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GENES: Structure and Expression

Edited by
A.M.Kroon

Horizons in Biochemistry and Biophysics

Volume 7

GENES
Structure and Expression

Volume Editor

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Preface

Volume 7 of Horizons in Biochemistry and Biophysics is completely devoted to gene structure and expression. Many books on this subject have been published in the past. Most of these are highly specialized and not in the first place directed to the interested colleague or student not yet involved in this field of science. Since Horizons in Biochemistry and Biophysics primarily aim to reach this group of scientists, the editors thought it useful to bring together some information concerning topics, which at first glance may appear rather distant, but which are all pertinent to the fascinating biological process of the flow of genetic information from the carrier of this information, the DNA, to the final functionally active gene products, the proteins. In this volume emphasis is put on the eukaryotic cell. An exception is made for the two chapters dealing with the ribosomal RNAs and proteins. This choice was made on the one hand because as yet much more relevant information is available for bacterial ribosomes than for eukaryotic cytoplasmic ribosomes and on the other hand since the observations most likely allow, at least to a certan degree, extrapolation to the latter type of ribosomes.

The chapters 1 and 2 deal with the structure and function of nucleosomes and chromatin, with the role of histones and with the possible mechanisms of activation for replication and transcription and of the selectivity of the information retrieval. The structure and function of ribosomal RNA and ribosomal proteins are described in the chapters 3 and 4. From sequence analysis quite detailed information on the various ribosomal building blocks is available and the way to the integration of this knowledge as to understand the complete architecture and functioning of the ribosomes in protein synthesis, seems open now. At the level of initiation of protein synthesis good progress has been made with respect to the sequential reactions of the various factors and the regulatory steps (chapter 5).

The major issues in euykaryotic gene expression concern the regulatory mechanisms. Of course also bacteria need their regulation processes, but the demands of eukaryotic cells, especially of multicellular organisms with different cell types and tissues, are much stronger. Firstly because of the considerable compartmentation of these cells and the presence of two (the nucleocytoplasmic and mitochondrial), in plants even three (also the plastidic) genetic systems. Secondly because of the high degree of differentiation of the various cell types in higher organisms. Seven chapters are dealing with various aspects of this regulatory processes. They do not give the final words on either of the topics treated, but they point to the problems, develop the possible approaches to tackle the various problems and summarize the observations and facts gathered so far. So at the end it is hoped that a clear picture has emerged, showing the needs for further research at the one side and a better understanding of the complexity of the overall process of gene expression at the other side.

The transport of proteins from the sites of genetic expression to their sites of functional expression is discussed in chapter 6. The many different membranes, each surrounding specific compartments of the cell, put strong barriers to many molecules, especially macromolecules like proteins. None the less there has to be a vivid protein traffic wthin the cell to enable the proteins to function properly. Besides via signal peptides, other conformational properties and thermodynamic conditions may play a role in this transport circuit.

As an example of differential expression of genes, hormonal regulation is the subject of chapter 7. It is well recognized these days that information about the signals on RNA and DNA responsible for differential expression must reside in specific base sequences. To analyse these sequences one may use the recombinant DNA technique. For this reason chapter 8 dealing with the strategies for optimizing foreign gene expression in *E. coli* is included.

The chapters 9 to 12 are focusing on various aspects of the biogenesis of mitochondria. The interplay between the different genetic systems in one cell is discussed for mitochondria. The type of problems seen in this context and the needs for concerted action of these two systems are essentially similar, although different in details for plastids.

The description of the mosaic genes of mitochondria has a more general range as well. Also many nuclear genes are mosaic and need proper processing of the primary transcripts. The way this processing is accomplished may be different in detail again but not in essence. The assembly of the mitochondrial proteins is rather well documented and adds further information to the phenomena described in chapter 6. Finally the non-universality of the genetic code is an unexpected finding, but interesting especially from an evolutionary point of view.

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THE STRUCTURE OF NUCLEOSOMES AND CHROMATIN

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INTRODUCTION

Chromatin is the nucleoprotein complex found in the nucleus of all eukaryotic cells. It undergoes major changes in morphology during the cell cycle, varying in form from the highly compact chromosomes of metaphase, which are readily visible even with the light microscope, to the much more diffuse structure present in the nucleus during many of the other phases of the cycle. However, even this "diffuse" structure must involve considerable compaction of the cellular DNA since the genome length of about a metre, for a typical organism, must be packed into a nucleus less than 10 μm in diameter.

