Histochem Cell Biol (2016) 146:237–238
DOI 10.1007/s00418-016-1468-x

EDITORIAL

In Focus in HCB

Douglas J. Taatjes[1] · **Jürgen Roth**[2]

Accepted: 15 July 2016 / Published online: 10 August 2016

Somatic cells in animals all possess the same genetic material obtained from the parental gametes, and their phenotypic cell-to-cell variability is mainly due to nongenetic factors. Single-cell genome-wide analysis techniques combining high-throughput molecular techniques and single-cell resolving microscopic analysis have provided important information about the mechanism of nongenetic cell-to-cell variability. In this issue of HCB, Golov et al. (2016) review recent progress in single-cell sequencing approaches and highlight their contributions to our current understanding of the term "cell type", and tissue homogeneity and plasticity. First, sources of cell-to-cell variability, the basics of epigenetics and their importance for the concept of cell types and the role of gene regulatory network architecture for cell identity are surveyed. The following sections then focus on single-cell transcriptome and epigenome analyses. Single-cell transcriptomics to establish cell type catalogs are discussed, as are the possibilities to acquire cellular spatial position data, to analyze cellular lineage trees, and to study the development and maintenance of tissue cell homeostasis. For single-cell epigenomics, currently available techniques are evaluated and applications are listed, including single-cell methylomes, chromatin status heterogeneity and accessibility, heterogeneity in DNA contacts with the nuclear lamina, or sites of specific histone modifications at the whole genome level.

✉ Douglas J. Taatjes
douglas.taatjes@uvm.edu

[1] Department of Pathology and Laboratory Medicine, The University of Vermont College of Medicine, Burlington, VT 05405, USA

[2] University of Zurich, 8091 Zurich, Switzerland

Chen et al. (2016) report on their work that dense small molecule labeling enables activator-dependent STORM by proximity mapping. For multi-channel 3D imaging, activator-dependent STORM has solved the problems caused by chromatic aberrations and errors of alignment. It is standard protocol that activators and reporters are coupled to a single antibody molecule, which has the disadvantage of providing only low labeling density, an important parameter for resolution in activator-dependent STORM. Chen and colleagues reasoned that reporter and activator do not necessarily need to be located on the same label, as long as the label is small enough to bind to its target with an interval of <3.5 nm. Such a design would be compatible with high labeling intensity and thus improve resolution. As an example of small-size label molecules, they successfully tested the F-actin-binding phalloidin and the membrane lipid bilayer-inserting mCling and obtained 3D super-resolved images. Based on measured differences of the proximity ratio for actin in lamellipodia as compared to stress fibers, activator-dependent STORM may provide an alternative to FRET to estimate molecular distance mapping proximity of 3–4 nm instead of 10 nm.

The paper by Marinović et al. (2016) addresses aspects of the regulation of actin cytoskeleton dynamics by small Rho GTPases. A new fluorescent probe for live cell imaging in highly motile *Dictyostelium discoideum* cells is reported. The probe consists of the GTP-binding domain of the DPAKa kinase fused to yellow fluorescent protein—DPAKa(GBD)–DYFP. Importantly, DPAKa(GBD)–DYFP was shown to bind exclusively to the active forms of Rac1 GTPases in *Dictyostelium* cells. As compared to a probe based on the GTP-binding domain of the PAK kinase, DPAKa(GBD)–DYFP provided superior specificity by showing a distinct labeling along the cell membrane in different types of polarization in vegetative cells

by confocal imaging. The average ratio of the local contrast of the plasma membrane and the cytoplasm was at least 4 and could be as high as 15. In addition, continuous recording of cells over a time period of 10 min with a frame rate of 0.3 Hz and an image pixel size of 230 × 230 nm^2 was achieved without loss of fluorescence and no evidence of phototoxicity. The observed finely graded intensity distribution permitted quantification of Rac1 activity in different segments of the plasma membrane and revealed spatiotemporal activity differences during chemotaxis, phagocytosis, and micropinocytosis, with probe enrichment in the leading edge and endocytic cups, respectively. During cytokinesis, the probe was observed to become enriched at the distal poles of the emerging daughter cells. In addition to the convincing properties of the new probe, the optimal design of the confocal imaging system—for instance, hybrid detectors with a quantum efficiency over 40 % in the region of DYFP emission spectrum as used in the current studies—was crucial.

