Chromosome Res (2015) 23:641–647
DOI 10.1007/s10577-015-9498-0

Chromosomes in the taiga

Alexey V. Pindyurin · Maurizio Gatti

Received: 6 October 2015 / Accepted: 7 October 2015 / Published online: 30 October 2015

The "Chromosome 2015" meeting was held at Akademgorodok (Novosibirsk, Russia) at the House of Scientists of the Siberian Branch of the Russian Academy of Sciences on August 24–28. This conference celebrated the 100th anniversary of the publication of "The Mechanism of Mendelian Heredity", a seminal book on the Chromosome Theory of Heredity, authored by T. H. Morgan, A. H. Sturtevant, H. J. Muller, and C. B. Bridges. The meeting was organized by the Institute of Molecular and Cellular Biology (IMCB, Novosibirsk, Russia) and the Novosibirsk State University, under the supervision of Igor F. Zhimulev and Alexander S. Graphodatsky. This meeting has a long history. In the Soviet Union, the first meeting focused on chromosomes (Symposium on the Structure, Reproduction, and Functions of Chromosomes of Multicellular Organisms) was organizes by Aleksandra Prokofieva-Belgovskaya in 1968, after the Lysenko-inspired ban on genetics was removed. After that, five additional chromosome conferences were held, but they did not continue after the collapse of the Soviet Union in the1990s. However, in 2009, the chromosome meeting was resurrected by professors Zhimulev and Graphodatsky, who organized "Chromosome 2009", which was followed by "Chromosome 2012" and now by "Chromosome 2015". All three "Chromosome" meetings were organized in Akademgorodok, a small town founded in the end of 1950s near Novosibirsk to create a research center of excellence in the Soviet Union. Akademgorodok is immersed in a beautiful pre-taiga forest and hosts more than 40 research institutions, the Novosibirsk State University and some high-tech companies, and is therefore also known with the nickname "Silicon Taiga".

Since "Chromosome 2009", the number of participants and non-Russian speakers increased steadily. "Chromosome 2015" acquired the dimension of an international meeting with many invited speakers from the USA and several European countries. In the following overview, we mention the main themes that were discussed and provide some selected highlights of the talks.

Responsible Editor: Conly Rieder

A. V. Pindyurin (✉) · M. Gatti (✉)
Institute of Molecular and Cellular Biology SB RAS, Novosibirsk 630090, Russia
e-mail: a.pindyurin@mcb.nsc.ru
e-mail: maurizio.gatti@uniroma1.it

A. V. Pindyurin
Novosibirsk State University, Novosibirsk 630090, Russia

M. Gatti
Department of Biology and Biotechnology, Sapienza, University of Rome, 00185 Rome, Italy

Organization and function of chromosomes during mitosis and meiosis

The meeting began with a series of talks focused on the kinetochore and its role in cell division. The opening lecture was by William C. Earnshaw (University of Edinburgh, UK), who talked about the epigenetic regulation of human kinetochores. He described his studies

using a synthetic human artificial chromosome that enables adjusting the chromatin composition within the centromere. He showed that centromeric DNA, although flanked by "deep" heterochromatin, transcribes non-coding sequences and that inhibition of this transcription by nucleating heterochromatin within the centromere rapidly leads to centromere inactivation. These studies highlighted the power of synthetic biology in the study of chromatin function at centromeres.

Next talk was by Conly Rieder (Rensselaer Polytechnic Institute, New York, USA). He gave an overview of the mechanisms of mitotic division using stunning high-resolution time-lapse video, phase-contrast, and DIC light microscopy. He focused on chromosome mono-orientation and its relationship to the spindle assembly checkpoint (SAC) and provided evidence that kinetochore-associated cytoplasmic dynein contributes to poleward chromosome movement.

Rieder's presentation was integrated and extended by Alexey Khodjakov (Wadsworth Center, Albany, USA), who described kinetochore dynamic transformations during mitosis. He showed that kinetochore shape plays an important role in spindle assembly and functioning. Large kinetochores increase the probability of interaction with microtubules (MTs), thereby accelerating spindle assembly. However, exceedingly large kinetochores are exposed to erroneous MT attachments, which might result in errors in chromosome segregation.

