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ON THE COVER

This year's edition of World Changing Ideas explores the leading ways that technology and innovation can create a healthier, cleaner, smarter world, from biologically inspired algorithms to vegetarian robots to a cheap nanotech-based water filter.
Photograph by Mark Hooper.

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34

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FEATURES

By adjusting the mechanical tension applied to DNA molecules the velocity with which a motor enzyme replicates DNA can be precision controlled.

Anita Goel is Chairman of Nanobiosym and a Harvard-MIT physicist and physician. Named as *MIT Technology Review's* "World's Top 35 Science-Technology Innovators," she has received multiple awards from agencies like DARPA, DOE, DOD, and AFOSR for her work in the emerging field of nanobiophysics.

BIOPHYSICS

Tuning DNA Strings

Precision Control of Nanomotors

Tools that can manipulate single molecules make it possible to observe and control the way molecular engines measuring ten billionth of a metre replicate, transcribe, and process information in DNA.

By Anita Goel

For the most part, throughout the twentieth century, biology and physics developed as wholly separate disciplines. Biologists and physicists lived in their own reductionistic silos, seldom communicating or collaborating on their respective research. Most physicists had assumed that our current laws of physics were essentially complete and biological systems were simply a subset of physical systems; thus there were no new physical principles needed to explain life or living systems. Yet some of the most prominent physicists of the 20th century questioned whether the laws of physics, developed in the context of inanimate matter, could ever fully explain life and living systems.

Erwin Schrödinger, the Austrian father of quantum mechanics, charted out a bold roadmap to explore this boundary between biology and physics. After receiving the 1933 Nobel Prize for mathematically describing the evolution of a quantum system over time, Schrodinger turned his attention to his lifelong personal quest. This resulted in his 1944 classic work entitled *What is Life?*, where he concluded:"We cannot expect that the 'laws of physics' derived ... [from the second principle of thermodynamics and its statistical interpretation] ... explain the behavior of living matter... We must be prepared to find a new type of physical law prevailing in it."

Schrödinger also wondered whether life, at its most fundamental level, could somehow be a quantum phenomenon or, at

IN BRIEF

Nature encodes genetic information for biological systems in DNA and other complex macromolecules. This biological information is replicated, transcribed, or otherwise processed by enzymes such as polymerases.

Such enzymes can be viewed as nanoscale bio-motors or molecular engines that convert chemical energy stored in nucleotides into mechanical work. These nanomachines can also be viewed as information processing machines that respond to cues in the environment as they replicate a strand of DNA.

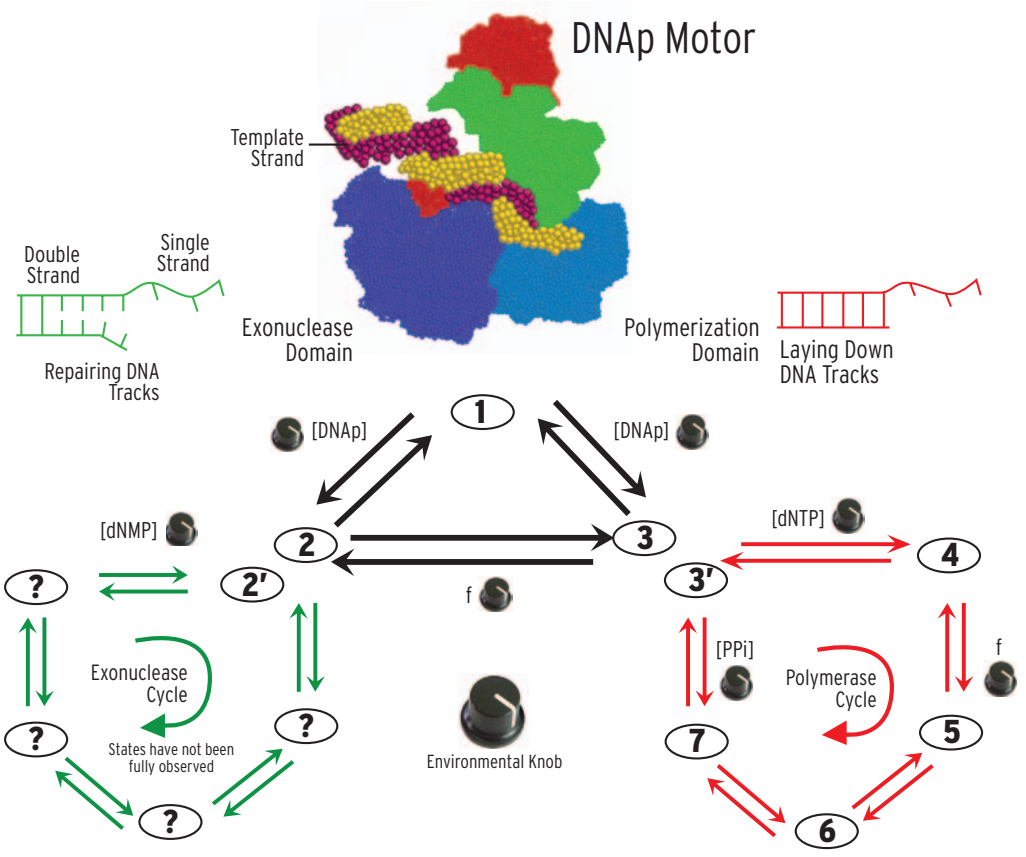
With the advent of new tools from physics and nanotechnology, we can precision control how these nanomachines read and write DNA, enabling a host of practical applications and shedding new light on fundamental scientific questions.

