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ORAL TALKS

POSTERS

Functional characterization of the conservative protein CG17337 in *Drosophila melanogaster*

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With the aim to understand the molecular mechanism of action of the Suppressor of Underreplication (SUUR) protein, which is known to modulate replication timing in *Drosophila* genome, we have previously used co-immunoprecipitation combined with mass spectrometry and identified the product of the ubiquitously expressed *CG17337* gene as one of putative SUUR partners. The function of CG17337 in *Drosophila* cells is so far unclear, but the protein might be a peptidase that is also involved in the carnosine pathway similarly to its human homologs CNDP1 and CNDP2. Here, we provide first insights on the role of the CG17337 protein in *Drosophila*. First, we have raised polyclonal antibodies specific to CG17337, which allowed us to visualize this protein in both cytoplasm and nucleus of cultured S2 cells. However, the nuclear localization of CG17337 was observed only in a fraction of analyzed cells suggesting its cell cycle-dependent manner. Indeed, we found that the CG17337 and PCNA (a marker of S-phase) proteins demonstrate almost complementary patterns of nuclear staining in S2 cells. Next, we demonstrated that RNAi-depletion of CG17337 in S2 cells leads to increase of proportion mitotic cells (the mitotic index was increased up to 1.4-fold) and accumulation of the Cyclin A and Cyclin B proteins. On the other hand, treatments of S2 cells by different reagents to arrest them at different stages of cell cycle did not result in any effect on the total amount of CG17337 protein. Then, we have detected the cell cycle-dependent association of the CG17337 protein with larval salivary gland polytene chromosomes, but its binding pattern is different from that of SUUR. After that, we found that CG17337 is able to interact *in vitro* with SUUR and the known partners of the latter, histone H1 and PCNA. Finally, using CRISPR/Cas-mediated homologous recombination, we generated null mutant of the *CG17337* gene, which appeared to be homozygous viable, but demonstrates about 2-fold increase of the mitotic index in larval brain cells and is characterized by a prolonged period of female fertility. Altogether, our results indicate that *Drosophila* CG17337 protein is a component of chromatin and cytoplasm and is necessary for proper mitosis progression.

The work was supported by RFBR grant #16-04-01598.

Functional dissection of Drosophila melanogaster SUUR protein influence on H3K27me3 profile

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During chromosome replication covalent histone modifications should be duplicated to ensure the proper gene regulation in daughter cells. Considering the diversity of histone marks discovered to date, reestablishment of chromosome domains could be achieved by different mechanisms. Indeed, the kinetics of different histone marks at the replication fork varies in a wide range. Molecular mechanisms that ensure reestablishment of different histone modifications during or after replication remain poorly understood. Existing evidence suggests that SUUR gene discovered in Drosophila contributes to the renewal of repressive histone marks during the heterochromatin replication. SuUR mutation makes heterochromatin replicate earlier than in wild type but reduces the level of repressive histone modifications. SUUR protein was shown to be associated with moving replication forks, apparently through the interaction with PCNA. The biological process underlying the effects of SUUR on replication and composition of heterochromatin remains unknown. We performed a functional dissection of SUUR protein effects on H3K27me2/3 level. Using Hidden Markow Model-based algorithm we revealed SuUR-sensitive chromosomal regions that demonstrated unusual characteristics: they do not contain Polycomb and require SUUR function to sustain H3K27me2/3 level. We tested the role of SUUR protein in the mechanisms that could affect H3K27me2/3 histone levels in these regions. We found that SUUR does not affect the initial H3K27me2/3 pattern formation in embryogenesis or Polycomb distribution in the chromosomes. We also ruled out the possible effect of SUUR on histone genes expression and its involvement in DSB repair. Obtained results support the idea that SUUR protein contributes to the heterochromatin maintenance during the chromosome replication. A model that explains major SUUR-associated phenotypes is proposed.

