ON THE BIOLOGICAL IMPLICATIONS OF CHROMATIN STRUCTURE

BRUCE ALBERTS,
ABRAHAM WORCEL and HAROLD WEINTRAUB

Department of Biochemical Sciences,
Princeton University, Princeton, New Jersey, U.S.A.

INTRODUCTION

As revealed by the talks at this Symposium, the general structure of mammalian chromatin now appears to be well established: the DNA is periodically folded by an octameric complex of the four histones H2A, H2B, H3 and H4 (Thomas and Kornberg, this Symposium), creating a string of particulate DNA-protein complexes called "nu bodies" or "nucleosomes" (75). These nucleosomes are in turn packed into regular higher-order structures possibly through the participation of histone H1 and other chromosomal proteins (e.g., see Bradbury, this Symposium; Fasman *et al.*, this Symposium).

The nucleosome is sometimes viewed as a purely structural element of chromatin, serving to condense the DNA without having any special role in the control of gene expression. However, there are several observations that suggest that this view may be incorrect. The 4 intranucleosome histones have a very strong DNA affinity, and several experiments in the literature suggest that they rarely (if ever) dissociate) from the particular DNA molecule to which they are bound within the cell (4, 15, 99). Thus, the *potential* exists for modulating gene activity via changes in nucleosome structure, as others have previously inferred (1, 2). Such a modulatory role for the nucleosome seems likely in view of the programmed changes in H2A and H2B primary structure during embryonic development (26), as well as the abundance and variety of observed histone modifications (acetylations, phosphorylations, and methylations). All of these mechanisms create a nucleosome heterogeneity which must have a central biological function, especially in view of the unique evolutionary stability of the

histone primary sequences upon which they impinge (see Delange and Smith (33) for reveiw).

A second less obvious potentiality follows directly from the first. Considering the fact that histones H2A, H2B, H3 and H4 are metabolically stable (45) and unlikely to leave the DNA during DNA replication, any alteration of nucleosome structure created in one cell generation is likely to be passed directly from parental to progeny genomes. In certain situations the structure of new nucleosome components, and thereby the potential for gene expression at a locus, may be principally determined by these old nucleosomes. In these cases, altered gene structures can be directly inherited in a clonal fashion, persisting long after the regulatory proteins which induced them are gone. Histones could thereby play a central role in cell determination and development despite their limited primary sequence heterogeneity (26, 96).

In this communication, we discuss these possibilities in the context of what is known about chromatin structure, beginning with a new general model for nucleosome structure based upon two symmetrically-paired "half-nucleosomes" (for details, see Weintraub *et al.* (104)). The view of the nucleosome to be presented is consistent with the idea that the nucleosome consists of DNA gently coiled about a spherical protein core (9, 59, 98) and suggests that its two halves might transiently unpair to uncoil the DNA and allow genetic readout without histone displacement. In addition, the dyad axis postulated for the entire particle (protein plus DNA) generates a potential set of intra- and internucleosome pairing interactions by which half-nucleosomes may be able to act as templates for information transfer, both across generations ("vertical templating" from parental to daughter chromatin) and along the same DNA helix ("horizontal templating" within a gene).

In what follows we wish to emphasize the idea that a system for direct communication between nucleosomes exists, which is likely to have profound implications for the mechanism of eukaryotic gene regulation. Such a system may cause the spreading of specific histone modification patterns in a cooperative fashion both within a gene and across generations in that same gene. We use the nucleosome model described and the existence of higher order structures to illustrate how this could occur, but wherever possible we shall stress the general validity of the principles rather than the details.

A NUCLEOSOME MODEL

It is simple and elegant to express the whole thing by saying,
"Well, there must be symmetry in the model".
J. Monod in: *The Creative Process in Science and Medicine (1975)*

The Nucleosome is Likely to be Constructed of Two Symmetrically-paired Half-nucleosomes

A nucleosome contains 140 base pairs of DNA (6, 89, 93) which must be condensed by 8 histones, 2 each of H2A, H2B, H3 and H4 (95; Thomas and Kornberg and this Symposium), into a roughly spherical particle with a radius of gyration of about 50 Å for the DNA and 30 Å for the protein (9, 79; Bradbury, this Symposium). Thus, most of the DNA must be wrapped about the protein core. As we shall discuss more fully later, a single left-handed DNA coil, about 95 base pairs in circumference, could account both for the dimensions of the particle and for the observed negative DNA superhelicity induced in closed-circular DNA by histone removal (39).

We are led by the occurrence of one pair of each histone (H2A, H2B, H3 and H4) per nucleosome to assume that the protein core is organized about a dyad axis. Thus, each octameric histone core is composed of two heterotypic tetramers, with each tetramer containing one each of the 4 histones. Such a tetramer has been observed in solution, and appears to display many of the biochemical properties of histones in chromatin (e.g. accessibility to iodination, trypsin digestion, and formaldehyde crosslinking; 100). The two heterotypic tetramers maximize their protein-protein interactions in a closed structure if each heterotypic tetramer is paired isologously with its partner (69). With the reasonable assumption that the DNA binds identically to each of the two equivalent heterotypic tetramers, the entire particle (including the coiled DNA) will be symmetrically disposed about the histone dyad axis.

It is clear that histone complexes greatly prefer to coil DNA so as to underwind rather than overwind the helix (39) and therefore, following our reasoning, they must induce a large excess of left-handed over right-handed toroidal DNA coils (see Figure 1 of Bauer and Vinograd (10) and Figure 13 of Worcel and Burgi). (106) It seems likely that the reason for this bias lies in specific histone DNA interactions. The

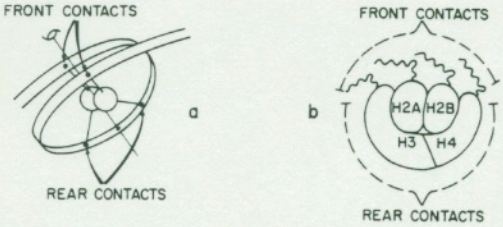

Figure 1: Schematic representation of a nucleosome model based upon two symmetrically paired half-nucleosomes. *(a) Outline of the minimal structure. Two identical heterotypic histone tetramers (shown as spheres) are paired about a dyad axis and make separate "front" and "rear" contacts to a symmetrically arranged, left-handed DNA coil. Note that the two rear contacts will determine the handedness of the DNA coiling. Seen from a rear view (see Figs 2b and 4) the left-handed DNA coil follows a path through the rear nucleosome contacts which goes from upper right to lower left. (b) A possible histone arrangement in the heterotypic tetramer. This cyclic arrangement maximizes the known pair-wise interaction patterns* (32). *The primary sequence of each individual histone indicates that H2A, H2B, H3, and H4 each contain 20-30 amino acids at the* NH_2 *terminal end which are highly enriched in basic residues. NMR data indicates that these* NH_2 *termini are unoriented when not bound to DNA* (16) *and since trypsin is capable of specifically removing them from the heterotypic tetramer* (101), *we assume that they extend as "fingers" outward from a globular core. It is likely that these fingers bind to negative sites on the DNA backbone* (16, 63, 101). *In addition, even though the trypsin-resistant portion of each histone molecule ("core") has an amino acid composition like that of most globular proteins* (51), *these cores likewise appear to bind to DNA* (17, 63, 101, 109). *One possible arrangement of histone DNA binding sites is shown in which the fingers from each of the 4 histones in the tetramer are grouped together to form a set of front contacts, arranged in a linear array. In this case, a separate region of each heterotypic tetramer composed predominantly of histones H3 and H4 would form the rear contacts, inasmuch as Camerini-Otero* et al. (20) *have elegantly demonstrated that these two histones alone can organize the DNA into nucleosome-like structures. This general type of arrangement is supported by data from combined nuclease and trypsin digestions of nucleosomes (102; Weintraub, in preparation).*

