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PRESENTING AUTHORS

ORAL TALKS

POSTERS

ORAL TALKS

DELETION OF CTCF SITES AT THE PDGFRA/KIT/KDR LOCUS IDENTIFIES NONESSENTIAL ROLES OF TAD ARCHITECTURE*

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At the submegabase level, the mammalian genome is divided into topologically associating domains (TADs). However, despite active research, the role of TADs in the regulation of gene activity is still not clear. In some cases, a violation of the structure of the TADs leads to a sharp change in the pattern/activity of genes, however, there are examples when removing the boundary of the TADs does not lead to detectable consequences. Apparently, for different loci, the role of TADs can be different, and new studies at new loci can help to understand links between 3D chromatin structure and gene regulation. For our study, we selected a locus containing three genes (Pdgfra/Kit/Kdr), each located in its TAD. All genes encode tyrosine kinase receptors, important for the differentiation and maintenance of cell types such as fibroblasts, mast cells, and endotheliocytes. Using CRISPR/Cas9, we created a set of five mouse lines carrying deletions of CTCF sites that determine the formation of TAD borders at this locus. We have shown that removing the boundaries of TADs does not lead to impaired devel-

* The reported study was funded by RFBR according to the research project № 18-29-07022.

opment of animals or any obvious phenotypic manifestations. However, we found that a large 300 kb deletion that removes the Kit gene and most of its TAD leads to mast cell depletion even in heterozygous animals. The mechanism of mast cell depletion remains to be explored. Kit is known to be one of the most active genes in mast cells; its transcription in mast cells is burst by a group of very active enhancers. We decided to study whether the removal of CTCF sites at the border of TADs would lead to incorrect activation of neighboring genes. However, mast cell RNA-seq analysis did not reveal any significant differences in the expression of the surrounding genes. Thus, the removal of TAD boundaries at the Pdgfra/Kit/Kdr locus does not affect gene expression.

DESIGN OF ALGORITHM FOR 3D CHROMATIN INTERACTIONS PREDICTION BASED ON EPIGENETIC GENOMIC FEATURES

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Spatial interactions between promoters and their regulatory sequences are required to maintain cell-type specific expression pattern. Importantly, enhancers should be physically close to their regulated promoters in the space of nucleus. However, enhancer-promoter (EP) interactions often span large genomic distances. Chromatin forms 3D macromolecular structures with loops and topologically domains, thereby allowing colocalization of distant genomic regions in nuclear space. There are several notable examples of pathological changes in EP contacts caused by chromosomal rearrangements in non-coding genomic regions. The 3D chromatin architecture plays an important role in these cases.

Chromatin spatial organization could be accessed using chromosome conformation capture technology; however, there are only several high-resolution dataset of chromatin interactions available. In contrast, the Encode consortium and independent laboratories generated plethora of epigenetic data for various cell types. Therefore, it is important to learn what epigenetic features underlie the spatial organization of chromatin and how to predict three-dimensional architecture based on these epigenetic data. This is especially important to resolve 3-dimensional organization of chromosomal rearrangements.

We aimed to develop an algorithm for prediction of spatial chromatin interactions, in particular promoter-enhancer interactions. We proposed an approach based on machine learning algorithm, which gives high-quality predictions of chromatin interactions using only information about gene expression and CTCF-binding. Using multiple metrics, we confirmed that our algorithm could efficiently predict 3-dimensional architecture of normal and rearranged genomes.

LARGE-SCALE SCREENING FOR GENE EXPRESSION REGULATORY DNA MOTIFS LOCATED DOWNSTREAM TRANSCRIPTION TERMINATION SITES *

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Precise processing of newly synthesized messenger RNAs (mRNAs) is one of most important checkpoints in the spatiotemporal and immediate regulation of gene activity in eukaryotic cells. The regulatory potential of 3'-downstream region of a gene has not yet been fully studied. Research within this field was mostly complicated for a long time due to the lack of methods that could allow systematic and large-scale identification of functional nucleotide motifs in 3'-downstream regions of genes (that are cut from polyadenylated transcripts during RNA maturation, but crucial for the cutting and polyadenylation processes). We have found that one-nucleotide deletion in the 3' region of the *eGFP* reporter gene causes two-fold increase in both its mRNA level and the eGFP protein production in cultured mouse as well as human cells. We demonstrated that this one-nucleotide deletion located 32 bp downstream of polyadenylation signal induces RNA stable cleavage site in 14 nucleotides downstream of the polyadenylation signal.