The earliest work on the structure of chromatin was concerned with defining its composition and elucidating the components. As found in the nucleus, chromatin contains approximately twice the mass of protein as of DNA. The protein consists of roughly equal amounts of two categories of protein, a number of very basic (i.e. positively charged) proteins collectively called "histones" and the other, so-called "non-histone", proteins which have a much wider range of properties and range from acidic to mildly basic in composition.

The high positive charge and abundance of the histones made them obvious candidates for interacting directly with the DNA, neutralising much of the negative charge and enabling the folding to occur which was essential for compaction. It was also once thought that they might well exercise a controlling role in the functioning of the DNA. The very nature of the histones made them extremely unfavourable for the separation techniques then available and there appeared to be tens of different components, allowing ideas of a moderate specificity for interaction with the DNA. However, steady improvements in fractionation and careful characterisation reduced the apparent number of individual histones so that, in a review in 1969, Stellwagen and Cole (1969) concluded that there were less than ten molecular species in any organism and hence that "the multiplicity of histones seems inadequate for them to be active repressors functioning individually, but it also seems too great for histones to have a very passive role". They were thinking in terms of a direction of histones by other proteins and

TABLE 1

The Histones

Histone	Molecular weight (M_r)	Degree of conservation
H3	15,400	Highly conserved
H4	11,340	Highly conserved
H2A	14,000	Moderate variation between tissues and species
H2B	13,770	Moderate variation between tissues and species
H1	21,500	Varies markedly between tissues and species
H1°	~ 21,500	Variable, mostly present in non-replicating cells
H5	21,500	Very variable, only present in transcriptionally inactive cells of some species

suggested that such direction could be "to specific genetic sites, or for specific or general derepression".

Within the next two years, the basic characterisation of the histones had been completed and there were known to be five types of histone, with a complete primary sequence for at least one member of each type. Moreover, in a review in 1971, De Lange and Smith could point out the extremely high conservation of sequence in both H3 and H4 and our general picture of the properties of the histones has not changed since then (Table 1). However, despite this successful characterisation of the histones, their function still was unknown and De Lange and Smith (1971) concluded that "the precise functions of histones, either collectively or individually, have not been established. It seems likely that histones function in the regulation of transcription and replication and as important structural components which may additionally serve to protect genetic material from various degradative processes".

The non-histone proteins have also been studied and several of the major ones isolated and sequenced (see Huntley and Dixon, 1972, for review). However, unlike the hisones, there are multiple non-histone proteins and individually they are present in much smaller amounts than any histone. Thus while the five histones are present in comparable molar amounts, even the major non-histone proteins are present at concentrations only about an order of magnitude lower and many of them are present in very small amounts indeed. Moreover, the non-histone proteins tend to be less tightly bound to the DNA than the histones and typically are washed off by 0.35 M salt, while the histones do not begin to come off significantly until above 0.45 M salt. This mode of

identification is, however, potentially unreliable - one protein identified as a histone in trout (H6) was later found by sequence analysis to be closely related to some of the major non-histone proteins and not to the authentic histones (Huntley and Dixon, 1972; Walker, Hastings and Johns, 1977; Goodwin, Walker and Johns, 1978).

Contemporaneously with this characterisation of the histones, the first steps were being taken to investigate the structural organisation within chromatin, using X-ray scattering from fibres (Wilkins, Zubay and Wilson, 1959; Pardon, Wilkins and Richards, 1967; Pardon and Wilkins, 1972). This showed a simple series of low angle reflections or bands at about 11, 5.5, 3.7, 2.7 and 2.2 nm and this pattern could be observed from intact nuclei as well as fibres of extracted chromatin. This meant that there was some repetitive sub-structure in chromatin, on the scale of about 11 nm. In the absence of any biochemical evidence for the likely structure, the X-ray pattern was interpreted as coming from a superhelical coiling of the DNA, with a diameter of about 10 nm and a helical pitch of 11 nm. The histones were thought to lie generally along the DNA, possibly in the major groove, and to induce the superhelical coiling by a torsion effect due to inexact matching of the spacings of the positively charged residues on the proteins and the negative phosphate groups of the DNA.

