



HAL
open science

Chromatin and gene regulation at the onset of embryonic development

Ap Wolffe

► **To cite this version:**

Ap Wolffe. Chromatin and gene regulation at the onset of embryonic development. *Reproduction Nutrition Development*, 1996, 36 (6), pp.581-606. <hal-00899927>

HAL Id: hal-00899927

<https://hal.science/hal-00899927v1>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Review article

Chromatin and gene regulation at the onset of embryonic development

AP Wolffe

Laboratory of Molecular Embryology, National Institute of Child Health and Human Development,
NIH, Bldg 18 T, Room 106, Bethesda, MD 20892-5431, USA

(Received 20 August 1996; accepted 2 September 1996)

Summary — Major transitions in chromosome and chromatin structure occur during development. Recent genetic and biochemical experiments demonstrate that these alterations in genome organization make significant contributions to establishing and maintaining states of differential transcriptional activity. Developmentally regulated changes in histone variants have a causal role in determining patterns of gene activity, directing the repression of specific eukaryotic genes. Chromosomal proteins such as Polycomb stabilize and maintain transcriptionally repressed states. Proteins regulating mitotic chromosome condensation have a role in determining the overall transcriptional activity of a chromosome. In this review, I place the developmental roles of these chromosomal constituents in a structural and functional context. Considerable insight now exists into the molecular mechanisms regulating chromosomal activity during development. Chromosomal architecture is emerging as a key controlling influence in the developmental regulation of gene expression. Recent genetic experiments using *Caenorhabditis elegans*, *Drosophila melanogaster* and the mouse have provided clear evidence for the functional differentiation of chromosomal structures during development. Chromosomes are visualized as highly specialized entities, within which the activity of particular domains is largely determined by defined structural proteins. At a more local level, the mechanisms regulating gene transcription during early embryogenesis in *Xenopus* and the mouse have been found to be dependent on the biochemical composition of individual nucleosomes. Thus, variation in the type and modification of chromosomal and chromatin structural proteins provides a dominant means of controlling the transcriptional activity of individual genes, individual chromosomal domains and of entire chromosomes. The focus of this review is to summarize these recent advances and to discuss their implications for developmental biology.

chromatin / nuclear organization / embryo / *Xenopus*

Résumé — Régulation de la chromatine et de l'expression des gènes au début du développement embryonnaire. Des modifications majeures de la structure des chromosomes et de la chromatine se produisent au cours du développement. Des expériences récentes de génétique et de biochimie montrent que ces changements de l'organisation du génome contribuent significativement à l'établissement et au maintien d'états d'activité transcriptionnelle différents. Des modifications dans les variantes d'histones, régulées en fonction du stade de développement, déterminent l'activité des

gènes, en contrôlant chez les eucaryotes la répression de gènes spécifiques des eucaryotes. Des protéines chromosomiques comme Polycomb stabilisent et maintiennent des états de répression de la transcription. Les protéines qui régulent la condensation des chromosomes mitotiques contrôlent l'activité transcriptionnelle générale d'un chromosome. Dans cette revue, les rôles de ces constituants chromosomiques dans le développement sont placés dans un contexte structural et fonctionnel. Les mécanismes moléculaires qui régulent l'activité des chromosomes pendant le développement (embryonnaire) commencent à être élucidés.

chromatine / organisation du noyau / embryon / xénope

CHROMOSOMAL STRUCTURES THAT IMPACT ON THE DEVELOPMENTAL CONTROL OF GENE EXPRESSION. AN OVERVIEW

There are three principle components to the regulation of differential gene transcription: the first is the availability of the specific *trans*-acting factors utilized by a particular gene required either for the recruitment of the basal transcriptional machinery (transcriptional activators) or the inhibition of this machinery (transcriptional repressors); the second is the availability of the basal transcriptional machinery itself, including the general transcription factors (TFIIB, TFIID, TFIIH, etc); and finally the chromosomal environment in which a gene is to function. The chromosomal environment can alternately facilitate or repress transcription. Only recently has it become possible to manipulate chromosomal structure *in vivo*, and to functionally test the significance of these directed alterations *in vivo* and *in vitro*.

Morphologically distinct regions of chromosomes have been visually discerned for many years: heterochromatin appears condensed throughout the cell cycle, while euchromatin is more diffuse during interphase (Heitz, 1928). The expression of a gene depends on whether it is placed within heterochromatin or euchromatin, which implies that the exact position of the gene within a chromosome influences function (Lewis, 1950; Lima-de-Faria, 1983). In *Drosophila*, the severity of this 'position

effect' is reduced by deletion of a fraction of the genes encoding the core histones (Moore et al, 1983) or by the post-translational modification of the core histones (Motus et al, 1980). These early experiments have been considerably extended by work in *Saccharomyces cerevisiae*. Yeast cells containing reduced levels of core histones arrest during the cell cycle at mitosis, with defects in both chromatin structure and nuclear segregation (Meeks-Wagner and Hartwell, 1986). Moreover, the initiation of transcription at several promoters is increased by this reduction in the levels of core histones and conversely position effects become less significant (Han et al, 1987; Clark-Adams et al, 1988; Aparicio et al, 1991).

Local chromatin structure: histone and nucleosomes

Genetic analysis has established that the core histones have an essential role in regulating the transcription of the vast majority of inducible genes in yeast (Grunstein et al, 1992; Herskowitz et al, 1992). The individual core histones are found to have architectural roles in the assembly of specific nucleoprotein structures including enhancers and promoters (McPherson et al, 1993; Schild et al, 1993). Histones also have regulatory roles through the contacts that they make with specific transcription factors targeted to particular *cis*-acting elements. These interactions can stabilize nucleosomal arrays leading to transcriptional repression,

or exert a disruptive influence on nucleosomes, thereby activating transcription (Hirschhorn et al, 1992; Roth et al, 1992).

The foundation of chromosomal architecture lies in the assembly of nucleosomes by the core histones and linker histones. Two molecules of each of the four core histones (H2A, H2B, H3 and H4) wrap 160 bp of DNA and, together with a single molecule of linker histone (H1), organize 180–190 bp of DNA into a nucleosome (fig 1). Each core histone has a structured histone fold domain involved both in specific heterodimerization with another core histone (H2A with H2B, H3 with H4) and in wrapping DNA. An amino (N-) terminal tail domain lies on the outside of the nucleosome; this positively charged tail influences the path of DNA and interacts with specific *trans*-acting factors (Garcia-Ramirez et al, 1992; Hecht et al, 1995). The N-terminal tail domains are also the sites of post-translational modifications such as acetylation and phosphorylation. These modifications reduce the positive charge of the tail and thereby alter both the association

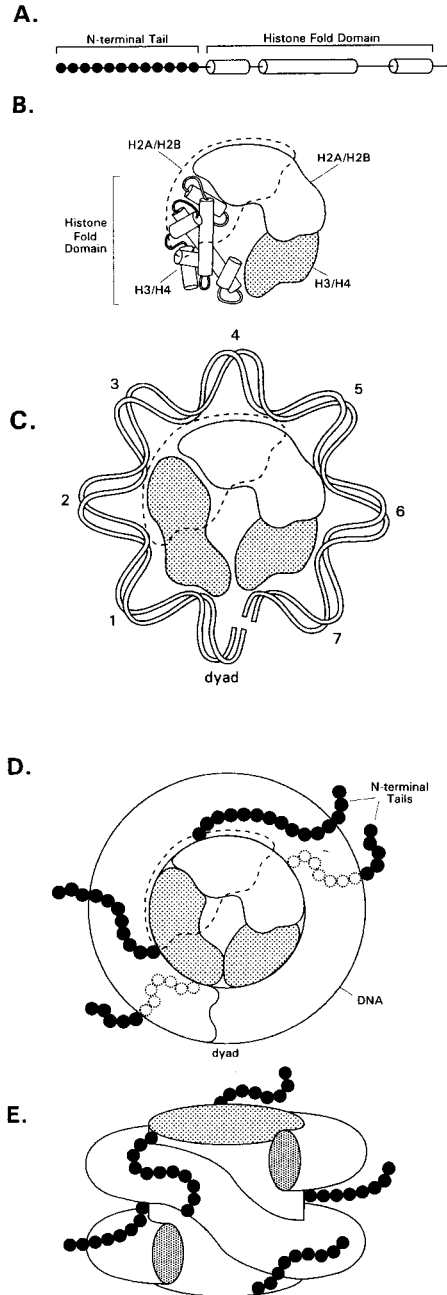


Fig 1. Histones and the nucleosome. **A:** A typical core histone containing an N-terminal tail (black) and the histone fold domain (white). The tail is the site of post-translational modifications. The histone fold domain is involved in protein–protein interactions and in wrapping DNA. **B:** The histone octamer is shown. One heterodimer of histones H3 and H4 is detailed, the other is indicated by the dotted portion. Two heterodimers, of H2A and H2B are indicated by the open portions enclosed by solid and dashed lines. One lies above and one lies below the two heterodimers of H3 and H4. **C:** Eighty base pairs of DNA are shown wrapped around the core histones from the dyad axis (dyad) to the edge of the nucleosome. The numbers indicate turns of DNA from the dyad axis. **D:** DNA is shown as a tube with the histone tails protruding towards the outside of the nucleosome. This view is along the superhelical axis of the nucleosome. **E:** A view of the nucleosome facing the dyad axis.

of this domain with other *trans*-acting factors and the path of DNA in chromatin destabilizing higher-order chromatin structures (Turner, 1993; Bauer et al, 1994; Garcia-Ramirez et al, 1995). Variants of the core histones exist in which the N-terminal tail domain has substantially diverged from that of the predominant core histone found in the chromatin of normal somatic cells.

Linker histones require DNA to be packaged by the core histones before they can bind tightly to chromatin (Hayes et al, 1994). These proteins have a structured domain that resembles the fokehead/HNF3 family of transcription factors (Clark et al, 1993) and two positively charged tail domains that together stabilize the internucleosomal interactions found in higher-order chromatin structures (Allan et al, 1981). Linker histones help the apparent coiling of nucleosomal arrays into the so-called 30 nm chromatin fiber (fig 2). One turn of this fiber contains 1–1.2 kb of DNA (fig 2). Linker histones, however, are not essential for the assembly of chromosomes or functional nuclei (Ohsumi et al, 1993; Dasso et al, 1994; Shen et al, 1995). They vary substantially in primary sequence; however, in metazoans they retain the same basic structure of a central domain flanked by basic N- and carboxy (C-) terminal tails. In *Tetrahymena* histone H1 the central domain is absent (Wu et al, 1986). The N- and C-terminal tails of linker histones or the entire *Tetrahymena* H1 are the sites of post-translational modifications such as phosphorylation that weaken the interaction of the protein with nucleosomal DNA (Hill et al, 1991). Phosphorylation of the linker histones might also be expected to destabilize higher-order chromatin structures.

Histones can package all DNA yet have marked selectivity in how they organize particular DNA sequences. Since histones bend DNA, they favor interaction with sequences that are intrinsically curved (Shrader and Crothers, 1989; Wolffe and Drew, 1989).