For the large-scale screening of 3'-downstream gene regions, we developed a modification of Massively Parallel Reporter Assay (MPRA). This approach allows us to simultaneously measure and individually identify the level of transcriptional activity of tens of thousands of similar transgenes that differ only within the region of interest. We found that various

* The work is funded by the Russian Science Foundation grant #16-14-10288.

mutations in the 3'-downstream gene region are capable to either severely suppress or enhance up to hundreds-fold the expression of the upstream *eGFP* reporter gene. Thus, we identified a spectrum of nucleotide motifs in 3'-downstream gene region that can substantially increase or decrease the level of gene expression.

SUPER-RESOLUTION MICROSCOPY REVEALS THE REPLICATION DYNAMICS IN *DROSOPHILA* POLYTENE CHROMOSOMES [*]

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At the level of extended chromatin domains, replication timing is accurately reproduced in subsequent cell generations. The issue of organizing replication at the intra-domain level is very far from its solution. Polytene chromosomes provide a unique opportunity to analyze the probabilistic nature of replication initiation, observing at the same time many carefully aligned DNA strands on the same chromosome. We visualized the very beginning of S phase in polytene chromosomes by super-resolution microscopy and revealed two different modes of replication initiation. There are local cites where very early simultaneous initiation of replication occurs on multiple DNA strands in the same chromosome site, indicating the presence of an early origin with very high efficiency. These sites correlate but not coincide with active transcription. Additionally, there are extended chromosomal areas with relatively even distribution of replication initiation events, corresponding to broad replication initiation zones. There is an evidence on coordinated initiation of replication within these zones. We confirmed the earlier model, according to which the spatio-temporal organization of replication in

[*] Funding: Russian Foundation for Basic Research #17-00-00284.

Drosophila chromosomes is in general closely related to the division of the genome into two types of domains well corresponding to polytene chromosome structures: the rb-bands (the most compact polytene chromosomes bands) and the intervals in between them (INTs, corresponding to alternating zones of loose bands and interbands). In cell cultures and in salivary glands cells INTs correspond to early replication initiation zones. Rb-bands are free of replication initiation events and replication in these bands goes from the edges to the middle, while replication leads to changes in the band shape.

DIVERSITY OF AUXIN RESPONSE ELEMENTS IN *ARABIDOPSIS THALIANA L.*[*]

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Phytohormone auxin regulates virtually every developmental process. Auxin responses are implemented via activation or inhibition of auxin response factors (ARFs). ARFs bind to TGTC-containing auxin response elements (AuxREs) in promoters of auxin-sensitive genes. However, the signalling beyond ARF-dependent response in largely unknown. Meta-analysis of 21 auxin-induced transcriptome datasets (microarray and RNA-Seq) was performed to search for single and bipartite elements enriched in upstream regulatory regions of Arabidopsis auxin-responsive genes. A list of cis-regulatory elements associated with auxin up- and down-regulation in early or late response was identified. Interestingly, instead of TGTC-containing elements the most highly overrepresented ones were bHLH- and bZIP- binding motifs and AT-rich elements (Cherenkov, Novikova et al., 2018). For 40 genes driven by the regulatory regions with predicted bipartite elements auxin-inducibility was supported by qPCR. Functionality of several predicted bipartite cis-elements was studied by mutagenesis in promoters of five genes. For three of these bipartite ele-

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ments we experimentally identified interactions with TFs and tested interactions of the latters with ARF proteins.

Thus, diversity of simple and bipartite AuxREs was described in detail. For half of potential cis-elements connection with auxin transcriptional regulation was shown first time. Meta-analysis results were confirmed experimentally and mechanism upstream three bipartite elements was investigated.

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MAKING THE MEIOTIC SPINDLE IN OOCYTES

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The bipolar spindle in oocytes is assembled in the absence of centrosomes, the major microtubule nucleation sites in mitotic cells. A crucial, yet unresolved question in meiosis is how the bipolar spindle is formed without centrosomes and only around chromosomes in the exceptionally large volume of oocytes. We use *Drosophila* oocytes to answer this question.

