessions, meals, and late-night pints in the Robinson College basement pub.

"I think the idea of doing work on the frontier, rather than work that's very good but relatively straightforward, has really been lived out here at this meeting. People seem to be trying to do what's difficult and challenging and not just following the general flow of their fields." Moreover, said Bargmann, "while its easy to meet people inside your own little field, it's quite a bit more difficult to meet a hand-picked group of people from outside your field who are very good, let alone meet people from Europe, Asia and North America all together. And I think you're the people who have the potential to really lead the international scientific community across countries and scientific fields through the connections you make at a unique meeting like this one in Cambridge."

Elke Deuerling: A Cradle for New Proteins - Discovering the First Direct Connection between Protein Translation and Folding

In her talk at the Cambridge Awardees meeting, entitled "Cytosolic Proteins at Birth," **Elke Deuerling (University of Heidelberg)** outlined the challenges a newborn polypeptide chain must overcome if it is to fold into its mature and functional three-dimensional form. The "birth canal of a protein" is the exit tunnel of the large 50s ribosomal subunit. But as the N-terminal end of the nascent protein emerges from the ribosomal tunnel in a largely unfolded state, the C-terminal end is still being translated and synthesized by other components of the ribosome: to extend Deuerling's analogy, while one end of a polypeptide is being born, the other end is still developing.

Deuerling explained that the N-terminal end of the nascent protein "exposes hydrophobic patches that are very prone to aggregation, especially in the crowded environment of the cytosol." Thus a newborn, unfolded protein is highly vulnerable to proteolysis or aggregation. Indeed, several human disease states, such as Alzheimer's, are associated with protein misfolding and/or aggregation. "So nascent proteins need molecular chaperones to assist folding to the native state," said Deuerling. "Chaperones are proteins that work by binding to unfolded, newly translated proteins, preventing aggregation or proteolysis," and providing a sheltered environment ("cradle") in which proper protein-folding can commence.

Deuerling and HFSP-supported collaborator **Nenad Ban (ETH Zurich)** focused their attention on bacterial Trigger Factor protein as a candidate for a chaperone acting at the earliest stages of protein synthesis: that is, at the ribosome itself. Deuerling showed that the primary Trigger Factor (TF) protein sequence has a modular architecture which includes an N-terminal ribosome binding domain, and a C-terminal domain whose function was extremely mysterious until recent structural studies of TF by Ban's group in Zurich. Genetic deletion of TF is viable in E. coli cells, because its function can be mostly compensated by another cytosolic chaperone system, DnaK. However, double TF-DnaK mutants are lethal, and lead to massive aggregation of over 340 different protein species. (Deuerling et al. (1999) *Nature* 400: 693-696).

Deuerling and her colleagues compared the N-terminal ribosome-binding domain of Trigger Factor in several different bacterial species and identified a highly-conserved "TF signature" motif. By mass spectrometry, she found that TF binds to two ribosomal proteins, including one called L23. The exquisite power of bacterial genetics enabled Deuerling to pinpoint the crucial amino acid sites within the TF signature motif that mediate binding to L23 (Deuerling made point mutations to the TF gene) and, in complementary fashion, the crucial amino acid sites on the L23 ribosomal protein to which TF binds (she expressed a mutant version of L23 on a plasmid under the control of a chemically manipulable (IPTG) promoter). Mutations at the site of the TF-ribosomal L23 interaction acted like deletions of the entire TF gene; in a DnaK deficient genetic background, cells died with an accompanying massive protein aggregation. (Kramer et al. (2002) *Nature* 419: 171-4).

These experiments, said Deuerling, provided "the first example demonstrating that two very basic processes in the cell are linked: protein translation and chaperone-assisted protein folding. And this link is provided by ribosomal protein L23, which is located right next to the polypeptide exit tunnel." Moreover, work in Deuerling's laboratory has indicated that bacterial TF can complement the function of analogous ribosome-associated chaperones in yeast, "pointing to conserved principles in higher eukaryotes."

