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CONFERENCE REPORT

The Czech Plant Nucleus Workshop 2021

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Abstract

The Czech Plant Nucleus Workshop 2021 (CPNW2021) took place during mid-September 2021 in Olomouc, Czech Republic. About 80 researchers and students working in the field of plant nuclear and chromosome biology in the Czech Republic gathered together to present and discuss their current research. The meeting revealed many plant models that are used to study plant genomes and their organization, and also a great diversity of topics including epigenetic regulation of gene expression, genome stability, telomere biology, or sex chromosomes. CPNW2021 provided a broad platform for establishing new research contacts and collaborations. Here, we summarize the main research directions and findings presented at the CPNW2021 meeting.

Keywords: chromatin, chromosome, DNA damage repair, DNA methylation, environmental responses, nucleus, sex- and B-chromosomes.

Introduction

The cell nucleus is a fascinating organelle. It contains chromosomes that periodically condense in preparation for cell divisions in proliferating cells, separate sister chromatids into daughter nuclei, and de-condense for the interphase during which the chromosomes replicate and the whole cycle repeats. Chromosomal DNA serves as a basic template for transcription, which is orchestrated at many levels by complex regulatory machinery and takes place in specific nuclear compartments. In parallel to all these functions, the cell nucleus is under constant surveillance for the mitigation of DNA lesions.

Academic institutions within the Czech Republic have a very long and fruitful history of plant cell nuclei and chromosome research. To share the latest achievements in the field and to provide a platform for establishing new collaborations, we organized a community-focused meeting

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"The Czech Plant Nucleus Workshop 2021" (CPNW2021). The meeting took place at Fort Science, an interactive science centre of Palacký University in Olomouc on 14th and 15th September 2021 and was attended by more than 80 participants from the majority of national plant research institutions. This included the Institute of Experimental Botany of the Czech Academy of Sciences (IEB), Biology Centre of the Czech Academy of Sciences (BC), Institute of Biophysics of the Czech Academy of Sciences (IBP), the Central European Institute of Technology (CEITEC), Charles University (CUNI), Masaryk University (MUNI), and Palacký University (UP).

Nuclear biology research benefits from the technological advancements

The keynote opening lecture was presented by Prof. J. Doležel (IEB), who gave a historical overview of the progress in nuclear and chromosome research and emphasized that many of the fundamental findings were based on a combination of excellent research ideas and the use of the new technologies. Several examples of new approaches were presented at the meeting. P. Cápal (IEB) used advanced environmental scanning electron microscopy (A-ESEM), which allows observing samples in high resolution in their native state, with the aim to investigate the surface structure of barley mitotic chromosomes. This revealed a topologically complex surface with numerous protrusions and regularly spaced inter-chromatid bridges. A complex study of the higher-order 3D structure of both metaphase and interphase chromosomes was presented by H. Šimková (IEB). By a combination of Hi-C and chromosome painting techniques, she demonstrated that sister chromatids of barley metaphase chromosomes have a helical structure, where one turn contains 20 - 38 Mbp chromatin-packed DNA, depending on the position on the chromosome arm (Kubalová *et al.* 2021a). Microscopy is a classical technique to study cell nuclei and chromosomes. However, understanding their 3D organization using classical microscopic techniques is hampered by the diffraction limit. E. Hřibová (IEB) and M. Franek (CEITEC/MUNI) introduced super-resolution microscopy techniques including structural illumination microscopy (SIM), stimulated emission depletion (STED) microscopy, and direct stochastic optical reconstruction microscopy (dSTORM), and discussed challenges in the selection of fluorochromes and preparation of the plant samples (Kubalová *et al.* 2021b).

Chromatin regulates plant development and environmental responses

Very high developmental plasticity represents a unique and integral component of plant environmental responses. Well-controlled dynamics of chromatin structure that ensures stable but responsive gene expression is therefore of utmost importance in orchestrating developmental and environmental cues. Among the crucial developmental and cell identity modulators in plants as well as animals are the Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2) that establish two key repressive histone post-translational modifications H2Aub and H3K27me3, respectively (Bieluszewski *et al.* 2021). A. Sharaf and V. Mallika (BC) presented the identification of the components of PRC2 complexes in the basal eukaryotes (Sharaf *et al.* 2021) and algae of the green lineage. M.G. Trejo Arellano (BC) presented an evolutionary study of H3K27me3 distribution in unicellular and multicellular eukaryotes. Several contributions also tackled the recently emerging view of PRC2 involvement in environmental sensing and response in plants (Shen *et al.* 2021, Kim *et al.* 2021). I. Mozgová and M. Zhou presented recent findings on the role of PRC2 in ambient light response and photoautotrophic growth in *Arabidopsis* and T. Konečný (BC) showed enhanced heterochromatin formation in PRC2 mutant plants during de-etiolation. Using *Physcomitrium patens*, K. Sobotková (BC) demonstrated that the function of PRC2 in fine-tuning primary metabolism during photoautotrophic growth might be evolutionarily conserved.