Firstly, we uncovered a novel oocyte-specific microtubule nucleation pathway. This pathway is mediated by the kinesin-6 Subito/MKlp2, which recruits the γ-tubulin complex to the spindle equator to nucleate microtubules. It is essential for assembling most spindle microtubules complementarily with the Augmin pathway. Away from chromosomes, Subito interaction with the γ-tubulin complex is suppressed by its N-terminal region to prevent ectopic microtubule assembly in oocytes. Therefore, microtubule nucleation regulated by Subito drives spatially restricted spindle assembly in oocytes.

Secondly, we uncovered a novel mechanism that spatially regulates crucial spindle proteins. Away from chromosomes, phospho-docking by

14-3-3 inhibits the microtubule binding activity of the minus-end directed motor Ncd (human HSET; kinesin-14) that is crucial for focusing of the spindle poles. Near chromosomes, further phosphorylation by Aurora B kinase releases Ncd from this inhibitory effect of 14-3-3. Therefore, 14-3-3 translates a spatial cue provided by Aurora B to target Ncd selectively to the spindle within the large volume of oocytes.

THE ROLE OF PATRONIN IN THE PROCESS OF KINETOCHORE-DRIVEN MICROTUBULE FORMATION IN *DROSOPHILA* S2 CELLS *

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Among many microtubule (MT)-associated proteins, there is a group of proteins which bind minus-end of MTs. It includes γ-tubulin and its interacting partners in γ-tubulin ring complexes (γ-TuRCs), multi-protein augmin complex (also known as HAUS8 complex), and the ASPM/Asp protein. Recently, a new family of MT minus-end binding proteins was discovered — the calmodulin-regulated spectrin-associated proteins (CAMSAPs/Patronin). *Drosophila* has only one CAMSAP-like gene — *Patronin/ssp4* (*CG33130*). Previously, we have explored the localization of Patronin during mitosis using *Drosophila* S2 cultured cells expressing the Patronin-GFP fusion protein. We showed that Patronin binds different populations of MTs within mitotic spindle and has a very dynamic behavior in living prometaphase cells [1].

Here, we performed MT regrowth experiments after cold or colcemid treatment to understand the role of Patronin in the process of kinetochore/chromosome-driven MT formation in S2 tissue culture cells. We analyzed the frequency and the intensity of MT regrowth in control and *Patronin* RNAi cells. Depending on the method of MT depolymerization (cold or colcemid treatment), we observed higher or lower frequency of MT regrowth in Patronin-depleted cells. However, we found that Patronin depletion has a strong effect on the MT regrowth intensity after colcemid

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treatment: in the absence of Patronin kinetochore-driven MT bundles incorporate less tubulin than their control counterparts. Then, we analyzed the localization of Patronin-GFP during mitotic spindle regrowth in fixed cells. Althought the first MT bundles start to form within 30 min after colcemid removal and localize around chromosomes, Patronin-GFP was detected only about one hour after colcemid removal on already growing MT bundles. Taken together, these data suggest that Patronin stabilizes kinetochore-driven MT bundles, but it does not participate in their nucleation.

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EPIGENETICS, HETEROCHROMATIN AND AGE REPROGRAMMING

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Heterochromatin represents cytologically-visible state of heritable (cell-to-cell) gene repression. Of the constituent non-histone chromosomal components of heterochromatin the most highly-conserved are those of the Heterochromatin Protein 1 (HP1)-class. Work in this laboratory showed that HP1 proteins are conserved in animals and plants, which in turn led to the discovery of mammalian HP1 orthologues of which there are three, called HP1α, HP1β and HP1γ. HP1 proteins are key epigenetic regulators that "decipher" the "histone code" by recognizing and binding the tri-methylated lysine 9 moiety of histone H3. Biophysical studies have shown that this binding is dynamic and dependent upon the differentiation status of the cell, with HP1 proteins being more dynamic in embryonic stem cells compared to their differentiated derivatives. Thus the mobility of HP1 proteins can be used as a quantitative measure of the differentiated state of a cell.

We have used HP1β mobility of test the hypothesis that two aspects of the reprograming process seen after introduction of "reprogramming" factors into senescent cells, namely "age" and "developmental" reprograming, are separable. Briefly, using senescent primary human fibroblasts and a single cell iPS cell technique we have shown that "age" and "developmental" reprogramming can indeed be disentangled, at least in terms of the chromatin dynamics of an essential epigenetic modifier, HP1β. Our observations show that "epigenetic rejuvenation" — where old cells are returned back to a youthful state without having to pass through an embryonic stage may be a viable alternative to current approaches to regenerative medicine.

