ABSTRACTS OF THE INTERNATIONAL MINI - CONFERENCE CHROMOSOMES AND MITOSIS

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Summaries of presentations are provided in the form submitted by authors with minimal formatting.

ORAL TALKS

New insights into role of HP1 in *Drosophila* chromatin and telomere maintenance

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This talk will be focused on the multiple functions of Drosophila HP1. HP1 is a conserved protein that plays essential roles in heterochromatin formation, positive and negative regulation of gene expression, and telomere capping. Most of HP1 functions are fulfilled through interactions with several well-known chromatin factors. I will present new genetic and molecular evidence revealing that HP1 works also in concert with Separase and Effete/UbcD1 to maintain telomere homeostasis.

Chromosomal organization of *Drosophila* genome

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Drosophila polytene chromosome display specific banding pattern; the underlying genetic organization of this pattern has remained elusive for many years. Recent progress in the annotation of the *Drosophila* genome and epigenome has made it possible to compare the banding pattern and the structural organization and activity of genes.

In the present work we analyze 32 polytene chromosome interbands mapped by cytology means. We demonstrate that interbands from polytene chromosomes correspond to 5'UTRs of housekeeping genes. As a rule, interbands display preferential "head-to-head" orientation of genes and are enriched for "broad" promoters characteristic of house-keeping genes as well as locations of proteins characteristic for open chromatin. Analysis of RNA-seq data (modEncode-FlyBase) indicates that the transcripts from interband-mapping genes are present in most tissues and cell lines studied. So, we propose that polytene chromosome interbands and their protein complexes are specific structures necessary for constant activity of housekeeping genes and for initiation of replication.

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Similarity in replication timing between polytene and diploid cells is associated with the organization of the *Drosophila* genome

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Replication timing in salivary gland polytene chromosomes of Drosophila melanogaster has been intensively studied by means of incorporation of labeled DNA precursors. The comparison of cytologically visible replication patterns led to the idea that replication timing in polytene chromosomes is quite different from that in diploid cells. Several comprehensive genome-wide replication profiles for drosophila cell lines were published over the past decade, yet these data were difficult to compare to the replication patterns observed cytologically in salivary gland polytene chromosomes. This was largely attributable to very limited data available on the exact correspondence between polytene chromosomes bands and the drosophila genome. Recently, we have mapped all large polytene chromosome bands along a single chromosome arm. This allowed us to compare replication timing between distinct types of chromosomes and to observe extensive similarity in the global replication patterns. In my report, I will discuss that replication timing in different drosophila tissues is substantially determined by the Drosophila genome organization as alternating domains with different properties. The first type of domains is characterized by high density of ubiquitously expressed genes and high concentration of replication origins; the second type includes predominantly gene-poor regions harboring tissue-specific genes.

Cell type-specific mapping of genomic binding sites of chromatin proteins using FLP-inducible DamID systems in *Drosophila*

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A systematic study of interactions of chromatin proteins with the genome is necessary for understanding the regulatory mechanisms governing DNA transcription, replication and repair in eukaryotic nuclei. The DNA adenine methyltransferase identification (DamID) approach is a powerful tool to identify genomic binding sites of chromatin proteins. It requires neither fixation of cells nor the use of specific antibodies. Recently, several inducible versions of DamID approach were developed, which allow cell type-specific profiling of chromatin proteins in heterogeneous *Drosophila* tissues. Notably, the inducible versions of DamID approach do not need sorting of cells of interest or their nuclei. Here, I will describe two FLP-inducible modifications of DamID approach, STOP#1-Dam and Dam^{inv}.

Genome-wide profiling of gene expression and transcription factors binding reveals new insights into the mechanisms of gene regulation during Drosophila spermatogenesis

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During cell differentiation, its fate switches from a proliferating stem cell to a specialized cell with distinct physiological function. This process involves several mechanisms that ultimately converge on the activation of genes required for future function of the cell and on the repression of the genes that are needed for stemness or function in other cell types. The present work uses Drosophila spermatogenesis as a model to study the steps in gene activation during cell differentiation. Two families of transcription factors regulate activation of about 1000 differentiation genes during Drosophila spermatogenesis. One family, tTAFs, is similar to TBP-associated factors and the members of the second family, tMAC, share homology with proteins of MMB-dREAM complex. Mutations in the members of tTAFs or tMAC lead to massive gene inactivation and the meiosis arrest.

