ADVANCES IN CELL AND MOLECULAR BIOLOGY

Edited by E. J. DuPraw

STANFORD UNIVERSITY
SCHOOL OF MEDICINE
STANFORD, CALIFORNIA

VOLUME 2

ACADEMIC PRESS New York and London 1972

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 73-163767

PRINTED IN THE UNITED STATES OF AMERICA

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INTRODUCTION

In planning this second volume of the *Advances in Cell and Molecular Biology*, I have tried once again to invite authors with exciting, creative ideas solidly founded in backgrounds of recognized scholarship and careful research. The result is a brand new collection that is both larger and more varied than last year's introductory volume, yet clearly continues the high standards of excellence established by our earlier contributors.

Three of the present articles deal with the basal body–cilia–flagella system. Taken together they provide a comprehensive, thorough updating of important new biochemical and ultrastructural advances in this rapidly moving field. First in the series is an article by Jason Wolfe, which presents his recent discoveries employing electron microscopy of unsectioned, negatively stained basal bodies in combination with acrylamide gel electrophoresis of ciliary and basal body proteins. Next comes Fred Warner's expert analysis of dynamic architecture in cilia and flagella; in this article, Warner integrates exact measurements taken from his elegant thin section electron micrographs into a series of precise mathematical equations, which describe in amazing detail the relative movements within and between the nine outer doublets and the two central tubules of the cilium. A more specialized article by Benjamin Bouck reviews the structure and role of mastigonemes, tiny hairlike projections from the flagella of certain algae; these structures appear to be synthesized in the ER, assembled in Golgi vacuoles, and then transported for attachment in some unknown way to the plasma membrane surrounding the flagellum.

Dealing with the cell nucleus, Carl Feldherr has provided an important review of the nuclear envelope itself, with special emphasis on his admirable micrurgical experiments and on biochemical analyses of

envelopes isolated in test tube amounts. Next, David Comings and Tadashi Okada present the newest and one of the most comprehensive in their prolific series of investigations exploring the organization of meiotic prophase nuclei. Their article begins with a carefully documented thin section analysis of meiotic prophase stages in mouse testes. They then proceed to integrate the thin section material with newer electron micrographs depicting the same stages in unsectioned, whole-mount preparations. The result provides exciting insights into the pairing mechanisms of meiosis. Of particular interest is their hypothesis that DNA pairing may depend on special protein molecules akin to the *lac* repressor, but containing *two* identical, sequence-specific DNA binding sites per molecule. Such pairing proteins could arise by unequal crossing over in a gene coding for a more conventional DNA-linked protein, such as a repressor or possibly a site-specific histone.

One of the most fascinating breakthrough areas in chromosomology at the present time deals with the relationships that link constitutive heterochromatin, various chromosomal satellite DNA's (repetitive DNA's), and the mechanisms which pack and control several meters of DNA double helix found in the chromosomes of each eukaryotic cell nucleus. Three articles in the present volume explore this field from different standpoints. The approach of the light microscope cytologist is elegantly presented by Jorge Yunis and Walid Yasmineh, who have been in the forefront of those developing and employing important new methods for detecting rapidly reannealing DNA fractions in metaphase squashes; generally these fractions are found to be highly localized in regions of constitutive heterochromatin, such as those around the centromere. Another type of data is necessary to find out which fractions of satellite DNA are localized in which chromosomal regions, requiring biochemical purification together with specific *in situ* hybridization of each fraction. This approach is emphasized by Peter Rae, whose article describes studies carried out with the giant polytene chromosomes of dipteran salivary glands.

The third article in the series represents an elegant application of flow microfluorometry by Paul Kraemer and his group at Los Alamos; using this new method, they have made the startling discovery that chromosome number often undergoes wide variation in heteroploid cells without correlated variations in the total amount of chromosomal DNA per cell. This finding has induced them to explore the poorly understood mechanisms which control DNA packing, and which thereby determine the number of chromosomes in the nuclei of healthy or malignant cells. Evidence brought forward by other investigators, including the Editor of this volume, indicates that very thin DNA fibers, generally visible only with the electron microscope, often interconnect nonhomologous

chromosomes within the same nucleus.[1] In some cells, including those of humans,[2] the entire diploid karyotype may assume a radial, or clock-face arrangement, which seems to be maintained by interchromosomal connectives at the centromeres. These connectives are strong enough to hold the chromosomes together even when they are pulled from the living cell with a micromanipulator.[3] Furthermore, in the opposum the connectives are sites of RNA synthesis.[4] To account for their group's experimental results, Kraemer and his colleagues have found it necessary to rely on the concept of interchromosomal DNA continuities, explaining how the same DNA can be packaged into varying numbers and types of chromosomes.

