6

Reversible Modifications of Nuclear Proteins and Their Significance

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This chapter covers a number of modifications of nuclear proteins, with an emphasis on reversible acetylation and phosphorylation, because these have been most extensively studied (and partly because methylation is covered elsewhere in this volume—see Chapter 7). Reversible acetylation occurs primarily on lysine residues in histones, and we discuss the enzymology and the likely structural and functional consequences of histone acetylation in chromatin. Two separate patterns of histone acetylation have been described, one associated with transcription and another associated with newly synthesized histones and chromosome replication. Phosphorylation is a much more widespread modification and its role in histones and non-histones is discussed. Phosphorylation of histone H1 is correlated with cell proliferation and chromosome condensation, while phosphorylation of histone H3 is correlated with metaphase. However, the precise function of these phosphorylations is not clear. Phosphorylation of non-histone structural proteins (HMG proteins and nuclear matrix proteins) and enzymes (RNA polymerase, ornithine decarboxylase, topoisomerase I) also occurs, but there is no clear description of the function of these phosphorylations except, to some extent, in the case of ornithine decarboxylase. We also mention acid-labile phosphorylation (amide phosphorylation) of histone H4. These areas provide extensive scope for further research.

I. Histone Acetylation and Function

A. Introduction

For a long time, histone acetylation has been correlated with active gene transcription (for a recent review, see Allfrey, 1977). For example, during early stages of gene activation by hormones, mitogens or during tissue regeneration, increased acetylation of histones precedes an increase in RNA synthesis. More recently, Kaneko (1983) has shown that protein acetylation is an early event in the stimulation of a human liver cell culture by epidermal growth factor. Deacetylation of histones was seen in maturing sperm cells that are transcriptionally inactive (Dixon *et al.*, 1975). The rate of acetylation of histones H3 and H4 in calf thymus lymphocytes was much higher in the diffuse, synthetically active euchromatin than in the inert heterochromatin (DeLange and Smith, 1971; Ruiz-Carrillo *et al.*, 1975).

These correlations led to a postulated mechanism. The acetylation occurred on lysines in the histones, converting the positively charged amino

groups into uncharged ϵ-N-acetylated lysines (Gershey *et al.*, 1968). All these reversible modifications occur on only a limited number of lysine residues that are clustered in the highly basic amino-terminal regions of four histones, H4, H3, H2A, and H2B (DeLange *et al.*, 1969a, 1972; DeLange and Smith, 1971). The basic histones were thought to be tightly binding to DNA in chromatin, especially through their highly basic regions, and histone acetylation would relax this binding and allow RNA polymerases to transcribe genes (Gershey *et al.*, 1968; DeLange and Smith, 1971). Chemical acetylation of histones produced template activation, supporting this idea (Allfrey *et al.*, 1964).

Newly synthesized histone H4 was found to be acetylated at internal lysines prior to its deposition onto chromatin, and the major part of the acetylation was lost during chromosome maturation. It seemed that for correct interaction between histone and DNA an initial decrease in the basic character of the histone was required (Marushige and Dixon, 1969; Candido and Dixon, 1972a,b).

In differentiating sperm cells, histone acetylation increased during the process of protamine deposition. The removal of histones from the DNA seemed to require a loosening of the interaction (Sung and Dixon, 1970; Candido and Dixon, 1972a,b).

Since these early studies of the 1960s, chromatin research has developed rapidly. Histones have been sequenced. The nucleosomal structure of chromatin was discovered. Transcriptionally active chromatin was found to be highly sensitive to nucleases. Histone acetyltransferases and deacetylases were localized in chromatin. Butyrate was shown to inhibit histone deacetylation and induce chromatin hyperacetylation.

In this chapter we want to re-examine the early ideas on the function of histone acetylation in the light of more recent studies. We will update the models used and pay special attention to the specific differences that individual histones display in their acetylation and thus in their function. In addition, we want to emphasize the possibilities for artifacts that are inherent when butyrate is used to study the functioning of chromatin.

B. Sequence location of the sites of acetylation

Amino acid sequence determination of histones from many sources has shown that conservation of sequence is very high (DeLange and Smith, 1971; von Holt *et al.*, 1979; Isenberg, 1979). This indicates that the histones interact with their surroundings in such specific ways that the smallest variation in primary sequence impairs interaction and function (Dixon *et al.*, 1975). However, the degree of conservation varies between histones and between regions within the histones. Three groups of se-

quences, differing in their degree of conservation, can be distinguished among the four core histones, H4, H3, H2A, and H2B.

The highest degree of conservation is seen in the basic amino-terminal region of H4, residues 1–20, and of H3, residues 1–30 (von Holt *et al.*, 1979; Isenberg, 1979; Ohe and Iwai, 1981; Waterborg and Matthews, 1983c; Waterborg *et al.*, 1983). With the single exception of the protozoon *Tetrahymena* (Glover and Gorovsky, 1979; Hayashi *et al.*, 1980), no residue substitution occurs (Table I). The selection against any variation in sequence indicates an extremely specific interaction and function.

A slightly less high degree of conservation is seen in the hydrophobic central and carboxy-terminal regions of all four histones. The conservation of sequence is very good, but point substitutions occur (von Holt *et al.*, 1979; Isenberg, 1979). These regions of the histones all form tight globular and hydrophobic protein structures that interact with each other and are involved in the formation of the nucleosomal core (Kornberg and Thomas, 1974; Whitlock and Simpson, 1977; Whitlock and Stein, 1978; Cary *et al.*, 1978).

The basic amino-terminal regions of histones H2A and H2B, while still considered conserved sequences, are more variable (von Holt *et al.*, 1979; M. S. Strickland *et al.*, 1978; W. N. Strickland *et al.*, 1980; Van Helden *et al.*, 1982; Mende *et al.*, 1983). Variations in length and sequence of the amino-terminal regions occur between phylogenetically distant groups, and residue substitutions and other smaller sequence variations are found between more closely related organisms. In many systems several variant forms of H2A and H2B occur together in a single cell (von Holt *et al.*, 1979). Apparently the amino-terminal regions of H2A and H2B interact with their surroundings in a much more variable way than the amino-terminal regions of H3 and H4.

The patterns of acetylation reflect these histone regions (Table I). H4 is reversibly acetylated at up to four internal lysines, and the first residue is a stable α-*N*-acetyl serine. The same four internal acetylated lysines are seen in *Tetrahymena* H4, which has a free amino-terminus, so the conservation of modification sites is even higher than that of the sequence itself (Isenberg, 1979; Waterborg *et al.*, 1983). H3 is generally considered to be acetylated at only four out of the possible five lysines in the region up to residue 27 (Isenberg, 1979; Waterborg and Matthews, 1983c). In many systems the most extensively modified form seen is tetra-acetylated, but in several studies apparently penta-acetylated H3 has been observed (Table I). All five lysines in the amino-terminus of calf H3 are acetylated *in vitro* by rat liver histone acetyltransferase (Thwaits *et al.*, 1976a). Thus in both histones maximal acetylation completely abolishes the positive

charge of the lysines in the amino terminal regions leaving only a limited number of charged arginine and histidine residues.

In the variant region of H2A, the initial 10–20 residues, generally one or two lysines are acetylated (Table I). This leaves several of the lysines charged even in highly acetylated H2A. The variant region of H2B, covering approximately the first 25 residues of calf thymus H2B, contains seven or eight lysines in calf, trout, and *Drosophila,* and up to as many as 11 in some sea urchin H2B forms (Isenberg, 1979). However, the maximum number of acetylations reported is four, or five in some extensively butyrate-treated mouse and human cells (Pantazis and Bonner, 1982; Bode *et al.*, 1983). This leaves several lysine residues charged as in H2A (Table I) (Isenberg, 1979; D'Anna *et al.*, 1980a; Nelson *et al.*, 1980; Nelson, 1982; Doenecke and Gallwitz, 1982; Pantazis and Bonner, 1982; Bode *et al.*, 1983).

C. Nucleosomal structure of chromatin

1. The nucleosome

The interaction of histones with DNA in chromatin is organized in clearly defined units called nucleosomes (e.g., Klug *et al.*, 1980; for reviews, see Felsenfeld, 1978; McGhee and Felsenfeld, 1980; Kornberg and Klug, 1981; Bradbury and Matthews, 1981; Igo-Kemenes *et al.*, 1982). Each nucleosome contains two copies each of histones H4, H3, H2A, and H2B in an octameric complex and one copy of histone H1. The eight "core histones" interact with their hydrophobic globular regions to form a particle that organizes 145 base pairs of DNA into 1.7 turns around its protein kernel. A tetramer of H3 and H4 forms the basic structure of the nucleosome that binds the DNA. H2A and H2B cannot do this, but they add additional stability to the core (Camerini-Otero *et al.*, 1976; Carter *et al.*, 1980; Daban and Cantor, 1982; Baer and Rhodes, 1983). The globular domain of H1 organizes about 23 additional base pairs of DNA, giving a total of two full turns and one negative superhelical twist (Crick, 1976; Gazit *et al.*, 1982) closely associated with histone (Simpson, 1978; Allan *et al.*, 1980). The length of DNA organized on the eight core histones is invariant (145 base pairs) irrespective of the nucleosome repeat length that varies from 150 to 240 base pairs among various species (Compton *et al.*, 1976).

Treatment of nucleosomes with trypsin yields a core particle of apparently unchanged stability containing 145 base pairs of DNA (Whitlock and Simpson, 1977; Grigoryev and Krasheninnikov, 1982). However, the his-

Table I

Sites of histone acetylation and methylation

Histone	Source	Sequence
H4[a]	Calf thymus	1 5 8 12 16 20 Ser.Gly.Arg.Gly.Lys.Gly.Gly.Lys.Gly.Leu.Gly.Lys.Gly.Gly.Ala.Lys.Arg.His.Arg.Lys.Val.Leu.Arg.... Ac Ac Ac Ac Ac Me
H4	*Tetrahymena*	1 4 7 11 15 20 Ala.Gly.Gly.Lys.Gly.Gly.Lys.Gly.Met.Gly.Lys.Val.Gly.Ala.Lys.Arg.His.Ser.Arg.Lys.Val.Leu.Arg.... NH_2 Ac Ac Ac Ac Me
H_3[b]	See footnote	1 4 9 14 18 23 27 Ala.Arg.Thr.Lys.Gln.Thr.Ala.Arg.Lys.Ser.Thr.Gly.Gly.Lys.Ala.Pro.Arg.Lys.Gln.Leu.Ala.Thr.Lys.Ala.Ala.Arg.Lys.Ser. NH_2 Ac/Me Ac/Me Ac Ac Ac Me
H2B[c]	Calf thymus	1 5 11 12 15 16 20 23 24 Pro.Glu.Pro.Ala.Lys.Ser.Ala.Pro.Ala.Pro.Lys.Lys.Gly.Ser.Lys.Lys.Ala.Val.Thr.Lys.Ala.Gln.Lys.Lys.Asp.Gly.. NH_2 Ac ( ) Ac Ac ( ) Ac ( ) ( )
H2B	Trout testis	1 5 9 10 13 14 18 22 Pro.Glu.Pro.Ala.Lys.Ser.Ala.Pro.Lys.Lys.Gly.Ser.Lys.Lys.Ala.Val.Thr.Lys.Thr.Ala.Gly.Lys.Gly.Gly.. NH_2 Ac ( ) Ac Ac ( ) Ac ( )
H2B	*Drosophila*	1 3 7 10 11 14 17 21 Pro.Pro.Lys.Thr.(Ser/Ala).Gly.Lys.Ala.Ala.Lys.Lys.Ala.Gly.Lys.Ala.Gln.Lys.Asn.Ile.Thr.Lys.Thr.Asp.. NH_2 Ac ( ) ( ) Ac Ac ( ) Ac
H2B	*Tetrahymena*	1 3 4 11 12 14 15 21 22 Ala.Pro.Lys.Lys.Ala.Pro.Ala.Ala.Ala.Glu.Lys.Lys.Val.Lys.Lys.Ala.Pro.Thr.Thr.Glu.Lys.Lys.Asn.. Me_3 Ac

Histone	Source	Sequence
		1 5 9 13 15
H2A	Calf thymus	Ser.Gly.Arg.Gly.Lys.Gln.Gly.Gly.Lys.Ala.Arg.Ala.Lys.Ala.Lys.Thr.Arg....
		Ac Ac Ac ( ) ( )
		1 5 9 13 15
H2A	Trout testis	Ser.Gly.Arg.Gly.Lys.Thr.Gly.Gly.Lys.Ala.Arg.Ala.Lys.Ala.Lys.Thr.Arg....
		Ac Ac ( ) ( ) ( )
		1 5 8 12 14
H2A[d]	Starfish	Ser.Gly.Arg.Gly.Lys.Gly.Gly.Lys.Ala.Arg.Ala.Lys.Ala.Lys.Ser.Arg....
		Ac Ac ( ) ( ) ( )

[a] Lysine residues may be acetylated (Ac) or methylated (Me) or may never be modified( ). The same sites of acetylation are also observed in calf thymus *in vitro* (Thwaits *et al.*, 1976b). *In vivo* the same sites are seen on an identical amino acid sequence in human spleen (Hayashi *et al.*, 1982), chicken erythrocytes (Wouters-Tyrou *et al.*, 1981), pea seedling (DeLange *et al.*, 1969b; Isenberg, 1979), trout testis (Candido and Dixon, 1971; DeLange and Smith, 1971; Isenberg, 1979; Doenecke and Gallwitz, 1982), cuttlefish testis (Wouters-Tyrou *et al.*, 1981), and *Physarum polycephalum* (Mende *et al.*, 1983; Waterborg *et al.*, 1983). Up to four sites of acetylation are also observed in other systems such as CHO cells (D'Anna *et al.*, 1980a), HTC cells (Nelson *et al.*, 1980), and yeast (Nelson, 1982). The acetylation of the amino-terminal serine is a stable modification that occurs in the cytoplasm during histone synthesis (DeLange and Smith, 1971; Marzluff and McCarty, 1970; Jackson *et al.*, 1975) and that is not considered a site of acetylation in the sense of potentially and reversibly acetylated lysines. In H4 generally only Lys-20 is methylated. However, in pea H4 Lys-20 is unmodified (DeLange *et al.*, 1969b) and in *Physarum* H4 an additional methylated lysine has been observed at residue 79 (Waterborg *et al.*, 1983).

