IMAGING

A brief history of nuclear organization

By marking protein-DNA interactions in real time, scientists track chromosomal rearrangements in the nuclei of living cells.

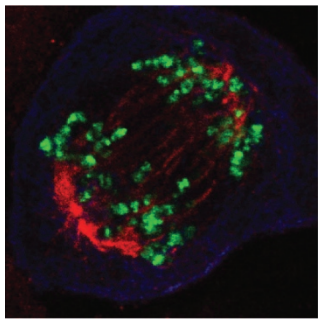
Eukaryotic nuclei are not merely haphazard jumbles of chromosomes, like some neglected storage drawer full of cables. Instead, the nucleus maintains three-dimensional organization via associations between an inner layer called the nuclear lamina (NL), composed of lamins and other proteins, and lamina-associated domains (LADs) sprinkled liberally throughout each chromosome.

Bas van Steensel's team at the Netherlands Cancer Institute in Amsterdam has previously conducted thorough surveys of LADs in diverse cell types. "They always comprise 30%–40% of the whole genome, even across different organisms," explains Jop Kind, a postdoc in van Steensel's group, "and they look pretty much the same in all the cells we've looked at so far." However, many questions remain about the LADs' dynamic behavior. Not all LADs are engaged with the NL simultaneously, and some studies hint that there may be a heritable component wherein mitotic cells pass along aspects of their nuclear organization to their 'daughters'.

Kind, van Steensel and their colleagues therefore devised a minimally intrusive system for charting DNA-protein interactions in living cells. In earlier work, the researchers devised a method called DamID, in which they fused a protein of interest to a bacterial enzyme that introduces an adenine-6-methylation (m6A) modification to any DNA sites with which it comes in contact. "This print is absent in higher eukaryotes, so you won't find it unless you express this protein," says Kind. The original DamID method involved breaking open cells and treating them with DpnI, an enzyme that selectively cuts DNA at m6A marks, to map modified genomic sites. Building on this approach in new work, Kind and colleagues fused the m6A-recognition domain from DpnI to enhanced green fluorescent protein (EGFP), thereby devising a genetically encoded "^{m6}A-Tracer" that detects these marks in living cells.

Kind and van Steensel generated cell lines that express lamin B1 fused to the DNA-labeling enzyme and ^{m6}A-Tracer. Each of these was regulated by a distinct conditional expression system so that the researchers could tightly control the timing and duration of labeling and tracing. This technique thus provided molecular-scale insight into lamin-chromosome interactions. "We know that a piece of DNA must have been within nanometer distance from lamin B1 to establish that print," says Kind. Equally importantly, this approach tracks interaction history such that a given LAD will retain the signature of its interaction with the NL even if it subsequently shifts further into the nucleus. This proved useful, as many tagged LADs were observed at distances up to a micrometer from the NL, which indicated clear reorganization over the course of the experiment. When they labeled cells undergoing mitosis, the researchers were surprised to note that the majority of the NL-interacting sites from the premitotic cell were situated deeper in the nuclear interior in the daughter cells, some distance away from the NL. "I had expected some mobility of LADs, but that they were completely rearranged—stochastically, it seems, as we couldn't find any heritable patterns," says Kind.

They also observed striking patterns of chromatin distribution. LADs are known to reside in dense regions of heterochromatin that repress gene expression, but the researchers learned that NL-interacting LADs are also especially prone to feature the repressive histone 3 lysine 9 dimethylation (H3K9me2) pattern. They fused ^{m6}A-Tracer to a viral protein that disrupts heterochromatin, thereby specifically targeting NL-interacting LADs. This resulted in reduced levels of H3K9me2 at these sites and a greater overall tendency for LADs to 'wander away' from the NL. This suggests a close connection between the presence of this repressive modification and LAD anchoring at the nuclear periphery, although the nature of this relationship remains unclear.



Lamina-associated domains (green) charted during mitosis. Reprinted from *Cell* with permission from Elsevier.

Kind believes single-cell studies will help address this and other unanswered questions. For example, this study focused on lamin B1, but there are other lamin proteins—including lamin A, which is found throughout the nuclear interior. Because all lamins appear to show the same chromosomal binding preferences, this could explain why so many LADs lie deeper within the nucleus than expected. "We think that lamin A specifically interacts with LADs in the middle of the cell," says Kind, "but this is pure speculation at this point." He also sees the potential to conduct similar live-cell labeling experiments to track the dynamics of chromosomal interactions with the nucleolus or large multiprotein regulatory complexes with this method, offering scientists a helpful tool to assign meaning to the seeming chaos of the nucleus.

Michael Eisenstein

RESEARCH PAPERS
Kind, J. *et al.* Single-cell dynamics of genome-nuclear lamina interactions. *Cell* **153**, 178–192 (2013).