Here, we investigated the binding of tMAC and tTAFs components to the chromosomes and studied their effects on the transcription and chromatin state as well as their interplay with repressive machinery. Specifically, we performed germline cell specific genome-wide profiling of the Cookie monster (Comr) protein representing tMAC, Mip40, which is a subunit shared by tMAC and MMB/dREAM, Cannonball (Can, tTAF) and Polycomb (PRC1). In our study, we investigated the effects of comr and can mutations on the chromatin by MNase-seq and H3K27me3 ChIP-seq.

The project is funded by the RFBR grants #16-04-01463 and #15-04-02264.

A point deletion in a region immediately downstream of GFP reporter 3′ UTR increases level of GFP transcription in mouse and human cells approximately two-fold

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In eukaryotes, the majority of protein-coding genes generate multiple mRNAs, partially due to variations in termination of transcription. The process of transcription termination appears to be complex and provides one of the levels of gene regulation. We explore the influence of transcription termination defects on protein-coding mRNA synthesis efficiency by RNA polymerase II in mammalian cells using the double-reporter model system. We have found that one-nucleotide deletion in a region immediately downstream of a GFP reporter causes two-fold increase in both the mRNA and protein production in cultured mouse as well as human cells. We demonstrated that deletion of a single C located 32 bp downstream of polyadenylation signal (AATAAA) provokes more stable cleavage of transcripts within 14 bases downstream of the signal with a preferable cleavage site within the palindromic sequence TGACTAGTCA. The reference reporter transcript is also cleaved at 31 bp and a number of other sites located between 14–31 bp downstream of the polyadenylation signal.

The project is funded by the Russian Science Foundation grant #16-14-10288. We thank S.V. Kulemzin and E.S. Reshetnikova for help with FACS analysis.

Adaptation of the piggyBac transposon system to study position effects in cultured *Drosophila* cells

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Our goal is the investigation of influence of the local chromatin environment on the activity of promoter elements of different types (constitutively active and inducible ones) in cultured *Drosophila* cells. The novel multiplexed TRIP (Thousands of Reporters Integrated in Parallel) approach based on piggyBac-mediated transposition of thousands of DNA-barcoded reporter constructs into the genome of cells of interest, which is followed by the identification of their genomic localization sites and the measurements of their transcriptional activity using high-throughput sequencing, is particularly suitable to address the issue. The aim of this study is the adaptation of the TRIP approach to cultured *Drosophila* cells, which are a convenient model system for understanding the regulation of gene expression.

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

Slbo-GAL4 driver in line 6458: a cautionary note on its use in *Drosophila* oogenesis

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This work is devoted to analysis of widely used line # 6458 from BDSC which is used for border cell analysis during Drosophila oogenesis. We faced with difficulties using this line, so we have established the genomic localization of transgenes in # 6458 line and their structure. As a result, we have shown that the line #6458 carries twice more transgenic constructs than it was stated. We also found that one of transgenes, carrying *slbo*-Gal4 driver, appears to be homozygous lethal allele of well-known *chickadee* gene. Thereby caution needs to be exercised when using *slbo*-Gal4 driver during border cell analysis since *chickadee* gene itself is involved in process of border cell migration. As *slbo*-Gal4 is a widely used driver, we received several new insertions of *slbo*-Gal4 in 2L regions that are potentially not involved in the cell movement. As a result six new independent lines were received. We tested all the lines for proper marking of border cells, established the genomic localization of new insertions and compared border cell migration index with the original line #6458.

The derivatives of laboratory strains from different laboratories are genetically heterogeneous

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Some *Drosophila melanogaster* strains are cultured in the laboratory from the beginning of the last century. The derivatives of old strains were distributed among the different researchers and were cultivated independently in different laboratories over the years. The question arises: are the strains with the same label from different laboratories genetically similar and experimental results reproducible? We tested five derivatives of CantonS strain and three derivatives of Harwich strain from different laboratories. These strains are used as marker strains in the system of intraspecies hybrid dysgenesis (HD). Despite the fact that these strains show identical dysgenic properties, they have different mitotypes, different Wolbachia infection status and have different pattern of *P* transposon and *blood* retrotransposon hybridization sites on the salivary gland polytene chromosomes. We discuss possible reasons of the genetic heterogeneity of CantonS and Harwich derivatives from different laboratories.

