50th Anniversary of the Nucleosome Discovery: a Brief Essay

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In January 1974, the first electron micrographs of swollen spread nuclei exhibiting chromatin "beads on a string" were published in Science [1]. These images represented a paradigm shift in our concept of the fundamental structure of nuclear chromatin (DNA+histones). For a more complete history of nucleosome discovery, see [2].

Prior to the discovery of nucleosome "beads", the consensus view of chromatin was that histone proteins "covered" DNA, generating irregular fibers with no apparent internal symmetries. The electron micrographs and subsequent biophysical studies established that the nucleosome beads represent a defined stoi-

(Cornell Univ. Press, New York, in press)]. Similarly, TYA may lack significant effects on platelet aggregation in vivo.

18. Agents used to induce platelet aggregation were adenosine diphosphate (Sigma), epinephrine (adrenaline chloride: Parke-Davis), bovine thrombin (Parke-Davis), and saline extract of human subcutaneous connective tissue (8).

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Spheroid Chromatin Units (ν Bodies)

Abstract. *Linear arrays of spherical chromatin particles (ν bodies) about 70 angstroms in diameter have been observed in preparations of isolated eukaryotic nuclei swollen in water, centrifuged onto carbon films, and positively or negatively stained. These bodies have been found in isolated rat thymus, rat liver, and chicken erythrocyte nuclei. Favorable views also reveal connecting strands about 15 angstroms wide between adjacent particles.*

The packaging of DNA within eukaryotic chromosomes continues to be a formidable structural problem. Packing ratios greater than 100/1 (DNA length/chromatid length) are not uncommon for metaphase chromosomes (*1*). The DNA concentrations within localized regions of interphase nuclei may approximate 200 mg/ml or more (*2*). Acutely aware of this problem, investigators have postulated multiple orders of coiling or folding of a fundamental nucleohistone molecule (*1, 3*). Several models have been derived from low-angle x-ray diffraction studies, including: four DNA molecules packed into a single nucleohistone fibril (*4*); a single DNA double helix and associated proteins folded into an irregular superhelix 80 to 120 Å in diameter and 45 Å in pitch (*5*); and a single DNA-protein fiber constrained into a superhelix 100 Å in diameter and 120 Å in pitch (*6*). Ultrastructural studies have also yielded a profusion of models. Spreading of chromosomes on a Langmuir trough frequently yields fibrils about 250 Å in diameter, although differences due to tissue type, presence of chelating agents, and method of dehydration and drying have been reported (*7*). Direct adsorption of sheared chromatin onto microscope grids has revealed a network of fibers approximately 100 Å wide with numerous side branches 80 to 200 Å in length (*5*). Spraying of chromatin onto a grid yields a network of fibers (*8*) and separated filaments (20 to 30 Å in diameter) containing numerous "nodular" elements about 150 Å in diameter (*9*). Thin sections of nuclei and chromosomes reveal fragments of threads frequently 100 to 200 Å wide (*3, 10, 11*). Bram and Ris (*5*) regard the 250-Å fiber as a folding (or doubling) of a superhelix, due to divalent metal ions, and interpret the thin-section data as artifacts of chelation by buffer ions. Lampert (*12*) views the 250-Å filament as a folding of the superhelix of Pardon and Wilkins (*6*), and explains the thin-section data in terms of shrinkage due to fixation. Despite this divergence of views, there is a consensus that multiple levels of coiling or folding are required to explain the observed variation in chromatin fiber widths.

We have attempted to visualize chromatin structure by methods different from those cited above. Interphase nuclei were isolated from fresh rat thymus (*13*), rat liver (*2*), and chicken erythrocytes (*2*), washed and centrifuged twice in CKM buffer (*14*) and once in 0.2*M* KCl, suspended in 0.2*M* KCl at a concentration of approximately 10^8 nuclei per milliliter, and diluted 200-fold into distilled H_2O. Nuclei were allowed to swell for 10 to 15 minutes, then made 1 percent in formalin (*p*H 6.8 to 7.0). Fixation proceeded for at least 30 minutes. All operations, up to this point, were at 0°

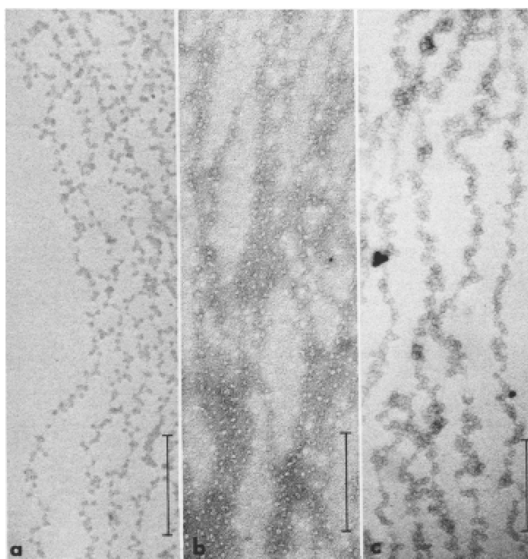

Fig. 1. Chromatin fibers spilling out of ruptured nuclei. The degree of fiber swelling and the proximity of individual ν bodies to each other varies within different regions of a single nucleus. Scale bars, 0.2 μm. (a) Rat thymus chromatin, positively stained with a mixture of 4 percent aqueous phosphotungstic acid and 95 percent ethanol (3 : 7), rinsed in 95 percent ethanol, and dried in air. (b) Rat thymus chromatin, negatively stained with 0.5 percent ammonium molybdate, adjusted to *p*H 7.4 to 8.0 with ammonium hydroxide. (c) Chicken erythrocyte chromatin, negatively stained as in (b). Clustering of ν bodies is most evident in (c), where groups of three or more are readily visualized. Connecting strands are most easily seen in (b).

