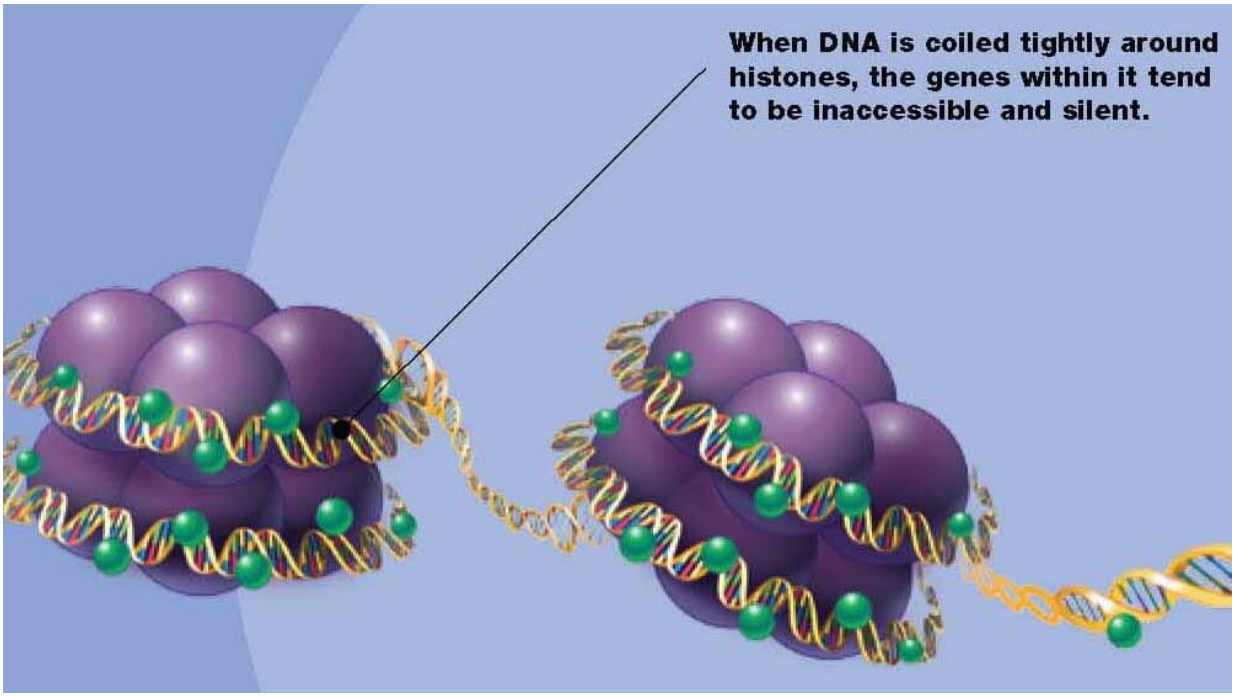
Using an egg 'soup' to understand how DNA is packed in the nucleus

December 19 2022, by Lily Neff



Histones spooling DNA. Credit: OIST (Okinawa Institute of Science and Technology Graduate University); CC BY 4.0 License

If you stretched the DNA found in one of your cells from end to end, it would extend approximately 2 meters or 6.5 feet. Every single cell in your body can pack away this much DNA by winding it around proteins called histones. The DNA is opened and closed when cells need access for normal processes such as cell division. However, many cancer cells

are extra sensitive to the packing and unpacking of DNA because they divide much faster than our healthy normal cells. Understanding which proteins specifically pack and unpack DNA could help us to target these cancer cells with inhibitors more accurately.

A Medical University of South Carolina (MUSC) research team led by David Long, Ph.D., reports in the *Journal of Biological Chemistry* that one protein, HDAC1, plays a larger role in packing DNA around histones than previously thought.

"Cells need to carefully unpack and read their DNA to create the different proteins found in your body," said Long.

To explain how DNA packing works, Colleen Quaas, Ph.D., first author of the article, offers an analogy.

"Think of histones like a reel and DNA like the hose that winds around it," she said. Quaas was a graduate student in Long's lab when she worked on the study. She is now a postdoctoral fellow in the lab of Tim Barnoud, Ph.D., at MUSC. Quaas's new research goals include developing novel therapies to treat pancreatic cancer.

Before this study, HDAC1 and HDAC2 were thought to play similar roles in packing away DNA by winding it around histone "reels," providing some built-in redundancy. Using a novel technique developed in Long's lab, the two researchers set out to explore and compare the functions of HDAC1 and HDAC2 proteins. They found that the story is far more complicated than that.

"There's more going on than you think, and so all those really simple, early interpretations are just waiting to be peeled back and explored further," said Long.

All the necessary information for creating proteins is written in your DNA. To extend Quaas's analogy, think of the process of using DNA to make proteins like watering your garden. You need to unwind the hose from the reel to water all of your plants, and when you're done watering, you wind the hose back up around the reel.

To understand better which proteins play a role in winding the DNA back up, Long developed a system using extract derived from the eggs of African clawed frogs. The extract system includes all of the proteins within the nucleus but removes the DNA.

"The extract is basically like a cell soup," Long said. "If you think of the nucleus as an orange, the extract is the juice that's squeezed out. We're looking at all the proteins that are 'squished out' of the eggs."

The extract system is unique in that it does not rely on cell cultures or animal models to answer scientific questions. Researchers can simply add the DNA of interest into the extract system of concentrated proteins and analyze how the DNA is packed or unpacked. Furthermore, they can determine which specific proteins interact with the DNA they add into the extract system.

"We can look at DNA in real time outside of a cell so it makes mechanistic studies of DNA much more accessible," said Quaas.

"We can do a lot of things in extract that you can't do in cells because you have to keep cells alive," said Long. "We can pull things out and add things back in, so it's very easy to manipulate the system."

Long and Quaas wanted to see what would happen when they used inhibitors that target different groups of HDAC proteins. Currently, multiple HDAC inhibitors have been approved by the U.S. Food and Drug Administration and are being used in clinical trials for cancer

treatment. The researchers decided to test several of these inhibitors and found that romidepsin specifically blocked DNA packing.

Romidepsin targets both HDAC1 and HDAC2, but it wasn't known previously which of the two HDACs was actually responsible for DNA packing. For their study, the researchers developed tools to target either HDAC1 or HDAC2, specifically. Given the prevailing wisdom, they thought that the effects on DNA packing would be the same, regardless of which protein they targeted. Instead, DNA packing was delayed only when HDAC1 was inhibited. This surprised the researchers, leading them to explore further how HDAC1 interacts with proteins other than HDAC2 to complete its job.

"We see that HDAC1 and HDAC2 have different roles in our system, and they're also found in different protein complexes" said Quaas.

The study's findings are important for several reasons. First, the extract system used in Long's lab is novel and can be used to answer questions that are difficult to address using traditional cell or animal models. Second, future cancer therapies could focus on developing inhibitors that specifically target individual HDAC proteins. The researchers found that HDAC1, not HDAC2, is the major driver of DNA packing, so targeting only HDAC1 could be more effective and have fewer side effects during treatment.

"These selective inhibitors are a great way to target cancer cells," said Long. "Cancer cells grow quickly, so if we can disrupt how they're able to pack and unpack DNA, that will make them more sensitive to cell death."

More information: Colleen E. Quaas et al, Transcription suppression is mediated by the HDAC1–Sin3 complex in Xenopus nucleoplasmic extract, *Journal of Biological Chemistry* (2022). DOI:

10.1016/j.jbc.2022.102578

Provided by Medical University of South Carolina