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"Identification of protein partners of lamin Dm and topoisomerase II under heat shock conditions in the *Drosophila melanogaster* model system"

Abstract

Lamins are the main structural and functional components of the cell nucleus. These proteins are classified as V-type intermediate filament proteins and form polymeric structures, called nuclear lamina, underlying the inner nuclear membrane. Lamins can be divided into two types depending on their expression pattern: A-type lamins (expressed only in differentiated cells) and B-type lamins (expressed in all cell types). All lamins, regardless of their type, undergo a series of post-translational modifications. Beyond maturation, post-translational modifications determine lamin-specific properties and functions. The best-known example of this is phosphorylation, which determines the degree of polymerization state of lamins, their nuclear transport and their abilities of binding to DNA and chromatin. Mutations within the *LMNA* gene are the cause of a heterogeneous group of disorders collectively known as laminopathies. Examples of these diseases are muscular dystrophies, cardiomyopathies, lipodystrophies, neuropathies, metabolic disorders and premature ageing syndromes. Very frequently, patient overall phenotypes result in a combination of muscular, skeletal, metabolic, cardiovascular and ageing abnormalities. Mutations in genes coding for proteins interacting with lamins (emerin, LAP2, LINC complex proteins) also give rise to rare genetic disorders with similar phenotypes. Additionally, lamin B knockout has been proven to be lethal at the early stages of development in *Metazoan*.

It has been believed that lamins and interacting proteins function as a mechanical support and are involved in all of the nuclear processes, signalling and nucleo-cytoplasmic traffic. Lamins play a critical role (directly or indirectly) in the maintenance and support of the cell nucleus and chromatin organization. They are important for the spatial organization of nuclear pore complex distribution, a connection of karyoskeletal structures with the cytoskeleton. Moreover, they play a critical role in mechanotransduction. Lamins are also important for the replication, transcription and modulation of intracellular signalling. Specific phosphorylation and dephosphorylation on so-called mitotic (Cdk1) sites in lamins are critical for nucleus disassembly at the beginning of cell division and for nucleus reassembly during mitosis.

Topoisomerase II (Top2) is an essential protein for cells and has been involved in processes such as replication, DNA repair, transcription, DNA topology and chromatin organization. As well topoisomerase II is involved in the condensation of chromosomes during mitosis and plays a structural role in a chromosomal scaffold.

In *Drosophila melanogaster* there have been discovered only two lamin genes: one coding for B-type lamin which has been named as lamin Dm protein and one coding single A-type lamin called lamin C protein. Additionally, there is only a single gene and protein for Top2. Therefore the system is relatively simple and seems to be a useful model for studies of lamin and topoisomerase II functions *in vivo*. *D. melanogaster* has been the very first model system to demonstrate and study the cellular response to heat shock (HS) and the correlation between heat shock and changes in gene expression due to the studies on chromosomal puffs (regions of active transcription) on salivary gland polytene chromosomes. Moreover, this model has been the first to expose the composition of nuclear matrix and nuclear scaffold structures

and their composition modulation by heat shock. It has been shown that heat shock increased the amount of lamins and topoisomerase II proteins interacting with chromatin in isolated structures of polytene chromosomes and oocytes and this has been accompanied by alteration in *in vivo* binding of topoisomerase II with chromatin. Several laboratories independently identified new *in vivo* Top2 binding sites on chromatin induced by heat shock. Some of them have been mapped to so-called heat shock loci (identified by chromosomal puffs appearance) which all together suggests that there has been a nice correlation between heat shock, chromatin rearrangement, gene expression modification, and rearrangement of lamin Dm and Top2 interactions. All the above observations have led to the research hypothesis related to this doctoral thesis based on the involvement of lamin Dm and topoisomerase II in the regulation of gene expression in response to heat shock.

The doctoral thesis aimed to identify new protein partners for lamin Dm, lamin C and topoisomerase II under standard conditions and after induction of a heat shock in the *D. melanogaster* model system, an analysis of potential changes in the composition of protein complexes interacting with lamins and Top2 under the influence of thermal shock, and determination of the potential function of lamin and topoisomerase II in response to heat stress. An additional goal was to verify changes in the level of solubility (potentially related to, among others, changes in phosphorylation and protein interactions) of lamin Dm and topoisomerase II in response to stress. For this purpose, protein level analyses were performed by fractionating the extracts using buffers with different ionic strengths. In the first stage of work on the main goal, it was decided to verify the physical interaction of lamin Dm and topoisomerase II. The second was to identify the lamin Dm, lamin C and topoisomerase II interactome in normal conditions and upon heat shock effect induction. Conducted experiments were analyzed by immunoprecipitation followed by LC-MS/MS analyses and analyses of obtained data using various bioinformatics tools. The third step of the dissertation was to correlate interactomes of both lamins and Top2 with themselves to identify potential common interactors and their modulation upon heat shock induction. The final step was the attempt to identify molecular processes in which these proteins have been involved in normal conditions and after stress induction. In conducted experiments, the model organism *D. melanogaster* and insect cell lines Kc (characterized by the presence of both types of lamins) and S2 (lack of endogenous lamin C) were used.