Both linker and core histones also have distinct sequence preferences. Variants of the core histones exist that direct their assembly into nucleosomes containing particular DNA sequences (Sullivan et al, 1994). Linker histones and specific *trans*-acting factors that contact both DNA and the core histones can also direct the precise positioning of histone-DNA contacts with respect to DNA sequence (Chipev and Wolffe, 1992; Roth et al, 1992; McPherson et al, 1993). This local position effect determines exactly how the histones organize regulatory DNA, including enhancers and promoters. It can facilitate transcription both by keeping key elements free of histone-DNA contacts and therefore accessible to *trans*-acting factors, and by bringing these elements together in three dimensions and thereby clustering the activation domains of bound transcription factors into an effective configuration (Schild et al, 1993). Specific nucleosomes can also impede transcription by restricting the access of transcription factors to regulatory DNA (Archer et al, 1992).

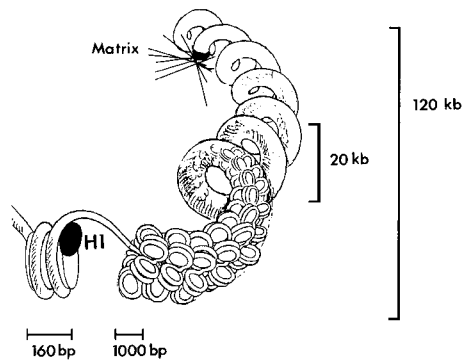


Fig 2. Chromatin higher-order structures. Ascending hierarchies of DNA compaction within chromatin. Each nucleosome contains 160 bp of DNA; each turn of the chromatin fiber contains at least 1 000 bp, and chromatin domains or loops contain at least 20 Kb of DNA. Chromatin domains are probably defined by the intrinsic topological constraints of coiling the chromatin fiber and by attachments to the nuclear matrix (adapted for Wolffe, 1996).

Other proteins of the HMG1 or 2 class can also influence transcription through their capacity to bend DNA (Tjian and Maniatis, 1994). HMG1 and 2 are small related basic proteins found in 5% of the chromatin within normal somatic cells (Goodwin et al, 1977). In yeast, isomorphous proteins such as SIN1 have a role in repressing inducible transcription (Kruger and Herskowitz, 1991; Baxevanis and Landsman, 1995). As we will discuss, during the early embryogenesis of several organisms, proteins of this type may functionally replace normal linker histones in directing the path of linker DNA (Dimitrov et al, 1994; Ner and Travers, 1994). This replacement will lead to specialized higher-order chromatin structures that might be less stable and thus more accessible to *trans*-acting factors than the normal chromatin found in somatic cells.

Long-range chromatin structure: domains and chromosomes

Arrays of nucleosomes associate with other chromosomal proteins that organize them into chromosomal domains. Experiments in yeast have clearly defined domains of transcriptional repression including the silent mating-type loci (Laurenson and Rine, 1992) and the telomeres (Gottschling et al, 1990). The efficiency of transcriptional silencing depends on the core histones, their post-translational modifications and *trans*-acting factors that bind to the core histones (Aparicio et al, 1991; Braunstein et al, 1993; Hecht et al, 1995). How the *trans*-acting factors involved in silencing organize chromatin structure has not yet been determined.

In *Drosophila*, the genetic search for suppressors of position effect has uncovered numerous chromatin-binding proteins (Reuter and Spierer, 1992; Henikoff, 1994). These proteins often act in a dose-dependent manner implying that they are stably sequestered into chromatin as structural

components (Locke et al, 1988). One family of proteins first identified in *Drosophila* from their capacity to influence position effect share a common domain involved in chromatin modification and organization known as the chromodomain. These proteins are conserved from yeast to man (Saunders et al, 1993; Singh, 1994). In *Schizosaccharomyces pombe*, a chromodomain protein *swi6* restricts both recombination and gene expression in the silent mating-type loci (Klar and Bonaduce, 1991; Lorentz et al, 1992, 1994). In *Drosophila*, the chromodomain protein heterochromatin protein 1 (HP1) binds selectively to heterochromatin predominantly at the centromere (James and Elgin, 1986; Eisenberg et al, 1990). More interestingly, the Polycomb Group of chromodomain transcriptional repressors bind to a large number of developmentally regulated genes (> 50) including the bithorax complex (Orlando and Paro, 1993; see later). Proteins of the Polycomb Group class are not DNA-binding proteins, yet have preferred sites of entry into chromatin (Pirotta and Rastelli, 1994). They form large multimeric complexes, exert their effects in a dose-dependent manner and show distinct boundaries in their association with particular functional chromosomal domains (Franke et al, 1992; Orlando and Paro, 1993). How these proteins recognize and modify particular chromatin structures is unknown (Messmer et al, 1992).

The boundaries of Polycomb group protein association with chromatin provide an example of the insulation of the structural and functional properties of adjacent chromatin domains. Several distinct DNA segments have been defined (\approx 1 000 base pairs long) that will insulate against position effects, such that a gene flanked by such sequences is expressed independent of its position within the chromosome (Kellum and Schedl, 1991; Chung et al, 1993; Roseman et al, 1993). Proteins that associate with such 'insulator elements' have been character-

ized previously (Parkhurst et al, 1988; Zhao et al, 1995). These include DNA-binding proteins that will also interfere with the capacity of an enhancer to function when they are placed between the enhancer and a responsive promoter (Geyer and Corces, 1992; Zhao et al, 1995). A current hypothesis is that insulators provide an architectural function that involves tethering genes to a nuclear matrix that prevents either transcriptional activation or repression (Wolffe, 1994a; fig 3).

Insulators mark boundaries not only of transcriptional activation or repression, but also of histone modification. In the chicken β -globin locus, histone hyperacetylation (in which the N-terminal tails of the core histones have three to four acetylated lysines) changes to hypoacetylation (in which these acetylated lysines are lost) in traversing an insulator element (Hebbes et al, 1994). Chromatin domains having distinct acetylation states have been extensively documented, ranging from hypoacetylation of the silent mating loci (Braunstein et al, 1993), and most of the inactive X chromosome in female mammals (Jeppesen and Turner, 1993), to hyperacetylation of the transcriptionally active male X chromosome in *Drosophila* (Bone et al, 1994) and of individual chromosomal domains in polytene nuclei (Turner et al, 1992). These results emphasize the highly regulated targeting of histone modifications to individual chromatin domains. Targeting might be accomplished either locally by enhancers or over an entire domain by locus control regions (Grosveld et al, 1993; Jenuwein et al, 1993; fig 3, see later). One hypothesis to explain the properties of these regulatory regions is that they serve several functions, including the recruitment of specific histone acetyltransferases.

Early experiments defined a chromosomal scaffold that tethered loops of nucleosomal chromatin containing from 20 kb to as much as 50–100 kb pairs of DNA (Laemmli et al, 1978; fig 2). Proteins within

this scaffold were proposed to pull the bases of the chromosomal loop domains together during chromosomal condensation at mitosis. The adjacent loop domains were thought to coil in a helical path along the axis of the chromosome (Rattner and Lin, 1985; Boy de la Tour and Laemmli, 1988; Saitoh and Laemmli, 1994; fig 2). Chromosome scaffold proteins remain in chromosomal preparations from which most nucleic acid and all the histones have been removed (Lewis and Laemmli, 1982). They include topoisomerase II, an enzyme essential for chromosome segregation (DiNardo et al, 1984), and a family of heterodimeric coiled-coil proteins that are important for mitotic chromosome condensation and segregation (Strunnikov et al, 1993; Hirano and Mitchison,

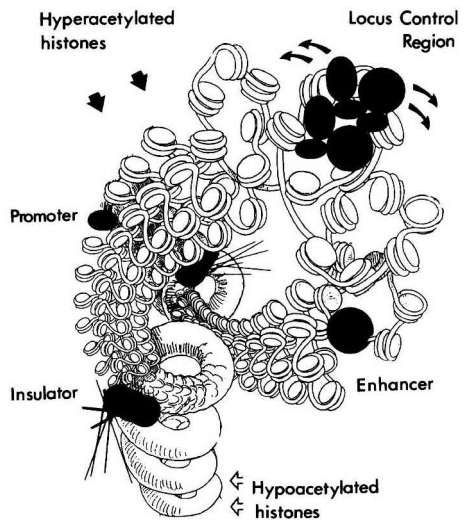


Fig 3. A model for a functional chromatin domain. The locus control region disrupts local chromatin structure as do nucleoprotein complexes associated with promoters and enhancers. The locus control region has the capacity to facilitate directly or indirectly chromatin structural changes such as histone acetylation. It can also make physical contact with promoter and enhancer complexes. The boundaries of locus control region function are defined by insulator elements which may make contact with the nuclear matrix.

1994; Saitoh et al, 1994). Proteins of this SMC (segregation of mitotic chromosome) family are also important for transcriptional control. *Dpy-27*, an essential dosage compensation gene in *Caenorhabditis elegans* is a member of this family. This protein preferentially associates with the *C elegans* X chromosome and selectively reduces transcriptional activity (Chuang et al, 1994).

The chromosomal environment is clearly revealed through these primarily genetic experiments to have major consequences for gene activity at multiple levels, ranging from the selective packaging of regulatory DNA into nucleosomes to the preferential modification of histones within entire chromosomes. The establishment and maintenance of patterns of differential gene activity during development will clearly be dependent on the properties of the chromosomes within which the genes are to be activated or repressed.

MODULATION OF THE CHROMOSOMAL ENVIRONMENT DURING DEVELOPMENT

Experiments predominantly using *S cerevisiae* that are briefly outlined in the preceding sections have established a key role for chromatin proteins in gene regulation. Nevertheless, with the exception of the silent mating-type loci, most genes in yeast require the capacity for continual regulation. Aside from the determination of mating type and the specialized process of sporulation, *S cerevisiae* cells do not display developmentally specific programs reflecting diverse stable patterns of gene activity, ie, differentiated states that are stably maintained through determinative mechanisms. In contrast, other unicellular eukaryotes such as the ciliate *Tetrahymena* exhibit several distinct patterns of differential patterns of gene activity associated with developmental events. Metazoans such as the common

experimental animals: *Drosophila melanogaster*, the sea urchin, *Xenopus laevis* and the mouse all exhibit global changes in gene activity during early development. These organisms rapidly establish cell lineage and tissue-specific patterns of gene expression early in embryogenesis. These committed states are stably maintained throughout adult life. In general, it appears that the global changes in gene activity seen during early embryogenesis are dependent on developmentally regulated changes in the type or modification of histone or other basic chromatin protein. In contrast, the selective repression of genes, in particular differentiated cell types, appears dependent on proteins influencing higher-order chromatin structure, such as those of the chromodomain class. This latter type of mechanism might also account for the phenomenon of imprinting or epigenetic determination in which genes of identical sequence are differentially expressed in the same cell dependent on the history of the chromosomes in which they are found.