However, the picture of Trigger Factor-mediated chaperone function remained rather murky. “Although we had learned a lot by studying this protein for seven years, we still had no idea how it sat on the ribosome and functioned,” said Deuerling after recently winning the 2005 FEBS Letters Award for Young Scientists. Then, in the spring of 2004, her HFSP-funded collaborator Nenad Ban’s laboratory solved the crystal structure of E. coli Trigger Factor along with the structure of the TF ribosome-binding domain bound to ribosomal protein L23. The picture that emerged, wrote Ban, Deuerling and their colleagues in *Nature* (431: 590-596,2004) is of a TF chaperone protein shaped like a “crouching dragon” which “hunches over the polypeptide exit [tunnel] of the ribosome,” thus “providing a shielded environment” for the nascent, unfolded protein. The “dragon-shaped” TF protein binds L23 by its “tail” (its N-terminal domain), while its previously-mysterious C-terminal domain makes up part of the dragon’s “back” and “arms” that help form the shielded environment, or “cradle for new proteins.”

The TF-L23 structure provides a vivid new picture of the first connection between newly-translated proteins and chaperones, and has stimulated a model of how TF binds to a nascent, unfolded protein and then releases it once the protein has either completed folding (in the case of some small proteins) or reached certain folding intermediate states. Moreover, the protein-cradle beneath the TF-dragon’s tail and arms seems relatively open to access by other cellular factors, such as those involved in protein targeting and secretion. In a conversation at the Cambridge meeting, Deuerling said that the HFSP Young Investigators Grant was “absolutely crucial” in starting her own laboratory at the University of Heidelberg, providing the critical seed funding for her research and allowing her to apply for and receive grant support from other sources. The HFSP-supported studies of a “molecular cradle for nascent proteins” has opened deep new biological paths, said Deuerling, “which we would like to extend to higher organisms, and also to other areas [of cellular function] such as protein targeting which we think may be closely linked to Trigger Factor function.”

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Alejandro Gardiol: Developing a New Model System for Probing Local Protein Synthesis at Synapses

HFSP Fellow Alejandra Gardiol (University of Cambridge, UK) was studying neurotransmitters as a graduate student at the Ecole Normale Superieure in Paris when a colleague in the lab made a fortuitous discovery: messenger RNA for the glycine receptor was localized at synapses in rat spinal cord neurons. This discovery, which immediately fascinated Gardiol, came amidst a sea-change in thinking about how the synthesis of new proteins might be locally regulated at individual synapses.

"Neurons are highly polarized cells," said Gardiol. "And for a long time, the dogma was that synaptic proteins, such as neurotransmitter receptors, were synthesized by ribosomes in the cell body and then transported to their final destination in axons and dendrites that could be quite far away in the cell." During the 1990s, however, this view was challenged by the discovery of polyribosomes and specific neural mRNAs localized adjacent to synaptic sites. [for review, see Steward and Schuman, Ann. Rev. Neurosci**.** (2001) 24: 299-325)]. It was possible that this local translation machinery at synapses existed simply to overcome the cellular cost of protein transport from the cell body: instead of having to ship proteins to synaptic terminals over long cellular distances (from many millimeters up to a meter in some axons), evolution simply placed protein-synthesizing factories at these terminals. But Gardiol and others were interested by a second possibility with deep intuitive appeal and major implications for the study of synaptic function.

"The general idea," said Gardiol in a conversation at the Cambridge meeting, "is that local mRNA translation could be a way to give a synapse independence from the cell body to regulate locally its own protein composition. Because every single synapse in a neuron is unique in terms of the pattern of activity it has experienced." Thus a neurons' nucleus would provide a pool of mRNAs encoding synaptic components, and these RNAs could be locally translated based on the synapse's unique history of external signals. Indeed, this process could be fundamental to forms of synaptic plasticity widely believed to underlie developmental tuning, learning and memory.

As a Ph.D. student in Paris, Gardiol spent many long hours doing electron microscopy in a basement room, contacting labs all over the world to obtain antibodies for different proteins involved in RNA translation and finding that they are localized at synapses. (Gardiol, Racca and Triller *J. Neurosci* (199) 19: 168-9). But at the same time, Gardiol felt rather frustrated with this purely morphological approach. She felt she was just seeing *where* RNA translation machinery was localized in neurons, not *how* they worked, and, even more importantly, what *function* they played in neural physiology. "But every time I thought about ways to address these mechanistic questions in our system, I came up against major technical barriers."

