GENOMICS

Native chromosome conformation

Isolation of nuclei in an isotonic buffer retains chromosome loops and allows the probing of intrinsic loop conformation.

Argyris Papantonis has a long-standing interest in genome architecture. "I did my first 3C the moment I started my post doc in 2008," he says, referring to chromosome conformation capture (3C) experiments he performed in Peter Cook's lab at the University of Oxford. His first project upon starting his own lab at the University of Cologne in 2013 had PhD student Lilija Brant working on a perennial challenge in the field—namely, that of capturing native chromosome conformation without crosslinking. "We want to understand the hierarchy of how folding takes place at the single loop level," says Papantonis.

In the classical 3C protocol, interacting chromatin domains are locked in place by a crosslinking agent; then the DNA is cut, and the ends are ligated, isolated and sequenced to derive contact maps that show the probability of any two loci interacting. Over the years there has been a lot of discussion as to whether crosslinking introduces bias in 3C experiments—Papantonis wanted to get around any possible crosslinking artifacts and detect interacting domains in their native conformation.

To develop intrinsic 3C (i3C), Brant modified the conventional protocol and used a very gentle isotonic buffer for the isolation of nuclei. A simple change in buffer sounds deceptively straightforward, but Papantonis stresses that it is essential for a successful i3C experiment. In a buffer with either too much or too little salt, nuclear components may aggregate or burst, but the nuclei isolated in their physiological buffer remain fully transcriptionally active; hence most of the chromatin structure will be preserved.

For background reduction Brant removed about 50% of the chromatin that was in the soluble, noninteracting fraction. Papantonis is not concerned that they are losing valuable information, since sequencing this soluble chromatin did not reveal any informative interactions.

Initial experiments with their i3C protocol confirmed known interactions, but when looking at the first maps done with iT2C—a capture-based 3C derivative that determines all loops emanating from a locus of interest—Papantonis felt deflated and initially thought of the experiments as a 'disaster'. Instead of the beautiful triangles denoting contacts that he had been used to seeing throughout his career, what the team now saw were dots (**Fig. 1**). It took them months of analyzing the data to believe that those dots represented true interactions and were actually very clean and focused data.

Papantonis explains the difference in the data: "When you crosslink two fibers you also get crosslinks further up and down the fiber even though they are not directly interacting, just because they are in close physical proximity." These 'bystander' interactions are not random and can be informative, but they are missing from the i3C data, which only report direct interactions. The background-free data allowed the team to examine regions with dense interactions that could not be resolved with crosslinking approaches. They examined a region with five enhancers and saw that only three of them contributed to interactions.

To validate their findings Papantonis first turned to Davide Marenduzzo at the University of Edinburgh, who does predictive polymer modeling to reproduce spatial chromatin organization based on known active and inactive regions. Their iT2C maps closely resembled the simulated maps. To also verify interactions experimentally, the team used the DNA-binding domain of a TALE fused to an adenosine methylase and targeted it to a particular enhancer. The enzyme methylates all adenosines in close proximity to the enhancer, and these modified bases were identified by a methylation-specific digest and confirmed the iT2C results.
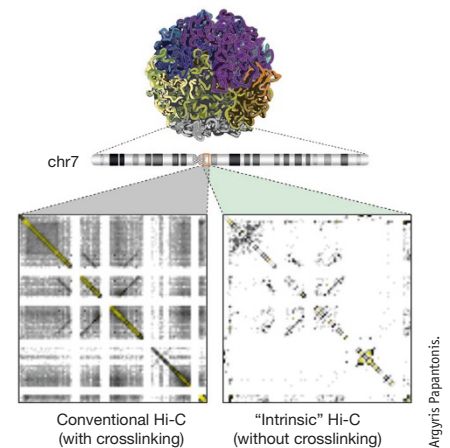
Comparison of typical contact maps from Hi-C approaches with and without crosslinking.

Conventional Hi-C (with crosslinking) — "Intrinsic" Hi-C (without crosslinking)

chr7

Argyris Papantonis.

But i3C methods are still a work in progress; one aspect to be addressed is data analysis. "We are happy to have the method out there," says Papantonis, "but computationally we are not there yet. We need a tool that can deal with sparse matrices." He also stresses that before adopting i3C methods researchers should consider the resolution they are interested in. To investigate larger structures, such as topologically associating domains (TADs)—which are on average a quarter of a megabase in size—conventional crosslinking methods are likely a better choice, but if one needs to resolve individual loops within the TADs, i3C methods are the way to go.

Papantonis' focus is on understanding the effect mutations in chromatin-binding proteins have on loops in prematurely aging and senescent cells. "We want to see what exactly changes in these cells and how this may explain changes in gene expression." i3C methods make crosslinking artifacts in this endeavor no longer a concern.

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RESEARCH PAPERS
Brant, L. *et al*. Exploiting native forces to capture chromosome conformation in mammalian cell nuclei. *Mol. Syst. Biol.* **12**, 891 (2016).