[b] In calf thymus and trout testis up to four sites of acetylation have been reported (Lys-4 is never modified) (Ogawa *et al.*, 1969; DeLange *et al.*, 1972; Candido and Dixon, 1972c; Isenberg, 1979; Doenecke and Gallwitz, 1982), as in human spleen (Ohe and Iwai, 1981) and chicken erythrocytes (Brotherton *et al.*, 1981). Four sites are also seen in butyrate-treated human and mouse cells (Pantazis and Bonner, 1982), at least four sites are found in yeast (Nelson, 1982), and five modified forms which are supposedly all based on lysine acetylation are seen in butyrate-treated CHO cells (D'Anna *et al.*, 1980a), HTC cells (Nelson *et al.*, 1980), toad (Truscello *et al.*, 1983), and *Tetrahymena* (Vavra *et al.*, 1982). Calf thymus H3 acetylated *in vitro* by rat liver histone acetyltransferase shows acetylation of all five lysines (Thwaits *et al.*, 1976a). The H3 sequence given is invariant except for residue 22 in yeast, which is serine (Isenberg, 1979). Methylation is generally observed at lysines 9 and 27 (DeLange *et al.*, 1972) but in human spleen residue 36 is methylated (Ohe and Iwai, 1981) and a low level of methylation is seen in Lys-4 in trout (Honda *et al.*, 1975a).

[c] The sequence of the other H2B histones is given to an identical position relative to the conserved core sequences.

[d] Two acetylation sites have been observed in calf thymus (Doenecke and Gallwitz, 1982), trout testis (Candido and Dixon, 1972a), CHO cells (D'Anna *et al.*, 1980a), HTC cells (Nelson *et al.*, 1980), yeast (Nelson, 1982), and in some H2A variants in L cells, while other variants in the same cells have only one site (Pantazis and Bonner, 1981, 1982). In butyrate-treated human lymphoma cells, three modified forms have been seen (Bode *et al.*, 1983).

tones in such a particle have lost part of their sequences, those sequences that are located on the surface and are accessible. Table II shows that the sequences digestible by trypsin include the complete amino-terminal regions of all the histones that can be acetylated. The relative sensitivity H3 = H4 > H2B = H2A (Weintraub and Van Lente, 1974) emphasizes the differences between the former two (H3 and H4) and the latter (H2A and H2B). However, the *Submaxillaris* arginine-specific protease used by Rill and Oosterhof (1982) shows a different sensitivity: H3 > H2B > H4 ⋙ H2A. The amino-terminal of H2A with the lowest number of possible acetylation sites of all the histones may be partially shielded and not accessible in the nucleosomal structure.

Two other approaches support the conclusion that the amino-terminal regions of the histones do not confer any significant stability on the isolated nucleosome. Hyper-acetylated histones and cores can be produced by extensive butyrate treatment of cells *in vivo*. If extensive ionic interactions between negatively charged phosphates on the DNA backbone and positively charged lysines in the amino-termini of the core histones would

Table II

Protease-sensitive and mobile regions in nucleosomal histones

	Protease-sensitive sequences			Mobile sequences	
Histone	Trypsin	Proteolytic self-digestion	Submaxillaris protease	^{1}H-NMR	^{13}C-NMR
H4	1–17, 1–19	1–16	1–17, 1–19	1–20	1–12
H3	1–26	1–20, 1–23	1–26	1–27	1–15
	130–135[b]		129–135		
H2B	1–20, 1–23		1–>13	1–30	1–30
				114–125	
H2A	1–9, 1–11		1–>3[c]	1–11	
	119–129			117–129	117–129
Reference[a]	A, B, C, D	C, E	F	G	F

[a] References A (Grigoryev and Krasheninnikov, 1982), B (Boehm *et al.*, 1980a), C (Boehm *et al.*, 1981), and D (Boehm *et al.*, 1982) all use chicken erythrocyte chromatin and nuclei. E (Brandt *et al.*, 1975) uses cycad pollen. F (Rill and Oosterhof, 1982) uses chromatin and nucleosomal cores of chicken erythrocytes, and G (Cary *et al.*, 1978) uses cores of calf thymus.

[b] A sensitive carboxy-terminal region is only seen during the digestion of chromatin (Boehm *et al.*, 1981) and not during the digestion of nucleosomal cores (Grigoryev and Krasheninnikov, 1982).

[c] Arginine 3 in H2A is very resistant to digestion (Rill and Oosterhof, 1982).

occur, negation of this interaction by acetylation would surely affect the thermal stability of the DNA. Only a minor effect is observed which could be due to non-specific electrostatic interactions between histone termini and the DNA phosphates under the experimental conditions that lack normal, physiological concentrations of counter ions (Vidali *et al.*, 1978; Bode *et al.*, 1980; Simpson, 1978; Grigoryev and Krasheninnikov, 1982; Yau *et al.*, 1982).

The other experimental approach that indicates that amino-terminal regions of core histones are mobile and not localized in or on the core particles, is proton or carbon-13 nuclear magnetic resonance (NMR) (Cary *et al.*, 1978; Rill and Oosterhof, 1982). This technique detects only those residues that are mobile under the conditions tested. Table II lists the sequences of the histones that are mobile in nucleosomal cores. In general, the NMR and protease sensitivity data agree well.

2. *The superstructure of chromatin*

The DNA length is reduced by a factor of approximately six by coiling it in a nucleosome. A further reduction is achieved by coiling the nucleosomes at approximately six per turn into a solenoid (superhelix), also called the 30-nm fiber (Suau *et al.*, 1979; Thoma *et al.*, 1979; Bates *et al.*, 1981; Marion *et al.*, 1981). Under certain conditions this fiber displays a knobby structure with "superbeads" (Azorin *et al.*, 1982). The main organizing element in the 30-nm fiber is histone H1 that through its amino- and carboxy-terminal regions complexes with the DNA of neighboring nucleosomes (Thoma *et al.*, 1979; Thoma and Koller, 1981; Allan *et al.*, 1981). The further condensation of this solenoid into mitotically condensed chromosomes is also dependent on histone H1. Such condensation is probably accomplished by a high level of phosphorylation of H1 termini during the last part of the cell cycle leading up to prophase and mitosis (Fig. 1) (see Section II).

The solenoid structure of chromatin is reversibly denatured by a decrease in the ionic strength from physiological to 10 mM. In electron micrographs the DNA in such chromatin appears to "zigzag" between the nucleosomes. Both strands exit at the same side of the nucleosome and they seem to cross. This shows that H1 is still present, binding linker DNA to its termini and closing off two full turns of DNA in the nucleosomal particle with its globular region. The selective removal of H1 causes further, irreversible, denaturation to a beads-on-a-string configuration in which the DNA exits at two opposite sides of the core and only 145 base pairs of DNA remain bound (Thoma *et al.*, 1979; Ruiz-Carrillo *et al.*, 1980).

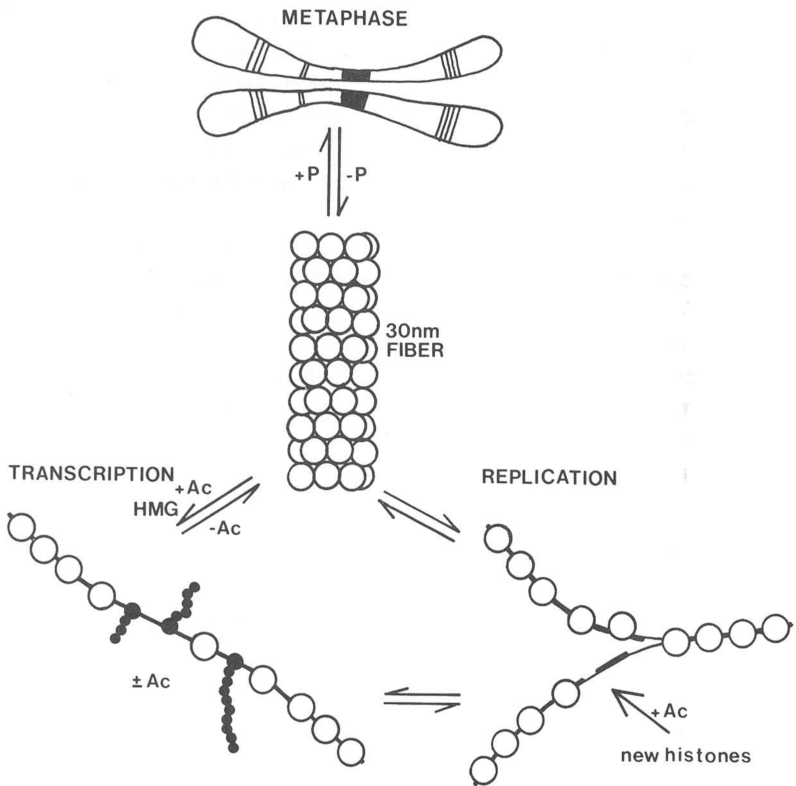

Fig. 1. Model representing major structural transitions in chromatin. The different structural states are shown in diagrammatic form only. Abbreviations: Ac, acetylation; HMG, high mobility group proteins.

However, H1 is not the only factor involved in the interactions of the nucleosomes in the 30-nm fiber (Thoma *et al.*, 1979; Thoma and Koller, 1981; Goyanes *et al.*, 1980; Marion *et al.*, 1982). While H1 is of major importance to solenoid formation, the basic core histone tails provide essential electrostatic shielding of the DNA of nucleosomes in adjacent solenoid turns. Thus, the absence of basic core histone termini prevents the formation of chromatin superstructure and locks the chromatin in an "open" conformation of beads-on-a-string (Fig. 1). This observation implies that removal of the positive charges in the nucleosome termini by acetylation of lysines will destabilize chromatin superstructure, as proposed by Chahal *et al.* (1980), and may prevent it completely (Allan *et al.*, 1982).

Studies employing peptides containing the amino-terminal region of H4

show that they form a complex with DNA at physiological ionic strength while multi-acetylation abolishes the binding of the peptides to DNA (Cary *et al.*, 1982). In the complex of non-acetylated peptides with DNA the mobility of the amino acid backbone of the sequence 1–16 of H4 is lost completely, as seen by NMR. The peptide backbone fits precisely in a groove on the DNA with mobile lysine–phosphate and immobile arginine–phosphate interactions (Couppez *et al.*, 1980; Cary *et al.*, 1982; Wachtel and Sperling, 1983).

Chemical acetylation of chromatin with acetic anhydride has been used to mimic the *in vivo* acetylation of histones. Although the template activity for transcription increased and the chromatin acquired a sensitivity to DNase I digestion similar to that seen for actively transcribing genes, these results must be viewed with caution. Lysine residues, different from the *in vivo* ones, had also been modified (Marushige, 1976; Wallace *et al.*, 1977; Tack and Simpson, 1979).

D. Transcriptionally active chromatin

1. *Conformations of active chromatin*

Chromatin active in transcription or replication is expected to be much more open in structure than inactive "bulk" chromatin to allow the enzyme complexes involved to follow the sequence of DNA. Electron microscopic studies support this notion, and the increased sensitivity to nuclease digestion of such active chromatin also points to a sterically open configuration (Mathis *et al.*, 1980; Scheer *et al.*, 1981; Bazett-Jones and Ottensmeyer, 1982; Johnson *et al.*, 1978a,b; Weisbrod, 1982a; Prior *et al.*, 1983). In the next sections we discuss both forms of active chromatin to see how histone acetylation is involved. The differences between these systems will be emphasized. However, in many studies on histone acetylation, asynchronous cell cultures have been used. This means that events of histone acetylation correlated with transcription are often mixed together with those correlated with replication in such a way that the separate processes cannot be distinguished.

DNase I generally digests active and potentially active gene sequences faster than bulk chromatin (Weintraub and Groudine, 1976; Garel and Axel, 1976); nucleosomes produced early during a DNase I digest are highly enriched in multi-acetylated histones (Nelson *et al.*, 1979; Davie and Candido, 1980; Mathis *et al.*, 1980; Weisbrod, 1982a,b; Doenecke and Gallwitz, 1982). Chemical acetylation (Shewmaker *et al.*, 1978) or butyrate-induced hyperacetylation of chromatin (Candido *et al.*, 1978; Sealy and Chalkley, 1978a; Boffa *et al.*, 1978; Vidali *et al.*, 1978) give increased

sensitivity to DNase I but additional or alternative factors are also involved (Gazit *et al.*, 1980; Weisbrod and Weintraub, 1979; Mathis *et al.*, 1980; Weisbrod, 1982a; Spiker *et al.*, 1983; Nelson *et al.*, 1979; Scheer *et al.*, 1981).

DNase II and micrococcal nuclease, enzymes which primarily cut between nucleosomes, also show a preference for actively transcribed genes (Gottesfeld and Butler, 1977; Johnson *et al.*, 1978a; Mathis *et al.*, 1980); again, nucleosomes released in early digests are enriched in acetylated histones (Davie and Candido, 1978; Kuehl *et al.*, 1980; Hutcheon *et al.*, 1980). Another characteristic of active chromatin, the irregular spacing of nucleosomes, is easily seen in early micrococcal nuclease digests of chromatin. The 200 base pair repeat pattern is blurred in nucleosomes containing active sequences, while the repeat pattern is distinct in bulk DNA (Wu *et al.*, 1979; Stalder *et al.*, 1979; Mathis *et al.*, 1980).

DNase I, micrococcal nuclease, and single-strand-specific nuclease S1 readily digest "hyper-sensitive sites" in chromatin. These sites are usually but not always located 5′ to the coding region of active genes and may lack nucleosomes (Weisbrod, 1982a; Lohr, 1983). Note, too, that nuclease digestion may select replicating chromatin as well as transcribing chromatin (Mathis *et al.*, 1980).