Patrizia Somma (IBPM/CBR, Rome, Italy) showed that two highly conserved splicing factors (SFs), Sf3A2 and Prp31, are required for chromosome segregation in both *Drosophila* cells and HeLa cells. Injections of anti-Sf3A2 and anti-Prp31 antibodies into *Drosophila* embryos disrupted mitosis within 1 min, arguing against a splicing-related mitotic function of these SFs. In addition, both SFs bind the spindle microtubules and the Ndc80 complex. These results collectively suggest that Sf3A2 and Prp31 have direct mitotic roles, promoting a stable association between the Ndc80 complex and the MTs during mitosis.

Related to the mechanisms of chromosome segregation, Leonid V. Omelyanchuk (IMCB, Novosibirsk) presented a new method for measuring MT flux during mitosis. He photobleached two large areas at both sides of the metaphase plate in *Drosophila* S2 cells expressing mCherry-tubulin and then filmed fluorescence recovery. This method allowed him to observe the flux for longer times compared to other extant methods and to obtain precise result on flux speed.

Lev Fedorov (Oregon Health & Science University, Portland, USA) studied another aspect of kinetochore-MT interactions. He used a mouse model to analyze the role of the SAC in recurrent pregnancy loss (RPL) in humans. He found that soon after implantation a large fraction of the cells of *BubR1*$^{-/-}$ morphologically defective mouse embryos was aneuploid and/or displayed premature sister chromatid separation. Because aneuploidy is considered the main cause of RPL, based on observations on the rare infant patients carrying *BubR1* mutations, he concluded that deficiency/insufficiency of BubR1 or other SAC components is responsible for RPL.

After many talks on monocentric chromosomes, Andreas Houben (IPK, Gatersleben, Germany) introduced the holocentric chromosomes. These peculiar chromosomes have been found in many species; the speaker described their evolution and their meiotic behavior. In some holocentric species, the unfused sister centromeres behave as distinct functional units at meiosis I, resulting in sister chromatid separation. Homologous non-sister chromatids then remain terminally linked by chromatin threads until metaphase II and separate at anaphase II. This inverted meiosis is likely to be an adaptation to handle holocentric chromosome architecture and behavior during meiosis.

The last talk in the session was on the PP2 phosphatase. The activity of this enzyme is high during interphase but is inhibited during mitosis to avoid premature reversion of CDK1-driven phosphorylation of mitotic proteins. Michael L. Goldberg (Cornell University, Ithaca, USA) discovered the molecular mechanism underlying PP2A inhibition during mitosis. He showed that the PP2A-B55 is inhibited by "unfair competition" with the small phosphoprotein pEndosulfine (pEndos). During M phase PP2A-B55 binds very tightly pEndos and dephosphorylates it more slowly than other substrates. As a result, PP2A-B55 is sequestered by pEndos and becomes available again only at M phase exit when pEndos is rapidly dephosphorylated. Intriguingly, myosin phosphatase (PP1-MYPT1) is downregulated with a similar mechanism by the pCPI-17 phosphoprotein, suggesting that "inhibition by unfair competition" is an ancient mechanism that allows PPP phosphatases regulation.

Interphase chromosome organization

The session was started by Igor F. Zhimulev (IMCB, Novosibirsk), who talked about the structural and

functional organization of chromatin domains in *Drosophila*. With the initial aim of defining at the molecular level the structure of the interbands of salivary gland polytene chromosomes, he developed a bioinformatic pipeline for the characterization of *Drosophila* chromatin. Following the identification of the genomic coordinates of a small set of interbands, he used the modENCODE ChIP-chip datasets for S2, Kc, BG3, and Cl.8 tissue culture cells to partition the *Drosophila* genome into four types of domains (named as the aquamarine, lazurite, malachite, and ruby chromatin) characterized by the enrichment in specific sets of proteins. Only the aquamarine chromatin corresponds to the polytene chromosome interbands; the other three types of chromatin correspond to the bands. In addition, each chromatin type displays characteristic genomic features. The aquamarine type is an "open" chromatin that tends to harbor the TSSs and 5'-UTRs of the protein-coding genes, non-coding RNAs, tRNA, and miRNA genes. The lazurite and malachite chromatin types are enriched in gene bodies and transposable elements, respectively; while the ruby domains correspond to a "closed" chromatin type devoid of tRNA and miRNA genes. In the next talk, Tatyana D. Kolesnikova (IMCB, Novosibirsk) discussed the relationship between these chromatin types and the DNA replication pattern in *Drosophila* polytene chromosomes. Her mapping studies also demonstrated that the vast majority of polytene chromosome bands that are visible at the light microscopy level are enriched in ruby chromatin.