Photo illustration by Kapil Kashyap

A Network Model

Nanomotors assemble DNA polymers by incorporating nucleotides as building blocks into a growing DNA replica strand. Shown here is a simple network model to describe our nanomachine. Each node of the network represents an internal microscopic state of the nanomachine and the topology of this network denotes the allowed transitions between these internal states. The DNA polymerase (DNAp) motor replicates one base for every polymerase cycle it completes. The polymerase cycle is illustrated here a by a red pentagon, where the nanomotor cycles through internal states or nodes (3->4->5-> 6->7->3') of the network to achieve the polymerization of 1 base pair [Goel et al. (PNAS, 2003)]. For instance, nodes 4 and 5 correspond to open and closed conformational states of the polymerase DNA complex as suggested by x-ray crystallography data. Likewise, the green pentagon denotes the exonuclease or error correction cycle in which the nanomotor unzips or removes 1 base pair from the double helix for each exonuclease cycle it completes. This network model provides a powerful conceptual framework for us to mathematically predict how various parameters or "knobs" in the environment couple into the dynamics of these nanomachines. These environmental parameters or "knobs" in the motor's environment include temperature, ambient concentrations of nucleotides [dNTP] and other biochemical agents [DNAp], [PPi], the amount of mechanical tension (f) or torsional stress on the DNA.

—A.G.



least, be influenced by quantum effects. Could complex molecules somehow store biological information in living organisms? Although many of these speculations have been dismissed by mainstream scientists, Schrödinger's book did influence the thinking of an entire generation of physicists delving into biology, including Watson and Crick for their Nobel Prize-winning discovery of the DNA double-helix.

Still, in some quarters of the physics community, the unorthodox idea persisted that physics itself might have to undergo some radical transformations in order to adequately describe life and living systems. The physics of the 20th century had been formulated in the context of nonliving matter. Its mathematical language dealt primarily with closed systems that were operating at or near equilibrium. Any interaction with the environment was considered, at best, to be a small perturbation to these closed systems. In contrast, living systems, are fundamentally open systems that continuously exchange matter, energy, and information with their environment. Despite the advent of thermodynamics, statistical mechanics, and quantum mechanics, physics had not yet developed adequate mathematical and conceptual tools to predict the behavior of non-equilibrium systems that are strongly coupled to their environment. Even Einstein, exasperated with this seeming inadequacy of modern physics, confessed to Leo Szilard that "One can best appreciate from a study of living things how primitive physics still is".

The Russian-Belgian physicist Ilya Prigogine realized that in order to describe the dynamics of open, dissipative systems that are far from equilibrium, physics would need new theoretical constructs and a mathematical machinery capable of predicting the dynamics of systems where the environment is strongly influencing, if not blatantly driving, its evolution. Prigogine, a 1977 Nobel Laureate, took issue not only with classical physics but also with quantum physics (including Schrödinger's equation), notably with the idea that fundamental processes were reversible and thus predictable. To him it was clear: the arrow of time was one-way and many systems—both physical and biological—are unstable and far from thermodynamic equilibrium. Some self-organize, while others dissipate and decay. Unstable systems resist prediction by deterministic equations. Instead, what unfolds through time depends on initial conditions as well as the continual influence of the surrounding environment. For example, the environmental milieu can determine why some tropical storms dissipate while others self-organize and strengthen into violent hurricanes. Thus, external environmental conditions are critical in determining the evolution of open systems that are operating far from equilibrium.