2. *Steady-state acetylation of histones in active chromatin*

The steady state of acetylation is a balance of acetylation and deacetylation reactions. Electrophoresis of isolated histones on acid–urea or acid–urea–Triton gels will separate histones that differ in their degree of acetylation (Panyim and Chalkley, 1969a; Bonner *et al.*, 1980; Davie *et al.*, 1981). In some organisms active and inactive chromatin have been distinguished without degradation to nucleosomes and in these cases, acetylation is correlated with active chromatin (Vavra *et al.*, 1982; Halleck and Gurley, 1982; Chahal *et al.*, 1980; Loidl *et al.*, 1983). In the yeast *Saccharomyces cerevisiae* the whole genome is in a DNase I–sensitive conformation, the whole genome is potentially active (Lohr and Hereford, 1979), and the average acetylation of H3 and H4 is very high (Davie *et al.*, 1981). In other cases, nucleosomes derived from transcriptionally active chromatin have been studied. Transcriptionally active chromatin isolated from trout testis early during testis differentiation (Christensen and Dixon, 1982) by limited nuclease digestion (Davie and Candido, 1978, 1980; Hutcheon *et al.*, 1980; Kuehl *et al.*, 1980) is enriched in multiacetylated histones H3 and H4 (Davie and Candido, 1978, 1980; Hutcheon *et al.*, 1980). Late during testis differentiation, acetylation occurs as a precursor to protamine deposition (Christensen and Dixon, 1982). Active

nucleosomes, isolated from chicken red blood cells and MSB cells on the basis of their affinity to HMG 14 and 17 proteins, clearly show higher steady state levels of acetylation for H4 and H3, and possibly for H2B, than inactive chromatin (Weisbrod, 1982b).

A correlation between DNase I sensitivity and histone acetylation has been shown in many different organisms after butyrate treatment. However, hyperacetylated chromatin in butyrate treated cells, while DNase I–sensitive, cannot be equated with transcriptionally active chromatin (see Section IH).

3. *Active reversible acetylation of histones in active chromatin*

The evidence that H4 and H3 in active chromatin are highly acetylated is significant but not overwhelming. In H2B only low levels of mono-acetylated forms are seen and in H2A very little modification is seen. This difference between H4 and H3 versus H2B and H2A also occurs when labelling studies with radioactive acetate are done. Pulse labelling with acetate highlights those histones that are rapidly turning over acetyl groups. Under these conditions, in *Physarum,* histones H4 and H3 incorporate acetate (Fig. 2) (Waterborg and Matthews, 1983a,b) if transcription is occurring but H2B and H2A are only labelled when histone synthesis also occurs (Section IE). *Physarum* lexosomes (Peak A particles, Johnson *et al.,* 1978a; Prior *et al.,* 1983) are enriched in H3 and H4 pulse-labelled with acetate (Waterborg and Matthews, 1983b).

In mouse mammary glands (Marzluff and McCarty, 1970) or rat liver (Jiakuntorn and Mathias, 1982), active chromatin is associated with acetate turnover exclusively on H4 and H3. In many organisms a clear preponderance for acetate labelling of the arginine-rich histones is seen under conditions where histone synthesis is low or non-existent (Jackson *et al.,* 1975; Vavra *et al.,* 1982; Nelson, 1982; Shepherd *et al.,* 1971; Moore *et al.,* 1979).

We have argued, on the basis of the data obtained with *Physarum,* that in active chromatin, acetate turnover exclusively occurs on H3 and H4. H2A and H2B, while possibly acetylated to a low level of steady-state modification, are clearly not available to the enzymes involved. This implies that the amino-terminal regions of H2A and H2B in the beads-on-a-string conformation of chromatin would be immobilized or buried (Waterborg and Matthews, 1983a). This idea is supported by the observation that even chemical acetylation of chromatin by acetyladenylate acetylates only H3 and H4 at their *in vivo* sites while H2A and H2B remain completely unmodified (Shewmaker *et al.,* 1978; Cohen *et al.,* 1980). The fact that only H3 and H4 are actively being acetylated in active chromatin may

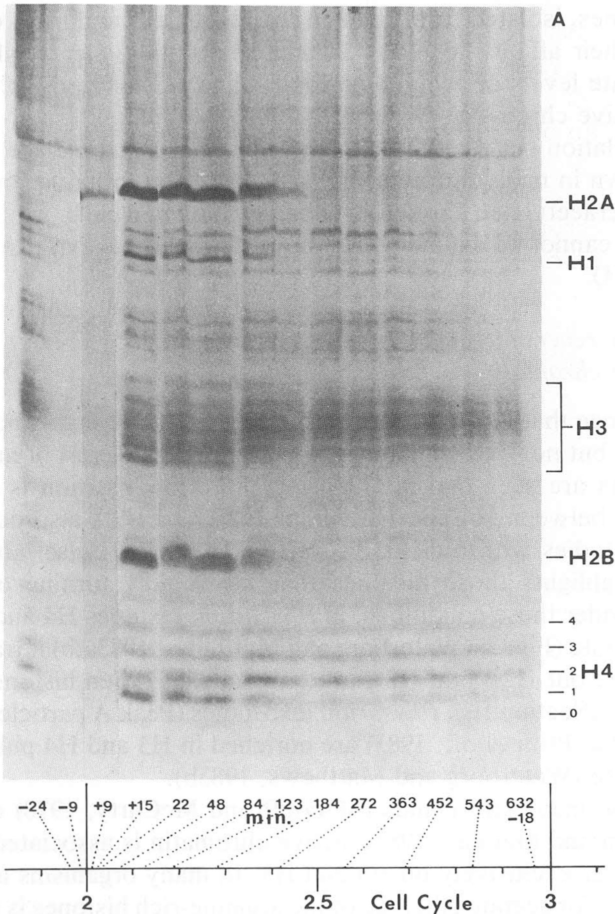

Fig. 2. Histone acetylation in the cell cycle of *Physarum polycephalum*. Macroplasmodia were labeled with [^{3}H]acetate for 3 min at the indicated positions of the 10.8-hr cell cycle between the second and the third metaphase after fusion of the microplasmodia. The histones were prepared, separated on acid–urea–Triton gel, and fluorographed (Mende *et al.*, 1983). At −24 min, prophase condensation is beginning; at −9 min, prophase condensation is clear; at +9 min there is telophase; at +15 min there is nucleolar reconstruction and decondensed non-nucleolar chromatin. The position for H2A, H1, H3, H2B, and H4, non- through tetra-acetylated, is indicated. (Reproduced from Waterborg and Matthews, 1983a.)

have bearing on the observation that destabilized active chromatin nucleosomes, such as peak A particles in *Physarum*, can be easily depleted of H3 and H4 under raised ionic strength conditions (Berkowitz and Doty, 1975; Johnson *et al.*, 1978a,b; Prior *et al.*, 1983).

The conclusion that only a minor part of histone H4 is actively turning over acetate may mean that in inactive chromatin H4 and the other core histones are frozen in their state of acetylation. Thus in the 30-nm fiber the amino-terminal regions of all four core histones are unavailable to the histone acetyltransferase and deacetylase enzymes (Waterborg and Matthews, 1983a).

Observations by Jackson *et al.* (1975, 1976) point to the existence of at least two different rates of histone acetate turnover in HTC cells under conditions of cycloheximide treatment, i.e., inhibited histone synthesis. In yeast, the turnover rate is fairly slow at several hours (Nelson, 1982) in contrast with other systems where it is rapid (e.g. Waterborg and Matthews, 1983a).

The fact that cordycepin, an inhibitor of transcription, significantly decreases the incorporation of acetate into G2-phase nuclei of *Physarum*, may point to an obligatory coupling between the movement of the RNA polymerase molecules and acetylation/deacetylation in active chromatin (Waterborg and Matthews, 1983b). In rat liver cells a similar effect has been observed with actinomycin D and cordycepin, while activation of transcription by spermine increased acetate labelling (Jiakuntorn and Mathias, 1981). However, acetylation was not sensitive to actinomycin D in HTC cells (Moore *et al.*, 1979) nor to actinomycin D, α-amanitin or rifamycin in duck erythroblasts (Ruiz-Carrillo *et al.*, 1976).

When chromatin condensation increases towards mitotic metaphase, RNA transcription slows down and stops. Both acetate turnover and the steady-state acetate content decrease at the same time (Fig. 2) (Waterborg and Matthews, 1983a; Chahal *et al.*, 1980; D'Anna *et al.*, 1977; Moore *et al.*, 1979). The shutdown of acetylation is not complete, possibly because some genes remain in a potentially active conformation even in metaphase chromosomes (Stalder *et al.*, 1978; Wilhelm *et al.*, 1982; Gazit *et al.*, 1982).

E. Replication and chromatin

1. *Histone synthesis and cytoplasmic acetylation*

Histone synthesis seems to be coordinated with DNA replication except for a basal level outside S phase (Wu and Bonner, 1981). The amino-terminal serine residue of histones H1, H2A, and H4 is irreversibly acety-

lated on the nascent polypeptide. The other histones have free amino-termini (for a review see Allfrey, 1977). However, in some H2A species no amino-terminal acetylation occurs, e.g., in *Physarum* (Mende *et al.*, 1983), while in at least one organism, *Tetrahymena,* the amino-terminal residue alanine is blocked by trimethylation (Nomoto *et al.*, 1982b). The function of the synthesis-dependent, irreversible acetylation of amino-termini is unknown.

In most organisms a generally higher level and rate of histone acetylation is seen in proliferating versus nonproliferating tissues and cell types (Allfrey, 1977). In the synchronous plasmodia of *Physarum* replication cannot be studied on its own because transcription occurs together with replication in an interdependent process (Pierron *et al.*, 1982). However, despite the presence of transcriptionally correlated patterns of acetylation, S phase–specific acetylation can be resolved (Figs. 2 and 3) (Waterborg and Matthews, 1983a, 1984b). H2A and H2B turn over acetate specifically in S phase and H3 and H4 have a specific pattern of acetate turnover in S phase. The S phase–specific acetylation is inhibited by cycloheximide but not by hydroxyurea or fluorodeoxyuridine, showing that histone synthesis but not DNA synthesis are required for acetylation (Waterborg and Matthews, 1983b, 1984b).

In some organisms, a specific form of H4 is observed that accumulates in cytoplasm and nucleus when DNA synthesis is inhibited. The accumulating form of H4 is newly synthesized and contains two reversible modifications: either two acetylated lysines in HTC cells, trout and rooster testis, and *Xenopus* oocyte nuclei (Jackson *et al.*, 1976; Cousens and Alberts, 1982; Dixon *et al.*, 1975; Oliva and Mezquita, 1982; Woodland, 1979); or a mixture of acetylated lysines and phosphorylated serine in duck erythroblasts and *Xenopus* oocyte cytoplasm (Ruiz-Carrillo *et al.*, 1975; Woodland, 1979). This accumulating form of H4 seems to be stable as long as DNA synthesis remains inhibited (Ruiz-Carrillo *et al.*, 1975; Woodland, 1979). In *Physarum,* the major form of S phase–specific H4 is mono-acetylated with lesser amounts of di- and non-modified H4 (Fig. 4). This pattern shifts towards increasing amounts of non-acetylated H4 when DNA synthesis is inhibited by fluorodeoxyuridine or hydroxyurea (Waterborg and Matthews, 1983b, 1984b).

2. *Histone deposition and chromatin maturation*

The observation that specific modified forms of newly synthesized H4 accumulate under conditions of inhibited DNA synthesis, suggests that this form of H4 is specifically required for the formation of new nucleosomes (Allfrey, 1977). H4 seems to be the first histone to associate with

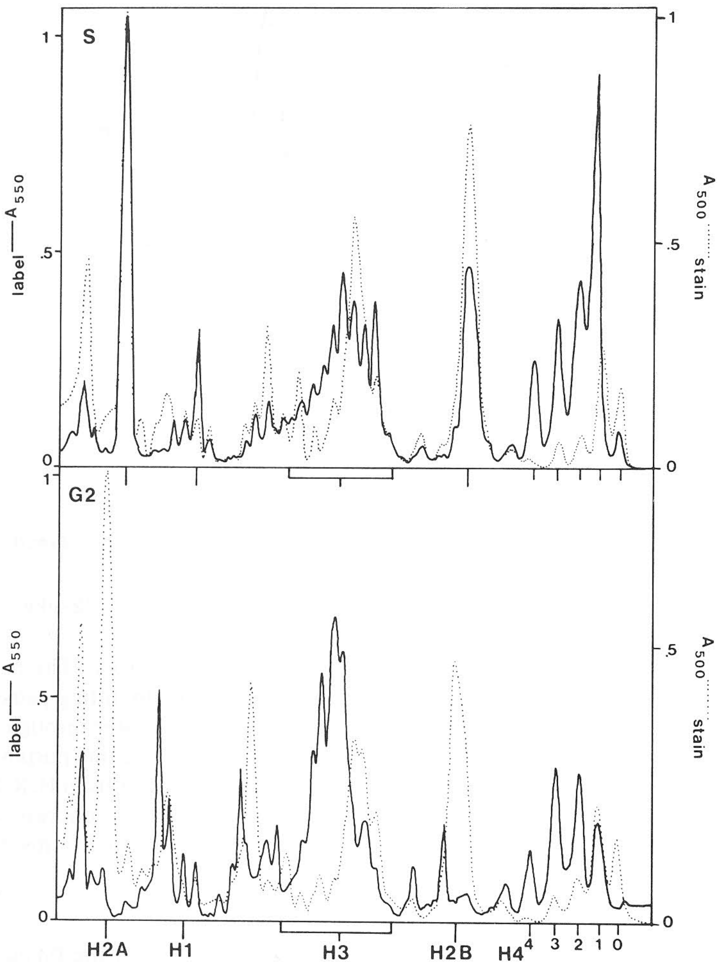

Fig. 3. Histone acetylation in S phase and G2 phase in *Physarum polycephalum*. Macroplasmodia were labelled with [^{3}H]acetate for 5 min at 20 min after metaphase 2 (S phase) and at 5 hr after metaphase 2 (G2 phase). The histones were prepared and separated on acid–urea–Triton gel (Mende *et al.*, 1983). The Coomassie-stained gel patterns (·········) are compared with the fluorogram (———). The position of H2A, H1, H3, H2B, and H4, non- through tetra-acetylated, is indicated. (From Waterborg and Matthews, 1984a.)