METHOD FOR HUMAN MITOCHONDRIAL DNA REPOPULATION USING CRISPR / CAS9-MEDIATED ELIMINATION OF DEFECT GENOME COPIES *

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Key words: mitochondrial DNA editing, cybrids, MitoCRISPR/Cas9

Often, the prevalence of mutant mitochondrial DNA (mtDNA) over wild — type molecules of the multicopy mitochondrial genome is the reason for the manifestation of many neuromuscular and neurodegenerative pathologies of humans. Removal of mutant mtDNA alleles by introducing a double-stranded break in defective copies is a potential way to shift the heteroplasmy level and therefore repopulate wild-type mtDNA molecules in the organelle. Mitochondria-imported restriction endonucleases, ZFN-and TALE-nucleases have already adapted for this task. However, the applicability of these methods is strictly limited by their low flexibility and low specificity. Meanwhile, the CRISPR/Cas9 system is much more flexible, easy to use and has more specificity. Here we propose a strategy for modifying the components of the well-known technology CRISPR/Cas9 to manipulate mitochondrial DNA (mtDNA) haplotype level in a cell. Previously, we obtained effectively import of SpCas9 nuclease into mitochondria by adding a mitochondrial localization signal to the 5'-end of the nuclease gene. We confirmed that MitoCas9 nuclease localized in the mitochondria by immunocytochemistry and western blot analysis on the transgenic cybrid cell lines — NARP3-1-MitoCas9 and NARP3-2-MitoCas9, stable expressed Mito-

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Cas9 from the nucleus. To delivery of the second component of the system, we made several modifications of guide RNA (gRNA) of CRISPR / Cas9, using known in the literature determinants of import of nucleic acids into mitochondria and confirmed the modifications did not affect on the functional activity of the system. Obtained results contribute to the further study of innovative technology of mitochondrial pathologies treatment — the MitoCRISPR/Cas9 system.

POSTERS

INVESTIGATION OF *LACERTA AGILIS* SEX CHROMOSOMES ORIGIN USING *LACERTA STRIGATA* FLOW-SORTED FLUORESCENCE PROBES*

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Reptiles have diverse mechanisms of sex determination, including many sex chromosome systems in different clades. *Lacerta agilis* is a widespread Eurasian lizard species from the family Lacertidae. Likewise other studied species in this family, *L. agili*s has ZZ/ZW sex chromosome system, but information about the origin of this chromosome pair is still contradictory.

In this study we have obtained metaphase chromosome suspensions from established fibroblast cell culture to prepare the spreads for fluorescence *in situ* hybridization (FISH). Then the fluorescence probes of telo-

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meric repeat and the flow-sorted chromosome-specific probes of *Lacerta strigata* (a close-related lizard species) were hybridized with these metaphase spreads.

The results shed some light to *L. agilis* sex chromosomes origin and could help to resolve this question. The sex chromosomes in *L. agilis* are most probably the same chromosomes as in *L. strigata*. This is consistent with the idea of stability of the lacertid ZZ/ZW sex chromosome system.

THE DREAM COMPLEX BINDING ON CHROMOSOMES OF MALE GERMLINE CELLS DURING *DROSOPHILA* SPERMATOGENESIS *

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Proper functioning of the multicellular organism is based on strict genetic control of the processes of cellular division and differentiation. There are many regulatory mechanisms that manage these processes and any defect in them can cause serious development problems.

Drosophila spermatogenesisis a convenient system for studying mechanisms controlling gene expression during cellular differentiation. One of the well-known transcriptional regulatory complexes in male germline cells is tMAC (**t**estis-specific **m**eiotic **a**rrest **c**omplex). Interestingly, tMAC is a counterpart of the broadly expressed complex dREAM (**D**rosophila **R**BF, **E**2F2 **a**nd **M**ip). The Mip40 and Caf1/p55 proteins are shared by dREAM and tMAC; some complexes subunits are paralogous (Mip120/Tomb, Mip130/Aly and dLin52/Wuc).

