

introduce newly optimized laser scanning microscopy-based techniques of detecting low level DNA damage, involving a novel method termed STRIDE, an ultra-sensitive fluorescence microscopy technique of detection of various types DNA lesions (including the ones induced by CRISPR/Cas9 nuclease and its nickase variant), and will discuss dynamics of the recruited repair proteins (XRCC1, 53BP1) and the architecture of repair foci in the context of repair efficiency and choice of the repair pathway.

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## Ultrastructural investigation of early replication in *Drosophila* polytene chromosomes

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In general, at the level of extended chromatin domains, replication timing is accurately reproduced in subsequent cell generations. Within these domains, different replication origins fire during each cell cycle, but the choice of origins for replication is probabilistic. *Drosophila* polytene chromosomes (PCh) are widely used as a model to delineate the functional organization of interphase chromosomes. Earlier we showed, that in general the spatio-temporal organization of replication in *Drosophila* chromosomes is closely related to

the division of the genome into two types of domains, the rb-bands (the most compact PCh bands) and the intervals in between them (INTs, corresponding to alternating zones of loose bands and interbands) (Kolesnikova *et al.*, 2018). In cell cultures and in salivary glands cells INTs correspond to early replication initiation zones. Rb-bands are free of replication initiation events and show a replication timing gradient. In order to verify the applicability of this generic model to individual chromosome regions, we visualized the very beginning of S phase in PCh by super-resolution microscopy. To visualize replication, we used EdU incorporation. In order to investigate the chromosomes at the very beginning of S phase, we used a *Drosophila* line carrying the hsp70-CycE transgene (Duronio, O'Farrell, 1995) allowing us to induce S phases by heat shock. 3D-structured illumination microscopy (3D-SIM) was used for the ultrastructural investigation of DNA replication. At the beginning of the S phase, no replication occurs inside of the rb-bands. We found EdU signals in INTs, but the intensity and distribution of the signal within the zones were varying. In some zones, a bright band-shaped signal perpendicular to the axis of the chromosome occurred. Usually the signals were laid directly in loose bands and puffs, i.e. in PCh structures commonly associated with the transcription of genes localized inside of them. For the distal part of chromosome 2L we found that the regions, labeled by bright band-shaped EdU signals correspond to high and wide peaks of "early origins" in S2 cells (MacAlpine *et al.*, 2010). The other INTs contain relatively evenly distributed EdU signals over their entire regions, i.e. we identified broad replication

initiation zones. Our data verify the previously proposed model of the spatio-temporal organization of replication in *Drosophila* chromosomes (Kolesnikova *et al.*, 2018) and indicate that the multi-stranded nature of PCh provides a unique opportunity to visualize in one chromosome the stochastic initiation of replication in some regions of the chromo-

somes, as well as the effective initiation of replication in other sites.

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## WORKSHOP IV NUCLEAR ORGANIZATION IN DISEASE

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### **Polyploidy reprograms regulatory pathways towards unicellular mode: the role in stress response, drug resistance, growth and cancer**

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Polyploidy (PLD) is a common event in development and aging [1]. Also, it may be induced by physiological and pathological stresses [2]. Currently, PLD attracts much attention because of its links to regeneration, tumor initiation and drug resistance [3-5]. Aim: To understand the evolutionary nature of these links, we investigated the effects of PLD on transcriptome in homologous human and mouse tissues differed by PLD (heart, liver and

placenta). Methods: The methods of bioinformatic data analysis and analysis of principal components (PCA), cross-species transcriptome comparison (TC), phylostratigraphy, protein interaction network (PIN) and gene module investigation were used. Results: We show that polyploidy exerts common effects on various cell types. The main PLD-related effects were the up-regulation of gene modules and PIN clusters increasing adaptation and transformation, including oncogene signaling, growth, development, drug metabolism, adaptation to stress and hypoxia and epithelial to mesenchymal transition. PLD-inhibited gene modules were involved mainly in differentiation and immunity. To find out whether PLD activates molecular programs of unicellular organisms that can trigger cancer, we applied the method of phylostratigraphy. The analysis revealed the enrichment of PLD-induced genes with genes from evolutionary ancient phylostrates (from procaryota, eucaryota, and opisthokonta). In particular, these genes were implicated in drug resistance and ABC transporters. Accordingly, PLD-inhibited genes were