Developmental regulation of nucleosomal structure

Tetrahymena

Changes in nucleosomal structure are a ubiquitous feature of developmental programs associated with the global control of nuclear activity. Excellent examples are found in the work of Gorovsky (1973) and Allis et al (1987), who have systematically characterized transitions in nucleosomal proteins associated with development in *Tetrahymena thermophila*, a ciliate protozoan. *T thermophila* has two types of nuclei: a diploid micronucleus that represents the germ line of the cell, and a polyploid somatic macronucleus that controls the phenotype of the cell (Gorovsky, 1973). During the sexual cycle known as conjugation, cells of opposite

mating types fuse. This leads the germ-line micronucleus to undergo meiosis, exchange and fertilization, resulting in the appearance of a diploid zygotic nucleus in each of the original mating cells (Nenny, 1953; Martindale et al, 1982). The prophase of meiosis for the micronuclei is characterized by meiotic DNA recombination and repair as well as a brief period of transcription (Allis et al, 1987). At the end of the mating process, the diploid zygotic nucleus divides to generate a new macro- and micronucleus. The decision to differentiate into a macro- or micronucleus depends on their exact positions in the cell. The pre-existing 'old' macronucleus is transcriptionally inactivated and eliminated, leading the transcriptional needs of the cell to be assumed by the developing 'new' macronuclei (Wenkert and Allis, 1984). Changes in nuclear function during this ordered developmental pathway are dramatically correlated with transitions in nucleosomal composition (table I).

Differences exist between the chromosomal composition of the macro- and micronucleus throughout the life cycle of *Tetrahymena*. Macronuclei contain histone H1, whereas micronuclei contain three distinct basic polypeptides, including an HMG1-like protein (Schulman et al, 1991; Wu et al, 1994). None of these proteins are essential for the assembly of functional nuclei

(Shen et al, 1995; see also Scarlato et al, 1995). Macronuclei are enriched in several components characteristic of transcriptionally active chromatin, including acetylated core histones (Chicoine and Allis, 1986), a histone H2A variant called hv1 containing a N-terminal tail domain similar to that of histone H4, and phosphorylated histone H1 (Roth et al, 1988; Stargell et al, 1993). These proteins and modifications are normally lacking in the micronucleus. In *Drosophila* the hv1 homologue known as H2A.vD is absolutely essential for early development, embryos in which this gene is 'knocked out' die (van Daal et al, 1988; van Daal and Elgin, 1992). The initial cell-cell interactions of *Tetrahymena* conjugation also trigger the rapid induction of a distinct HMG 1-like protein, HMG B, which accumulates both in the 'old' macronucleus and in the micronucleus (Schulman et al, 1991; Wang and Allis, 1992). The appearance of HMG B and some hv1 in micronuclei correlate with their brief period of transcriptional activity during meiotic prophase. All these modifications are predicted to destabilize nucleosomes and chromatin. This would lead DNA within the chromosome to be more accessible to transcription factors and more easily traversed by processive enzyme complexes such as DNA and RNA polymerases. Finally, during the senescence of 'old' macronuclei at

Table I. Variation in the chromatin composition of *Tetrahymena* nuclei.

<i>Macronucleus</i>		<i>Micronucleus</i>
<i>Transcriptionally active</i>	<i>Senescent, transcriptionally inactive</i>	
Phosphorylated H1	Dephosphorylated H1	Specialized linker binding protein
Acetylated H4	Tailless core histones	Unmodified core histones
hv1 (H2A.Z)		No hv1
Acetylated hv1		
HMG B		

the end of conjugation, the core histone N-terminal tails are proteolytically removed and histone H1 is dephosphorylated. Removal of the core histone tails will prevent any interaction with regulatory proteins. Dephosphorylation of the linker histone tail domains will stabilize their binding to nucleosomal DNA and facilitate the assembly of higher-order chromatin structures (fig 2). These events correlate with the inhibition of transcription and the condensation of macronuclear chromatin (Lin et al, 1991).

These observations indicate the plasticity of chromatin composition; although some nucleosomal proteins are selectively incorporated into chromatin during nuclear replication, others exchange into chromatin independent of the replication process (Wu et al, 1988; Wang and Allis, 1992), others are modified independent of replication (Lin et al, 1991). It is clear that changes in the chromosomal environment, especially in the micronucleus early in conjugation and in the senescent macronucleus, are tightly coupled to states of nuclear activity. Comparable changes occur during metazoan development.

Xenopus and sea urchin

In the sea urchin and *Xenopus laevis*, stores of core histones are sequestered in the egg to facilitate the efficient assembly of chromatin during the rapid cell division events following fertilization (Salik et al, 1981; Adamson and Woodland, 1974). The stored histones also serve to remodel sperm chromatin once it enters the egg cytoplasm (Poccia et al, 1981; Poccia and Green, 1992; Dimitrov et al, 1994). Sperm chromatin is highly specialized and the paternal genome is packaged by a wide variety of proteins, including specialized histones (Strickland et al, 1980; Jutglar et al, 1991), small core histone-like proteins (Abé and Hiyoshi, 1991) and protamines (Marushige and Dixon,

1969). All that these proteins have in common is their capacity to reversibly compact DNA into a transcriptionally inert state. They are all removed and replaced by core histones within the egg cytoplasm. In *Xenopus* the replacement of sperm-specific basic proteins by histones H2A and H2B is facilitated by molecular chaperones such as nucleoplasmin (Philpott et al, 1991; Philpott and Leno, 1992).

Histone acetylation appears to have a functional role in coupling chromatin assembly to the replication process (Kaufmann and Botchan, 1994). Histone H4 is stored in the diacetylated form in both the *Xenopus* and sea urchin egg. During early embryogenesis these stores of histone H4 are utilized for chromosome assembly and the diacetylated form is progressively converted to the deacetylated form (Dimitrov et al, 1993; Chambers and Shaw, 1984). The level of deacetylation of histone H4 appears to be inversely related to the rate of nuclear assembly and cell division during development. Acetylation of histone H4 in early embryogenesis may reduce chromatin condensation within the chromosomes of embryonic nuclei, accounting in part for the large nuclear volume (Gurdon, 1976).

Histone hyperacetylation has been shown to facilitate the access of *trans*-acting factors to DNA (Lee et al, 1993). Since the level of acetylation of the core histones is controlled by an equilibrium between histone acetyltransferases and deacetylases (Turner, 1993), it is normally possible to induce histone hyperacetylation using inhibitors of deacetylation such as sodium butyrate (Candido et al, 1978) or Trichostatin A (Yoshida et al, 1995). In *Xenopus*, however, hyperacetylated histones only accumulate in the presence of these inhibitors for the first time during early gastrulation (Dimitrov et al, 1993). This implies that histone acetyltransferase activity is developmentally regulated. Hyperacetylation of the core histones correlates with the capacity to

selectively induce transcriptional activity (Khochbin and Wolffe, 1993) and leads to a delay in the completion of gastrulation (Almouzni et al, 1994). *Xenopus* embryos maintained in Trichostatin A have defects in mesoderm formation. In the starfish *Asterina pectinifera*, the inhibition of histone deacetylation leads to the arrest of development at the early gastrula stage before mesenchyme formation (Ikegami et al, 1993). Thus, the capacity to modulate histone acetylation levels appears important in establishing stable states of differential gene activity during gastrulation (Almouzni et al, 1994).

In the growing *Xenopus* oocyte, transcription is very active. A large fraction of RNA synthesis occurs within specialized lampbrush chromosomes (Anderson and Smith, 1978; Callan et al, 1987). Following oocyte maturation into an egg, transcription from the endogenous chromosomes is inhibited (La Marca et al, 1975). Fertilization initiates 11 cycles of cell division, which occur in the absence of zygotic transcription (Newport and Kirschner, 1982a). At the mid-blastula transition (4 000 cell stage), the cell cycle lengthens and a complex program of transcription is initiated (Krieg and Melton, 1987; Rupp and Weintraub, 1991; Lund and Dahlberg, 1992). Various models have been proposed to explain this global control of gene expression. The simplest model is that the DNA synthesized during embryogenesis titrates an inhibitor component of chromatin, perhaps the large excess of core histones stored in the embryo, and that once the histones are all sequestered onto DNA, transcriptional programs will initiate (Newport and Kirschner, 1982b; Prioleau et al, 1994). Substantial evidence suggests that more complex mechanisms also contribute to this major developmental transition. Injection of exogenous DNA, or arrest of DNA cleavage does not influence the temporal control of snRNA, tRNA or rRNA synthesis (Takeichi et al, 1985; Shiokama et al, 1989;

Lund and Dahlberg, 1992). The transcriptional activation of exogenous DNA by titration of histones is a gene-specific phenomenon, indicative of a key role for particular transcription factors in the process (Almouzni and Wolffe, 1995). A limitation in transcription factor activity in the early embryo is also suggested from experiments that indicate chromatin assembly to have a key role in repressing basal but not activated transcription (Almouzni and Wolffe, 1993, 1995). Early experiments actually failed to observe a heat-shock response in *Xenopus* oocytes (Bienz, 1986) simply because basal transcription was not repressed through chromatin assembly (Landsberger et al, 1995).

The chromosomal environment in the early *Xenopus* embryo is also unusual in many other respects. The entire genome is being replicated every 30 min and chromatin assembly is coupled to replication, which tends to repress basal transcription (Almouzni et al, 1990, 1991; Almouzni and Wolffe, 1993, 1995), DNA replication will also disrupt pre-existing transcription complexes (Wolffe and Brown, 1986). The reassembly of transcription complexes has a considerable lag time (Bieker et al, 1985). Mitotic events that influence transcription will also occur every 30 min. Mitosis leads to the modification of the transcriptional machinery (Hartl et al, 1993), condensation of the chromosome into a transcriptionally incompetent environment (Chuang et al, 1994; Hirano and Mitchison, 1994), and the displacement of both transcription factors and RNA polymerase (Prescott and Bender, 1962; Hershkovitz and Riggs, 1995). Thus, many events in the nucleus have the potential to influence transcription. These variables may have distinct consequences for transcription from genes within the endogenous chromosomes compared to those within exogenous plasmid DNA microinjected into the egg cytoplasm (Landsberger and Wolffe, 1995).

In the sea urchin, stores of nuclear components do not apparently inhibit zygotic transcription. Both the maternal and paternal pronuclei are transcriptionally active following fertilization (Brandhorst, 1980; Poccia et al, 1985). The cleavage nuclei are also actively transcribed (Wilt, 1963, 1964; Kedes and Gross, 1969). However, the pattern of gene expression changes dramatically between the early blastula and gastrula. The majority of mRNA expressed during sea urchin embryogenesis begin to accumulate at very early blastula stages and are then spatially restricted during gastrulation (Kingsley et al, 1993). A comparable restriction occurs in *Xenopus* between the mid-blastula transition (MBT) and gastrulation. At the MBT many cell lineage specific genes are pleiotropically transcribed; only during gastrulation are cell lineage specific patterns established (Wormington and Brown, 1983; Rupp and Weintraub, 1991). These transitions in gene expression are associated with major changes in the type of histone present within the chromosome (Newrock et al, 1977; Dimitrov et al, 1993, 1994).