Long non-coding RNAs (lncRNAs) are emerging as important players in chromatin modulation. For instance, the introduction of H3K27me3 by PRC2 in the FLC locus is aided by a lncRNA called COLDAIR (Xu and Chong 2018), and lncRNA APOLO has been implemented in PRC2-associated repressive chromatin looping at the PINOID locus, that encodes a major regulator of polar auxin transport (Ariel *et al.* 2014). J. Hajný (IEB) introduced a newly identified lncRNA that is expressed in the root protophloem and regulates the transcription of a xylem-expressed leucine-rich receptor-like kinase. This gene in turn controls the relationship between longitudinal anticlinal divisions in the endodermis and the stele area. The presented findings uncover an intriguing mechanism of cell division plane specification by long-distance coordination of lncRNA production and associated target gene expression. A.J. Wiese (IEB) demonstrated that upon heat stress, two bZIP transcription factors, bZIP18 and bZIP52, undergo dephosphorylation and relocate into the nucleus. Here, bZIP18 and bZIP52 regulate the transcription of several common genes, including lncRNA genes that are elevated in response to heat treatment (Wiese *et al.* 2021). These results demonstrate that phosphorylation can mediate extra-nuclear sequestration of transcription factors that orchestrate heat stress response, and perhaps suggest a more general mechanism of stress response attenuation under optimal conditions but rapid transcriptional response upon exposure to adverse environmental conditions.

Vernalization is a key trait regulating flowering time in plants relative to the winter or extended period of cold conditions. Among the well-described mechanisms is the vernalization response in *Brassicaceae* governed by PRC2, whereby the flowering inhibitor locus *FLC* is subjected to H3K27me3-mediated stable repression upon extended periods of cold (Xu and Chong 2018). Interestingly, the mechanism of vernalization response differs in monocot grasses including crops, where the release of transcriptional repression of the flowering

MADS-box activator *VERNALIZATION 1* (*VRN1*) is required for the induction of flowering. Analysis of over 100 hexaploid bread wheat cultivars by B. Strejčková (IEB) revealed several known as well as new vernalization insensitive *VRN1* alleles, supporting earlier evidence that *VRN1* is the major breeding locus in cereals (Strejčková *et al*. 2021). J. Šafář (IEB) showed that genetic and epigenetic regulation of VRN1, including a putative role of the recently identified bread wheat PRC2 components (Strejčková *et al*. 2020), remains unknown. Therefore, future plans towards the development of modifier and reporter VRN1 lines were presented.

Cereal grains are complex structures harbouring diploid embryo, triploid endosperm, and diploid seed coats of maternal origin. Cereal grain development starts with fertilization and consists of several phases including syncytium, cellularization, maturation, and desiccation (Nowicka *et al*. 2021). A. Pečinka (IEB) presented a transcriptomic meta-analysis of the embryo, endosperm, and seed maternal tissues from developing barley grains (Kovacik *et al*. 2020). This atlas of barley seed expression provides ample marker genes, indicates local and temporal specificity of biological pathways, and points to the dynamic role of epigenetic pathways.

DNA methylation - the guardian of the heterochromatic genome fraction

Methylation of cytosines is an important epigenetic mark used by plant cells to label chromatin for distinct functions. It is mostly present in the transcriptionally inactive chromatin where it occurs together with specific histone marks. *De novo* DNA methylation of native loci is driven by small RNAs (sRNAs) (Zhang *et al.* 2018). L. Fischer (CUNI) showed that the potential of sRNAs to induce DNA methylation depends not only on the level of sRNAs but also on their origin and likely also on the epigenetic state of the target locus (Čermák *et al*. 2020). Analysis of the dynamics of the initial phases of DNA methylation, using an experimental system for inducible production of sRNAs in a homogeneously responding tobacco BY-2 cell line, demonstrated that *de novo* cytosine methylation can occur already 12 h after the exposure to sRNAs (Přibylová *et al*. 2019). Furthermore, A. Přibylová (CUNI) showed that changing the chromatin state by *de novo* DNA methylation could affect the activity of the CRISPR/Cas9 editing tool and the subsequent repair of double-strand DNA breaks.

