tion, have proven extremely fruitful for computer science. Artificial neural networks (ANNs)—which have myriad uses, including classification, prediction, and sequential decision-making—are based on complex biological networks of neurons, axons, dendrites, and synapses. The learning rules used by ANNs, which nonlinearly combine signals from different nodes into outputs that are fed to other nodes or interpreted as computational results, have grown more sophisticated over time (*3*). They can still be traced in spirit, however, to a theory introduced by neuropsychologist Donald Hebb in the 1940s, sometimes described as "neurons that fire together wire together" (*4*). Discovery of the molecular basis of natural evolution ultimately inspired John Holland (*5*) and other pioneers of genetic algorithms to solve complex problems in combinatorial optimization by using computers to generate populations of "individuals" that represented solutions, allowing them to "breed" and then selecting for the "fittest," or most optimal, solution.

Biological analogies have been especially instructive for developing artificial immune systems (AISs). Like their biological namesakes, computer viruses replicate by attaching themselves to a host (a program, or a computer) and coopting the host's resources to copy themselves. The analogy worked well at a macroscopic level, where it provided insights into computer virus epidemiology and helped explain the evolution of different types of viruses as different storage media and operating systems rose and fell in prevalence. It also worked at a microscopic level, where it became very natural to explore how the detailed workings of the vertebrate and invertebrate (or "innate") immune system could provide computer virus remedies. During the 1990's, for instance, IBM Research (*6*, *7*) and the University of New Mexico (*8*) developed computer analogs of the immune system that employed "know thyself" or "know thine enemy" strategies to recognize unknown pathogens (computer viruses or intrusions).

Many of these past computational approaches sought to apply biological ideas that had been known for decades or centuries. In contrast, Afek *et al*. sought to simultaneously solve a pair of contemporary problems: one computational and one biological. The computational problem, known as distributively selecting a Maximal Independent Set (MIS), is to develop a mechanism by which nodes of a large computer or sensor network can work together without any central coordination to identify a subset of "leader" nodes that form a backbone through which messages can be routed efficiently. The biological problem is to discover the distributed mechanism by which, during development of a fly's nervous system, certain precursor cells are selected to differentiate into sensory bristles. After showing that the two processes are analogous, Afek *et al*. conducted a series of experiments that revealed the details of a protein-based inhibition system that results in a regular pattern of bristles. Based on biological plausibility arguments and experimental results, the authors postulated a small set of possible selection mechanisms, and then ran computer simulations that revealed that just two of those tested could explain the observed results. They then selected the one that was more plausible for use in computation, and showed it could produce an MIS with high probability.

The jury is still out on whether the algorithm derived by Afek *et al*. accurately mirrors the biological system, but what matters from a computer science perspective is that it works. Indeed, it has more attractive properties than any that had been conceived by decades of computer scientists. This result hearkens back to IBM's decision to pattern its AIS on an outmoded "template" theory of antibody generation, rather than on modern theories of negative and clonal selection, because the outmoded idea worked better for computer viruses. In both cases, harnessing a biological analogy—and treating it with both respect and some skepticism—led to very effective computer algorithms. Apparently, to truly profit from the lessons of Mother Nature, we must be judicious, not slavish, in applying analogies.

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CELL BIOLOGY

The Lives of Proteins

Joshua B. Plotkin

A new method to measure protein dynamics in living cells reveals how protein removal regulates cell physiology.

Biologists have traditionally studied gene regulation through a transcriptional lens. This is due in part to the influence of Jacob and Monod's early work on transcription and, more recently, to the availability of high-throughput messenger RNA (mRNA) quantification by microarrays and sequencing. Libraries of fluorescently tagged proteins (*1*) and advances in mass spectrometry (*2*) have since allowed quantification of protein abundances, and revealed that as much as 30% of the variation is governed by posttranscriptional processes (*3*). Still, most systematic studies have focused on steady-state amounts of mRNAs and proteins. A new technique called "bleach chase," described by Eden *et al*. (*4*) on page 764 of this issue, accurately quantifies protein half-lives in living cells, an innovation that shifts the focus to the dynamic role of protein removal in regulating cell physiology.

There are two distinct mechanisms of protein removal: dilution by cell division and active degradation (see the figure). Whereas dilution decreases all proteins simultaneously, degradation can be highly specific to individual proteins (such as by the ubiquitin-proteasome pathway). Dilution is dominant in fast-dividing cells, such as bacteria, whereas degradation dominates in slow-dividing cells. As a result, the relative importance of synthesis versus removal in regulating the stoichiometry of the proteome likely varies by cell type, because rapid cell division decreases the potential for specific removal mechanisms to control protein amounts. To elucidate the balance between dilution and degradation in moderately growing cells, Eden *et al*. applied the bleach-chase technique to quantify the half-lives of 100 proteins in human lung cancer cells that divide daily.