Evolutionary analysis of 3-dimentional genome architecture

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Availability of high-resolution genome-wide methods allows studying 3-dimentional genome architecture of multiple species across the tree of life. Several pioneer researches comparing 3D genome organization of closely related species (e.g. mammalians) or evolutionary distant species (e.g. mammalians and insects) describe both shared and taxon-specific principles of genome organization. We have generated of several Hi-C datasets from different taxa of animals: mammalians (mouse fibroblasts and sperm cells), vertebrates (chicken fibroblasts and erythrocytes) and invertebrates (several species of Anopheles genus and an insect *Polypedilum vanderplanki*). We have found similar features in all investigated species: genome compartmentalization; areas of local chromatin contacts enrichment; exponential dependence of DNA-DNA contacts frequencies from distance between loci in linear molecule. However, on a finer scale each species shows its specific contacts pattern. For instance, mouse cells harbor topologically associated domains (TADs), which often appear to be conserved between different cell types. In chicken TADs exist and are often located in accord with mammalian TADs, but show different size comparing to mammals. Moreover, TADs disappear in mature and immature chicken erythrocyte. In insects, TADs are much smaller than in vertebrates, and according to recently published data, may be formed by different mechanisms.

To support further analysis of species-specific changes in contact frequencies, we propose a novel computational approach of between-species contact frequencies comparison.

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The role of *pendolino* (*peo*) in the epigenetic regulation of *Drosophila* telomeres

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Drosophila telomeres are capped by the terminin complex that localizes and function exclusively at telomeres, and by a number of non-terminin proteins that do not serve telomere-specific functions. *pendolino* (*peo*), encodes a non-terminin protein homologous to the E2 variant ubiquitin-conjugating enzymes. *peo* mutants exhibit frequent telomeric fusions (TFs) that preferentially involve the heterochromatin-associated telomeres (the Y, XR and 4th chromosome telomeres), a TF pattern never observed in any of the other telomere capping mutants so far characterized. In *peo* mutants both DNA synthesis and PCNA recruitment are compromised, and Peo physically interacts with SuUR that negatively regulates DNA replication in heterochromatic regions. Mutations in *SuUR* lower the frequency of TFs in a *peo* mutant background, suggesting that the fusigenic lesions in *peo* telomeres are generated by defects in DNA replication. We also found that mutations in *peo* strongly reduce di- and tri-methylation at lysine 9 (K9) of histone H3 in both heterochromatin and telomeres, and that mutations in *Su(var)3-9* dominantly enhance the TF frequency in *peo* mutants. This effect is highly specific, because mutations in the other *Drosophila* HMTases coding genes, *G9a* and *eggless* (*egg*), do not genetically interact with mutations in *peo*. Finally, our preliminary data suggest that mutations in *peo* lead to a reduction in histone ubiquitination, a post-translational modification that is known to regulate histone methylation. Collectively, our results suggest that when reduction of K9H3 methylation at *Drosophila* telomeres falls below a critical threshold, telomeric DNA replication is severely disturbed resulting in the formation of fusigenic lesions at chromosome ends. Because methylated H3K9 is particularly enriched in heterochromatin, we also envisage that heterochromatin-associated telomeres accumulate more fusigenic lesions than their euchromatin-associated counterparts. We will also present some recent studies on the biological roles the human and mouse orthologues of *peo* (*AKTIP* and *Ft1*), which are involved in telomere maintenance and aging.

Untangling spatial genome organization: detailed insights from single-cell DamID

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The eukaryote genome is tightly organized to fit within the confines of the nucleus. This organization is non-random and accumulating evidence indicates that the positioning of genomic regions within the three-dimensional space of the nucleus contributes to the regulation of gene activities. The nuclear lamina (NL), situated at the nuclear periphery, provides a large nuclear scaffold that is believed to aid the spatial organization of genomic regions. Genome-wide profiling has revealed that ~35% of the genome of different species associates with the NL in large domains of transcriptionally inactive chromatin. These lamina-associated domains (LADs) are variable between cell types in accordance with lineage-specific gene expression signatures. Hence, insight in LADs is key to understanding how phenotypic variation arises from the heritable acquisition of cell type-specific gene expression programs during lineage specification events. The first lineage specification event in development, involves the commitment of cells to either the extraembryonic tissue (tropectoderm), or the embryo proper (ICM). However, due to the limited cell material, little is known about the spatial organization of the genome at the different cleavage stages and the role LADs may play in directing lineage specification events. To obtain detailed insight in LAD-organization during preimplantation development, we have applied the previously developed single-cell DamID (sc-DamID) technique to profile LAD-organization during the first cleavage events of preimplantation development. Here I will discuss our preliminary findings of these experiments, and in addition present an extensively modified version of the sc-DamID that allows to obtain simultaneous measurements of LADs and transcriptomics genome-wide from the same single cell.