The unchecked presence of reactive oxygen species (ROS) in cells and tissues can lead to DNA damage, potentially resulting in a variety of pathologies and eventually cell death. The cellular response to genomic attack by ROS takes the form of a variety of DNA damage response (DDR) mechanisms. During prolonged meiotic prophase in mammalian oocytes, the exposed DNA is susceptible to attack by environmental ROS, leading to a myriad of genetic abnormalities and fertility issues. In addition to DDR, cells produce a variety of antioxidant enzymes to protect against ROS oxidative damage. Among these antioxidant enzymes, the presence of catalase (CAT; converts highly reactive hydrogen peroxide to oxygen and water) in mouse oocytes is somewhat controversial. In this issue of HCB, Park et al. (2016) sought to investigate whether CAT is indeed expressed in mouse oocytes. Using multilabel fluorescence microscopy, quantitative ROS fluorescence microscopy, and immunoblotting, they detected the presence of CAT in mouse oocytes, surprisingly associated with the chromosomes. In somatic cells, CAT presence is restricted mostly to peroxisomes, leading CAT to be a universally accepted marker protein for the identification of peroxisomes. Therefore, its interesting association with oocyte chromosomes during meiosis suggests its important role in defending the genetic material from oxidative damage during meiotic maturation.

Organ development also represents a spatially and temporally complex interaction of cells and molecules. During embryonic lung development, epithelial and vascular networks secrete factors responsible for orchestrating proper organ development. Seo et al. (2016) have now investigated the enhancer-based regulation of the transcription factor forkhead box f1 (*Foxf1*), expressed by lung mesenchyme and thought to influence pulmonary angiogenesis during development. They found that during mouse pulmonary development, *Foxf1* was regulated by three enhancers, two of which were determined to be long-range enhancers. The two long-range enhancers demonstrated regional specificity in the lung: one was expressed in the proximal and distal pulmonary blood vessels, while the other was only active in distal blood vessels. Interestingly, these enhancers were evolutionarily conserved, and they were included in a regulatory region deleted in several human samples from patients with pulmonary defects, suggesting their importance in pulmonary blood vessel development. Additionally, in other experiments, these authors showed that the sonic hedgehog (*Shh*) signaling molecule may influence the regulation of *Foxf1* in distal pulmonary blood vessels in a Gli transcription factor binding-dependent mechanism.

References

Chen Y, Gu M, Gunning PW, Russels SM (2016) Dense small molecule labeling enables activator-dependent STORM by proximity mapping. Histochem Cell Biol. doi:10.1007/s00418-016-1451-6

Golov AK, Razin SV, Gavrilov AA (2016) Single cell genome-wide studies give new insight into nongenetic cell-to-cell variability in animals. Histochem Cell Biol. doi:10.1007/s00418-016-1466-z

Marinović M, Šoštar M, Filić V, Antolović V, Weber I (2016) Quantitative imaging of Rac1 activity in *Dictyostelium* cells with a fluorescently labelled GTPase-binding domain from DPAKa kinase. Histochem Cell Biol. doi:10.1007/s00418-016-1440-9

Park YS, You SY, Cho S, Jeon H-J, Lee S et al (2016) Eccentric localization of catalase to protect chromosomes from oxidative damages during meiotic maturation in mouse oocytes. Histochem Cell Biol. doi:10.1007/s00418-016-1446-3

Seo H, Kim J, Park G-H, Kim Y, Cho S-W (2016) Long-range enhancers modulate *Foxf1* transcription in blood vessels of pulmonary vascular network. Histochem Cell Biol. doi:10.1007/s00418-016-1445-4