My own quest to understand the physics of living systems is driven in part, by an inner, intuitive conviction that there must be an underlying unity or wholeness in nature. The deeper I went in my academic pursuits of physics and biomedicine at Stanford, MIT, and Harvard, the more keenly aware I became of just how deep the modern scientific divide is between these seemingly orthogonal disciplines. My early childhood exposure, while growing up in rural Mississippi, to Eastern philosophy and the Vedanta had instilled in me a worldview that we should be able to understand far reaches of the universe and the living systems around us with one integrated, holistic conceptual framework that is self-consistent and mathematically rigorous. Nanotechnology provided the practical tools and conceptual platform to bring the seemingly divergent worlds of physics and biomedicine under one common roof. I founded Nanobiosym as a research institute and idea lab to advance new scientific and technological innovations at the nexus of physics, biomedicine, and nanotechnology—an emerging field which we call "nanobiophysics."

Tools such as optical tweezers, magnetic tweezers, atomic force microscopes, and other nanoscale detection and manipulation methods, have unleashed a new frontier in probing the real-time single molecule dynamics of biological systems. In particular, the advent of such nanotechnology tools has enabled us to probe the detailed single molecule dynamics of enzymes like polymerases as they read and write DNA, providing unprecedented insight into their context-dependent function.

Biological information is replicated, transcribed, or otherwise processed by enzymes such as polymerases. Such enzymes can be viewed as nanoscale bio-motors or molecular engines that convert chemical energy stored in nucleotides into mechanical work. Over 15 years ago, I became fascinated with these biological nanomachines that read and write information into molecules of DNA. I hypothesized that the dynamics of a molecular motor would depend not only on the DNA sequence it reads, but also on the environmental milieu in which it operates. Simply put, I wondered whether the environment influences the way cells process the information encoded within DNA. Could cancer-causing mutations result, in part, from environmental stresses on the motor as it reads DNA? Armed with new experimental tools from nanotechnology and conceptual tools from physics, I set out to elucidate how various changes in the environment of a molecular motor could influence its actions along the DNA template.

Our framework suggests that the information content or number of bits stored in a DNA-motor system is larger than conventionally assumed.

I conjectured that these DNA nanomachines would provide an excellent laboratory to experimentally probe the dynamics of a biological system that was operating out-of-equilibrium and openly exchanging matter, energy, and information with its environment. These nanomotors convert chemical free energy stored in nucleotides (matter) into mechanical work as they copy biological information stored in a DNA molecule. These motors can be thought of as information processing machines that use information embedded in their environment to evolve or adapt the way they read out DNA. I hypothesized that information from their environment could couple into and modulate the dynamics of these nanomachines as they replicate or transcribe genetic information.

USING NETWORKS TO MODEL OPEN SYSTEMS

Our aim has been to develop a self-consistent physics framework to quantitatively describe how various cues from the environment can directly couple into the dynamics of the nanomotor. By understanding how these various environmental conditions affect the molecular motor's characteristic dynamics, we can develop a more holistic picture of their context-dependent function. While studying the physics of networks at the Santa Fe Institute, I developed a simple network model to describe our nanomachine. Each node of the network represents an internal microscopic state of the nanomachine and the topology of this network denotes the allowed transitions between these internal states. The DNA polymerase (DNAp) motor replicates one base for every polymerase cycle it completes. The nanomotor cycles through internal states or nodes (3->4->5->6->7->3') of the network to achieve the polymerization of 1 base pair. Likewise, the nanomotor unzips or removes 1 base pair from the double helix for each exonuclease or error correction cycle it completes.

This network model provides a powerful conceptual framework for us to mathematically predict how various parameters or "knobs" in the environment couple into the dynamics of these nanomachines. These environmental parameters or "knobs" include temperature, ambient concentrations of nucleotides and other biochemical agents, the amount of mechanical tension or torsional stress on the DNA, etc. [Goel et al. (PNAS, 2003)]. Hence, we now have a language to model these nanomachines as open biological systems that are operating far from equilibrium and are strongly coupled to information embedded in their environment.