Both in *DNA and Chromosomes* and in a recent symposium,[5] I have speculated about possible patterns of DNA folding which might account for interchromosomal connectives as they are observed with the electron microscope. One difficulty is that supposedly identical sister chromatids often show significant differences with regard to the number of connecting fibers attached to each, as well as in the precise places along the arms where the interchromosomal fibers enter. If the connecting DNA were linearly continuous with the DNA of the chromosome arms, it would be predicted that these connectives should be symmetrical for two identical sister chromatids. Further analysis of this problem has focused attention on the possible occurrence of phospho*tri*ester linkages within the DNA of eukaryotes; such chemical groups could give rise to asymmetrical branch points along double-stranded DNA molecules, which in principle would be almost as structurally sound as the main phosphodiester DNA chains themselves.

In the literature of cell and molecular biology, inadequate attention has been given to the possibility that short or long chemical sidechains may attach to any phosphorus atom in a DNA or RNA molecule by forming a third ester bond along the phosphodiester chain. The general configuration is portrayed by the diagram on page xvi. In addition to P—O—C linkages, there is no physicochemical reason why P—O—P or P—O—N bonding configurations may not occur as well. Several relatively simple phosphotriester compounds are sufficiently stable to be employed as pharmacological agents, such as *diethyl 4-nitrophenyl phosphate, tetraethyl pyrophosphate*, and the insecticide Parathion.[6] If triester side-

[1] DuPraw, E. J. (1972). "DNA and Chromosomes," Chapter 11. Holt Rinehart & Winston, New York.

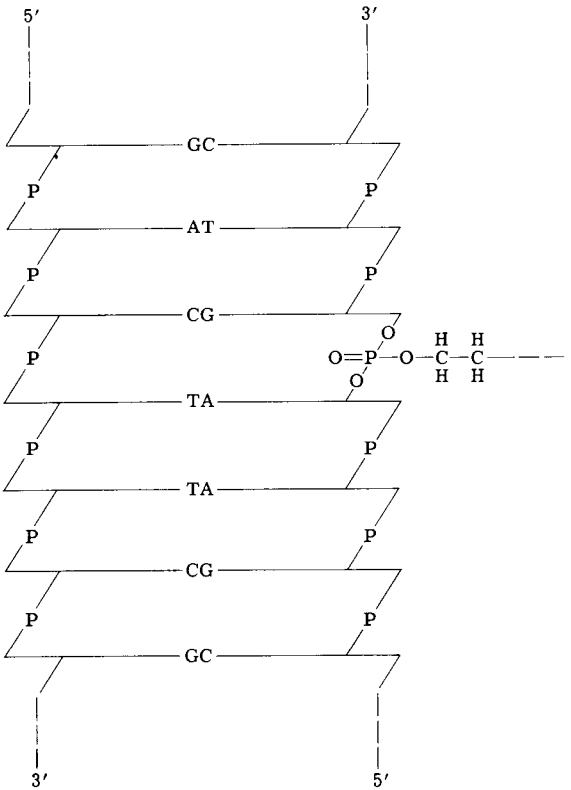
[2] Heneen, W., and Nichols, W. (1966). *J. Cell Biol.* **31**: 543.

[3] Diacumakos, E., Holland, S., and Pecora, P. (1971). *Nature (London)* **232**: 33.

[4] Schneider, L. K. (1972). *J. Cell Biol.* (in press).

[5] DuPraw, E. J. (1972). *Brookhaven Symp. Biol.* **23**: 230.

[6] Goodman, L., and Gilman, A. (1970). "The Pharmacological Basis of Therapeutics," 4th ed., p. 449. Macmillan, New York.