The analysis of epigenetic marks including DNA methylation is challenging in the repetitive genomic regions. Mapping DNA methylation in repeats is problematic when averaging cell populations or analyzing clusters of repeats in single-cell analysis. This problem can be overcome by analyzing individual DNA/chromatin fibres by an optimized method introduced by A. Kilar (CEITEC/MUNI). This DNA fibre extension technique combines immunofluorescence and fluorescence *in-situ* hybridization signals detected using super-resolution microscopy followed by the quantitative evaluation of DNA methylation levels using an image analysis approach (Franek *et al*. 2021).

Chromosome organization and regulation in large and polyploid genomes

Large grass genomes are generally thought to display the Rabl chromosome organization with centromeres and telomeres clustered at opposite nuclei poles (Rabl 1885). A. Doležalová (IEB) investigated the relationship between DNA replication, chromosome organization, and genome size in *Poaceae*. While there was a Rabl genome organization in *Brachypodium distachyon*, *Hordeum vulgare,* and *Triticum aestivum*, the non-Rabl organization was found in *Oryza sativa* and *Zea mays*. Prevailing replication of telomeric sequences was observed in the early and middle S phase, in contrast to centromeric sequences which underwent replication during the middle and late S phase (Němečková *et al*. 2020). Using FISH against major repetitive DNA sequences on isolated embryos and endosperm barley nuclei, A. Nowicka (IEB) showed striking differences in chromosome organization. While embryo nuclei showed typical Rabl configuration at all times, endosperm nuclei progressively lost Rabl organization in age and nuclear DNA content-dependent manner.

D. Kopecký (IEB) provided an overview of the research questions connected to interspecific hybridization and polyploidy with a particular focus on the allopolyploid genome evolution and stability and the phenomenon of genome dominance (Glombik *et al*. 2020). J. Majka (IEB) further corroborated this topic on the examples of *Allium roylei* × *Allium cepa* and *Festuca pratensis* × *Lolium multiflorum* hybrids and suggested that the shifts in genome composition towards one parent could be due to uneven behaviour of parental chromosomes during meiosis in these hybrids. There is a continuous debate on the effects of hybridization and polyploidization on gene transcription. M. Glombik (IEB) showed that in allopolyploids of *Festuca pratensis* × *Lolium multiflorum*, the overall transcript profile is much closer to that of *Lolium* parent, suggesting it as a transcriptionally dominant genome, presumably *via trans*-acting regulatory factors (Glombik *et al*. 2021). K. Perničková (IEB) analyzed the 3D nuclear positioning of a pair of rye chromosomes introgressed to the hexaploid wheat genome. She reported an occasional lack of contact between telomeres of the additional chromosomes and nuclear envelope, which could be responsible for the observed less efficient chromosome pairing in meiosis and transmission into subsequent generations (Perničková *et al*. 2019).

Unravelling the mysteries of plant sex- and B-chromosomes

R. Hobza (IBP) showed that separated sexes evolved independently and repeatedly in about 5 % of plant species. In many species, dioecy has evolved recently, so

these plants provide an excellent model for studying the early stages of sex chromosome divergence, which later in evolution leads to gradual sex chromosome degeneration (Hobza *et al*. 2018). Z. Kubát (IBP) showed that some TEs proliferate preferentially either in the male or in the female germline which can be connected with specific circumstances affecting TE management and activity in male and female plants and during gametophyte formation (Jesionek *et al*. 2021). The process of evolutionary diversification of sex chromosomes is also connected with specific chromatin modifications of both histones and cytosines in *Silene latifolia* as demonstrated by M. Hubinský (IBP) (Rodríguez Lorenzo *et al*. 2020). In addition to the pivotal role of *S. latifolia* in studying plant sex chromosome evolution, V. Hudzieczek (IBP) with colleagues aims to establish the genus *Silene* as a common model to study also other aspects of plant ecology, evolution, and development. For this purpose, they established a set of methods for *S. latifolia*, including *Agrobacterium*-mediated gene delivery, genome editing by TALENs and CRISPR/Cas9 or gene silencing *via* RNAi.
Certain genomes contain specific supernumerary chromosomes that carry a generally low number of protein-coding genes but often harbour molecular toolkits for their preferential inheritance. J. Bartoš (IEB) introduced plant B chromosomes and presented maize and *Sorghum* as promising models to study molecular mechanisms of non-disjunction, preferential fertilization, and chromosome elimination (Blavet *et al.* 2021, Karafiátová *et al*. 2021).