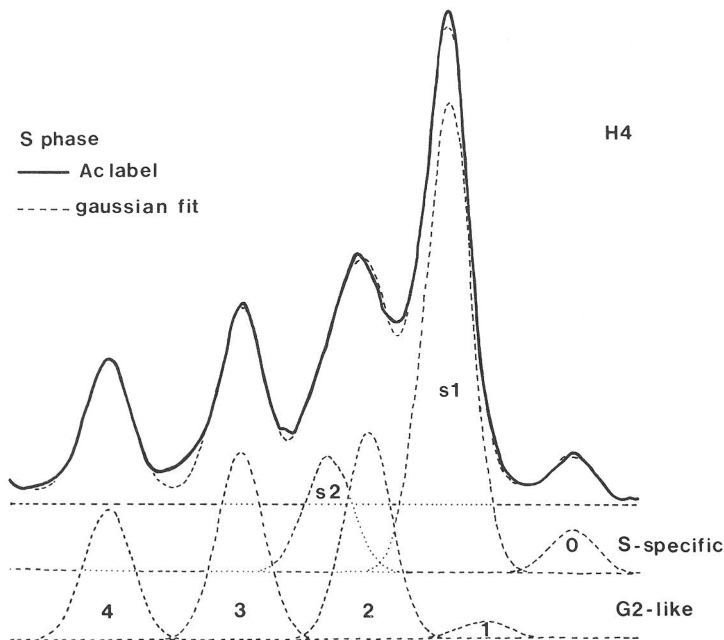

Fig. 4. Acetylation pattern of *Physarum* histone H4 in S phase. The pattern of acetylation of H4 in an S-phase fluorogram was analyzed by fitting gaussians to the scan data, collected into a Hewlett-Packard 9845S computer through a 16-bit parallel interface, using an interactive program developed for this purpose (Matthews, 1984). (This program, in BASIC, is available on request from H.R.M.) The G2-like acetylated forms and the non-acetylated form co-electrophorese with the bulk forms of H4. The S-phase–specific mono- and di-acetylated forms display a reduced mobility.

the DNA after replication and a decrease of the charge of the basic amino-terminal region of H4 may be required for the initial histone–DNA interaction (Candido and Dixon, 1972b). When nucleosomes can form, i.e. when DNA synthesis is normal or if its inhibition is lifted, a rapid deacetylation to non-modified H4 is seen which is essentially complete in 20–30 min (Fig. 5c) (Ruiz-Carrillo *et al.*, 1975; Jackson *et al.*, 1976; Cousens and Alberts, 1982). This length of time is similar to that seen for the maturation of newly made chromatin (see below). The deacetylation is followed by an increase in acetylation to the steady-state level specific for inactive or transcriptionally active chromatin (Ruiz-Carrillo *et al.*, 1975; Jackson *et al.*, 1976) (Section ID).

Newly synthesized H3 may have the same steady state level of acetylation as pre-existing H3 (Ruiz-Carrillo *et al.*, 1975, Woodland, 1979) but

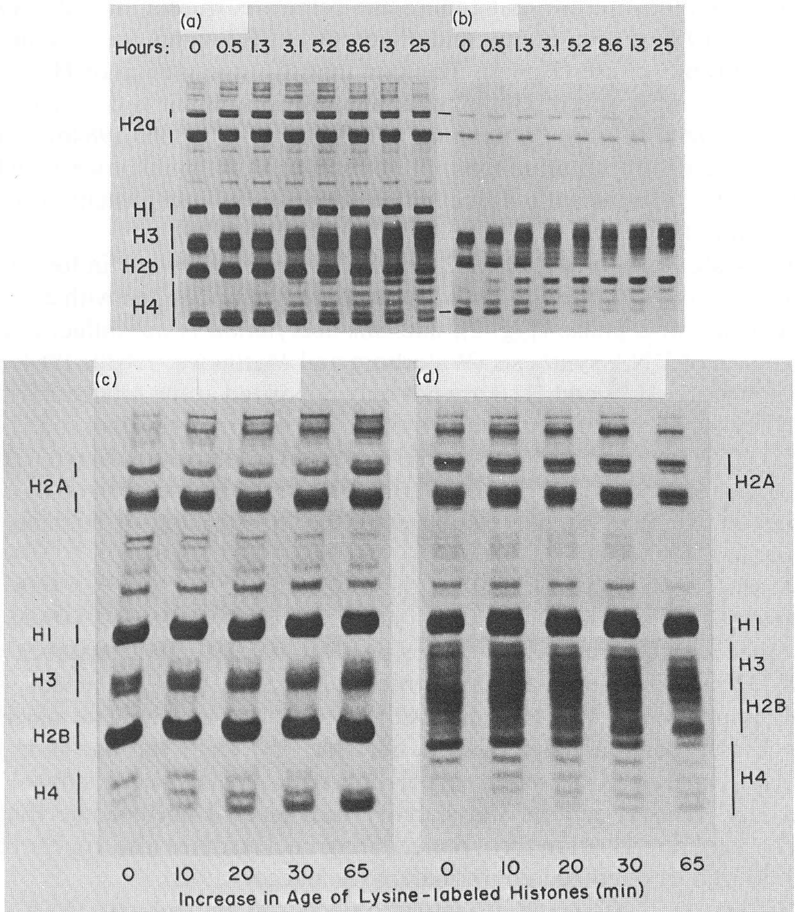

Fig. 5. The effect of butyrate on histone acetylation in HTC cells. During a chase incubation of HTC cells in 50 mM butyrate, [^{3}H]acetate–prelabelled histones reach higher levels of acetylation, and respond more rapidly, than does the total population of histones. (a) Coomassie-stained acid–urea–Triton gel with equal amounts of protein per lane. (b) Fluorographed gel with equal amounts of ^{3}H label per lane. The length of the chase incubation is indicated in hours above each gel lane. (Reproduced from Cousens and Alberts, 1982 and from Cousens *et al.*, 1979, with kind permission from the authors.) HTC cells, pulse-labelled with [^{3}H]lysine, were chased for the indicated times in unlabelled medium to increase their age. The histones were (c) isolated directly, analyzed on acid–urea–Triton gel, and fluorographed or (d) the cells were grown for an additional 16 hr in unlabelled medium containing 50 mM butyrate prior to histone isolation and analysis. (Reproduced from Cousens and Alberts, 1982, with kind permission from the authors.)

acetate labelling shows that replication-correlated forms may also exist for H3. In *Physarum,* mono- and di-acetylated H3 forms are specifically observed in S phase (Fig. 3). The fact that this acetylation of H3 is depressed by inhibition of DNA synthesis indicates that it is dependent on nucleosome assembly (Waterborg and Matthews, 1983b, 1984b). Since new H3 does not separate from pre-existing H3, it is unknown whether H3, like H4, is transiently deacetylated during chromatin formation (Jackson *et al.,* 1976).

The state of acetylation of H2A and H2B prior to chromatin formation is unknown. In *Physarum,* both histones are highly labelled with acetate, specifically in S phase (Fig. 2), and this acetylation is not influenced by inhibition of DNA synthesis (Waterborg and Matthews, 1983b, 1984b). It is known that H2A and H2B are not only deposited at the replication fork into new nucleosomes but also elsewhere in the chromatin (Jackson *et al.,* 1981). This fact makes it impossible to decide whether the acetylation of H2A and H2B occurs prior to, during or after the assembly of nucleosomes or whether the acetylation could be independent of nucleosome formation.

At the replication fork, half of the DNA can be re-packaged into nucleosomes without the formation of nucleosomes from newly synthesized histones and the remainder requires newly synthesized nucleosomes. H4 and H3 probably form the first structural element of new nucleosomes (DePamphilis and Wassarman, 1980) but this nucleosome-like particle, which may contain some H2B, is unstable in 0.45 M salt and is highly trypsin-sensitive. Only after association with new or pre-existing H2A and H2B does it acquire the stability of a mature nucleosome (Seale, 1981). At the replication fork, pre-exisiting nucleosomes also appear transiently in a destabilized conformation (Schlaeger, 1982).

Histone acetylation and deacetylation occur in parallel with DNA synthesis and nucleosome assembly. However, it is not known whether the acetylation that is seen on the newly synthesized histones also occurs on the histones of the pre-existing nucleosomes, i.e. whether the acetylation is required for nucleosome formation or whether it could be due solely to a non-specific activity of histone acetyltransferase(s). These enzyme(s) could possibly reach nucleosomal histones in the sterically open replicational chromatin prior to maturation into the 30-nm fiber. The absence of a distinct S phase–specific form of H4 in *Physarum* that has the mobility of pre-existing H4 (Fig. 4) suggests that at least the major part, if not all, of the S phase–specific acetylation is restricted to the newly synthesized histones (Waterborg and Matthews, 1983b, 1984b).

Newly replicated chromatin is highly sensitive to micrococcal nuclease

and DNase I and shows an abnormal nucleosome spacing (Mathis *et al.*, 1980; DePamphilis and Wassarman, 1980; Seale, 1981; Annunziato and Seale 1982a, 1983; Annunziato *et al.*, 1982). Maturation takes 15–30 min and requires histone deacetylation (Annunziato and Seale, 1983). At the replication fork (Moyne *et al.*, 1981; Seale, 1981), the steady-state level of acetylation is as low as that seen in the bulk of the chromatin. Also, in *Physarum*, the S phase–specific acetylation of H3 and H4 produces only mono- and di-acetylated forms and there is no multi-acetylation (Waterborg and Matthews, 1983b, 1984b).

F. Replacement of histones by protamine

Early during spermatogenesis in spermatogonia, active transcription and replication are observed with histone acetylation levels correlated with these processes. For instance, in trout testis, spermatogonia H4 is multi-acetylated (three or four modified lysines) at a steady-state level of approximately 3% (Christensen and Dixon, 1982). After meiosis to spermatocytes, replication stops and transcription decreases rapidly to zero in spermatids. However, a dramatic increase in histone acetylation is observed. In trout, the steady state level of multi-acetylated H4 rises to 30 or 40%. The acetylation levels of all histones are increased but H4 is increased to the highest degree (Christensen and Dixon, 1982) and is subject to a very rapid turnover (Oliva and Mezquita, 1982). In late spermatids in rat testis, the level of multi-acetylation, almost exclusively in H4, reaches levels of 60% and probably higher (Grimes and Henderson, 1983). It is unknown whether an increasing level of histone acetyltransferase activity or the induction of new forms of this enzyme causes the increase in the steady-state levels of acetylated histones during spermatogenesis. The observation of high turnover rates of the modification suggests that decreasing histone deacetylase levels are probably not a major factor (Christensen and Dixon, 1982).

The increase in histone acetylation correlates with the onset of protamine deposition on the DNA, and the most acetylated histone, H4, is the first histone to be displaced. This suggests, as noted previously (Candido and Dixon, 1972a,b,c), that histone acetylation is required to weaken the interaction between DNA and histone so that the protamines can displace the nucleosomal histones. Histone acetylation alone is insufficient for the complete displacement of the histones. A controlled process of proteolysis seems also to be required (Christensen and Dixon, 1982).

The function of histone acetylation in spermatogenesis, as described for trout, seems to be general. It has also been observed in the testis of rat

(Grimes *et al.*, 1975; Grimes and Henderson, 1983), locust (Bouvier and Chevallier, 1976), and cuttlefish (Wouters-Tyrou *et al.*, 1981). Histone acetylation does not increase during spermatogenesis in species where protamine replacement does not occur, such as carp (Christensen *et al.*, 1982).

The gradual shift in cellular differentiation from spermatogonia to spermatozoa results in a highly heterogeneous cell population in testis, so that observations on the relationship between acetylation and transcription become suspect (Section D). Steady-state levels of multi-acetylated H4 in total nuclei clearly above a few percent, and acetylation of H4 clearly much higher than that of H3, should be taken as indicators for testis late in development (e.g., Levy-Wilson *et al.*, 1979). Therefore it is disappointing to note that proof is lacking that nuclease-sensitive nucleosomes from trout testis, with their high level of histone acetylation, are indeed derived from transcriptionally active chromatin (see Section ID2) (Davie and Candido, 1978, 1980; Hutcheon *et al.*, 1980; Kuehl *et al.*, 1980).

G. Histone-acetylating enzymes

1. Histone acetyltransferase

Histone acetyltransferase (or acetylase) acetylates lysine residues in the amino-terminal regions of histones with acetyl-CoA as coenzyme (McCarty *et al.*, 1982). The enzyme activity is generally determined by incorporation of label from radioactive coenzyme into histones. In many cases a mixture of histones in solution has been used as substrate, but attempts have been made to determine a more realistic substrate specificity with nucleosomes or chromatin as substrate. The number of acetyltransferase studies is limited because the enzymes are generally low in activity, very heat-labile, present in several types or complexed forms requiring rigorous salt extraction procedures (Boehm *et al.*, 1980b; Sures and Gallwitz, 1980; Belikoff *et al.*, 1980; McCarty *et al.*, 1982) and in several instances naturally occuring inhibitors have been found (Cano and Pestana, 1976; Libby, 1980; Belikoff *et al.*, 1980). Also, the rapid hydrolysis of coenzyme in crude cellular or nuclear preparations, e.g. in *Physarum* (J. H. Waterborg and H.R. Matthews, unpublished results), makes study of nuclear histone acetyltransferases difficult.

In Table III the substrate specificity of observed acetyltransferase activities has been compiled, but it should be noted that the substrate specificities are highly dependent on the conditions of the assay such as ionic strength, divalent cations, and polyamines (Dod *et al.*, 1982).

