encyclopedia of knowledge that can be drawn upon for formulating incisive experiments to illuminate the disease process.

2) Model organisms will increasingly be used for the direct investigation of medical problems that seemingly have little to do with them. For example, the misfolding or aggregation of proteins implicated in the process of neurodegeneration in disorders like Alzheimer's disease, Parkinson's disease, and Huntington's disease, can be recapitulated in yeast, worms, and flies. In addition, other components discovered in these organisms may be important in the disease process. Analysis of aging in simple models is turning up genes that play analogous roles in more complex organisms. Model organisms will provide further insights into the cell cycle and cancer, glucose metabolism and diabetes, chromosome segregation and mental retardation, protein glycosylation and lysosomal storage diseases, mechanisms of drug action and resistance, and much more. Studies of *S. cerevisiae* will help us to unravel the workings of its pathogenic cousins such as *Candida albicans*; studies of *D. melanogaster* will reveal secrets of the *Anopheles* mosquito.

3) Model organisms will remain at the forefront for the foreseeable future in efforts to sort out biological complexity and achieve a more quantitative understanding of life processes, which is needed to unravel the network of molecular interactions that constitute an organism as complicated as a human. For example, it is with yeast that biologists first will elucidate how DNA binding proteins, DNA sequence elements, components of the transcriptional machinery, chromatin structure, and signaling pathways combine in the circuitry of gene regulation. The resulting comprehension of biological networks that will result will bestow upon biologists the predictive powers and design capabilities long held by physicists and engineers. Such insights will require the application of multiple technologies, the confluence of individual investigator's experiments and genomewide data sets, and the intense collaboration of experimentalists and computational biologists. Learning how to carry off this ambitious project is itself a lofty goal of model organism research.

4) Model organisms offer the best hope for coming to grips with the breadth of genetic diversity and the depth of its consequences. Most of the variance among individuals of a species is due to small differences in multiple genes, and it is with model organisms that we will first learn how to analyze and understand complex quantitative traits. Such an understanding will provide the principles and procedures for predicting disease susceptibilities in humans and tailoring optimal methods for prevention and treatment. Genetic diversity is the grist for the mill of natural selection that produced the remarkable diversity of life on Earth, and model organisms should continue to teach us about the origin of the species.

5) Model organisms will remain the proving ground for developing new technologies, which typically spread quickly throughout the research community. For example, our skills in isolating and manipulating genes were won while studying bacteria and bacteriophages. Many other technologies got their start or achieved their apogee in yeast, including two-hybrid analysis, high-throughput protein purification and localization, genomewide epistasis analysis (synthetic lethality), gene expression profiling, protein arrays, and genomewide chromatin immunoprecipitation (ChIP). Worms and flies have been the test beds for large-scale RNA interference screens. We don't see these developments abating. Indeed, the more the fund of knowledge of simple organisms grows, the more useful they become for subsequent technological innovation.

But will an organism like yeast be able to maintain its seat on the Security Council? Not indefinitely. And just as yeast has led the way in many areas of research, we expect that its fate as an experimental organism will foreshadow that of the rest of the council. Does this mean that the end of biology is near? Hardly. We will still be a long way from a comparably deep-seated understanding of humans and our afflictions. How do cells and organs regenerate after damage? How do eukaryotic parasites, which are so different from model organisms, wreak havoc with fatal diseases like malaria, African sleeping sickness, and Chagas' disease? How do strange bacteria and viruses elude our immune systems and stymie our best efforts at drug therapy? How do genes and the environment interact in behavioral diseases like schizophrenia or autism? What is the basis of memory and consciousness?

The reductionist approach of biologists has enabled remarkable achievements by causing us to focus on just a few experimentally tractable organisms, but it also has tended to restrict our vision. There is much to learn about the many organisms that populate our planet, most in ways we can't yet begin to fathom. How do creatures survive in extreme environments? How do some manage to metabolize bizarre substrates? How do individuals organize themselves into incredibly complex communities? This list of questions seems endless (as seemed the list of genes in model organisms not so long ago). Providing adequate answers to these and many other questions is certain to occupy us for a long time. And the knowledge and sophisticated analytical tools that model organism research has laid at our feet bring the entire General Assembly of organisms within our reach, enabling us eventually to answer a question that has framed our enterprise from its beginning: What is life?