The transition from these early ideas to our current more specific structural picture came as a result of the application of nuclease digestion to probe the organisation of chromatin (Clark and Felsenfeld, 1971; Hewish and Burgoyne, 1973; Burgoyne, Hewish and Mobbs, 1974). Thus, Hewish and Burgoyne (1973) reported that an endogenous nuclease in the nuclei isolated from rat or mouse liver would digest chromatin to produce multimers of 200 base pair lengths of DNA. This result was rapidly confirmed and also shown to be produced by other nucleases, in particular micrococcal nuclease (Noll, 1974), showing that it was not a property of the specific enzyme but rather of the substrate. In other words, it could be concluded that there was a basic repeating pattern in the organisation of the majority of chromatin. While the length of this repeat has since been shown to vary somewhat not only between types of nucleus but also within an individual nucleus, the overall picture suggested by Kornberg (1974), with a repeating unit of approximately 200 base pairs of DNA, two each of the four histones H2A, H2B, H3 and H4 and a single molecule of histone H1, is still the basis of our current ideas.

GROSS ORGANISATION

One problem with the folding and compaction of DNA lies in its stiffness. This can be measured by the "persistence length", which gives the distance apart of two regions on the rod-like DNA double helix for it to have bent sufficiently that the directions of the rod in these two places are not correlated with each other. The more flexible the rod, the shorter will be the persistence length. For double helical DNA the persistence length is largely

independent of ionic strength above 1 mM and equals about 50 nm (Hagerman, 1981). This corresponds to approximately 150 base pairs of DNA and correlates well with the shortest lengths of DNA which can readily circularise, by having their ends joined to give covalently closed circles (Shore, Langowski and Baldwin, 1981). A circle with a circumference of 50 nm would have a radius of about 8 nm and this therefore is the smallest radius of curvature around which free DNA will easily bend.

In practice, electron micrographs of chromatin show a variety of appearances from "beads-on-a-string", with the "beads" about 10 nm diameter (Olins and Olins, 1974), through a rather uniform fibre of 10 nm diameter (Ris and Kubai, 1970) to a less uniform 30 nm fibre (Finch and Klug, 1976) (these different forms are discussed below). The length of the DNA will not allow it to be simply stretched straight along the 10 nm fibres, so it must be folded with a maximum radius of curvature of about 5 nm. Such compaction can only come about by a close interaction with the histones, with a binding energy sufficiently high to overcome the unfavourable bending of the DNA which is necessary. Moreover, in order to minimise this bending the DNA needs to lie on the outside of the fibre. While this idea was generally unexpected when it was first suggested (Kornberg, 1974), as it is in marked contrast to the situation in viruses to which chromatin had been likened, with hindsight it has the obvious advantage of allowing relatively unencumbered access to the DNA for the many enzymes and regulatory proteins which must interact with it if it is to function, even while the DNA is in its folded state.

The size of the DNA in a chromosome suggests that it is impossible for it to be folded in a unique and specific fashion, but rather it must be packaged into a repeating structure, as indicated by the X-ray diffraction pattern, so that the same motif can be used many times. This is the obvious cause of the nuclease digestion pattern (Hewish and Burgoyne, 1973; Burgoyne, Hewish and Mobbs, 1974; Noll, 1974; Kornberg, 1974) and, as already described, the repeating unit is the 200 base pairs of DNA with nine histone molecules. This subunit is called the "nucleosome" (Oudet, Gross-Bellard and Chambon, 1975) and appears to be the fundamental unit for the structure of all chromatin. The picture is, however, complicated by the fact that not all nucleosomes are identical, even within a given nucleus. One obvious variable is the exact length of the DNA in the nucleosome. While the general nature of the nucleosomal repeating structure was being established, it rapidly became clear that the repeat length varied from about 198 base pairs, as found in many cells, up to 210 base pairs in some metabolically less active nuclei (e.g. the nucleated erythrocytes of birds or amphibia) and down to 163 base pairs in more active nuclei (e.g. yeast cells or the neuronal cells from brain) (Morris, 1976; Thomas and Thompson, 1977; Lohr et al., 1977; Weintraub, 1978). More recently a range of repeat length has even been found within individual nuclei (Prunell and Kornberg, 1982), although the general repeat is still characteristic of the particular cell type.

