3D Super-resolution imaging of localization of H3K27me3, Lamin A/C and DNA

Histone molecules wound by genomic DNA undergo chemical modifications such as methylation and acetylation, which affect the regulation of gene expression. Therefore, in order for proliferating cells to show the same traits as their original cells, it is considered that the chemical modifications of parental histones need to be accurately replicated when DNA is replicated. However, the molecular mechanisms involved therein remain largely unclear.
In this Application Note, we introduce examples of imaging (using the N-SIM super-resolution microscope) performed by pharmaceutical scientist Dr. Tsukasa Oikawa of the Department of Molecular Biology, Hokkaido University Graduate School of Medicine. In the absence of p53, the product of a tumor suppressor gene TP53, K27-trimethylation of histone H3 (H3K27me3) was observed to occur at the perinuclear regions during DNA replication. The researcher then wanted to know the positional relationship between H3K27me3, the nuclear membrane represented by Lamin A/C, and DNA.

Analysis of the methylated histone dynamics in the nucleus

Replication of a nucleosome structure consisting of DNA and histone complexes is thought to require the recycling of parental histones and the chemical modifications of newly synthesized histones with parental histones as templates. Dr. Oikawa discovered that antagonizing regulation of EZH2, the catalytic subunit of the Polycomb group protein complex (PRC2) and p53 is involved in the maintenance and transition of H3K27me3 (Oikawa et al., *Sci. Rep*. 2018). Furthermore, during the course of investigating the molecular mechanism therein involved, it is suggested that the antagonism between p53 and EZH2 occurs when the histone variant H3.1 forms a complex with other histone molecules in the nucleus. The histone variant H3.1 is newly synthesized in the cytoplasm during DNA replication and transported through the nuclear membrane into the nucleus. Dr. Oikawa aims to clarify the mechanisms of the maintenance and transition of cell traits over cell division by clarifying the dynamics of histone methylation during DNA replication.

3D super-resolution imaging of intracellular colocalization using N-SIM

While H3K27me3 was mostly distributed throughout the nucleus, it was relocated to the perinuclear regions during DNA replication when p53 was depleted, which was revealed by confocal microscopy. Dr. Oikawa then took advantage of the 3D super-resolution imaging of the N-SIM, which enabled him to localize H3K27me3, Lamin A/C and DNA with much higher resolution. Observation using the N-SIM made it possible to determine whether H3K27me3 is located on the cytoplasmic side or in the nuclear interior across the nuclear membrane and, if it is located in the nuclear interior, whether it is in contact with Lamin A/C or colocalized with DNA.

Visualization of H3K27me3 which does not consistently colocalize with DNA

With the super-resolution images obtained, it was found that the deletion of p53 causes H3K27me3 to be located in proximity with Lamin A/C inside the nuclei during DNA replication. On the other hand, H3K27me3 was not consistently colocalized with DNA. (Figure 1)

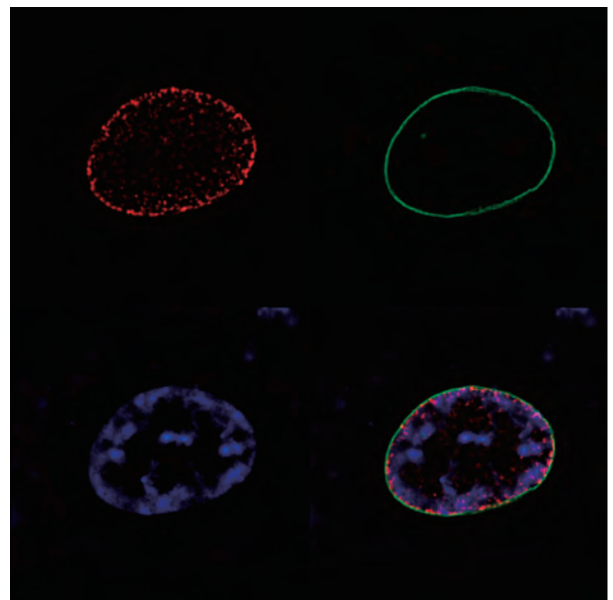

Figure 1: N-SIM image of human lung cancer cell line A549. Its wild-type p53 was knocked down by RNAi
Red: H3K27me3, Green: Lamin A/C, Blue: DNA
Antibodies: anti-H3K27me3 (Cell Signaling, rabbit monoclonal, #9733), anti-Lamin A/C (Cell Signaling, mouse monoclonal, #4777), anti-Lamin B1 (Santa Cruz, mouse monoclonal, #365214), TO-PRO-3 Iodide (for DNA stain, Thermo Fisher Scientific, #T3605)
Measurement condition: PFA-fixed samples were embedded by Prolong Diamond (Thermo Fisher Scientific) after immunostaining.
Magnification of objective: 100X
Acquisition mode: 3D-SIM mode (Z-stack)
Images courtesy of: Dr. Tsukasa Oikawa, Department of Molecular Biology, Hokkaido University Graduate School of Medicine. Images were captured at the Nikon Imaging Center at Hokkaido University.