minimal structure with a dyad axis of symmetry in which the protein core is able to distinguish coil handedness is illustrated in Fig. 1a. Each heterotypic tetramer (which, together with the associated DNA, makes up the asymmetric unit of the nucleosome henceforth referred to as "half-nucleosome") is shown bridging the DNA coil to form links to two different regions of DNA helix; we denote the DNA binding domain towards the end of the DNA coil as the "front contacts", and the binding domain closer to the center of the coil as the "rear contacts" of each half-nucleosome. Note that the tetramer must be rigid enough to keep rear contacts out of the plane passing between the two half-nucleosomes, and thus produce a left-handed "curvature" in the DNA binding site, since without this handedness in the protein core, left- and right-handed DNA coils should be generated in equal numbers by an octamer (see legend for Fig. 1).

The structure of the heterotypic tetramer which must form the protein core for each half-nucleosome can only be approximated at this point in our knowledge. We have used the pairwise histone interaction patterns (32) and the crosslinking data on chromatin bound histones (46) to construct the cyclic heterotypic tetramer ⌐H2A-H3-H4-H2B¬ schematically illustrated in Fig. 1b. This heterotypic tetramer was first proposed by D'Anna and Isenberg (32), and is likely to be the major structure observed in solutions of high NaCl by Weintraub, Palter and Van Lente (100). Rather than the limited DNA contacts minimally required (Fig. 1a), both front and rear DNA contacts are shown as extending all around the tetramer in Fig. 1b. This is suggested both by the presence of multiple DNA binding sites on each histone, and by the fact that most of the DNA wound about each nucleosome core is protected from staphylococcal nuclease digestion (6, 89, 93).

Several views of a schematic space filling model (non-atomic) that incorporates the main features of the basic nucleosome structure we propose are illustrated in Fig. 2. This model uses the heterotypic tetramer and DNA contacts just described (Fig. 1b). The side view of the intact nucleosome (Fig. 2a) is oriented to show the front contacts from each histone, each of which is shown as a finger which follows the DNA helix for a single turn. The rear view (fig. 2b) shows how the pairing of half-nucleosomes could generate an H3-H4 tetramer which organizes and covers the rear of the DNA coil. An essential role for such a tetramer in organizing the nucleosome was postulated by R. Kornberg (59) and demonstrated by the chromatin reconstitution studies of Camerini-Otero *et al.* (20).

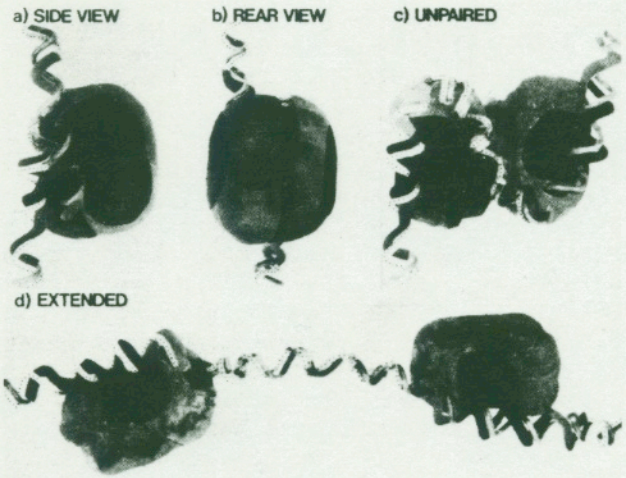

Figure 2. A scale 3D model of the general type of nucleosome structure based upon two symmetrically paired half-nucleosomes. *The model was constructed by adjusting for the known volume of DNA and histones. The DNA mass (140 base pairs) was multiplied by its partial specific volume* ($\bar{\nu}$ = *0.53). The known mass of each histone was multiplied by its partial specific volume* ($\bar{\nu}$ = *0.75). One DNA strand is darker; the other, lighter, is dotted. Note the proposed single strand preference of the histone fingers in each half-nucleosome and the two-fold rotational axis of symmetry in the whole particle (protein plus DNA) which is apparent in all of these views.*

Histone code in increasing shades of darkness: histone H4 < histone H3 < histone H2B < histone H2A. It should be noted that the heterotypic tetramer (see Fig. 1b) is oriented so as to place the NH_2 *terminal fingers of histone H3 at the ends of the 140 base pair DNA coil. This assignment of the H3 fingers is chosen to account for their unusually high protease sensitivity* (25, 72, 101) *and for the observation that nucleosomes from which the H3 fingers have been predominantly removed with trypsin produce a staphylococcal nuclease limit-digest pattern that is similar to that obtained from control nucleosomes, except for the absence of DNA fragments of 140 and 130 base pairs (H. Weintraub, in preparation; see also Fig. 4 of Weintraub and Van Lente* (101)*).*

DNA Supercoiling

When nucleases cut *within* the nucleosome, they produce a series of cleavages at intervals of 10 bases (20, 70). These results, as well as the X-ray diffraction data (105), suggest that the coiled nucleosomal DNA is in the B-conformation with 10 base pairs per turn of helix.

The DNA coiling shown in Figs 1 and 2 will result in superhelical turns in protein-free DNA. Recent data on the SV40 minichromosome reveal a ratio of about 1.2 negative superhelices per nucleosome after histone removal (30, 44, 58, 90), and studies of *Drosophila* indicate that this chromatin is similarly coiled (12). This value represents the result of DNA coiling about the nucleosome, as well as possible superhelical turns introduced as a consequence of the regular higher order packing of nucleosomes (21, 37, 76). Ignoring this latter term, and assuming that the DNA within the nucleosome remains in the B-conformation, the symmetrically paired half-nucleosome model suggested can readily account for anywhere between 0.8 and 1.5 DNA superhelical turns per nucleosome, the exact value being a function of the angle which the DNA entering the nucleosome on one side makes with the DNA leaving the nucleosome on the other side (see Fig. 1a). Electron microscopic observations (75, 77) indicate that the internucleosome DNA fiber is not significantly bent as it passes through the nucleosome, in which case the actual number of DNA turns per nucleosome will be about 1.0, as shown in Figs 1a and 2a.

The Unpairing of Half-nucleosomes

The 4 intranucleosome histones are metabolically stable (45) and probably rarely (if ever) dissociate from the DNA molecule to which they are bound within the cell (4, 15, 99). Yet it seems unlikely that the tightly compacted DNA within a nucleosome can serve as a template for either RNA or DNA polymerase directly. Instead there must be some transient change in the nucleosome which first allows the DNA to uncoil within it, and then permits the two DNA strands to separate for transcription and replication processes. In the symmetrically-paired half-nucleosome model, DNA could in principle uncoil either by complete release of the front histone-DNA contacts (see Fig. 1a), or by half-nucleosome unpairing accompanied by at least partial disruption of the rear contacts.

The half nucleosome unpairing mode of opening is diagrammatically illustrated in Fig. 2. Two different unpaired

conformations of half-nucleosomes are shown (Figs 2c, d). The fully extended form is illustrated in Fig. 2d. Notice, however, that unpairing may take place without complete disruption of the rear contacts, and that this might suffice for genetic readout (Fig. 2c).

