In our original application we proposed to study within the duration of the award of this grant (March 1997 - December 1999) 1) the structure of chromatin and chromosomes by neutron and x-ray scatter and atomic force microscope; 2) the architecture of human sperm and the structure of sperm by atomic force microscopy (AFM); 3) genome-architecture and higher-order structures in human sperm nuclei; and 4) the effects of histone modifications on the structure of nucleosomes by protein DNA crosslinking) method.

1. **Effect of histone acetylation on the structure of assembled 195 bp nucleosome particles by neutron and X-ray scatter**

   Fractionation of individual acetylated histones from 400 liters of Butyrate treated HeLa cells. In collaboration with Dr. Joe Gatewood at LANL, milligram quantities of individual histones with different levels of acetylated were isolated. These histones will be used for reconstituting nucleosomes and chromatin for structural studies.

**Neutron scatter experiment at LANSCE**

   Study of reconstituted nucleosomes containing trypsinized octamers by neutron scatter. Since all the reversible acetylation sites of the histones are located on the NH₂ termini, chromatin reconstituted with limited trypsinized octamers are routinely used to simulate the structure of fully acetylated chromatin. In July 1997, we were allocated 10 days of neutron beam time at LANSCE to study the structure of reconstituted nucleosomes containing trypsinized octamers as a preliminary study for the reconstituted fully acetylated nucleosomes. Unfortunately the sample precipitated in the final dialysis step. This experiment has been postponed and rescheduled to a future date in the next beam cycle.

**Recloning of the original nucleosome positioning sequence**

   We have been using the original plasmid (p5S-207-18) from Dr. Robert Simpson containing the nucleosome positioning sequence for our work up to now. When used to reconstitute with the histone octamer, this DNA leaves asymmetrical ends on the nucleosome. We have recloned this sequence making the nucleosome symmetrically positioned on the DNA. This DNA will be used for future studies involving chromatin reconstituted with H1 and fully acetylated histones.

2. **AFM Studies of Native Chromatin, assembled chromatin and Sperm Nuclei**
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**Study of DNA/protamine structure by AFM (Reference 1)**

A novel method for reconstituting sperm chromatin was used to investigate how protamine 1 condenses DNA. Complexes formed in vitro using linearized plasmid DNA were imaged and measured by atomic force microscopy (AFM). The structures formed were found to be highly dependent on the sample preparation method used for reconstitution. Interstrand, side-by-side fasiculation of DNA and toroidal-like structures only 1-2 DNA diameters thick were observed for complexes formed in solution following direct mixing of the DNA and protamine. Large chromatin aggregates were also observed on the mica. However, if the DNA was first allowed to attach to the mica prior to addition of the protamine, well-defined toroidal complexes were formed without any observed DNA fasiculation or aggregate formation. The diameter of the toroids measured 30.6-50.2 nm (mean 39.4 nm). The dimensions of these structures indicate that the condensed DNA is stacked vertically by four to five turns, with each coil containing as little as 360-370 bp of 'B'-form DNA. This approach for preparing and imaging DNA-protamine complexes permits the analysis of intermediate structures 'trapped' on the mica as partially formed toruses of nucleoprotamine.

**Study of bovine sperm by AFM (Reference 2)**

We used atomic force microscopy (AFM), which utilizes a novel 3D image-contrast mechanism, to obtain nanometer-resolved, topographic data images of the natural surface structures of untreated bovine sperm cells. Freshly ejaculated, thawed, sonicated, and demembranated bovine sperm were adsorbed passively or by motility from suspension onto a coverglass substrate and directly imaged in normal air and saline environments without damaging the cells. Our AFM images of the surface structures of unfixed sperm imaged in normal air were consistent with previous electron microscope results on frozen or fixed sperm, demonstrating that the accurate preservation of small cellular structures is achievable using greatly simplified AFM sample preparation and imaging environments. Our AFM results also indicate that imaging sperm in physiologic buffer provides more native views of sperm due to the retention of cytoplasmic structures easily disrupted by drying forces. In addition, the AFM images show that numerous nanometersized subcellular structures of the sperm head and tail regions could be clearly visualized on rapidly prepared, unfixed, intact cells. Consequently, AFM should be considered a new tool for studying sperm structure abnormalities and monitoring the specific effects of, or damage caused by, various chemical reactants or other treatments on the structures of metabolically active or partially demembranated sperm. AFM is now emerging as an important new structural technique for imaging hydrated cells and organelles and, in addition, has the capabilities to physically "interrogate" them with the local probe.

3. **Architecture of Human Sperm Nuclei**

*Genome architecture and higher-order chromatin structure in human sperm nuclei*
Establishment of the sperm-specific genome architecture
Recently we demonstrated (3,4) an unique and surprisingly well-ordered chromosome packaging in human sperm nuclei. Sperm chromosome architecture and chromatin organization are very different from those of somatic cells (for review see 5). In this current grant, we proposed to study the establishment of sperm-specific genome architecture during spermatogenesis in humans. To this end we localized telomeres in different cell types on sections of testis of males 7 months, 24 months and 50 years old showed that higher-order telomere-telomere interactions into dimers and tetramers were absent in spermatogonia but became well-pronounced in spermatocytes. During the same developmental stages telomeres relocalized towards the nuclear periphery and became associated with the nuclear membrane. These new data (6) suggest that sperm-specific genome architecture in human reflects spatial reorganization which occurs during meiotic progression. Therefore, “sperm-specific” chromosome packaging and localization may be important for meiotic recombination in addition to their role in fertilization.