Alteration of rRNA expression levels in patients with intellectual disability and enlarged p-arms on acrocentric autosomes

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Intellectual disability (ID) is an important medical and social problem that can have different causes. One genetic factors connected to ID could be rDNA amplification and changes in rRNA expression and maturation. We described a family case of abnormal chromosome 13 with an enlarged p-arm and rDNA amplification, which was inherited by an ID child from his healthy father. Therewith, the child had essentially higher rRNA expression levels, but both his healthy parents revealed those not exceeding corresponding levels in a healthy reference control with normal karyotype. Further investigation in ID patients and healthy people showed elevated rRNA expression and shifted 28S/18S ratio in ID patients who had NOR-bearing chromosomes with enlarged p-arms compared to healthy donors, therewith, frequencies of NOR-bearing chromosomes p-arms enlargements did not differ between ID patients and healthy people. These results could point on disrupted regulation of rRNA expression and processing in ID patients.

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Large fraction of heterochromatin in *Drosophila* neurons is simultaneously bound by B-type lamin and HP1a

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In most mammalian cell lines, chromatin located at the nuclear periphery is condensed, according to both microscopy observations and mapping of lamina-associated domains (LADs), and is enriched in dimethylated Lys9 of histone H3 (H3K9me2). On the contrary, LADs do not significantly overlap either with H3K9me2 or with Heterochromatin Protein 1a (HP1a) domains in cultured Kc167 cells, which are the only *Drosophila* cell type with mapped LADs. Here, we used inducible DamID systems to perform genome-wide mapping of LADs, HP1a and Pc domains in the central brains, Repo-positive glia and Elav-positive neurons of the central brains, as well as in fat body of *Drosophila* third instar larvae. We found that LADs, HP1a and Pc domains correspond to silent or low-expressed genes, and that genes residing in the HP1a-bound LADs are expressed at the lowest level. Yet, a fraction of HP1a domains harbors actively expressed genes. The inter-LAD regions that are shared by all studied cell types are enriched in ubiquitously expressed genes, whereas the conserved LADs are less abundant than in mammals. Importantly, in the central brains and in neurons, which constitute the majority of cells in the brains, we found strong overlap of HP1a domains with LADs both in the chromosome arms and in pericentromeric regions. Consistent with these findings, centromeres appear to reside closer to nuclear lamina in neurons than in Kc167 cells. Overall, the results imply that mechanisms of heterochromatin compaction and attachment to nuclear lamina may be similar in *Drosophila* and mammals.

The experimental work of the study was partially done in the laboratory of Prof. B. van Steensel at the Netherlands Cancer Institute (Amsterdam, the Netherlands).

How our clocks are evolving? Latitude-driven polygenic adaptation in favor of evening preference might occur by small allele frequency shifts spread across numerous loci in different circadian genes on different chromosomes

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The mechanism of the molecular circadian clocks is currently understood as a transcription/translation feedback loop involving more than ten genes on different chromosomes. Genetic variation in these genes might be shaped by adaptation to environmental factors. To examine whether the out-of-African dispersal of our species to higher latitudes of the Eurasian continent led to significant shifts in allele frequency at polymorphic loci in circadian genes, 559 polymorphisms in 10 such genes were analyzed. Polymorphisms in "athletes' genes" served as controls. Additional analyses included 26 polymorphisms in the same 10 circadian genes for which positive vs. negative findings on their association with morning-evening preference were reported in candidate gene studies by our and other groups. The results suggested that the out-of-African expansion seemed to be accompanied by the latitude-driven polygenic selection against morning preference that occurred by small allele frequency shifts spread across many loci in circadian genes on different chromosomes.