Table III

Histone acetyltransferase (AT) enzyme activities[a]

Source	AT type	Substrate specificity on: Histone solution	Substrate specificity on: Nucleosomes/chromatin	Reference
Calf thymus	A	H4 > H3 > H2A = H2B = H1	N.D.	Sures and Gallwitz (1980)
	B	H4 ≫ H2A (not H3, H2B, H1)	N.D.	Sures and Gallwitz (1980)
	C	H4 > H3 > H2A = H2B = H1	N.D.	Sures and Gallwitz (1980)
Calf thymus nuclei	A	H4 > H3 > H2B ≫ H2A	H4 > H2A = H2B ≫ H3	Garcea and Alberts (1980)
Calf thymus cytoplasm	B	H4 (no other histones)	N.D.	Horiuchi and Fujimoto (1972)
Calf liver nuclei	A	H4 > H2A = H3 > H2B > H1	N.D.	Libby (1978)
	B	H4 = H3 > H2A > H2B > H1	N.D.	Libby (1978)
Bovine lymphocyte nuclei	DB	H3 > H4 > H2B > H2A (not H1)	H2B, H3, H4 ≫ H2A (not H1)	Boehm *et al*. (1980b)
SV40 chromatin	DB	N.D.	H3 = H4 ≫ H2B (not H2A, H1)	Otto *et al*. (1982)
Hog liver nuclei	A	H3 > H4 > H2B > H2A	H4 = H2B > H2A ≫ H3	McCarty *et al*. (1982)
Rat liver nuclei	—	H4 > H2B = H2A = H3 > H1	N.D.	Lue *et al*. (1973)
	—	N.D.	H4 ≫ H3, H2B, H2A (not H1)	Fukushima *et al*. (1980)
	—	N.D.	H4 > H3 > H2A (not H2B, H1)	Horiuchi *et al*. (1978)
	A	H3 > H2B = H2A > H4 (not H1)	N.D.	Libby (1980)
	A[b]	H3 > H2A > H2B > H4 > H1	N.D.	Libby (1980)
	A	N.D.	H4 > H3 > H2A = H2B	Dod *et al*. (1982)
	A	N.D.	H3 = H4 ≫ H2A, H2B[c]	Dod *et al*. (1982)
	A/DB	H3 > H4 ≫ H2B = H2A > H1	N.D.	Wiktorowicz and Bonner (1982)
	—	N.D.	H3 (no other histones)	Delpech *et al*. (1982)
Rat brain	—	H3 ≫ H4 ≫ H2B, H2A (not H1)	N.D.	Sarkander *et al*. (1975)
Drosophila	B	H4 (no other histones)	No histones	Wiegand and Brutlag (1981)
Artemia nauplii	A	N.D.	H4 = H3 > H2A, H2B (not H1)	Estepa and Pestana (1981)
	A	N.D.	H3 ≫ H4 ≫ H2A, H2B[c]	Estepa and Pestana (1981)

[a] N.D., not determined; —, not isolated and characterized.

[b] This enzyme is called "B" in the paper but is apparently type "A" (Libby, 1980).

[c] Substrate preference in the presence of spermidine.

Acetyltransferase A is the major nuclear enzyme, and it acetylates all five histones if present in solution. Even H1, which is not modified *in vivo,* is slightly acetylated. Analysis of the sites of acetylation on histones in solution showed several additional ones in addition to those *in vivo* (Sures and Gallwitz, 1980). Acetyltransferase A is a chromatin-localized enzyme and raised salt concentrations are generally employed for solubilization, but it has been shown that this can be avoided (Wiktorowicz and Bonner, 1982). Under some conditions it is found in a high molecular weight form called C (Sures and Gallwitz, 1980). Acetyltransferase A is sometimes isolated by affinity chromatography on DNA and is then called DB for DNA-binding (Boehm *et al.*, 1980b; Garcea and Alberts, 1980).

Acetyltransferase B specifically acetylates H4. The low *in vitro* activity on H2A is probably based on the identity of the amino-terminal sequence of H4 and H2A (Table I) (Sures and Gallwitz, 1980). It is inactive on nucleosomal histones, even H4 (Garcea and Alberts, 1980; Wiegand and Brutlag, 1981). Although isolated as a nuclear enzyme it can also be prepared from cytoplasm. This localization combined with the specificity for H4 suggests that it is acetyltransferase B that acetylates nascent H4 to the specific modified form that accumulates during inhibition of DNA synthesis (Section IE) (Horiuchi and Fujimoto, 1972; Ruiz-Carrillo *et al.*, 1975; Wiegand and Brutlag, 1981).

The substrate characteristics of acetyltransferase A show generally a preference, but not a specificity, for H4 and H3 over H2B and H2A in mixtures of histones and in nucleosomes, chromatin or nuclei. The differential inhibition of acetyltransferase A by a transition analogue of acetyl-CoA if acting on H4 and H3 versus H2B and H2A (Cullis *et al.*, 1982) might suggest the presence of an enzyme specific for H4 and H3, i.e., specific for transcriptionally active chromatin. However, such an enzyme has not been found. The data available suggest that acetyltransferase A is active in nuclear histone acetylation both in transcriptional and in replicational chromatin. Some indications exist that the chromatin structure and not the enzyme characteristics determine the actual specificity of the acetyltransferase for histones in both types of active chromatin. In soluble chromatin, H2A and H2B are weak acceptors for acetate. When the conformation of the chromatin is changed by H1 addition or by spermidine, exclusively H4 and H3 act as substrates for acetyltransferase A (Dod *et al.*, 1982). Factors influencing the enzyme specificity through their action on chromatin have also been found in calf thymus. During the purification of acetyltransferase A from calf thymus or hog liver nuclei, a factor is lost that is essential for activity of the enzyme on H3 in nucleosomes, while the enzyme retains its activity on H3 in solution (Garcea and

Alberts, 1980; McCarty *et al.*, 1982). The factor could have been spermidine which increases the relative acetylation of H3 in chromatin (Estepa and Pestana, 1981; Dod *et al.*, 1982).

2. *Histone deacetylase*

The detection of histone deactylase activity has been based on solubilization of acetate from a mixture of histones acetylated *in vitro* with labelled acetyl-CoA and purified histone acetyltransferase or *in vivo* with labelled acetate (Buerger, 1976; Candido *et al.*, 1978; Cousens *et al.*, 1979; Reeves and Candido, 1980; Libby and Bertram, 1980; Hay and Candido, 1983). The heterogeneity of the substrate mixture and the difficulty of its preparation has severely limited studies on histone deacetylase. The adsorption of the substrate histones to DNA or histones, especially in crude enzyme preparations, also complicates the detection of deacetylase activity (Hay and Candido, 1983). Only the enzyme from calf thymus nuclei has been studied in any detail (Inoue and Fujimoto, 1970; Vidali *et al.*, 1972; Buerger, 1976).

An alternative approach that has been used is pulse labelling of cells *in vivo* with acetate followed by a chase period (Jackson *et al.*, 1975). However, the kinetics of the decrease of histone-bound acetate are very complex and difficult to interprete in terms of enzyme activity. The location of the acetate labelling within the chromatin and the conformation of the chromatin, rather than the level of deacetylase activity, are the major factors that determine the rate of acetate turnover (Moore *et al.*, 1979).

Recently a new assay has been developed that employs an amino-terminal peptide of calf thymus histone H4 (Waterborg and Matthews, 1982a). H4 peptide 1–23 can be completely acetylated radiochemically to a very high specific activity at *in vivo* sites of acetylation only. This ensures substrate fidelity and high sensitivity for the assay. The peptide made from calf thymus H4 can be used for a very wide variety of organisms based on the extreme degree of sequence conservation of H4 (Section IB), and it is known that the deacetylase is active on small peptide substrates (Kervabon *et al.*, 1979a,b). The substrate peptide does not bind to DNA or histones, so that in crude extracts or even in isolated nuclei, histone deacetylase activity can be quantified. Using this assay, it has been found that the level of nuclear deacetylase activity in the cell cycle of *Physarum polycephalum* is constant under conditions where steady-state levels of histone acetylation fluctuate (Waterborg and Matthews, 1982b).

In all organisms surveyed, including calf thymus, which was studied in

some detail, the presence of a single nuclear histone deacetylase activity is indicated. The enzyme is chromatin-localized (Libby and Bertram, 1980) and may be associated with the nuclear matrix (Hay and Candido, 1983; Waterborg and Matthews, 1984a). Attempts at enzyme isolation and purification have often shown multiple forms with molecular weights as high as 600,000 Da, suggesting it is active in heterogeneous complexes (Kikuchi and Fujimoto, 1973). Purified deacetylase retains a high affinity for chromatin (Vidali *et al.*, 1972). No clear specificity for certain acetylated histone species over others has been found. The general preference observed for H3 and H4 could be real or could be based on the fact that these two histone substrates are often labelled to higher specific activities than the other histones (Vidali *et al.*, 1972; Inoue and Fujimoto, 1972; Buerger, 1976).

The observation that histone deacetylase activity could be inhibited by high mobility group proteins HMG 14 and 17 in Friend cells (Reeves and Candido, 1980) is very suggestive. HMG 14 and 17 may be enriched in transcriptionally active chromatin which also displays a higher level of histone acetylation (Section ID). HMG 14 and 17 could specifically inhibit histone deacetylase activity in active chromatin. This hypothesis may not be valid since very large amounts of HMGs are required for a significant inhibition of deacetylase activity (Reeves and Candido, 1980) and similar high concentrations of homologous HMGs in rooster testis (Mezquita *et al.*, 1982), or of calf thymus HMG 17 in *Physarum* (Waterborg and Matthews, 1982b), have no effect on deacetylase activity. However, possibly only certain modified forms of the HMGs—i.e., acetylated ones (Sterner *et al.*, 1981)—are effective in inhibiting deacetylase activity.

A very effective noncompetitive inhibitor of all known histone deacetylase activities is *n*-butyrate (Cousens *et al.*, 1979). Although other short-chain fatty acids also inhibit the enzyme, butyrate is most potent (Sealy and Chalkley, 1978b; Cousens *et al.*, 1979; Truscello *et al.*, 1983). Calf thymus deacetylase is inhibited more than 95% by less than 5 mM butyrate, while 50% inhibition is observed at 60 μM butyrate (Cousens *et al.*, 1979). Butyrate is now often included in procedures for histone isolation to prevent loss of the histone acetate (Davie *et al.*, 1981). However, especially in lower organisms such as *Tetrahymena, Physarum* or yeast, concentrations of 50–100 mM butyrate have to be used for complete protection against histone deacetylation (Davie *et al.*, 1981; Nelson, 1982; Vavra *et al.*, 1982; Waterborg and Matthews, 1982a). Apparently the inhibition of deacetylase activity in these organisms requires higher concentrations of butyrate. The isolated deacetylase of *Physarum* is only 50% inhibited by 30 mM butyrate (Waterborg and Matthews, 1982a).

H. Butyrate-induced hyperacetylation of chromatin

1. *Butyrate effects* in vivo *on cells and chromatin*

Butyrate treatment *in vivo* has many effects. Some of these may be related to hyperacetylation of chromatin; others may be unrelated. Most of the effects are readily or gradually reversed upon withdrawal of butyrate. Butyrate is known to cause gross changes in cellular morphology, to block cell cycle progression in G1 phase, and to alter the rate of cell cycle progression through G2, M, and S phase, thereby indirectly reducing DNA synthesis rates and obliterating the normal correlation between acetylation levels and the cell cycle (Hagopian *et al.*, 1977; Rastl and Swetly, 1978; Candido *et al.*, 1978; D'Anna *et al.*, 1980b; Littlefield *et al.*, 1982; Boffa *et al.*, 1981). Butyrate also causes induction of cellular differentiation in erythroleukemic cells, of new species of RNA and proteins including histone H1 and $H1_0$-like proteins (Leder and Leder, 1975; Leder *et al.*, 1975; Prasad and Sinha, 1976; Riggs *et al.*, 1977; Candido *et al.*, 1978; Rubinstein *et al.*, 1979; D'Anna *et al.*, 1980a,b), reduction in cellular histone acetyltransferase levels, dephosphorylation of H1, phosphorylation of H3 and HMG 14 and 17, and stimulation of poly(ADP-ribose)polymerase and many other enzyme activities (Candido *et al.*, 1978; Rastl and Swetly, 1978; Whitlock *et al.*, 1980; D'Anna *et al.*, 1980a,b, Levy-Wilson, 1981; Covault *et al.*, 1982).

Histone acetylation is affected by butyrate through competitive inhibition of deacetylase (Riggs *et al.*, 1977; Hagopian *et al.*, 1977; Sealy and Chalkley, 1978b; Cousens *et al.*, 1979; Boffa *et al.*, 1978). The inhibition of histone deacetylase activity causes a progressive increase in the acetylation level of all four core histones, which is termed hyperacetylation (Fig. 5). After butyrate treatment, chromatin contains rows of nucleosomes containing hyperacetylated histones (Vidali *et al.*, 1978; Sealy and Chalkley, 1978a; Simpson, 1978; Mathis *et al.*, 1978; Nelson *et al.*, 1978, 1979, 1980; Kitzis *et al.*, 1980; Perry and Chalkley, 1981, 1982; Georgieva *et al.*, 1982). These nucleosomes are DNase-1–sensitive, which has resulted in the use of chromatin from butyrate-treated cells as a model for transcriptionally active chromatin. However, DNase I sensitivity in such a system should not be taken as indicating that the chromatin is transcriptionally active (Vidali *et al.*, 1978; Nelson *et al.*, 1978; Reeves and Cserjesi, 1979).

Another problem is that extensive hyperacetylation of H2B occurs to a degree not seen in untreated, active chromatin (Fig. 5) (Sealy and Chalkley, 1978a; Nelson *et al.*, 1979; Cousens *et al.*, 1979; Cohen *et al.*, 1980; Schroeter *et al.*, 1981; Cousens and Alberts, 1982; Georgieva *et al.*,

1982; Bode *et al.*, 1983) and the maximum number of acetylated lysines increases with extensive butyrate treatment from four to five for H3 and H2B, and from one to two or three for H2A (Table I). Hyperacetylation has been shown to have only minor effects on measured properties of nucleosomes or chromatin, including *in vitro* transcription (Mathis *et al.*, 1978, 1980; Lilley and Berendt, 1979; Dobson and Ingram, 1980) and physical properties (Simpson, 1978; Bode *et al.*, 1980, 1983; Vidali *et al.*, 1978; Yau *et al.*, 1982).

2. *Butyrate as a probe for chromatin structure and function*

Butyrate is a useful tool for preparing acetylated histones for reconstitution studies. Its usefulness *in vivo* is probably limited to very early effects that precede the drastic deformations produced by extensive treatment.