FISH studies of chromosomes folding
The structure of elementary nucleoprotamine complexes, DNA loop domains and gross genome architecture in mammalian sperm have just started to emerge. However, a series of gaps exist between known features of these levels of organization. In the current grant we proposed to fill in these gaps by studying the folding and pathways of individual chromosomes in sperm.
We have performed two color FISH with different combinations of micro-dissected DNA probes corresponding to different regions of chromosomes 6. Each combination consists of 2 - 4 probes with known localization on metaphase chromosomes. Each probe was labeled either with digoxigenin or biotin and detected using FITC or Texas Red. Using this approach we were able to study relative localization of define chromosomal segments in sperm nuclei. The data obtained indicate:
1. Long-range looping of chromosome 6 in sperm, observed as frequently opposed positioning of p and q chromosomal ends. These data support our earlier model of nuclear architecture in human sperm (4).
2. Numerous backfolds and loops along chromosome fiber.
3. Elements of rod-like, linear structures.
Thus, the higher-order chromatin organization in sperm shows much more elements of order and constrictions than that in interphase nuclei. In the latter, overall chromosome conformation is described by a random-walk/giant loop model (7).

4. Effect of histone modifications on histone-DNA contacts in nucleosomes by protein-DNA crosslinking
Nucleosome histone-DNA contacts at different phases of the cell cycle.
It is well known that chromosomes undergo major structural changes throughout the cell cycle. Although recent studies imply that the nucleosome itself plays a regulatory role in this process, the nucleosome structure/function relationship
through the cell cycle remains unclear. To understand the mechanism of this process, it is important to study the structure of the nucleosome at the level of histone-DNA interactions and the effect of histone’s posttranslational modifications at different phases of the cell cycle. Recently, we have shown that the level of chromatin compaction in vitro correlates with alterations of histone-DNA contacts in the nucleosomal core (8,9). This suggests that the changes in the level of chromatin compaction leads to structural changes in the nucleosome. Because chromosomes undergo major structural transitions during the cell cycle ranging from the dispersed functional state in S phase to the fully condensed inactive state of metaphase chromosomes, one of the goals of this research project is to study the structural features in nucleosomes at the level of histone-DNA interactions at the different phases of the cell cycle.

To address one of the specific aims, chemically induced zero length protein-DNA crosslinking in nuclei isolated from butyrate treated HeLa cells, was used to map core histone-DNA contacts in chromosomal domains comprising very different levels of histone acetylation. Certain well-defined core histone-DNA contacts identified in the hypoacetylated chromosomal domains were absent in the hyperacetylated domains. These altered contacts involve mainly the histone structured domains which are not subjected to acetylation and occur largely in the middle of the 146 bp nucleosomal core, whereas the contacts at the end of the nucleosomal core remain unchanged. This indicates that the observed alterations in core histone-DNA contacts in the hyperacetylated chromosomal domain are due not to the partial unwinding of the 146 bp DNA from the histone octamer but are the result of conformational changes within the nucleosomal core. The protein compositions of the two analyzed chromosomal domains also showed that the hyperacetylated domain was significantly depleted of histone H1, particularly in the H1A subtype. In addition, the difference in the composition of other chromosomal proteins observed in the analyzed chromosomal domains suggests that histone acetylation may be the major but not the only factor which plays a key role in the cooperative mechanism affecting chromatin and nucleosome conformation. This study provides direct evidence for structural polymorphism of the nucleosome in different chromosomal domains comprising different levels of condensation and histone acetylation and suggests that nucleosome conformation could be an essential element of chromatin function (Usachenko, S. I. and Bradbury, E. M., submitted for publication).

To study the features of nucleosome structure as a result of chromosome condensation/decondensation at the different phases of the cell cycle the conditions for large scale preparation of HeLa cells arrested in S and M phase are currently being worked out. Microscopic analysis shows a significant difference in chromatin morphology between viable interphase cells and nuclei isolated by procedures available from current literature. Conditions for S phase nuclei isolation are currently being worked out to preserve the chromatin morphology. These conditions will be used for zero length protein-DNA crosslinking to map histone-DNA contacts in the nucleosomes within intact nuclei. Mild micrococcal nuclease digestion is also being used to study the
structure of the nucleosomes in S and M phase cells. Different patterns of micrococcal nuclease digestion observed at the level of mononucleosomes may be an indication of the difference in the arrangement of histone H1 which plays a key role in chromatin condensation. To address this specific aim the conditions for the isolation of biochemical amounts of H1 containing nucleosomes from crosslinked interphase nuclei and mitotic chromosomes are also being worked out using preparative polyacrylamide gel electrophoresis. These nucleosomes will be used to map the location of histone H1 on the nucleosomal DNA.

References:
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