However, the first paper Gardiol read upon the lab's discovery of synaptically localized neurotransmitter mRNA was a paper describing the pioneering work on local RNA translation in Drosophila oocytes, *the* model system for studying local translation control. Indeed, Gardiol read the paper with some measure of envy. "I thought: This is a really great system." Then one day in 1999, Gardiol read that a mammalian homologue to the Drosophila Staufen protein, well-known to bind to and regulate the translation of localized RNAs in Drosophila oocytes and embryos, had been discovered at synapses of the mammalian hippocampus - a brain region crucial for long-term memory formation. "And this day I said to myself: 'Now I am going to Drosophila!'" remembers Gardiol with a laugh, pounding her fist on a table to emphasize how strongly she felt about this need for a change of systems.

Most of her colleagues were extremely sceptical, however. Gardiol was a neuroanatomist with scant experience in molecular biology and none at all in genetics. Colleagues told here that studying synapses in the tiny central nervous system of the adult fruit fly brain was extremely difficult. "They said to me: it's an insane idea [to move from vertebrate neuroanatomy to Drosophila genetics], you will fail. But I thought to myself: it's my first post-doc, I can fail once. I can take three years of my life, and spend them following a crazy idea."

At the Cambridge meeting, Gardiol gave a mid-fellowship report indicating that this idea was far from crazy. The HFSP fellowship enabled her to develop a new model system to study mRNA localization in synapses, working in the Cambridge laboratory of Daniel St. Johnston, a world-leader in investigating RNA localization in Drosophila oocytes. Working in the relatively large neuro-muscular junction (NMJ) synapses of Drosophila third instar larvae, she discovered that Staufen RNA-binding protein was present. This led to two intriguing findings. When she first looked at NMJ's in a well-studied *Staufen* mutant, she was rather disappointed to see that Staufen protein was still present at these synapses. Then she looked at a second *Staufen* mutant allele that deleted most of the gene, and found that synaptic Staufen protein was abolished, suggesting that perhaps a specific neural isoform of Staufen might be encoded in the Drosophila genome, a possibility she is pursuing.

Even more dramatically, she found that in the *Staufen* mutant, the size of the neuromuscular junction looked abnormal: it was significantly smaller. She did a quantitative comparison between NMJ synaptic boutons in wild-type and *Staufen* mutants and found about a 30% reduction: in *Staufen* mutants these synapses are far less "exuberant". Thus Staufen protein appears to play an important role in regulating the size of the NMJ. Indeed, one known mechanism of neural plasticity in fly larvae involves the regulation of NMJ size. Gardiol also discovered that *oskar* mRNA, whose localization to the posterior pole of oocytes is crucial to Drosophila body-axis development, is present in the NMJ. However, NMJ synapses are normal in *oskar* mutants, indicating that the Oskar protein is not Staufen's partner at the NMJ. Gardiol is intensively studying other candidate mRNAs and RNA-binding proteins, as well as contemplating new genetic screens to dissect local protein synthesis at the NMJ synapse, employing the powerful genetic and molecular tools that had drawn her to Drosophila in the first place.

"I applied for this fellowship because HFSP was also looking along this line, encouraging you to try risky projects," Gardiol said. Already, the fellowship has enabled her to become part of the superb community of Drosophila biologists working in and around Cambridge, "who are really, really helpful, as is the Drosophila community in general." And if the highly promising first fruits of Gardiol's Drosophila project are any guide, "I will very happy, I will have years and years of new questions to explore."

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Dae-Shik Kim: Imaging Brain Functions and Connectivity Simultaneously

In 1998, neuroscientist **Dae-Shik Kim** had a bold new idea. Kim was then working at the frontier of functional Magnetic Resonance Imaging (fMRI), the spectacular methodology for visualizing the location of specific neural functions in living brains in a non-invasive fashion. Yet Kim was frustrated by a major limitation of fMRI. fMRI imaged *where* in the brain neural activity occurs when a human subject performs a specific task. But fMRI could not illuminate how this pattern of neural activity in specific brain regions is achieved. For a brain region's functional activity pattern is ultimately determined by the anatomical pattern of neural connections feeding into and out of the region: anatomical information about neural circuitry to which conventional fMRI is blind.