One of the earliest observations made after butyrate is added to cells is that chromatin active in acetate turnover is much more rapidly hyperacetylated than the bulk of the chromatin (Fig. 5a,b) (Cousens *et al.*, 1979; Nelson *et al.*, 1980; Covault and Chalkley, 1980; Schroeter *et al.*, 1981; Bode *et al.*, 1983). This indicates that transcriptionally and replicationally active chromatin is the first target for hyperacetylation.

Cousens and Alberts (1982) have used brief butyrate treatment to study the maturation of chromatin. New histones near the replication fork are very rapidly hyperacetylated, this feature disappearing subsequently as the chromatin matures (Fig. 5c,d) (Cousens and Alberts, 1982) until after extensive butyrate treatment no differences are seen between old and new histones (Sealy and Chalkley, 1978a). These observations account for the presence of all DNA sequences in hyperacetylated chromatin from asynchronous proliferating cells (Perry and Chalkley, 1982). In chicken erythrocytes, terminally differentiated non-dividing cells, most of the chromatin does not become hyperacetylated (Brotherton *et al.*, 1981).

II. Phosphorylation of Histones and Nuclear Non-histone Proteins

Phosphorylation is a major modification of nuclear proteins, occurring on many protein substrates and catalyzed by many protein kinases (e.g. Allfrey, 1980). Nuclear protein kinases were recently reviewed (Matthews and Huebner, 1983), so this contribution will concentrate on the phosphoproteins and the possible significance of their phosphate groups. Most work with nuclear proteins in this area has been concerned with phosphorylation of serine and threonine. *O*-Phosphoserine and *O*-phos-

phothreonine are resistant to mild acid hydrolysis but lose the phosphate group in mild alkaline hydrolysis or strong acid hydrolysis. This type of phosphorylation can be termed alkali-labile phosphorylation. In addition, two types of alkali-stable phosphorylations are known, namely *O*-phosphotyrosine and amide-linked phosphates found in *N*-phosphohistidine, *N*-phospholysine, and *N*-phosphoarginine. Most studies of nuclear proteins, especially histone studies, have not included the amide phosphorylations because they are very acid-labile and are often destroyed by the conditions of protein isolation or analysis. Most studies of phosphotyrosine have been with non-nuclear proteins, but a recent report (Atmar and Kuehn, 1983) of phosphotyrosine in ornithine decarboxylase (to be discussed further) suggests phosphotyrosine may be found in nuclear proteins, and Henry and Hodge (1983) have reported the presence of phosphotyrosine in nuclear matrix proteins. There appears to be some coupling between nuclear protein phosphorylation and polyadenosine diphosphoribosylation (poly ADP-ribosylation) (Wong *et al.*, 1983; Tanigawa *et al.*, 1983a,b).

A. Histones

Phosphorylation has been observed in each of the five histones *in vivo*, but the major alkali-labile phosphorylation occurs on histones H1, H2A, and H3. The role of phosphorylation is probably different in each case, so they are considered separately next. Alkali-stable phosphorylation has been studied in H4.

1. Histone H1

Histone H1 binds to the outside of the nucleosome core particle in chromatin, stabilizing the DNA at the entry and exit points to the core particle (Allan *et al.*, 1980; Igo-Kemenes *et al.*, 1982) and taking part in the higher order structure of chromatin (Littau *et al.*, 1965; Noll and Kornberg, 1977; Worcel and Benyajati, 1977) (Section IC2). H1, like the core histones, has distinct structural domains; the amino-terminal region (residues 1–40 approximately) is a random coil domain in free solution; residues 41–120 approximately fold into a conserved globular structure; and the *C*-terminal 90 residues approximately comprise a second random coil domain (Hartman *et al.*, 1977; Chapman *et al.*, 1976, 1978; Bradbury *et al.*, 1975; Rall and Cole, 1971; Jones *et al.*, 1974; Matthews, 1980a). The globular domain is required to stabilize the nucleosome (Allan *et al.*, 1980); the precise function of the random coil domains is unknown, although it is believed that they interact with DNA to control the higher

order coiling of nucleosomes (Langmore and Paulson, 1983; Thoma *et al.*, 1979). The domain structure and the amino acid sequence of the globular domain are highly conserved in evolution. However, there are substantial variations in H1 sequences between organisms. Even within one cell type there is normally a number of H1 molecules differing in sequence, and the pattern of sequence microheterogeneity varies between cell types. The sequence microheterogeneity may affect phosphorylation sites as described below, but the function of different H1s is unknown (Cole, 1977; Blumenfeld *et al.*, 1978; Hohmann *et al.*, 1983).

Figure 6 shows that the phosphorylation sites in histone H1 are distributed thoughout the random coil domains of the protein, with the exception of Ser-106 (or -108, depending on the individual H1 histone) in the globular domain which can be phosphorylated *in vitro* by a cyclic AMP-independent histone kinase but has not been found to be phosphorylated *in vivo* (Langan, 1978; Langan *et al.*, 1981; Romhanyi *et al.*, 1982). Similarly, the phosphorylation site specific for the cyclic GMP-dependent protein kinase has only been observed *in vitro* (Hashimoto *et al.*, 1976; Zeilig *et al.*, 1981).

The sites that are known to be phosphorylated *in vivo* fall into two groups: Ser-37 (or -38), which is phosphorylated by the cyclic AMP-dependent protein kinase and the cyclic GMP-dependent protein kinase; and the group of sites known as the growth-associated sites (including Thr-16, Thr-136, Thr-153 and Ser-180), that are phosphorylated by a cyclic-nucleotide–independent protein kinase (Lake, 1973; Lake and Salzman, 1972; Schlepper and Knippers, 1975; Quirin-Stricker and Schmitt, 1981; Zeilig and Langan, 1980; Schmitt *et al.*, 1982; Langan, 1978; Langan *et al.*, 1981; Chambers *et al.*, 1983). It is interesting that two of these

```
                 N-terminal        /        globular              /      C-terminal

                   P              P                                  P                 P           P              P            P
acetyl ser.......thr...........ser...............................ser...............thr........thr.............ser.........ser.....COOH
         1        16              37                                 106               136         153            180                 212
                growth           cAMP                                in               growth      growth         growth       cGMP
                                 cGMP                                vitro
```

Fig. 6. The diagram shows the histone H1 molecule stretched out in a straight line, with the known sites of phosphorylation (P) identified and their approximate positions in the sequence given (16, 37, 106, 136, 153, 180, and one near the COOH-terminus). The three structural domains of H1 are identified (*N*-terminal, globular, and *C*-terminal). The kinase responsible for phosphorylating each site is indicated as follows: growth, growth-associated kinase, both *in vivo* and *in vitro;* cAMP, cyclic-AMP–dependent protein kinase, both *in vivo* and *in vitro;* cGMP, cyclic-GMP–dependent protein kinase, *in vitro* only; in vitro, histone kinase II, *in vitro* only.

phosphorylation sites are not conserved among H1 histones. The Ser-37 site is replaced by alanine in one rabbit H1 (Langan *et al.*, 1971), and the Thr-16 (or -17) site is apparently missing in a major HeLa cell histone H1 (Ajiro *et al.*, 1981b) and in H1 subcomponent 3 from rat thymus and Novikoff rat hepatoma cells (Langan, 1982).

Phosphorylation of Ser-37 occurs in non-growing cells and is stimulated by cyclic AMP and by hormones that use cyclic AMP as a second messenger (Langan, 1968, 1969a,b; Mallette *et al.*, 1973; Harrison *et al.*, 1982). There is substantial evidence that the cyclic AMP-dependent protein kinase, or possibly its catalytic subunit, is translocated from cytoplasm to nucleus in response to cyclic AMP (Palmer *et al.*, 1974; Jungmann *et al.*, 1981; Laks *et al.*, 1981; Matthews and Huebner, 1983). This could account for the increased phosphorylation of Ser-37 on H1. There is speculation that phosphorylation of chromosomal proteins including H1 may enhance transcriptional activity (e.g., Stein *et al.*, 1974), but there is little direct evidence for this attractive hypothesis, which is discussed in more detail by Jungman *et al.* (1981) and by Matthews and Huebner (1983). There are also some cell cycle-dependent changes in the activity of cyclic AMP-dependent protein kinase, but their significance is unclear (Laks *et al.*, 1981; Hardie *et al.*, 1976; Mitchelson *et al.*, 1978; Chambers *et al.*, 1983; Matthews, 1980a; Matthews and Huebner, 1983).

Phosphorylation at the growth-associated sites is correlated with cell growth rate (Balhorn *et al.*, 1971, 1972a,b,c; Marks *et al.*, 1973). Most of this phosphorylation occurs in late G2 phase and early mitosis in the cell cycle and is followed by a rapid dephosphorylation in late mitosis and early G1 phase [Bradbury *et al.*, 1973; Gurley *et al.*, 1978a,b; Ajiro *et al.*, 1981a; Zeilig and Langan, 1980; but see Fischer and Laemmli (1980) for an alternative view]. The growth-associated sites are phosphorylated at a very low level in G1 phase (approximately one phosphate per molecule depending on the subcomponent), are phosphorylated slightly during S phase to approximately 2 phosphates per molecule, and then the major phosphorylation in G2 phase increases the phosphate content to 3.5–7 phosphates per molecule (Gurley *et al.*, 1978a; Ajiro *et al.*, 1981a; Dolby *et al.*, 1981). The question of whether all the sites are involved at each stage of the cell cycle is not resolved (Langan *et al.*, 1981; Langan, 1982; Hohmannn *et al.*, 1975; Wilkinson *et al.*, 1982; Ajiro *et al.*, 1981b; Dolby *et al.*, 1981). However, there clearly are differences in H1 phosphorylation patterns between cell types and these do not appear to be solely due to different H1 sequences in those cells (Hohmann *et al.*, 1975; Wilkinson *et al.*, 1982; Hohmann and Sykes, 1983; Sherod *et al.*, 1975).

The correlation of H1 phosphorylation with mitosis has led to the general hypothesis that H1 phosphorylation is involved in the chromosome

structural changes that take place in mitosis (Bradbury *et al.*, 1973, 1974a,b; Inglis *et al.*, 1976; Gurley *et al.*, 1978a; Traub and Traub, 1978; Block and Atkinson, 1979; Boffa *et al.*, 1981; Matthews, 1980a,b; Hohmann, 1983).

H1 phosphorylation has been correlated with chromosome condensation in a mouse mutant, temperature-sensitive for H1 phosphorylation (Matsumoto *et al.*, 1980), and in systems where premature chromosome condensation occurs (Krystal and Poccia, 1981; Ajiro *et al.*, 1983) but other experiments suggest that H1 phosphorylation is not a sufficient condition for chromosome condensation (Tanphaichitr *et al.*, 1976; Krystal and Poccia, 1981). *Tetrahymena* may behave differently (Gorovsky *et al.*, 1974; Gorovsky and Keevert, 1975; Allis and Gorovsky, 1981).

Phosphorylation has been shown to reduce the strength of binding of H1 to DNA (Rattle *et al.*, 1977; Adler *et al.*, 1971, 1972; Fasy *et al.*, 1979; D'Anna *et al.*, 1979; Langan, 1982; Lennox *et al.*, 1982) and this effect leads to an increase in aggregation of DNA by H1 or a decrease depending on the sites phosphorylated (Matthews and Bradbury, 1978; Corbett *et al.*, 1980; Matthews, 1980a,b).

The effect of H1 phosphorylation on mobility in gel electrophoresis and ion-exchange chromatography has been interpreted in terms of two conformational states of H1 and the interpretation of H1 histone gel patterns may be very complex (Panyim and Chalkley, 1969a; Sherod *et al.*, 1970; Balhorn and Chalkley, 1975; Billings *et al.*, 1979; Glover *et al.*, 1981; Langan, 1982; Balhorn *et al.*, 1972c; Gurley *et al.*, 1978a; Ajiro *et al.*, 1981a; Laemmli, 1970; Fischer and Laemmli, 1980; Blumenfeld, 1979; Lennox *et al.*, 1982; Joseph *et al.*, 1981; Kincade and Cole, 1966).

Histone H1 is a good substrate *in vitro* for the calcium-activated phospholipid-dependent protein kinase (Isawa *et al.*, 1980).

In conclusion, H1 phosphorylation is a complex subject and it seems likely that the process may serve more than one function, depending on the sites of phosphorylation and the number of phosphates per molecule.

2. *Other very lysine-rich histones*

In many cell types there are new histones synthesized when proliferation ceases. In the case of ovarian erythrocytes this is histone H5, while in mammalian cells it is histone $H1_0$. (Walker *et al.*, 1980; Gjerset *et al.*, 1982; Pehrson and Cole, 1982). Both these histones have three structural domains with random coil regions at the ends and a globular region (residues 22–100 in H5) between them (Aviles *et al.*, 1978; Cary *et al.*, 1981). The amino acid sequences of $H1_0$ and H5 show major homology with each

other and lesser homology with H1 (Smith and Johns, 1980). $H1_0$ is present in growing cells although it accumulates in growth-inhibited cultures (Panyin and Chalkley, 1969b; Pehrson and Cole, 1980). In growing cells, $H1_0$ is phosphorylated in parallel with histone H1. In cells in G1 arrest, there is very little phosphorylation of $H1_0$ (D'Anna *et al.*, 1980b, 1981). This type of phosphorylation pattern has not been reported for H5, but the overall pattern of H5 phosphorylation during erythropoesis suggests H5 phosphorylation may follow a similar pattern. In early stages of erythropoesis, histone H5 is synthesized and becomes highly phosphorylated. H5 is then dephosphorylated in the mature erythrocyte (Seligy and Neelin, 1973; Tsuzuki and Loeb, 1974; Sung *et al.*, 1977). The phosphorylation sites *in vivo* on H5 have not been determined but phosphorylation *in vitro* with a cyclic AMP-dependent protein kinase phosphorylated mainly Ser-145 with lesser sites at Ser-22, -20, and -166 (Martinage *et al.*, 1980). Phosphorylation *in vitro* with a cyclic AMP-independent protein kinase modified mainly serines 117 and 148 with minor sites at serines 3, 7, 104, and 117 (Martinage *et al.*, 1981).

