**THE STRUCTURAL ANALYSIS OF THE DNA FRAGMENTS ASSOCIATED WITH THE NUCLEAR LAMINS IN DROSOPHILA MELANOGASTER.**

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To understand better the role of extrachromosomal karyoskeletal proteins in the organization of the interphase nucleus, we investigated the interaction of lamins with amino acids *in vivo*; previously studies have been focused on *in vitro* interaction. *Drosophila* tissue culture cells were grown in the presence of bromodeoxyuridine (BrdU) and exposed to 366-nm light or were not treated with BrdU and UV. Cells were then lysed, treated with micrococcal nuclease, and lamin was immunopresipitated with specific antibodies. Nucleic acids fragments covalently cross-linked to proteins and coimmunopresipitated with lamin were labeled by incubation with T4 polynucleotide kinase and gamma32-P ATP. It was found that the DNA-lamin complexes isolated from cells grown without BrdU and UV treatment also incorporated gamma32-P ATP, although their labeling intensity was 3.1 times lower. Further purification using HAP chromatography showed that labeled materials associated with lamin noncovalently. However, good labeling of lamin-associated material isolated and purificated under standard conditions (without HAP chromatography) indicated that the lamin molecules conformationally bound to DNA did not lyse when boiled in SDS excess. Using the above approach, DNA-lamin complexes from the BrdU and UV treated as well as untreated cells were isolated in preparative amount. After denaturation and DOP PCR master amplification, it was found that the NrdU and UV treated fragments were not amplified. At the contrary, the amplification of DNA fragments from untreated complexes was successful. That amplified material was cloned into pSK vector. As a result, the library consissting of 31 clones was generated. All these were sequenced. Eleven clones (46-267 bp in size) met the requirements of the cloning scheme. Sequence analysis demonstrated that DNA of all the 11 clones was M/SAR. A pool of homologous blocks (up to 25 bp in size) was identified in structure of the clone sequences. More than half of the blocks occurred in different combinations in all the cloness. Based on presence of a common consensus, the blocks were divided into 6 groups. A part of the sequences contained the TopoII recognition site and also the lamin-binding sites described in the literature. DNA of the 10 clones demonstrated the abnormal mobility in 6% PAAG. R value (mobility reduction ratio) estimated as 0.67-1.10.

These 12 DNA sequences binding the lamin proteins were also aligned multiply with using the standard Gibbs Free Energy algorithm. In this way the both positive and negative DNA chains were taken into consideration, and, as result, the best alined of them was selected. The longest 15 bp consensus nucleus of the multiple alignment obtained was characterized by the weigh matrix given in Table 1 and by its information content value,http://www.bionet.nsc.ru/meeting/bgrs/thesis/66/index1.gif. The consensus nucleotides having their probability values over 0.67 observed were significant statistically withhttp://www.bionet.nsc.ru/meeting/bgrs/thesis/66/alpha.gif<0.01 are **bold-faced**, and those over 0.583 with http://www.bionet.nsc.ru/meeting/bgrs/thesis/66/alpha.gif<0.05 are *italicized*. This consensus resulted was*T***AAA**x**AA**xx**T**x**AA**x**T** (here, x is for any nucleotide without preference) One can see, this consensus is the pattern of which the T/A-rich dinucleotides multiply repited and separated from one another by the several positions without nucleotide preferred. This is the commonly accepted pattern of the nucleosome core binding sites in DNA which is also characterized by the B-DNA bending at the consensus T/A-rich dinucleotides observed. This both interpretatins of the consensus TAAAxAAxxTxAAxT obtained are consistent with the biological function of the lamin binding site (DNA packing) and this site abnormal mobility in 6% PAAG described above.

For independent verification of these both interpretation, the difference between all the 15 bp DNA sequence fragments multiply aligned and the 500 random sequences of the same length were tested in significant by using the 37 well known conformational and physico-chemical B-DNA properties. The 5 most significant difference are exemplified in Table 2. One can see, the highest probability to be binding nucleosome core was, indeed, obtained within this control test to be significant for the consensus TAAAxAAxxTxAAxT DNA sequences. Also, the highest mobility to bend toward Major Groove for this consensus sequences was detected by the same control test, too. Thus, the independent control test was confirmed the preliminary results that the lamin binding sites can be accompannied by the DNA regions with highest bend and probability to be binding nucleosome core.

**Table 1.** The longest 15 bp consensus nucleus of the lamin binding DNA sequences aligned multiply using the standard Gibbs Free Energy algorithm and its the weigh matrix obtained.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Position numbers | | | | | | | | | | | | | | |
| Code | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| A | 0.25 | **0.75** | **0.75** | **0.92** | 0.08 | **1.00** | **0.67** | 0.42 | 0.08 | 0.25 | 0.50 | **0.92** | **1.00** | 0.50 | 0.08 |
| T | *0.58* | 0.00 | 0.08 | 0.08 | 0.17 | 0.00 | 0.17 | 0.25 | 0.50 | **0.67** | 0.42 | 0.00 | 0.00 | 0.25 | **0.92** |
| G | 0.17 | 0.08 | 0.17 | 0.00 | 0.25 | 0.00 | 0.17 | 0.25 | 0.42 | 0.08 | 0.00 | 0.08 | 0.00 | 0.17 | 0.00 |
| C | 0.00 | 0.17 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.08 | 0.00 |
| **Cons** | *T* | **A** | **A** | **A** | **-** | **A** | **A** | **-** | **-** | **T** | **-** | **A** | **A** | **-** | **T** |

**Table 2.** The 5 most significant difference between all the 15 bp DNA sequence multiply aligned and the 500 random sequences in their conformational and physico-chemical B-DNA properties averaged.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Significant B-DNA property averaged on a given region | | | | Range | | Mean ± s.d. values | | |
|  | Region | Name of the B-DNA property | Unit | Min | Max | Site | Random | Utility |
| 1 | 2 - 13 | Rise | Angstrom | 3.16 | 4.08 | 3.36± 0.05 | **3.52± 0.09** | 0.97 |
| 2 | 0 - 13 | Slide | Angstrom | -0.40 | 1.60 | 0.13± 0.07 | **0.40± 0.13** | 0.93 |
| 3 | 2 -13 | Mobility to bend via Major Groove | relative units | 0.98 | 1.18 | **1.10± 0.02** | 1.05± 0.02 | 0.92 |
| 4 | 2 - 12 | Major Groove Width | Angstrom | 12.15 | 15.49 | 12.71± 0.25 | **13.47± 0.41** | 0.92 |
| 5 | 2 -12 | Probability to bind nucleosome | % | 1.1 | 18.4 | **14.20± 0.94** | 11.23± 1.49 | 0.90 |

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