Sequence-specific fluorescent probes for nucleic acid visualization in cells

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Design of sequence-specific fluorescent probes for nucleic acid imaging in fixed and living cells for imaging intracellular RNA and DNA is the issue of the day. In the case of double-stranded DNA, sequence-specific polyamide oligo(N-methylpyrrole/N-methylimidazole) minor groove binders are promising as the basis for fluorescent probes. Synthetic minor groove-binding pyrrole-imidazole polyamides labeled by fluorophores and targeted to the repeated sequences from mouse major satellites were synthesized and tested.

In the case of intracellular RNA, modified oligonucleotides provide sequence-specific imaging. Several types of fluorescent probes targeted to 28S ribosomal RNA were used: linear fluorescent probes based on 2'-O-methyl RNA analogs, molecular beacons and binary fluorescent probes. Pyrene that possesses a high quantum yield and able to form excimer with appearance a new band in fluorescence spectrum was used as a fluorophore. The suitability of these probes for DNA and RNA visualization were demonstrated.

Financial support by the RFBR-CNRS grant 12-04-91053.

Mitotic alterations in human microcephaly: the crucial role of Citron kinase

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The human brain is composed of approximately 90 billion neurons, which are generated during embryonic life starting from neural stem cells. If too few neurons are produced or too many neurons die during development, the brain volume is compromised, leading to microcephaly. Microcephaly can be the result of rare genetic disorders. The characterization of genes involved in primary microcephaly has revealed that most of them act during cell division and are involved in the control of different mitotic processes. The seminar will illustrate the exemplar case of Citron kinase, a human microcephaly protein expressed in mitotic cells and involved in control of cytokinesis, spindle orientation and genomic stability.

The evidence for reciprocal aneuploidies as a consequence of mitotic errors in the human blastocysts

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The origin of mitotic aneuploidy during embryo development is still underestimated due to fast elimination of cells with changes in chromosome number. The presence of cell free DNA in the blastocoele fluid provides the possibility to discover the karyotype of cell undergoing apoptosis. Fifteen, Day 5, human blastocysts were studied by molecular karyotyping using aCGH and NGS. The DNA samples from the blastocoele fluid, inner cell mass and trophectoderm were analyzed separately. This approach allowed us for the first time to detect the reciprocal aneuploidies (RAs), when the trisomy and monosomy of specific chromosome were present within the single blastocyst. RAs were observed in 26.7% (4/15) of blastocysts. The origin of RAs is related to mitotic non-disjunction in the euploid cell. Therefore, the RAs as a marker of mitotic *de novo* non-disjunction event can explain the 21% (16/76) of all detected aneuploidies.

This study was supported by RFBR (№ 15-04-08265).

Non-disjunction of chromosomes causes abnormal sperm formation in *Anopheles* hybrids

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The *Anopheles gambiae* complex contains several morphologically indistinguishable sibling species. In this complex, *An. gambiae* and *An. coluzzii* are the world's most important vectors of human malaria. *Anopheles merus* is a minor vector in eastern coastal region of Africa. Based on the crossing experiments, most of the species in this complex confirm Haldane's rule that sterility or inviability of F1 offspring from two different species is found in heterozygous sex individuals. Using X-specific and Y-specific FISH probes we followed the process of meiosis in individual species and their F1 hybrids. We found that indeed Haldane's rule applies to hybrids between lab strains *An. gambiae Zanu* or *An. coluzzii Mopti* with *An. merus MAF*. Abnormal chromosomal activities in meiosis I produce X+Y bearing sperms leading to hybrid male sterility.

Kinetochore-microtubule interactions in *Drosophila*: the roles of splicing factors and Int6

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This talk will be focused on the roles of two splicing factors (SFs) and of the oncogenic Int6 protein in the regulation of kinetochore-microtubule interactions. We found that the two highly conserved SFs, Sf3A2 and Prp31, bind the spindle microtubules (MTs) and the Ndc80 kinetochore complex, directly mediating proper kinetochore-MT attachment during mitotic division. We also found that loss of Int6 prevents ubiquitin-mediated degradation of the Klp67A MT depolymerase, leading to enhanced turnover of MT plus ends, kinetochore deformation and metaphase arrest.