Whatever the cell type, digestion with micrococcal nuclease reduces the DNA length from the full-length monomer, through a brief plateau around 165 base pairs, to an end product of 147 base pairs (Fig. 1). Only substantially more extensive digestion causes further degradation of the DNA of these "nucleosome cores", which are therefore relatively stable. The cores contain this discrete length of DNA and two molecules each of the four "core histones", H2A, H2B, H3 and H4 (Sollner-Webb and Felsenfeld, 1975; Axel, 1975; Bakayev et al., 1975; Shaw et al., 1976). H1 appears to be lost concomitantly with digestion below 165 base pairs (Noll and Kornberg, 1977), and the particle containing this length of DNA, the eight core histone molecules and one molecule of H1 has been described and called a "chromatosome" (Simpson, 1978). The extra DNA in the nucleosome repeat, which joins the nucleosome cores or, perhaps more precisely, the chromatosomes, is frequently referred

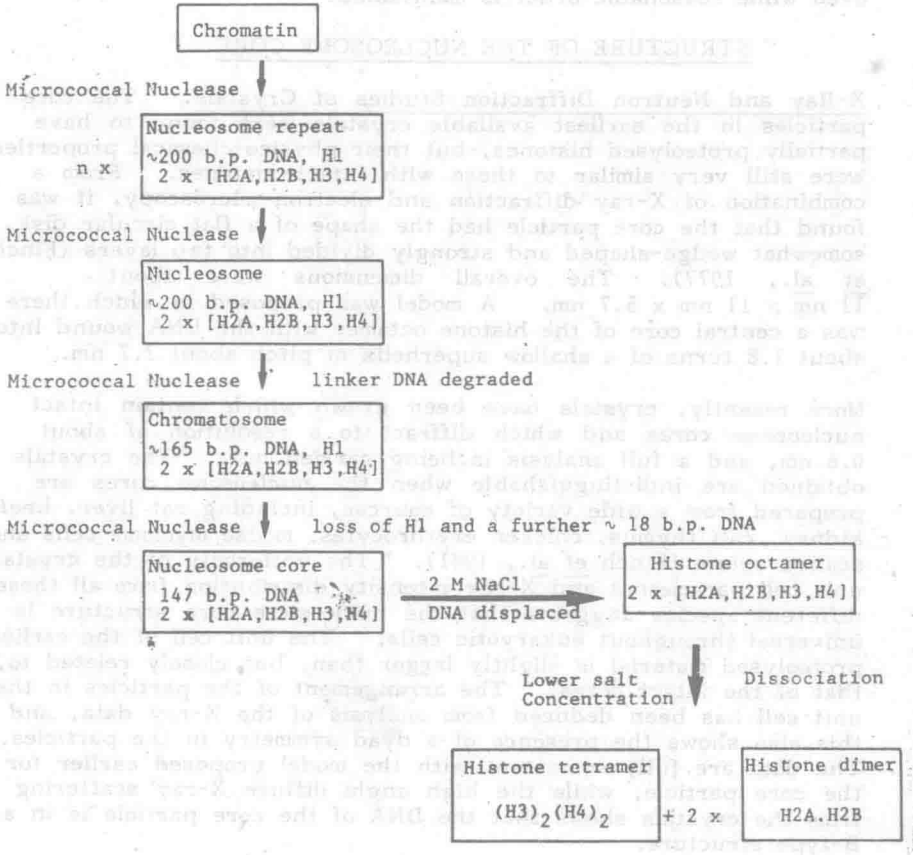

Fig. 1 Schematic diagram of breakdown of chromatin to subunits by nuclease digestion and dissociation of subunits with salt.

to as "linker DNA". However, in native chromatin at normal ionic strengths it certainly is not stretched out, but is structured into the 10 nm fibres. It appears to be variation in the length of this linker DNA which allows the differing repeat lengths.

The closely packed nucleosomes form the 10 nm fibre, which can then be further folded to give the 30 nm fibres, as discussed below, and at some stages of the cell cycle to even higher levels of structure up to the chromosomes. Little is known about these highest levels of structure, but it is apparent that, even by the 30 nm fibre, although the structures present are ordered, they are not essentially completely regular, as can be deduced from the fact that the bands in the X-ray patterns are relatively broad. Thus while the nucleosome cores, and perhaps also the chromatosomes, appear to have an unvarying structure, the varying lengths of the linker DNA mean that the nucleosome repeat is not constant and the regularity falls off further on going to higher levels of structure, even while reasonable order is maintained.