This mode of DNA uncoiling by half-nucleosome unpairing seems preferable to the alternative in which the DNA is uncoiled by releasing the front contacts of the octamer, since it can produce a structure in which the bound histones need not interfere with helix opening or templating processes. This would require that, once unpaired, each histone heterotypic tetramer be bound primarily to only one strand of the DNA helix, so as not to cross-link the two DNA strands. In addition, the two DNA strands must be free to rotate about each other, which means that the persisting histone-histone contacts in the tetramer must be wrapped around the helix, following the contour of the bound strand (not shown in the schematic model in this communication). Alternatively, strand rotation might be achieved by the action of the chromatin-bound nicking closing enzyme (7, 57) or by a transient rotation of the individual histones in the heterotypic tetramer relative to each other as the polymerase passes, without their leaving the DNA. In any case, a clear precedent for genetic readout over bound protein has been experimentally established with DNA-unwinding proteins, which bind very tightly to DNA single strands without covering the bases or interfering with templating processes (3, 50, 91).

The above nucleosome model allows for DNA uncoiling and recoiling with minimal loss of the histone-DNA and histone-histone interactions. A local unwinding of the DNA helix, such as the one generated by the transcribing RNA polymerase (85) will uncoil the left-handed toroidal DNA supercoil and tend to open the nucleosome (12). We therefore suggest that nucleosomes tend to remain closed until the polymerase approaches, and once it has passed, they will close again.

Internucleosomal "Spacer" DNA

Each pair of half-nucleosomes will be separated from the neighboring pairs by an internucleosome spacer DNA. A spacer about 60 base pairs long is required in order to give a total value for the nucleosome repeat distance of 200 base pairs (140 base pair core plus 60 base pair spacer), as reported by Hewish and Bourgoyne (47) and Noll (71). The spacer length, presumably determined by the interaction of the nucleosomes with each other and with histone H1 and other chromosomal

proteins, is not defined by the basic nucleosome model and need not be universal (see Chambon, this Symposium; Thomas and Kornberg, this Symposium).

The nuclease susceptibility of the internucleosomal spacer DNA indicates that it must be much more accessible than the DNA within the nucleosome (6, 89, 93, Zachau *et al.*, this Symposium). Proteins which recognize specific DNA sequences should find them most readily in such exposed spacer regions. Thus, unless nucleosomes are uniquely phased relative to the DNA sequence (which seems unlikely: 81; Yaniv, personal communication), some nucleosome sliding would seem necessary *in vivo* to release from within the nucleosome all specific DNA sequences which are presumably recognized by control proteins. Our nucleosome model in principle allows the DNA helix to slide through the protein portion of each nucleosome without major disruption of nucleosome structure. As the DNA rotates relative to the histones, internucleosome "spacer" DNA enters the DNA coil inside the nucleosome, while an equal length of DNA ·aves the nucleosome to enter the spacer region behind.

Implications of Symmetry

The 2-fold rotational axis of symmetry of the entire particle (protein plus DNA) must coincide with a dyad axis of the DNA helix as it passes through the back side of the DNA coil, and must bisect this coil into two equal halves (this is required to make the specific DNA-histone contacts identical in each half-nucleosome). Because of the observed radius gyration of 50 Å for the DNA (9, 79, Bradbury, this Symposium), we have utilized in Fig. 2 a circumference of 95 base pairs as an approximation, chosen to create a ring of DNA in which the 20 Å wide helix sits between 40 Å and 60 Å from the center of mass (see Fig. 5 of Pardon *et al.* 79). Since an extra 45 base pairs of DNA can be protected by the front histone contacts on either side of the symmetry axis, this type of model can readily account for the observed monomer core particle of 140 base pairs protected from nuclease digestion (6, 39, 93, see Fig. 2, Weintraub *et al.*).

The 2-fold rotational axis of symmetry has profound structural implications. First of all, it demands that unpaired half-nucleosomes strictly alternate in their polarity along the extended DNA helix (see Fig. 2d and Fig. 6, below). Thus, each half-nucleosome has the choice of pairing with either of two neighbors. The two paired structures, designated as "flip" and "flop" nucleosomes respectively, are similar, with ·e "flip" spacer DNA becoming intranucleosomal in the "flop"

state and vice versa. However, the sense of the DNA coil is reversed: the "flip" nucleosome is the native form previously described and has the left-handed DNA coil seen in Figs 1 and 2; the "flop" nucleosome, in contrast, contains a right-handed DNA coil (see Fig. 4). Because of its different sense of DNA coiling, the "flop" nucleosome has different histone-DNA rear contacts, and therefore will not be energetically favored. Nevertheless, evolution may have selected for a half-nucleosome which can transiently switch to the flop state in order to provide a direct means of communication between adjacent nucleosomes. As discussed below, this flip-flop transition could have important biological implications, serving to propagate site-specific signals through adjacent regions of chromatin.

A second implication of the symmetry axis is illustrated in Fig. 2 (see also Fig. 2 of Weintraub *et al.*, 104). If each heterotypic tetramer binds predominantly to only one of the 2 strands of DNA helix as we have suggested, the two heterotypic tetramers which make up each particle will be preferentially bound to opposite DNA strands (i.e., one tetramer to the white dotted strand and the other to the grey unmarked strand in Fig. 2). Given this assumption, and the alternating polarity of the heterotypic tetramers along the chromatin just described, the *strand preference* of the heterotypic tetramers will alternate all along an extended DNA helix (see Fig. 2d and Fig. 6, below).

HIGHER ORDER CHROMATIN STRUCTURES

> *We believe a gene - or perhaps the whole chromosome fibre - to be an aperiodic crystal.*
>
> *E. Schrödinger, What is Life? (1944)*

One Gene - One Microcrystalline Array of Nucleosomes?

As discussed by others, in this Symposium and elsewhere (21, 37, 76), nucleosomes tend to stack on each other to form regular helices. These helices may be further folded upon each other in regular arrays in chromatin, in which neighboring helices may interact side by side. Helices are themselves in essence two-dimensional crystals, as is most clearly seen by their representation on planar "helical nets" (23). Direct interactions between nucleosomes in these regular arrays would occur on the nucleosome outer surfaces, which could help

explain why even the amino acid sequences which must correspond to this surface of the nucleosome are highly restricted (as witnessed by the unique stability of the entire histone sequence during evolution; 33).

In the giant chromosomes of Dipteran insects, each of the 5,000 discretely-folded DNA bands corresponds to roughly one unit of function or "gene" (48, 55). A remarkable literature exists on these fascinating bands, which we believe to be of general relevance to the present discussion (reviewed in Ashburner 5 and Beerman 11). Most strikingly, different bands not only can be distinguished and mapped (18), but in addition they differ in their detailed structure (11, 94) and in their sensitivity to chemically-induced unfolding. Thus, for example, the chromatin packing in some bands is disrupted by exposure to high concentrations of (Mg^{2+}); for other bands high (K^{+}) is effective while still other bands are unravelled by high (Na^{+}) (reviewed in Lezzi and Robert 62). This chemical uniqueness to the banding pattern implies a certain homo-neity of chromatin structure within each band, despite the on-homogeneous DNA sequence which each band must contain.