3. *Sperm-specific proteins*

Spermatogenesis often involves replacing histones with small arginine-rich proteins called protamines, and this process has been studied in rainbow trout by Dixon's group (Dixon *et al.*, 1975; Louie *et al.*, 1973; Louie and Dixon, 1972a,b,c; Ingles and Dixon, 1967). Serine residues in protamines become highly phosphorylated when they appear in the nucleus during early stages of spermatogenesis, and the protamines are then slowly dephosphorylated as chromosome condensation increases to the final stage of highly condensed nucleoprotamine. Dixon has proposed that protamines bind to DNA in their phosphorylated state, the reduced net charge on the protamine giving it sufficient mobility on the DNA to locate its specific binding sites. Dephosphorylation would then "lock" the protamine in position and possibly also enhance condensation (Louie and Dixon, 1972a,b,c).

In a different fish, the winter flounder, protamines do not occur, but a set of high molecular weight basic nuclear proteins is synthesized late in spermatogenesis and is found in mature sperm together with histones. As in the case of protamines, the high molecular weight basic nuclear proteins are initially very highly phosphorylated and are then dephosphorylated during chromosome condensation. The dephosphorylation is correlated with dephosphorylation of the *N*-acetyl serine of histones H2A and H4 and with the loss of HMG proteins. Hence, phosphorylation and

dephosphorylation appear to be important in spermatogenesis although the mechanisms involved are still a matter of speculation (Kennedy and Davies, 1981).

4. *Histone H3*

Histone H3 may be phosphorylated *in vivo* in a number of tissues (reviewed by Gurley *et al.*, 1978b) and the site of phosphorylation is Ser-10 (Dixon *et al.*, 1975). Histone H3 may be phosphorylated *in vitro* by a cyclic AMP-independent protein kinase that is highly specific for H3 but phosphorylates threonine-3 (Shoemaker and Chalkley, 1978, 1980). Whitlock *et al.* (1980, 1983) reported a calcium-dependent phosphorylation of H3 in isolated HeLa cell nuclei which was increased by pretreatment of the cells with butyrate. However, the *in vivo* significance, if any, of these results is not clear. Histone H3 can also be labelled with thiophosphate in HeLa cells (Sun *et al.*, 1980; Sun and Allfrey, 1982).

Gurley *et al.* (1974) showed that H3 phosphorylation occurred during mitosis in Chinese hamster ovary (CHO) cells. The phosphorylation appeared to occur on all H3 molecules (Gurley *et al.*, 1978a) and in contrast to H1 phosphorylation was entirely restricted to mitosis (Gurley *et al.*, 1981). Joseph *et al.* (1981) also reported phosphorylation of histone H3 in CHO cells but found it was concentrated in one variant of H3. Jackson *et al.* (1975) found a rapid turnover of phosphate on H3 in growing (non-synchronous) cells. Allis and Gorovsky (1981) found H3 phosphorylation only in the mitotically dividing micronuclei and not in the amitotically dividing macronuclei. In micronuclei, only the processed form of H3 was phosphorylated, with the H3 precursor (which has a 6-amino acid leader sequence; Allis *et al.*, 1980a,b) being unphosphorylated. Allis and Gorovsky (1981) interpret their data as supporting a role for H3 phosphorylation in chromosome condensation in mitosis as originally proposed by Gurley *et al.* (1978a). In a temperature-sensitive mutant of baby hamster kidney cells, premature chromosome condensation occurs when the cells are shifted from the temperature permissive for growth to the non-permissive temperature. Phosphorylation of H3 is tightly coupled to the occurrence of premature chromosome condensation (Ajiro *et al.*, 1983).

The *in vivo* correlations suggest that phosphorylation of histone H3 is associated with the condensed state of chromatin at metaphase. However, Paulson and Langmore (1983) failed to observe any difference in X-ray diffraction patterns of metaphase chromosomes whether H3 was phosphorylated or not. The amino acid sequence of the amino-terminus of calf thymus histone H3 is given in Table I. This region of H3 forms a random coil domain in free histone (Bradbury *et al.*, 1981). It seems likely

that there is a highly specific interaction of Ser-10 in chromatin if it is important in condensing chromatin.

5. *Histone H2A*

Phosphorylation of histone H2A, unlike that of H1 and H3, does not change during a cell's progress through the cell cycle (Gurley *et al.*, 1975, 1978c) and is similar in proliferating and quiescent cells (Prentice *et al.*, 1978). Only a small proportion of the H2A molecules are phosphorylated at a given time and different H2A sub-fractions may be phosphorylated to different extents (Balhorn *et al.*, 1972b,c; Jackson *et al.*, 1975; Dolby *et al.*, 1979; D'Anna *et al.*, 1980a; Halleck and Gurley, 1980; Pantazis and Bonner, 1981; Joseph *et al.*, 1981). H2A may be phosphorylated when it is conjugated with ubiquitin in protein A24 (Goldknopf *et al.*, 1979). H2A phosphorylation may play a structural role in transcriptionally active chromatin, but the evidence is very indirect (Prentice *et al.*, 1982; Ruiz-Carrillo *et al.*, 1976; Allis and Gorovsky, 1981; Neuman *et al.*, 1978; Boffa *et al.*, 1981) and sometimes contradictory (Gurley *et al.*, 1978c; Halleck and Gurley, 1980).

6. *Histone H4*

Histone H4 can be phosphorylated on the amino-terminal serine, which is also acetylated, giving α-*N*-acetyl-O-phosphoserine. Although the α-*N*-acetyl group is added irreversibly, the O-phosphate group is a reversible modification. In erythrocytes, newly synthesized H4 is phosphorylated and then dephosphorylated soon after synthesis and may be involved in maturation of the nucleosome (Ruiz-Carrillo *et al.*, 1975). In trout testis, phosphorylation and dephosphorylation appear to take place over a longer period (Louie and Dixon, 1972b; Candido and Dixon, 1972a,b; Honda *et al.*, 1975a,b; Dixon *et al.*, 1975). H4 phosphorylation occurs in mammalian cells (Jackson *et al.*, 1975, 1976; Joseph *et al.*, 1981; Ajiro *et al.*, 1983) but not in the lower eukaryotes *Physarum* (Mende *et al.*, 1983) or *Tetrahymena* (Allis and Gorovsky, 1981). The role of this phosphorylation is unknown.

Histone H4 is also subject to phosphorylation of histidines. This involves a P–N phosphoramidate bond with either N^1 or N^3 of the histidine. This bond is very acid-labile and is usually destroyed during histone isolation. However, there is evidence that, in nuclei, it is quantitatively as important as acid-stable phosphorylation (Chen *et al.*, 1977; Zetterquist and Engström, 1966). Phosphohistidine is formed by nuclear kinases that have been detected in several rat tissues and a Walker 256 carcinosarcoma cell line (Smith *et al.*, 1973, 1974) and in *Physarum* (Matthews and

Huebner, 1984b). In regenerating rat liver, nuclear kinase increased in activity in parallel with DNA synthesis. Phosphohistidine was isolated from total H4 in regenerating rat liver but not from newly synthesized H4. It has been reported that both histidines in H4 may be phosphorylated *in vitro* (Fujitaki *et al.*, 1981) and *in vivo* (Bruegger, 1977). More work is clearly required in this area (Matthews and Huebner, 1984a).

B. Non-histones

Phosphorylation of non-histone chromosomal proteins has been regarded as a potential mechanism for controlling transcription for many years (e.g., Gershey and Kleinsmith, 1969), and the phosphorylation of these proteins changes during growth, development, hormone stimulation, or aging (Teng *et al.*, 1971; Borun and Stein, 1972; Ruiz-Carrillo *et al.*, 1974; Karn *et al.*, 1974; Yeoman *et al.*, 1975; Kleinsmith, 1975; Neuman *et al.*, 1978; Kahn *et al.*, 1982), due to the action of a variety of protein kinases (Kish and Kleinsmith, 1974; Jungmann and Kranias, 1977; Christmann and Dahmus, 1981; Matthews and Huebner, 1984a). In a few cases, to be discussed in this section, phosphorylation of specific non-histone proteins has been studied, but there is still little understanding of the actual role or roles of non-histone protein phosphorylation in general. For example, Saffer and Coleman (1980) purified a DNA-binding protein of molecular weight 55,000, termed D-55, that stimulated transcription of reconstituted nucleosomes by *E. coli* RNA polymerase, and the stimulation was abolished by incorporation of 1 mole phosphate per mole D-55. More recently, Murdoch *et al.* (1982) found a specific nuclear protein that was phosphorylated in response to cyclic AMP. This protein co-migrated with histone H1 in SDS gel electrophoresis but not in Triton–acid–urea gel electrophoresis.

Nuclear enzymes that can be phosphorylated include topoisomerase I (Mills *et al.*, 1982), RNA polymerase II (to be discussed) and ornithine decarboxylase (to be discussed). A number of structural proteins may also be phosphorylated (e.g. Song and Adolph, 1983). The chromosomal conjugate protein (A24 or uH2A), formed by linking the carboxy terminus of ubiquitin to the ε-amino group of Lys-119 of histone H2A (Goldknopf and Busch, 1977), is phosphorylated in Novikoff hepatoma cells on a serine in the H2A part of the molecule (Goldknopf *et al.*, 1979).

If non-histone protein phosphorylation modulates gene activity, then hormone receptors might be expected to possess protein kinase activity. Such activity has been found in two components of the chicken oviduct progesterone receptor. One of these components, a protein of molecular weight 110,000, has been found in the nucleus as well as in the cytoplas-

mic receptor. Its protein kinase activity requires Mg^{2+} and results in autophosphorylation in the absence of other substrates. It will phosphorylate histone substrates. The *in vivo* role of the kinase activity may include phosphorylation of chromatin regulatory proteins but this has yet to be demonstrated (Garcia *et al.*, 1983).

1. HMG proteins

HMG proteins constitute a group of proteins that can be extracted from nuclei with 0.35 M NaCl, are soluble in 2% trichloracetic acid and contain a high proportion of acidic and basic residues (Johns, 1982). They suddenly became fashionable when two of them, HMG 14 and HMG 17, appeared to be associated with active chromatin and to be at least partly responsible for the DNase-I sensitivity of active chromatin (Levy-Wilson *et al.*, 1977; Weisbrod and Weintraub, 1979; Weisbrod *et al.*, 1980). In the rush to report key initial experiments, a number of mistakes were made and the field is only now beginning to settle down. The discussion below attempts to give the current view of HMG phosphorylation, but several apparent disagreements in experimental results remain unresolved.

HMG 1 and HMG 2 are not phosphorylated *in vivo* in HeLa cells (Bhorjee, 1981; Walton and Gill, 1983), Ehrlich ascites cells, L1210 cells (Saffer and Glazer, 1980), P388 leukemia cells, human colon carcinoma cells (HT-29), or CHO cells (Saffer and Glazer, 1982). However, an HMG-1–like protein in *Tetrahymena* is phosphorylated (Levy-Wilson *et al.*, 1983). HMG 1 was thiophosphorylated *in vitro* using the cyclic AMP-dependent protein kinase (Sun *et al.*, 1980), but phosphorylation of HMG 1 or 2 by the same kinase was not observed by Walton *et al.* (1982).

HMG 14 is extensively phosphorylated in all cell types studied (Halegoua and Patrick, 1980; Hasuma *et al.*, 1980; Saffer and Glazer, 1980, 1982; Levy-Wilson, 1981; Bhorjee, 1981; Bhorjee *et al.*, 1983; Levy-Wilson *et al.*, 1983; Walton and Gill, 1983; Paulson and Taylor, 1982; Cooper *et al.*, 1982; Arfmann *et al.*, 1981). The level of phosphorylation is sensitive to factors that change the activity of cells. Saffer and Glazer (1982) found that phosphorylated HMG 14 was preferentially released after brief digestion of nuclei by micrococcal nuclease but not by brief digestion with DNase 1.

Mammalian HMG 14 can be phosphorylated *in vitro* at Ser-6 by cyclic-nucleotide–dependent protein kinases (Walton *et al.*, 1982; Taylor, 1982; Palvino *et al.*, 1983), but Palvino *et al.* (1983) failed to phosphorylate HMG 14 from ovarian erythrocytes with a cyclic GMP-dependent protein kinase. This is due to the absence of the major phosphorylation site (Ser-6) in ovarian erythrocyte HMG 14. This site is phosphorylated in

bovine thyroid slices stimulated by TSH, probably by the cyclic AMP-dependent protein kinase. However, this is not the site phosphorylated during the HeLa cell cycle. Casein kinase II (Hathaway and Traugh, 1982; Matthews and Huebner, 1984a) phosphorylates one major site on HMG 14 which does correspond to the site phosphorylated *in vivo* in growing HeLa cells (Walton and Gill, 1983). HMG 14 can also be phosphorylated *in vitro* by the Ca^{2+}-phospholipid-dependent protein kinase (Kikkawa *et al.*, 1982; Wise *et al.*, 1982; Ramachandran *et al.*, 1984).

Phosphorylation of HMG 17 has been reported by several groups (Saffer and Glazer, 1980, 1982; Bhorjee, 1981; Arfmann *et al.*, 1981; Levy-Wilson, 1981; see also Lund *et al.*, 1981). However, these results have been questioned by Bhorjee and colleagues (Bhorjee *et al.*, 1983; D'Anna *et al.*, 1983) and the phosphorylation of HMG 17 *in vivo* needs to be investigated more rigorously. HMG 17 is a substrate for protein kinase N II *in vitro* (Inoue *et al.*, 1980). Recently, Ramachandran *et al.* (1984) have shown that HMG 17 is a high-affinity substrate for the Ca^{2+}-phospholipid-dependent protein kinase *in vitro* and suggest that this kinase may phosphorylate HMG 17 *in vivo*, but this remains to be demonstrated. If Ca^{2+}-phospholipid-dependent phosphorylation of HMG 14 and 17 occurs *in vivo* then it could link physiological stimuli that affect the phosphatidylinositol cycle and hence diacyl glycerol formation (Michell, 1975) with changes in chromosome structure leading to modified gene expression.

