Measuring the number of protein molecules inside cells

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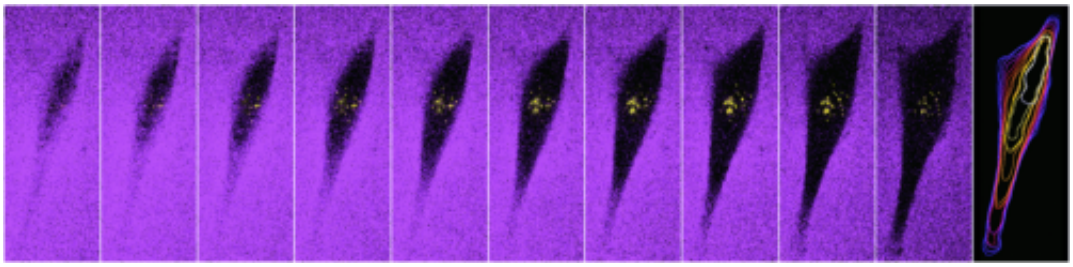

These images show the different planes of a cell (black) expressing CENP-A (yellow dots). Credit: Dani Bodor (IGC).

The identification of the genes and proteins involved in a biological process, as well as the way they interact, are essential for the understanding of that process. However, often little is known about the dimensions of molecular biological structures. Knowing how many molecules make up a structure and are required for its function are essential for our understanding of biological mechanisms. Yet, quantifying molecules of infinitesimal size poses a difficult challenge. Now, in a breakthrough study, Lars Jansen and his team from Instituto Gulbenkian de Ciência (IGC, Portugal) were able to measure the amount of protein molecules in living human cells required to form an important structure of the chromosome, the centromere. This study, published this week in the open access scientific journal *eLife**, presents new methodologies that may also be used to unveil other biological problems.

Centromeres are protein structures present at chromosomes. These structures recruit the necessary molecular machinery that drives the segregation of chromosomes into the daughter cells, a process essential for cell division. If the location of centromeres is changed or if the proteins that compose these structures are impaired, abnormal cell divisions may arise. Lars Jansen's laboratory, together with other research groups, have identified the components of centromeres and found one protein, called CENP-A to be central to centromere function. What has been lacking thus far is a measure of how many of these molecules are present which is important to understand how centromeres are built and maintained.

Dani Bodor, PhD candidate at Jansen's laboratory and first author of this study, explains the context of this study: "We knew the CENP-A protein was playing a crucial role in the formation of centromeres. Previous studies showed that without this protein, cells failed to divide properly, with consequences in the number of chromosomes transmitted to the daughter cells. But exactly how much CENP-A was required to form a centromere? We needed to find a way to count CENP-A molecules, that have a size in the order of nanometers (1.000.000 times smaller than 1 millimeter)."

The research team set to develop tools that allow for such a measurement. Using modern genetic engineering they fused a gene that codes for a fluorescent protein to the CENP-A gene. By using this genetic trick, all CENP-A proteins produced by cells became fluorescent. Next, the researchers observed these cells under the microscope, and were able to quantify the total amount of fluorescence present in the cell and the fraction of fluorescence at centromeres. Ultimately, these measurements allowed them to determine that approximately 400 molecules of CENP-A are present on the centromeres of human cells.

Dani Bodor says: "We were inspired by a methodology used in yeast. But until now, no one had used it to measure molecules in more 'complex' cells. Yeast cells have more or less the same shape and volume, but human cells differ in shapes and volumes which increases the degree of complexity when this kind of techniques are used."

To confirm their calculations were accurate, the researchers used two other techniques. Their results showed that independently of the technique used they would always reach a number around 400.

Lars Jansen says: "Centromeres need to be very stable structures to ensure the faithful transmission of chromosomes to the daughter cells during cell division. When cells divide, the CENP-A proteins are distributed to the daughter cells, and the number of molecules that each cell receives may vary. By having 400 molecules the cell can assure that a sufficient number of CENP-A is passed to form the centromeres. The calculation of the number of CENP-A molecules allows us to propose a mechanistic framework that can explain the formation and inheritance of centromeres".

When asked about the technical difficulties faced during this study Lars says: "We took 5 years to conduct this work, and for sure we would not be able to have done it 10 years ago. We need to develop new techniques continuously to be able to go further and answer novel questions, even to old biological problems. We have arrived at a time in biology where more and more laboratories will start looking at the quantitative aspects of the biological problem they are studying. The techniques we have employed can be quite helpful for that."

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