CHROMOSOME STRUCTURE AND FUNCTION

Impact of New Concepts

Edited by

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and

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Drs. Ernie and Lotti Sears have devoted many decades of their lives toward the study of cereal genetics and chromosome manipulation. We would like to dedicate this volume to them in recognition of their lifelong commitment to science, and to the generous sharing of their knowledge and germplasm. Their doors have always been open to students and scientists from around the world. We are sure their future research will continue to be an inspiration to us all. They will always remain our best of friends.

FOREWORD

A Historical Perspective on the Study of Chromosome Structure and Function

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"Modern physical science gives us no model to explain the reduplication of the gene-string in each cell generation, or to explain the production of effective quantities of specific enzymes or other agents by specific genes. The precise pairing and interchange of segments by homologous gene-strings at meiosis also suggest novel physical properties of this form of matter".

Stadler (1954)

The very strong influence of reductionism in the history of understanding chromosome structure and function is evident in the above quotation from Stadler's 1954 paper, "The gene". Early observations on the constancy of the cytological appearance of chromosomes and their regular behaviour in cell division led to speculation on their biological importance. As genetics became more refined in the early decades of the 20th century the genes-on-a-string model of chromosomes developed and greater emphasis was placed on the further dissection of these structures. As a result, in the 1980's the reductionist approach is reaching a crest as extensive regions of the genetic material are being sequenced. However, although high levels of structural resolution have been attained, several aspects of the statement by Stadler remain unsolved. Problems such as the nature of the chromosome pairing at meiosis serve as a reminder that a process of building up a concept

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of the chromosome as a whole, from the parts generated by the genetic and the molecular/biochemical analyses, remains an important challenge.

The present symposium covers a broad spectrum of studies on chromosome structure and function and it is useful to view progress in this area with an appreciation of the history on which many of the studies are built; the following is a brief survey of the historical developments in our understanding of chromosome structure and function.

Era	Chromosome feature	Impact
1900 and before	Chromosome cytology. Primary and secondary constrictions as well as nucleolus organiser regions are clearly recognised. General chemical nature of RNA and DNA are understood.	Constancy of appearance and behaviour at cell division suggest chromosomes are of basic importance in cell biology.
1900-1930's	The chromosome as the bearer of genes: changes in chromosome structure (cytological) are correlated with genetic and phenotypic changes. Linkage of genes and crossing-over are found to occur in chromosomes.	Understanding of aneuploidy, polyploidy, translocations, inversions, and deficiencies. Specific phenotypic effects are associated with specific chromosome segments.
	Mitotic and meiotic (pachytene) chromosomes define euchromatin and heterochromatin in plants and animals. Salivary gland chromosomes of _Drosophila_, in particular, reveal fine detail in euchromatin.	The combination of salivary gland cytology and genetics in _Drosophila_ with the pachytene cytology and genetics in corn provide a new level of appreciation of genetic material. Heterochromatin introduces the new concept of apparently genetically inert material which can influence gene expression by its position in the chromosome.
1930's-1940's	Misdivision of centromeres is described.	Provides a rationale for one level of change in chromosome structure (translocations involving

		whole chromosome arms) observed in later experiments (1950-1970's).
	Microscope spectrophotometry measures the chemical composition of cell components directly. Biochemical studies show nucleoprotein to be composed of deoxyribonucleic acid and histone protein. The chemical structure of the components making up DNA are defined.	Provide a starting point for studies on how DNA is organised into the interphase nucleuc and chromosomes.
1940's	Genetic instability in maize is demonstrated and reinforces earlier observations of genetic instability in other systems.	Concept of a unit in the chromosome which can move to create mutations. Concepts of regulatory and structural elements.
1950's	The overall structure of DNA and its importance as the genetic material is generally accepted. Semiconservative replication is demonstrated cytogenetically and biochemically.	New appreciation of the transmission of genetic information and the understanding of recombination and mutations.
	Polytene chromosomes in Chironomus correlate chromosome puffs and gene activity.	Concept of structural change in the chromosome related to gene activity
	Successful manipulation of cereal chromosomes to introduce leaf rust into wheat. Homoeologous pairing mutation is described in wheat.	One product of chromosome engineering finds its way into a commercial wheat variety.
	Operon, and repressor protein, interpretation of bacterial gene expression.	Concept of specific regions of DNA to accomplish control of gene expression.
1960's	Development of preparatory techniques for examining unsectioned chromosomes by electron microscopy.	Concept of a chromosome comprised of folded or looped chromatin fibers develops.