We suggest that each band in a giant chromosome is generated by a different microcrystalline array of nucleosomes, with differences between bands being generated both by variation in nucleosome structure (including changes in the covalent modification pattern of the histones) and by differences in the structural non-histone proteins (see, for example, Goodwin and Johns, this Symposium). In this view, each band contains a single type of repeating unit, which is slightly different from the repeating unit in neighboring bands, and which causes it to prefer a different packing arrangement of nucleosomes. By utilizing *combinations* of variations in the histone and non-histone proteins to construct each band, the diversity in band structure (postulated to be generated by 5000 different DNA sequence-specific structural proteins by Mayfield and Ellison (68)) can be generated with relatively little genetic investment. The fact that identical chromosome bands on two homologous polytene chromosomes have a strong mutual affinity for each other (34, 68, 78) is then simply a manifestation of some of the same crystal packing forces (i.e., the regular side by side packing of homologous helices) which gave the band its condensed morphology in the first place.

How many different microcrystalline structures might exist in chromatin? At one extreme each of the 5000 polytene chromosome bands in *Drosophila* might be different, but this hardly seems necessary or reasonable. At the other extreme, there might be as few as 10 different types of band structures, in

which case ectopic pairing maps (27, 56, 92) might be useful for identifying homologous crystalline arrays.

Cooperativity

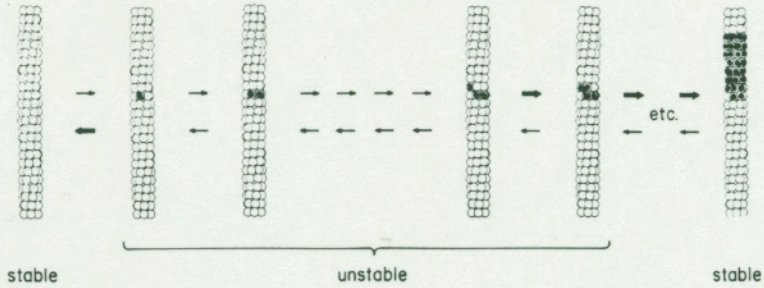

Figure 3. The cooperative nature of changes in chromatin. *A helix of nucleosomes of arbitrary dimensions is illustrated. Modified nucleosomes are shown as black dots and are assumed to perturb the regular packing of white nucleosomes, while generating a black-black interaction. The relative width of the paired arrows indicate relative rates of forward and backward reactions, the actual rates depending on the exact type of modification being introduced; in this case, we have assumed that the black-black interactions are more stable than the white-white interactions and/or that the white to black modification process for each isolated nucleosome is energetically favorable. Those changes could be initiated by any non-histone protein which interacts with DNA and/or histones. For simplicity, spreading of the white to black modifications in only one direction from the initial perturbation is shown; in actuality, a bidirectional spread seems probable.*

If each gene in a higher eukaryote is analogous to a tiny crystal (with different genes containing nucleosomes packed together in distinctly different regular arrays) most changes in nucleosome structure and packing accompanying biological function will be resisted by lattice forces. As schematically illustrated in Fig. 3, altering a single nucleosome in a region is predicted to be quite difficult, since this will destroy regular interactions with neighbors on all sides. Moreover, not until at least one entire helical turn of nucleosomes has been altered does continuing the process reach a point of minimal difficulty. For example, with n nucleosomes

per turn, the white to black change diagrammed in Fig. 3 might disrupt four white to white contacts for the first altered nucleosome; 3 contacts for the second through nth nucleosome, and only 2 contacts per nucleosome thereafter. Thus, chromatin structure should always change in a highly cooperative manner, i.e. changes will be very difficult to initiate, and where successful will encompass multiple helical turns of contiguous nucleosomes.

GENE REGULATION BY CONTROL OF CHROMATIN STRUCTURE

> *The nature of the changes responsible for (the "spreading effect") along the chromosome is of considerable importance for an understanding of the manner by which controlling elements can induce their effects.*
>
> *B. McClintock* in: *Cold Spring Harbor Symposia on Quantitative Biology, (1956)*

Gene Structure Will Influence Gene Expression

Control of gene expression in prokaryotes is built upon two basic principles elucidated by the pioneering work of Jacob and Monod (53): (a) freely diffusible proteins exist in prokaryotes which recognize and bind tightly to specific DNA sequences; and (b) by affecting the RNA polymerase these DNA-bound regulatory proteins either block early stages of transcription (lac, gal, tryp, λ, cro) or make possible the initiation of transcription (CAP, ara C) of adjacent genes located downstream. It is not known whether these mechanisms also operate in eukaryotic cells, but several lines of evidence suggest that additional principles are involved. First, eukaryotic DNA, unlike prokaryotic DNA, is tightly packaged and covered by histones, as previously discussed. Second, eukaryotes have 10 to 100 times the amount of DNA needed to code for their essential proteins (48, 55, 74); this extra DNA is likely to have some, as yet undefined, role in the control of gene expression (19, 31). Third, many eukaryotic messenger RNAs appear not to be synthesized as unit length, finished chains as in prokaryotes, but rather as part of a much longer heterogeneous nuclear RNA precursor molecule.

These eukaryotic features may be correlated with a mechanism of gene regulation based upon differences in gene structure. Different classes of regulatory proteins may bind to the DNA either (a) at a specific DNA sequence, (b) where the

chromatin has a particular microcrystalline structure, or (c) in a completely non-specific manner. In each of these cases, the regulatory proteins might immediately induce a local modification of the chromatin where they are bound. However, only if the particular gene structure in the vicinity of the chromatin-ligand complex is receptive, would they then cause some more extensive structural change which affects that region's potential for active RNA transcription.

Following this reasoning, the extra DNA in eukaryotes may be needed to set up a microcrystalline array of nucleosomes in each genetic region. One might speculate that the longer the DNA in a given "gene", the more reliable the control based on differential gene structure will be; in contrast, control based solely on DNA sequence specificity will be hampered as the DNA length increases because of the competing non-specific DNA binding sites added.

Cell determination may consist of an ordered series of changes in the structure of crucial controlling genes, which leads to cells with different gene structures and therefore different genetic potentialities. Thus, in the extreme view, two levels of control would exist in eukaryotes: the first, that which McClintock has termed gene "*setting*" (67), occurs relatively early in development and determines cell potentialities; while the second level of control involves the activation of a preset program, and results in actual cell differentiation and the production of recognizable tissue-specific proteins. The general underlying principles by which these two levels of control operate may be similar, with a cascading series of intermediate control levels between the two extremes. For this reason, we have not attempted to distinguish between gene setting and gene activation in the following discussion, even though the two mechanisms could be somewhat different.

A Spreading Effect via "Horizontal Templating"

For reasons discussed previously, the lattice forces in highly organized regions of chromatin will resist the distortion in nucleosome structure required for RNA polymerase readthrough. Thus, gene activation may require a loosening of the higher order chromatin structure which *precedes* polymerase travel as originally suggested by Allfrey and collaborators (2, 82). Covalent histone modifications, as well as the interaction of nucleosomes with other non-histone proteins, could modulate gene activity by making possible the *initiation* of RNA polymerase travel through the first helical turn of nucleosomes. Thereafter, the polymerase might be able to continue

on its own. Alternatively, the polymerase might *not* be able to transcribe through normal nucleosomes, even after transcription through the first helical turn of nucleosomes has facilitated a continued change in higher order chromatin packing (Fig. 3). In this case, special nucleosomes would be required throughout the entire genetic region to be transcribed. By either hypothesis, RNA synthesis would be automatically turned off except in genetic regions where regulatory proteins have altered a stretch of contiguous chromatin to an "active patch" conformation. In general, RNA polymerase readthrough could be facilitated both by a reduction in the strength of higher order interactions, and by a modification of the nucleosome itself which directly promotes the unpairing of half-nucleosomes (104). In contrast to this behaviour of the RNA polymerase, the DNA replication apparatus and its associated DNA polymerase must be specially designed so as to be able to open up even the most tightly packed chromatin structures as replication proceeds.