Then Kim learned that a related magnetic resonance methodology, called Diffusion Tensor Imaging (DTI), was being adapted to non-invasively visualize patterns of axonal fibers in living brains. And Kim thought: why not combine fMRI and DTI? Why not perform these two complementary magnetic resonance imaging techniques in the same brain in the same experiment, enabling the investigator to visualize not only the regions of the brain activated during a specific brain function, but the pattern of neuronal connections linking these regions to each other and to the rest of the brain?

"This was the idea I had, and I thought it was quite logical," Kim remembers. Kim, then a young faculty member at the University of Minnesota, applied for an NIH grant to turn this logical idea into experimental reality. But there was one major problem with this grant. "I had almost zero preliminary data."

This is the Catch-22 of most conventional grant proposals. The investigator typically must have a body of preliminary data supporting the validity of the idea being proposed; he or she must already be well on the way along the research path for which funding is sought. But if the idea is truly new, as in Kim's case, where is he to get the funds to embark along this new path, to begin to collect the body of preliminary data convincing enough to demonstrate that a truly novel idea can be translated into reality?

Kim's initial NIH grant was rejected as too risky, since it was not yet supported by a body of experimental results. Fortunately, Kim learned of the then-new HFSP Young Investigator grants program, and HFSP's guidelines supporting well-argued, high-impact project proposals even in the absence of preliminary data. Kim assembled a world class international and interdisciplinary team consisting of himself as primary investigator; Johns Hopkins physicist Susumu Mori; and University of Maastricht (The Netherlands) computational expert Rainer Goebel. With an HFSP grant extending to May 2005, the three young investigators have been able to demonstrate the feasibility of Kim's novel idea for a new hybrid imaging methodology. Moreover, with these proof-of-principle results in hand, the Kim team has obtained significant new funding to extend their groundbreaking studies to such vanguard biomedical problems as the neural basis of human face recognition, and the nature of the abnormal outgrowth of axonal connections associated with autism.

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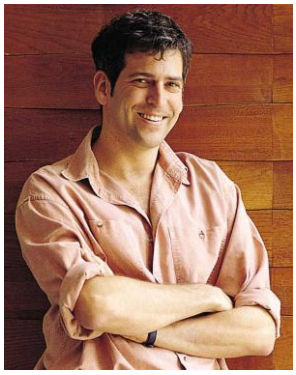
Uri Alon: Network Motifs - The Basic Building Blocks of Complex Networks



Uri Alon (Weizmann Institute, Israel) opened his talk at Cambridge with an overhead image of a densely crowded and intricately interconnected graph of nodes and lines. "Across biology we are confronted we these monsters: networks in cells, proteins interacting with proteins, with DNA," said Alon, a physicist turned biologist. "There is not just an arrow between [molecules] X and Y but an entire network of interactions. How can we ever understand complexity in something as complicated as this?"

Is the network of molecular interactions of a complex biological process such as a cell's gene transcription program irreducibly complex, so that the only adequate description of this network is a complete, fine-grained graph of all its arrows of interactions and their weights? Or can biological networks be broken into building blocks or recurrent subgraphs, what Alon calls "network motifs" by analogy with the sequence motifs used to analyze DNA and proteins sequences?

"Sequence motifs are pieces of protein sequences that usually have a specific function," said Alon, such as the ability to catalyze a particular enzymatic reaction or bind to a particular DNA regulatory sequence or protein domain. "Are there [analogous] pieces of a network that appear much more frequently than in randomized networks? Do they exist and if so, do they have a particular biological function?"

Using the well-characterized E. coli gene transcription network as an initial model system, Alon and his colleagues designed an algorithm to identify network motifs - molecular circuit-elements or sub-graphs. The algorithm built all possible sub-graphs involving different numbers of molecular nodes, than compared the frequency with which each sub-graph appeared in randomly-generated, and actual E. coli transcription networks, identifying sub-graphs or network motifs that were most over-represented in E. coli.