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MOLECULAR BIOLOGY

Signal Processing in Single Cells

Farren J. Isaacs, William J. Blake, James J. Collins

Consider a high-tech version of the "telephone game" in which you and a group of your friends attempt to transmit a message via your cell phones. One person in the chain has a phone from the 1990s, which is very noisy. Another person is standing in the middle of Times Square in New York City. It would not be surprising if the message received by the person at the end of the chain, or cascade, were corrupted as a result of noise intrinsic to the old phone and the noise arising from the Times Square environment. In this issue, Rosenfeld *et al.* on page 1962 (*1*) and Pedraza and van Oudenaarden on page 1965 (*2*) investigate a living-cell version of this game by exploring how signals are transmitted through gene cascades in noisy cellular environments.

Cell phones consist of multiple, interacting components. Engineers characterize the performance of such devices by determining quantitatively the input-output relationships, or transfer functions, of the respective components. Rosenfeld *et al.* present a new method for calculating the transfer function for the expression of a single gene. Specifically, they investigate the relationship between the concentration of active transcription factor (input) and the rate at which target protein is produced (output) in

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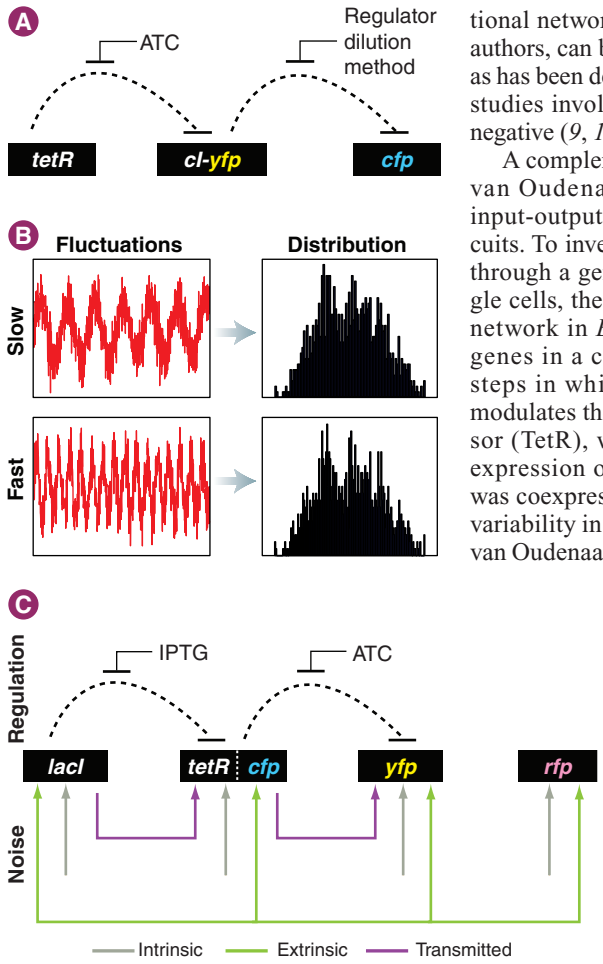
single cells. They define this relationship as the gene regulation function. To experimentally measure the gene regulation function, they constructed a synthetic "λ-cascade" system (see the figure) in *Escherichia coli*, in which the expression of a target, or output, gene (CFP, cyan fluorescent protein) is modulated by the activity of an input transcription factor (CI-YFP, λ repressor fused to yellow fluorescent protein). This scheme allows simultaneous measurement of both input and output protein levels in vivo, and is well suited to quantitatively derive the gene regulation function.

To vary the concentration of the CI-YFP input protein, the authors used a "regulator dilution method" in which a given amount of regulator is systematically decreased through successive cell divisions. As the level of CI-YFP decreases, the production of the CFP target increases. By analyzing binomial errors in protein partitioning, the authors obtain the relative fluorescence intensity of individual protein molecules, which allows them to determine the apparent number of molecules per cell. This information was used to determine the gene regulation functions for individual cells, from which a mean gene regulation function was calculated for a select population.

The gene regulation function was subsequently used to extract a number of system-specific properties. For example, the mean gene regulation function was fit to a Hill function to calculate in vivo values of several biochemical parameters. These values were used to provide a molecular-level representation of a transcriptional input-output relationship in individual cells. Interestingly, these values were comparable to previously calculated in vitro estimates.

