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for young scientists

"Molecular Control of Gene Expression"

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Invited speakers

Dr. Judith Kassis is the head of the Section on Gene Expression, Program in Genomics of Differentiation, NICHD (USA). Dr. Kassis obtained her PhD in 1983 at the University of Wisconsin, Madison. She did postdoctoral research at the University of California, San Francisco and then joined the Center for Biological Research and Evaluation (CBER), FDA as a tenure-track investigator in 1987. At CBER she conducted research on the control of gene expression and transposon homing using the model system Drosophila and was a product reviewer and license chair for biological products. She joined the Laboratory of Molecular Genetics, NICHD as a Senior Investigator in 1999.
Dr. Kassis studies epigenetic silencing by the Polycomb group of transcriptional repressors using Drosophila as a model system. She is also interested in how transcriptional enhancers, often located 50kb or more away from the promoters they regulate, achieve their specificity. The laboratory uses two approaches to understand PRE function. First, the group identifies DNA sequences required for PRE function and has made considerable progress towards identifying the complex array of DNA binding proteins required for the activity of one PRE. Second, in an effort to understand the role of PREs in the context of other regulatory DNA, the group studies PRE activity at the PcG target gene engrailed.

Dr. Paul Schedl is the head of lab at Princeton University (USA). Prof.Schedl has made important contributions to the fields of molecular biology, developmental biology and genetics. As a graduate student he identified temperature sensitive mutations in genes required for tRNA biosynthesis and the processing of tRNA precursor RNAs. As a post-doctoral fellow, he and his co-workers generated one of the first plasmid based Drosophila genomic libraries and was one of the first to molecularly isolated and characterized specific RNA Pol III and RNA Pol II genes. After completing a post-doc in Basal Switzerland, Paul joined the Biology Department at Princeton University as an Assistant Professor in the fall of 1978. Accomplishments as a faculty member at Princeton cover a wide range of topics. In the area of chromatin, his lab, together with the lab of Abe Worcel, was one of the first to show that nucleosomes are not

randomly distributed along the DNA. His laboratory was amongst the first to discover chromatin insulator or boundaries, to develop in vivo assays to test for insulator or boundary activity and to identify proteins/factors that confer insulator/boundary activity. Other chromatin work includes studies on Polycomb dependent silencing, trithorax group genes and transvection. In the field of sex determination his lab together with the lab of Tom Cline analyzed the role of the master-regulatory gene Sex-lethal in choosing and remembering the female or male identity. In the field of translational regulation, his lab discovered one of the first localized mRNAs, the product of the orb gene. orb is a member of the CPEB family of translational regulators, and was the first CPEB gene to be molecularly isolated and characterized. Studies on germline development showed that nanos and polar granule component function to impose transcriptional quiescence on newly formed primordial germ cells (PGCs) and that this is critical in the specification of PGC identity.

Dr. Rakesh Mishra is the Senior Principal Scientist and Group Leader (Genome Organization and Nuclear Architecture lab) in Center for Cellular and Molecular Biology (Hyderabad, India). Dr. Mishra received hid PhD in 1986 from the University of Allahabad. His research postdoctoral research was devoted to study of non-B DNA conformations and DNA topology at Molecular Biophysics Unit, Indian Institute of Science, Bangalore. Later in Center for Cellular and Molecular Biology Dr. Mishra studied transcription initiation in prokaryotes, chemical recognition and cleavage of DNA. In 1992-1996 he investigated antisense oligonucleotide mediated knock down against protozoan parasites at the University of Bordeaux, France and small nucleolar RNAs in Xenopus oocytes to study their role in rRNA maturation at Saint Louis University School of Medicine, USA. In 1996 to 2001 Dr. Mishra was engaged in studies of chromatin organization and regulation of homeotic gene complex of Drosophila melanogaster at the University of Geneva, Switzerland.
In 2001 Dr. Mishra joined CCMB. His work is devoted to understanding the role of genome organization and nuclear architecture in epigenetic mechanisms and hence the gene expression during development. Work in laboratory of Dr. Mishra is conducted in the following principal directions: structure and function of boundary elements, organization and regulation of

Hox gene complexes in animals, comparative and functional genomics of non-coding DNA, epigenetic regulation in development, nuclear matrix.

Dr. Vasily Studitsky is a Principal Investigator in Fox Chase Cancer Center, Philadelphia (USA). He received his PhD in 1988 in Institute of Molecular Biology RAS, Moscow. Early work of prof. Studitskiy focused on analysis of the pol III-related mechanism of transcription through chromatin that was discovered in his studies. In early 2000s his lab has developed an experimental system for analysis of the mechanism of transcription through chromatin by RNA polymerase II (Pol II) in vitro. This system faithfully recapitulates numerous features of transcribed chromatin described in vivo and allows their molecular analysis in vitro. Using this system, a novel Pol II-specific mechanism of transcription through chromatin (the "nucleosomal cycle") has been discovered and solved on molecular level. After establishing the system it has become clear that additional factors are required to fully recapitulate the rates of transcription and nucleosome fate observed in vivo. Since then the molecular mechanisms of action of chromatin-specific elongation factors, histone chaperones and factors involved in cancer development and aging FACT, TFIIS and nucleolin have been determined. Since early 2000s, prof. Studitskiy also is interested in analysis of the mechanisms of distant communication between regulatory elements and their targets (mechanisms of enhancer/insulator action) on DNA and in chromatin. His lab developed a highly purified experimental system for quantitative analysis of distant enhancer action on DNA and in chromatin in vitro. Chromatin components (histone "tails" & histone-free DNA gaps) and chromatin-interacting factors (linker histone H1 & HMGN5 protein) modulating the distant communication between enhancers and target promoters have been identified. Mechanisms of distant action of DNA regulatory elements ("slithering" and "slithering barrier" mechanisms for enhancers and insulators, respectively) have been discovered. Insulators blocking enhancer action over a distance in vitro and in vivo have been rationally designed, constructed and analyzed.

Thursday, June 18, 2015

11:00 – 11:10 Welcome remarks

11:10 – 11:50 **Polycomb response elements: architecture, redundancy, and function**
Judith Kassis
Section on Gene Expression, NICHD/NIH, Bethesda, MD, USA

11:50 – 12:30 **Genomic packaging, chromatin domain boundaries and epigenetic regulation of genes**
Rakesh K Mishra
CCMB, Uppal Road, Hyderabad, India

12:30 – 13:00 Coffee break

13:00 – 13:20 **ZAD domains of insulator proteins Pita, Zw5 and ZIPIC support specific distance interactions in *Drosophila***
Oksana Maksimenko
Institute of Gene Biology RAS, Moscow, Russia

13:20 – 13:40 **Opbp protein involved in the differential expression of some divergently-paired genes in *Drosophila***
Nickolay Zolotarev
Institute of Gene Biology RAS, Moscow, Russia

13:40 – 14:00 **Early-late genes of ecdysone cascade as models for transcription studies**
Marina Mazina
Institute of Gene Biology RAS, Moscow, Russia

14:00 – 15:00 Lunch

15:00 – 17:00 Poster session & discussion

Friday, June 19, 2015

15:00 – 15:40 **A common set of developmentally restricted factors confer the constitutive insulator activity of the BX-C Fab-7 and Fab-8 boundaries**
Paul Schedl [1,2]
Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA,
Institute of Gene Biology RAS, Moscow, Russia

15:40 – 16:20 **Structure of Transcribed Chromatin is a Sensor of DNA Damage**
Vasily Studitsky
Department of Pharmacology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA
Biology Faculty, Lomonosov Moscow State University;Moscow, Russia.
Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

16:20 – 16:40 Coffee break

16:40 – 17:00 **The mechanism of heat stress – induced premature cellular senescence**
Omar Kantidze
Institute of Gene Biology RAS, Moscow, Russia