In accord with the above view of RNA transcription requirements, it has recently been convincingly demonstrated that active and inactive regions of the eukaryotic genome differ in their structure. The active regions (constituting perhaps 10-20% of the total genome) contain a restricted subset of the total DNA sequences in a more open, more accessible conformation, as judged by their preferential accessibility to nuclease digestions (41, 103). The active and inactive regions contain similar amounts of histones H2A, H2B, H3 and H4 (80), and both appear to contain DNA regularly folded by histone into the particulate nucleosomes (60). Recent data suggests that there is some alteration of the structure of the nucleosome itself in active regions (42, 103), and that these alterations occur in large "patches" averaging 6000-7000 contiguous DNA base-pairs, which approach the size of an average hnRNA transcription unit (43).

What is the event which alters the conformation of contiguous nucleosomes, many of which must be far (a 6000-7000 base-pair active patch would contain 30 to 35 nucleosomes) from the site occupied by a regulatory protein? Some special mechanism must exist in chromatin to transduce the original signal from nucleosome to nucleosome along the DNA (103, 107). A relatively trivial explanation for this "spreading effect" would be that RNA polymerase itself alters the nucleosomes as it passes by, i.e. that RNA transcription comes first, with all changes in the nucleosome being secondary to transcriptional control processes. We prefer the opposite view, that at least some nucleosome alterations must precede transcription, for several reasons: (a) the direct effects of steroid

hormone receptor proteins on chromatin structure (14, 40, 64) appear to be much more extensive than their direct effects on *in vivo* RNA transcription. This suggests that many non-productive changes in chromatin structure precede productive gene transcription (reviewed in Yamamoto and Alberts (107)); (b) once activated, the hemoglobin gene sequences remain preferentially accessible to nuclease digestion even in mature chick erythrocytes, where hemoglobin mRNA synthesis has been shut off (104); (c) analysis of several experimentally-induced puffing events in *Chironomus* giant chromosomes has suggested that some change in structure of the puffs precedes the onset of RNA synthesis (13); (d) if, as we now propose, the spreading effect generates the type of structural diversity seen in the chromatin of the giant chromosome bands, there must be many *different* types of structures which can propagate from nucleosome to nucleosome along the DNA helix; in contrast, transcription-induced alterations would be expected to be similar for all genes.

How might a specific perturbation of chromatin structure spread in the absence of RNA synthesis? We suggest that covalent modifications of histones are involved, including the striking acetylation of histone N-termini (2, 52, 65, 84). It is suggestive in this regard that histones H3 and H4 of SV40 and polyoma virus chromatin (perhaps examples of active genes) are unusually highly acetylated (86). Likewise, in tetrahymena, the histones of the macronucleus, but not those of the transcriptionally-inactive micronucleus, are acetylated (54), analogous to the difference between the sea urchin embryo and its transcriptionally-inactive histone-containing sperm chromatins (35). Since even minor changes in the amino acid sequence of the 4 histones of the nucleosome are comparatively rare, the change in nucleosome charge and conformation caused by the known covalent modification of these histones (see Delange and Smith (33) for review) must play a central role in nucleosome function.

To conceptualize how such a spreading mechanism could work, we might postulate that steroid receptor proteins and other gene activators act as "triggering proteins" which serve to distort a nucleosome immediately adjacent to their DNA binding sites. This distortion exposes the nucleosome to enzymes which covalently modify histones, with the particular pattern of modification which occurs depending on the type of distortion. To explain the spreading effect, one then postulates that these modified nucleosomes act in turn to destabilize their immediate neighbors, and, provided that the structure of the particular gene is receptive to such a change, a cooperative wave of identical modification of nucleosomes will be set

off in the vicinity of the initial event. The cooperativity would arise from a nucleosome modification altering the regular tertiary interactions which pack nucleosomes into regular helical arrays, as previously discussed (Fig. 3).

The information as to which type of nucleosome modification is to be spread could come from direct like-with-like packing interactions between nucleosomes at adjacent lattice positions in the helix, possibly modulated by histone H1 and accessory non-histone proteins in the structure. Alternatively, since we believe that nucleosomes consists of paired "half-nucleosomes", neighboring nucleosomes may be able to communicate with each other by a transient flip-flop exchange of their halves, as schematically illustrated in Fig. 4. To explain the spreading effect in this scheme, "hybrid nucleosome modification" enzymes are proposed which act to restore

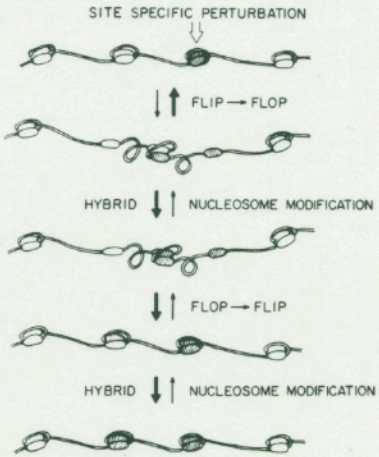

Figure 4. A possible specific mechanism for horizontal templating based on the nucleosome model shown in Fig. 2. *A site specific perturbation introduced by a "triggering protein" initially leads to the modification of a single nucleosome. A modified nucleosome is shown shaded. This perturbation is then propagated by a series of transient flip-flops and hybrid nucleosome modifications as described in the text. Note the opposite handedness of the DNA coil in the flip and flop nucleosome conformations (see also Fig. 2 in Weintraub* et al. (104)*).*

symmetry to a hybrid nucleosome (defined as a nucleosome with one of its two halves already modified). The modified half nucleosome distorts the structure of the nucleosome and makes

the modification of the other half much more likely. In this view, the same drive towards symmetry which makes ligand binding by oligomeric proteins cooperative (69) provides the specificity which ensures that the type of modification which spreads throughout the nucleosomes is identical to that established by the initial triggering protein.

We shall refer to the spread of homologous protein structure along a single DNA helix as "horizontal templating". We propose that, whatever the mechanism, this process may be responsible for the generation of a varied gene structure (i.e., the bands seen in polytene chromosomes) in all cells of higher eukaryotes. To prevent confusion, special DNA sequences must exist at intervals (at the boundary between band and interband) which act as barriers that limit the spreading effect to well-defined regions, thereby segregating the continuoue DNA helix into discrete units of function. Each such unit would tend to become homogeneously folded under the influence of triggering proteins, with folding diversity along the genome generated during cell development by cascades of such elements. Note that we would predict that polytene chromosomes from different cells of the same organism should differ in the details of their band structure at crucial genetic loci. Although there have been reports in the literature to this effect (5, 11, 87), the issue is not clearly resolved.

The folding diversity generated in different genes would serve the biological role of determining which genes are to be activated in the particular cell type as a function of environmental conditions and cell age. Such an intimate linkage between gene structure and gene function is clearly operative for special cases, including the examples of facultative heterochromatinization to be discussed below, and underlies many previous views of the genome (2, 28, 31, 38, 108).