PRECISION CONTROL WITH "KNOBS"

For the past several years, my lab has been seeking to identify and experimentally characterize the various "knobs" in a motor's environment that can exert control on its single molecule dynamics as it replicates or transcribes the genetic code. These environmental inputs can directly couple into the internal state transitions of the nanomachines (See the nodes in the network diagram) to influence their overall translational dynamics as they move along a DNA molecule. By increasing the mechanical tension applied to a DNA template, we can "tune" the velocity at which the motor enzyme DNA polymerase (DNAp) replicates DNA. Note at zero tension, the nanomotor replicates DNA at 100-150 bases per second. As the mechanical tension knob is ramped up from 0 to about 25-35 pN (picoNewtons), the nanomotor starts to slow down its rate of polymerization until it reaches a screeching halt at around above 35 pN. If we then increase the mechanical force on the DNA molecule about ~35 pN, the nanomotor switches gears and starts running backwards unzipping DNA, in exonuclease or error correction mode [Goel and Vogel, *Nature Nanotech* (2008)]. The mechanical tension f couples into key internal state transitions within the nanomachine to effect the overall external dynamics of the nanomotor along DNA. This prototypically illustrates precision control of the nanomotor by tuning various external knobs in the motor's environment. Thus, our network model describes the dynamics of nanomachines at a level commensurate with single-molecule data and provides a framework to control these nanomotors by controlling various knobs in their environment. Macroscopic knobs to precision-control the motor's movement along DNA tracks can be identified by probing how the motor's dynamics vary with each external control knob (varied one at a time). Efforts are currently under way in my lab to control even more precisely the movement of these nanomotors along DNA tracks by tightly controlling the parameters in the motor's environment (see www.nanobiosym.com). Principles of fine-tuning and robustness in networks can be extended to describe the sensitivity of nanomotors to various external control parameters. Much like traffic signals can direct macroscopic motors along a highway, environmental signals can influence and even control the dynamics of nanomotors along DNA tracks.

Learning how to control and manipulate the performance of nanomotors externally is another critical hurdle in harnessing nanomotors for *ex vivo* applications. By finding or engineering appropriate external knobs in the motor or its environment, its nanoscale movement can be tightly regulated, switched on and off, or otherwise manipulated on demand. To achieve external control over the nanoscale movement of biological motors, it is important to identify the correct external parameters that can be used to control their dynamics. These external modulators of motor function ("handles") can be either naturally occurring or somehow artificially engineered into the motor to make it susceptible to a particular external control knob.

This network model provides a powerful conceptual framework for us to mathematically predict how various parameters or "knobs" in the environment couple into the dynamics of these nanomachines. These environmental parameters or "knobs" include temperature, ambient concentrations of nucleotides and other biochemical agents, the amount of mechanical tension or torsional stress on the DNA, etc. [Goel et al. (PNAS, 2003)]. Hence, we now have a language to model these nanomachines as open biological systems that are operating far from equilibrium and are strongly coupled to information embedded in their environment.

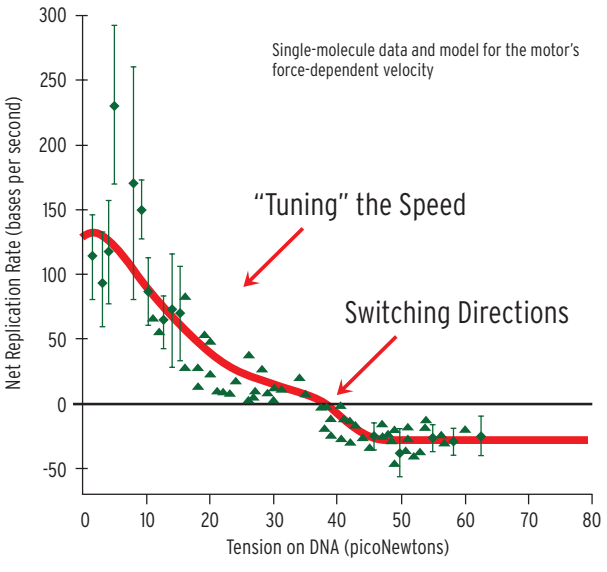
INFORMATION PROCESSING AT THE NANOSCALE

There is fervent interest in developing technologies that can store, retrieve, and process information at the nanoscale. Biological systems have already evolved the ability to efficiently process remarkable amounts of information at the nanoscale. By understanding how such external environmental perturbations affect the dynamics of a molecular motor, we hope to develop a more holistic picture of their context-dependent function. New conceptual and experimental tools are elucidating how the nanomotor's dynamics are intrinsically linked with its exchange of information, energy, and matter with its environment. Likewise, viewing a molecular motor as a complex adaptive system that is capable of utilizing information in its environment to evolve or

TUNING DNA TENSION

Fiddling With Knobs

Increasing the mechanical tension applied to a DNA template can "tune" the velocity at which the motor enzyme DNA polymerase (DNAp) replicates DNA. At zero tension, the nanomotor replicates DNA at 100-150 bases per second. As the mechanical tension knob is ramped up from 0 to about 25-35 (picoNewtons), the nanomotor starts to slow down its rate of polymerization until it reaches a screeching halt at around 35 pN. If we then increase the mechanical force on the DNA molecule about above ~35 pN, the nanomotor switches gears and starts running backwards unzipping DNA, in its exonuclease or error correction mode. The red curve denotes the net tension-dependent steady state flux Jnet(f), where f denotes the mechanical tension on the DNA template. Thus, we can demonstrate precision control of the nanomotor by tuning various external knobs in the motor's environment [Goel and Vogel, *Nature Nanotech* (2008)].