STRUCTURE OF THE NUCLEOSOME CORE

X-Ray and Neutron Diffraction Studies of Crystals. The core particles in the earliest available crystals were found to have partially proteolysed histones, but their physico-chemical properties were still very similar to those with intact histones. From a combination of X-ray diffraction and electron microscopy, it was found that the core particle had the shape of a flat circular disk, somewhat wedge-shaped and strongly divided into two layers (Finch et al., 1977). The overall dimensions were about 11 nm x 11 nm x 5.7 nm. A model was proposed in which there was a central core of the histone octamer with the DNA wound into about 1.8 turns of a shallow superhelix of pitch about 2.7 nm.

More recently, crystals have been grown which contain intact nucleosome cores and which diffract to a resolution of about 0.6 nm, and a full analysis is being carried out. The crystals obtained are indistinguishable when the nucleosome cores are prepared from a wide variety of sources, including rat liver, beef kidney, calf thymus, chicken erythrocytes, mouse myeloma cells and scallop sperm (Finch et al., 1981). The uniformity of the crystal unit cell parameters and X-ray intensity distribution from all these different species suggests that the nucleosome core structure is universal throughout eukaryotic cells. The unit cell of the earlier proteolysed material is slightly larger than, but closely related to, that of the intact cores. The arrangement of the particles in the unit cell has been deduced from analysis of the X-ray data, and this also shows the presence of a dyad symmetry in the particles. The data are fully consistent with the model proposed earlier for the core particle, while the high angle diffuse X-ray scattering from the crystals shows that the DNA of the core particle is in a B-type structure.

Nucleosome core particles are very suitable for study by neutron scattering using the contrast variation method (described in the

next paragraph), as they contain approximately equal proportions of protein and DNA. Several groups have investigated the scattering from solutions of core particles (Richards et al., 1977; Hjelm et al., 1977) and the model described above is consistent with the solution scattering data (Pardon et al., 1978; Suau et al., 1977). However, in solution scattering all of the three-dimensional information is compounded into one-dimensional data and this has the great disadvantage that solution scattering does not show the orientational relations in the particle of the different features which give rise to the diffraction pattern. This problem does not arise in crystal diffraction and a low angle neutron diffraction study of crystals of nucleosome cores has recently been reported (Finch et al., 1980; Bentley, Finch and Lewit-Bentley, 1981).

At low resolution (to about 2 nm) the protein, DNA and solvent components of the crystals scatter neutrons (or X-rays) as uniform domains of different contrast. Thus by changing the D_2O content of the solvent, its scattering power for neutrons can be varied relative to those of the protein and DNA. Hence at 39% D_2O the solvent scattering matches that of protein and only the DNA is effectively scattering, while at 65% D_2O only the protein effectively scatters. Fourier maps have been calculated, to a resolution of about 2.5 nm, for the three principal projections of the nucleosome core, at contrasts of 39% and 65% D_2O, to show the distributions of the DNA and protein components separately (Finch et al., 1980; Bentley, Finch and Lewit-Bentley, 1981). The maps at 39% D_2O

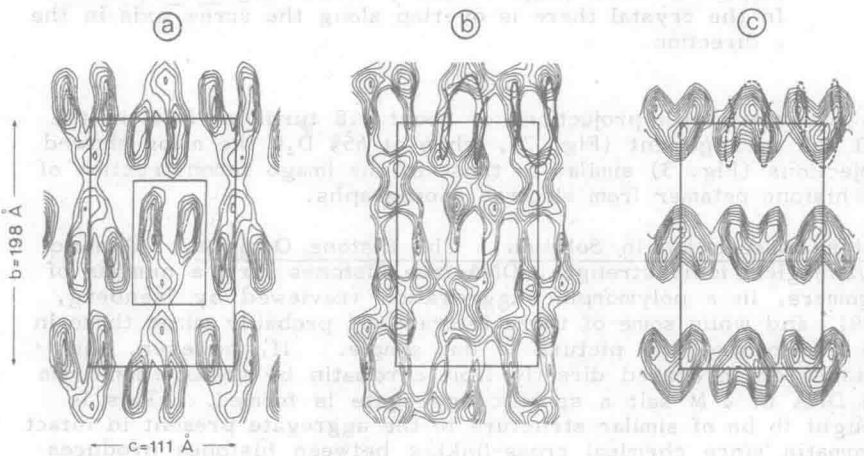

Fig. 2 Fourier projection maps of nucleosome cores. (a) map from X-ray data, with both DNA and protein visible; (b) the DNA component (neutron scattering in 39% D_2O), with the path of the superhelix superimposed; (c) the protein core component (neutron scattering in 65% D_2O). From Finch et al., 1980.