This work was supported in part by a grant from the Ministry of Education and Science of Russian Federation #14.Z50.31.0005.

Microtubule dynamics in S2 cell mitosis: an ultrastructural overview

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Drosophila S2 cell culture is a very useful model to study dynamics of microtubules and their interactions with kinetochores during different stages of mitosis. Despite numerous data obtained on mitosis of these cells with the help of light microscopy, the fine structure of microtubules and their organization during division of a cell remains virtually unknown.

In our study, we describe the dynamics of microtubules from early prometaphase to late telophase and analyze in detail fine organization of single microtubules and microtubule bundles as well as their interactions with chromatin and nuclear envelope of S2 cells.

This work was supported by a grant from the Ministry of Education and Science of Russian Federation #14.Z50.31.0005.

Measuring Microtubule Flux using the modification of a standard photobleaching-based method

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The spindle microtubule (MT) flux is the continuous translocation of MTs toward the spindle poles caused by MT polymerization at plus ends coupled to depolymerization at minus ends. Poleward flux is observed in both mitotic and meiotic spindles; it is evolutionarily conserved and contributes to the regulation of spindle length and anaphase chromosome movement. MT photobleaching is a tool frequently used to measure poleward flux. Spindles containing fluorescently-tagged tubulin are photo-bleached to generate a non-fluorescent stripe, which moves towards the spindle poles allowing a measure of the flux. However, this method only permits rapid measurements of the flux, because the fluorescence of the bleached stripe recovers rapidly due to the spindle MT turnover. Here we describe a modification of the current photobleaching-based method for flux measurement.

This work was supported by a grant from the Ministry of Education and Science of Russian Federation #14.Z50.31.0005.

Analysis of the involvement of kinesin-like proteins in cell division of *Drosophila* S2 cells

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The spindle is a microtubule-based highly dynamic molecular machine that mediates precise chromosome segregation during both mitosis and meiosis. Understanding the molecular mechanisms of spindle assembly is one of the key goals of modern cell biology. We analyzed mitotic spindle reassembly upon depletion of microtubule-destabilizing kinesins Klp10A, Klp59C and Klp67A after cold- and colcemid-induced depolymerization of microtubules in *Drosophila* S2 cells.

This work was supported by a grant from the Ministry of Education and Science of Russian Federation #14.Z50.31.0005.

Role of Eb1, Mars, Mast and Mei-38 proteins in kinetochore-driven microtubule growth in *Drosophila* S2 cells

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Kinetochore-driven microtubule formation is essential for proper spindle assembly. To dissect the mechanisms underlying this process we analyzed spindle microtubule regrowth after cold- or colcemid-induced microtubule depolymerization in *Drosophila* S2 cells depleted of individual spindle components. Specifically, we used RNAi to deplete Eb1, Mars (HURP), Mast and Mei-38 (TPX2). We found that the kinetochore-driven microtubule regrowth is substantially reduced by the loss of each of these proteins.

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Role of the minus-end binding proteins in kinetochore-driven microtubule growth

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To form a mitotic spindle, centrosome-containing cells exploit 3 classes of microtubules (MTs): MTs nucleated by the centrosomes, MTs nucleated near the chromosomes/kinetochores and MTs nucleated from preexisting MTs through the augmin-based pathway. Here we studied the role of the minus-end binding proteins Asp and Patronin in spindle re-assembly after MT depolymerization in *Drosophila* S2 cells. In addition, we analyzed the effects of depletion of the MBD-R2 and Rcd1, components of the non-specific lethal (NSL) multiprotein complex, on spindle formation and functioning.

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Functions of *Drosophila* septins in cell division of neural ganglia

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Septins are a family of conservative GTPases. The septin genes were originally discovered through genetic screening for budding yeast mutants defective in cytokinesis and were later found in all eukaryotes except for higher plants. Active investigation of the septins structure and function held since their discovery, confirmed their essential role in cytokinesis. Septins are also involved in other processes, such as the establishment of cell polarity, the formation of a diffusion barrier, chromosome segregation, and vesicular traffic.

