The MIT Physical Science–Oncology Center

Tackling Cancer Biology with Fundamental Physics

by Alexander van Oudenaarden

As physics makes increasingly important contributions to biology and medicine, physicists who once analyzed fluctuations in electrical circuits are turning to the exploration of fluctuations in gene expression in living cells—a complex system ideally suited to the physicist's toolkit. Alexander van Oudenaarden, MIT Professor of Physics and Biology, is directing a new Physical Science-Oncology Center at MIT, where interdisciplinary teams of physicists and biologists apply physics and its principles to problems in cancer biology.

IN THE VAN OUDENAARDEN LAB, researchers combine the physics methodologies of quantitative and predictive modeling with the powerful experimental tools of modern biology to analyze important problems in genetics and cell biology. By applying statistical physicists' methods of probability theory and mathematical tools to biology's enormous stash of quantitative data, researchers can test biological theories and potentially challenge accepted theories about cancer and its microenvironment.

Statistical physics, in particular, is being applied to a wide variety of fields with an inherently stochastic, or random, nature. Historically, statistical methods were first applied to the motion of particles or objects subjected to forces. A cell contains molecules interacting with one another while governed by Brownian motion, bouncing around in random directions like billiard balls. Statistical mechanics also can provide a framework that clarifies the abstract properties of biological networks such as intracellular and protein signaling systems. Physics helps researchers identify how specific networks elucidate the broad classes of activities that take place in a cell. In signal transduction networks, for example, proteins on a cell's surface detect information from outside the cell. That signal is transduced to other proteins inside the cell and ultimately modifies gene expression.

Shaking the Black Box

The mechanisms that cells use to sense and respond to environmental changes include complicated systems of biochemical reactions. Although a system may involve hundreds of reactions, often only a few of them dictate the system dynamics. Unfortunately, identification of the dominant processes is often difficult. Quantitative modeling of a biological pathway normally involves intense computer simulations to crunch all available data on the dozens of relevant reactions in the pathway, producing a detailed interaction map. These simulations are difficult to perform and interpret because many model parameters are not, or cannot be,

experimentally measured. Moreover, because there are so many interconnected components in the network, it's difficult to make reliable predictions.

Recently the van Oudenaarden lab took an alternative, physics-inspired approach to tackle this problem. Suppose somebody hands you a black box and you want to figure out what's in it without opening it. Just by shaking the box you can learn a lot. Comparing the response when you shake it fast to when you shake it slowly reveals important information about the physical properties of the object in the box. The van Oudenaarden lab took an analogous approach by considering a biological cell as a black box.

In this work, a complex signaling pathway in yeast was excited with periodic inputs—in this case, the salt concentration of the media—and corresponding output of the pathway (concentration of a protein in the cell nucleus) was monitored at different frequencies of the excitation. By analyzing which frequencies propagate through the network and which frequencies are highly damped, it was possible to determine the basic topology of the underlying biochemical network of reactions. This approach is widely used in physics, *e.g.*, Fourier analysis, but has rarely been applied to biological pathways. The technique could be applied to any cellular pathway with measurable inputs and outputs.

Counting Dots

Another interest of the van Oudenaarden lab is stochastic gene expression. Stochastic fluctuations in gene expression can lead to the fascinating phenomenon of non-genetic individuality: two genetically identical individuals, such as identical twins, who exhibit different protein concentrations and therefore have different phenotypes, that is, they do not look alike because of the stochastic nature of protein synthesis. Using a combination of statistical physics and biology, the "noise" in a single gene, cell or organism can be predicted theoretically and measured experimentally.

The van Oudenaarden lab uses budding yeast, the roundworm *C. elegans* and mammalian cells as experimental model systems to explore stochastic gene expression. Gene expression is a two-step process. In the first step, called *transcription*, a protein machine reads the DNA code and synthesizes a new molecule: messenger RNA (mRNA). In the second step, called *translation*, another molecular machine reads the mRNA code and synthesizes the final protein.

A variety of techniques are available to determine the presence and abundance of an mRNA in a tissue or cell sample. The latest methods developed at MIT provide accurate counts, as well as the intracellular locations of the mRNAs, in individual cells. The van Oudenaarden lab's technique for imaging individual mRNA molecules in fixed cells uses oligonucleotide probes that bind to separate mRNA species, causing them to fluoresce (see *Figure 1*). This makes each mRNA molecule visible through a fluorescence microscope as a glowing dot, allowing researchers to see and count single mRNA molecules within individual cells.

The data are collected by automated fluorescence microscopes that scan different parts of the tissue for different colors of fluorescence. The data is then digitized and analyzed with image-processing software developed specifically for this application.

