

The aim of the project is the verification of the working hypothesis that lamins and topo II participate in epigenetic regulation of transcription in model system of heat shock induction and *Drosophila melanogaster* as an animal model system. In fruit fly heat shock induction results in total transcription shutdown of all loci except 6, so called heat shock loci which get highly activated for transcription. This gives us an unique opportunity to analyse the global changes in transcription and associated mechanisms in which lamins and topo II proteins (as we believe) take part and simultaneously to analyse particular activated and silenced loci on polytenic chromosomes of salivary glands of the third instar larvae. Our working hypothesis was formulated on our experimental data both already published and unpublished preliminary data. Our preliminary data indicated that heat shock induction dramatically increase the topo II binding to chromatin (DNA, RNA and histones) – about 6-7 times.

Lamin Dm increased binding to chromatin about 3 times. Both proteins revealed preferences in relocation to AT-rich sequences. This is in full agreement with previous published reports that heat shock induces new binding sites in vivo for topo II. All of four identified heat shock sites had an AT-rich context. Our unpublished data suggest that most of topo II protein and lamin Dm relocates during heat shock suggesting that systemic reorganization of karyoskeleton structure during heat shock.

We previously reported that regulation of lamin function such as polymerization and chromatin binding depends upon specific phosphorylation. Phosphorylation also regulates topo II chromatin binding, solubility and activity.

The major aim of the project is to verify a working hypothesis that lamins and topoisomerase II plays a role in epigenetic regulation of transcription during heat shock in *Drosophila* model system. The aim will be reached through several partial tasks.

The first aim of the project is to address the question of direct interaction between lamins and topo II protein and identification of other potential protein complex components associated with each protein. Preliminary studies indicated that both topo II and lamin Dm associate in protein complexes with many different proteins including transcription factors, LEM domain proteins, bicaudal, belle, Hsp70 and Hsp90 and many others. We aim at identification of these proteins and identification of lamin Dm and topo II domains involved in interactions with themselves. Changes in protein composition of the complexes during heat shock and recovery will be also analysed.

The second aim of the project is to identify in vivo changes in association of protein complexes associated with lamins and topo II on chromatin in response to heat shock induction of transcription shutdown and global changes in organization of chromatin. We also plan to analyze global association of lamins, topo II, modified histones, heterochromatin protein 1 (HP1), PcG protein and active and inactive fraction of polymerase II with chromatin and particular active and inactive genes using ChIP-seq method.

The aim of the third part of the project is to demonstrate the changes in association of proteins (lamins, topo II, fractions of pol II, association of nucleoporins of the FG repeat type, heterochromatin proteins with particular selected active and inactive loci on third instar larvae salivary gland polytenic chromosomes upon heat shock induction and during recovery.

A large variety of different methods from cell biology, molecular biology, genetics, biochemistry, developmental biology and proteomics will be used. The animal model system for study will be *Drosophila melanogaster*, the secondary model will be tissue cultured cells from *Drosophila* (Kc, S2). Typical methods which will be used are: immunoprecipitation (IP), IP with photo-crosslinking and labeling, mass spectrometry, ChIP with anti lamin Dm and anti topo II antibodies combined with analyses of nucleic acids (sequencing) and proteins (mass spectroscopy), 3D deconvolution of confocal images of IF labeled tissues and cells, combination of FISH/RISH with IF, image analyses and data processing, western blot, SDS PAGE, PCR, FACS, autoradiography, culture and crossing of fly stocks, isolation of salivary glands, imaginal discs, ovaries and staged egg chambers etc. It is essential to mention that our experimental approach would give more valuable and precise data on current physical lamin (or topo II) interaction with DNA than Dam methylase-tag approach used so far for this purpose.