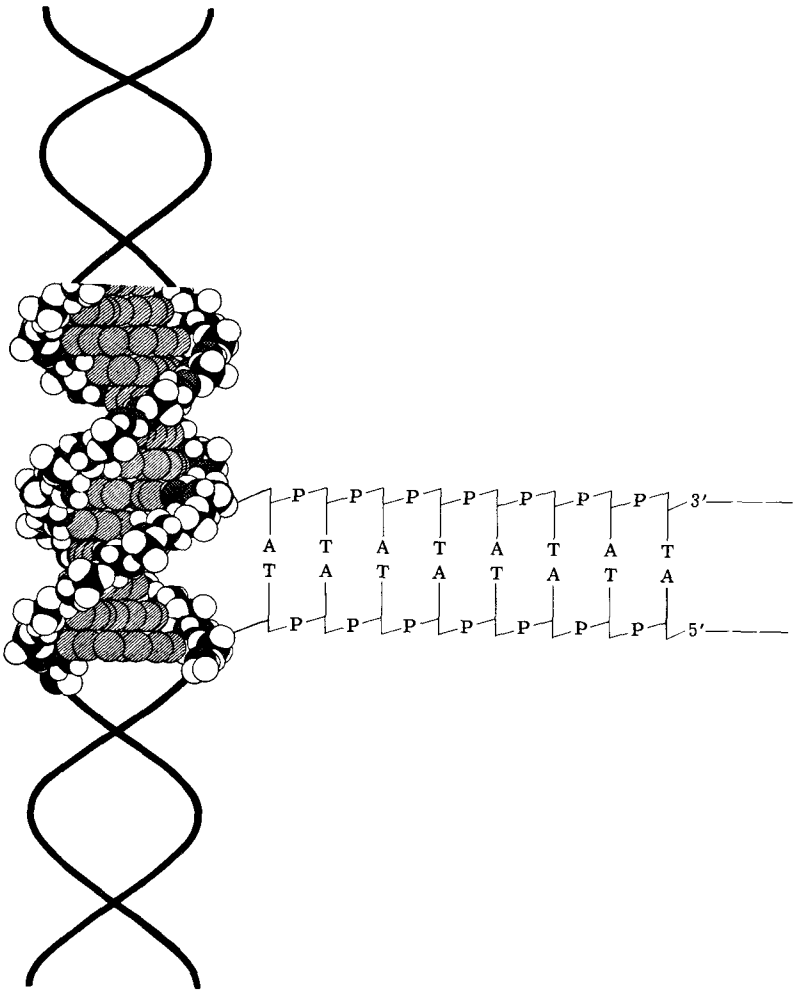


chains should occur even occasionally in eukaryotic chromosomal DNA, they might be postulated to play a role in cuing specific DNA-linked proteins, such as those which mediate the initial tight supercoiling of DNA to constitute the chromosomal fibers.[7] Another intriguing possibility is that the spindle fibers may attach to the chromosomes at their kinetochores by forming triester bonds between the centromeric DNA and molecules of microtubule protein.

Assuming that phosphotriester sidechains can occur at all, then in principle they would be able to provide one mode of attachment between chromosomal DNA and the interchromosomal DNA connectives. This pattern would differ from any proposed in my earlier publications, because the connectives would not be linear extensions of the chromosomal DNA itself, but would be related to it as structural branches. Such DNA branches would not be expected to generate branched gene maps, provided that the DNA connectives were genetically inert, e.g., composed of a highly repetitive chromosomal satellite DNA. Interestingly, there seems to be no steric difficulty to prevent the formation of *paired* triester link-

[7] Watanabe, M., and Tanaka, N. (1972). *Japan. J. Genet.* **47**: 1.

ages, holding together two separate double helices in a sidearm configuration; whereas the diameter of the phosphorus atoms in the two interwound chains of one DNA molecule is about 18.1 Å, the distance separating the two DNA chains across the major groove on the surface of the other molecule is about 18.8 Å (B configuration). Such a linkage between two DNA helices might or might not act as a covalent crosslink to control the unwinding and replication of the two complementary chains, depending on still unknown details of the mechanism which accomplishes unwinding during DNA synthesis *in vivo*. Some models postulate repetitive nuclease scissions of one or both chains, and in this case the sidearm configuration might replicate, branch point and all.



Obviously, DNA cross-connections of this type could not constitute a major fraction in eukaryotic DNA without being detected by X-ray diffraction, in quantitative analyses of DNA base composition, or in studies documenting nearly a 1:1 ratio of counterions to DNA phosphates. However, in the role of labile, interchromosomal connectives, only one or two hundred triester configurations would be required in a nucleus containing billions of nucleotide units. Such a small number would easily escape detection in conventional studies. It is of interest that enzymes capable of handling phosphotriesters have been demonstrated in rat liver, at least in connection with the metabolic degradation of insecticides. Inadequate though our present data may be, the mere fact that DNA branches and interchromosomal connectives are seen with the electron microscope, while the possibility of phosphotriester linkages has been neither excluded nor seriously investigated by previous students of the subject, makes the concept doubly intriguing.

