# Cell-free protein synthesis sheds light on intracellular dynamics

**Primarily a tool to produce large amounts of protein quickly, the cell-free system is being adapted to the study of complex biological processes.**

**The chemistry of living things** is complicated. To synthesize a protein molecule from the information encoded in a single gene, a cell must first transcribe the DNA into messenger RNA; then the mRNA is translated to produce a protein. Additional reactions inactivate the mRNA and degrade the protein when the cell no longer needs them. Altogether, more than 100 enzymes and other molecules are involved in the reactions, and the translation process is especially complicated, as shown schematically in the figure. Gaining quantitative insight into the reaction dynamics by observing living cells is a daunting challenge: Many of the molecules are also involved in other reactions going on at the same time, so it is difficult to look at the synthesis of one protein in isolation.

But now Vincent Noireaux (University of Minnesota), Roy Bar-Ziv (Weizmann Institute of Science in Israel), and their colleagues have carried out a complete gene-expression reaction in a biochemical system that contains no living cells.[1] Their cell-free system was designed to mimic the protein synthesis in *Escherichia coli* bacteria. And they've developed a simple model that quantitatively describes their results.

## Breaking out of the cell

Cell-free protein synthesis itself is not new. It's been used for decades to produce proteins for various applications in research and medicine. But because the goal is to produce a lot of protein quickly, typical cell-free systems, which are available commercially, are not ideal for reproducing reactions as they occur in vivo. For one thing, they combine components from different organisms and viruses; in particular, the RNA polymerizing enzyme found in certain viruses is much more efficient than the forms of the same enzyme found in bacteria or other organisms. For another thing, the systems don't allow any control over the rates of mRNA inactivation and protein degradation—those reactions are biologically relevant, but when the aim is to maximize protein yield, they're mere hindrances.

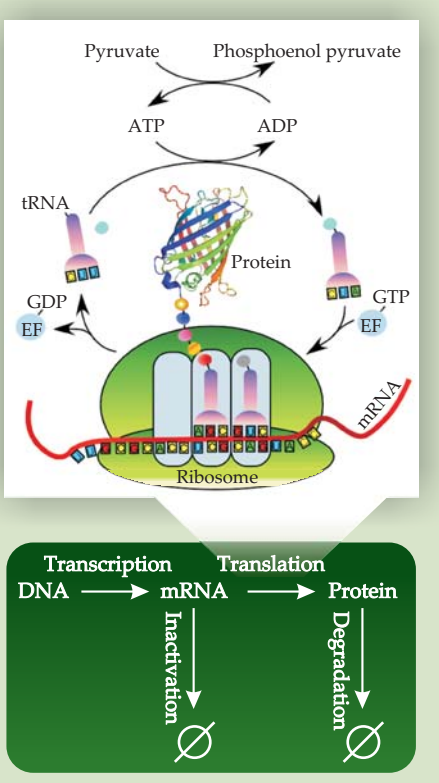
In the early 2000s, Noireaux and Bar-Ziv were postdocs together under Albert Libchaber at the Rockefeller University in New York. There they became interested in the possibility of using cell-free systems to create gene circuits, systems of several genes that interact—the expression of one could enhance or inhibit the expression of others.[2] But as Noireaux explains, "We were using commercial systems at that time, which had too many limitations to develop this approach."

Once Noireaux had set up his own lab in Minnesota, he and his student Jonghyeon Shin developed a new cell-free system that resolved many of the difficulties.[3] They used only molecules extracted from *E. coli*, including enzymes for mRNA inactivation and protein degradation. Using Noireaux and Shin's cell-free system and preliminary data, Bar-Ziv and his student Eyal Karzbrun carried out thorough experiments, tracking the protein concentration as a function of time and of the concentrations of various components.

## A simple model

Together, the researchers found that they could describe the main features of their results with a coarse-grained model that treats each of the four processes—transcription, translation, mRNA inactivation, and protein degradation—as if it were catalyzed by a single enzyme and parameterized by one kinetic constant. In contrast, "a fine-grained model would include all the biochemical reactions and would have a much longer list of parameters," explains Karzbrun. "It would only be doable by computer modeling."

One surprising aspect of the results was the scaling behavior of the protein-degradation reaction. The researchers expected the degradation rate to be proportional to the protein concentration—that is, a first-order reaction. Instead, they found that except for very low protein concentrations, the reaction was zeroth order: The degradation rate was independent of protein concentration. "Most other reported experiments show first-order degradation," Bar-Ziv says, "so it took us some time until we believed our results."




**A gene-expression system** comprising machinery for transcription, translation, messenger RNA inactivation, and protein degradation involves more than 100 different molecules; translation, represented by the reactions in the white box, is especially complicated. But the main features of the system's behavior are reproduced by a model that treats each of the four processes as if it were catalyzed by a single enzyme.

There are two possible reasons for the discrepancy. The first reason is that some of the other experiments looked at ensembles of cells. If each cell exhibited zeroth-order dynamics, but with a different rate constant, the ensemble measurement could appear to be first order.[4]

The other reason is that every enzymatic reaction has a zeroth-order regime and a first-order regime, depending on whether the time it takes an enzyme molecule to find a new reactant is small or large compared to the time it takes the enzyme to convert reactants to products. Adding more reactant decreases the first time scale but

not the second, so when conversion of reactants to products is the rate-limiting step, the reaction is zeroth order. In *E. coli*, an "affinity-enhancing protein" helps the protein-degradation enzymes find the proteins they degrade at the end of the translation process. Both Noireaux and Shin's system and the researchers' model include that protein, but some other experiments did not and would only regard protein degradation as a first-order reaction.

The next step for the researchers is to extend the experiment to study more complicated systems, including gene circuits. Using a biochip developed by Bar-Ziv and his group for immobilizing DNA on a surface,[5] they'd also like to develop a quantitative approach to study the spatial patterns that can form in gene-expression systems.

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## References


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# Room-temperature source delivers record-power terahertz beam

**The nonlinear optics device could help to resolve one of astronomy's lingering blind spots.**

**The universe teems** with terahertz radiation. A byproduct of thermal motions of atoms and molecules, it is shed in abundance by cool interstellar dust, protostars, and other celestial objects. A blackbody at 30 K, for example, radiates most strongly at frequencies near 1 THz. Along with the adjacent far-IR—which ranges roughly from 5 to 20 THz—terahertz radiation is estimated to account for 98% of all the photons that have been emitted since the Big Bang.[1]

Here on Earth, however, you'd never know. That's partly because terahertz, or submillimeter, radiation is resonant with the vibrations and rotations of atmospheric molecules; most of it is absorbed and never reaches the ground. It's also quite tricky to produce in the lab, with the swath between about 0.8 and 4 THz—known as the terahertz gap—being particularly elusive. A no man's land in the heart of the electromagnetic spectrum, the terahertz gap encompasses frequencies just below the reach of optics technologies and just above the reach of electronics.

The few devices that have encroached into the gap have eked out beams of just a few microwatts or so. That's enough power to serve some spectroscopy purposes—say, detecting trace amounts of hydrogen cyanide in a plume of smoke[2]—but not enough to drive the arrays of heterodyne receivers that might scan the skies for protostars. Some pulsed sources attain larger powers, but with relatively poor frequency resolution.

Now Jerome Moloney of the University of Arizona in Tucson and a team of US and German researchers have designed a continuous, room-temperature source that delivers narrowband, milliwatt beams at terahertz-gap