17:00 – 17:20 **Analysis of chromatin spatial structure in four *Drosophila melanogaster* cell lines of different origin**
Sergey Ulianov
Institute of Gene Biology RAS, Moscow, Russia
Biological Department; Lomonosov Moscow State University, Moscow, Russia

17:20 – 17:40 **The role of crowding forces in juxtaposing genome regulatory elements**
Arkadiy Golov
Institute of Gene Biology RAS, Moscow, Russia

17:40 – 18:00 **Studying PRE/TRE activity switch in *Drosophila***
Daria Chetverina
Institute of Gene Biology RAS, Moscow, Russia

18:00 – 19:00 Poster session & discussion

ABSTRACTS

TALKS

Polycomb response elements: architecture, redundancy, and function

Judith Kassis, *J. Lesley Brown, Payal Ray, and Sandip De*

Section on Gene Expression, NICHD/NIH, Bethesda, MD

Polycomb group proteins (PcG) are a group of transcriptional repressors that act in protein complexes to repress or silence gene expression by modifying chromatin. Many PcG-regulated genes encode transcription factors that regulate development. Polycomb group response elements (PREs) are DNA-elements that recruit PcG complexes to chromatin in Drosophila. PREs are made up of binding sites for multiple DNA binding proteins, but the number, order, spacing, and composition of sites vary in different PREs.Functionally, PREs render transgenes PcG-responsive and are easily recognizable in genome-wide chromatin-immunoprecipitation experiments as large peaks of PcG-protein binding over discrete DNA fragments. We have recently deleted all the known PREs from the *invected-engrailed* PcG target genes and find that these genes are still PcG-regulated; this suggests that PcG-target genes have multiple, seemingly redundant ways to recruit PcG proteins. Similarly, there are a large number of PRE-DNA binding proteins, and loss of a single PRE-DNA binding protein has little effect on PcG-protein recruitment. We suggest that the extreme redundancy of PcG-recruitment reflects the need for robust regulation of PcG-target genes.

Genomic packaging, chromatin domain boundaries and epigenetic regulation of genes

Rakesh K Mishra

Center for Cellular and Molecular Biology, Uppal Road, Hyderabad, India

Large proportion of the non-coding DNA is emerging as key to the packaging of the genome in the nucleus that is critical for chromosomal organization and gene regulation. We, however, do not know what the '*packaging code'* of genome is, that allows as many packaging options as the number of cell types in higher eukaryotes. What is clear is that packaging restricts enhancers/silencers that are capable of functioning over long distances, to interact with only appropriate promoters. Boundary elements that define topologically independent chromatin domains facilitate this by flank the gene or gene complex that is differentially regulated. We identified a large number of boundary elements using variety of approaches. We find that the elements that are involved in the regulation genes by higher order chromatin structure are conserved across species - from flies to mouse. Our studies also highlight long-range interactions involved in regulation of genes by means of genomic packaging in cell type specific manner. We propose that accumulation of non-coding DNA, including at least some of the repetitive elements, with the evolution of complexity is the consequence of the regulatory function embedded in this part of genome.

ZAD domains of insulator proteins Pita, Zw5 and ZIPIC support specific distance interactions in *Drosophila*

Maksimenko O., *Kyrchanova O., Fedotova A., Zolotarev N., Bonchuk A., Georgiev P.*

Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia

In recent years, considerable progress has been made in understanding chromosome organization. High-resolution chromosome conformation capture techniques have provided evidence that chromosomes in the genomes of human, mouse, and *Drosophila* are partitioned into a series of discrete topologically associating domains (TADs). However, we still do not have a clear mechanistic picture of how long-range interactions between distant regulatory regions are established and maintained through the cell cycle. In the past few years, a concept has been formed that there is a special class of architectural proteins that are responsible for global chromosome architecture as well as for local regulation of enhancer–promoter interactions. Many transcription factors that are involved in the activity of insulators were attributed to architectural proteins. Here we tested three Drosophila insulator proteins, Zw5, Pita and ZIPIC, for ability to support distance interactions. A zinc finger-associated domain (ZAD) that is restricted to dipteran and closely related insect genomes is located at the amino-termini of these proteins. The ZAD domain is characterized by a conserved constellation of four cysteines, which form a zinc-coordinated fold. The crystal structure of the ZAD of Grauzone protein provides evidence that two ZAD molecules interact in a head-to-tail mode to form a dimmer. We have shown that ZAD domains of the tested insulator proteins form homodimers. No interaction between different ZAD domains was observed *in vitro*, yeast two-hybrid assay and co-immunoprecipitation experiments. In transgenic Drosophila lines, binding sites for the same ZAD protein can support distance interaction between GAL4 activator and reporter gene. However, we did not observe functional interaction between different ZAD proteins. Thus, ZAD proteins can support specific distance interactions. At the end, ZIPIC facilitated distance stimulation of reporter gene by GAL4 activator in yeast. Deletion analysis showed that ZAD domain is required for this activity of ZIPIC. These results suggest that ZAD insulator proteins are directly involved in organization of distance specific interactions in Drosophila chromatin.

Opbp protein involved in the differential expression of some divergently-paired genes in *Drosophila*

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32% of D. melanogaster genes are divergently-paired genes (DPGs). These genes are transcribed in opposite directions from the TSSs separated less than 1 kbp. A significant part of DPGs have different expression profiles within a pair. Promoters regions of DPGs are often bound by insulator proteins (CP190, BEAF-32, dCTCF). Probably these proteins can form a boundary between paired genes, allowing differential expression.
From yeast two-hybrid assay and GST pull-down we found that transcription factor Opbp is a partner of the insulator protein CP190. From EMSA experiments we found that Opbp can bind specific DNA sequence. We also performed ChIP-seq and GO analysis for Opbp that showed a preferential localization of Opbp at promoters of DPGs with a significant enrichment of pairs where one gene is a highly expressed ribosomal gene and another one is a tissue-specific gene.
We performed mutation of biding site and Opbp knock down and demonstrated that Opbp is a important for the differential expression of DPGs.We are interested to further explore the mechanisms of DPG differential expression regulation and the role of Opbp in this process.

Early-late genes of ecdysone cascade as models for transcription studies

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DHR3 and *Hr4* early-late genes of ecdysone cascade are described as convenient models for transcription study in drosophila cells. Set of experiments revealed these genes as powerful systems for research in physiological conditions upon 20-hydroxyecdysone induction. *DHR3/Hr4* genes transcription is characterized by fast activation kinetics, which enables transcriptional studies without influence of indirect effects. This finding is supported with the fact of highly selective activation of genes in drosophila cells by ecdysone (only 73 genes are induced upon ecdysone addition). Expression of DHR3 and Hr4 genes is completely controlled by ecdysone titer and decreases within several hours after hormone withdrawal. Within 20 minutes after induction, promoters of *DHR3* and *Hr4* genes became functional, which make them useful tools for investigation of early activation process. Their transcription is controlled by RNA polymerase II pausing mechanism, which is spread through drosophila genome. Uniform transcription activation of *DHR3* and *Hr4* genes in cell population was confirmed on the level of RNA and protein. That makes transcriptional research in DHR3/Hr4 system independent on changes in cells ratio.