2. *RNA polymerase*

Three distinct RNA polymerases are present in nuclei. RNA polymerase I (or A) is responsible for synthesizing the large ribosomal RNA precursor; RNA polymerase II (or B) synthesizes HnRNA and some small nuclear RNAs; and RNA polymerase III (or C) synthesizes transfer RNA precursor and 5S ribosomal RNA (Losick and Chamberlin, 1976). The process of synthesizing an RNA molecule involves many stages, including correct initiation, elongation on the correct strand, and correct termination. However, studies of phosphorylation of RNA polymerase to date have used non-selective assay systems where aberrant types of RNA synthesis may have predominated. This limits the validity of conclusions drawn from *in vitro* measurements of RNA polymerase activity (e.g., Matthews and Huebner, 1984a).

It is clear that RNA polymerases are phosphoproteins (Bell *et al.*, 1977; Dahmus, 1981b) but the sites of phosphorylation have not been determined. Treatment of RNA polymerase I with phosphatase did not affect the polymerase activity, as assayed by a non-selective assay system (Bell *et al.*, 1977). All three polymerases can be phosphorylated *in vitro* and some reports describe a stimulation of activity (Jungmann *et al.*, 1974;

Kranias *et al.*, 1977; Hirsch and Martelo, 1976; Dahmus, 1976; Duceman *et al.*, 1981) but this has not been confirmed (Bell *et al.*, 1977; Dahmus, 1981b) in different systems. Bell *et al.* (1977) studied yeast RNA polymerases and a kinase that initially co-purified with RNA polymerase I and was similar in its subunit composition to casein kinase II (Matthews and Huebner, 1984a; Hathaway and Traugh, 1982). This kinase phosphorylated several RNA polymerase subunits (Bell *et al.*, 1977). The co-purification of RNA polymerase I and casein kinase II was later confirmed in mammalian cells by Rose *et al.* (1981), who suggested that casein kinase II was a subunit of RNA polymerase I holoenzyme. This view has been refuted by Dahmus (1981a), who separated casein kinase II and RNA polymerase I and found no similarities between their respective polypeptides.

RNA polymerases are phosphorylated *in vivo* and are substrates *in vitro* for cyclic AMP-dependent protein kinase and the cyclic AMP-dependent casein kinases I and II, but the role, if any, of phosphorylation in the function of RNA polymerase is quite unclear.

3. Ornithine decarboxylase

Ornithine decarboxylase is regulated by reversible phosphorylation and dephosphorylation in the slime mold *Physarum polycephalum* (Daniels *et al.*, 1981; Kuehn *et al.*, 1981; Atmar and Kuehn, 1981). The phosphorylated form of ornithine decarboxylase is inactive. The kinase that phosphorylates active ornithine decarboxylase requires the polyamines spermidine and spermine for activity (Atmar *et al.*, 1978). These molecules are the end-products of the pathway initiated by ornithine decarboxylase. The kinase is inhibited by putrescine, an intermediate in the pathway. This system provides feedback control or end-product inhibition for the synthesis of polyamines. Recent results suggest that the kinase is also involved in cellular responses to interferon and Ca^{2+}-mediated agents, since the polyamine-dependent protein kinase can be activated by interferon (Kuehn and Atmar, 1982a,b) or inhibited by autophosphorylation in the presence of Ca^{2+}-calmodulin. The polyamine-dependent protein kinase phosphorylates tyrosine residues (Atmar and Kuehn, 1983), and other polyamine-dependent kinases have been reported (Ahmed *et al.*, 1983).

The phosphorylated form of ornithine decarboxylase, while enzymatically inactive, has an intriguing effect on transcription of the genes for ribosomal RNA in *Physarum* (Kuehn *et al.*, 1979; Atmar *et al.*, 1980). These genes are found on DNA molecules of size 60,000 base pairs located in the nucleolus. Each of these DNA molecules behaves like a mini-

chromosome, with two copies of the genes for ribosomal RNA arranged palindromically near the ends of the mini-chromosome (Molgaard *et al.*, 1976; Seebeck *et al.*, 1979) with a large central "spacer" sequence containing inverted repeats (Ferris and Vogt, 1982) and 5-methylcytosine (Cooney *et al.*, 1984). The phosphorylated form of ornithine decarboxylase binds to the central spacer region and stimulates transcription of the ribosomal RNA genes (Kuehn *et al.*, 1979; Atmar and Kuehn, 1981). The initiation of transcription occurs at the inner ends of the ribosomal RNA genes (Sun *et al.*, 1979) but the binding site for ornithine decarboxylase appears to be far upstream from the transcription initiation sites. The activation does not appear to be due to a direct effect of phosphorylated ornithine decarboxylase on RNA polymerase I (Atmar *et al.*, 1980).

4. *Structural proteins of the nucleus*

The cell nucleus is thought to be enclosed by a pair of concentric membranes separated by the perinuclear space. The membranes and perinuclear space are interrupted by nuclear pore complexes that pass through from the cytoplasmic face of the outer nuclear membrane to just beyond the inner nuclear membrane. The nuclear pore complexes are connected by a fibrillar network called the lamina. Usually, the nuclear pores and lamina are isolated together as the pore–lamina complex or with the nuclear membrane as the nuclear envelope. The lamina is located near the surface of the inner nuclear membrane, between it and the peripheral chromatin. Within the nucleus, in interphase, there is another structural framework, the nuclear matrix. These interphase structures change dramatically in mitosis; the lamina is dispersed, and the matrix appears to rearrange itself to form the "scaffolds" on which metaphase chromosomes are aligned (Paulson and Laemmli, 1977; Gerace and Blobel, 1980; Bekers *et al.*, 1981).

The major protein constituents of the lamina are called lamins A, B, and C. These components have molecular weights in the region of 60,000–70,000. For example, in rat liver the molecular weights are 69,000, 67,000, and 62,000 for A, B, and C, respectively. Lamins A and C are closely related in primary structure, while lamin B is different (Kaufmann *et al.*, 1983). The fate of the lamins in mitosis was investigated by Gerace and Blobel (1980), who obtained mitotic CHO cells by mechanical shake-off and compared them with interphase cells. All three lamins were phosphoproteins and their phosphate contents were measured using long-term labelling with ^{3}H-leucine and ^{32}P-phosphate *in vivo*. Lamins A and C were found to be threefold higher in phosphate content during mitosis compared with interphase, while lamin B was sixfold higher. In early G1

phase, all lamins had an intermediate phosphate content. The increases in phosphate content were correlated with loss of the lamin structure and solubilization of lamins A and C. Lamin B remained associated with membrane components during mitosis (Gerace and Blobel, 1980). Phosphorylation of lamina proteins also occurs *in vivo* with an endogenous kinase that phosphorylates a polypeptide of molecular weight 68,000 (Allen *et al.*, 1977; Lam and Kasper, 1979; Agutter *et al.*, 1979; Krohne *et al.*, 1981). Lamins A and C show multiple spots in isoelectric focussing, consistent with heterogeneity of phosphorylation, but lamin B shows a single spot. These isoelectric focussing results were obtained from rat liver, a non-dividing tissue (Kaufmann *et al.*, 1983); it would be interesting to compare them with a dividing tissue since Gerace and Blobel (1980) found a major change in phosphorylation of lamin B during mitosis.

The proteins of the nuclear matrix are less well characterized than lamins A, B, and C of the lamina, but they are phosphorylated. Cell cycle studies in HeLa cells indicate an increase in phosphorylation of nuclear matrix proteins in S phase and pre-mitosis, with a lower level of phosphorylation in G1 phase. Song and Adolph (1983) identified a polypeptide of molecular weight 119,000 as the major phosphorylated species in dividing HeLa cells. Henry and Hodge (1983) identified phosphoserine as the major phosphorylated amino acid, but they also found significant amounts of phosphothreonine and phosphotyrosine. In spite of the indications that the nuclear matrix may form the chromosome scaffolds during mitosis (Bekers *et al.*, 1981), Song and Adolph (1983) found low levels of phosphorylation of proteins from isolated metaphase chromosomes, in contrast to high levels in nuclear matrix proteins pre-mitosis. It seems likely that there is a complex series of protein phosphorylations and dephosphorylations as cells prepare for and go through mitosis. Additional phosphorylation events are apparently associated with S phase (Song and Adolph, 1983).

III. Other Modifications of Histones and HMG Proteins

A. Histone polyADP-ribosylation

PolyADP-ribosylation (poly adenosinediphosphoribosylation; for a recent review, see Mandel *et al.*, 1982), a reversible modification of proteins including histone H1, has been functionally correlated with DNA repair synthesis in chromatin. The modification only occurs on a minor fraction of H1 but the level of modification may be very high, yielding polymers of H1 connected through the modification groups (Aubin *et al.*, 1982b;

Holtlund *et al.*, 1983; Wong *et al.*, 1983). Electron microscopic studies have suggested that polyADP-ribosylation of H1 can convert a small region of a 30-nm fiber into a beads-on-a-string conformation (Poirier *et al.*, 1982). In such a sterically open structure, repair of patches of damaged DNA can take place. DNA repair can also be facilitated *in vitro* by a similar structural change, which occurs in chromatin that has been hyperacetylated by extensive butyrate treatment (Smerdon *et al.*, 1982). *In vivo*, chromatin active in transcription or replication is not known to contain polyADP-ribosylated histones. The apparent preference for modification of condensed chromatin (Levy-Wilson *et al.*, 1979) correlates well with the preferential localization of the polyADP-ribose polymerase in the oligonucleosome fraction of isolated chromatin (Aubin *et al.*, 1982a).

Although *in vivo* H1 is the major histone acceptor, some modification of the core histones may also occur (Holtlund *et al.*, 1983) as observed *in vitro* (Levy-Wilson, 1983).

B. Ubiquitin modification of histones H2A and H2B

Modification of histone H2A, and to a lesser extent H2B, by the protein ubiquitin through an isopeptide linkage of the terminal carboxyl group of ubiquitin to a lysine near the carboxy terminus of the histone, is reversible (for a recent review, see Busch and Goldknopf, 1981). A specific isopeptidase is required for the removal of the modification (Matsui *et al.*, 1982). Although this histone modification has no clear function (Goldknopf *et al.*, 1978), it has recently been suggested that the ubiquitin–H2A histone analogue helps the structural transition of chromatin from a condensed to a open configuration, and vice versa (I. L. Goldknopf, personal communication). Specific loss of the ubiquitin moiety from H2A has been observed for CHO cells entering mitosis, and reformation of ubiquitinated H2A for the transition from mitosis into G1 phase (Matsui *et al.*, 1979). The ubiquitin moiety apparently has no effect on nucleosomal stability or on acetylation and phosphorylation of the histone (Goldknopf *et al.*, 1979).

C. Histone methylation

Methylation of specific lysine residues in H4 and H3 is a form of irreversible histone modification that starts after histone synthesis but is completed only by the beginning of mitosis (Thomas *et al.*, 1975). Thus the major part of histone methylation occurs in G2 phase within assembled chromatin, but loss of function in undermethylated chromatin has never been reported. This has led to the suggestion that methylation may be

required for mitotic condensation (Honda *et al.*, 1975b; Camato and Tanguay, 1982). However, there is no proof for this function of lysine methylation, and no indication of the function of the rare methylation of histidines and arginines that may also occur in histones (DeLange and Smith, 1971; Duerre and Chakrabarty, 1975).

Lysine methylation occurs in or near amino-terminal regions of H3 and H4 (Table I). Recently, a new site for lysine methylation has been observed in *Physarum* H4 at residue 79 (Waterborg *et al.*, 1983). The extent of modification of lysines varies widely from incomplete with mono-, di-, and trimethylated forms in *Physarum* H4 to complete with mono- and dimethylated forms in H4 and mono-, di-, and trimethylated forms in H3 in mammalian organisms (Table I). It also ranges from no modified residues per histone H4 in pea and one in calf thymus (DeLange *et al.*, 1969a,b) to as high as 44% trimethylation of all lysines in wheat germ H4 (Motojima and Sakaguchi, 1981). Although generally H4 and H3 are the only methylated histones (Hempel *et al.*, 1968; Duerre and Chakrabarty, 1975), sometimes only H2A is methylated as in *Tetrahymena* (Levy-Wilson, 1983), or H2B in addition to H3 and H4 in *Drosophila,* where methylation of H4 is not affected by heat-shock treatment, that of H3 is reduced, and that of H2B is increased (Camato and Tanguay, 1982).

D. Modifications on HMG proteins

In addition to phosphorylation of HMG proteins (Section II) (Weisbrod, 1982a), other reversible and irreversible modifications are known, in particular on HMG 14 and 17.

Reversible acetylation of lysines in the amino-terminal region of these proteins is observed *in vivo* in duck erythrocytes (Sterner *et al.*, 1981). Calf thymus or hog liver histone acetyltransferase will acetylate the HMGs *in vitro,* and histone deacetylase is active on acetylated HMGs (Sterner *et al.*, 1981; McCarty *et al.*, 1982). The change in HMG character due to acetylation seems only minor in the highly charged non-structured proteins. The acetylation sites are found in the basic amino-terminal region just as in histone (Sterner *et al.*, 1981). Since the function of HMG 14 and 17 proteins is not clear, the function of HMG acetylation remains also unknown. However, it may be correlated with the preferential localization of HMG 14 and 17 in transcriptionally active chromatin, where they may inhibit histone deacetylase activity (Section IG2) (Mathis *et al.*, 1980; Reeves and Candido, 1980; Weisbrod, 1982a).

Another function of the HMGs is suggested by the observation that they are irreversibly glycosylated (Reeves *et al.*, 1981). Through their carbohydrate moieties they may interact with the nuclear matrix of the

cell, while interacting at the same time with the chromatin, possibly preferentially the active chromatin, so that they could function as anchors of the chromatin within the nucleus (Reeves and Chang, 1983; Waterborg and Matthews, 1984).

PolyADP-ribosylation and methylation are additional modifications observed on HMG proteins without any functional correlation (Wong *et al.*, 1977; Boffa *et al.*, 1979; Mathis *et al.*, 1980; Tanuma and Johnson, 1983; Levy-Wilson *et al.*, 1983).

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