Previously the role of Mip40 in transcriptional regulation during spermatogenesis had been investigated using DamID-seq profiling. The data obtained showed that localization of studied protein dramatically changes during cell differentiation. But Mip40 DamID profile likely represents a superposition of two profiles, since Mip40 is shared by dREAM and tMAC. To distinguish between tMAC and dREAM localization, we performed an additional DamID profiling of specific subunit of dREAM — Mip130 protein.

Thus we obtained the binding profiles of the Mip130 with chromosomes in *D. melanogaster* male germline cells at different stages of spermatogenesis, separated tMAC and dREAM binding sites and also we established target genes specificity of these complexes.

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CHROMOSOMAL ORGANIZATION AND PHYSICAL MAPPING OF SIBERIAN STURGEON GENOME (*ACIPENSER BAERII*) *

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The Siberian sturgeon (*Acipenser baerii*) has a complex karyotype containing around 250 chromosomes, resulted from two rounds of whole genome duplication (WGD). Thus it represent a challenge for cytogenetic and genomic studies due to the high number of chromosomal elements and presence of almost identical paralogous chromosomes. We applied the acipenserid minisatellite-specific and sterlet (*A. ruthenus*)-derived whole chromosome-specific probes to analyze the Siberian sturgeon chromosomes. We showed that the last WGD event in the Siberian sturgeon was accompanied by the simultaneous expansion of several repetitive DNA families. Some of these elementsmay serve as good cytogenetic markers that allow distinguishing paralogous chromosomes and detecting ancestral syntenic regions. We demonstrate that some minisatellites are specific for chromosome size groups in the Siberian sturgeon, as we previously observed in the sterlet genome. Thus, we provide an initial physical chromosome map of the Siberian sturgeon genome based on molecular markers, which will facilitate further genomic studies in acipenserids.

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REPETITIVE DNA CHARACTERIZATION IN CYPRINID SPECIES WITH DIFFERENT PLOIDY LEVEL

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Polyploid species represent a challenge for both cytogenetic and genomic studies due to high diploid number and similarity between paralogous chromosomes, derived from WGD (whole genome duplication) event. Here using low coverage NGS sequencing we identified 14 most abundant tandemly arranged repetitive elements in the tetraploid genome of the crucian carp (*Carassius carassius*, 2n = 100) and applied them as probes for molecular cytogenetic studies. As a result, 8 probes turned out to be paralog-specific and marked on a single pair (mostly p-arms) in the *Carassius carassius* karyotype. In the triploid *Carassius gibelio* (3n = 150 ± 2) some probes labelled 3 chromosomes of similar morphology. Four probes produced multiple signals on many centromeric regions in both species. Our data indicate that the repetitive elements are very conserved in cyprinid species and may serve a good cytogenetic and genomic marker for distinguishing paralogous chromosomes.

THE ROLE OF HETEROCHROMATIN FACTORS IN THE PATERNAL GENOME INACTIVATION DURING THE EMBRYOGENESIS OF *PLANOCOCCUS CITRI* [*]

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Genomic imprinting is an epigenetic process that leads to changes in the activity of genetic material depending on its parental origin. Genes, chromosomes, and even set of chromosomes can be imprinted.

Planococcus citri shows a clear manifestation of sexual dimorphism at the imago stage that presumably is associated with genomic imprinting. In the *P. citri* males paternal haploid set of chromosomes becomes heterochromatic at early developmental stages. At the same time the set of chromosomes obtained from the mother remains active. In females, both parental sets of chromosomes stay euchromatic. It is assumed that the heterochromatization of the paternal chromosome set in males occurs as a result of a cascade of interactions between the HP1 protein and modified histones H3K9me3 and H4K20me3.

Bioinformatic analysis of *P. citri* genome revealed two homologues of HP1, eight homologues of H3K9-specific histone methyltransferase (2 genes for SetDB1, 2 genes for G9a and 4 genes for Su(var)3-9) and two homologues of H4K20-specific histone methyltransferase (HMT). Each of these genes was analysed for the presence of functional domains. Homologues of HP1 contain both chromo- and chromoshadow domains with

[*] The study was supported by the grant from the Ministry of Education and Science of Russian Federation #14.Y26.31.0024.

conservative amino acids consensus. All of HMTs homologues contain SET domain with three conservative amino acids consensus, except for one of SetDB1 homologue. Previously, knocking down one of SetDB1 homologues resulted in 1,5-fold increase of females/male ratio in embryos compared with control experiment. Knockdown of the second gene didn't show any effects and that probably is related to the absence of consensus in the SET domain. Simultaneous knockdown of two H4K20-HMT homologues demonstrated more intensive alteration of the sex ratio. At the same time knocking down one of HP1 homologues didn't affect sex ratio but led to weak decondensed chromatin in nuclei of male embryos.