Plant nuclei functions and chromatin organization during DNA damage repair

DNA is constantly exposed to a variety of genotoxic factors that may alter its chemical and/or physical structure and result in DNA lesions. DNA damage response (DDR) is therefore a key mechanism contributing to genome stability. DDR is a very complex process, ultimately leading to DNA damage repair or cell death. After DNA damage is sensed, the local chromatin environment must be reorganized and histones are displaced. In accordance, FASCIATA1 (FAS1), a subunit of the H3-H4 histone chaperone complex CHROMATIN ASSEMBLY FACTOR 1 (CAF1), is important for genome stability and DNA damage repair (Kolářová *et al*. 2021). In her talk, M. Nešpor Dadejová (CEITEC/MUNI) introduced a newly developed *in vivo* method employing laser microirradiation-induced DNA damage. Using the PCNA1-GFP marker line (Yokoyama *et al*. 2016), this technique allows observing immediate relocation of PCNA1 during DNA damage response in wild type cells.

The Structural Maintenance of Chromosomes (SMC) complexes are key components of higher-order chromatin structure. The SMC5/6 complex is involved in homologous recombination, replication fork stability, and DNA damage repair and its basic structure is evolutionarily conserved (Díaz and Pecinka 2018). J. Paleček (MUNI) presented a detailed architectural analysis of the SMC5/6 complex in humans and yeast (Adamus *et al*. 2020), highlighted this SMC complex as the most ancestral in eukaryotes, and showed various models of its possible DNA processing activity. His laboratory identified the SMC5/6 complex subunits in the moss *Physcomitrium patens* and is planning to reveal the SMC5/6 complex architecture in plants. M. Holá (IEB) presented functional characterization of SMC5/6 in *P. patens*. Sensitivity assays revealed a critical role of SMC5/6 in double-strand-break (DSB) repair and proposed that the circularization of SMC5/6 complex by the kleisin subunit NSE4 is indispensable for the *P. patens* SMC5/6 function (Holá *et al*. 2021).

DNA-protein crosslinks (DPC) represent a specific type of DNA damage, caused by the covalent trapping of virtually any protein to DNA (Stingele and Jentsch 2015). E. Dvořák Tomaštíková (IEB) presented a forward-directed genetic screen that aimed to identify factors involved in the repair of zebularine-induced DPCs in *Arabidopsis* (Procházková *et al*. 2022). Besides several unknown factors undergoing characterization, the SMC5/6 complex was identified as an important DPC repair factor, representing a new DPC repair pathway.

Maintaining genome stability over generations is a key issue for all living organisms. F. Yang (IEB) explained how SMC5/6 complex functions contribute to normal male meiosis in *Arabidopsis* (Yang *et al*. 2021). Loss-of-function mutants in SMC5/6 subunits generate unreduced microspores. The diploid pollen leads to an unbalanced maternal and paternal genome dosage in endosperm, which is responsible for a frequent seed abortion but in about 10 - 15 % of seeds leads to the production of triploid offspring. Thus, SMC5/6 has an important role in the maintenance of gametophytic ploidy in *Arabidopsis*.

Maintenance of the chromosome ends

Telomeres consist predominantly of non-coding repetitive tandem repeats and protect the ends of linear eukaryotic chromosomes from progressive shortening and erroneous recognition as unrepaired chromosome breaks. A rosette-like organization of chromosomes, where telomeres show persistent clustering at the nucleolus in interphase nuclei while centromeres associate with nuclear envelope was observed in *A. thaliana* (Fransz *et al*. 2002). M. Kubová (CEITEC/MUNI) showed that the rosette-like configuration is not a universal model for interphase genome organization in *Brassicaceae*. In species with large-genome (≥ 1 Gb), centromeres and telomeres adopt either the Rabl-like configuration or a dispersed distribution in the nuclear interior, with telomeres being only rarely anchored to the nucleolus (Shan *et al*. 2021).