Distinct core histones and linker histones accumulate in chromatin as embryogenesis proceeds in the sea urchin. Maternal pools of cleavage stage (CS) histones CSH1, CSH2A and CSH2B replace the specialized sperm histones following fertilization (Poccia et al, 1981). After the 16-cell stage, the CS variants are replaced with newly synthesized 'a' or 'early' histone H1, H2A and H2B variants (Newrock et al, 1977). In the sea urchin, the rate of histone biosynthesis is equivalent to that necessary to package newly synthesized DNA into chromatin; thus, pre-existing histones are rapidly diluted during the early cell cycles (Moav and Nemer, 1971; Goustin and Wilt, 1981). The late *b*, *g* and *d* sea urchin histone genes begin to be transcribed in the blastula and continue to be active in adult cells, whereas transcription of the *a* histone genes

is repressed (Maxson et al, 1983; Knowles and Childs, 1984). Thus, *d* and *g* variants of histone H2B, *b*, *d* and *g* variants of H2A and *b* and *g* variants of H1 accumulate in chromatin as development proceeds. The expression of CS and early histone gene sets during oogenesis and early embryogenesis is uncoupled from DNA replication, whereas the expression of the late histone genes is temporally linked to DNA replication.

A common theme in sea urchin development is the variation in histones H1, H2A and H2B compared to histones H3 and H4. This reflects the essential structural and signal transduction role of histones H3 and H4 in the nucleosome (Wolffe, 1995). Histones H1, H2A and H2B readily exchange out of the nucleosome in vivo (Louters and Chalkley, 1985). These proteins do not influence the rotational positioning of DNA on the surface of the histone-octamer, but they do influence the accessibility of nucleosomal DNA to *trans*-acting factors (Wolffe, 1989; Dong and van Holde, 1991; Hayes et al, 1991; Hayes and Wolffe, 1992; Lee et al, 1993; Ura et al, 1995). Thus, it can be expected that modulation in the structural properties of the nucleosome through variation in the structure of the H2A/H2B dimer and of H1 may effect nuclear functions such as transcription and replication in early sea urchin development. Conclusive proof for such an essential role for a linker histone variant (H1 in *Xenopus*, see further down) and for a histone H2A (H2AvD in *Drosophila*, see earlier) has been obtained.

In *Xenopus*, changes in histone gene expression are much more modest than those in the sea urchin. It was found that the bulk of *Xenopus* core histone genes are constitutively active in the oocyte and in the developing embryo following the mid-blastula transition (Perry et al, 1986). Oocytes do accumulate large quantities of the specialized variant histone H2A.X (Kleinschmidt and Steinbeisser, 1991; Dimitrov et al,

1994). This protein accumulates in early embryonic chromatin but is rapidly diluted as cell division proceeds. Linker histones show major alterations in abundance during *Xenopus* embryogenesis (Khochbin and Wolffe, 1994). The *Xenopus* egg stores large quantities of HMG1 and a linker histone B4 (Kleinschmidt et al, 1985; Smith et al, 1988; Dimitrov et al, 1994). HMG1 can functionally replace linker histones in binding to nucleosomal DNA. HMG1-like proteins are also abundant in early embryonic chromatin from *Drosophila* (Ner and Travers, 1994). Aside from a structural role in chromatin HMG1 can also influence the functionality of the basal transcriptional machinery (Ge and Roeder, 1994; Shykind et al, 1995). The transcriptional quiescence in the early *Xenopus* and *Drosophila* embryo (Brown and Littna, 1966; Edgar and Shubiger, 1986) might therefore be a consequence of the enrichment of HMG1 within chromatin. Linker histone B4 is oocyte-specific in expression (Cho and Wolffe, 1994) and is much less basic than somatic histone H1 (Dimitrov et al, 1993). Nucleosomal arrays containing histone B4 would be expected to be less stable than those containing histone H1. Histone B4 accumulates in early embryonic chromatin, but is progressively diluted by somatic histone H1 as development proceeds (Bouvet et al, 1994). This somatic histone H1 is synthesized from large maternal stores of histone H1 mRNA that are only released for translation following fertilization (Flynn and Woodland, 1980; Tafuri and Wolffe, 1993; Bouvet and Wolffe, 1994). Somatic histone H1 begins to substantially replace histone B4 at the mid-blastula transition; this replacement is complete at the end of gastrulation (Dimitrov et al, 1993; Hock et al, 1993).

The progressive accumulation of histone H1 in *Xenopus* embryonic chromatin correlates with the transcriptional repression of many genes between the mid-blastula transition and neurulation (Wolffe, 1989;

Andrews et al, 1991; Bouvet et al, 1994). The precocious expression of histone H1 will cause a large family of oocyte specific 5S rRNA genes to become transcriptionally repressed (Bouvet et al, 1994). Inhibition of H1 synthesis sustains 5S rRNA gene transcription (Bouvet et al, 1994; Kandolf, 1994). This is an important result since it establishes that changes in chromosomal composition can directly influence the transcriptional activity of specific genes during metazoan development. The structural basis for this specific regulatory role for histone H1 appears to be the selective recognition of DNA sequence leading to the establishment of positioned nucleosomal arrays including the oocyte-specific 5S rRNA genes (Chipev and Wolffe, 1992; Ura et al, 1995). However, the stability of transcription complexes assembled on the promoter elements of these genes also has an important regulatory role (Wolffe and Brown, 1988). A family of somatic type 5S rRNA genes form very stable transcription complexes, whereas the oocyte specific 5S rRNA genes form unstable transcription complexes. Limitation in transcription factor availability as development proceeds can serve to establish a state of differential 5S rRNA gene transcription where the somatic 5S rRNA genes remain active and the oocyte 5S rRNA genes are inactivated. During normal development the assembly of a specific repressive chromatin structure on the oocyte-specific 5S rRNA genes dependent on histone H1 has a major contribution towards maintaining differential 5S rRNA gene expression (Wolffe, 1994b). The accumulation of histone H1 in *Xenopus* chromatin also correlates both with the restriction in cell lineage-specific gene expression through gastrulation (Rupp and Weintraub, 1991) and in the capacity of embryonic nuclei to revert to totipotency (Gurdon, 1962, 1963). It is therefore possible to speculate that somatic linker histones have an essential role as repressors of transcriptional programs in maintaining stable states of gene activity.

The mouse

Considerable circumstantial evidence supports an important role for chromatin structure in the global control of transcriptional regulatory mechanisms during early embryogenesis in the mouse. Extensive changes in nuclear activity occur during early mammalian embryogenesis (Telford et al, 1990). Immediately following fertilization, early development in the mouse is directed by maternally inherited mRNA and proteins. However, the zygotic genome is transcriptionally active as early as G2 of the first cell cycle (Latham et al, 1992; Ram and Schultz, 1993; Matsumoto et al, 1994; Christians et al, 1995). Nuclear transplantation experiments indicate that changes in chromatin structure occur immediately following the two-cell stage. This is because nuclei of early two-cell mouse embryos will readily support normal embryonic development when transplanted into enucleated one-cell embryos, whereas nuclei from more advanced embryos do not (McGrath and Solter, 1984; Robl et al, 1986; Howlett et al, 1987). Thus, beyond the two-cell stage chromatin begins to differentiate. In an interesting parallel to comparable events in *Xenopus* (see earlier) somatic histone H1 is first detectable in the mouse embryo at the four-cell stage (Clarke et al, 1992).

The major transcriptional activation of the endogenous mouse chromosomes begins in two-cell embryos at the end of G1, concurrent with DNA replication. A further significant increase in transcription occurs during G2 when maternal mRNA are degraded. This pattern of gene activity is controlled by a zygotic clock (Schultz, 1993), which is largely independent of cell division or the ratio of DNA to cytoplasm. It regulates the transcriptional activity of exogenous DNA microinjected into mouse embryos as well as that of the endogenous chromosomes (Martinez-Salas et al, 1989; Wiekowski et al, 1991). One function of the zygotic clock may

be the regulation of nuclear architecture. Core histone acetylation changes dramatically within chromatin during the first few cell divisions of mouse embryogenesis. Chromatin containing acetylated histone H4 becomes enriched at the nuclear periphery when the zygotic genome is strongly activated at the two-cell stage (Worrada et al, 1995). Inhibition of histone deacetylase using Trichostatin A increases the efficiency of gene expression. Acetylated chromatin localizes with RNA polymerase II, suggesting that it represents the site of active transcription. This localization of acetylated chromatin to the nuclear periphery is lost in the four-cell embryo and during subsequent development. An important conclusion from these experiments is that the functional compartmentalization of the nucleus occurs very early in mouse embryogenesis (Thompson et al, 1995).

De Pamphilis and colleagues examined the transcriptional activity of exogenous DNA microinjected into developing mouse embryos. They found that the promoters of microinjected plasmid DNA are strongly repressed in two-cell embryos, but that this repression can be relieved by embryo-specific enhancers (Martinez-Salas et al, 1989; Mélin et al, 1993). Promoter activity can also be relieved by the inhibition of histone deacetylases using sodium butyrate. If histones are hyperacetylated then the need for an enhancer to achieve full promoter activity is substantially reduced (Wiekowski et al, 1991; Majumder et al, 1993). This result implicates histone acetylation as a regulatory factor in the two-cell mouse embryo.

Further evidence for an influence of nuclear environment on gene function follows from the microinjection of templates into paternal and maternal pronuclei. Promoters are repressed when injected into the paternal pronucleus. This transcriptional repression is not relieved by the inhibition of histone deacetylases. In contrast, although the same templates are repressed

following injection into maternal pronuclei, transcription is stimulated by histone hyperacetylation (Wiekowski et al, 1993). Thus, rather like the macro- and micronuclei of *Tetrahymena* (see earlier), the paternal and maternal pronuclei provide very different environments for transcription. A challenge for the future is to determine the potential role of these different nuclear environments in the imprinting of paternally and maternally derived chromosomes (Pfeifer and Tilghman, 1994).

Developmental regulation of long-range chromatin structure

The interrelationship between changes in chromosomal composition and function is less clear for long-range chromatin organization than for nucleosomal DNA. This is simply because the nature of long-range chromosomal structure is currently much less well defined than nucleosomal organization. It is also important to recognize that the changes in nucleosomal organization outlined earlier will also have direct consequences for overall nuclear structure and function. The most dramatic example of this phenomenon occurs during erythropoiesis in birds and amphibians when the whole erythrocyte nucleus becomes heterochromatinized due to the accumulation of linker histone variants (H5 in chicken, H1 in *Xenopus*, Appels and Wells, 1972; Rutledge et al, 1988; Khochbin and Wolffe, 1993). The accumulation of these linker histone variants leads to the arrest of transcription (Hentschel and Tata, 1978) and of cell proliferation (Sun et al, 1989; Aubert et al, 1991).

Xenopus

During early *Xenopus* embryogenesis there are major changes in the long-range organization of chromosomes. As already dis-

cussed, the oocyte genome is organized into loop domains (Callan et al, 1987). A loop domain organization is maintained through early development; however, the size of the loops changes dramatically. There is a progressive thickening and shortening of metaphase chromosomes as development proceeds from the blastula embryo to the swimming tadpole (Micheli et al, 1993). Concomitant with this change in chromosomal morphology the average length of DNA loops increases by more than 60%. Moreover, an analysis of chromosome length values at different developmental stages suggests that chromosomal shortening is simultaneous for all chromosomes in an individual cell, but occurs asynchronously in different embryonic cell types. These cell-specific phenomena might reflect the differentiation of chromosomal structures concomitant with determinative events. The size of DNA loops correlates with the size of replicons (ie, the distance between adjacent origins of replication) (Buonigiorno-Nardelli et al, 1982). During *Xenopus* embryogenesis, prior to the mid-blastula transition replication, origins are closely spaced. Once the cell cycle lengthens, origins become more dispersed (Graham and Morgan, 1966). It has been proposed that the attachment of DNA loops to the chromosomal scaffold corresponds to their association to replication factories (Cook, 1991). Changes in chromosomal organization during early embryogenesis would offer a practical system to explore the developmental control of replicon size and of replication. These observations illustrate the dramatic changes in chromosome loop domain organization which can occur during early development.