A common set of developmentally restricted factors confer the constitutive insulator activity of the BX-C Fab-7 and Fab-8 boundaries

Paul Schedl [1,2]

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Chromatin boundary elements or insulators are cis-regulatory elements that play an important role in the 3-dimensional organization of the eukaryotic chromosomes. In addition to their architectural activities, insulators also have a role in gene regulation, functioning to direct enhancers and silencers to their appropriate gene targets. In the Drosophila Bithorax complex (BX-C) insulators have two functions. The first is to insure the functional autonomy of the 9 BX-C *cis*-regulatory domains. The second is to facilitate interactions between the *cis*-regulatory domains and their target homeotic genes. We've been studying two of the insulators associated with the *Abdominal-B* gene, *Fab-7* and *Fab-8*. These studies have focused on the identification of the *cis*-elements and *trans*-acting factors that are required for their insulator activity during development. Previous studies have shown that *constitutive* insulator of *Fab-7* is generated by cis-elements whose insulator activity is developmentally restricted. One of these sub-elements, pHS1 has insulator activity early in development, and we've shown that this insulator activity is conferred by a heterotrimeric complex that is present in early embryos. A second sub-element, dHS1, has its primary insulator activity from mid-embryogenesis onwards. Consistent with this stage specificity, dHS1 has multiple binding "sites" for a large, 700 kD complex called the LBC that is enriched in nuclear extracts from "late" embryos. The LBC has unusual sequence recognition properties and contains at least three different proteins, the GAGA factor, Mod(mdg4) and en(y)2. Like *Fab-7*, *Fab-8* relies on developmentally restricted factors for its insulator activity. We show that it has binding sites for the early Elba factor and the late LBC.

Structure of Transcribed Chromatin is a Sensor of DNA Damage

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† Equal contribution.

Early detection and repair of damaged DNA is essential for cell functioning and survival. Although multiple cellular systems are involved in repair of single-strand DNA breaks (SSBs), it remains unknown how SSBs present in non-template strand (NT-SSBs) of DNA organized in chromatin are detected. Here the effect of NT-SSBs on transcription through chromatin by RNA polymerase II was studied. NT-SSBs hidden in nucleosome structure can induce nearly quantitative arrest of RNA polymerase. The location of the arrest sites on nucleosomal DNA suggests that formation of small intranucleosomal DNA loops causes the arrest. This mechanism likely involves relief of unconstrained DNA supercoiling accumulated during transcription through chromatin by NT-SSBs. The data suggest existence of a novel chromatin-specific mechanism allowing detection of NT-SSBs by the transcribing enzyme.

The mechanism of heat stress – induced premature cellular senescence.

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Here, we demonstrate that heat stress induces p21-dependent cellular senescence. Notably, only early S-phase cells undergo premature senescence in response to heat stress. The encounter of DNA replication forks with topoisomerase I-generated single-stranded DNA breaks (SSB) was found to be a primary cause of heat stress-induced senescence in these cells. Moreover, different SSB-inducing agents were found to induce similar changes (i.e. senescence-like phenotype) in early S-phase cells. This study of heat stress-induced cellular senescence elucidates the mechanisms underlying the exclusive vulnerability of early S-phase cells to ultra-low doses of SSB-inducing agents.

Analysis of chromatin spatial structure in four *Drosophila melanogaster* cell lines of different origin

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Using a Hi-C experimental approach we characterized the spatial organization of interphase chromosomes in four cultured cell lines of Drosophila melanogaster, S2, Kc167, DmBG3-c2 и OSC. Partitioning of chromosomes into topologically-associated domains (TADs) was found to be very similar, although not identical in these cell lines. Inter-TAD regions were found to be rich in active chromatin marks and RNA polymerase II, transcriptome analysis has demonstrated that these regions are highly transcribed. In contrast, H1 and core histones were underrepresented in inter-TADs, and deposition sites of insulator proteins, dCTCF and Su(Hw) were almost randomly distributed between TADs and inter-TADs. Based on the above observations we propose that TADs are self-organized structures stabilized by interactions between nucleosomes while inter-TADs are the regions where inter-nucleosomal interaction is compromised by high level of histone acetylation linked to high transcription activity.
The research was supported by RFBR grants 13-04-40086-H, 14-04-00010, 13-04-40088-H, 13-04-40089-H).

The role of crowding forces in juxtaposing genome regulatory elements

Golov A.K.[1], *Gavrilov A.A.*[1], *Razin S.V.*[1,2]

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Now it is widely accepted that an extremely high concentration of macromolecules in living cells can dramatically increase the level of intermolecular attraction. This effect is usually referred to as «macromolecular crowding». It has been previously shown that crowding forces support the integrity of nucleoli and PML and Cajal bodies in eukaryotic cell nuclei. The contribution of these forces to self-organization of other nuclear compartments is widely discussed. One type of such compartments are so-called active chromatin hubs in which remote regulatory elements of large genomic regions are spatially juxtaposed. Here we experimentally addressed the possible role of crowding forces in stabilization of active chromatin hubs. Using chromosome conformation capture technique and mouse β-globin domain as a model, we showed that spatial proximity between remote regulatory elements can be reversibly changed upon manipulations with the concentration of macromolecules. In addition to proving the role of crowding forces in shaping interphase chromatin, our results suggest that the folding of chromatin fiber is a major determinant in juxtaposing remote genomic elements.

Studying PRE/TRE activity switch in *Drosophila*

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During development of multicellular organisms, individual patterns of gene expression are established in each cell type and stably transmitted through many cell divisions. The Polycomb group proteins maintain repression, while the antagonistically acting Trithorax group members control activation of gene transcription. In *Drosophila*, Polycomb group and Trithorax group proteins are assembled on the DNA regulatory sequences termed Polycomb response elements (PREs) or Trithorax response elements (TREs), respectively. At least some of PREs/TREs are able to switch their activity between repression and activation. In present study, we created model system in which GAL4 activator can switch PRE activity. We show that PcG proteins remain bound to unrepressed PRE even in case of passing through the PRE transcription. The molecular basis of this phenomenon will be discussed.

This study was supported by RFBR № 15-34-20581 and RFBR 15-04-04208-a to D.C.

POSTERS

Studying the role of orb in local mRNA translation

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Our laboratory studies CPEB protein family of Drosophila. These proteins bind to target sequences in 3' UTRs and regulate mRNA translation by modulating poly(A) tail length. Drosophila has two CPEB genes, orb and orb2. Orb expression is only detected at high levels in the germline and has critical functions in oogenesis but not spermatogenesis.Orb protein autoregulates its own synthesis by binding to orb mRNA and activating its translation.

The determination of cell fate and the establishment of polarity axes during Drosophila oogenesis depend upon pathways that localize mRNAs within the egg chamber and control their on-site translation. Orb plays a central role in regulating on-site translation of mRNAs.

This study was supported by the program “Molecular and Cell Biology” of the Russian Academy of Sciences, and grant of the Russian Federation Ministry of Education and Science (14.B25.31.0022).

Relative-of-WOC (ROW) protein is involved in activity of MSL complex

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Drosophila HP1c regulates gene expression and localizes to active chromatin domains, where it extensively colocalizes with the poised form of RNApolymerase II. HP1c also interacts with transcription factor named Relative-of-WOC (ROW). This protein contains different zinc-finger domains that are involved in protein-protein interactions. We have identified that ROW directly interacts with MSL1, protein involved in Drosophila dosage compensation. The domains involved in this interaction were mapped in the yeast two-hybrid and GST pull down. In addition ROW directly interacts with number of DNA binding transcription factors. The genome-wide mapping of ROW binding sites in embryos is in progress. Our preliminary model suggests role of ROW in recruiting of MSL complex to gene promoters.

Purification and characterization of insulator protein complex containing factor GAGA (GAF) of Drosophila melanogaster

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GAF is a well-known chromatin protein, which exerts multiple molecular functions. Its DNA-binding sites were mapped, however, its protein partners are poorly studies. Here we describe the method for purification of GAF-containing protein complex from nuclear embryonic extract. We found GAF to be a component of 700 kD complex, which contains multiple proteins, including insulation factors, proteins of TrxG and PcG families, Zn finger proteins, transcription factors, etc.

