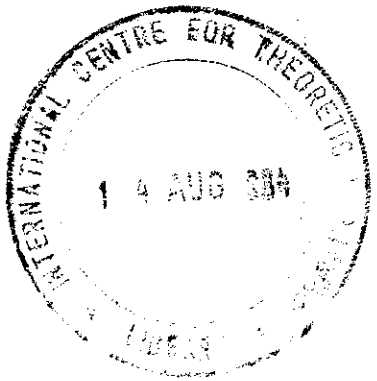

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SECOND SUMMER COLLEGE IN BIOPHYSICS

30 July - 7 September 1984

Lecture 3: Eucaryotic Chromatin Structure I - the Nucleosome.

Lecture 4: The 300Å Fiber.

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J. Widom Lecture III 8 August 1984, Trieste

Lecture III: Eucaryotic Chromatin Structure I - The Nucleosome

The nucleosome is the fundamental repeating unit of chromatin structure. The nucleosome "core" particle contains ~146 bp of DNA wrapped on the outside of a protein core which contains 2 copies each of the four "core" histones: H2A, H2B, H3, H4. It is common to all eucaryotic cells, and represents the first stage in a hierarchy of folded structures which eventually bring about a ~10,000 fold linear reduction in length of the double-stranded DNA which makes up chromosomes.

Early x-ray and electron microscope work showed that the nucleosome was flat, ~110Å × ~110Å × ~57Å, and somewhat wedge-shaped. Neutron diffraction (in protein-matching or DNA-matching solvents) of crystals of nucleosomes showed, after certain assumptions were made about the phases, that both the DNA and the protein core had dyad symmetry axes, which were coincident, and that the DNA was most likely wrapped 1.75 turns in a left-hand superhelix about the protein core.

Later work (A. Klug et al, Nature 287 p509-516, 1980) used the methods of 3-dimensional image reconstruction to solve the structure of tubular aggregates of nucleosomal-protein-cores ("the octamer") to 20Å resolution. The octamer has a distinctive morphology: dyad symmetry, and a helical ridge or ramp which is located symmetrically on the dyad and has sufficient length to bind 1 3/4 turns of DNA in the manner previously proposed.

From a consideration of histone-DNA chemical crosslinking data and histone-histone crosslinking data the position of each of the 8 proteins of the octamer (2 each, H2A, H2B, H3, H4) were located on the electron-density map. A DNA molecule on the octamer makes the following series of protein contacts along its length, starting at one end: $H2A^1$, $H2B^1$, $H4^1$, $H3^1$, - $H3^2$, $H4^2$, $H2B^{\prime 2}$, $H2A^{\prime 2}$; the superscripts distinguish symmetry-related copies, and the hyphen separates the two symmetry-related halves.

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Recently (T. Richmond et al, Nature in press, 1984) have solved the structure of the nucleosome (octamer + DNA) to 7Å resolution.

The large size (200,000 m.w.) required the development of new x-ray techniques: in particular, heavy metal clusters were used to solve the phase problem.

The results confirm previous work, and afford many new insights including (1) the DNA is right-handed in the B-form, wrapped in a left hand superhelix; this is accomplished by bending the DNA sharply at several points, each adjacent to but not at sites of extensive protein contact; (2) H3 and H4 organize the central turn of DNA, and interact via the minor groove of the DNA; (3) Deformation of the DNA appears to be delocalized over several base-pairs

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Lecture IV : The 300Å fiber

One isolates a string of nucleosomes (perhaps 10-200 nucleosomes per "string"), each nucleosome containing one molecule of the fifth histone, H1. If one then adds ~60-70 mM Na^+ or ≈1-5 mM Mg^{2+} the string of nucleosomes folds up to give a 300Å (wide) fiber. Finch & Klug have proposed that the fiber is a "solenoid" or helix of nucleosomes, with ~6 nucleosomes per turn and a pitch of 110Å, determined by the 110Å nucleosomal diameter.