Telomeric repeats across the *Eukaryotes* typically follow the formula $(T_xA_yG_z)_n$ (Schrumpfová and Fajkus 2020). In plants, telomeres are mostly composed of the *Arabidopsis*-type $TTTAGGG_n$ repeats (Richards and Ausubel 1988). However, recent studies revealed significant variability in telomere sequences in lower and also higher plants (Peska and Garcia 2020). Moreover, telomeric or telomeric-like repeats are found also at multiple intra-chromosomal regions (Uchida *et al.* 2002, Majerová *et al*. 2014). This

phenomenon observed in many plant families is most likely caused by genome rearrangements during evolution. Interestingly, larger genomes in gymnosperm species than in angiosperms were previously reported to be associated with a larger proportion of repetitive sequences (Novák *et al*. 2020). A wide survey in gymnosperms, namely in the *Cycadaceae* family by R. Vozárová (IBP), has shown that canonical *Arabidopsis*-type telomeric repeats are located predominantly at chromosome ends, while pericentromeric blocks comprise other telomeric variants. Telomeric repeats are a natural target of epigenetic regulation and are traditionally considered heterochromatic regions. Recent studies show that telomeres in *A. thaliana* possess both euchromatic (H3.3, H3K4me3) and heterochromatic (H3K9me2, H3K27me1, H3K27me3) marks (Procházková Schrumpfová *et al*. 2019). Histone H3 deposition is maintained by histone chaperone proteins. A. Machelová (CEITEC/MUNI) focused on H3 chaperones and showed that HISTONE CELL CYCLE REGULATOR (HIRA) and ANTI-SILENCING FUNCTION 1 (ASF1) are required for telomere maintenance in *A. thaliana*, as their simultaneous depletion causes a lethal phenotype.

TELOMERE REPEAT BINDING (TRB) family represents a rare example of proteins with confirmed *in vivo* telomere localization in plants (Schrumpfová *et al*. 2014). These proteins bind telomeric DNA through the MYB-like domain and they possess plant-specific protein-domain organization (Peska *et al*. 2011). Conserved features of TRB proteins in *P. patens* and *A. thaliana* were discussed by A. Kusová (MUNI). Apart from binding telomeric sequences, TRB proteins directly interact with the protein catalytic subunit of telomerase (Schrumpfová *et al*. 2014). Telomerase adds telomeric repeats to the ends of chromosomes and consists of telomerase RNA (TR) and Telomerase reverse transcriptase (TERT) subunits. In humans, the expression of TERT is strictly controlled at the transcript level, but TR is ubiquitously expressed. Interestingly, the expression of the TR subunit follows a tissue-specific pattern similar to that of TERT expression in plants, and the plant *TR* gene is transcribed by RNA Polymerase III (Pol III), but not by Pol II, as in mammals or yeasts (Fajkus *et al*. 2019). All these features as well as the evolution of both subunits of telomerase were presented by P. Procházková Schrumpfová (SCI, MUNI) (Schrumpfová and Fajkus 2020; Fajkus *et al*. 2021). K. Konečná (IBP, CEITEC, MUNI) presented characterization of the Armadillo (ARM) repeat type plant-specific protein found as an interactor with TERT (Dokládal *et al*. 2018). The results suggested that ARM cellular activity is independent of the telomerase canonical function and implied the involvement of ARM protein in response to a drug, biotic, and abiotic stimuli.

Summarizing remarks

The CPNW2021 showed a remarkable diversity of the topics broadly linked to plant nuclear and chromosome biology that were studied in the Czech Republic. Particularly prominent were the topics of 3D genome organization, chromatin modifications, epigenetic regulation of gene expression, sex chromosomes, and genome stability. A variety of plant model species was also introduced (only selected species are listed here) *Arabidopsis thaliana*, *Silene latifolia*, *Nicotiana tabacum*, *Allium* sp., temperate cereals (*Triticum aestivum*, *Hordeum vulgare*), *Zea mays,* or *Physcomitrium patens*. This provided unique opportunities to discuss research questions and to establish new research connections. At the end of the meeting, the young researchers (under 35 years) were awarded in several categories. The awards for the best poster presentations were given to K. Kaduchová (IEB) and M.G. Trejo Arellano (BC). M. Glombik (IEB) and A. Přibylová (CUNI) were awarded for the best Ph.D. student talks and Mingxi Zhou (BC) for the best post-doc talk. Finally, it was announced that the next CPNW meeting will be held in 2023 in Brno.