This study was supported by the program “Molecular and Cell Biology” of the Russian Academy of Sciences, and grant of the Russian Federation Ministry of Education and Science (14.B25.31.0022).

Role of dCTCF in regulation of the bithorax complex

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CTCF is main insulator protein in higher eukaryotes that contains a conserved eleven zinc finger central DNA-binding domain and divergent N and C termini. While CTCF was suggested to be the main architectural protein that supports distance interactions, its structural domains have been poorly characterized. We mapped the dimerization domain at the N-terminus of Drosophila CTCF (dCTCF) that is essential its functional activity. Previously association of dCTCF with CP190 was reported. By GST pull down and the yeast two hybrid assay we determined region in the C-terminus of dCTCF that interacts with the BTB dimmer of CP190. Deletion derivative of dCTCF lacking the ability to interact with CP190 *in vivo* can complement a null dCTCF mutation suggesting that interaction with CP190 is not critical for functional activity of dCTCF. Inactivation of dCTCF in the null mutation lead to some homeotic transformations in the first generation and embryonic lethality in F1, suggesting that dCTCF is predominantly required during embryo development. Role of CTCF in regulation of the bithorax complex will be discussed.

The new DNA-binding protein factors of Msl-complex in Drosophila melanogaster

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Dosage compensationin *Drosophilamelanogaster* occursby increasingthe transcription level from the single male X-chromosomeequating X-gene products between males (1X) and females (2X). The key regulator of dosage compensation in Drosophila is the male-specific lethal (MSL) complex, which includes at least five proteins (MSL1, MSL2, MSL3, MOF, MLE) and long noncoding RNA (roX1, roX2). A central question is how Msl-complex distinguishes the male X-linked genes by autosome genes. It is assumed that the main role in this process is played by the regulatory DNA sequences which specifically attach (recruit) Msl-complex. However, the way in which these non-homologous regulatory elements provide efficient binding of Msl-complex on the X chromosome is still unknown.

We proposed that the DNA-binding ZnF-proteins with C2H2-domain could recruit Msl-complex to X-chromosome. Therefore, we looked for Msl1/Msl2 partners among ZnF-proteins of *Drosophila melanogaster* by means of yeast two-hybrid system. As a result,a number ofproteins interactingwith Msl1 and Msl2 were identified.Nextwewere localizedbinding sitesof the proteinsrequired forprotein-protein interactionswithMsl-proteins.Thus, we haveidentifieda DNA-binding transcription factorsthat are able to providespecific binding Msl-complex to regulatory elementson the X-chromosome of males.Furtherstudy ofgenome-widedistribution ofthese proteinswill helpto understand themechanism ofDCCattraction to the X-chromosome of *Drosophila melanogaster* males.

Role of Su(Hw) protein in *Drosophila* gene transcription

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Chromatin insulators are DNA regulatory elements able to modulate enhancer action and protect genes from negative influence of repressors. The best-studied insulators in *Drosophila* require Suppressor of Hairy wing (Su(Hw)) protein for their activity. The whole-genome studies identified several thousands of Su(Hw) binding sites in *Drosophila*. Transgene reporter assays suggest that Su(Hw) insulators act mainly as modulators directing enhancers to target promoters. However, the functional role of Su(Hw) binding sites in their genome environment remains poorly studied. The Su(Hw) binding sites could be placed in intergenic and intragenic regions, often near promoters and terminators.

The aim of this study was to test the role of Su(Hw) binding sites in the transcription of nearby placed genes. The X-ChIP analysis was used to detect the formation of insulator complexes in wild-type flies and in flies with mutations in *su(Hw)* gene. In parallel we used RT-qPCR to detect the transcription of selected genes. As a result we show that absence of Su(Hw) near terminators didn't affect transcription and termination of studied genes. However, the transcription of several genes containing Su(Hw) binding sites near promoters was sensitive to *su(Hw)* mutations resulting in activation of their transcription levels. Thus, Su(Hw) negatively controls transcription of these genes. Using different deletion of Su(Hw) protein we identified a core part of Su(Hw) responsible for the repression.

This study was supported by RFBR 15-04-03973-a to M.E.

Stabilizing activity of enhancers and silencers in *Drosophila* transgenic systems

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Level of gene transcription in high eukaryotes depends on DNA regulatory elements: enhancers that activate gene transcription and silencers that control gene repression. Within transgenic constructs both types of regulatory elements are affected by genome environment that leads to changes in level of reporter gene transcription. Search of conditions capable to protect activities of regulatory elements are required to ensure their stable action within transgenic systems and to maintain reporter gene expression at appropriate levels. In this study, we demonstrate that terminators of transcription from SV40 flanking transgene effectively protect *Drosophila* enhancers and silencers stabilizing their activities.

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Study of repressor protein INSV in Drosophila melanogaster

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Drosophila Insensitive (Insv) promotes sensory organ development and has activity as a nuclear corepressor for the Notch transcription factor Suppressor of Hairless [Su(H)]. Insv lacks domains of known biochemical function but contains a single BEN domain. INSV binds to TCYAATHRGAA as dimmers. In embryos INSV was found to co-localize with the insulator protein CP190 that is common partner for all know Drosophila insulator proteins. The CP190 protein contains several domains: homodimerization BTB/POZ domain, aspartic acid rich (D-rich) domain, a centrosomal targeting domain (M domain), four C2H2 zinc fingers, and C-terminal glutamic acid rich (E-rich) domain (Oliver et al. 2010). Using in virto approaches and the yeast two-hybrid assay we found that INSV contains dimerization domain at the amino-terminus. INSV directly interacts with CP190 protein by two regions located near the dimmerization domain. Interestingly, that CP190 interacts with INSV by the N-terminal BTB domain and the M-domain. Using transgenic lines we are testing role of CP190-interacting domains in Insv activity as repressor.

A novel zinc-finger protein CG9890 interacts with components of the *Drosophila* transcription and replication machinery *in vivo*.

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DNA replication begins from multiple sites distributed throughout the genome, named replication origins. The origin recognition complex (ORC) binds to the replication origins and plays a critical role in the initiation of DNA replication by creating a platform for pre-RC complex assembly and replication origins firing. Despite increasing data on the properties of replication origins, molecular mechanisms underlying origin recognition complex (ORC) positioning in the genome are still poorly understood. We show that Su(Hw) interacts with ENY2 and recruits the histone acetyltransferase complex SAGA and chromatin remodeler SWI/SNF to Su(Hw)-dependent insulators, which gives rise to regions with low nucleosome density and creates conditions for ORC binding. We suggest that the key determinants of ORC positioning in the genome are DNA-binding proteins that constitute different DNA regulatory elements, including insulators, promoters and enhancers. Su(Hw) is the first example of such a protein.

Aiming to identify new DNA-binding factors of ORC positioning that interact with ENY2, we carried out a yeast two-hybrid screen of the Drosophila cDNA library with an ENY2 fusion as a bait. A novel zinc-finger protein CG9890 was identified. The interaction of CG9890 and ENY2 was confirmed by co-immunoprecipitation. We carried out ChIP-seq analysis of the CG9890 distribution in the *Drosophila* genome and found its preferential localization to the promoters of annotated genes. We found a significant enrichment of the ORC, SAGA and SWI/SNF subunits on CG9890 binding sites. To confirm physical association of Su(Hw) with SAGA, SWI/SNF and ORC in vivo, we performed co-immunoprecipitation of the nuclear extract from Drosophila embryos. The anti-CG9890 antibody precipitated CG9890 and co-precipitated subunits of these complexes. These results indicate that CG9890 do interact *in vivo* with SAGA, SWI/SNF and ORC complexes.

