Cytologia focus:

FISH Is in the Limelight Again As More Than a Cytogenetical Technique for Metaphase Chromosomes

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Received February 9, 2016; accepted February 12, 2016

Summary Fluorescent *in situ* hybridization (FISH) has been a powerful technique to reveal chromosomal loci and distribution of DNA sequences in metaphase chromosomes. Three-dimensional FISH (3D-FISH) has also revealed the chromosome territory of interphase chromosomes in the nucleus. Recently, 3D-FISH has played a supportive or complementary role to confirm the genome organization and topologically associated domains, which are provided by chromosome conformation capture techniques.

Key words FISH, Banding, Hi-C, Chromosome, Topologically associated domain, Chromosome conformation capture, Chromosome territory, Genome organization.

Cytogenetical techniques for mitotic chromosomes have revealed karyotypes and chromosomal arrangements (Maneechot *et al.* 2015, Phimphan *et al.* 2015). Chromosome banding techniques contribute not only to karyotyping but also to the identification of specific chromosomes (Afroz *et al.* 2013, Alam *et al.* 2013a, 2013b, Sultana *et al.* 2013, Lombello and Pinto-Maglio 2014, Mahbub *et al.* 2014, Manzum *et al.* 2014, Tung-kajiwangkoon *et al.* 2015). Some chromosome banding techniques, including C-banding and G-banding, are strongly dependent on heterochromatin distribution in mitotic chromosomes (Sumner 2003, Bansal and Kaur 2015). Recent chromosome banding with fluorescent stains can represent the difference of GC content along mitotic chromosomes. Chromosome banding with chromomycin A₃ can reveal GC-rich regions, whereas that with 4',6-diamidino-2-phenylindole can highlight AT-rich regions (Akter *et al.* 2015, Hizume 2015, Hizume and Kan 2015, Imran *et al.* 2015).

Fluorescent *in situ* hybridization (FISH) can identify mitotic chromosomes and reveal specific genomic loci or distribution of DNA sequences in more detail (Hizume *et al.* 2013, Ikeda *et al.* 2013, Kuroki *et al.* 2013, Ruan *et al.* 2013, Yokomi *et al.* 2013, Kantek *et al.* 2015). FISH procedures include the preparation of metaphase chromosomes, labeling of probes, DNA denaturation, hybridization, detection of probes with fluorescent antibodies and observation by fluorescent microscopy (Shibata and Hizume 2015). Moreover, multiplex FISH with the mixture of each genome from a single pair of

homologous chromosomes as multicolor probes, which is also called chromosome painting, can identify each chromosome and elucidate chromosome synteny and translocation (Suto *et al.* 2003, 2012, 2013).

FISH has also been challenged to answer the question of how decondensed chromosomes are arranged in the nucleus. Interphase chromosomes are spatially compressed into subnuclear regions with territorial organization (Rabl 1885, Boveri 1909, Cremer *et al.* 1982). This spatial occupation of interphase chromosomes is called chromosome territory (Cremer *et al.* 1996, Cremer and Cremer 2010; Fig. 1). Three-dimensional FISH (3D-FISH) is performed by confocal microscopy. 3D-FISH visualized the location of interphase chromosomes in nuclei and revealed conserved rules of chromosome territory and chromosome positioning (Dernburg *et al.* 1996, Solovei *et al.* 2002).

In the last decade, several chromosome conformation capture techniques, which can quantify chromatin contacts, have disclosed the folded state of genomes in interphase nuclei (Dekker *et al.* 2013, Fraser *et al.* 2015). In particular, a chromosome conformation capture technique in combination with deep sequencing, Hi-C, can reveal the spatial genome organization in the whole genome through the frequency of the DNA interaction with each other. This genome organization has been confirmed by 3D-FISH (Markenscoff-Papadimitriou *et al.* 2014, Feng *et al.* 2014, Grob *et al.* 2014). Chromosome conformation capture techniques also proposed a chromosomal module at sub-Mb scale, which is termed a topologically associated domain (TAD) (Fig. 1; Nora *et al.* 2013). TADs are more discrete chromatin structures consisting of chromosome territory (Gibcus and Dekker

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DOI: 10.1508/cytologia.81.3

Fig. 1. Hierarchy of three-dimensional structures of chromatin in the nucleus.

2013). The distribution of TADs is also supported by 3D-FISH (Nora *et al.* 2012). However, some genome organization revealed by chromosome conformation capture techniques could not be confirmed by 3D-FISH (Williamson *et al.* 2014). The inconsistency would be derived from the technical difference between FISH and Hi-C. FISH is a hybridization-based technique strongly dependent on the length of probes and the compaction state of the targeted chromatin. Although Hi-C can reveal DNA sequence-based data with high resolution, it tends to offer more intrachromosomal contacts than interactions between different chromosomal DNAs (Lieberman-Aiden *et al.* 2009). Beyond the technical difference, the inconsistency may be explained by the ploidy state including endoreduplication (Matsunaga and Umeda 2011, Matsunaga *et al.* 2013, Hayashi *et al.* 2013) and aneuploidy (Gordon *et al.* 2012). In addition, FISH discloses genome organization at a single nuclear level, whereas most Hi-C data were provided using a vast number of nuclei isolated from cell populations. However, Hi-C using a single cell (Nagano *et al.* 2013, 2015, Kind *et al.* 2015) or in combination with a 3D-FISH-calibration software (Shavit *et al.* 2014) will fill the gap between 3D-FISH and conventional Hi-C data.

Acknowledgements

This work was supported by CREST grants from the Japan Science and Technology Agency to S. M. and MEXT/JSPS KAKENHI to S. M. I thank Mr. Takeshi Hirakawa for his generous support to prepare Fig. 1.

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