References

Adamus, M., Lelkes, E., Potesil, D., Ganji, S.R., Kolesar, P., Zabrady, K., Zdrahal, Z., Palecek, J.J.: Molecular insights into the architecture of the human SMC5/6 complex. - J. mol. Biol. **432**: 3820-3837, 2020.

Ariel, F., Jegu, T., Latrasse, D., Romero-Barrios, N., Christ, A., Benhamed, M., Crespi, M.: Noncoding transcription by alternative RNA polymerases dynamically regulates an auxin-driven chromatin loop. - Mol. Cells **55**: 383-396, 2014.

Bieluszewski, T., Xiao, J., Yang, Y., Wagner, D.: PRC2 activity, recruitment, and silencing: a comparative perspective. - Trends Plant Sci. **26**: 1186-1198, 2021.

Blavet, N., Yang, H., Su, H.D., Solansky, P., Douglas, R.N., Karafiatova, M., Simkova, L., Zhang, J., Liu, Y., Hou, J., Shi, X.W., Chen, C., El-Walid, M., McCaw, M.E., Albert, P.S., Gao, Z., Zhao, C.Z., Ben-Zvi, G., Glick, L., Kol, G., Shi, J.H., Vrana, J., Simkova, H., Lamb, J.C., Newton, K., Dawe, R.K., Dolezel, J., Ji, T.M., Baruch, K., Cheng, J.L., Han, F.P., Birchler, J.A., Bartos, J.: Sequence of the supernumerary B chromosome of maize provides insight into its drive mechanism and evolution. - Proc. nat. Acad. Sci. USA **118**: e2104254118, 2021.

Čermák, V., Tyč, D., Přibylová, A., Fischer, L.: Unexpected variations in posttranscriptional gene silencing induced by differentially produced dsRNAs in tobacco cells. - Biochim. biophys. Acta - Gene Regul. Mech. **1863**: 194647, 2020.

Díaz, M., Pecinka, A.: Scaffolding for repair: understanding molecular functions of the SMC5/6 complex. - Genes (Basel) **9**: 36, 2018.

Dokládal, L., Benková, E., Honys, D., Dupľáková, N., Lee, L.-Y., Gelvin, S.B., Sýkorová, E.: An armadillo-domain protein participates in a telomerase interaction network. - Plant mol. Biol. **97**: 407-420, 2018.

Fajkus, P., Peška, V., Závodník, M., Fojtová, M., Fulnečková, J., Dobias, Š., Kilar, A., Dvořáčková, M., Zachová, D., Nečasová, I., Sims, J., Sýkorová, E., Fajkus, J.: Telomerase RNAs in land plants. - Nucl. Acids Res. **47**: 9842-9856, 2019.

Fajkus, P., Kilar, A., Nelson, A.D.L., Holá, M., Peška, V., Goffová, I., Fojtová, M., Zachová, D., Fulnečková, J., Fajkus, J.: Evolution of plant telomerase RNAs: farther to the past, deeper to the roots. - Nucl. Acids Res. **49**: 7680-7694, 2021.

Franek, M., Kilar, A., Fojtík, P., Olšinová, M., Benda, A., Rotrekl, V., Dvořáčková, M., Fajkus, J.: Super-resolution microscopy of chromatin fibers and quantitative DNA methylation analysis