Stanford, California
September, 1972

E. J. DuPraw

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MODEL FOR MAMMALIAN CONSTITUTIVE HETEROCHROMATIN

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I. Introduction

Heterochromatin was defined by Heitz (1928, 1933) as the chromosomes or chromosome regions in liverworts and mosses which are condensed at interphase and prophase, and which do not unravel like the rest of the chromosomes at telophase. This definition still holds true for most organisms. For example, in mammals two main types of heterochromatin are recognized: (1) constitutive heterochromatin, or the heterochromatin present in homologous chromosomes (which fits Heitz's definition best); and (2) facultative heterochromatin, the heterochromatin that results

from inactivation of one of the two X chromosomes in females (Brown, 1966). Facultative heterochromatin comprises about 2.5% of the genome and takes on the condensed form of the sex chromatin body very early in embryogenesis (Ohno, 1967). Genetic evidence indicates that it is metabolically inactive, providing an effective mechanism for equalizing X-linked genes between the two sexes (Lyon, 1961, 1966).

Concepts of constitutive heterochromatin have often been quite vague and filled with contradictions. However, recent observations carried out mainly with mammalian cells have begun to clarify its biochemical composition (Yasmineh and Yunis, 1969, 1970, 1971a; Yunis and Yasmineh, 1970), its location within the various phases of the cell cycle (Schmid, 1967; Lima-de-Faria, 1969; Lee and Yunis, 1971a,b), and its expression in various cell types during development (Lee and Yunis, 1971a,b). This has also permitted formulation of a model of constitutive heterochromatin, which brings together new information with older concepts that have evolved since the time of Heitz (Swanson, 1957).

An important new approach was initiated by Yasmineh and Yunis (1969), who showed that preparations of constitutive heterochromatin isolated from the nuclei of mouse liver and brain are composed primarily of a highly repetitive DNA; this DNA differs in a number of respects from the bulk of the nuclear DNA (Yasmineh and Yunis, 1969, 1970). Repetitive DNA was first described in the early 1960's by a number of investigators working on mouse DNA preparations (Kit, 1961; Chun and Littlefield, 1963); originally it was termed satellite DNA (S-DNA) because in CsCl gradients it bands at a different density from that of the bulk of nuclear DNA, and it represents only a small portion of the genome (about 10% in the mouse). Confirmation of these results was provided by the work of Jones (1970) and by Pardue and Gall (1970), who showed that mouse S-DNA hybridizes *in situ* with DNA in the regions of the centromere and nucleoli; both these regions are known to be heterochromatic (Ohno *et al.*, 1957) and late replicating (Church, 1965).

Our original observations in the mouse were soon thereafter extended to a number of mammalian species, including guinea pig, calf, *Microtus agrestis*, kangaroo rat, and man (Yunis and Yasmineh, 1970, 1971; Yasmineh and Yunis, 1971a,b; Yunis *et al.*, 1970; Mazrimas and Hatch, 1970). In all these species, it was shown that constitutive heterochromatin is rich in S-DNA's which differ drastically from one another and are composed of repeated sequences that appear to be characteristic for each species (Table I). These results challenged the belief of many investigators that constitutive heterochromatin is a state of chromatin, rather than a specific entity (Brown, 1966; Lima-de-Faria, 1969).