	Definition, by amino acid sequencing, of the conserved character of the histones.	Concept of a DNA-protein combination providing the basic building blocks of a chromosome devlops.
	Ribosomal DNA genes are shown to be located in the nucleolus organiser region.	The first detailed understanding of a classic chromosome landmark.
	High content of repetitive sequences in eukaryotic chromosomes is demonstrated by Cot curves.	New questions are raised regarding the function of DNA and how genes are arranged in chromosomes.
1960's-1970's	The structure of highly purified 18S-26S ribosomal DNA and 5S DNA is determined. Ribosomal DNA transcription is visualised by electron microscopy.	Gene-spacer-gene-spacer etc., structure provides a detailed insight into eukaryotic genome structure.
	Banding techniques on mitotic chromosomes are developed.	New era of diagnostic work using mitotic chromosomes is initiated. In the analysis of human chromosomes this is particularly significant.
1970's	Specific classes of highly repetitive DNA sequences are shown to be located in heterochromatin. Genetic analyses demonstrate a low density of genes in heterochromatin.	An explanation of the apparent genetic inertness of heterochromatin is revealed.
	The repeated folding structure of chromatin is discovered.	The nucleosome concept develops and progress is made toward understanding how the DNA is arranged in chromosomes.
	A segment of 18S-26S ribosomal DNA from *Xenopus* is cloned into bacteria.	A new era of the high resolution analysis of gene structure is started.
	Demonstration that each chromosome of *Drosophila* contains only a single	The composition of chromosomes is clarified.

	continuous DNA strand, and that the length of it may increase by translocations but is unaffected by inversions.	
1980's	Major progress in the linear analysis of DNA.	Understanding the structure of transposable elements and how they cause genetic instability. Promoters, enhancers, and other controlling elements are identified for many genes. The exon/intron structures of many genes are determined.
	Specific eukaryote chromosomal proteins are identified. X-ray crystallography of DNA-protein complexes.	Details of how DNA interacts with protein molecules are revealed.
	Transformation systems in eukaryotes are developed.	Factors affecting the structure and function of chromosomes can be analysed with a great degree of precision.
	Cloning of yeast centromeres.	Reconstruction of a small chromosome to determine its essential features.
	Use of DNA markers are combined with earlier work (from 1960's) on isozyme markers in constructing genetic maps.	Major expansion of genetic maps from many organisms.

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J. P. Gustafson
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THE ANALYSIS OF CHROMOSOME ORGANIZATION BY EXPERIMENTAL MANIPULATION

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INTRODUCTION

Chromosomes have occupied a pivotal position in genetics ever since their involvement in heredity became firmly established in the early years of the twentieth century. There has consequently been a longstanding and fervent desire to understand how chromosomes are organized, and in particular, to ascertain how the hereditary material is arranged and regulated at the molecular level.

Historically, the evolution of concepts concerning chromosome organization has often occurred in a step-wise fashion, with major advances and shifts in knowledge following closely upon the heels of new technological advances. As in many other areas of genetics, the rapid development of innovative technology during the past few years has provided the opportunity for increasingly sophisticated approaches to the study of chromosome organization. The application of an armamentarium of new cytogenetic, morphological, and molecular biological techniques has led to radical and sometimes controversial changes in our views about the way in which chromosomes are constructed.

The purpose of this review is to highlight emerging concepts of chromosome organization. More specifically, two fundamental but interrelated aspects of this organization will be addressed: 1. the higher-order arrangement of chromatin in mitotic chromosomes, and 2. the underlying molecular and functional organization revealed by the chromosome banding techniques. In order to place recent

research in its proper perspective, the problems and pitfalls of chromosome research are considered, and alternative possibilities of chromosome organization are discussed. References have been chosen to illustrate specific points as opposed to providing a comprehensive literature review. For the most part, the emphasis will be on the organization of mammalian chromosomes since these have been studied in the greatest detail.

QUESTIONS, APPROACHES AND PROBLEMS IN CHROMOSOME RESEARCH

Major questions relating to chromosome organization include: 1. How is DNA arranged in the chromosome? 2. How are the other molecular components (e.g. proteins) of chromosomes involved in this organization? 3. How is the structural organization of the chromosome related to its functional organization?

In spite of intensive study, the answers to these questions have been elusive. The reason for this is apparent from the following considerations. A human diploid nucleus contains about 6.4 pg of DNA which corresponds to a combined total length of about 1.74 meters. This DNA is subdivided amongst the 46 chromosomes and, based on studies in other organisms (Laird, 1971; Kavenoff and Zimm, 1973; Molitor et al., 1974), exists as a single uninterrupted molecule within each chromatid. At metaphase, a chromosome only a few μm long may contain anywhere from 1.4 to 7.3 cm of DNA packaged into each sister chromatid. This translates into DNA and histone concentrations of approximately 25 mg/ml each in a condensed chromosome while the nonhistone protein concentration may be as high as 50-75 mg/ml (Okada and Comings, 1980). Such incredibly high concentrations mean that the chromosomes are chock-full of highly-condensed chromatin, effectively hindering any and all attempts by morphological approaches to examine the manner in which constituent chromatin fibers are organized in native metaphase chromosomes.

The vast majority of morphological studies on mammalian metaphase chromosomes have utilized light or electron microscopy. For light microscopy, used in most cytogenetic studies, the mitotic cells are exposed to a hypotonic salt solution (usually 0.075 M KCl), fixed in a mixture of acetic acid and methanol (1:3), and dried on slides. This is the only method known that gives good chromosome morphology and adequate spreading. Additional treatments are usually required to produce chromosome banding. It is obvious that an understanding of the effects of these preparatory procedures and banding treatments on chromosome constituents is required in order to fully comprehend what the observed morphological effects mean in terms of normal chromosome organization.

