Researchers show how cells solve biochemical challenges as they get bigger

June 11 2015, by Evan Lerner

In any textbook diagram, a group of red blood cells, skin cells or nerve cells will typically be identical in size. But, just as no two people are quite the same height and weight, in a population of real cells there are larger and smaller individuals.

A team of University of Pennsylvania researchers has now shown how the two copies of nuclear DNA in most cells' chromosomes can serve a cell of any size.

While cells of a given type can differ widely in size, their DNA content remains the same. This observation has puzzled biologists because it means that larger cells have a lower concentration of DNA than smaller cells, akin to having the same sized library serving two towns of vastly different populations. All of a cell's functions depend on transcribing DNA's contents—like checking out a certain book from the library—to produce the proper concentration of molecular products. It was unclear how the DNA of larger cells could keep up with increased demand.

"Within a population of cells that are identical in terms of function but vary in volume, how do they deal with having the same amount of DNA?" said Arjun Raj, a professor of bioengineering at the University of Pennsylvania School of Engineering and Applied Science. "This is an important question to consider in many aspects of cell analysis."

Now Raj and a team of collaborators believe they have solved the mystery. Through a series of laboratory experiments, the researchers

have shown that, as cells grow in volume, DNA is transcribed more quickly into mRNA, the messenger molecule that takes orders to cells' protein-producing factories. This allows larger cells to maintain the correct concentration of all biomolecules.

Furthermore, the team discovered two distinct mechanisms that underpin changes in the rate of mRNA transcription. One mechanism compensates for size differences across cells, while another mechanism governs changes in mRNA production when the load on a cell's DNA increases. These insights could help researchers pinpoint the cellular mechanisms of cancer and shed light on the early stages of embryo formation.

Doctoral candidate Olivia Padovan-Merhar of Penn's Department of Physics and Astronomy in Penn Arts & Sciences was lead author on the study, which also included contributions from former bioengineering postdoc Gautham Nair, cell and molecular biology graduate student Shawn Foley of Penn's Perelman School of Medicine and Penn undergrads Andrew Biaesch and Steven Scarfone.

Computer scientist Abhyudai Singh of the University of Delaware; biochemist Angela Wu, then of Stanford University and geneticists Stirling Churchman and Andreas Mayer of Harvard University's School of Medicine also contributed to the work.

The study was published in *Molecular Cell*.

Biologists have known for decades that not all cells of a given type are created equal. Despite having the same underlying genetic code, some amount of random variability exists from cell to cell. For instance, volume can differ as much as six-fold between cells that are functionally identical.

This creates a puzzle: If a large and a small cell contain the same number of DNA copies and if both cells produce and recycle mRNA at the same rate, then the larger cell should have lower concentrations of proteins and other essential molecules. This, in turn, would cause the rate of metabolic reactions to vary dramatically with cell size.

But larger cells apparently compensate for their size by stockpiling more mRNA relative to their complement of DNA.

"We know that a bigger cell needs more RNA from the same amount of DNA molecules," Raj said. "We wanted to characterize exactly how the cell is doing this."

In biology, simple questions often contain multiple layers. The first layer of the question the researchers decided to tackle related to the production of mRNA itself.

"In bigger cells, there are two ways that you could get more RNA from DNA," Raj said. "The cell could transcribe RNA faster, or the RNA could degrade more slowly."

By incorporating a fluorescently-tagged molecule into individual cells, Raj and his colleagues were able to watch RNA production and degradation in real time. They discovered that larger cells transcribed RNA from DNA more quickly and degraded it at the same rate.

Next, the researchers sought to establish a causal relationship between transcription rates and cell size. Just because cell size and RNA production are correlated doesn't mean one factor triggers the other.

"We know that bigger cells have more RNA," Raj said. "What we don't know is, if I were to make a bigger cell, would it start making more RNA? Or, is a cell bigger because it has more RNA? Or, is there

something else entirely that controls both of these?"

To find out, the researchers fused small cells to large cells, producing new cells that were well more than twice the size and contained double the DNA load of the original ones.

"If volume itself can dictate transcription, then we should see the absolute number of RNA molecules go up, and that's what we saw," Raj said. "When we fuse these cells, their nuclei begin producing more RNA because they can sense the extra volume. This is the first time anyone has shown a causal relationship between volume and RNA copy number."

Through their cell fusion experiments, Raj's team discovered another wrinkle to the story: Rather than simply detecting a change in volume, cells precisely monitor shifts in the ratio of size to DNA load.

"You could imagine there's something that tells the DNA, you're in a cell of size 5, transcribe RNA accordingly," Raj said. "But that's not how it works. When we fuse two cells together, we produce a cell that's twice as large but also now has double the DNA."

Rather than transcribing four times the original amount of RNA, which would happen if both sets of DNA blueprints began transcribing at double their original rate, the larger, fused cells were able to spread their increased transcription needs across the two copies of their genome.

Learning that the cell can modify RNA production based on both volume and DNA load led Raj and his colleagues to suspect there may be several mechanisms at play. They confirmed this hunch by watching cells undergo mitosis, or cell division. During division, the cell first produces an extra copy of all of its genes, before dividing into two cells.

"During mitosis we have the case that, for a period of time, there's twice as much DNA in the same sized cell," Raj said. "How does the cell ensure that it has half as much transcription from each DNA copy? We found that transcription occurs in pulses, and, when there's more DNA present, these pulses occur less frequently."

By contrast, Raj and his team found that when cells vary in size but not DNA content, pulses of transcription don't change in frequency, they simply become larger or smaller.

What specific molecules govern the cell's ability to finely tune its mRNA production based on size and DNA content are not yet known. Raj suspects that a mixture of transcription enzymes and histones, small molecules that bind to DNA and influence mRNA production, are at play.

Insights from this study could help researchers pinpoint the cellular basis for diseases such as cancer, where the ratio of volume to DNA load seems to be important. The study could also offer insights into how cells maintain proper biochemistry during the early stages of embryo formation.

"During early embryogenesis, you start with one large cell, which then rapidly divides into many smaller cells," Raj said. "The cells need to have mechanisms in place to account for these huge changes in volume."

There are likely to be many other aspects of cell biology and biochemistry that these fundamental insights can inform.

"Our study shows, if a cell is bigger or smaller, how it solves all sorts of concentration problems that go along with that size change," Raj said. "Anyone who is interested in how transcriptional rates relate to cell volume could find this knowledge useful."

More information: "Single Mammalian Cells Compensate for Differences in Cellular Volume and DNA Copy Number through Independent Global Transcriptional Mechanisms." DOI: dx.doi.org/10.1016/j.molcel.2015.03.005

Provided by University of Pennsylvania