Direct Inheritance of Gene States via "Vertical Templating"

Within a gene. For many if not most activated genes, removal of the triggering protein would be expected to lead to the eventual decay of the nucleosomes in the region back to their original non-modified state, accompanied by loss of gene activation (i.e., in thermodynamic terms, we might assume that the initial shift to a patch of modified nucleosomes represents a shift in the minimum free energy of the system, attributable to the increased binding energy of the triggering protein when the nucleosomes next to its specific DNA site are modified). However, it is clear that such reversible regulation via diffusible triggering proteins fails to account for several well-

known examples of cell determination in which a differential activity of one of two otherwise identical chromosomes is faithfully inherited through cell lineages. Here we include the apparently similar phenomena of X-chromosome inactivation in mammals (22, 66), position-effect variegation in *Drosophila* (8, 61), and asymmetric chromosome imprinting in the coccids (24). These examples of so-called "facultative heterochromatinization" imply that the eukaryotes have developed a type of gene control which allows cell memory to be passed by directly replicable changes in local chromatin structure. A similar mechanism may well regulate the expression of developmentally important genes, and be responsible for both the clonally inherited nature of cell determination and the stability of the differentiated state. Analogous conclusions have been reached by others (29, 49).

In order for an altered gene structure to be directly inherited, the fully modified chromosomal band, once established, must persist even though the triggering proteins are removed. Packing forces could make this possible, generating situation where both the fully unmodified gene and the fully modified gene represent local free energy minima, with mixed states very unstable. Thus, certain partially modified states generated by new histone deposition following DNA replication could always rapidly "recrystallize" back to the fully modified form, the directly inherited "old" nucleosomes providing both the driving energy and the information required for new nucleosome conversion (Fig. 5).

The nucleosome model proposed previously suggests how the inheritance of gene structure might be achieved via the upper pathway shown in Fig. 5. Assuming that each self-complementary heterotypic tetramer is bound primarily to a single-strand on the DNA duplex, as required for efficient genetic readout, each half of a parental nucleosome should segregate to a different DNA strand, as illustrated in Fig. 6. Each of these will then pair with a second heterotypic histone complex from the cell cytoplasm to regenerate the full nucleosome structure. If, and only if, the parental histone was modified, the same hybrid-nucleosome modifying enzymes introduced in Fig. 4 will convert the new histones to match (Fig. 5, upper pathway).

The particular scheme illustrated in Fig. 6 predicts that old histones should tend to segregate with old DNA strands in a Meselson-Stahl type of experiment. However, this preferential strand association need not be permanent, and might decay towards randomness between successive S-phases of the cell cycle. Conflicting data have thus far been obtained in this type of experiment, with some reports showing no preferential strand association of old histones (52b) and others indicating

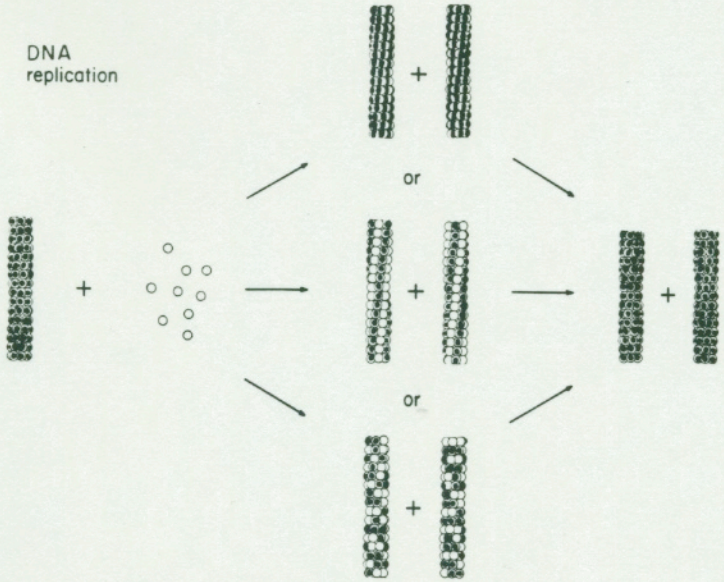

Figure 5. Possible pathways for direct inheritance of gene states for special genes. *A patch of black, modified nucleosomes such as that created in Fig. 3 is shown during a later DNA replication cycle when the non-histone proteins which induced the modification have been diluted out or decayed. New histones (unmodified) are shown as white, and are assumed to make up half of the total histone on each daughter gene. Three different possible histone segregation patterns are shown; top: a regular segregation of half-nucleosomes (see Fig. 6); middle: a regular segregation of whole nucleosomes; bottom: a random segregation of whole nucleosomes. Note that, in all cases, packing forces favoring the mutual interaction of black nucleosomes could drive nucleosome modification equilibria to regenerate the all black state.*

some preference (97 O. Smithies, personal communication). A possible objection to Fig. 6 is that an inherent instability of unpaired half-nucleosomes must be postulated to explain why whole, rather than half, nucleosomes accumulate on the pulse-labelled DNA made in the absence of new histone synthesis (88, H. Weintraub, in preparation).

As shown by the two lower pathways in Fig. 5, inheritance of chromatin structure need not require hybrid nucleosomes as intermediates. Regardless of how old histones are distributed within a gene following DNA replication, the cooperative

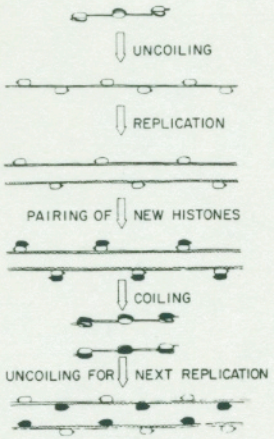

Figure 6. Possible half nucleosome segregation mechanism ısed on the specific nucleosome model shown in Fig. 2. *Old ,stones are shown as white, new histones as black. Note the alternating orientation and strand preference of half-nucleosomes in the uncoiled conformation, as required by the dyad axis of symmetry (see text). This alternating strand preference will cause the segregation of every other half-nucleosome to a different DNA daughter helix as indicated, unless the basic asymmetry of the replication fork with leading and lagging strands overrides the nucleosome symmetry. Note that this scheme leads to the top pathway on Fig. 5.*

horizontal templating processes previously discussed (Figs 3 and 4) could drive the structural chromatin changes toward the right in Fig. 5, and thus result in direct inheritance of gene structure. The *minimal* requirement is that the majority of the old histones in each gene remain with that gene following DNA replication.

Note that occasional individual errors in vertical templating will be corrected by the horizontal templating process, which will drive non-matching nucleosomes back to the majority type in each genetic region. The prediction that *horizontal and vertical templating are in reality reflections of the same chemical process in chromatin* is one of our strongest statements, and it allows the direct inheritance of gene structure to be a highly faithful process in theory.