Terry Orr-Weaver (Massachusetts Institute of Technology, Cambridge, USA) exploited the ovarian follicle cell gene amplification system to investigate the regulation of fork progression during *Drosophila* DNA replication. By studying the firing of ectopically inserted amplification origins, she showed that origin activation is regulated by the surrounding chromatin, which can inhibit or enhance the process. Interestingly, she showed that the chromatin at one genomic locus blocks origin activation downstream of localization of the ORC, Cdc6, and DUP (Cdt1), preventing binding of the MCM helicase to the origin. In addition, she showed that double-strand breaks that are produced at the amplicon sites with multiple replication forks are repaired by nonhomologous end joining rather than homologous recombination, the mechanism normally employed to repair and restore replication forks.

In the subsequent talk, Stepan N. Belyakin (IMCB, Novosibirsk) highlighted the involvement of the SuUR protein in the regulation of chromatin renewal during DNA replication. He proposed that in the H3K27me-/H3K9me-enriched repressive chromatin, replisome progression is delayed by a SuUR-containing complex, until the appropriate histone marks are properly placed on the newly-synthesized DNA strands. This model provides insight into the mechanisms of epigenetic inheritance and can be tested by further analysis of the interplay between local enrichment of repressive histone modification and fork progression.

Daria V. Kopytova (Institute of Gene Biology, Moscow, Russia) demonstrated that several *Drosophila* ORC subunits, besides their very well-known role in DNA replication, are also directly involved in mRNA export from the nucleus to the cytoplasm. She purified the TREX-2/AMEX general mRNA export complex and found ORC among its components. The ORC subunits were also shown to interact with messenger ribonucleoprotein particles. Some ORC subunits were detected at the nuclear periphery, where they colocalize with the nuclear pore complex (NPC). Finally, she showed that ORC knockdown disturbs the mRNA association with Nxf1, the major mRNA export receptor, interfering with the mRNA export process.

Related to nuclear RNA export, Elena V. Kiseleva (Institute of Cytology and Genetics, Novosibirsk) investigated the nuclear envelope (NE) and NPC biogenesis in growing *Xenopus* oocytes using transmission, scanning, and immune electron microscopy (EM). Through a series of beautiful EM images, she illustrated the NE expansion and nascent NPC insertion during interphase and was able to subdivide the process into nine steps.

Yuri Ya. Shevelyov (Institute of Molecular Genetics, Moscow) studied the role of nuclear lamina in chromatin architecture in *Drosophila* S2 cells. These cells lack tissue-specific A-type lamin (LamC), but contain ubiquitous B-type lamin (Dm0). He showed that depletion of lamin Dm0 by RNA interference results in *en masse* detachment of chromatin from nuclear envelope and in an increased chromatin density. Hi-C analysis confirmed that depletion of lamin Dm0 results in highly compacted topologically associating domains. These results suggest that release of chromatin from the nuclear lamina leads to its spontaneous compaction by macromolecular crowding.

Margarete M. S. Heck (University of Edinburgh, UK) talked about invadolysin, a zinc-metalloprotease that plays multiple roles in both *Drosophila* and humans. Invadolysin is required for proper chromosome

condensation, progression through the cell cycle, and cell migration. Invadolysin localizes to lipid droplets, interacts with mitochondrial ATP synthase subunits and with an ubiquitin protease that targets histone H2B, and plays a role in angiogenesis. In addition, it has been recently discovered that a secreted form of invadolysin is present in vertebrate serum and *Drosophila* hemolymph. These findings suggest that the secreted form of invadolysin has a conserved and potentially very important physiological activity, which will be addressed in future studies.