The *Su(var)* gene complement and new chromatin functions in Drosophila

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Heterochromatin is important for genome integrity, maintenance of cell identity and stability of gene expression programs. During the last 15 years several epigenetic factors controlling heterochromatin formation and maintenance have been described. In particular, screens for *Su(var)* mutations of PEV (position effect variegation) in Drosophila have proven instrumental in identifying heterochromatin components conserved in higher eukaryotes. However until now only 25% of the about 100 *Su(var)* loci have been molecularly defined.

For systematic genetic and molecular dissection of unknown *Su(var)* genes we isolated more than 300 new EMS induced *Su(var)* mutations in sequenced isogenic backgrounds allowing a genome-wide sequencing approach allowing rapidly mapping of novel *Su(var)* genes.

First, complementation analysis allowed identification of new alleles for known SU(VAR)s including the H3K9 methyltransferase SU(VAR)3-9, the H3K9me2/3 binding protein SU(VAR)2-5 (HP1), the H3K4 demethylase SU(VAR)3-3 (LSD1) and the SU(VAR)3-7 zinc finger protein. Sequence analysis identified interesting new mutations including the first point mutation within the SU(VAR)3-9 chromo domain.

Combining genetic complementation and mapping analysis with genome-wide sequencing, caps marker mapping and candidate gene analysis allowed identification of more than 15 novel *Su(var)* genes. These genes encode factors involved in a plethora of molecular and epigenetic processes including cell signaling, control of histone and protein modification, a new central regulator of genome wide histone deacetylation, several band and interband specific proteins as well as functions putatively involved in RNA dependent processes.

Most of the *Su(var)* mutations were selected in a sensitized genetic background allowing identification of mutations modifying different alternative silencing processes. Analysis of their effect on a series of PEV rearrangements revealed significant differences in epigenetic control of alternative silencing processes within the Drosophila genome.

Identification of novel *Su(var)* genes will not only contribute to further understanding of the molecular basis of heterochromatin and gene silencing in Drosophila because many of the newly characterized pathways might also operate in higher eukaryotes.

The role of the *ADAMTS1, THBS1, WHSC1, RBFOX2* genes in the radiation-induced human somatic cellular response to DNA damage

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Despite the direct mechanisms of DNA repair are largely known, the role of other indirect participants of the radiation-induced cell response in maintaining the stability of the genome is still insufficiently explored. Our microarray analysis of gene expression on human peripheral blood lymphocytes allowed to reveal the correlation between the level of spontaneous γH2AX foci and the frequency of radiation-induced micronuclei and the expression of *ADAMTS1*, *WHSC1*, *THBS1* and *RBFOX2* genes, which was subsequently confirmed in extraembryonic mesoderm fibroblasts. Mutations of these genes in HeLa cell lines created using the CRISPR/Cas9, lead to changes in clonogenic survival, levels of spontaneous and radiation-induced γH2AX foci and the histone H3K27 and H3K9 methylation levels. Thus, genes affecting the efficiency of DNA double-strand break repair and cell survival were revealed. Perhaps, this effect is realized by epigenetic mechanism through a change in the level of H3 histone methylation.

The interplay between chromosome-nuclear envelope attachments and chromosome-chromosome contacts