It was relevant to find out whether CG9890 genome localization is mediated by its own ability to bind DNA. To do this, we applied DIP-qPCR (DNA-immunoprecipitation followed by qPCR) technique and demonstrated that CG9890 is capable of DNA binding in a sequence-specific manner. Using various bioinformatics approaches, we identified a DNA motif that is enriched on CG9890 binding sites. We

showed that the CG9890 protein recognizes this motif and this recognition accounts for its ability to bind DNA in the DIP-qPCR experimental system.

Future experiments, elucidating the effects of CG9890 knockdown on the SAGA, dSWI/SNF and ORC complexes recruitment, are crucial to further clarify the role of this protein in coordinated positioning of the replication and transcription machinery at the subset of *Drosophila* promoters.

RNA-binding proteins of CPEB family in gene expression regulation

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Cytoplasmic Polyadenylation Element Binding (CPEB) proteins are translational regulators that can either activate or repress translation depending on the target mRNA and the specific biological context. There are two CPEB subfamilies and most animals have one or more genes from each. Our laboratory studies CPEB of Drosophila. Drosophila has a single CPEB gene, orb and orb2, from each subfamily. Orb expression is only detected at high levels in the germline and has critical functions in oogenesis but not spermatogenesis. By contrast, orb2 is broadly expressed in the soma; and previous studies have revealed important functions in asymmetric cell division, viability, motor function, learning, and memory.

Orb2 is also expressed in the adult male germline and that it has essential functions in programming the progression of spermatogenesis from meiosis through differentiation. Amongst the orb2 differentiation targets are orb and two other mRNAs, which are transcribed post-meiotically and localized to the tip of the flagellar axonemes. Additionally, analysis of a partial loss of function orb2 mutant suggests that the orb2 differentiation phenotypes are independent of the earlier arrest in meiosis.

Orb2 is required to properly localize and activate the translation of apkc mRNAs in polarizing spermatid cysts. orb2 functions not only in orienting cyst polarization with respect to the apical-basal axis of the testis, but also in the process of polarization itself. One of the orb2 targets in this process is its own mRNA. Moreover, the proper execution of this orb2 autoregulatory pathway depends upon apkc.

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Poly(A)-binding protein – interacting protein 2 (paip2) participates in sequentialevents of mRNA biogenesis

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Poly(A)-binding protein – interacting protein 2 (paip2) represses mRNA translation in cytoplasm. It was shown to be important in regulation of synthesis of specific key proteins in spermatogenesis, as well as in regulator of long-term synaptic plasticity in mouse. We study function of this protein in Drosophila. Our data indicate that paip2 is involved in mRNA biogenesis starting from its synthesis in cell nucleus. Using several techniques we have detected paip2 on early stages of mRNA synthesis. Thus, molecular functions of this factor is wider that it was known previously.

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ORC interacts with TREX-2 and promotes Nxf1 association with mRNPand mRNA export in *Drosophila*

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The Origin Recognition Complex (ORC) of eukaryotes associates with the replication origins and initiates the pre-replication complex assembly. We have purified Drosophila TREX-2/AMEX general mRNA export complex by the methods of conventional chromatography. Our results revealed that TREX-2 is associated with a fraction of origin recognition complex (ORC). The results of pull-down assays confirm the interaction between TREX-2 and ORC, suggesting that it is likely to involve several subunits of these complexes. Thus, Xmas-2, the scaffold subunit of TREX-2, interacts with the Orc3 and Orc6 subunits of ORC, ENY2 has been shown to interact with Orc3 and Orc6. The PCID2 subunit associated with N-terminal region of Xmas-2 interacts with Orc4, in addition to Orc3. The results of co-immunoprecipitation and pull-down experiments indicate that Orc3 subunit of ORC complex which is likely to be more strongly involved in the interaction with TREX-2. Double immunostaining with antibodies against Orc3 or Orc4 and Xmas-2 demonstrated that the pattern of ORC subunits significantly overlapped with that of Xmas-2 both inside the nuclei and at their periphery. The similar result was observed for ORC subunits and Nxf1 nuclear export receptor. Such a result indicated that some ORC is colocalized with mRNA export factors in the nucleus.

RNA immunoprecipitation (RIP) was performed from the nuclear extract of *Drosophila* S2 cells prepared in the presence of RNase inhibitors, as described. In experiments with antibodies against ORC subunits a high level of coprecipitation was observed for Orc3, Orc4, and Orc5, providing evidence for the interaction of ORC and mRNP complex.

We performed the RNAi knockdown of Orc5, since this subunit is required for the proper assembly of the ORC complex. The overall level of Orc5 in cell decreased up to three times that has led to 60% decrease of Orc5 association with *ras2* mRNA assayed by RIP. In line with this about twofold decrease in the association of Orc3 and Orc4 association with the *ras2* mRNA was also observed.

Next, the role of TREX-2 in the ORC–mRNP interaction was studied using the RNAi knockdown of Xmas-2, the central TREX-2 subunit. This treatment did not change the overall level of ORC subunits in the cells, but it strongly interfered with the association of Orc3 and, to some extent, Orc4 and Orc5 with *ras2* mRNA, indicating that TREX-2 is at least partially involved in the mRNP–ORC interaction.

Since ORC interacts with TREX-2, it is conceivable that it also associates with some other components of the mRNA export machine. Interestingly, we observed that Orc3 was co-precipitated by antibodies against Nxf1 from *Drosophila* embryo extract. In pull-down assay with the anti-HA or anti-FLAG antibodies, Nxf1 co-precipitated with Orc3 but not with Orc5, suggesting that Orc3 and Nxf1 directly interact with each other. We investigated the influence of ORC depletion on the Nxf1 interaction with mRNP complex. An RNAi knockdown of Orc5 was performed. RIP experiments showed that this treatment resulted in a significant (60%) drop in the association of Nxf1 with the *ras2* mRNA.

Also, we showed, that RNAi ORC subunits disturbs mRNA export of mRNA from nucleus to cytoplasm. RNAi knockdown of Orc3 and Orc5 was performed, and the numbers of cells having mRNA export defects were scored and compared with the control (Xmas-2 RNAi). The proportion of affected cells in the positive control reached up to about 80%, as observed previously, and it also proved to be high (about 60%) after Orc3 or Orc5 RNAi knockdown.

Thus, we demonstrate that in *Drosophila,* ORC interacts with the mRNA export machinery and promotes the assembly of the mRNA export complex. A significant fraction of ORC directly binds to the TREX-2 general mRNA export complex and this interaction is essential for the ORC recruitment to mRNP. The Orc3 subunit directly interacts with Nxf1, a general mRNA export receptor, and ORC depletion disrupts the association of Nxf1 with mRNP, thereby interfering with mRNA nuclear export.

Identification of long-distance interactions mediated by Homie insulator and core sequences responsible for insulator proteins binding to it in *Drosophilamelanogaster*

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Eukaryotic genomes are organized into a series of structurally and functionally autonomous domains. This subdivision depends upon a special class of regulatory elements called insulators or boundaries. Insulators were initially identified on the basis of their ability to block communication between enhancers and a target promoter when interposed between them. Additionally, many of the known insulators display barrier activity that enables them to protect genes against heterochromatic silencing by stopping the spread of heterochromatin.

The even skipped (eve) locus is a well-defined Pc domain based on genome-wide analysis, and is regulated by PcG genes. An insulator flanks its well-characterized regulatory region, which includes the eve PRE at its 3' end. Thus, this insulator is in a position to separate both positive and negative eve regulatory elements from the constitutively expressed neighboring gene TER94, and/or to prevent ectopic activation of eve by TER94 enhancers. This insulator was shown to have 3 distinct activities in model transgene assays. In addition to enhancer blocking, it causes homing of P-element transgenes to the endogenous eve neighborhood, for which it was nicknamed Homie (Homing insulator at eve). Furthermore, from within a several megabase region flanking endogenous eve, it causes long-range interactions of transgenic promoters with endogenous eve enhancers. Genome-wide analysis showed that most known insulator proteins bind to the Homie region.