Rosenfeld and colleagues noted significant deviations of single-cell gene regulation functions from the calculated mean gene regulation function. The authors detected and quantified the effects of factors such as cell cycle phase and gene copy number on these deviations. After compensating for these sources of noise in the analysis, the authors examined whether the variability of the gene regulation functions arises from random fluctuations in other cellular factors that globally affect gene expression (extrinsic noise), or from fluctuations in the biochemical processes involved in the expression of an individual gene (intrinsic noise). Consistent with their previous work (*3*), they detected only a minor noise contribution from intrinsic factors, implicating extrinsic factors as the prominent noise culprits.

In contrast to previous experiments that collected steady-state snapshots of gene expression (*3*–*6*), Rosenfeld *et al*. measured temporal changes in the rates of input (CI-YFP) and output (CFP) production levels. The authors noted that fluctuations occurring on different time scales can lead to identical steady-state distributions (see the figure). They also found that intrinsic noise decays rapidly, whereas single-cell responses depart from the mean gene regulation function over long time periods (about one cell cycle), indicating that extrinsic noise can occur on slow time scales. The authors suggest that cells would have to integrate signals over long time periods to account for such noise, which means that there may be a trade-off between accuracy and response time of a transcriptional network. Feedback, as noted by the authors, can be used to affect this trade-off, as has been demonstrated experimentally in studies involving both positive (*7*, *8*) and negative (*9*, *10*) feedback loops.



Synthetic gene network cascades. (**A**) Gene network used to determine gene regulation function (*1*). TetR represses the expression of a CI-YFP fusion protein, which, in turn, represses the expression of CFP. The chemical anhydrotetracycline (ATC) modulates repression by TetR, and the "regulator dilution" method was used to control the concentration of CI-YFP. (**B**) Time course of slow (top) and fast (bottom) fluctuations can both result in similar population distributions (right). (**C**) Regulatory connections and noise sources for a synthetic gene cascade used to study noise propagation in gene networks (*2*). LacI represses the expression of TetR, which, in turn, represses the expression of YFP. CFP and YFP fluorescent reporters were used to measure noise properties of the network. Unregulated expression of red fluorescent protein (RFP) was used to measure variability due to global sources of noise not influenced by the properties of the network.

A complementary study by Pedraza and van Oudenaarden (*2*) investigated the input-output relationships of genetic circuits. To investigate how noise propagates through a gene regulatory cascade in single cells, the authors designed a synthetic network in *E. coli*. They arranged three genes in a cascade with two regulation steps in which the lac repressor (LacI) modulates the expression of the tet repressor (TetR), which, in turn, regulates the expression of YFP (see the figure). CFP was coexpressed with TetR to measure the variability in TetR expression. Pedraza and van Oudenaarden systematically perturbed the effective regulation along this cascade by using the chemical inducers IPTG (isopropyl-β-D-thiogalactopyranoside) and ATC (anhydrotetracycline). This method allowed them to investigate how interactions among network components affect the transmission of noise along the cascade (see the figure).

As the repressive effect of LacI was decreased by the addition of IPTG, its target, CFP, responded with an increase in expression. Expression of the downstream YFP target in the next regulatory step was inversely correlated to the CFP signal, but it exhibited a much steeper response. These results illustrate that genetic cascades can display sharp, switchlike responses to input signals, a recognized property of analogous protein kinase systems (*11*).

The experiments also showed that the two fluorescent reporters in the cascade, both regulated by single repressors, display strikingly different levels of noise in their response to varying strengths of regulation. To interpret these results, Pedraza and van Oudenaarden developed a stochastic model of their cascade that distinguished between various sources of noise influencing the expression of each gene (see the figure). Importantly, the authors included in the model contributions from transmitted noise, that is, intrinsic and extrinsic noise influences transmitted from a regulator gene to its target.

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The model demonstrated that expression variability of a target gene in the cascade was most influenced by noise transmitted from the upstream regulator. This interesting finding suggests that network connectivity can have a greater effect on the variability of expression than noise intrinsic to the expression of a gene itself. Remarkably, the authors' model was able to accurately predict the noise properties of single genes within the cascade, as well as correlations between each of their fluorescent reporters, demonstrating that such behavior can be described in a quantitative manner.