For contiguous genes. Facultative heterochromatinization

appears to be a more extreme (and therefore more easily detected) example of the horizontal and vertical templating mechanisms already described. Both X-chromosome inactivation in mammals and position-effect variegation in *Drosophila* can determine function of essentially any gene (and thus work independently of specific DNA nucleotide sequences (8, 22, 36, 61, 66)). It is striking that both processes display a long range spreading effect (horizontal templating) analogous to that predicted to operate within a gene. For example, in translocations of the mouse X-chromosome to an autosome, a zone of stably-inherited gene inactivation spreads linearly down the adjacent autosomal DNA (22, 35), and in *Drosophila* the inactivation spreads along adjacent euchromatic chromosomal regions from translocated heterochromatin (8, 61). In both cases, many genes are covered, and the extent of spread initially differs from cell to cell. Once a genetic region has been recruited into heterochromatin, this change is clonally inherited (vertical templating) through all subsequent cell progeny. Note that, rather than "loosening" the chromatin structure as proposed for gene activation, the spreading effect seen in facultative heterochromatinization is just the reverse: a transmitted modification resulting in a *tight* chromatin structure which turns off genes. Since this horizontal templating can traverse many genes, it must pass the barriers postulated to separate different genetic domains. Perhaps this is due in part to the fact that this spreading occurs very early in embryonic development (73), when many of these barriers may not yet have been established.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health, the National Science Foundation, and the American Cancer Society. We gratefully acknowledge many long hours of discussion with Dr. Barbara McClintock, during which some of the general ideas expressed here were generated. In addition, Dr. Walter Kauzmann helped us to realize the advantages of using crystal packing forces to stabilize gene states. We also thank our colleagues at Princeton University for their critical comments on the manuscript, and Dr. H. Holtzer for introducing one of us (H.W.) to these problems.

REFERENCES

1. Allfrey, V.G. (1964). *Can. Cancer Conf. 6*, 313-335.

2. Allfrey, V.G. (1971). In "*Histones and Nucleohistones*", (Ed. D.M.P. Phillips), pp. 241-294, Plenum Press London and New York.
3. Alberts, B. and Frey, L. (1970). *Nature, Lond. 227*, 1313-1318.
4. Appels, R., Bolund, L. and Ringertz, N.R. (1974). *J.Mol. Biol. 87*, 339-355.
5. Ashburner, M., Chihara, C., Meltzer, P. and Richards, G. (1973). *Cold Spring Harbor Symp.Quant.Biol. 38*, 655-662.
6. Axel, R. (1975). *Biochemistry 14*, 2921-2925.
7. Baase, W.A. and Wang, J.C. (1974). *Biochemistry 13*, 4299-4303.
8. Baker, W.K. (1968). *Adv.Genet. 14*, 133-169.
9. Baldwin, J.P., Boseley, P.G., Bradbury, M. and Ibel, K. (1975). *Nature, Lond. 253*, 245-249.
10. Bauer, W.R. and Vinograd, J. (1968). *J.Mol.Biol. 33*, 141-171.
11. Beerman, W. (1972). In "*Results and Problems in Cell Differentiation*" (W. Beerman, ed.) Vol. 4, Springer-Verlag, Berlin and New York, p. 1.
12. Benyajati, C. and Worcel, A. (1976). *Cell*, (in press).
13. Berendes, H.D. (1972). In "*Results and Problems in Cell Differentiation*" (W. Beerman, ed.) Vol. 4, Springer-Verlag, Berlin and New York, pp. 181-207.
14. Borthwick, N.M. and Smellie, R.M.S. (1975). *Biochem.J. 147*, 91-101.
15. Bourgaux, P. and Bourgaux-Ramoisy, D. (1972). *Nature, Lond. 235*, 105-107.
16. Bradbury, E.M. and Crane-Robinson, C. (1971). In "*Histones and Nucleohistones*", (D.M.P. Phillips, ed.), Plenum Press, London and New York, pp. 85-134.
17. Bradbury, E.M., Molgaard, H.V. and Stevens, R.M. (1972). *Eur.J.Biochem. 31*, 474-482.
18. Bridges, C.B. (1935). *J.Heredity 26*, 60.
19. Britten, R.J. and Davidson, E.H. (1969). *Science, N.Y. 165*, 349-357.
20. Camerini-Otero, R.D., Sollner-Webb, B. and Felsenfeld, G. (1976). *Cell* , 333-347.
21. Carpenter, B.G., Baldwin, J.P., Bradbury, M. and Ibel, K. (1976). *Nucl.Acid Res. 3*, 1739-1746.
22. Cattenach, B.M. (1974). *Genet.Res. 23*, 291-306.
23. Caspar, D.L.D. and Klug, A. (1962). *Cold Spring Harb. Symp.Quant.Biol. 27*, 1-24.
24. Chandra, H.S. and Brown, S.W. (1975). *Nature, Lond. 253*, 165-168.
25. Chatterjee, S. and Walker, I.O. (1973). *Eur.J.Biochem. 34*, 519-526.

26. Cohen, L.H., Newrock, K.M. and Zweidler, A. (1975). *Science, N.Y. 190*, 994-997.
27. Cohen, M. (1976). *Chromosoma 55*, 349-357.
28. Cook, P.R. (1973). *Nature, Lond. 245*, 23-25.
29. Cook, P.R. (1974). *Biol.Rev. 49*, 51-84.
30. Cremisi, C., Pignatti, P., Crosissant, O. and Yaniv, M. (1976). *J.Virol. 17*, 204-211.
31. Crick, F. (1971). *Nature, Lond. 234*, 25-27.
32. D'Anna, J.A. and Isenberg, I. (1974). *Biochemistry 13*, 4992-4997.
33. DeLange, R.J. and Smith, E.L. (1975). *Ciba Symp.Structure and Function of Chromatin 28*, 59-70.
34. Dobzhansky, T. and Sturtevant, A.H. (1938). *Genetics 23*, 28-64.
35. Easton, D. and Chalkley, R. (1972). *Exptl.Cell Res. 72*, 502-508.
36. Eicher, E.M. (1970). *Adv.Genet. 15*, 175-260.
37. Finch, J.T. and Klug, A. (1976). *Proc.Nat.Acad.Sci. (U.S.A.) 73*, 1897-1901.
38. Frenster, J.H. (1969). *Handbook of Molecular Cytology* (A. Lima de Faria, ed.), North Holland Press, pp. 251-276.
39. Germond, J.E., Hirt, B., Oudet, P., Gross-Bellard, M. and Chambon, P. (1975). *Proc.Nat.Acad.Sci.(U.S.A.) 72*, 1843-1847.
40. Glasser, S.R., Chytil, F. and Spelsberg, T.C. (1972). *Biochem.J. 130*, 947-957.
41. Gottesfeld, J.M., Garrard, W.T., Bagi, G., Wilson, F.R. and Bonner, J. (1974). *Proc.Nat.Acad.Sci.(U.S.A.) 71*, 2193-2197.
42. Gottesfeld, J.M., Murphy, R.F. and Bonner, J. (1975). *Proc.Nat.Acad.Sci.(U.S.A.) 72*, 4404-4408.
43. Gottesfeld, J.M., Bagi, G., Berg, B. and Bonner, J. (1976). *Biochemistry*, (in press).
44. Griffith, J.D. (1975). *Science, N.Y. 187*, 1202-1203.
45. Gurley, L.R. and Hardin, J.M. (1969). *Arch.Biochem. Biophys. 130*, 1-6.
46. Hardison, R.C., Eichner, M.E. and Chalkley, R. (1975). *Nucl.Acid Res. 2*, 1751-1769.
47. Hewish, D.R. and Bourgoyne, L.A. (1973). *Biochem.Biophys. Res.Comm. 52*, 504-510.
48. Hochman, B. (1973). *Cold Spring Harb.Symp.Quant.Biol. 38*, 581-589.
49. Holliday, R. and Pugh, J.E. (1975). *Science, N.Y. 187*, 226-232.
50. Huberman, J., Alberts, B. and Kornberg, A. (1971). *J.Mol. Biol. 62*, 39-52.