1. Why 60-70 mM Na^+ or ≈ mM Mg^{2+} ? i.e. what is the role of the cation in chromatin folding?
Cations must act as general DNA counterions (see lecture I), reducing repulsion between DNA segments which neighbor in space in folded chromatin.
∴ Predictions : a) higher-valent cations especially effective (e.g. $Co(NH_3)_6^{3+}$)
b) Concentration of $Co(NH_3)_6^{3+}$ or Mg^{2+} etc required to induce folding will be dependent on the concentration of Na^+ and other buffer cations, e.g. $Tris^+$...
Both predictions verified.

2. When is chromatin folded? Results of survey of Mg^{2+}/Na^+ concentration-plane by electron microscopy (with and without glutaraldehyde fixation), sedimentation velocity and low angle x-ray scattering yield the following facts:

a) x-ray reflections at ~37Å are characteristic of both folded and unfolded chromatin (300Å fibers and string-of-nucleosomes); the 37Å reflection arises from scattering within a nucleosome. Reflections at ~200 Å, (& ~70Å?) characteristic for string-of-nucleosome (unfolded) state. Reflections at ~110-120Å and ~60 or ~55Å are characteristic of folded chromatin (300Å fiber); a reflection at 300-500Å may appear, and is due to packing of 300Å fibers.

b) In the absence of any Mg^{2+}, patterns diagnostic for 300Å fiber appear at ≈ 50 mM Na^+; addition of Mg^{2+} or further amounts of Na^+ leads to a sharpening of the pattern indicating an increase in internal order of the 300Å fiber.

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c) Na^+ and Mg^{2+} are in competition with each other for binding; thus to reach any particular folded state, an increase in the concentration of Na^+ requires an increase in the concentration of Mg^{2+}.

3. Any model proposed for the structure of the 300Å fiber must be consistent with the observed x-ray solution patterns; additionally, I have obtained partially oriented patterns from folded chromatin, which place much stronger constraints on possible models;

a) ~110Å reflection is the equilibrium direction (hence certainly the fiber direction) and the ring is markedly elliptical.
b) Broad peak from ~27-37Å perpendicular to this direction.

The "solenoid" and certain other models are consistent with these data; many other models are not.

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Lecture V : The metaphase chromosome.

In the metaphase chromosome (the most compact form of the chromosome) the linear double-stranded DNA has been compacted ~10,000 fold. This is done by (a) winding DNA on nucleosomes
(b) winding string of nucleosomes into 300Å fiber
(c) further folding of 300Å fiber.

Several lines of reasoning suggest that the next order of folding involves attaching the 300Å fiber at frequent intervals to a scaffold, creating a multiply-looped structure ~~@@@@@@~~ where each loop is a contiguous piece of DNA in the 300Å fiber conformation.

a) removal of all histones creates a visible "scaffold" with numerous loops of DNA, ≈ 30-100 kbp (1000 base-pairs) in length. (10-30μ in length)

b) metaphase chromosomes swollen in EDTA (which unwinds the 300Å fiber back to the string of nucleosomes) show (in cross-section) loops emanating from a central core; the loops are ~3-4μ in length, which is roughly the value expected for 30-100 kbp of DNA in a closely packed nucleosomal arrangement

c) metaphase chromosomes swollen in Mg^{++} show short, stubby loops; the chromatin is folded in the 300Å fiber state, and the loops are ~0.6μ in length, as expected for "solenoid"-folding of close-packed strings of nucleosomes

d) "Lampbrush Chromosomes" from amphibia oocytes have many regions of the chromosome that are very active in transcription; these regions of the DNA are not packaged in nucleosomes. Large loops are visible: their contour length is ≈ 20-30μ, rather like in "a" above.

At least one, and possibly several, orders of subsequent folding are still required to take a string of 300Å fiber-loops into a metaphase chromosome.

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There are several possible biochemical approaches to this problem:

1. The "scaffold" can be purified and appears to have a definite protein composition, principally 2 major species of proteins.

2. Factors are present in metaphase cells, but not in cells at other stages of the cell cycle, that cause a dramatic compaction of interphase chromatin.

3. Light microscopy of chromosomes in dilute K^+SCN^- (a mild denaturant) shows a possible uncoiling of a fixed stage in chromosomal folding; this could possibly turn out to be a reversible reaction.

4. Purified 300Å fiber-chromatin folds back on itself to yield short loops reminiscent of those in metaphase chromosomes in Mg^{++}.

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