Drosophila melanogaster has five septin proteins, named Peanut (Pnut), Sep1, Sep2, Sep4, and Sep5, whose functions are not yet well understood. In 1994 Pnut was discovered and its participation in cytokinesis of neuroblasts was shown. Later, we found that mutations in *pnut* caused not only cytokinesis abnormalities, but also defects in centrosome and chromosome segregation which led to ploidy abnormalities. Here, we studied the role of all five *Drosophila* septins in neuronal ganglion cell division using 69B-Gal4-driven RNA-interference. Despite the fact that the functional form of septins is considered to a heteromeric protein complex Pnut-Sep2-Sep1-Sep1-Sep2-Pnut, depletion of each septin resulted in different consequences. Cytological analysis of mitosis in neural ganglia of *Drosophila* larvae with depleted Sep1 demonstrated the most strong aneuploidy among septins, shortening of prometaphase duration and some delay at anaphase stage. Sep2 RNA interference led to the hypocondensation of chromosomes and cell accumulation at prometaphase stage. Pnut knockdown as well as null-allele mutation of *pnut* gene resulted in cytokinesis abnormalities, defects in centrosome and chromosome segregation; all these anomalies were more pronounced in *pnut* null allele larvae. Depletion of Sep4 did not lead to any significant alterations on the mitotic stages distribution. Surprisingly, knockdown of ovary-specific Sep5 resulted in metaphase shortening and some delay at mitosis exit. Our findings suggest that *Drosophila* septins have different pleiotropic roles in somatic cell division.

References:

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2. Akhmetova, K.A., F.S. Fedorova. The *peanut* gene mutations effects on somatic and germ line cell division in *Drosophila melanogaster*. Russian Journal of Genetics: Applied Genetics, 2012, 2(3): 229-234.

Interactions of endoplasmic reticulum and the nuclear envelope during mitosis in *Drosophila* early embryo and S2 cells: an ultrastructural analysis

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The endoplasmic reticulum (ER) is a huge interconnected network of tubular and cisternal domains, which spans the entire cytoplasm and participates in many essential cellular events including the nuclear envelope (NE) disassembly and reassembly during mitosis. The outer membrane of the NE is biochemically and functionally similar to the ER and can be considered as its specialized subdomain, while the inner membrane contains a specific set of integral membrane proteins that mediate interaction of the NE with chromatin and the nuclear lamina. The NE dynamics in mitosis are based on cell cycle dependent phosphorylation - dephosphorylation events and the concomitant destabilization of protein-protein or protein-membrane interactions. The NE membrane-associated components are absorbed into the ER after NE breakdown in prophase and new NE reemerges from the intact ER after sister chromatids separation. Despite the data already obtained on NE dynamics, the mechanism of its assembly/disassembly is still controversial and we are far from understanding all the details of mitotic process and its regulation. Recently, a lot of new factors involved in ER and NE reformation during mitosis such as reticulons, nucleoporins gp210 and gp210, Nup 107, Sun proteins and others have been identified (Schlaitz, Bioessays, 2014; Phillips and Voeltz, 2015). We have used the transmission and scanning electron microscopy for investigation of ER and NE structural dynamics and their interactions during mitosis in Drosophila early embryo and S2 cells. New details of this process, in particular the formation of nuclear stacked (quadruple) membranes at early prometaphase of dividing S2 cells and reformation of ER membranes during assembly of nascent NE fragments on chromosomes in syncytial embryos at anaphase, will be demonstrated.

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POSTERS

Overcoming challenges in the generation of large-scale barcoded plasmid libraries for post-genomic studies

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In this project we generated DNA-barcoded plasmid libraries to study the position effects in cultured *Drosophila* cells using the TRIP (Thousands of Reporters Integrated in Parallel) approach. The barcode is a short random DNA sequence (18 nucleotides in length) located upstream of the polyadenylation signal of the reporter gene allowing the parallel monitoring of thousands of integrated reporters throughout the genome simultaneously. A TRIP reporter library should fulfill two main requirements: 1) the complexity of the barcoded library should be at least a few hundred thousands of clones and 2) the non-barcoded reporter plasmid in the library should be absent. The complexity of the TRIP library (a number of uniquely barcoded clones) arises from the quality of the barcoded primer used for PCR product amplification, the efficiency of its cloning procedure, and the efficiency of bacterial transformation. Here we developed a simple protocol to check the quality of the barcoded primer by Sanger sequencing and describe the most efficient procedure to clone barcodes into the host reporter vector using Gibson assembly.