THE ROLE OF TRANSCRIPTION FACTORS PPAR, LXR IN THE DEVELOPMENT OF EXPERIMENTAL METABOLIC SYNDROME WITH FATTY LIVER DISEASE IN THE ISIAH RAT STRAIN

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Metabolic syndrome (MS) is a complex of abnormalities that increase the risk of cardiovascular disease. A major risk factor for cardiovascular disease is obesity, which is often associated with hypertension and metabolic disorders such as insulin resistance and dyslipidemia. MS can lead to the development of nonalcoholic fatty liver disease (NAFLD). High population frequency of MS causes an increased interest to the MS experimental modeling, and study the mechanisms of MS development, and new approaches to it treatment and prevention. The complex of MS symptoms suggest systemic impairments in lipid and carbohydrate metabolism, having a common basis at the level of appropriate genes expression. The expression of genes involved into lipid and carbohydrate metabolism is regulated by transcription factors belonging to the superfamily of nuclear hormone receptors: peroxisome proliferator-activated receptors (PPAR) and liver X receptors (LXR). The endogenous PPAR and LXR are ligand-activated transcription factors. Ligands of PPAR are fatty acids, eicosanoids, prostaglandins and prostacyclins. Ligands of LXR are oxidized cholesterol derivatives (oxysterols). It was shown that functional activity of PPAR and LXR was higher in the liver of hypertensive ISIAH rats compared to the normotensive WAG rats, that demonstrates initial abnormalities of lipid and carbohydrate metabolism in ISIAH rats. Fructose load in ISIAH rats led to increase of the body fat content in 2.5 times (284 %) as well as to fatty degeneration of the liver that demonstrate development of fatty liver disease. Thus, hypertensive

ISIAH rat strain has the signs of a genetic predisposition to MS. Fructose load led to the MS development, accompanied by fatty hepatosis in ISIAH rat strain, but not in normotensive Wistar rat strain. Thus, the hypertensive ISIAH rats loaded by fructose can be considered as an experimental model for studying molecular and genetic mechanisms of MS development, and bring a good opportunity to search for new biomarkers and approaches to treatment of MS, accompanied by NAFLD.

THE ROLE OF CP190 IN GENETIC REGULATION OF *DROSOPHILA* SPERMATOGENESIS

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CP190 is essential component of Drosophila melanogaster chromatin, which is necessary for enhancer-blocking and barrier function of insulators and also serves as a linker between various transcription factors and their genomic targets. Despite its significance for genetic regulation, the role of CP190 in development remains elusive. To investigate the role of CP190 in cell differentiation we, first, analyzed the dynamics of CP190 chromatin binding through the development of adult male germline using germ-line specific DamID-seq. We found that the distribution of CP190 significantly changes in the course spermatogenesis, demonstrating a tendency of CP190 to associate with transcriptionally active genes. It was found that CP190 was recruited to genes necessary for spermatocyte differentiation after onset of terminal differentiation of spermatosytes. We also found significant colocalization of CP190 with transcription factors tTAF and tMAC, both are necessary for the onset of terminal differentiation. Second, we analyzed the effect of the CP190 inactivation on male germline development. For this purpose, we used Cre recombinase based germline-specific inactivation of CP190 gene, which led to significant decrease of CP190 in early spermatocytes and in the following stages. However, males with CP190 depletion were found to have neither significant changes in germline morphology nor fertility disorders. The transcriptome analysis of testes subjected to germline-specific inactivation of CP190 showed that the downregulation of CP190 resulted in increased expression of spermatocyte differentiation genes. Our results indicate the participation of CP190 in the genetic regulation of terminal differentiation of male germline.