of DNA fiber preparations. - J. Cell Sci. **134**: jcs258374, 2021.
Fransz, P., De Jong, J.H., Lysak, M., Castiglione, M.R., Schubert, I.: Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. - Proc. nat. Acad. Sci. USA **99**: 14584-14589, 2002.
Glombik, M., Bačovský, V., Hobza, R., Kopecký, D.: Competition of parental genomes in plant hybrids. - Front. Plant Sci. **11**: 200, 2020.
Glombik, M., Copetti, D., Bartos, J., Stoces, S., Zwierzykowski, Z., Ruttink, T., Wendel, J.F., Duchoslav, M., Dolezel, J., Studer, B., Kopecky, D.: Reciprocal allopolyploid grasses (*Festuca* × *Lolium*) display stable patterns of genome dominance. - Plant J. **107**: 1166-1182, 2021.
Hobza, R., Hudzieczek, V., Kubat, Z., Cegan, R., Vyskot, B., Kejnovsky, E., Janousek, B.: Sex and the flower - developmental aspects of sex chromosome evolution. - Ann. Bot. **122**: 1085-1101, 2018.
Holá, M., Vágnerová, R., Angelis, K.J.:. Kleisin NSE4 of the SMC5/6 complex is necessary for DNA double strand break repair, but not for recovery from DNA damage in physcomitrella (*Physcomitrium patens*). - Plant mol. Biol. **107:** 355-364, 2021.
Jesionek, W., Bodláková, M., Kubát, Z., Čegan, R., Vyskot, B., Vrána, J., Šafář, J., Puterova, J., Hobza, R.: Fundamentally different repetitive element composition of sex chromosomes in *Rumex acetosa*. - Ann. Bot. **127**: 33-47, 2021.
Karafiátová, M., Bednářová, M., Said, M., Čížková, J., Holušová, K., Blavet, N., Bartoš, J.: The B chromosome of *Sorghum purpureosericeum* reveals the first pieces of its sequence. - J. exp. Bot. **72**: 1606-1616, 2021.
Kim, J., Bordiya, Y., Kathare, P.K., Zhao, B., Zong, W., Huq, E., Sung, S.: Phytochrome B triggers light-dependent chromatin remodelling through the PRC2-associated PHD finger protein VIL1. - Nat. Plants **7**: 1213-1219, 2021.
Kolářová, K., Nešpor Dadejová, M., Loja, T., Lochmanová, G., Sýkorová, E., Dvořáčková, M.: Disruption of NAP1 genes in *Arabidopsis thaliana* suppresses the *fas1* mutant phenotype, enhances genome stability and changes chromatin compaction. - Plant J. **106**: 56-73, 2021.
Kovacik, M., Nowicka, A., Pecinka, A.: Isolation of high purity tissues from developing barley seeds. - J. visualized Exp. **164**: e61681, 2020.
Kubalová, I., Câmara, A.S., Cápal, P., Beseda, T., Rouillard, J., Krause, G.M., Toegelová, H., Himmelbach, A., Stein, N., Houben, A., Doležel, J., Mascher, M., Šimková, H., Schubert, V.: Helical metaphase chromatid coiling is conserved. - bioRxiv: 2021.09.16.460607, 2021a.
Kubalová, I., Němečková, A., Weisshart, K., Hřibová, E., Schubert, V.: Comparing super-resolution microscopy techniques to analyze chromosomes. - Int. J. mol. Sci. **22**: 1903, 2021b.
Majerová, E., Mandáková, T., Vu, G.T.H., Fajkus, J., Lysak, M.A., Fojtová, M.: Chromatin features of plant telomeric sequences at terminal vs. internal positions. - Front. Plant Sci. **5**: 1-10, 2014.
Němečková, A., Koláčková, V., Vrána, J., Doležel, J., Hřibová, E.: DNA replication and chromosome positioning throughout the interphase in three-dimensional space of plant nuclei. - J. exp. Bot. **71**: 6262-6272, 2020.
Novák, P., Guignard, M.S., Neumann, P., Kelly, L.J., Mlinarec, J., Koblížková, A., Dodsworth, S., Kovařík, A., Pellicer, J., Wang, W., Macas, J., Leitch, I.J., Leitch, A.R.: Repeat-sequence turnover shifts fundamentally in species with large genomes. - Nat. Plants **6**: 1325-1329, 2020.
Nowicka, A., Kovacik, M., Tokarz, B., Vrána, J., Zhang, Y., Weigt, D., Doležel, J., Pecinka, A.: Dynamics of endoreduplication in developing barley seeds. - J. exp. Bot. **72**: 268-282, 2021.
Perničková, K., Koláčková, V., Lukaszewski, A.J., Fan, Ch., Vrána, J., Duchoslav, M., Jenkins, G., Phillips, D., Šamajová, O., Sedlářová, M., Šamaj, J., Doležel, J., Kopecký, D.: Instability of alien chromosome introgressions in wheat associated with improper positioning in the nucleus. - Int. J. mol. Sci. **20**: 1448, 2019.