In similar recent work, Hooshangi *et al.* (*12*) constructed gene regulatory cascades of varying lengths in *E. coli* to explore the effects of cascade length on the timing, variability, and sensitivity of the network's response. They found that the sensitivity of the network's response to changes in an input signal increased with cascade length, similar to the results discussed above (*2*, *11*). Moreover, they showed that longer cascades, which are more sensitive, amplify cell-cell variability, especially at intermediate signal levels. Noise due to high levels of expression in genetic cascades can also have dynamic effects, with phenotypic consequences. For example, an earlier study involving a synthetic gene regulatory cascade in yeast (*5*) showed that increased variability in an upstream regulator can cause a cell population to display prolonged bistable states of gene expression.

To date, studies of synthetic gene regulatory networks have relied primarily on qualitative models that describe general system behavior (*13*). The work by Rosenfeld *et al.* (*1*) and Pedraza and van Oudenaarden (*2*) represents an important advance toward a more quantitative synthetic biology. These studies offer insights into gene regulation, and, together, provide a framework for the further characterization of input/output relationships among regulators and their targets. These quantitative approaches can be applied to natural gene networks and used to generate a more comprehensive understanding of cellular regulation. This will enable a better characterization of individual genetic components and modules, opening up the possibility of designing more complex synthetic gene networks. Such networks could be engineered with specific properties that filter unwanted noise from signaling networks or exploit noise-induced switching to sample more diverse phenotypes.

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ATMOSPHERIC SCIENCE

Something in the Air

Daniel M. Murphy

Aerosol particles in the atmosphere play important roles in climate change and human health. Some of the largest uncertainties in how human activities affect climate come from the effects of aerosols (*1*). Epidemiological studies have shown significant excess mortality from particle pollution (*2*). In both cases, particles less than 1 or 2 μm in diameter are particularly influential, because they efficiently scatter light, influence cloud formation, and can penetrate into human lungs. In recent years, new instrumentation has led to important advances in the detection and characterization of these particles.

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The measurement of aerosol composition poses substantial analytical challenges. Sample sizes are small: A particle with a diameter of 200 nm weighs about 6 fg (6×10^{-15} g), and outside of polluted regions, a liter of air may contain just 1 ng (10^{-9} g) of particles. This material is typically a complex mixture of inorganic and organic compounds. Some compounds are so volatile that pressure and temperature changes during sampling cause evaporation, biasing the results. Others are so sticky that it is nearly impossible to recover them from a filter or other substrate without changing their composition.

The evaporation and recovery issues have pushed new instrumentation toward techniques that analyze particles directly from the air. Such "on-line" techniques are also attractive because measurements need to be made every few minutes to track changing wind conditions or sample from mobile platforms such as aircraft. The cost of handling filters or other off-line samples acquired at these short time intervals quickly becomes prohibitive (*3*). Furthermore, on-line samples can be more easily compared to gas-phase measurements, as shown in the figure, where the combined analysis of gas-phase and particle chemistry provides important insights into the sources of different substances.

Because of its sensitivity and fast response time, mass spectrometry is an important technique for the on-line analysis of aerosols (*4*, *5*). At least 20 mass spectrometers have been developed for this purpose. In many of these instruments, a nozzle brings nearly all particles into a vacuum chamber in a highly collimated beam while reducing the gas pressure by seven orders of magnitude (*4*, *6*). Some instruments examine one particle at a time, whereas others analyze an ensemble. Most single-particle instruments ionize material with a pulsed laser, and then use a time-of-flight mass spectrometer to obtain a complete mass spectrum of the abated material. The most common ensemble configuration evaporates particles with a hot filament, then ionizes the gas-phase material with electron impact or chemical ionization. There are trade-offs between the detailed information from single-particle instruments and the better quantitation of organics, sulfate, and nitrate achieved by ensemble instruments.

Other techniques for on-line aerosol composition analysis include automated samplers that feed directly into ion chromatographs, and instrumentation for detecting specific chemical groups such as nitrate (*7*). Off-line analyses use increasingly advanced versions of electron microscopy, atomic force microscopy, and accelerator-based nuclear techniques.

The analytical techniques discussed above have contributed to a paradigm shift in atmospheric science: the recognition of the global role of organic compounds. Before 1995, global models of aerosols generally did not include organics (*1*). Since then, traditional and on-line techniques have found that organics are of com-

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