Karyotypic and molecular cytogenetic characterization of the reedfish (*Erpetoichthys calabaricus*)

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The reedfish (*Erpetoichthys calabaricus*) belongs to the family Polypteridae, the only extant family in the Polypteriformes order. Despite its basal position among all contemporary ray-finned fishes, the order still remains poorly investigated by molecular cytogenetic approaches.

In this work we established a fibroblast tissue culture of *Erpetoichthys calabaricus* and analyzed the reedfish karyotype using conventional cytogenetic methods (including GTG-banding) and fluorescent *in situ* hybridization (FISH) with repetitive sequences and chromosome specific probes. The Spl101 repeat obtained from sterlet (*Acipenser ruthenus*, Acipenseriformes) was localized on the reedfish metaphase spreads by FISH and it marked subtelomeric regions of each reedfish chromosome. We also performed FISH using several sterlet microdissection chromosome-specific painting probes and revealed regions labeled by conserved repetitive sequences. The results suggest that there are some highly conserved repeats between reedfish and Acipenseriformes, despite the ancient divergence dated over 400 mln years ago.

The work was supported by RSF grant № 14-14-00275

Evolution of satellite DNA sequences in sterlet (*Acipenser ruthenus*)

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Satellite DNAs constitute a considerable fraction of fish genomes. Acipenseriformes represent a clade phylogenetically basal to Actinopteri. Despite a high interest to this fish group, only limited studies have been accomplished on isolation and characterization of repetitive DNA and sex chromosomes are still to be identified.

Here we applied new generation sequencing and cluster analysis to characterise major fractions of sterlet (Acipenser ruthenus) satellite DNAs. Using fluorescence in situ hybridization (FISH) we mapped these sequences on sterlet chromosomes and found that some chromosomes and chromosomal regions are enriched in different satellite DNAs.

Most sterlet satellite sequences seem to be evolutionary conserved and might be used as chromosome specific markers to identify acipenserid chromosomes. Biased accumulation of repetitive DNAs in particular chromosomes makes them especially interesting for search of sex chromosomes.

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X chromosome evolution in Ruminantia

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X chromosome in Ruminantia is variable by morphology and suffered multiple rearrangements. We investigate evolution of this sex chromosome to reveal in detail changes in chromosome structure and order of syntenic segments. We also check whether ‘centromere repositioning’ (recently described phenomenon of evolutionary new centromere (ENC)) had taken place in X-chromosome evolution of Cetartiodactyl. We have selected 25 evolutionary conserved BAC clones from CHORI-240 library evenly distributed along cattle X chromosome. High resolution BAC maps of X chromosome on a representative range of Ruminantia species from key families: Java mouse deer (Tragulidae), Siberian musk deer (Moschidae), giraffe (Giraffidae), Siberian roe-deer (Cervidae), musk ox and cattle (Bovidae) and gray whale (Cetacea) as out group. We identify conservative syntenic blocks and describe key intrachromosome rearrangements. We have reconstructed putative ancestral Ruminantia X chromosome and hypothesize about chromosome morphology and centromere position changes.

Investigation of polyploidy in Acipenseridae family using sterlet fluorescent chromosome-specific microdissection probes

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Acipenseriformes is an order of ray-finned fishes, comprising 27 extant species of sturgeons and paddlefishes inhabiting waters of the Northern Hemisphere. The order is characterized by many specific morphological and genomic features, including high diploid chromosome numbers, various levels of ploidy between species, unclear sex determination, and propensity to interspecific hybridization. Minimal diploid chromosome number among extant Acipenseridae is about 120 chromosomes, which is believed to be of a paleotetraploid origin. The aim of the investigation was to reconstruct genome evolution in the sterlet (Acipenser ruthenus, Linnaeus, 1758; ARUT; 2N=120), Siberian sturgeon (Acipenser baerii Brandt, 1869; 2N=240) and the bester (hybrid between beluga (Huso huso, Linnaeus, 1758) and sterlet; 2N=120) by a set of ARUT whole chromosome-specific probes generated by microdissection. The studied genomes represent a complex mosaic structures and consist of both diploid and tetraploid chromosome segments. This may be regarded as a transition stage from paleotetraploid (functional diploid) to diploid genome condition.

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