Graph courtesy Goel et al, PNAS (2003) and Nature Nanotech (2008)

Darwinian or Lamarckian?

Our experiments suggest that by changing environmental parameters at the nanoscale like the amount of mechanical tension in DNA or the ambient concentration of nucleotides, we can measurably alter the detailed dynamics by which the polymerase nanomotor replicates a DNA strand. By changing the environmental knobs, we can alter both the speed and accuracy with which polymerases replicate DNA. These empirical observations open up deep philosophical questions. Can biological information be embedded on many levels not only in the DNA but also within the nanomotor's environment? Could the environment be somehow deeply entangled with the dynamics of these molecular motors as they move along DNA? Could information embedded in the motor's environment somehow modulate or influence its information processing, and hence how it reads the DNA bases? Could the environment somehow be selectively driving evolution and if so could it be that evolution, at least at the molecular level, is more Lamarckian than it is Darwinian? What implications does this have on the nature vs. nurture debate? For example, if identical twins each carry the same oncogene, yet one develops cancer and the other one does not, how did the environment determine which twin expressed the oncogene? Could these nanomachines be processing information quantum mechanically? If so, futuristic quantum information technologies could perhaps find their best realization as of yet in the context of biomolecular motors. Schrodinger was the first to speculate that quantum mechanical fluctuations could give rise to mutations. In more recent times, McFadden [McFadden and Al-Khalili (1999)] describes how quantum mechanics may provide a mechanism for understanding "adaptive mutations"—i.e. mutations that are not purely random but are driven by environmental pressures. Could quantum noise or fluctuations perhaps give rise to mistakes made during the motor's copying of the DNA? As fields like nanotech, biotech, and quantum information processesing come together and new fields like quantum biology are born, it will become more fashionable to ask such questions and increasingly possible to experimentally address them.

—A.G.

learn may shed new light on how information processing and computation can be realized at the molecular level.

Conventionally, information in DNA is seen as being stored in the DNA bases itself. However, our framework suggests the information content or number of bits stored in a DNA-motor system is much larger than conventionally assumed. In our approach, the DNA, the replicating motor, and its environment comprise a dynamic and complex information-processing network with dramatically higher information storage and processing capabilities. This increase in information storage density results, in part, from the motor itself having several internal microscopic states. Each node on the network represents a decision point in the nanomotor's trajectory. As the nanomachine moves along DNA it must therefore process information and integrate environmental inputs from multiple levels to determine exactly how it reads the DNA.

APPLICATIONS

At Nanobiosym, we are harnessing these nanomachines for a variety of practical applications. These range from portable diagnostics like GeneRADAR®, next generation methods to sequence whole genomes with ultra-high precision and accuracy, and molecular manufacturing of biopolymers, to biological computation, nanoscale information storage in biomaterials, and ultra-efficient energy transduction schemes. Nanotechnology-enabled DNA readers like our Gene-RADAR aim to empower people to diagnose their own disease by taking the ability of disease detection outside of a hospital or pathology lab bringing it into doctor's offices, patient's homes, and even rural remote villages in the developing world. Nanomanufacturing processes, much like macroscopic assembly lines, urgently need procedures that offer precise control over the quality of the product, including the ability to recognize and repair defects. By controlling these nanomachines, we can artificially increase their error correction activity, resulting in higher precision and quality control when manufacturing DNA molecules. We have illustrated above the built-in mechanism used by the polymerase (DNAp) motor to repair mistakes made during the process of DNA replication. When the DNAp motor misincorporates a base while replicating the template DNA strand, it slows down and switches gears from the polymerase to the exonuclease cycle. Once in exonuclease mode, it will excise the mismatched base pair and then rapidly switch back to the polymerase cycle to resume forward replication. Living systems use numerous quality control procedures to detect and repair defects occurring during the synthesis and assembly of biological nanostructures. Deciphering the underlying engineering design principles of damage surveillance and error correction mechanisms in biological systems will inevitably allow better quality-control procedures to be integrated into nanoengineered systems of the future. ■

MORE TO EXPLORE

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