They found three such motifs or interaction patterns in E. coli "that occur much more often than they do in the randomized network. And each of these has a specific biological function that you can study theoretically and experimentally."

The first such motif is called a Feed-forward Loop (FFL), in which protein *X* activates protein *Y* which together are necessary to turn on target gene *Z*. Theoretical and experimental studies (the latter using a novel and highly-sensitive system for systematically measuring temporal patterns of gene activation) show that Feed-forward Loops perform a specific information-processing function. This network motif acts as a "persistence detector," filtering out transient signals and turning on gene *Z* only in response to signals of certain duration. "And the minimal input pulse duration that can be passed by this filter can be tuned by the molecular factors involved" (e.g., by their binding affinity). Moreover, the architecture of this network motif promotes rapid shut-off of gene *Z* transcription once the input signal ceases.

A second network motif is called a Single Input Module (SIM), whereby a single molecular regulator *A* activates the transcription of many downstream genes *B*, *C, D* etc. Alon and his colleagues' experimental studies have shown that this network motif architecture promotes a temporal program in the expression of genes encoding the protein components of many biochemical pathways, such as arginine biosynthesis, flagellar assembly and DNA repair.

"The genes turn on in an order," said Alon, "with minutes different between them, and it turns out that the order is [typically] the same as the order of [protein component] function in the pathway." It

seems that evolution has worked to tune the binding-site affinities of the downstream genes responding to regulator *A* so that the cell "doesn't make a protein before it needs it. It's not *essential* to the system, it's an economizing principle."

The third network motif is called Dense Overlapping Regulators (DOR). This network architecture, which like the other two network motifs, FFL and SIM, is highly over-represented both in E. coli and yeast transcription circuits, appears to "hard-wire combinatorial logic decisions based on many inputs. And to understand this, we need to understand the combinatorial logic encoded by the promoters of each of the downstream genes of this system, which is a project we're working on in the lab."

Indeed, Alon and fellow HFSP Young Investigator **Michael Surette (University of Calgary, Canada)** have shown that the intensively-analyzed promoter of the classical *lacZYA* operon of E. coli performs an "unexpectedly" intricate computational function, more complex that the simple logic gate that had been previously defined (Setty, Mayo, Surette, Alon *PNAS* 100: 7702-7 (2003)). This complexity appears to have been evolutionarily selected to enable cells to survive under a wider range of nutritional regimes than that subserved by a "purer" AND-like promoter logic gate. A similar approach has been applied to quantitatively describe and reprogram the genetic network mediating E. coli flagellar biosynthesis, described by Alon and colleagues in an article featured on the June 11, 2004 cover of *Cell* (117: 713-20). An accompanying commentary concludes: "The remarkable aspect of this work is that the experimental methods are widely used and can be readily extended to most model organisms. In principle this makes it possible to build detailed models of any transcriptional network whose connectivity is known."

At the Cambridge Awardee Meeting, Alon said the same motif-identification approach can be applied to any network of known connectivity. "Twenty-first century science is obsessed with networks in almost every field; ecology, physics, engineering, the World Wide Web, cell biology, neurobiology. And in each case when we applied this algorithm we found that networks from nature [and technology] contain their own set of motifs that are highly characteristic of the type of network" examined. (see "Superfamilies of evolved and designed networks", Milo et al. *Science* 303: 1538-42 (2004)).

Biological evolution has been famously described by François Jacob as a tinkerer, not an engineer, modifying and reconfiguring inherited molecular bits and pieces gradually over time rather than designing whole new systems from scratch. Yet a new generation of biologists such as HFSP Young Investigators Alon and Surette, who were trained in mathematics, engineering, and the physical sciences, are finding some remarkable parallels between the tinkering of biological evolution by natural selection and the work of human engineers. Indeed, both biological evolution and human engineers may have converged on the use of modular architecture such as recurring network motifs and circuit-elements for rather similar reasons. "Modular networks can be readily reconfigured to adapt to new conditions," Alon has written (*Science* 301: 1866-7 (2003). "The similarity between the creations of tinkerer and engineer also raises a fundamental scientific challenge: understanding the laws of nature that unite evolved and designed systems."

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