Tracking the footprints of protein synthesis

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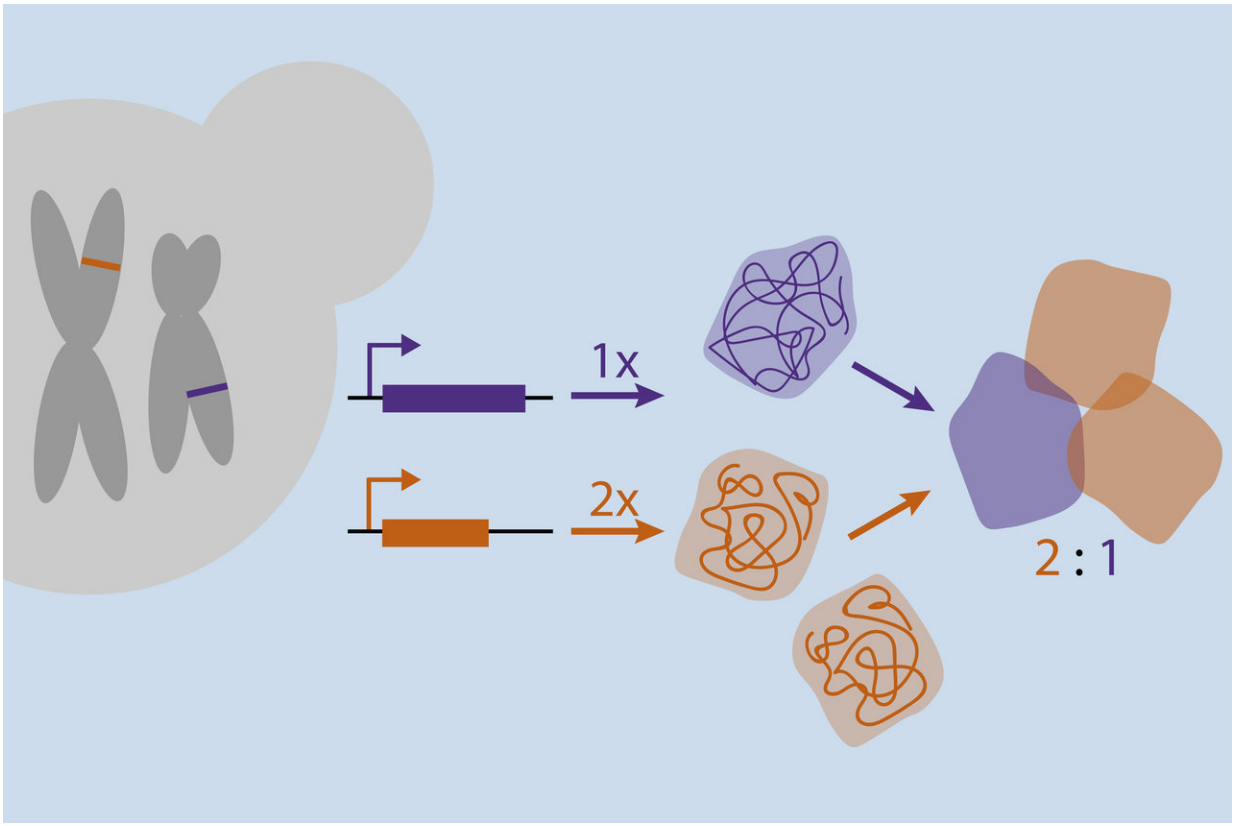

Cells have evolved to produce the components of their multi-protein complexes in precise ratios. Credit: Massachusetts Institute of Technology

To trace which proteins are produced and when, researchers say, just follow the ribosome "footprints."

Researchers are tracking these large molecular machines, following their

trails of protein synthesis to determine how precisely cells produce their protein components. Building too few might upset growth, metabolism, and maintenance, while too many might be wasteful and potentially toxic. Whether eukaryotic cells tune their gene expression to produce just enough of each protein remains a longstanding question.

Bacteria appear to generate the exact levels needed to function—no more, no less. However, more complex organisms have different metabolic needs, means to control gene expression, and ways to eliminate unwanted proteins, perhaps engendering a different strategy to ensure correct protein levels. Using a combination of their own experiments and open access databases, a duo of scientists from the MIT Department of Biology aimed to establish how precisely cells from organisms like budding yeast, zebrafish, mice, and humans tune their protein production. Do these eukaryotic cells generate precise amounts of protein, or do they make roughly the correct amount and rely on processes like degradation to trim the excess?

To answer this question, the researchers refined an existing technique, known as ribosome profiling, to quantify the protein synthesis rates by tracking the ribosome footprints. They were intrigued to find that, for the proteins they studied, eukaryotes precisely tuned their protein production just like bacteria. Despite the fundamental differences between these organisms, they shared a basic strategy.

"We tend to think of gene expression as a production line, transforming genetic information into well-defined protein machines," says Gene-Wei Li, an assistant professor of biology and senior author of the study. "But in actuality, it's not yet clear whether all organisms or all cells operate under the same principles of protein production. The impetus of this study was to understand if proteins are made as precisely in eukaryotes as they are in bacteria, while also resolving ambiguities in existing methods for measuring protein synthesis rates."