Drosophila

The majority of the evidence for higher-order chromatin structures having a role in the

determinative events of development is based on genetic analysis. We have discussed the role of the histones, HP1 and proteins of the Polycomb group in position effect, and the evidence for the existence of insulators that separate functional domains of chromatin. Here we discuss how chromodomain proteins might function in a developmental context. Proteins of the Polycomb group have important roles in homeotic gene regulation. The homeotic genes of the *Antennapedia* complex and the bithorax complex need to be differentially expressed in the developing *Drosophila* embryo in order to establish correct segmental identity (Lewis, 1978; Kaufman et al, 1980). The segmental identity of the nuclei within a *Drosophila* embryo begins to be established even before the transition from syncytial to cellular blastoderm (Illmensee, 1978; Kauffmann, 1980). It is at this time that heterochromatin and associated chromodomain proteins can first be cytologically detected. Homeotic genes are initially activated in the correct spatial domains by the interplay of transcriptional regulators, encoded by maternally expressed genes and early acting segmentation genes (Harding and Levine, 1988; Irish et al, 1989). Having once established a pattern of differential *Antennapedia* and bithorax gene complex expression within the *Drosophila* embryo, however, the transacting factors decay away. Nevertheless, the homeotic genes need to remain differentially active throughout development in order to ensure proper segmental identity (Morata et al, 1983). The Polycomb group of proteins is an essential part of the maintenance mechanism that ensures segmental identity by maintaining silent homeotic genes in the repressed state through the rest of development (fig 4). The Polycomb group proteins are not required to establish normal patterning of homeotic gene expression early in embryogenesis (Kuziora and McGinnis, 1988; Franke, 1991). However, at later embryonic stages when homeotic

genes should be maintained in a silent state, mutation of the Polycomb gene leads to relief of homeotic gene repression (Beachy et al, 1985; Carroll et al, 1986; Celnikier et al, 1990). Regulation of the *Drosophila* homeotic genes by the Polycomb group is similar to the regulation of *Xenopus* 5S rRNA genes by histone H1 in that differential transcription factor association establishes a pattern of differential gene activity that is then maintained through the assembly of a specific chromatin structure.

Although the exact structural consequences of the association of the Polycomb group proteins with chromatin are not known, modifiers of position effect influence the homeotic genes regulated by the Polycomb group (Grigliatti, 1991). A model for heterochromatin formation proposes that

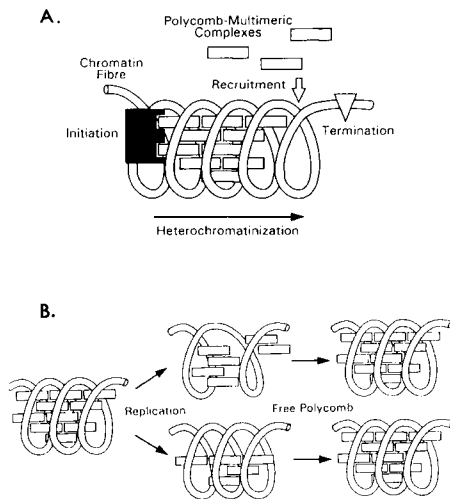


Fig 4. Models for the function of Polycomb group proteins. **A:** Model for the initiation and termination of Polycomb group protein changes in chromosomal organization. **B:** Model for the maintenance of repressive chromosomal structures by the Polycomb group proteins. Replication disperses Polycomb-group proteins to both daughter chromatids which then sequester-free Polycomb group proteins from the nucleoplasm to reassemble repressive chromosomal structures.

the component structural proteins cooperate in large complexes which can self-assemble to package large chromosomal domains (Locke et al, 1988). Consistent with this hypothesis, the Polycomb protein assembles into multimeric complexes (Franke et al, 1992) and is associated with inactive chromatin over large chromosomal domains (Orlando and Paro, 1993). In contrast, the Polycomb protein is absent from transcribed regions.

Immunofluorescent techniques have been used to examine the distribution of proteins within chromosomal domains in the interphase nucleus. These studies complement the more structural and genetic analysis of domain organization (Laemmli et al, 1978; Saitoh and Laemmli, 1994). Clear evidence emerges for the existence of specific compartments within the nucleus (see also Cook, 1991) at which particular proteins are clustered into foci. The Polycomb protein is found to be present within such foci within the nuclei of *Drosophila* tissue culture cells (Messmer et al, 1992). This subnuclear localization depends on the integrity of the chromodomain, and if localization is lost, so is the repressed state of the homeotic genes. Thus, the Polycomb protein might function by assembling a repressive chromatin domain that is tethered to the nuclear matrix in a transcriptionally incompetent nuclear compartment.

Opposing the repressive effects of Polycomb group proteins on homeotic gene expression are the trithorax group of activators (Kennison, 1993). Experiments on the gene *brahma*, a member of the trithorax group, lend additional support to the hypothesis that alterations in chromatin structure have an important maintenance role in sustaining patterns of homeotic gene transcription. Mutations in *brahma* strongly suppress mutations in *Polycomb*. It appears that *brahma* is necessary to relieve the repressive influence of *Polycomb* on homeotic gene expression after embryoge-

nesis (Kennison and Tamkun, 1988; Tamkun et al, 1992). The *brahma* protein has similarities to the yeast SWI/SNF general activator complex. This complex of more than ten proteins (total of 2×10^6 Da) assists a wide variety of *trans*-activators in facilitating the transcriptional activation process (Cairns et al, 1994). However mutations within the histone fold domain of the core histone proteins H3 and H4 relieve the requirement for the SWI/SNF complex (Herskowitz et al, 1992; Winston and Carlson, 1992; Wolffe, 1994c). This suggests that one role of SWI/SNF and potentially the *brahma* protein is to disrupt locally repressive chromatin structures.

The mammalian embryo

Although the long-range chromatin organization of the mammalian embryo is even less well understood than that of either *Drosophila* or *Xenopus*, considerable evidence suggests that aspects of chromosomal structure will influence gene expression during early mammalian development (Fundele and Surani, 1994). We have already discussed how nucleosomal composition changes during early mouse development, the differences in nuclear environment between paternal and maternal pronuclei and the correlations with transcriptional activity. Related epigenetic phenomena are the silencing of one of the two wild-type copies of a gene within a diploid nucleus during early development: these include X chromosomal inactivation in females and the parental imprinting of selected autosomal genes (Pfeifer and Tilghman, 1994). Much attention has focused on differential DNA methylation as a marker for imprinting; however, it is unclear whether DNA methylation acts as a primary determinant of differential gene activity (Groudine and Conkin, 1985) or whether it simply reflects changes in chromatin structure that determine dif-

ferential activity and that restrict the access of DNA methyltransferases to genes (Gottschling, 1992). DNA-binding proteins exist that can selectively recognize methylated DNA and direct transcriptional repression (Boyes and Bird, 1991, 1992). Alternatively, the association of histones and the assembly of repressive nucleosomal structures might differ between methylated and unmethylated DNA (Kass et al, 1993).

Many genes in the inactive X chromosome are heavily methylated, in contrast to those of the active X chromosome (Grant and Chapman, 1988). However, the kinetics with which X-linked genes become methylated during the differentiation of embryonic female somatic cells do not always correlate with the timing of transcriptional inactivation (Lock et al, 1987). Other mechanisms must therefore supplement any direct influence of DNA methylation on transcription within the inactive X chromosome, ie, methylation might serve to maintain a state of repression, but not to establish repression. In contrast, if methylation is impaired in the developing mouse embryo by restricting the activity of DNA methyltransferase, major changes occur in the expression pattern of imprinted autosomal genes (Li et al, 1993). A reduction in DNA methylation leads to the activation of the normally silent paternal allele of *H19*, the repression of the normally active paternal allele of the *Igf2* gene and the repression of the normally active maternal allele of the *Igf2r* gene. These results dramatically demonstrate a key role for DNA methylation in imprinting; however, they also show that there is no simple relationship between the methylation state of a gene and transcriptional activity. Determination of the nucleoprotein organization of imprinted autosomal genes having different levels of methylation will help establish the molecular basis for the imprinting phenomenon.

Considerable progress has been made concerning the nucleoprotein organization of

the active and inactive X chromosome. Promoters in the inactive X chromosome appear to be incorporated into positioned nucleosomes, whereas promoters in the active X chromosome are free of such structures and have transcription factors bound to them (Riggs and Pfeifer, 1992). Thus, specific chromatin structures clearly appear to have a role in regulating differential gene activity between the two chromosomes. Moreover, nucleosomes on the active X chromosome contain predominantly acetylated histones, whereas those on the inactive X chromosome are not acetylated (Jeppesen and Turner, 1993). Since parental nucleosomes will be segregated to the daughter chromatin during chromosomal duplication (Perry et al, 1993), differential acetylation may play an important role in the maintenance of differential gene activity (Wolffe, 1995). Therefore, the packaging of regulatory DNA within the X chromosomes has considerable potential not only for modulating the transcriptional activity of genes, but also for propagating states that are permissive or restrictive for transcription.

CONCLUSION AND PERSPECTIVE

Chromosomes are highly differentiated structures. Their precise structure varies depending on the functional requirements of the nucleus and cell. A unifying feature of early metazoan development is the remodeling of chromatin structure from fertilization to the terminal differentiation of particular cell types. Chromatin assembled immediately following fertilization contains specialized proteins (HMG1, and variant core and linker histones) or post-translational modifications (acetylated core histones) that are known to assemble less stable nucleosomal structures or nucleosomal arrays. This may facilitate both *trans*-acting factor access to regulatory sequences and

the rapid movement of replication forks during the early cleavage cycles. However, there are no simple rules concerning the contribution of these specialized chromatin structures to the transcription process. In *Xenopus* and *Drosophila*, transcription in the early embryo is very low. In the sea urchin and the mouse, transcription is much more active. These variations will depend not only on the quantity and modification of chromatin structural proteins, but also on comparable variations in general and specific transcription factors. Nuclear organization and cell division frequency will also have an important influence on the transcription process especially the timing between replication and mitotic events.

As embryogenesis proceeds patterns of gene activity become progressively more restricted. This restriction correlates with the appearance of the histone variants found in normal somatic cells. Particular histone variants can potentially be causal for the repression of particular genes. These developmental transitions that correlate with cell determinative events may be established in large part by variation in *trans*-acting factor abundance or activity between different cell types, but they appear to be 'locked in' or maintained by transitions in chromatin structure. This stabilizing role might be dependent on histone H1, Polycomb or DNA

methylation dependent on the repressed gene and organism (fig 5). It is important to emphasize that the nucleoprotein complexes regulating gene expression will be specific; whether or not these complexes involve histones, HMG or elements of higher-order chromatin structure will depend on the particular gene examined.