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Fig. 1. The first page of the publication "Spheroid chromatin units (v bodies)" from Science (1974).

PERSPECTIVES

TIMELINE

Chromatin history: our view from the bridge

Donald E. Olins and Ada L. Olins

Thirty years ago, our conception of chromatin structure underwent a total metamorphosis as the nucleosome era began. In Kurosawa's classic movie 'Rashomon' (1951), each participant had a different perspective of the same pivotal event. This review outlines our perception of history.

J. J. Grandville's 'Le pont des planètes' (FIG. 1) provides an artistic metaphor for our view of chromatin. The history of chromatin can be said to begin with W. Flemming, who suggested the name 'chromatin'[1–3] (see TIMELINE). At the time (~1880), Flemming's research was focused on nuclear division ('mitosis' was another term suggested by him). Cell biology had achieved a level of technical and conceptual maturity with the development of microscopes with minimal optical aberrations[4], the increased availability of fixatives and stains[4], improvements in preparative techniques[5], and with the beginning of the chemical characterization of nuclear substances[3,6,7].

During Flemming's lifetime, seminal descriptions of DNA and histones were emerging from biochemical studies (see TIMELINE). F. Miescher and A. Kossel, both students of E. Hoppe-Seyler, laid the crucial groundwork for the characterization of chromatin components. Miescher, as is well known, developed methods for the isolation of nuclei from pus leukocytes and, in 1871, described a strong phosphorus-rich acid, which he called 'nuclein'[8]. Later, he described acidic 'nuclein' and basic 'protamin' from the isolated sperm heads of the Rhine salmon.

Kossel, encouraged by Hoppe-Seyler, continued the investigations, describing, in 1884, the 'histon' in acidic extracts from avian erythocyte nuclei[9]. He developed the intriguing, although now rejected, notion that protamines arose as breakdown products from the wasting muscle mass of the migrating salmon. Flemming, influenced by H. Zacharias' microscopy studies of protease-digested isolated nuclei (1881), which showed a resistance of 'nuclein' to degradation, wrote: "...in view of its refractile nature, its reactions, and above all its affinity to dyes, is a substance which I have named chromatin. Possibly chromatin is identical with nuclein, but if not, it follows from Zacharias' work that one carries the other. The word chromatin may stand until its chemical nature is known, and meanwhile stands for that substance in the cell nucleus which is readily stained." (REFS 1,3).

And so the name 'chromatin' still stands, and is likely to remain into the future.

Chromatin — the dark ages

The first half of the twentieth century revealed great strides in the emerging field of genetics, but was largely devoid of advances in understanding the structure of chromatin. Well known are the rediscovery of Mendelian principles by H. de Vries (1900), the development of gene theory and the principle of linkage by T. H. Morgan (1910), the identification of a 'transforming principle' by F. Griffith (1928), and the demonstration that this 'principle' is DNA by O. Avery, C. MacLeod and M. McCarty (1944)[10]. However, little was accomplished in characterizing the basic proteins of chromatin, other than their extraction in strong acid[11]. The 1941 Cold Spring Harbor Laboratory Symposium — entitled 'Genes and Chromosomes: Structure and

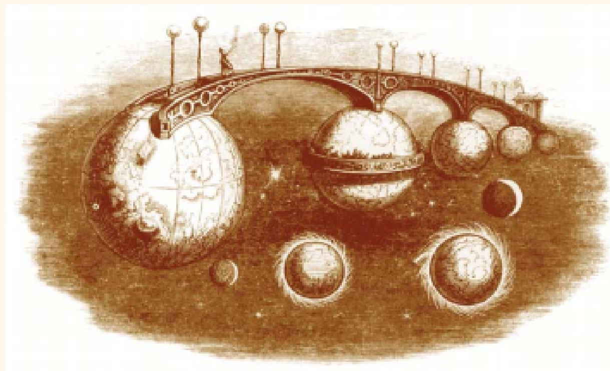

Figure 1 | **A fanciful view of chromatin structure.** 'Le pont des planètes'. Reproduced from REF. 67.

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Fig. 2. The first page of the publication "Chromatin history: our view from the bridge" from Nature Reviews Molecular Cell Biology (2003).

chiometry of histones (duplicates of histones H3, H4, H2A & H2B) on the "inside", with ~146 bp of DNA coiled on the "outside" of the histone core. One histone (H1) is associated with the nucleosome bead and the "linker" DNA connecting the string of beads. To the initial discoverers of this structure, these images and derived concepts represented an amazing simplification of chromatin structure.

This apparent simplicity has not lasted very long. The discoveries of histone variants and histone epigenetic modifications has illustrated how evolution utilizes a stable structural motif (e.g., consider the DNA double helix and the nucleosome, both with a defined dyad axis) to generate incredible diversity in genetic information and utilization of this information. Chromatin research today concentrates on higher levels of structure and regulation of changes.

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