in this process, in particular the IF and the tubulin network. The latter is organised from the centrosome positioned at the nuclear membrane. - Prior to cell division the centrosomes divide and move to opposite poles of the cell from where the spindle is formed in mitosis; this determines the direction of cellular division in space. However, prior to daughter cell formation the spindle may change its position within the cellular space. Programs of *spindle (re-)orientation* define the direction of subsequent cell divisions; combined with allowed numbers of divisions this fixes primary morphogenesis, prior to cell-cell interaction and selective apoptosis. Spindle re-orientation happens at critical steps of differentiation and morphogenesis and is based on the internal topological organisation of the mitotic cell. Thus, 3D organisation of the human genome, transcripts and gene expression may link DNA polymorphism and supra-cellular morphogenesis in individuals, as a paradigm of, e.g., definition of facial patterns obviously linked to genetics.

Workshop III DNA replication, repair and cancer

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Unrepairable analogous of nucleotide excision repair substrates as a potential anti-cancer drugs

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In the previous studies we have demonstrated that DNA with the bulky Fap-dC derivative is a hardly repairable substrate for the cellular NER system [1]. Such type of compounds could be of particular interest as possible selective inhibitors of the NER system, considerably reducing the potency of DNA repair due to competitive immobilization of protein factors involved in this process. Tumor-initiating stem cells (TISCs) which are capable to internalize exogenous DNA [2, 3] could be the potential target for such synthetic analogues of NER substrates. In the current work the process of DNA internalization was considered as an approach to deliver the model DNAs into TISCs in order to reduce the reparative potential of cancer cells. Methods: Enzymatic DNA synthesis, PCR, RT-PCR, NER-competent cell extract preparation, *in vitro* NER assay, lab animals breeding, fluorescence microscopy, laser scanning microscopy. Results: 756 bp PCR product containing bulky photoactivable dC adducts inhibits the nucleotide excision repair system. The Krebs-2 ascites cells were

shown to natively internalize such modified DNA. The basic parameters for this DNA probe internalization by the murine Krebs-2 tumor cells were characterized. Upon internalization, the fragments of DNA undergo partial and non-uniform hydrolysis of 3′ ends followed by ligation into a ring. The degree of hydrolysis, assessed by sequencing several clones with the insertion of specific PCR product, was 30-60 nucleotides. Conclusions: Results of the current investigation suggest the possibility to use Fap-dC-modified DNA fragments for further analysis of both membrane-associated and intracellular factors mediating the internalization of eDNA by Krebs-2 cells. Capability of Fap-dC-DNA to impair the NER process presumes their possible applicability in antitumor therapy.

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A CRISPR view of the genome in living cells: cell cycle and genomic distance dependent dynamics of chromosomal loci

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In contrast to the well-studied condensation and folding of chromosomes during mitosis, their dynamics during interphase are less understood. We deployed our newly developed, brightness-enhanced CRISPR-based DNA imaging system (CRISPR-Sirius, Ma *et al.*, 2018) to track the dynamics of genomic loci situated kilobases to megabases apart on a single chromosome. Two distinct modes of dynamics were resolved: local movements as well as translational movements of the entire domain within the nucleoplasm. The magnitude of both of these modes of movements increased from early to late G1, whereas the translational movements were reduced in early S. The local fluctuations decreased slightly in early S and more markedly in mid-late S. These results (Ma el al. J. Cell Biol., in press) suggest an ongoing compaction-relaxation dynamic of the interphase chromosome fiber, operating concurrently with changes in the extent of overall translational movements of loci in the 4D nucleome. The former possibility was anticipated some time ago (Pederson, 1972) and the latter shortly thereafter (Crick, 1978). It is too soon to know the full meaning of these interphase chromosome dynamics but all the tools to access this dimension of genome biology are fortunately now at hand.

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