Integrated imaging approach to the study of dynamics of chromatin

Chromatin remodelers are responsible to regulate gene expression of eukaryotic cells by dynamic modification of chromatin architecture. Arp4, a member of actin related protein family, is reported to serve as a regulator of chromatin remodeling possibly through its direct binding to histone. However, the detailed mechanisms are still elusive.

Aiming to clarify the dynamics of Arp4 in chromatin remodeling, we conducted quantitative imaging analysis in the nucleus. FRAP analysis revealed two components in the movements of Arp4. Single-molecule analysis by HILO microscopy discriminated the movements between Arp4 and chromatin. These results of the integrated imaging approach suggest dynamic interactions of Arp4 with either chromatin-remodeling complexes or chromatin.

Elucidation of construction and function of heterochromatin through HP1 binding proteins
Chikashi Obuse (Grad. Sch. Life, Hokudai)

HP1 is thought to play a role in heterochromatin formation by binding to K9 trimethylated histone H3 (H3K9me3) and its interacting proteins. By proteomic analysis, we identified 82 HP1 binding proteins (HPBPs) in human cells [Nature Cell Biol. 12: 719, 2010]. An uncharacterized HPBP was enriched in inactive X chromosomes (Xi) in association with SMCHD1, and thus we named it HBIx1. Cytological and epigenomic analyses revealed that HBIx1 and SMCHD1 mediate the compaction of Xi to form heterochromatin structure, by linking the H3K9me3 domains and the XIST/H3K27me3 domains [Nature Struct. & Mol. Biol. 20: 566, 2013]. These results enable us to discuss a molecular mechanism how epigenetic marks are translated into higher order chromatin structure.

Monitoring histone and RNA polymerase modification dynamics in living cells and organisms
Hiroshi Kimura (Grad Sch Frontier Biosci., Osaka Univ.)

In eukaryotes, post-translational modifications on histone and RNA polymerase II (RNAPII) play an important role in gene regulation. However, how these modifications are regulated in vivo remains largely unknown. To reveal the dynamics and function of these modifications, we developed two live-cell tracking systems by using specific antibody binding fragments (Fab). Another system uses genetically encoded single-chain variable fragments tagged with the fluorescent protein. This technique allows us to monitor modification dynamics in living organisms. I will present our recent data on the kinetics of histone and RNAPII modifications in response to gene activation.