Peska, V., Garcia, S.: Origin, diversity, and evolution of telomere sequences in plants. - Front. Plant Sci. **11**: 117, 2020.
Peska, V., Prochazkova Schrumpfova, P., Fajkus, J.:Using the Telobox to search for plant telomere binding proteins. - Curr. Protein Pept. Sci. **12**: 75-83, 2011.
Přibylová, A., Čermák, V., Tyč, D., Fischer, L.: Detailed insight into the dynamics of the initial phases of *de novo* RNA-directed DNA methylation in plant cells. - Epigenetics Chromatin **12**: 54, 2019.
Procházková, K., Finke, A., Dvořák Tomaštíková, E., Filo, J., Bente, H., Dvořák, P., Ovečka, M., Šamaj, J., Pecinka, A.:. Zebularine induces enzymatic DNA-protein crosslinks in 45S rDNA heterochromatin of *Arabidopsis* nuclei. - Nucl. Acids Res. **50**: 244-258, 2022.
Procházková Schrumpfová, P., Fojtová, M., Fajkus, J.: Telomeres in plants and humans: not so different, not so similar. - Cells **8**: 58, 2019.
Rabl, C.: Über Zelltheilung. - Morphologisches Jahrbuch **10**: 214–330, 1885.
Richards, E.J., Ausubel, F.M.: Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. - Cell **53**: 127-136, 1988.
Rodríguez Lorenzo, J.L., Hubinský, M., Vyskot, B., Hobza, R.: Histone post-translational modifications in *Silene latifolia* X and Y chromosomes suggest a mammal-like dosage compensation system. - Plant Sci. **299**: 110528, 2020.
Schrumpfová, P.P., Fajkus, J.: Composition and function of Telomerase-A polymerase associated with the origin of Eukaryotes. - Biomolecules **10**: 1425, 2020.
Schrumpfová, P.P., Vychodilová, I., Dvořáčková, M., Majerská, J., Dokládal, L., Schořová, S., Fajkus, J.: Telomere repeat binding proteins are functional components of *Arabidopsis* telomeres and interact with telomerase. - Plant J. **77**: 770-781, 2014.
Shan, W., Kubová, M., Mandáková, T., Lysak, M.A.: Nuclear organization in crucifer genomes: nucleolus-associated telomere clustering is not a universal interphase configuration in *Brassicaceae*. - Plant J. **108**: 528-540, 2021.
Sharaf, A., Vijayanathan, M., Oborník, M., Mozgová, I.: Phylogenetic profiling suggests early origin of the core subunits of Polycomb Repressive Complex 2 (PRC2). - bioRxiv: 2021.07.16.452543, 2021.
Shen, Q., Lin, Y., Li, Y., Wang, G.: Dynamics of H3K27me3 modification on plant adaptation to environmental cues. - Plants (Basel) **10**: 1165, 2021.
Stingele, J., Jentsch, S.: DNA-protein crosslink repair. - Nat. Rev. mol. cell. Biol. **16**: 455-460, 2015.
Strejčková, B., Čegan, R., Pecinka, A., Milec, Z., Šafář, J.: Identification of polycomb repressive complex 1 and 2 core components in hexaploid bread wheat. - BMC Plant Biol. **20**: 175, 2020.
Strejčková, B., Milec, Z., Holušová, K., Cápal, P., Vojtková, T., Čegan, R., Šafář, J.: In-depth sequence analysis of bread wheat *VRN1* genes. - Int. J. mol. Sci. **22**: 12284, 2021.
Uchida, W., Matsunaga, S., Sugiyama, R., Kawano, S.: Interstitial telomere-like repeats in the *Arabidopsis thaliana* genome. - Genes Genet. Syst. **77**: 63-67, 2002.
Wiese, A.J., Steinbachová, L., Timofejeva, L., Čermák, V., Klodová, B., Ganji, R.S., Limones-Mendez, M., Bokvaj, P., Hafidh, S., Potěšil, D., Honys, D.: *Arabidopsis* bZIP18 and

bZIP52 accumulate in nuclei following heat stress where they regulate the expression of a similar set of genes. - Int. J. mol. Sci. **22**: 530, 2021.

Xu, S., Chong, K.: Remembering winter through vernalisation. - Nat. Plants **4**: 997-1009, 2018.

Yang, F., Fernández-Jiménez, N., Tučková, M., Vrána, J., Cápal, P., Díaz, M., Pradillo, M., Pecinka, A.: Defects in meiotic chromosome segregation lead to unreduced male gametes in *Arabidopsis* SMC5/6 complex mutants. - Plant Cell **33**: 3104-3119 2021.

Yokoyama, R., Hirakawa, T., Hayashi, S., Sakamoto, T., Matsunaga, S.: Dynamics of plant DNA replication based on PCNA visualization. - Nat. Publ. **6**: 29657, 2016.

Zhang, H., Lang, Z., Zhu, J.-K.: Dynamics and function of DNA methylation in plants. - Nat. Rev. Mol. Cell Biol. **19**: 489-506, 2018.