Analysis of changes in protein solubility level after induction of heat shock showed statistically significant changes for both lamin Dm and topoisomerase II. Among others, it has been shown an increase in the level of these proteins after stress induction in comparison to standard conditions in fractions extracted with buffers with higher salt concentrations. The demonstrated data suggest a change in the phosphorylation pattern, a change in the localization or rearrangement of protein complexes interacting with lamin Dm and topoisomerase II in response to thermal stress. The studies carried out as part of the next stage of the work confirmed the direct interaction of lamin Dm with topoisomerase II and showed that after stress induction the number of observed Lam-Top2 complexes increases. During the next stages of work, it was discovered that lamin Dm interactome changes significantly upon stress induction and we managed to identify a group of proteins which are specific solely for HS interaction or are statistically significantly increased in HS, among others involved in transcription regulation, mRNA transport and splicing. Results obtained for the interactome

of lamin C did not show a similar trend, moreover, the statistical analysis of quantitative changes in lamin C – associated proteins after heat shock showed a global decrease in the number of protein complexes (which was an opposite observation to lamin Dm experiments, where the number mainly increase). These suggest the direct involvement of lamin B- type in the processes mentioned above. Besides, it has been observed that protein complexes associated with lamin Dm after HS are involved in stress response, gene regulation, RNA metabolism and proteins involved in transcription and translation silencing in response to stress. Identified proteins suggest the role of lamin Dm in the organization or regulation of structures tightly connected to stress response and inhibition of protein synthesis designated as stress granules. Since lamins are strictly nuclear (despite the small fraction of newly synthesized proteins), this also suggests the formation of stress granules inside the cell nucleus (those structures have been considered so far as mostly cytoplasmic). A more detailed study focused on interactions of lamin Dm with stress granule marker – Fmr1 using two-way immunoprecipitation confirmed data from interactome analyses. Laser confocal imaging also revealed the formation of stress granules not only in the cytoplasm but also at the cell nucleus compartment, with lamin Dm colocalization with Fmr1, as well relocation of lamin Dm induced by HS (after induction of heat shock, a signal from Lam Dm-specific antibodies was observed not only within the nuclear envelope but also a certain pool dispersed within the nucleoplasm).

In the next step, it has been shown, that proteins identified as topoisomerase II interactome in normal conditions are composed of chromatin interacting proteins, proteins involved in the regulation of transcription and mRNA transport. Upon stress induction interactions of topoisomerase II increased statistically significantly (in comparison to standard conditions) in the group of proteins associated with chromatin remodelling complex components, gene expression regulation, replication, nuclear export and RNA processing. Common elements for lamin Dm and topoisomerase II after heat shock induction (which changes statistically significant in both interactomes) were proteins involved in transcription regulation, chaperones, and proteins involved in mRNA transport and splicing.

Therefore analyses of lamins and topoisomerase II interactome fully confirms the working hypothesis stated in the project that these proteins do take part in chromatin reorganization and gene expression modification upon stress induction. All of the above data which have been obtained during the work on the project fully confirm the working hypothesis of the doctoral project and grant proposal thesis (OPUS 11) of the involvement of lamin Dm and topoisomerase II in the formation of many different multi-protein complexes which have been remodelled upon heat shock induction. All identified protein groups are involved in important nuclear processes attributed to lamins and topoisomerase II. In conclusion, the results obtained in this dissertation enabled to create a model of verified protein interactions for lamin Dm, lamin C and topoisomerase II, which reflect the actual interactions nicely interconnected with each other. Moreover, the presented results have illustrated the summary of a new network of interactions discovered in global interactome analyses of lamins and topoisomerase II as well as their modifications upon heat shock induction. In addition, this dissertation, by demonstrating changes in the protein complexes interacting with lamins and topoisomerase II in response to heat shock, provides a good basis for broader research on the involvement of these proteins in the regulation of the cellular stress response.