There is much to be discovered concerning how chromatin and chromosomal environments influence gene expression during development. The developmental literature contains many experiments involving the introduction of exogenous template DNA into embryos. In general, this exogenous DNA only partially recapitulates the pattern of gene expression obtained from the same gene within the endogenous chromosomes (Davidson, 1986). These discrepancies are not due to differences in regulatory sequences, but are most probably due to differences in the packaging of those regulatory sequences into nucleoprotein complexes (see Archer et al, 1992). This should be seen as an opportunity to determine the influence of chromosomal packaging on gene expression by attempting to recreate aspects of the chromosomal environment on exogenous DNA. This might require the physiological packaging of DNA into chromatin during replication or it might require chromodomain proteins such as Polycomb

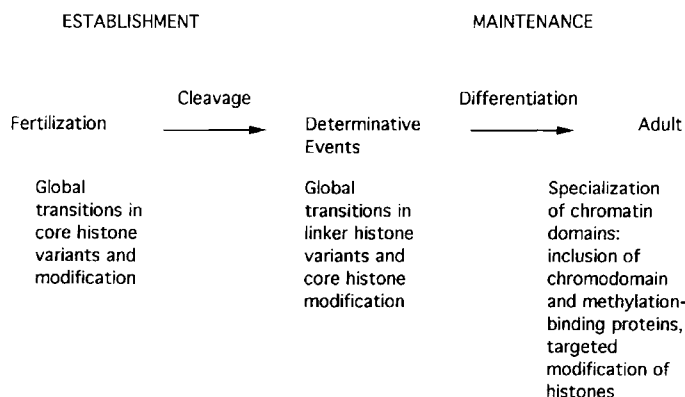


Fig 5. Model for changes in nuclear activity and structure during development.

or specialized linker histone variants to be made available. Having established a repressive chromosomal architecture, other molecular machines like those containing the *brahma* protein or the replication fork might be necessary to relieve this repression and activate transcription.

An appreciation of the impact of chromatin and chromosomal structure does not make the understanding of the developmental control of gene expression necessarily easier; however, it does provide an additional range of regulatory mechanisms. The substantial evidence presented in this review demonstrates that chromosomal architecture has a significant impact on developmental processes. The future offers considerable promise for reconstructing and thus understanding the correct developmental regulation of genes in a natural chromosomal context.

REFERENCES

- Abé SI, Hiyoshi H (1991) Synthesis of sperm-specific basic nuclear proteins (SPs) in cultured spermatids from *Xenopus laevis*. *Exp Cell Res* 194, 397-414
- Adamson ED, Woodland HR (1974). Histone synthesis in early amphibian development. Histone and DNA syntheses are not coordinated. *J Mol Biol* 88, 263-285
- Allan J, Cowling GJ, Harborne N, Cattani P, Craigie R, Gould H (1981) Regulation of the higher-order structure of chromatin by histones H1 and H5. *J Cell Biol* 90, 279-288
- Allis CD, Colavito-Shepanski M, Gorovsky, MA (1987) Scheduled and unscheduled DNA synthesis during development in conjugating *Tetrahymena*. *Dev Biol* 124, 469-480
- Almouzni G, Wolffe AP (1993) Replication coupled chromatin assembly is required for the repression of basal transcription in vivo. *Genes Dev* 7, 2033-2047
- Almouzni G, Wolffe AP (1995) Constraints on transcriptional activator function contribute to transcriptional quiescence during early *Xenopus* embryogenesis. *EMBO J* 14, 1752-1765
- Almouzni G, Méchali M, Wolffe AP (1990) Competition between transcription complex assembly and chromatin assembly on replicating DNA. *EMBO J* 9, 573-582
- Almouzni G, Méchali M, Wolffe AP (1991) Transcription complex disruption caused by a transition in chromatin structure. *Mol Cell Biol* 11, 655-665
- Almouzni G, Khochbin S, Dimitrov S, Wolffe AP (1994) Histone acetylation influences both gene expression and development of *Xenopus laevis*. *Dev Biol* 165, 654-669
- Anderson DM, Smith LD (1978) Patterns of synthesis and accumulation of heterogenous RNA in lampbrush stage oocytes of *Xenopus laevis* (Daudin). *Dev Biol* 67, 274-286
- Andrews MT, Loo S, Wilson LR (1991) Coordinate inactivation of class III genes during the gastrula-neurula transition in *Xenopus*. *Dev Biol* 146, 250-254
- Aparicio OM, Billington BL, Gottschling DE (1991) Modifiers of position effect are shared between telomeric and silent mating-type loci in *S cerevisiae*. *Cell* 66, 1279-1287
- Appels R, Wells JRE (1972) Synthesis and turnover of DNA-bound histone during maturation of avian blood cells. *J Mol Biol* 70, 425-434
- Archer TK, Lefebvre P, Wolford RG, Hager GL (1992) Transcription factor loading on the MMTV promoter: a bimodal mechanism for promoter activation. *Science* 255, 1573-1576
- Aubert D, Garcia M, Benchaibi M, Poncet D, Chebloune Y, Verdier G, Nigon V, Samarut J, Murra CV (1991) Inhibition of proliferation of primary avian fibroblasts through expression of histone H5 depends on the degree of phosphorylation of the protein. *J Cell Biol* 11, 497-506
- Bauer WR, Hayes JJ, White JH, Wolffe AP (1994) Nucleosome structural changes due to acetylation. *J Mol Biol* 236, 685-690
- Baxeavanis A, Landsman D (1995) The HMG-1 box protein family: classification and functional relationships. *Nucleic Acids Res* 23, 1604-1613
- Beachy PA, Helfand, SL, Hogness DS (1985) Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature* 313, 545-551
- Bieker JJ, Martin PL, Roeder RG (1985) Formation of a rate limiting intermediate in 5S RNA gene transcription. *Cell* 40, 119-127
- Bienz M (1986) A CCAAT box confers cell-type-specific regulation of the *Xenopus* hsp70 gene in oocytes. *Cell* 46, 1037-1042
- Bone JR, Lavender J, Richman R, Palmer MJ, Turner MB, Kuroda ML (1994) Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes Dev* 8, 96-104
- Bouvet P, Wolffe AP (1994) A role for transcription and FRGY2 in masking maternal mRNA in *Xenopus* oocytes. *Cell* 77, 931-941
- Bouvet P, Dimitrov S, Wolffe AP (1994) Specific regulation of chromosomal 5S rRNA gene transcription in vivo by histone H1. *Genes Dev* 8, 1147-1159

- Boy de la Tour E, Laemmli UK (1988) The metaphase scaffold is helically folded: sister chromatids have predominantly opposite helical handedness. *Cell* 55, 937-944
- Boyes J, Bird A (1991) DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell* 64, 1123-1134
- Boyes J, Bird A (1992) Repression of genes by DNA methylation depends on CpG density and promoter strength: evidence for involvement of a methyl-CpG binding protein. *EMBO J* 11, 327-333
- Brandhorst BP (1980) Simultaneous synthesis, translation and storage of mRNA including histone mRNA in sea urchin eggs. *Dev Biol* 52, 310-311
- Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR (1993) Transcriptional silencing in yeast is associated with reduced histone acetylation. *Genes Dev* 7, 592-604
- Brown DD, Littna E (1966) Synthesis and accumulation of low molecular weight RNA during embryogenesis of *Xenopus laevis*. *J Mol Biol* 20, 95-112
- Buonigiorno-Nardelli M, Micheli G, Carri MT, Marilley M (1982) A relationship between replicon size and supercoiled loops domains in the eukaryotic genome. *Nature* 298, 100-102
- Cairns, BR, Kim, YJ, Sayre, MH, Laurent, BC, Kornberg RD (1994) A multisubunit complex containing the SWIA/ADR6, SWI2/SNF2, SWI3/SNF5 and SNF6 gene products isolated from yeast. *Proc Natl Acad Sci USA* 91, 1950-1954
- Callan HG, Gall JG, Berg CA (1987) The lampbrush chromosomes of *Xenopus laevis*: Preparation, identification, and distribution of 5S DNA sequences. *Chromosoma (Berl)* 95, 236-250
- Candido EPM, Reeves R, Davie JR (1978) Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* 14, 105-115
- Carroll SB, Layman RA, McCutcheon MA, Riley PD (1986) The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. *Cell* 47, 113-122
- Celniker SE, Sharma S, Keelan DJ, Lewis E (1990) The molecular genetics of the *bithorax* complex of *Drosophila*: *Cis* regulation of the Abdominal-B domain. *EMBO J* 9, 4277-4286
- Chambers SAM, Shaw BR (1984) Levels of histone H4 diacetylation decrease dramatically during sea urchin embryonic development and correlate with cell doubling rate. *J Biol Chem* 259, 13458-13463
- Chicoine LG, Allis CD (1986) Regulation of histone acetylation during macronuclear differentiation in *Tetrahymena*. Evidence for control at the level of acetylation and deacetylation. *Dev Biol* 116, 477-485
- Chipev CC, Wolffe AP (1992) Chromosomal organization of *Xenopus laevis* oocyte and somatic 5S rRNA genes in vivo. *Mol Cell Biol* 12, 45-55
- Cho H, Wolffe AP (1994) Characterization of the *Xenopus laevis* B4 gene: an oocyte specific vertebrate linker histone gene containing introns. *Gene* 143, 233-238
- Christians E, Campion E, Thompson EM, Renard JP (1995) Expression of the *HSP 70.1* gene, a landmark of early zygotic activity in the mouse embryo is restricted to the first burst of transcription. *Development* 112, 113-122
- Chuang PT, Albertson DG, Meyer BJ (1994) DPY-27: a chromosome condensation protein homolog that regulates *C elegans* dosage compensation through association with the X chromosome. *Cell* 79, 459-474
- Chung JH, Whiteley M, Felsenfeld G (1993) A 5' element of the chicken β -globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila*. *Cell* 74, 505-514
- Clark DJ, O'Neill LP, Turner BM (1993) Selective use of H4 acetylation sites in the yeast *Saccharomyces cerevisiae*. *Biochem J* 294, 557-561
- Clark-Adams CD, Norris D, Osley MA, Fassler JS, Winston F (1988) Changes in histone gene dosage alter transcription in yeast. *Genes Dev* 2, 150-159
- Clarke HJ, Oblin C, Bustin M (1992) Developmental regulation of chromatin composition during mouse embryogenesis: somatic histone H1 is first detectable at the 4-cell stage. *Development* 115, 791-799
- Cook PR (1991) The nucleoskeleton and the topology of replication. *Cell* 66, 627-635
- Dasso M, Dimitrov S, Wolffe AP (1994) Nuclear assembly is independent of linker histones. *Proc Natl Acad Sci USA* 91, 12477-12481
- Davidson EH (1986) *Gene Activity in Early Development*. 3rd edn. Academic Press, San Diego, CA, USA
- DiNardo S, Voelkel K, Sternglanz R (1984) DNA topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. *Proc Natl Acad Sci USA* 81, 2616-2620
- Dimitrov S, Almouzni G, Dasso M, Wolffe AP (1993) Chromatin transitions during early *Xenopus* embryogenesis: changes in histone H4 acetylation and in linker histone type. *Dev Biol* 160, 214-227
- Dimitrov S, Dasso MC, Wolffe AP (1994) Remodeling sperm chromatin in *Xenopus laevis* egg extracts: the role of core histone phosphorylation and linker histone B4 in chromatin assembly. *J Cell Biol* 126, 591-601
- Dong F, van Holde KE (1991) Nucleosome positioning is determined by the (H3-H4)₂ tetramer. *Proc Natl Acad Sci USA* 88, 10596-10600
- Edgar BA, Schubiger G (1986) Parameters controlling transcriptional activation during early *Drosophila* development. *Cell* 44, 871-877

