GENOMICS

Micro-C maps of genome structure

A method using micrococcal nuclease for chromatin fragmentation gives a high-resolution view of 3D genome structure.

The packaging of vast tracts of DNA into the tiny space of the nucleus in a way that allows gene regulation and other vital activities to proceed smoothly is still not fully understood. At the primary level of organization, it is known that DNA is wrapped around histones to form nucleosomes. But what happens after that, one level of organization up, is still hotly debated. The so-called 30-nm fiber seen in electron micrographs of *in vitro* samples has been questioned, for example, in part because it has not actually been detected within cells.

One of the reasons for the gaps in knowledge, according to Oliver Rando at the University of Massachusetts Medical School in Worcester, is that there is a blind spot in the available methodology. Techniques like chromatin immunoprecipitation sequencing (ChIP-seq) and its variants can map protein binding to the genome at a resolution of one to a few hundred base pairs. On the other hand, the chromatin conformation analysis (3C) method and its variants, which measure the frequency of contacts between different genomic positions in the linear sequence, cannot resolve structures smaller than about 1 kb because they rely on restriction enzymes for fragmentation (an exception is the DNase HiC method reported earlier this year). What was needed was a less biased method with a resolution somewhere in between.

To meet this need, Rando and colleagues developed Micro-C, a procedure in which the cross-linked genome is cut with micrococcal nuclease (MNase) instead of restriction enzymes, yielding mononucleosomes and generating maps of genome structure at nucleosome resolution. The researchers applied Micro-C to budding yeast.

As has been previously reported in some organisms, but not in yeast, Rando and colleagues do observe internucleosomal organization using Micro-C. The reported chromosome interacting domains (CIDs) are substantially shorter than the topologically associated domains seen in mammalian cells. Interestingly, because yeast CIDs typically span 1–5 genes, the size of these domains is conserved if one scales by gene number rather than sequence length (as yeast genes are much shorter than mammalian ones).

The boundaries of CIDs are enriched in the upstream regions of highly transcribed genes. Treatments that perturb transcription, such as diamide stress or RNA polymerase inhibition, affect genome compaction in a manner consistent with an anticorrelation with transcription activity. From mutant analysis, the researchers further implicate the Mediator complex and the RSC chromatin remodeling complex, among others, in different aspects of genome organization.

Although Rando and colleagues do not see evidence for the extended tracts of a 30-nm fiber using this approach, their data do support a tri- or tetranucleosome folding motif, which is not inconsistent with a zigzag fiber (one of the two dominant 30-nm fiber models). Notably, they may be unable to see a more extended organization because they used formaldehyde, a short-distance cross-linker, which may not efficiently link more distant contacts even if they do exist.

Unlike restriction enzyme–based methods, Micro-C does not effectively capture known long-range interactions, such as those between centrosomes. The methods are thus complementary, with Micro-C able to access shorter-range interactions (between 200 bp and 4 kb) but at higher resolution. The DNase HiC method, which uses DNase I to fragment the chromatin, also substantially improves mapping resolution. Rando predicts that this method may be more suitable for probing interactions between regulatory elements given that DNase I preferentially cleaves nucleosome-depleted regions. Indeed, so far, DNase HiC has been used in combination with sequence capture to analyze interactions between the promoters of about 1,000 lncRNA genes; future work will be needed to probe its capabilities in a global analysis of genome folding.

Needless to say, the problem of how the genome is organized in space is a sufficiently complex and interesting one that the more tools we have, the merrier.

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RESEARCH PAPERS

Hsieh, T.H. *et al*. Mapping nucleosome resolution chromosome folding in yeast by Micro-C. *Cell* **162**, 108–119 (2015).

Ma, W. *et al*. Fine scale chromatin interaction maps reveal the *cis*-regulatory landscape of human lincRNA genes. *Nat. Methods* **12**, 71–78 (2015).

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