ULTRASTRUCTURAL FEATURES OF THE DIVISION OF *DROSOPHILA MELANOGASTER* NEUROBLASTS [*]

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Neural stem cells play a central role in the development of the nervous system. As the rule, their division is asymmetric and leads to appearing of various types of neurons, which subsequently control the complex behavior of animals. Drosophila's neuroblasts are a popular model for studying asymmetric stem cells division and the mechanisms underlying their transformation into cancer cells. Neuroblasts' division is well-described at the light microscopy level. However, nowadays there is no work characterizing asymmetric neuroblasts division at the ultrastructural level. This knowledge is very important for researches of defects in cell division including investigation of tumorigenesis mechanisms.

The aim of this work was to perform an ultrastructural analysis of mitosis of *Drosophila melanogaster* neuroblasts using a transmission electron microscope (TEM). We concentrated on the dynamics of intracellular membranes (nuclear envelope and endoplasmic reticulum) and the mitotic spindle.

To reach the goal we fixed 20 samples of the central nervous system of drosophila 3rd instar larvae. Then we investigated dividing neuroblasts within sections of the brain under TEM JEOL1400. During the work, the data of internal structure of 152 neuroblasts on different stages of the cell cycle was obtained.

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The database of received photos of neuroblasts ultrastructure can be used for further studies about division defects or their comparison with other living organisms neuroblasts.

Electron microscopic analysis made it possible to characterize the fine organization and the dynamic of neuroblasts organelles and reveal the criteria for identifying the certain stage of the cell cycle.

Thus, for the first time, we proposed the model of asymmetric Drosophila larval neuroblasts division by taking into account features of their inner structure at each cell cycle stage.

IDENTIFICATION OF THE CE REGION IN THE *DE NOVO* ASSEMBLED GENOME OF *S. COPROPHILA* *

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Differential inactivation and/or elimination of chromosomes as a mechanism of sex determination is found in few groups, such as Coccoidea, Cecydomyiidae and Sciaridae. The work focuses on a particular case of sex determination based on paternal X-chromosome elimination that occurs in the early development stages in sciarid flies (Diptera, Sciaridae) which are commonly known as fungus gnats. This pattern of sex chromosome behavior is a result of chromosome imprinting. The nature of this phenomenon is poorly understood but probably epigenetic mechanisms are implicated. The controlling element (CE) which located in the heterochromatin part of X chromosome short arm plays the main role in elimination of sex chromosomes. The sequence of the CE is unknown, one of the first objectives is therefore to identify and describe it with the aim of further studying of the chromosome imprinting mechanisms in sciarid, which are probably evolutionary conservative.

Using the microdissection method, we excised the chromosome area that contains CE from polytene chromosomes of *Sciara coprophila* and eventually sequenced it. Thereby we created the microdissection library and used it for CE identification in the *de novo* assembled genome of *S. coprophila*. We have found three scaffolds that probably belong to CE area and now we are verifying it by FISH method.

* This work was supported by Government of the Russian Federation grant №14.Y26.31.0024.

AN AUTOSOME-BORNE GENE IS EXTENSIVELY AMPLIFIED IN THE W CHROMOSOME OF *EREMIAS VELOX* (LACERTIDAE, REPTILIA) *

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Heteromorphic sex chromosomes frequently accumulate repetitive sequences. The W chromosome of *Eremias velox* is heterochromatic and larger than the Z chromosome, whereas the W chromosomes of related species are small. In the meiotic prophase I (lampbrush stage) the W chromosome is decondensed, which indicates transcriptional activity. To study the repeat content of the *E. velox* W chromosome in more detail and identify putative transcribed sequences, we performed sequencing of the microdissected W chromosome library, using the Illumina MiSeq platform.

The resulting reads were aligned to the reference genome of *Podarcis muralis* using BWA-MEM. We identified high coverage in a 17 kbp fragment of the gene ATF7IP2, located on chromosome 14.

We designed a primer pair for a 900 bp fragment of this gene basing on the consensus sequence. The fragment was amplified from the genom-

* The work was supported by the grant #18-34-00182 from the RFBR.

ic DNA of *E. velox* and was fluorescent-labelled by TAMRA-dUTP. Then, FISH was performed both on lampbrush and metaphase chromosomes.

FISH showed that the copies of this gene are present throughout the whole length of the chromosome. At the lampbrush stage, there was no preferential hybridization in the loops or in the chromomeres. This result shows that the W chromosome of *E. velox* exhibits high degree of internal sequence homogenization, probably due to gene conversion and non-equal recombination. Perhaps, this process also contributed to the relatively large size of the *E. velox* W chromosome.