51. Isenberg, I. and Van Holde, K.R. (1976). "*Critical Reviews in Biochemistry*", Chemical Rubber Co, (in press).
52. Jackson, V., Shires, A., Chalkley, R. and Granner, D.K. (1975). *J.Biol.Chem. 250,* 4856-4863.
52b. Jackson, V., Granner, D.K. and Chalkley, R. (1975). *Proc. Nat.Acad.Sci.(U.S.A.) 72,* 4440-4444.
53. Jacob, F. and Monod, J. (1961). *J.Mol.Biol. 3,* 318-356.
54. Johman, C.A. and Gorovsky, M.A. (1976). *Biochemistry 15,* 1249-1256.
55. Judd, B.H. and Young, M.W. (1973). *Cold Spring Harb.Symp. Quant.Biol. 38,* 573-579.
56. Kaufmann, B.P. and Iddles, M.K. (1963). *Port.Acta.Biol. 7,* 225-248.
57. Keller, W. (1975a). *Proc.Nat.Acad.Sci.(U.S.A.) 72,* 2550-2554.
58. Keller, W. (1975b). *Proc.Nat.Acad.Sci.(U.S.A.) 72,* 4876-4880.
59. Kornberg, R.D. (1974). *Science, N.Y. 184,* 868-871.
60. Lacy, E. and Axel, R. (1975). *Proc.Nat.Acad.Sci.(U.S.A.) 72,* 3978-3982.
61. Lewis, E.B. (1950). *Adv.Genet. 3,* 73-115.
62. Lezzi, M. and Robert, M. (1972). In "*Results and Problems in Cell Differentiation*" (W. Beerman, ed.) Vol. 4, Springer-Verlag, Berlin and New York.
63. Li, H.J. (1975). *Nucl.Acid Res. 2,* 1275-1289.
64. Libby, P.R. (1972). *Biochem.J. 130,* 663-669.
65. Libby, P.R. (1973). *Biochem.J. 134,* 907-912.
66. Lyon, M.F. (1974). *Proc.Roy.Soc. B 187,* 243-268.
67. McClintock, B. (1965). *Brookhaven Symp.Biol. 18,* 162-182.
68. Mayfield, J.E. and Ellison, J.R. (1975). *Chromosoma 52,* 37-48.
69. Monod, J., Wyman, J. and Changeux, J.P. (1965). *J.Mol. Biol. 12,* 88-118.
70. Noll, M. (1974a). *Nucl.Acid Res. 1,* 1573-1578.
71. Noll, M. (1974b). *Nature, Lond. 251,* 249-252.
72. Noll, M., Thomas, J.O. and Kornberg, R.D. (1975). *Science, N.Y. 187,* 1203-1206.
73. Ohno, S., Geller, L.N. and Kan, J. (1976). *Cell 1,* 175-181.
74. Ohta, T. and Kimura, M. (1971). *Nature, Lond. 233,* 118-119.
75. Olins, A.L. and Olins, D.E. (1974). *Science, N.Y. 183,* 330-332.
76. Olins, A.L., Breillat, J.P., Carlson, R.D., Senior, M.B., Wright, E.B. and Olins, D.E. (1976). In "*Molecular Biology of the Mammalian Genetic Apparatus*", (Ed. Paul Ts'o)

Elsevier-Excerpta Medica, North Holland (in press).
77. Oudet, P., Gross-Bellard, M. and Chambon, P. (1975). *Cell 4,* 281-300.
78. Painter, T.S. (1934). *Genetics 19,* 175-188.
79. Pardon, J.F., Worcester, D.L., Wooley, J.C., Tatchell, K., Van Holde, E. and Richards, B. (1976). *Nucl.Acid Res. 2,* 2163-2176.
80. Pederson, T. and Bhorjee, G.S. (1975). *Biochemistry 14,* 3238-3242.
81. Polisky, B. and McCarthy, B. (1975). *Proc.Nat.Acad.Sci. (U.S.A.) 72,* 2895-2899.
82. Ruiz-Carrillo, A., Waugh, L.J. and Allfrey, V.G. (1975). *Science, N.Y. 190,* 117-128.
83. Sahasrabuddhe, C.G. and Van Holde, K.E. (1974). *J.Biol. Chem. 249,* 152-156.
84. Sanders, L.A., Schechter, N.M. and McCarty, K.S. (1973). *Biochemistry 12,* 783-791.
85. Saucier, J.M. and Wang, J.C. (1972). *Nature New Biol. 239,* 167-170.
86. Schaffhausen, B.S. and Benjamin, T.L. (1976). *Proc.Nat. Acad.Sci.(U.S.A.) 73,* 1092-1096.
87. Schultz, J. (1965). *Brookhaven Symp.Biol. 18,* 116-147.
88. Seale, R.L. (1975). *Nature, Lond. 255,* 247-249.
89. Shaw, B.R., Herman, T.M., Kovacic, R.T., Beaudreau, G.S. and Van Holde, K.E. (1976). *Proc.Nat.Acad.Sci. (U.S.A.) 73,* 505-509.
90. Shure, M. and Vinograd, J. (1976). *Cell 8,* 215-226.
91. Sigal, N., Delius, H., Kornberg, T., Gefter, M. and Alberts, B. (1972). *Proc.Nat.Acad.Sci.(U.S.A.) 69,* 3537-3541.
92. Slizynski, B.M. (1945). *Proc.Roy.Soc.Edinb. B 62,* 114-119.
93. Sollner-Webb, B. and Felsenfeld, G. (1975). *Biochemistry 14,* 2915-2920.
94. Sorsa, V., Green, M.M. and Beerman, W. (1973). *Nature New Biol. 245,* 34-37.
95. Thomas, J.O. and Kornberg, R.D. (1975). *Proc.Nat.Acad. Sci.(U.S.A.) 72,* 2626-2630.
96. Tsanev, R. and Sendov, B. (1971). *J.Theoret.Biol. 30,* 337-393.
97. Tsanev, R. and Russev, G. (1974). *Eur.J.Biochem. 43,* 257-263.
98. Van Holde, K.E., Sahasrabuddhe, C.G. and Shaw, B. (1974). *Nucl.Acid Res. 1,* 1579-1586.
99. Weintraub, H. (1973). *Cold Spring Harb.Symp.Quant.Biol. 38,* 247-256.
100. Weintraub, H., Palter, K. and Van Lente, F. (1975).

Cell 6, 85-110.
101. Weintraub, H. and Van Lente, F. (1974). *Proc.Nat.Acad. Sci.(U.S.A.) 71*, 4249-4253.
102. Weintraub, H. (1975). *Proc.Nat.Acad.Sci. (U.S.A.) 72*, 1212-1216.
103. Weintraub, H. and Groudine, M. (1976). *Science, N.Y.* (in press).
104. Weintraub, H., Worcel, A. and Alberts, B. (1976). *Cell*, (in press).
105. Wilkins, M.H.F., Zubay, G. and Wilson, H.K. (1959). *J.Mol.Biol. 1*, 179-185.
106. Worcel, A. and Burgi, E. (1972). *J.Mol.Biol. 71*, 127-147.
107. Yamamoto, K.R. and Alberts, B. (1976). *Ann.Rev.Biochem. 45*, 721-746.
108. Yunis, J.J. and Yasmineh, W.G. (1971). *Science, N.Y. 174*, 1200-1209.
109. Ziccardi, R. and Shumaker, V. (1973). *Biochemistry 12*, 3231-3235.