- Eissenberg JC, James TC, Foster HDM, Hartnett T, Ngan V, Elgin SC (1990) Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position effect variegation in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 87, 9923-9927
- Flynn JM, Woodland HR (1980) The synthesis of histone H1 during early amphibian development. *Dev Biol* 75, 222-230
- Franke A (1991) The Polycomb protein: genetic and molecular analyses of interactions with other Polycomb-group genes in *Drosophila melanogaster*. PhD Thesis, University of Heidelberg, Heidelberg, Germany
- Franke A, DeCamillis M, Zink D, Cheng N, Brock H, Paro R (1992) *Polycomb* and *polyhomeotic* are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. *EMBO J* 11, 2941-2950
- Fundele RH, Surani MA (1994) Experimental embryological analysis of genetic imprinting in mouse development. *Dev Genet* 15, 515-522
- Garcia-Ramirez M, Dong F, Ausio J (1992) Role of the histone 'tails' in the folding of oligonucleosomes depleted of histone H1. *J Biol Chem* 267, 19587-19595
- Garcia-Ramirez M, Rocchini C, Ausio J (1995) Modulation of chromatin folding by histone acetylation. *J Biol Chem* 270, 17923-17928
- Ge H, Roeder RG (1994) The high mobility protein HMG1 can reversibly inhibit class II gene transcription by interaction with the TATA-binding protein. *J Biol Chem* 269, 17136
- Geyer PK, Corces VG (1992) DNA position-effect repression of transcription by a *Drosophila* zinc-finger protein. *Genes Dev* 6, 1865-1873
- Goodwin GH, Woodhead L, Johns EW (1977) The presence of high mobility group non-histone proteins in isolated nucleosomes. *FEBS Lett* 73, 85
- Gorovsky MA (1973) Macro- and micronuclei of *Tetrahymena pyriformis*: a model system for studying the structure and function of eukaryotic nuclei. *J Protozool* 20, 19-25
- Gottschling DE (1992) Telomere-proximal DNA in *Saccharomyces cerevisiae* is refractory to methyltransferase activity in vivo. *Proc Natl Acad Sci USA* 89, 4062-4065
- Gottschling DE, Aparicio OM, Billington BL, Zakian VA (1990) Position effect at *S cerevisiae* telomeres: reversible repression of pol II transcription. *Cell* 63, 751-762
- Goustin AS, Wilt FH (1981) Protein synthesis, polyribosomes, and peptide elongation in early development of *Strongylocentrotus purpurulus*. *Dev Biol* 82, 32-45
- Graham CF, Morgan RW (1966) Changes in the cell cycle during early amphibian development. *Dev Biol* 14, 439-460
- Grant SG, Chapman VM (1988) Mechanisms of X-chromosome regulation. *Ann Rev Genet* 22, 199-233
- Grigliatti T (1991) Position-effect variegation: an assay for non histone chromosomal proteins and chromatin assembly and modifying factors. *Methods Cell Biol* 35, 587-627
- Grosveld F, Antoniou M, Berry M, de Boer E, Dillou N, Ellis J, Fraser P, Hurst J, Iman A, Meijer D, Philippen S, Pruzina S, Strouboulis J, Whyatt D (1993) Regulation of human globin gene switching. *Cold Spring Harbor Symp Quant Biol* 58, 7-15
- Groudine M, Conkin KF (1985) Chromatin structure and de novo methylation of sperm DNA: implications for activation of the paternal genome. *Science* 228, 1061-1068
- Grunstein M, Durrin LK, Mann RK, Fisher-Adams G, Johnson LM (1992) Histones: regulators of transcription in yeast. In: *Transcriptional Regulation* (S McKnight, K Yamamoto, eds), Cold Spring Harbor Press, New York, 1295-1315
- Gurdon JB (1962) Adult frogs derived from the nuclei of single somatic cells. *Dev Biol* 4, 256-276
- Gurdon JB (1963) Nuclear transplantation in amphibia and the importance of stable nuclear changes in promoting cellular differentiation. *Q Rev Biol* 38, 54-78
- Gurdon JB (1976) Injected nuclei in frog eggs: fate, enlargement and chromatin dispersal. *J Embryol Exp Morphol* 36, 523-540
- Han M, Chang M, Kim UJ, Grunstein M (1987) Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication and transcription. *Cell* 48, 589-597
- Harding K, Levine M (1988) Gap genes define the limits of *Antennapedia* and *bithorax* gene expression during early development in *Drosophila*. *EMBO J* 7, 205-214
- Hartl P, Gottesfeld J, Forbes DJ (1993) Mitotic repression of transcription in vitro. *J Cell Biol* 120, 613-624
- Hayes JJ, Clark DJ, Wolffe AP (1991) Histone contributions to the structure of DNA in the nucleosome. *Proc Natl Acad Sci USA* 88, 6829-6833
- Hayes JJ, Pruss D, Wolffe AP (1994) Histone domains required to assemble a chromosome including the *Xenopus borealis* somatic 5S rRNA gene. *Proc Natl Acad Sci USA* 91, 7817-7821
- Hayes JJ, Wolffe AP (1992) Histones H2A/H2B inhibit the interactions of transcription factor IIIA with the *Xenopus borealis* somatic 5S RNA gene in a nucleosome. *Proc Natl Acad Sci USA* 89, 1229-1233
- Hebbes TR, Clayton AL, Thorne AW, Crane-Robinson C (1994) Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken β -globin chromosomal domain. *EMBO J* 13, 1823-1830
- Hecht A, Laroche, T, Strahl-Bolsinger S, Gasser SM, Grunstein M (1995) Histone H3 and H4 N-termini

- interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* 80, 583-592
- Heitz E (1928) Das Heterochromatin der Moose. *I Jahrb Wiss Bot* 69, 762-819
- Henikoff S (1994) A reconsideration of the mechanisms of position effect. *Genetics* 138, 1-5
- Hentschel CC, Tata JR (1978) Template-engaged and free RNA polymerases during *Xenopus* erythroid cell maturation. *Dev Biol* 65, 496-507
- HersHKovitz M, Riggs AD (1995) Metaphase chromosome analysis by ligation-mediated PCR: heritable chromatin structure and a comparison of active and inactive X chromosomes. *Proc Natl Acad Sci USA* 92, 2379-2383
- Herskowitz I, Andrews B, Kruger W, Ogas J, Sil A, Coburn C, Peterson C (1992) Integration of multiple regulatory inputs in the control of HO expression in yeast. In: *Transcriptional Regulation*, vol 2, (S McKnight, K Yamamoto, eds), Cold Spring Harbor Press, New York, Vol. 2, 949-974
- Hill CS, Rimmer JM, Green BN, Finch JT, Thomas JO (1991) Histone-DNA interactions and their modulation by phosphorylation of Ser-Pro-X-Lys/Arg-motifs. *EMBO J* 10, 1939-1948
- Hirano T, Mitchison TJ (1994) A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell* 79, 449-458
- Hirschhorn JN, Brown SA, Clark CD, Winston F (1992) Evidence that SNF2/SWI2 and SNF activate transcription in yeast by altering chromatin structure. *Genes Dev* 6, 2288-2298
- Hock R, Moorman A, Fischer D, Scheer U (1993) Absence of somatic histone H1 in oocytes and preblastula embryos of *Xenopus laevis*. *Dev Biol* 158, 510-522
- Howlett SK, Barton SC, Surani MA (1987) Nuclear cytoplasmic interactions following nuclear transplantation in mouse embryos. *Development* 101, 915-923
- Ikegami S, Ooe Y, Shimizu, Kasahara T, Tsuruta T, Kijima M, Yoshida M, Beppu T (1993) Accumulation of multiacetylated forms of histones by trichostatin A and its developmental consequences in early starfish embryos. *Roux's Arch Dev Biol* 202, 144-151
- Illmensee K (1978) *Drosophila* chimeras and the problem of determination. In: *Genetic Mosaics and Cell Differentiation* (WJ Gehring, ed) Springer Verlag, Berlin, 51-69
- Irish VF, Martinez-Arias A, Akam M (1989) Spatial regulation of the *Antennapedia* and *Ultrabithorax* homeotic genes during *Drosophila* early development. *EMBO J* 8, 1527-1537
- James TC, Elgin SC (1986) Identification of a non histone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* in its gene. *Mol Cell Biol* 6, 3862-3872
- Jenuwein T, Forrester WC, Qui RG, Grosschedl R (1993) The immunoglobulin *m* enhancer core establishes local factor access in nuclear chromatin independent of transcriptional stimulation. *Genes Dev* 7, 2016-2032
- Jeppesen P, Turner BM (1993) The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* 74, 281-291
- Jutglar L, Borell JI, Ausio J (1991) Primary, secondary and tertiary structure of the core of a histone H1-like protein from the sperm of *Mytilus*. *J Biol Chem* 266, 8184-8191
- Kandolf H (1994) The H1A histone variant is the in vivo repressor of oocyte-type 5S gene transcription in *Xenopus laevis* embryos. *Proc Natl Acad Sci USA* 91, 7257-7260
- Kass SV, Goddard JP, Adams RLP (1993) Inactive chromatin spreads from a focus of methylation. *Mol Cell Biol* 13, 7372-7379
- Kauffmann SA (1980) Heterotopic transplantation in the syncytial blastoderm of *Drosophila*: evidence for anterior and posterior nuclear commitments. *Archiv Dev Biol* 189, 135-145
- Kaufman TC, Lewis R, Wakimoto B (1980) Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosome interval 84A-B. *Genetics* 94, 115-133
- Kaufmann PD, Botchan MR (1994) Assembly of nucleosomes: do multiple assembly factors mean multiple mechanisms? *Curr Opin Genet Dev* 4, 229-235
- Kedes LH, Gross PR (1969) Synthesis and function of messenger RNA during early embryonic development. *J Mol Biol* 42, 559-675
- Kellum R, Schedl P (1991) A position-effect assay for boundaries of higher order chromosomal domains. *Cell* 64, 941-950
- Kennison JA (1993) Transcriptional activation of *Drosophila* homeotic genes from distant regulatory elements. *Trends Genet* 9, 75-79
- Kennison JA, Tamkun JW (1988) Dosage-dependent modifiers of *Polycomb* and *Antennapedia* mutations in *Drosophila*. *Proc Nat Acad Sci USA* 85, 8136-8140
- Khochbin S, Wolffe AP (1993) Developmental regulation and butyrate inducible transcription of the *Xenopus* histone H1 promoter. *Gene* 128, 173-180
- Khochbin S, Wolffe AP (1994) Developmentally regulated expression of linker-histone variants in vertebrates. *Eur J Biochem* 225, 501-510
- Kingsley PD, Angerer LM, Angerer RC (1993) Major temporal and spatial patterns of gene expression during differentiation of the sea urchin embryo. *Dev Biol* 155, 216-234
- Klar AJS, Bonaduce MJ (1991) *swi6*, a gene required for mating-type switching, prohibits meiotic recom-