Thin-section electron microscopy, which has been extremely informative in demonstrating the intricate structure of other

cellular organelles, has been notably unproductive in revealing chromatin organization in mitotic cells. The chromosomes have a homogeneous, electron-dense appearance in thin-sections (DuPraw, 1970). Short segments or cut ends of fibers can be seen, suggesting a densely-packed, tangled network of chromatin, but there is no evidence of any kind of regular organization or internal structure. In fact, the only revealing aspect of chromosome structure seen in thin-sections is the kinetochore (Comings and Okada, 1971; Roos, 1973). This is the plate-like structure, located in the centromeric region on the superficial surface of each sister chromatid, to which the spindle microtubules are attached.

The dirth of information obtained from thin-section electron microscopy has necessitated the development of alternate ultrastructural methods for examining chromatin organization in chromosomes. Without exception, these methods all involve some kind of experimental manipulation designed to loosen or disaggregate the typical condensed chromosome structure so that internal detail can be observed microscopically. In some instances, this involves hypotonic shocks and spreading the chromosomes on aqueous hypophases; in others, it involves the isolation of mitotic chromosomes, followed by their exposure to a variety of chemical treatments. Unfortunately, these approaches are problematic because they can unknowingly modify the chromosomes so that the observed morphology does not accurately reflect the organization that exists _in vivo_. The fibrous nature of the chromosomes renders them highly susceptible to the formation of artifacts by any kind of manipulative procedure. This appears to be reflected in the diversity of results, opinions, and models that have appeared and disappeared over the years.

In view of these inherent problems, it is imperative to constantly question the effect of the experimental manipulations on the results obtained. Caution must also be exercised in extrapolating the implications of the results to the _in vivo_ organization of chromosomes until the conclusions can be verified by other independent approaches. Surprisingly, in spite of the problems, an integrated concept of chromosome organization is slowly emerging.

ORGANIZATION OF THE CHROMATIN FIBER

Although many of the fine details have yet to be worked out, it has been convincingly established that nuclear DNA is organized into several hierarchical levels within chromatin (reviewed by Felsenfeld, 1978; McGhee and Felsenfeld, 1980; Igo-Kemenes et al., 1982). The fundamental unit of chromatin organization is the nucleosome, consisting of a well-protected core and a nuclease-sensitive linker region which varies in length but is approximately 60 base-pairs long. The core consists of 146 base-pairs of DNA

wrapped around the outside of a histone octamer comprised of two molecules each of histones H2A, H2B, H3, and H4. One molecule of histone H1 is located at the site where DNA enters and exits the core particle, effectively closing two full turns of DNA around the histone octamer. The linker DNA interconnects adjacent nucleosomal DNA, producing a nucleosome chain containing a single continuous DNA molecule. As seen by electron microscopy of chromatin prepared under conditions of low ionic strength, this chain has a typical beads-on-a-string appearance.

In vivo, the elemental chromatin fiber consists of a linear chain of closely-packed nucleosomes without any apparent internucleosomal space, forming a 10-11 nm filament. The next level of order is the 25-30 nm chromatin fiber characteristically seen in most electron microscopy studies. This thicker fiber appears to form through a continuous coiling of the 10 nm fiber, giving rise to a solenoid with 6-7 nucleosomes per turn. It is thought that histone H1 may be involved in stabilizing the thick fiber, perhaps by crosslinking adjacent turns of the coil (Felsenfeld, 1978). Moderate ionic strengths are required to preserve the stability of this fiber, and it can be reversibly converted to the 10 nm fiber by successively lowering and raising the ionic strength.

The manner in which nonhistones may associate with the 10 or 30 nm fibers is largely unknown. However, the high mobility group (HMG) proteins 14 and 17, which appear to be preferentially associated with transcriptionally-active chromatin, have been shown to reversibly bind to mononucleosomes (reviewed by Igo-Kemenes et al., 1982). Additional details of how these and other nonhistones bind to chromatin, and the implications of these associations, must await further study.

CONSIDERATIONS FOR THE ORGANIZATION OF THE MITOTIC CHROMOSOME

During the transition from interphase to mitosis, there is no change in the basic structure of nucleosomes or their organization into chromatin fibers; the 10 and 30 nm fibers of interphase chromatin are also found in mitotic chromosomes (Rattner and Hamkalo, 1978a, 1978b; Labhart et al., 1982). On the other hand, there is a major rearrangement of the 30 nm fibers during prophase to form the compact, condensed structure of the mitotic chromosomes. This higher-order organization of chromatin has been remarkably refractory to elucidation.

Out of the myriad of chromosome models that have appeared over the years, variations on three major themes are predominant: 1. an organization based on chromatin folding; 2. an organization based on higher levels of helical coiling; or 3. an organization based on a central core that dictates chromatin arrangement. Each of these

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