Epigenetic regulation of chromatin structure and function

The first three talks in this session were given by researchers from the Institute of Gene Biology (Moscow) and touched on the mechanisms of action of Su(Hw)-dependent insulators in *Drosophila*. Anton K. Golovnin studied the interactions between the main components of the insulator Su(Hw)/Mod(mdg4)-67.2/CP190 complex and delineated the protein domains responsible for the formation and activity of the complex at different genomic loci. Specifically, he identified the portions of the Su(Hw) protein that mediates dimerization. Next, Larisa S. Melnikova described how the protein EAST, a component of the nucleoskeleton, modulates the activity of the insulator proteins. Finally, Aleksey N. Krasnov demonstrated that the properties of Su(Hw)-binding sites do not depend on the type of surrounding chromatin and that Su(Hw) recruits SAGA, BAP, and ORC to the gene promoters.

Jorgen Johansen (Iowa State University, Ames, USA) reported his studies on the JIL-1 kinase, which is responsible for histone H3S10 phosphorylation. An analysis of the salivary gland transcriptome revealed that in *JIL-1* null mutants most genes that are normally active are repressed, whereas most normally inactive genes are activated. In the absence of H3S10 phosphorylation, the H3K9me2 repressive mark redistributes according to the new gene expression pattern. In addition, using an antibody to the double H3S10phK9me2 mark, he demonstrated that this mark is present in pericentric heterochromatin of wild-type polytene chromosomes with little or no labeling on the chromosome arms. Thus, the pericentric heterochromatin and the 4th chromosome contain the double H3S10phK9me2 mark, in contrast to the chromosome arms where the single marks are likely to reside on separate histone tails.

Vladimir A. Gvozdev (Institute of Molecular Genetics, Moscow) talked about the role of the Piwi protein in the nucleolus. He showed that Piwi nucleolar localization is dynamic and responds to various types of stress. Piwi colocalizes with three nucleolar proteins: Fibrillarin, Nopp, and the Udd regulatory subunit of Pol I. In addition, Piwi is physically associated with the Pol I complex, and its nucleolar localization is prevented by the Pol I inhibitor ellipticine. At the functional level, loss of nucleolar Piwi causes an increase in the R1 and R2 transcripts, pointing to a Piwi function in the repression of the rDNA copies carrying these transposons. An involvement in the modulation of rRNA biogenesis suggests a role of Piwi in the germinal cell fate and proliferation.

Gunter Reuter (Martin Luther University Halle-Wittenberg, Halle/Saale, Germany) reported a study on the properties of *Drosophila* germline chromatin. He examined the effects of several basic chromatin proteins on the silencing of transgenes containing the germ line-specific *fs(1)K10* gene. This analysis identified Su(var)2-1 as a new chromatin protein that controls histone acetylation through the recruitment of histone deacetylases. He also performed a chromosome wide crossover analysis in strains containing mutations or additional genomic copies of genes encoding different epigenetic factors. The results of these analyses suggest that chromatin accessibility affects the crossover frequencies.

Organization and function of telomeres

This session was focused on *Drosophila* telomeres. In *Drosophila*, telomerase is absent and telomeres are elongated by transposition of three specialized retrotransposons, called HeT-A, TART, and TAHRE (collectively abbreviated as HTT). *Drosophila* telomeres are epigenetic structures assembled independently of the sequence of terminal DNA; they are capped by terminin, a complex composed of fast-evolving proteins not conserved outside Drosophilidae. It has been proposed that terminin is functionally analogous to the shelterin complex that protects human telomeres.

Alla L. Kalmykova (Institute of Molecular Genetics, Moscow, Russia) showed that depletion of proteins such as the deadenylase complex components Ccr4 and Not, the Woc and Trf2 transcription factors, and RNA-

binding Ars2 results in the accumulation of excessively polyadenylated *HeT-A* transcripts in ovaries. This in turn leads to an abnormal concentration of *HeT-A* transcripts at the chromosome ends and around the centrosomes in early embryos, accompanied by mitotic defects. Although the majority of human TERRA (telomeric repeat-containing RNA) is not polyadenylated, these results suggest that the transcripts of telomeric DNA remain associated with chromosome ends in both *Drosophila* and humans.