The activities of insulators have been extensively documented. On the other hand, not much is known about the mechanisms responsible for their different activities. With aim of gaining some additional insight into how these elements function, we will try to clarify details of intramolecular interactions mediating assembly of Homie insulator by finding the minimal regions that mediate interaction of Homie with proteins and check the model of chromosomal looping by visualization of this process in the living cell.

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Role of boundaries in regulation of the Abd-B gene

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Boundary elements have been found in the regulatory regions of the Drosophila melanogaster abdominal-A (abd-A) and Abdominal-B (Abd-B) genes, which is subdivided into a series of iab domains. The Mcp, Fab-7 and Fab-8 boundaries showed enhancer blocking activity in transgenic lines. Previously, using the inability of the yeast GAL4 activator to stimulate the white promoter when its binding sites are separated by a 5-kb yellow gene, we found that the Fab-6, Fab-7 and Fab-8 boundaries interact with each other and the regulatory region located upstream of the Abd-B promoter. At the same time, the Mcp boundary involved in regulation of abd-A does not interact with Abd-B promoter region. Based on these results, the model was suggested that the boundaries have two activities: separate regulatory domains (barrier) and support specific distance interaction (communicator) between the iab enhancers and the appropriate promoters. Using transgenic lines, the enhancer blocking and communicator activities were mapped in 337 bp region of Fab-8 and in 858 bp region of the Fab-7 and 340 bp region of the Mcp. Next, we checked whether these elements can fulfill their activity in vivo. Indeed, the 337 bp Fab-8 and 858 bp Fab-7 can completely substitute functional activity of the 1.7 kb Fab-7. At the same time, the 340 bp Mcp insulator inserted instead of the 1.7 kb Fab-7 insulator displays only barrier activity. These results showed high correlation between results obtained in transgenic lines and in vivo.

Studying the role of factors gcl and SAYP in forming embryonic stem cells of *Drosophila*

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Embryonic stem cells possess unique properties, which make them an interesting object both for fundamental, and applied studies. We study the molecular role of two conservative factors, gcl and SAYP, in sustaining of the status of stem cells in Drosophila embryo. Presence of both proteins is important for normal development of stem cells in early Drosophila embryo. We investigate the effect of mutations in genes encoding gcl and SAYP on fate of these cells and looking for the signaling pathways mediating their role.

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Identification of DNA-binding partners of PcG proteins in *Drosophila*

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Gene repression by PcG complexes is required to control tissue and stage specific gene expression. In *Drosophila*, genes controlled by PcG proteins have PcG response elements (PREs) to which these proteins are recruited. However, the mechanism of PcG recruitment on PREs remains elusive. The important role in PcG recruitment is suggested for DNA binding factors. Indeed, a number of DNA-binding factors involved in PcG repression were found. However, the combination of DNA-binding sites for known factors fails to reconstruct functional PRE.

In this study, we performed an Y2H search for DNA-binding factors that can interact with components of PcG complexes. We identified several C2H2-type ZnF partners of PcG proteins. One of them is PRP1[Polycomb recruiter protein 1] protein that interacts with dSfmbt. We demonstrate that PRP1 protein colocalize with PcG proteins on *bxd* PRE. The domains important for interaction between PRP1 and dSfmbt were analyzed.

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SWI/SNF complexes of various subunit compositions participate at different stages ofgene transcription activation

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SWI/SNF chromatin remodeling complex participation in recruitment of the RNA polymerase II to the promoters of the genes was demonstrated in various organisms. Rather recently it was shown that SWI/SNF complex's influence on transcription is not limited to its role in initiation, but also includes participation at further stages, particularly elongation. In the current work we describe subunit composition of the SWI/SNF complexes participating in initiation, in preparations for the elongation and in the elongation of hsp70 gene transcription in Drosophila melanogaster. Obtained data indicate high mobility of SWI/SNF complex composition during hsp70 gene transcription process. We suggest a model describing the process of sequential SWI/SNF complex formation during the transcription of hsp70 gene.

Transcription of Yersinia pseudotuberculosis mcc-like operon

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Growing need of a new antibacterial drugs demands extensive research activity in a field of development of a new semisynthetic and synthetic antibiotic compounds. Those, in turn, are based on the studies of the synthesis, regulation and mechanism of action of natural antibiotics.

Microcin C (McC) is a small ribosomally synthetized post-translationally modified antibacterial peptide produced by *E. coli* strains harbouring a plasmid-borne *mcc* operon. McC is active against *E. coli* strains that lack the *mcc* operon as well as other enteric bacteria. *Mcc* gene cluster consists of six genes transcribed from two promoters: *mccA*, encoding the peptide part of antibiotic, as well as genes from *mccB* to *mccE*, responsible for both post-translational modifications and self-immunity, are transcribed from the single promoter P_{mcc} while *mccF* gene, responsible only for self-immunity to McC, is transcribed from the second promoter (fig. 1A). Transcription from a P_{mcc} promoter directs the synthesis of two transcripts: a longer minor transcript containing *mccA* and downstream ORFs and a short highly abundant (~20-fold) transcript containing the *mccA*. The short transcript is generated when RNA polymerase terminates transcription at an intrinsic terminator located in the intergenic region between the *mccA* and *mccB* genes.

Recently *mcc*-like operons were found in many bacterial species including both Gram-negative and Gram-positive bacteria as well as cyanobacteria. The structure of *mcc*-like gene clusters varies in the number of genes, in the position of each gene within the cluster, and in their putative function. The long intergenic region in *Yersinia pseudotuberculosismcc*-like gene cluster (fig. 2B) downstream of *ymccA* contains putative termination hairpin.

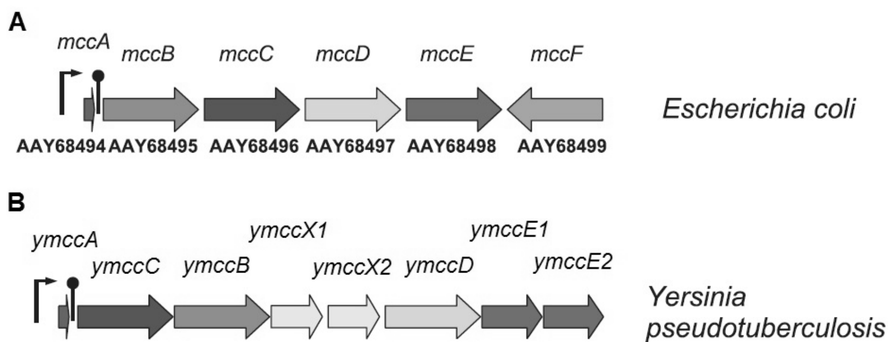

Fig. 1. Structural organization of *mcc*-like operons from *E. coli* (A) and *Y. pseudotuberculosis* (B). Genes are indicated by arrows whose direction refers to gene transcription. Homologous genes are shown in the same color. Transcription terminators are shown as hairpins. Figures under genes indicate NCBI Entrez numbers (Severinov et al., 2007).