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Spatial organization of chromosome territories is important for maintenance of genomic stability and regulation of gene expression. However, the interplay between interactions of chromosomes with the nuclear envelope and with each other is not well understood. We used a combined experimental and computational approach to study the effects of chromosome-nuclear envelope attachments on the 3D genome organization of *Drosophila melanogaster* salivary gland nuclei. We investigated if and how the presence and the number of chromosome-nuclear envelope attachments affect several key characteristics of 3D genome organization: chromosome territories and chromosome-chromosome contacts. Our results demonstrate that chromosome-nuclear envelope attachments increase the probability of intra-chromosomal contacts and decrease the probability of inter-chromosomal contacts. We also conducted a thorough analysis of tissue-specific features of the X chromosome and nucleolus nuclear position in follicular epithelium and nurse cells of the malaria mosquito *Anopheles atroparvus* and chromosome organization in nurse cells and salivary glands of *An. gambiae*. The analysis in *An. atroparvus* was conducted with TANGO — a software for a chromosome spatial organization analysis. We show that the volume and position of the X chromosome have tissue-specific characteristics. The position of the X chromosome changes with respect to the nuclear envelope in the process of nucleus growth: it moves toward the nuclear interior in follicular epithelium and toward the nuclear periphery in nurse cells. We used oligonucleotide probes to study three-dimensional aspects of chromosome organization in the polytene nuclei of *An. gambiae* ovarian nurse cells and salivary glands. We found the reverse relationship between the frequency of nuclear envelope attachments and the frequency of inter-chromosomal contacts in salivary glands and nurse cells. For example, arms 2L and 3R have more interactions with the nuclear envelope in salivary glands than in nurse cells. The same arms interact with each other less frequently in salivary glands than in nurse cells. It is likely that a higher number of nuclear envelope attachments in salivary glands limits the inter-chromosomal contacts in this cell type. We propose that chromosome-nuclear envelope attachments may facilitate or prevent tissue-specific chromosome-chromosome contacts where actively transcribed genes may co-localize and share sites of transcription.

Identification of loss of heterozygosity in miscarriages from families with recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is defined by two or more failed clinical pregnancies and affects 3–5% of women. In this study, we investigated the loss of heterozygosity (LOH) in 16 paired first-trimester miscarriages with normal karyotypes from 8 women with RPL. Search of LOH was performed using microarray SurePrint G3 Human CGH+SNP 4 × 180K (Agilent). A total of 30 regions with LOH were found in 10 miscarriages. Several LOH were observed in more than one embryo: four LOH were found in two embryos and one LOH – in three embryos. One of the mechanisms of implementation of the negative effect of LOH may be the presence of imprinted genes. In regions with LOH identified, there were five genes, predicted to be imprinted according to the Geneimprint Database: *OBSCN*, *HIST3H2BB*, LMX1B, *CELF4*, and *FAM59A*.

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Endoplasmic reticulum and mitochondria behavior during *Drosophila* S2 cell mitosis

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Mitosis is a highly dynamic process involving constant moving, multiplication and reorganization of different cellular organelles. One of its major tasks is to equally distribute DNA material as well as other cytoplasmic constituents among dividing cells. Studying the organelle inheritance and their distribution during different stages of mitosis is of great interest since any distortion in this process might lead to severe consequences. Besides the enormous amount of information about chromosomes distribution, there are not so many data describing organelle movement during cell division at ultrastructural level. *Drosophila* S2 cell culture represents a very useful model for investigating this subject.

With the help of transmission electron microscopy, we described in details the dynamics of endoplasmic reticulum (ER) membranes and mitochondria localization at different stages of S2 cell mitosis. We showed that ER forms a unique quadruple membrane structure around the nucleoplasm at early prometaphase, which presumably serve as a protective layer to keep chromosomes' area free of organelles. We observed dissociation of this structure at late prometaphase and formation of ER stacks around the nucleoplasm. Quantification of ER membrane length revealed that in comparison to early stages of mitosis there is a 1.5 fold increase in membrane content at metaphase and anaphase stages, which correlates with cell requirements to build new plasma membrane after division.

Mitochondria were always observed outside of the nucleoplasm during early prometaphase whereas seen also in nucleoplasm area starting at late prometaphase. This data indicated the transition from closed to more open spindle envelope in S2 cells. We also deciphered tight association of mitochondria with ER membranes at late prometaphase until telophase, which most likely maintain their inheritance during cytokinesis.

In general, our results demonstrated a highly dynamic nature of ER and mitochondria behavior in S2 cell mitosis and deciphered the formation of unique quadruple membrane structure at early prometaphase. It is of great interest in the future to compare the data obtained in this study on other animal cell cultures and in vivo models.