The ATF7IP2 gene, which originates from another chromosome, was probably brought to the W chromosome by a mobile genetic element.

It remains unclear whether this gene is active. More detailed analysis of the W chromosome sequence is required to determine the structure and context of the amplified ATF7IP2 copies, and RNA-seq is required to study its possible transcription and functional role.

THE ROLE OF CONDENSIN COMPLEXES IN INTERPHASE CHROMOSOME ORGANIZATION IN MOUSE EMBRYONIC STEM CELLS

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Abstract

In mammalian cells, genomic DNA adopts a complex three-dimensional conformation within the nucleus. By virtue of recent advances in sequencing and imaging technologies, the basic features of chromatin organization at both chromosomal and local levels has been expanded. These include A/B compartments, topologically associating domains (TADs) and long-range chromatin loops. Spatial genome organization plays critical role in many biological processes, such as transcriptional activity, chromosome segregation during cell divisions, genome integrity etc.

Throughout interphase, according to the loop extrusion model, cohesin organizes chromosomes into loops spatially brings together enhancer and promoter regions. During mitosis, 3D organization of chromosomes changes dramatically: cohesin mostly dissociates from the chromosomes and condensin complexes, in analogy to cohesin, refold chromatin into helically arranged arrays of high-density nested loops. Although most studies are mainly focused on the role of condensin in the formation of mitotic chromosomes, these proteins are also shown to play important roles in the organization of interphase chromatin. We analyzed the published data of the spatial contacts in multiple cells lines and noticed that mouse embryonic stem cells (ES) display an unique characteristic — the increased number of distant (>10Mb) chromosomal contacts. This trait was previously reported for metaphase chromosomes and is thought to

originate from programmed chromosome folding mediated by condensins. Based on these observations we have supposed that condensin complexes is likely have a wider role in genome organization of ES. We are creating genetically modified ES lines with auxin-inducible depletion of subunits of the cohesin complex and condensin complexes I, II, or both. We aimed to study the chromatin interactions and the gene expression profile in ES lines with depleted condensin levels. We expect that obtained results will be important for understanding the details of the interphase genome architecture of pluripotent cells.

NOVEL NUCLEOLAR PROTEIN 3 IS ESSENTIAL FOR *DROSOPHILA* PERICENTRIC HETEROCHROMATIN FORMATION*

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Nucleolar proteins are primarily known to be involved in ribosome biogenesis. However, there are several sources of evidence that nucleolar proteins play crucial roles in other important cellular processes. A set of nucleolar proteins directly take a part in mitosis, particularly in spindle assembly.

One of such moonlighting proteins is Novel Nucleolar protein 3 (Non3) that belongs to the highly conservative BRIX (biogenesis of ribosomes in Xenopus) domain protein family. Its yeast and human orthologs, RPF2 proteins, participate in the pre-ribosomal RNA processing and assembly of the 60S ribosomal subunit. It was previously shown that depletion of Non3 in *Drosophila* S2 cells affects mitotic spindle formation.

In the present work, we have generated and characterized *Drosophila* fly lines for ectopic expression of the recombinant Non3-eGFP and eGFP-Non3 proteins under the control of the upstream activation sequence (UAS). Ubiqioutus ectopic expression of Non3 appeared to be nontoxic for fly viability, whereas loss of Non3 function was shown previously to be lethal. Further, we have shown that HP1 protein is weakly detected in the pericentric chromosome regions of *Non3* mutants most probably due to the disruption of histone H3 lysine 9 (H3K9) methylation. These histone H3 modifications are required for HP1 binding and establishing of peri-

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centric chromatin structure. Using the In(1)w^{m4h} inversion, we found that *Non3* gene is a suppressor of position effect variegation (PEV). Next, we revealed that lack of Non3 due to gene deletion leads to increase of meiotic recombination level in pericentric regions of chromosomes. Consistent with these findings, we showed that reparation of double-strand breaks (DSBs) is delayed in ovaries of flies carrying one copy of *Non3* deletion (null-mutation).

Our results suggest that *Non3* is a haploinsufficient gene and lack of Non3 protein alter the structure of the pericentric heterochromatin both in soma and germ line cells. Observed disruption of HP1 localization and H3K9 methylation in pericentric chromosome regions may lead to an impairment of kinetochore-dependent assembly of the mitotic spindle in *Non3* mutants.

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