INTERACTIONS OF *Drosophila melanogaster* LAMIN Dm WITH NUCLEIC ACIDS AND TOPOISOMERASE II *IN VIVO*

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Substantial evidence suggests that *in vitro*, interphase lamins can bind DNA in a sequence (AT-rich) specific manner. Binding is not covalent and probably depends on the polymerisation state. Results of previous studies suggested that the vertebrate nuclear envelope and/or lamins also interacted with DNA *in vivo*. Lamins can also bind chromatin *in vitro* where interactions through histones H2A and H2B were shown. We developed previously a \[^{32}\text{P}]\gamma\text{-ATP}\) strategy to study the interactions in tissue culture cells between proteins and nucleic acids. Interphase and mitotic nuclear lamins from *Drosophila melanogaster* Kc cells. After *in vivo* photo-crosslinking it was found that interphase lamins were associated with DNA and RNA. Interaction with mitotic lamin Dm\textsubscript{mit} was not observed. *In vivo* photo-crosslinking in the presence of antibiotics: distamycin and/or chromomycin suggested that interphase lamins interacted with both A-T-rich and G-C-rich DNA; the letter predominated.

In contrast with lamin, DNA topoisomerase II (topo II) was first identified as a DNA-metabolizing enzyme. Topo II is an enzyme that mediates breakage and religation of double stranded DNA, thereby catalysing movement of one duplex past another for review see:. *In vivo* topo II is essential at mitosis where participation in chromatin condensation and in formation of the mitotic chromosome scaffold were implicated. It is similarly important in meiosis. The precise role of topo II in maintaining mitotic chromosome structure remains uncertain. A structural role of topo II in organizing interphase nuclei was also suggested. During meiosis as well as mitosis in *Drosophila*, a substantial portion of topo II was non-nuclear and was located throughout the cell; see also:. Lately we found that during interphase most topo II was insoluble (chromatin bound). Upon entry into mitosis topo II was apparently solubilized (did not associate with condensed chromosomes). Using our *in vivo* photo-crosslinking method we demonstrated that interphase topo II was associated mostly with DNA. Mitotic (non-chromosomal) topo II was primarily associated with RNA. These nucleic acid interactions occurred through poorly understood C-terminal domain of the protein. Interphase topo II was highly active enzymatically. Activity was reduced by treatment with phosphatase(s). In
contrast mitotic topo II was relatively inactive but activity could be increased by phosphatase(s). Mitotic topo II was more heavily phosphorylated then interphase topo II. In both only the C-terminal domain of protein was detectably modified. Our observation suggest that cell cycle-dependent changes in the distribution, nucleic acid interactions and enzymatic activity of topo II are regulated at least in part, by phosphorylation and dephosphorylation.

In order to play a role in structural organization and regulation of the structure of interphase nuclei, seemed likely to us that topo II would interact stably with various macromolecules, both proteins and nucleic acids. We anticipated that at least some of these associations would be dynamic, changing with changes in cell physiology. Native interactions between topo II and lamin, major nuclear structural (karyoskeletal) protein, were studied by immunoprecipitation performed after NaCl extraction of karyoskeletal proteins from nuclease treated nuclei. To our knowledge, Drosophila is uniquely suited to perform these studies since only in Drosophila can major karyoskeletal proteins be solubilized from such nuclei without the need for protein denaturation. Interactions of both topo II and lamin with nucleic acid were studied by immunoprecipitation performed after in vivo photo-crosslinking of proteins to nucleic acids and SDS denaturation of total cellular protein. Studies were executed with normally growing cells as well as during recovery after heat shock. We conclude that topo II and lamin interact directly with each other, that they also interact indirectly through nucleic acid and that interactions between proteins and nucleic acid change dramatically as well as reversibly during heat shock. We also demonstrated that vast majority of lamin Dm and topo II bound to nucleic acid is easily extractable from normal cells. In contrast to normal cells, in heat shock treated cells vast majority of lamin Dm- and topo II- nucleic acid interaction are found in nuclear matrix-lamina-pore complex fraction. In heat shock treated cells interactions with A-T-rich DNA prevailed.

These results have clear implications for elucidating nuclear organization under conditions of normal growth and after heat shock.