FIGURE I

Counting the hundreds of dots in three different colors in individual embryos allows the researchers to reconstruct the dynamics of gene expression in single cells. As the number of dots peaks and falls, they can see gene expression dynamics in unprecedented detail. This absolute measure of mRNA concentration clarifies which signalling molecules were responsible for the specific gene expression th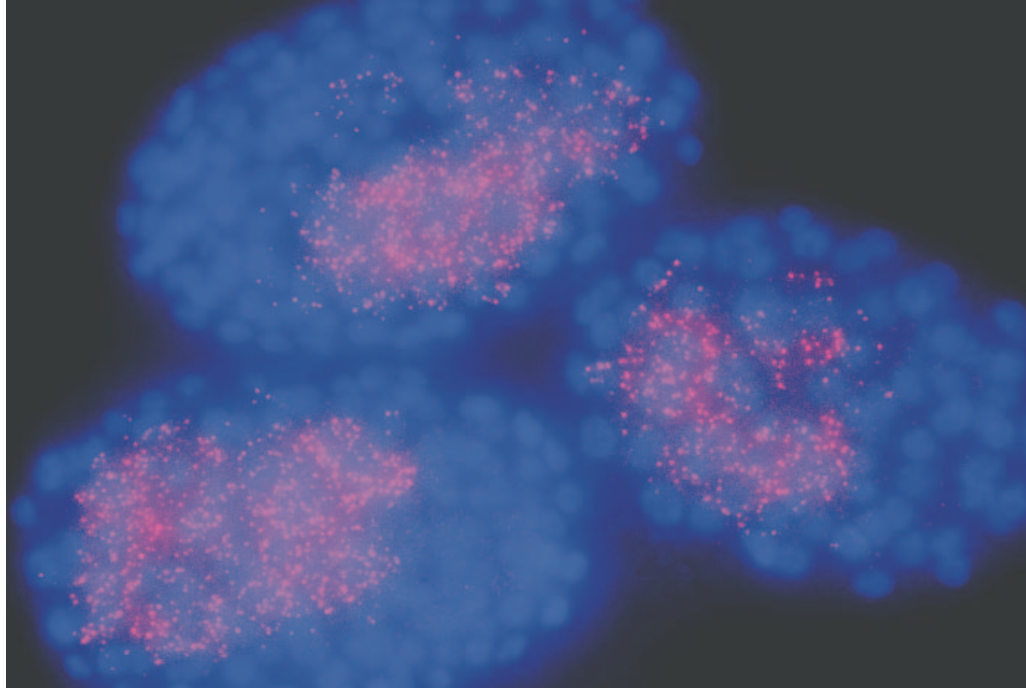at took place within the cell. The MIT team has successfully applied this method to cells in yeast, nematodes and fruit flies, as well as to mammalian cell lines and neurons.

Recently the van Oudenaarden lab applied this novel technology to obtain a quantitative understanding of a phenomenon called *incomplete penetrance*. For years, biologists have sought to understand why every person who carries a mutated gene does not express the trait or condition associated with the mutation. Of the tens of thousands of genes in our bodies, not all are switched on at the same time and many are never switched on at all. The function and expression patterns of many genes are unknown. It is becoming increasingly apparent that gene expression in individual cells deviates significantly from the average behavior of cell populations.

At a molecular level, proteins called *transcription factors* work like switches that turn gene expression on and off as needed. During an organism's early development, a multitude of extremely complex switching takes place. With variables such as genetic variation, fluctuating environments and random mutations, it's a wonder that any creature develops normally. But evolution has ensured that there are fail-safe mechanisms in place so that even if one switch is flipped in error, others will get it right. Cancer is an example of a process gone wrong. In cancer, some switches are flipped, suspending cells in a state in which they grow too fast or cannot regulate their growth, leading to the formation of tumors.

Incomplete penetrance exists in a wide range of organisms, including humans. Gene mutations linked to certain cancers, Parkinson's disease and Type 1 diabetes, for instance, are incompletely penetrant. Not everyone carrying the mutation will develop the disease. This may be due to environmental factors or the influence of other genes, but even genetically identical organisms living in the same environment can show variability in some incompletely penetrant traits. Possessing the identical genetic makeup and growth environment are not enough, apparently, to ensure that two cells will develop the same phenotypes.

Genes are subject to the random way molecules bounce around and find each other probabilistically. Within the confines of individual cells, minute changes in the concentration or spatial arrangement of molecular species can produce substantial effects. For example, a transcription factor equally prevalent in two

cells with identical sets of genes might be bound to a gene in one and unbound in another. Protein production would consequently begin in one cell and not the other, amplifying the fluctuation, and propelling each cell to a different fate.