Yikang S. Rong (Sun Yat-sen University, Guangzhou, China) described a new *Drosophila* protein, Tea, required to prevent telomere fusions (TFs). He showed that Tea localizes specifically at telomeres and forms a complex with the terminin components Moi and Ver. The in vitro purified Moi-TEA-Ver (MTV) complex binds and protects ssDNA in a sequence-independent manner. In addition to its telomere capping function, MTV appears to regulate end-elongation by participating in the recruitment of telomeric transposon to chromosome ends. Thus, MTV shares functional similarities with both the CST and the Tpp1-Pot1 subcomplexes of telomerase-maintained organisms.

Maurizio Gatti (Sapienza, University of Rome; and IMCB, Novosibirsk) described the role of the *pendolino (peo)* gene in *Drosophila* telomere protection. Mutations in *peo* cause TFs that preferentially involve the telomeres juxtaposed to constitutive heterochromatin (the Y, XR, and 4L telomeres), a TF pattern never observed in other telomere-capping mutants. *peo* encodes an E2 variant ubiquitin-conjugating enzyme that interacts with terminin. In *peo* mutants, DNA replication and PCNA recruitment are defective, and TF formation is partially suppressed by mutations in the *SuUR* gene, which facilitate heterochromatic DNA replication. This suggests that DNA replication in Peo-depleted cells results in fusigenic lesions specifically concentrated in heterochromatin-associated telomeres.

Giovanni Cenci (Sapienza, University of Rome) showed that mutations in the *Drosophila* Separase-coding gene *Sse* not only lead to endoreduplication as previously described but also to TFs. Sse physically binds both terminin and HP1, a non-terminin protein required to prevent TFs. Loss of Sse strongly reduced the HP1 level, and HP1 overexpression in *Sse* mutants suppressed TF formation, suggesting that TFs are due to a reduction of HP1. A catalytically inactive Sse failed to restore the HP1 levels and to reduce the TF frequency, indicating that Sse-endopeptidase activity is required for telomere protection. These results highlight a telomeric role of separase, suggesting that this protein contributes to telomere maintenance by regulating HP1 recruitment at chromosome ends.

Kent Golic (University of Utah, Salt Lake City, USA) spoke about a topic closely related to telomeres. He examined the characteristics of dicentric chromosome breakage during anaphase. He generated dicentric chromosomes by inducing a sister chromatid exchange within a ring X chromosome. These recombinant rings form a double bridge in mitosis that breaks during anaphase, generating linear chromosomes. An examination of these linear X chromosomes showed that one half of the breaks were in the heterochromatin; the euchromatic breaks were clustered in hotspots that coincide with markers of polytene chromosome intercalary heterochromatin (which is characterized by late replication, under-replication and fragility). These results indicate that the structure of polytene chromosomes translates to mitotic chromosomes.

Genome evolution

The session was opened by Alexander S. Graphodatsky (IMCB, Novosibirsk), who reviewed the studies on heterochromatin from an evolutionary point of view. He first discussed cases of closely related species that live in similar environments but display substantial differences in the heterochromatin. He then recalled studies aimed at the identification of the possible relationships between the amount of heterochromatin in the human genome and social behavior. The importance of the fast-evolving non-coding portion of the eukaryotic genome was further analyzed in the subsequent talks of Dmitrii I. Ostromyshenskii, Olga I. Podgornaya (both from Institute of Cytology, St. Petersburg, Russia), and Alsu F. Saifitdinova (St. Petersburg State University, St. Petersburg).

The presentation of Daniel A. Barbash (Cornell University, Ithaca, USA) was also related to heterochromatin. He talked about the genetic basis of hybrid incompatibilities between *Drosophila melanogaster* and *Drosophila simulans.* In crosses of *D. simulans* males to *D. melanogaster* females, F1 hybrid males die as larvae. This lethality is caused by the *Hybrid male rescue* (*Hmr*) and *Lethal hybrid rescue (Lhr)* genes, which encode interacting proteins that localize to the heterochromatin and repress transposable elements and satellite DNAs.

Although the roles of the Hmr and Lhr are still poorly understood, these results highlight a role of heterochromatin in the speciation process and in the maintenance of the interspecific barrier.