DNA methylation in inherited copy number variations with incomplete penetrance

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Chromosomal diseases caused by inherited copy number variations (CNVs) are frequently characterized by incomplete penetrance, but its mechanisms remain unexplored. Transgenerational epigenetic modifications may be one of such mechanisms. Here, we report about DNA methylation of several gene promoters in families with inherited CNVs. We have analyzed seven probands with intellectual disability using aCGH with subsequent analysis of identified CNVs by real-time PCR and bisulfite NGS of CpGs within CNV regions. For all analyzed genes (*SMCHD1*, *METTL4*, *CNTN6*, *GEMIN4*, *ACAD10*, *ASTN1*, *GRPEL2*), the DNA methylation in CpG-islands differed <10% between probands, healthy sibling without microduplication and parents. However, several genes (*SMCHD1*, *CNTN6* and *ASTN1*) had higher differences (>10%) between parents and proband outside the CpG-islands. Therefore, DNA methylation of genes may be the cause of their incomplete penetrance in some inherited CNVs.

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DNA damage in uncharted waters of M phase

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DNA damage is widespread and perpetual. Eukaryotic cells possess multiple extensively studied systems for DNA repair, damage response, and damage tolerance operating in G_0/G_1, S and G_2 phases of the cell cycle. In contrast, very little is known about the effects of DNA damage after the cell entry into mitosis. Here we summarize the current status of knowledge about various kinds of DNA damage in condensed mitotic and meiotic chromosomes. Components of condensed chromosomes, such as condensins, cohesins and SMC5/6 complex, are also involved in DNA repair in the interphase. Checkpoints in the process of spindle assembly are used to prevent DNA lesion passing into daughter cells. Extensive damage to genomic DNA leads to controlled separation of the damaged DNA into micronuclei. On the other hand, in cancer cells the mitotic DNA damage response pathways are subverted to propagate structural and numerical chromosomal instabilities.

New pathway of cytosine demethylation based on off-target base excision repair

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Cytosine methylation in CpG dinucleotides is a well-known way of gene activity regulation at the level of transcription, forming the basis of many cases of epigenetic inheritance, the transfer of information on the gene activity from parent cells or organisms to their progeny. Although a large body of literature is devoted to the appearance of epigenetic methylation marks in DNA, much less is known about their removal. We have shown that in the course of OGG1-initiated repair of oxoGua, APEX1 can remove mCyt 5' to the oxoGua base from DNA. To unequivocally establish that a new pathway of active demethylation may be based on such non-targeted DNA, using substrates mimicking various repair intermediates (AP sites, gap, etc.) we have characterized the ability of the human BER nuclease APEX1 to remove mCyt from DNA.

Genome-wide analysis of chromatin position effects in cultured *Drosophila* Kc167 cells

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To reveal the influence of local chromatin environment on gene expression level in *Drosophila*, we adopted the recently developed TRIP (Thousands of Reporters Integrated in Parallel) approach for cultured Kc167 cells. The approach is based on simultaneous integration of multiple DNA-barcoded reporter constructs at random positions in the genome of cultured cells. Typically, each individual cell in the obtained transgenic pool carries a few integrated reporters; however the analysis of the entire pool can provide information about genomic location and transcriptional activity of several thousand integrated reporters. The presence of the DNA barcodes allows tracing all these reporters at once without the need to establish enormous number of stable transgenic cell lines each bearing a unique transgene. First, we found optimal conditions for effective transgenesis of cultured Kc167 cells with piggyBac transposon-based transgenes. We used temporary activated tamoxifen-regulated form of the piggyBac transposase to minimize the translocation of once integrated in the genome barcoded reporters to other genomic loci. Such conditions ensure that each individual DNA barcode will be associated with a unique genomic location. Next, we selected several constitutive and inducible promoters of *Drosophila* genes for the TRIP analysis and characterized their episomal activity in cultured Kc167 cells. Then, we prepared a set of piggyBac transposon-based plasmid reporter libraries encoding eGFP under the control of the chosen promoters. Each of these constructs contains additional DNA tag ("promoter index") next to the barcode, which identifies the cloned promoter sequence. After that, using an equal molar mix of these plasmid libraries, we established transgenic Kc167 cell pools each carrying about 7000 integrated reporters. Finally, we have prepared DNA samples and subjected them to Illumina high-throughput sequencing to identify genomic location of each integrated reporter and measure its transcriptional activity.

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