What if incomplete penetrance was controlled by random fluctuations in gene expression? To understand key cellular mechanisms at play in incomplete penetrance, the researchers looked at the intestinal embryonic development of *C. elegans*, a small worm whose genetic makeup is well-known. Even though the DNA in all the individual worms was identical, gene expression was not. *C. elegans*'s digestive tract starts out as a single cell and eventually becomes 20 cells in the adult worm. That process is initiated by a gene called *skn-1*, which activates a series of other genes. Most of those genes code for transcription factors, which bind to DNA and turn on additional genes. The team first characterized normal progression of intestine development, then applied the method to worms with a mutation in *skn-1*. They found that some of the worms developed normal digestive tracts while others failed to develop any digestive tract at all. It appears that the controlling factor is the number of copies of mRNA produced by a gene called *end-1*, one of the genes activated by *skn-1*. The number of *end-1* mRNA strands varied greatly in embryos with the mutation: in those with a number above a certain threshold, development proceeded normally; if the number was below the threshold, no digestive tract developed. Evolution has apparently produced networks of genes that smooth out the effects of such fluctuations, which are revealed only when there's a mutation in the molecular pathway of a particular gene.

Switching Off Cancer

The intestines—in humans as well as in worms—contain fast-changing tissues. The entire lining of the intestines is replaced every four or five days. The new cells are generated by stem cells buried deep in indentations called crypts between nutrient-absorbing, finger-like protrusions lining the intestinal walls.

Stem cells divide rapidly into new cells, each pushing the next up the sides of the protrusions like a conveyor belt. If a cell on one of the protrusions is cancerous, it quickly gets purged with the rest of the sloughed-off cells. On the other hand, a cancerous stem cell in the colon is disastrous. It churns out new cells, all carrying the original mutation. To contribute to a better understanding of how stem cells in the colon become cancerous, MIT biologists have developed ways of studying this process in mice.

Ideally, scientists would like to understand how transcription levels of individual cancerous cells change in its metamorphosis from a stem cell into a differentiated colon cell. Traditionally, this might involve analyzing cancerous cells from the mouse at different stages in the progression of the disease. These snapshots in time are helpful, but van Oudenaarden plans to use mRNA detection to study mammalian colon stem cells in hopes of figuring out whether random fluctuations in gene expression influence the mutations that can cause cancer. This powerful visualization technique will allow his laboratory to detect potentially cancerous scenarios within and among cells. Seeing these dynamics at the transcription level

will be an invaluable tool, potentially elucidating exactly at what point in the transformation from stem cell to cancer cell switches are flipped in the wrong direction.

These approaches from the physical sciences are necessary to understand cancer, as it is known that some individuals carry gene mutations associated with certain cancers, yet never develop the disease. Scientists may learn, for instance, that mutations are only harmful under certain conditions, or that there is one point in the process where—if the proper intervention can be devised—the cell can be rescued or destroyed before it leads to tumors. Studying the human genome has shown which gene mutations might make an individual vulnerable, but if researchers also understand the factors at play in penetrance, they can design drugs or therapies targeted to those factors that would make that particular gene less variable, and ultimately make the carrier of the gene less vulnerable to disease.

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ALEXANDER VAN OUDENAARDEN *is a Professor of Physics and Biology at MIT. His research focuses on how single cells use gene and protein networks to accurately process intra- and extracellular signals. Professor van Oudenaarden's laboratory made pioneering contributions to understanding stochastic gene expression and systems biology at the single-cell level. The current efforts in the van Oudenaarden group are focused on an integrated theoretical and experimental approach to understand the role of stochastic gene expression during development and differentiation. Professor van Oudenaarden's Ph.D. research in the field of experimental solid state physics was performed at Delft University of Technology in the Netherlands. He obtained his Ph.D. degree in 1998 (with highest honors) and received the Andries Miedema Award for best Ph.D. research in the field of condensed matter physics in the Netherlands. From 1998 to 1999, he was a postdoctoral fellow at Stanford University, collaborating with Drs. Steven Boxer and Julie Theriot. Professor van Oudenaarden joined the MIT faculty in January 2000. In 2001, he was named an Alfred Sloan Research Fellow, a Keck Career Development Professor in Biomedical Engineering, and received the NSF CAREER award. He was promoted to Associate Professor with tenure in 2004. Since 2001, Prof. van Oudenaarden has been teaching a graduate level course in systems biology at MIT for which he received the MIT School of Science Prize for Excellence in Graduate Teaching in 2007. In 2008 he was promoted to full Professor of Physics at MIT and received a John Simon Guggenheim Fellowship and the NIH Director's Pioneer Award. Professor van Oudenaarden is the director of the NIH/NCI-funded Physical Sciences-Oncology center at MIT.*