We studied transcription of the *Yersinia pseudotuberculosis mcc*-like operon. Our results shows the presence of the short transcript containing *ymccA* ORF which indicates that the mechanism of transcription regulation in *Y. pseudotuberculosis* is similar to that in *E. coli*. The level of *Y. pseudotuberculosis mcc*-like operon gene expression depending of the growth phase was evaluated via RT-qPCR. We revealed that *ymccE2* gene, responsible for self-immunity, is expressed mainly in stationary phase while for other genes the level of expression depends on the growth phase to a lesser extent. The level of *ymccE2* expression is much higher in comparison to other genes. These results suggest the presence of an additional promoter located directly upstream of *ymccE2* gene. Probably, its biological role is to independently provide the immunity to external McC-like antibiotic. It's also worth mentioning, that heterological expression of *Y. pseudotuberculosis mcc*-like operon under control of the natural promoter in *E. coli* cells was activated only in *Δhns* strains.

Studying the mode of Elba functioning

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Elba is a protein factor mediating insulation in early Drosophila embryo. One reason why the Elba factor is active in early embryos but not later in development is that two of the genes encoding Elba proteins, elba1 and elba3, are only transcribed during the mid-blastula transition. However, it is possible that other mechanisms are also important in preventing this insulator complex from being active at other stages of development. We will study the issue using S2 cell culture, yeast 2-hybrid system and biochemical purifications. We will find the partners of Elba, mediating its recruitment onto chromatin and action in embryogenesis.

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Regulation of gene expression by insulator proteins

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Eukaryotic genomes are organized into a series of structurally and functionally autonomous domains containing individual genes together with their corresponding regulatory elements, clusters of co-expressed genes, or groups of genes that share common regulatory elements. This organization protects genes in one domain from adverse effects of regulatory elements in neighboring domains and is critically important for ensuring that eukaryotic genes are expressed in the appropriate cell types, developmental stages or even at the correct point in the cell cycle. This subdivision of the genome into discrete domains depends upon a special class of regulatory elements called insulators or boundaries. Insulators or insulator-like elements have been discovered in a wide range of organisms ranging from single cell eukaryotes like yeast and Plasmodium falciparum to complex multicelullar animals such as Drosophila, frogs, mice and humans

The activities of insulators have been extensively documented. On the other hand, not much is known about the mechanisms responsible for these different activities. With aim of gaining some additional insight into how these elements function, we will try to identify the proteins or proteins complexes that insulators interact with and recruit onto chromatin.

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Influence of expression E.coli mcC operon on hosts proteins' expression

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Microcins are a group of peptide antibiotics synthesized by *Enterobacteria* [1,2]. These compounds are produced under stress conditions and are effective against bacteria closely related to the producing strain. Microcins are characterized by their small size (<10 kDa) and synthesis on the ribosomes. One of them, microcin C is produced by *Escherichia coli* cells carrying plasmids with the *mccABCDEF* gene cluster. The peptide moiety of microcin C is encoded by *mccA*, a 21 bp-long gene [3]. The *mccB*, *mccD* and *mccE* genes are responsible for the synthesis of antibiotic. The products of *mccC*, *mccE* and *mccF* make cells immune to both endogenously produced and externally added microcin C [4]. The *mccABCDE* genes constitute an operon and are transcribed from a single promoter activated by nutrient deprivation [4,5]. The divergent *mccF* gene is transcribed separately and expressed constitutively [4, unpublished data].

It was shown previously in our laboratory that GadA protein level is significantly decreased in microcin C producing cells. GadA is known to confer resistance to extreme acid stress. Its absence in microcin C producing cells could indicate significant differences in intracellular acidity compared to non-producing cells. To address this we performed RNAseq analysis of cells carrying mcc plasmid grown on rich media (LB), comparing them with non-producing cells. We used the same strain but grown on LB media with addition of glucose as non-producing control. It was previously shown [5] that mccABCDE promoter activity is suppressed upon high glucose level.

RNAseq data analysis revealed differential expression of several gene groups between microcin C producing and non-producing cells. We observed differential expression of genes related to metabolism of sulfur (*suf*, *tau, cys*) and aspartate (*asn*) and also of *gad* genes, involved in intracellular pH regulation. However glucose itself influence expression of many genes and also is known to decrease intracellular pH. To distinguish the effects of glucose and microcin C synthesis we performed qPCR analysis of gene expression in the cells carrying a wider range of plasmids. Based on RNAseq data we chose 14 genes from different groups for analysis (*dppA, asnA, carB, bsmA, gadA, gadE, cysP, cysC, moaB, asnB, ansB, pyrB, aspA, iaaA*). We compared these genes expression between the cells, carrying the plasmid with deleted mcc

operon, with intact mcc operon or with mcc operon, carrying inactivating mutation of mccABCDE promoter. We also compared same strains grown on LB with or without glucose to address effects of glucose. qPCR data analysis revealed that *dppA, carB, bsmA, asnA, asnB, ansB, aspA, iaaA* are up-regulated and *gadA, gadE, cysP, cysC, moaB, pyrB* are down-regulated in microcin C producing cells.

Our qPCR data suggest that the pathway of intracellular pH regulation is switched in microcin C producing cells. There are two interconnected pH-maintaining systems in the bacterial cells, glutamate- and aspartate-dependent. According to our qPCR data, genes responsible for glutamate system activity (*gadA, gadE, pyrB*) are expressed at high levels in non-producing cells. In microcin C producing cells levels of these genes are decreased, whereas levels of aspartate-dependent system genes (*carB, asnA, asnB, ansB, aspA, iaaA*) are increased. These data suggest that unusual switch from glutamate-to aspartate-dependent pH maintenance system happens in microcin C producing cells. Possible function of this switch will be the subject of further investigation.

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SAGA complex participates in snRNA transcription in Drosophila melanogaster

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Using chromatin immunoprecipitation method we found that components of the complex SAGA - Ada2b, GCN5, Sgf11, ENY2 are present on snRNA genes, which are transcribed by both RNA polymerase II (snRNA U1, snRNA U2), and RNA polymerase III (snRNA U6). Enrichment for all components of the SAGA complex obtained on genes snU1 snU2 is about three times less than the values obtained on the snU6 genes.

Since the complex SAGA is currently known as transcriptional coactivator of the RNA polymerase II transcription, the question arose whether SAGA complex participate in the transcription by RNA polymerase III. To test this possibility, we performed chromatin immunoprecipitation reaction using antibodies against RNA polymerase II. We showed that RNA polymerase II is present on promoters of snU1 and snU2, but it is not on the genes of snU6, as expected.

To test the interaction between components of SAGA complex and RNA polymerase III machinery, we raised antibodies against Brf1, a component of the RNA polymerase III complex. The immunostaining of Drosophila larval salivary gland polytene chromosomes was performed using rabbit anti-Brf1 and mouse anti-Sgf11, and showed that Sgf11 and Brf1 bound to numerous interband sites, which was indicative of their association with actively transcribed chromatin.

Using chromatin immunoprecipitation method, we also showed that Sgf11 and Brf1 are located on the promoter region of the snRNA U6, and other promoters of RNA polymerase III-dependent genes. Antibodies against either Sgf11 Gcn5, ENY2 co-immunoprecipitated Brf1 from the cell extract of Drosophila S2 cells. Thus, SAGA complex is present on the promoter of RNA polymerase III - dependent genes and interacts with its transcription machinery.

To test the interaction of coactivator complex SAGA with snRNA transcription apparatus, antibodies against the component of the complex snRNA SNAP - Snap43 were obtained. The immunostaining of Drosophila larval salivary gland polytene chromosomes by Snap43 and Sgf11 antibodies showed that Sgf11 and Snap43

colocalized on actively transcribed chromatin. Using CHIP assay we also showed that Snap43 located on the promoter region of snRNA, as expected. Also, the subunits of SAGA complex Sgf11, Gcn5, ENY2 immunoprecipitated Snap43 from cell extract of Drosophila S2 cells. Thus, SAGA complex is not only present on snRNA promoters, but also interacts with the SNAP.