TABLE I: CHARACTERISTICS OF S-DNA FROM MAMMALIAN CONSTITUTIVE HETEROCHROMATIN

	(1)	(2)		(3)	(4)				(5)	(6)	
	Density of main DNA in neutral CsCl (main peak) (gm/cm³)		Density of S-DNA in neutral CsCl (gm/cm³)	Density of S-DNA strands in alkaline CsCl (gm/cm³)	Base composition of S-DNA				A + T/G + C of S-DNA		
					%A	%T	%G	%C	From base composition	From density in CsCl	Ref.[a]
Mouse	1.700		1.691[b]	1.722	44.8	20.2	22.0	13.0	1.84	2.17	1, 2
				1.742	19.2	45.8	14.0	21.4			
Guinea pig	1.699	I	1.705[b]	1.722	39.7	21.1	3.1	36.0	1.56	1.18	3
				1.800	21.8	39.6	35.7	2.9			
		II	1.705[b]	1.722	27.7	28.2	13.5	30.6	1.30	1.18	
				1.752	29.0	27.2	31.9	11.9			
Calf	1.699	I	1.706[b]	1.770					1.17	1.13	4
		II	1.713[b]	1.773					0.82	0.85	
				1.783							
Microtus agrestis	1.699	I	1.700[c]						2.00[c]	—	5
		II	1.717[c]						1.03[c]	—	6
Man	1.700	I	1.687[d]	1.707					—	2.62	7
				1.738							
		II	1.693[e]	1.740					—	1.88	
				1.750							
		III	1.696[de]						—	—	

[a] Key to references: 1. Yasmineh and Yunis (1970); 2. Corneo *et al.* (1968); 3. Yunis and Yasmineh (1970); 4. Yasmineh and Yunis (1971a); 5. Yasmineh and Yunis (1971b); 6. Yunis *et al.* (1970); 7. Corneo *et al.* (1970).

[b] Initially isolated in Cs_2SO_4-Ag^+.

[c] Determined after denaturation of sheared DNA, reassociation, and isolation on hydroxyapatite.

[d] Initially isolated in Cs_2SO_4-Hg^{2+}.

[e] This DNA, isolated by Corneo *et al.* (1970) and termed "homogeneous DNA" may correspond to a G + C-rich fraction isolated in our laboratory by denaturation of sheared DNA [45,000 pounds per square inch (psi) in a French pressure cell], reassociation, and fractionation on hydroxyapatite. The fraction comprises about 8% of the total DNA and sediments at 1.715 mg/cm³ in CsCl. After further shearing (60,000 psi) and density gradient centrifugation in CsCl, two additional subfractions sedimenting at densities of 1.725 and 1.729 g/cm³ were observed. The three subfractions show a much steeper melting profile than the original fraction sheared at 45,000 psi.

This belief was partly based on the facts that: (1) heterochromatin could not be visualized cytologically in some lower animals in early embryogenesis (Brown, 1966); and (2) it seemed to vary quantitatively in cytological expression in various cell types of the same species (Brown, 1966; Schmid, 1967; Lima-de-Faria, 1969). The controversy led us to make a thorough cytological study of the European field vole, *Microtus agrestis*, in which constitutive heterochromatin is located primarily within the sex chromosomes, which consequently appear as two large, conspicuous masses in the interphase nucleus of many cells (Lee and Yunis, 1971a,b). The results (discussed in Section III,C) indicated that the two giant chromosomes are condensed as early as the zygote and germ cells and they remain condensed in all cell types throughout development. Any variation in the cytological expression of constitutive heterochromatin is due basically to the degree of folding and compactness of the two condensed chromosomes, which may appear on the one extreme as compact round masses, and on the other as extended heterochromatic fibers.

Other considerations that have aided in the formulation of a model for mammalian constitutive heterochromatin relate to its possible function. For example, genetic and biochemical evidence suggests that constitutive heterochromatin is mainly inactive in protein synthesis (Heitz, 1933; Swanson, 1957; Hsu, 1962; Brown, 1966; Schmid, 1967; Leppert *et al.*, 1968; Yunis and Yasmineh, 1970; Sieger *et al.*, 1970; E. M. Southern, 1970); cytological evidence indicates that it is involved in nuclear organization (Doutreligne, 1933; Vanderlyn, 1948, 1949; Wagenaar, 1969); genetic evidence indicates that it has a lower frequency of crossover than euchromatin during meiosis (Darlington, 1937; Callan, 1941; Hyde, 1953; Gropp and Citoler, 1969; D. I. Southern, 1970); other evidence indicates that at least one region with which constitutive heterochromatin is normally associated, i.e., the nucleolar organizer, has undergone very little change throughout evolution (Loening, 1968; Ohno, 1970). All these lines of investigation were important factors in the development of our model.

II. Model for Mammalian Constitutive Heterochromatin

It is proposed that constitutive heterochromatin contains sequences of DNA which are usually highly repetitive and unique for each species. These sequences are not transcribed into RNA for protein synthesis, and consequently may be partly dispensable. They are gen-