Graduate student James Taggart was the first author of this study, which appears in the journal *Cell Systems* on Dec. 12.

A clear case of proportional synthesis

In 2014, when Li was still a postdoc at the University of California at San Francisco, he and his colleagues set out to measure protein synthesis rates in bacteria. They examined the subunits of multi-protein complexes in Escherichia coli, and showed that the bacteria operated under laws of proportional synthesis—meaning they were generating proteins in the exact ratio needed for cellular function. If bacteria produce too much for some reason, a degradation pathway is activated to break down those subunits, but this process constitutes more of a failsafe than the primary means to regulate protein abundance.

In the present study, Li and Taggart aimed to make similar measurements in eukaryotes, starting with budding yeast. They used the same technique as Li in 2014—ribosome profiling—but with a few modifications.

Developed in 2009, ribosome profiling permits researchers to capture a snapshot of which mRNAs are being translated at a single moment in time. By using drugs that literally stop ribosomes in their tracks, scientists can freeze these molecular machines in place and destroy any un-protected mRNA that is not occupied (and thus shielded) by a stagnant ribosome. The fragments of mRNA—the ribosome footprints—can be sequenced to provide a barcode identifying which proteins were being made. The density of such footprints reveals the synthesis rates of each protein relative to the others.

Although ribosome profiling revolutionized our ability to gauge protein synthesis across the entire genome, it is sometimes difficult to map each mRNA fragment back to its original location within the genome and the

protein product to which it corresponds. A portion of one gene may have a sequence that's identical to another gene that encodes an entirely separate protein.

Here's where Taggart tweaked the approach slightly: he excluded these ambiguous mRNA fragments in his analysis, and only counted the unique ribosome footprints that he could trace back to specific proteins. He then divided the number of ribosome footprints by the length of the gene, minus the ambiguous sequences and non-coding intron regions. While more common analytic approaches fail to accurately account for these ambiguous footprints, Taggart only considered what he calls "meaningful" footprints that he could map to specific regions on the genome. As a result, his modifications generated more precise synthesis rates.

He ultimately curated a comprehensive list of roughly 500 proteins in yeast, comprising about 100 different protein complexes. As he monitored the yeast's protein output, it appeared they produced just the right amount of subunits to complete the complex—no more, no less. It was a clear case of proportional synthesis.

Protein overload

Once they fine-tuned their method of quantifying protein production, the researchers wondered what would happen if they upset the cell's careful synthesis balance. Do eukaryotes have a widespread mechanism to regulate the amount of protein they produce?

Although yeast normally only have 16 chromosomes, with the help of Angelika Amon's lab, the researchers duplicated each of them, one at a time, so the cell would have the capacity to build twice as many proteins using the genetic information from that extra chromosome. In humans, this kind of imbalance, known as aneuploidy, can lead to disorders like

Down syndrome.

Rather than sensing the excess protein and subsequently reducing production, the yeast did not initiate any internal communication to shut down operations at the level of transcription or translation. This runs counter to what is observed in some bacteria.

"It was interesting to see that bacteria and yeast both make the exact amount of protein they need," Taggart says, "although the ways they ensure precise synthesis are different. Many bacterial genes possess the negative feedback loops that yeast appear to lack."

Using data from open access databases, the researchers also identified proportional synthesis in higher eukaryotes, including zebrafish, mice, and humans for the subunits they examined from three large, highly conserved protein complexes. Despite the clear physiological and genetic difference between organisms, the complexes were produced in just the right ratios. The only exception was during zebrafish embryonic development, when the researchers concluded that protein production may not be proportional. This signified that the requirements for proportional synthesis might vary over the course of an organism's lifetime, depending on age, nutrient availability, and stress.

"Perhaps this precision is something we can learn from biology," Li says. Once researchers fully understand how cells fine-tune their protein production, he explains, they can apply that knowledge to designing their own molecules and pathways.

Eduardo Torres, an assistant professor of molecular, cell and cancer biology at the University of Massachusetts Medical School, says these requirements are conserved from bacteria to humans, "suggesting that the evolutionary pressure to produce protein amounts efficiently is a fundamental aspect of cell biology."

"The next step would be to understand the mechanisms behind the balanced synthesis of protein complexes," says Torres, who was not involved in the study. "Future studies integrating knowledge of several aspects of the regulation of gene expression will be necessary to understand how cells fine-tune the expression of each subunit of a particular complex."

Taggart also finds their findings compelling from an evolutionary perspective. "It appears eukaryotes have also evolved under pressure to achieve this proportional synthesis even though they're different from bacteria in so many other ways," he says. "In all domains of life, protein synthesis is both an engine for proliferation and a hub for regulation."

More information: James C. Taggart et al. Production of Protein-Complex Components Is Stoichiometric and Lacks General Feedback Regulation in Eukaryotes, *Cell Systems* (2018). DOI: 10.1016/j.cels.2018.11.003

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