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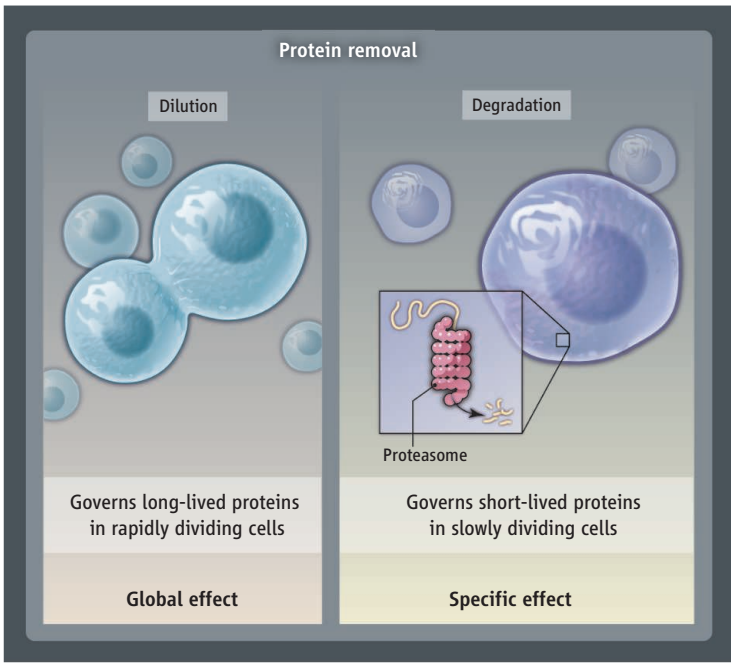

Protein removal. Proteins are either diluted by cell division or actively degraded (by the proteasome, for example). The balance of synthesis versus removal in regulating the stoichiometry of the proteome may vary with cellular division timing.

Bleach chase is a broadly applicable method to quantify protein turnover in living cells without the use of compounds to inhibit protein expression (translation), antibodies, or radioactive labels. The amount of a protein that is tagged with a fluorescent protein is monitored by time-lapse microscopy in a population of several hundred cells after a pulse of light renders some of the fluorescent protein irreversibly nonfluorescent. The relevant signal is the deficit of fluorescence in the "bleached" population relative to a nonbleached control population. This fluorescence deficit decays to zero as bleached proteins are removed. Thus, bleach chase is an elegant inversion of the classic pulse-chase technique (*5*), whose (positive) signal consists of a radioactive marker that decays as the tagged protein is removed.

The bleach-chase technique offers several advantages. Although mass spectrometry can increase the throughput of the pulse-chase approach (*6*), it does not allow real-time monitoring of proteins in living cells. Likewise, techniques based on translation inhibition involve perturbation of the cell and produce relatively noisy estimates of individual half-lives (*1*), especially for long-lived proteins. Techniques based on activatable fluorophores (*7*) cannot take advantage of existing libraries of proteins tagged by standard fluorophores. Dual-fluorescent techniques share many advantages of bleach chase, but their typical high-throughput applications have not yet produced quantitative estimates of half-lives (*8*). However, the relative accuracy of mean protein turnover rates measured by Eden *et al*. in a population of asynchronous cells comes at a price—sacrificing the ability to study variation in protein decay rates across individual cells (which can be done using a dual-fluorescent method) (*8*).

The protein half-lives estimated by Eden *et al*. in cancer cells range from roughly 1 hour to 1 day, versus a mean half-life of 45 min in yeast cells (*1*), which divide more rapidly. Removal was dominated by active degradation for about half of the proteins studied, whereas removal was dominated by dilution for the 10% of proteins that were very long-lived; both dilution and degradation played substantial roles for the remaining proteins. These results quantify the balance between the fundamental mechanisms of protein removal in these cells.

How do protein half-lives change when cell growth is arrested? To study this, Eden *et al*. treated cells with either drugs or nutrient starvation. In all treatments, protein half-lives typically increased. Moreover, long-lived proteins showed the most substantial increases, because the removal of such proteins is governed primarily by the rate of dilution. Remarkably, treated cells did not compensate for the change in division rate by increased protein degradation, and indirect evidence suggests only partial compensation by decreased synthesis. As a result, growth arrest may lead to pathogenic proteome imbalance (*9*), especially in cells that are dividing quickly before treatment. Eden *et al*. suggest this mechanism to explain why some anticancer drugs preferentially kill tumor cells. At the same time, the view that perturbing cell division is more detrimental to rapidly dividing cells, as opposed to differentiated cells that divide rarely, is also a convincing explanation.

Therapeutic implications aside, measurements of protein dynamics raise intriguing questions about evolutionary strategies for robust cell regulation. Although energetically costly, high levels of synthesis combined with active, specific degradation might reduce noise in protein abundances and produce steady-state amounts that are also more robust to changes in the rate of cell division. Such features would be desirable in genes that require precise temporal regulation. Indeed, Eden *et al*. observed enrichment for cell division cycle proteins among the degradation-dominated short-lived proteins, similar to findings in yeast (*1*). This is somewhat analogous to strategies that reduce noise in protein synthesis found among essential yeast genes and among complex-forming proteins that require strictly regulated stoichiometry (*10*). Furthermore, if the abundance of a protein must respond rapidly to a stimulus, then this demand will select for more dramatic transcriptional regulation in a long-lived protein than in a short-lived protein—a trend that has been observed statistically in yeast (*1*) but has not yet been extended to mammals.

The roles of protein synthesis, degradation, and dilution in providing a robust cellular physiology remain topics of active research. Recent work in mammals has uncovered remarkable bursts of transcription (*11*), raising the possibility that long half-lives are selected to buffer protein amounts against overdispersion, especially in essential or complex-forming proteins. Other work, by contrast, emphasizes the adaptive value of noisy expression for bet-hedging against unpredictable environments in microbial populations (*12*, *13*) and for cell differentiation in multicellular organisms (*14*). However these questions are resolved, measurements of protein dynamics will undoubtedly improve our understanding of how cells operate.

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