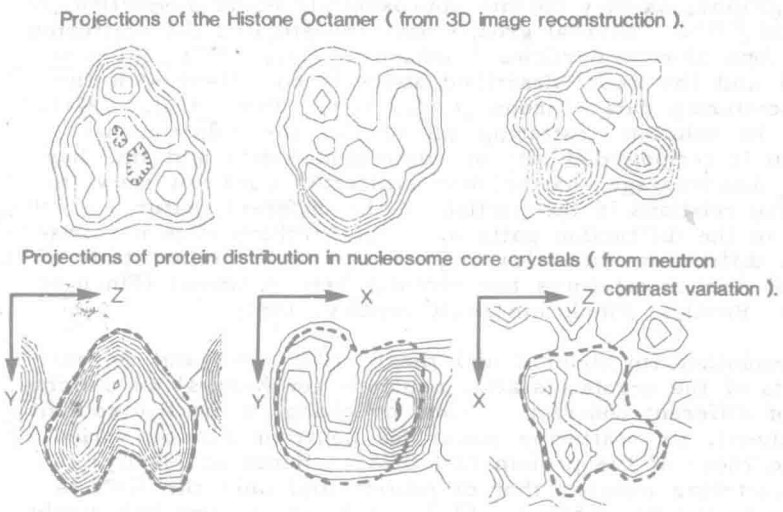

Fig. 3 Comparison of projections of the protein core in the directions of the crystal axes obtained from neutron scattering (65% D_2O; from Bentley et al., 1981) and those of the three-dimensional image reconstruction of the histone octamer (Fig. 5 below; from Klug et al., 1980). In the crystal there is overlap along the screw axis in the c direction.

are consistent with projections of about 1.8 turns of DNA in the unit cell arrangement (Fig. 2), while at 65% D_2O the maps showed projections (Fig. 3) similar to those of the image reconstruction of the histone octamer from electron micrographs.

Histone Association in Solution - The Histone Octamer. Around physiological ionic strength, DNA-free histones form a number of oligomers, in a polymorphic aggregation (reviewed by Isenberg, 1979), and while some of these interactions probably mimic those in the nucleosome, the picture is not simple. If, however, the histones are prepared directly from chromatin by dissociation from the DNA in 2 M salt a specific aggregate is formed. This is thought to be of similar structure to the aggregate present in intact chromatin since chemical cross-linking between histones produces very similar patterns in both cases (Thomas and Kornberg, 1975a). Moreover, the cross-linked aggregate can be reassociated with free DNA to regenerate many of the properties of chromatin (Stein, Bina-Stein and Simpson, 1977).

The earliest cross-linking studies, in which the aggregate free in solution in 2 M salt was shown to be similar to the state in native chromatin, showed that during a time course, the monomeric

histones became cross-linked in an octameric aggregate and so it was proposed that the histones were associated as an octamer (Thomas and Kornberg, 1975a). Moreover, it was shown by use of a cleavable cross-linking agent, that the octamer contained two molecules of each of the core histones, H2A, H2B, H3 and H4, and the pattern of dimeric contacts between them was also determined (Fig. 4) (Thomas and Kornberg, 1975b). The existence of this octamer free in solution was however questioned (Weintraub, Palter and Van Lente, 1975), and it was suggested to be an artefact caused by the cross-linking together of two "heterotypic tetramers", each containing a single copy of each core histone.

Such a "heterotypic tetramer" has been invoked in a number of models for the action of chromatin (Weintraub, Palter and Van Lente, 1975; Weintraub, Worcel and Alberts, 1976). Its existence appeared to be supported by other studies, either as the only

	H2A	H2B	H3	H4
H2A		dms zl ma	t-Pt	dms
H2B	dms zl ma			dms zl ma t-Pt
H3	t-Pt		dms S-S ma	dms zl
H4	dms	dms zl ma t-Pt	dms zl	

dms dimethyl suberimidate (12 Å)
ma methyl acetimide (~ 4 Å)
zl "zero-length" linker
S-S disulphide by oxidation
t-Pt trans isomer of $Cl_2(NH_3)_2Pt(II)$

Fig. 4 Pattern of the histone-histone cross-linking with short cross-linking agents. (References to the individual experiments are given by Klug et al., 1980, and also Lippard and Hoeschele, 1979.)