These data suggest the following possibilities as to the identity of H3K27me3 that is distributed unevenly toward the perinuclear region:
(1) The perinuclear H3K27me3 is the histone variant H3.1 which is synthesized in the cytoplasm during DNA replication, imported through the nuclear membrane and K27-trimethylated before forming a nucleosome inside the nucleus.
(2) The perinuclear H3K27me3 is a H3K27me3 excluded from a pre-existing nucleosome during its reorganization associated with DNA replication, and which was trapped near the nuclear membrane for some reason.

Subsequently, a Proximity Ligation Assay (PLA) was performed, followed by observation with the N-SIM, confirming that the proximity of H3K27me3 and Lamin A/C occurs on another nuclear membrane lining molecule Lamin B1 (Figure 2).

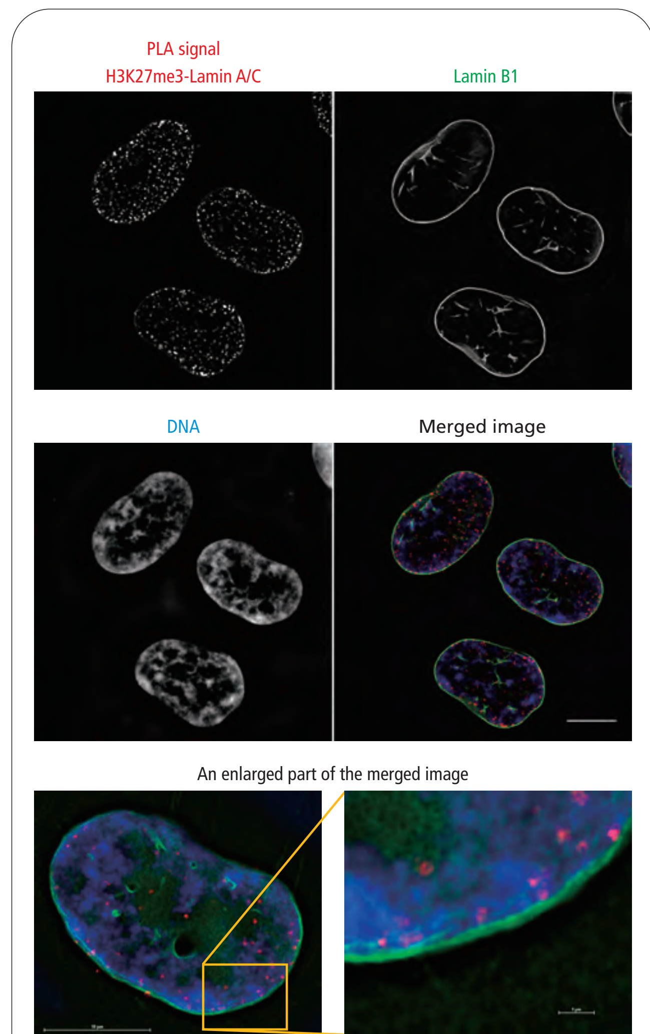

Figure 2: p53-null human lung cancer cell line H1299
Antibodies: anti-H3K27me3 (Cell Signaling, rabbit monoclonal, #9733), anti-Lamin A/C (Cell Signaling, mouse monoclonal, #4777), anti-Lamin B1 (Santa Cruz, mouse monoclonal, #365214), TO-PRO-3 Iodide (for DNA stain, Thermo Fisher Scientific, #T3605)
Measurement condition: PFA-fixed samples were embedded by Prolong Diamond (Thermo Fisher Scientific) after immunostaining.
Magnification of objective: 100X
Acquisition mode: 3D-SIM mode (Z-stack)
Images courtesy of: Dr. Tsukasa Oikawa, Department of Molecular Biology, Hokkaido University Graduate School of Medicine. Images were captured at the Nikon Imaging Center at Hokkaido University.

Getting closer to the identity of H3K27me3 distributed unevenly toward the perinuclear region

Further, additional PLA followed by N-SIM observation was performed to examine the proximity of H3K27me3 with other histones or with EZH2. These observations suggested that at least a part of H3K27me3 at the perinuclear region does not form complexes with other histones and was ectopically methylated by EZH2. Therefore, the perinuclear H3K27me3 is thought to be derived from newly synthesized H3.1 as described (1) above.

When conducting this research, a resolution of at least several hundred nanometers was required to understand the positional relationship and the proximity state of molecules in a micro region, i.e. in the vicinity of the nuclear membrane. With N-SIM, a high resolution of several hundred nanometers was obtained not only in the XY direction but also in the Z direction, at the same time it was possible to carry out the steps from preparation to observation of samples with a simple procedure similar to that of a confocal microscope.

Product Information

N-SIM S Super Resolution Microscope

The N-SIM S utilizes Structured Illumination Microscopy (SIM) technology to capture the minute structures within a specimen at twice the resolution of conventional light microscopes. Its larger field of view allows it to acquire large-area images of neurons at a high throughput.

- Lateral resolution: 115 nm (3D-SIM mode), 86 nm (TIRF-SIM mode)
- Axial resolution: 269 nm (3D-SIM mode)
- Field of view: Up to 66 μm x 66 μm (with a 100X objective)