Several presentations were about comparative genomics. Among those, Polina L. Perelman (IMCB, Novosibirsk) gave an overview of recent progress of the Genome 10K project in sequencing and assembly of avian genomes and described the requirements for platinum genome assembly: high quality DNA, large insert libraries for sequencing, long reads, and physical maps. Denis M. Larkin (Royal Veterinary College, London, UK) focused on chromosomal evolution of amniotes by comparing mammals with birds. He showed that chromosome rearrangements in animals are likely to be caused by transposable elements and the presence of other repetitive sequences, and that multispecies homologous synteny blocks are enriched in genes related to ancestral- or clade-specific phenotypes. Moreover, evolutionary conserved breakpoint regions tend to rearrange genes related to species-specific and adaptive features.

Vladimir A. Trifonov (IMCB, Novosibirsk) talked about the evolutionary genomics of sturgeons (Acipenseridae), famous for providing the delicious black caviar. Most sturgeon species are under severe threat from overfishing, water pollution, and damming of rivers, and many species are at the verge of extinction. One of the unsolved problems in sturgeon biology is their sex determination system. Genome sequencing revealed a difference in the content of some repetitive elements between male and female starlet (*Acipenser ruthenus*). In addition, the genome of sterlet appears as a mosaic of diploid and tetraploid components.

Anna V. Kukekova (University of Illinois at Urbana-Champaign, USA) talked about the tame and aggressive fox strains (*Vulpes vulpes*) developed by long-term selection in Novosibirsk. These foxes offer a unique opportunity to study genetic regulation of complex social behaviors. She described the status of the sequencing and assembly of the fox genome and the preliminary results obtained by sequencing ten foxes from each of three populations (tame, aggressive, and conventionally farm-bred). The comparative alignment of the regions with increased homozygosity pinpointed possible genomic regions implicated in behavioral differences between the tame and aggressive strains.

A group of participants in the meeting had the unique opportunity to visit the famous fox farm and to see the striking differences between the aggressive and tamed foxes. These latter foxes were surprisingly friendly and behaved like dogs.

Chromosomal disorders

In the last part of the conference dedicated to the chromosomal abnormalities resulting in congenital disorders, Thomas Liehr (Institute of Human Genetics, Jena, Germany) described microscopic and submicroscopic copy number variations (CNVs) in humans. CNVs can be either connected with a disease or just be one of the many possible genetic variants without phenotypic consequences. He gave a detailed overview of the differences between submicroscopic and microscopically visible CNVs and about their phenotypic consequences. An example of the deleterious effects of CNVs was provided by Igor N. Lebedev (The Research Institute for Medical Genetics, Tomsk, Russia), who identified specific CNVs in several patients with intellectual disability and developmental delay.

Poster session and prizes

Three young scientists received a prize for their presentations. The prizes, consisting in book vouchers generously provided by Chromosome Research/Springer, were given to two posters and to an oral presentation. The winners of the poster awards, selected from the 36 posters presented at the meeting, were Elizaveta I. Radion (Institute of Molecular Genetics, Moscow) and Irina V. Grischenko (IMCB, Novosibirsk). Elizaveta studied the *Drosophila* telomeric and subtelomeric piRNA clusters. She used transgenic construct insertions to monitor chromatin structure and piRNA/siRNA production in *Drosophila* ovaries. She found that telomeric regions that generate mainly piRNAs are enriched in the HP1 paralog Rhino; in contrast, siRNA-producing regions are mainly enriched in HP1. Irina analyzed the in vivo formation of DNA G-quadruplexes in a number of immortalized B lymphocyte cell lines obtained from patients with the fragile X syndrome. Her preliminary results suggest the presence of G-quadruplexes in the FRAXA fragile site.

The winner for the oral presentation was Veniamin S. Fishman (Institute of Cytology and Genetics, Novosibirsk). He used the Hi-C approach to study genome-

wide chromatin interaction frequencies in mouse sperm cells and embryonic fibroblasts. Both cell types showed a high degree of similarity, although they displayed statistically significant differences in the contact probabilities of defined loci. The overall conclusion of this study was that the spatial structure of the genome is transmitted through generations without dramatic changes in sperm cells.

Acknowledgments We apologize to those whose presentations were not included in this report due to space limitations. The organization of "Chromosome 2015" and the preparation of this report were supported in part by a grant from the Ministry of Education and Science of Russian Federation (14.Z50.31.0005) to MG.