The SAGA complex is a coactivator of the transcription of many genes. In order to verify whether SAGA association influence on snRNA gene transcription was performed knockdown of some subunits of the SAGA complex: Nonstop, and Sgf11. Number snRNA transcripts in cells after RNAi knockdown of Nonstop and Sgf11 compared with the amount of transcripts in cells to which was added dsRNA GFP. RNAi knockdown of Nonstop and Sgf11 decreases the number of snRNA transcripts on average by 30%. Thus, we have shown that reducing the number of components of the SAGA complex in the cell leads to disruption of the transcription of snRNA.

Interplay of transcription factors SAYP, Brahma and Mediator on promoters

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Transcription coactivator SAYP has been previously described in our laboratory. This factor has been found to function in many tissues during all developmental stages of drosophila and has homologues among all metazoans. PHF10, the human homologue of SAYP, has been proved to be indispensable for cell proliferation and associated with tumorogenesis. Mutations in gene encoding SAYP lead to decrease in viability, malfunction of cell cycle and organs development (Shidlovskii et al., EMBO J., 24: 97-107, 2005). SAYP acts as a component of stable coactivator supercomplex BTFly which also includes chromatin remodeler Brahma and general transcription factor TFIID (Vorobyeva et al., Proc Natl Acad Sci U S A, 106: 11049-54, 2009).

Critical role of SAYP in JAK/STAT signaling pathway and ecdysone cascade activation has been proved (Vorobyeva et al., Cell Cycle, 10: 1821-7, 2011; Panov et al, Nucleic Acids Res., 40: 2445-53, 2011). According to our model of SAYP action, gene-specific activators bound to promoters interact with BTFly which in turn recruits RNA-polymerase II (Pol II). Moreover, SAYP is supposed to be a universal transcription factor for many signaling pathways.

We study interplay of SAYP, Brahma and Mediator on several model promoters and enhancers. We study interdependence in their recruitment onto active genes and their joint effect on gene expression.

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Analysis of *mcc*-like operonencoded in *Bacillus amyloliquefaciens* genome

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Microcin C (McC) is ribosomally synthesized peptide-nucleotide antibiotic produced by some strains *Escherichia coli* and active against closely related strains. McC is produced by cells, carrying *mccABCDEF* operon (**fig. 1A**) on a plasmid.

The heptapeptide precursor of McC is encoded by *mccA* gene. Products of *mccB*, *mccD* and *mccE* genes are responsible for McC maturation, *mccE* and *mccF* genes provide self-immunity against McC. Mature McC is exported outside the producing cell by *mccC* encoded pump. After import into sensitive cell, McC is processed by cellular peptidases with release of nonhydrolyzable aspartyladenylate analogue. This substance binds non-reversible to aspartyl-tRNA synthetase, resulting in translation inhibition [1].

Bioinformatic analysis revealed a set of *mcc*-like operons in a wide range of bacterial species, including gram-positive, gram-negative and cyanobacteria [2]. Here we analyze *mcc*-like operon encoded by the chromosome of gram-positive bacteria *Bacillus amyloliquefaciens* (**fig. 1B**). This operon lacks *mccD*, *mccE* and *mccF* genes, but encodes for an extended two-domain MccB form, BMccBX_0,and for an additional protein BMccX_1. BMccBX_0 and BMccX_1 modify 19-aminoacid peptide BMccA, making mature microcin C (BMcC).Also unlike *E. coli* operon, *BmccA* gene is located in the middle of the operon, preceding *BmccC*. *BmccA* encodes for a precursor peptide and should be expressed in high levels, so it is possible that *BmccA* expression is regulated by a separate promoter.

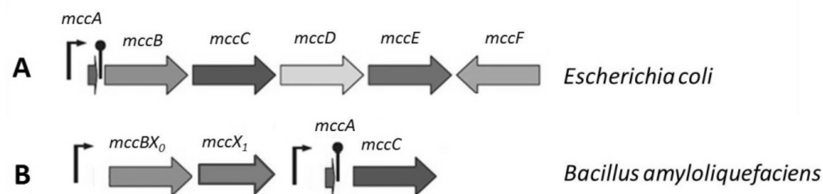

Fig.1. Structure of *E.colimcc* operon (A) and *Bacillus amyloliquefaciens mcc*-like operon (B).
Inverted repeats are shown by lollipop signs.

We were unable to detect *Bmcc* expression or BMcC synthesis using natural isolate of *B.amyloliquefaciens* containing single *Bmcc* copy on the chromosome. The

reason for this can be low *Bmcc* promoters activity in the conditions we tested. For further investigation of *Bmcc* operon closely related to *B.amyloliquefaciensB. subtilis* cells, carrying a multicopy pHT expression vector with *Bmcc* operon were used. In these cells we were able to detect production of BMcC (unpublished results).We performed extension of the primers complementary to *mccBX$_0$* and *BmccC* genes on RNA from BMcC producing cells. Thus we confirmed and mapped two promoters, one regulating expression of *BmccBX$_0$X$_1$* operon, and the other – of *BmccAC* operon.

There is an intergenic region with inverted repeat downstream *E. colimccA*. This region is important for effective McC synthesis. Our previous study shows that regulated transcription termination happens in this region, resulting in a very effective synthesis of a short transcript. This abundant and stable short transcript contains only *mccA* open reading frame, and its translation results in peptide precursor quantities enough to establish high-level McC production [3].

Bmcc operon also contains intergenic region with inverted repeat, located between *BmccA* and *BmccC* genes. Such operon structure suggests possibility of *mccA* expression regulation similar to that observed in *E. coli* operon. To address this we performed Northern-blot hybridization of RNA from BMcC producing *B. subtilis* cells with labeled oligonucleotide complementary to *BmccA* gene, and detected a short transcript, containing only *BmccA* gene. These results demonstrate similarity between mechanisms of *mccA* and *BmccA* expression regulation despite the differences in operon organization between *E. coli* and *B.amyloliquefaciens*.

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Studying the role of cis-regulatory elements in localization of mRNA in Drosophila spermatids

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Cell polarity is a common phenomenon, which plays an important role during ontogenesis. Establishing of cell polarity occurs via conservative mechanisms, which require proteins of PAR family. Asymmetrical mRNA localization is one of the ways to establish cell polarity.

Mature Drosophila sperm is a useful model for studying cell polarization. Sperm are highly polarized cells on one side is a nearly 2 mm long flagellar tail that comprises most of the cell, while on the other is the sperm head, which carries the gamete's genetic information. The polarization of the sperm cells commences after meiosis is complete and the 64-cell spermatid cyst begins the process of differentiation. The spermatid nuclei cluster to one side of the cyst, while the flagellar axonemes grows from the other. The elongating spermatid bundles are also polarized with respect to the main axis of the testis; the sperm heads are always oriented basally, while the growing tails extend apically. This orientation within the testes is important for transferring the mature sperm into the seminal vesicles.

We are studying the role of cis-elements in mRNA (CPEs), which are important for its localization during spermatogenesis. Using artificial constructs carrying different sets of CPEs in 3'-UTR, we establish their importance in the indicated process.

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Proper localization of aPKC mRNA is regulated by orb2 protein

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Orienting cyst polarization with respect to the main axis of the testis in Drosophila depends upon atypical Protein Kinase C (aPKC), a factor implicated in polarity decisions in many different biological contexts. When apkc activity is compromised in the male germline, the direction of cyst polarization within this organ is randomized. Significantly, the mechanisms used to spatially restrict apkc activity to the apical side of the spermatid cyst are different from the canonical cross-regulatory interactions between this kinase and other cell polarity proteins that normally orchestrate polarization. The asymmetric accumulation of aPKC protein in the cyst depends on an mRNA localization pathway that is regulated by the Drosophila CPEB protein Orb2.

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