- bination in the *mat2-mat3* 'cold spot' of fission yeast. *Genetics* 129, 1033-1042
- Kleinschmidt JA, Steinbeisser H (1991) DNA dependent phosphorylation of histone H2A.X during nucleosome assembly in *Xenopus laevis* oocytes: involvement of protein phosphorylation in nucleosome spacing. *EMBO J* 10, 3043-3050
- Kleinschmidt JA, Fortkamp E, Krohne G, Zentgraf H, Franke WW (1985) Coexistence of two different types of soluble histone complexes in nuclei of *Xenopus laevis* oocytes. *J Biol Chem* 260, 1166-1176
- Knowles JA, Childs GJ (1984) Temporal expression of late histone messenger RNA in the sea urchin *Lytechinus pictus*. *Proc Natl Acad Sci USA* 81, 2411-2415
- Krieg PA, Melton D (1987) An enhancer responsible for activating transcription at the mid-blastula transition in *Xenopus* development. *Proc Natl Acad Sci USA* 84, 2331-2335
- Kruger W, Herskowitz I (1991) A negative regulator of HO transcription, SIN1 (SPT2), is a non specific DNA binding protein related to HMG1. *Mol Cell Biol* 11, 4135-4146
- Kuziora MA, McGinnis W (1988) Different transcripts of the *Drosophila Abd-B* gene correlate with distinct genetic sub-functions. *EMBO J* 7, 3233-3244
- La Marca MJ, Strobel-Fidler MC, Smith LD, Keem K (1975) Hormonal effects on RNA synthesis by stage 6 oocytes of *Xenopus laevis*. *Develop Biol* 47, 384-393
- Laemmli UK, Cheng SM, Adolph KW, Paulson JR, Brown JA, Brumbach WR (1978) Metaphase chromosome structure: the role of non histone proteins. *Cold Spring Harbor Symp Quant Biol* 42, 351-360
- Landsberger N, Wolffe AP (1995) Chromatin and transcriptional activity in early *Xenopus* development. *Semi Cell Biol* 6, 191-200
- Landsberger N, Ranjan M, Almouzni G, Stump D, Wolffe AP (1995) The heat shock response in *Xenopus* oocytes, embryos and somatic cells: an essential regulatory role for chromatin. *Dev Biol* 170, 62-74
- Latham KE, Solter D, Schultz RM (1992) Acquisition of a transcriptionally permissive state during the 1-cell stage of mouse embryogenesis. *Dev Biol* 149, 457-462
- Laurenson P, Rine J (1992) Silencers, silencing and heritable transcriptional states. *Microb Rev* 56, 543-560
- Lee DY, Hayes JJ, Pruss D, Wolffe AP (1993) A positive role for histone acetylation in transcription factor binding to nucleosomal DNA. *Cell* 72, 73-84
- Lewis CD, Laemmli UK (1982) Higher order metaphase chromosome structure: evidence for metalloprotein interactions. *Cell* 29, 171-181
- Lewis EB (1950) The phenomenon of position effect. *Adv Genet* 3, 73-115
- Lewis EB (1978) A gene complex controlling segmentation. *Nature* 276, 565-570
- Li E, Beard C, Jaenisch R (1993) Role of DNA methylation in genomic imprinting. *Nature* 366, 362-365
- Lima-de Faria A (1983) Processes of directing expression, mutation and rearrangements. In: *Molecular Evolution and Organization of the Chromosome* (A Lima de Faria, ed), Elsevier Science Publishers, Amsterdam, The Netherlands, 507-604
- Lin R, Cook RG, Allis CD (1991) Proteolytic removal of core histone amino termini and dephosphorylation of histone H1 correlate with the formation of condensed chromatin and transcriptional silencing during *Tetrahymena* macronuclear development. *Genes Dev* 5, 1601-1610
- Lock LF, Takagi N, Martin GR (1987) Methylation of the *hprt* gene on the inactive X occurs after chromosome inactivation. *Cell* 48, 36-46
- Locke J, Kotarski MA, Tartof KD (1988) Dosage dependent modifiers of position effect variegation in *Drosophila* and a mass action model that explains their effect. *Genetics* 120, 181-188
- Lorentz A, Heim L, Schmidt H (1992) The switching gene *swi6* affects recombination and gene expression in the mating-type region of schizosaccharomyces pombe. *Mol Gen Genet* 233, 436-442
- Lorentz A, Ostermann K, Fleck O, Schmidt H (1994) Switching gene *swi6* involved in the repression of silent mating-type loci in fission yeast, encodes a homolog of chromatin associated proteins from *Drosophila* and mammals. *Gene* 143, 1-8
- Louters L, Chalkley R (1985) Exchange of histones H1, H2A and H2B in vivo. *Biochemistry* 24, 3080-3085
- Lund E, Dahlberg JE (1992) Control of 4-8S RNA transcription at the midblastula transition in *Xenopus laevis* embryos. *Genes Dev* 6, 1097-1106
- McGrath JD, Solter D (1984) Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro. *Science* 226, 1317-1319
- McPherson CE, Shim EY, Friedman DS, Zaret KS (1993) An active tissue-specific enhancer and bound transcription factors existing in a precisely positioned nucleosomal array. *Cell* 75, 387-398
- Majumder D, Miranda M, De Pamphilis M (1993) Analysis of gene expression in mouse preimplantation embryos demonstrates that the primary role of enhancers is to relieve repression of promoters. *EMBO J* 12, 1131-1140
- Martindale DW, Allis CD, Bruns PJ (1982) Conjugation in *Tetrahymena thermophila*: a temporal analysis of cytological stages. *Exp Cell Res* 140, 227-236
- Martinez-Salas E, Linney E, Hassell J, De Pamphilis ML (1989) The need for enhancers in gene expression first appears during mouse development with formation of the zygotic nucleus. *Genes Dev* 3, 1493-1506

- Marushige K, Dixon GH (1969) Developmental changes in chromosomal composition and template activity during spermatogenesis in trout testis. *Dev Biol* 19, 397-414
- Matsumoto K, Anzai M, Nakagata N, Takahashi A, Takahashi Y, Miyata K (1994) Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. *Mol Reprod Dev* 39, 136-140
- Maxson R, Mohun T, Gormezano G, Childs G, Keddes L (1983) Distinct organization and patterns of expression of early and late histone sets in the sea urchin. *Nature* 301, 120-127
- Meeks-Wagner D, Hartwell LH (1986) Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. *Cell* 44, 53-63
- Mélin F, Miranda M, Montreau N, De Pamphilis ML, Blangy D (1993) Transcription enhancer factor-1 (TEF-1) DNA binding sites can specifically enhance gene expression at the beginning of mouse development. *EMBO J* 12, 4657-4666
- Messmer S, Franke A, Paro R (1992) Analysis of the functional role of the Polycomb chromo domain in *Drosophila melanogaster*. *Genes Dev* 6, 1241-1254
- Micheli G, Luzzatto ARC, Carri MT, de Capoa A, Pellicca F (1993) Chromosome length and DNA loop size during early embryonic development of *Xenopus laevis*. *Chromosoma* 102, 478-483
- Moav B, Nemer M (1971) Histone synthesis: assignment to a special class of polyribosomes in sea urchin embryos. *Biochemistry* 10, 881-889
- Moore GD, Sinclair DA, Grigliatti TA (1983) Histone gene multiplicity and position-effect variegation in *Drosophila melanogaster*. *Genetics* 101, 327-344
- Morata G, Botas J, Kerridge S, Struhl G (1983) Homeotic transformations of the abdominal segments of *Drosophila* caused by breaking or deleting a central portion of the *bithorax* complex. *J Embryol Exp Morphol* 78, 319-341
- Mottus R, Reeves R, Grigliatti TA (1980) Butyrate suppression of position effect variegation in *Drosophila melanogaster*. *Mol Gen Genet* 178, 465-469
- Nenny DL (1953) Nucleocytoplasmic interaction during conjugation in *Tetrahymena*. *Biol Bull* 105, 133-148
- Ner SS, Travers AA (1994) HMG-D, the *Drosophila melanogaster* homologue of HMG1 protein, is associated with early embryonic chromatin in the place of histone H1. *EMBO J* 13, 1817-1822
- Newport JW, Kirschner MW (1982a) A major developmental transition in early *Xenopus* embryos. 1. Characterization and timing of cellular changes at the mid blastula stage. *Cell* 30, 675-686
- Newport JW, Kirschner MW (1982b) A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell* 30, 687-696
- Newrock KM, Alfageme CR, Nardi CV, Cohen LH (1977) Histone changes during chromatin remodeling in embryogenesis. *Cold Spring Harbor Symp Quant Biol* 42, 421-431
- Ohsumi K, Katagiri C, Kishimoto T (1993) Chromosome condensation in *Xenopus* mitotic extracts without histone H1. *Science* 262, 2033-2035
- Orlando V, Paro R (1993) Mapping Polycomb-repressed domains in the bithorax complex using in vivo formaldehyde cross-linked chromatin. *Cell* 75, 1187-1198
- Parkhurst SM, Harrison DA, Remington MP, Spana C, Kelley RL, Coyne RS, Corces VG (1988) The *Drosophila su(Hw)* gene, which controls the phenotypic effect of the *gypsy* transposable element, encodes a putative DNA binding protein. *Genes Dev* 2, 1205-1215
- Perry CA, Allis CD, Annunziato AT (1993) Parental nucleosomes segregated to newly replicated chromatin are underacetylated relative to those assembled de novo. *Biochemistry* 32, 13615-13623
- Perry M, Thomson GH, Roeder RG (1986) Major transitions in histone gene expression do not occur during development in *Xenopus laevis*. *Dev Biol* 116, 532-538
- Pfeifer K, Tilghman SM (1994) Allele-specific gene expression in mammals: the curious case of the imprinted RNAs. *Genes Dev* 8, 1867-1874
- Philpott A, Leno GH (1992) Nucleoplamin remodels sperms chromatin in *Xenopus* egg extracts. *Cell* 69, 759-767
- Philpott A, Leno GH, Laskey RA (1991) Sperm decondensation in *Xenopus* egg cytoplasm is mediated by nucleoplamin. *Cell* 65, 569-578
- Pirotta V, Rastelli L (1994) *White* gene expression, repressive chromatin domains and homeotic gene regulation in *Drosophila*. *BioEssays* 16, 549-556
- Poccia DL, Green GR (1992) Packaging and unpackaging the sea urchin genome. *Trends Biochem Sci* 17, 223-227
- Poccia D, Salik J, Krystal G (1981) Transitions in histone variants of the male pronucleus following fertilization and evidence for a maternal store of cleavage stage histones in the sea urchin egg. *Dev Biol* 82, 287-299
- Poccia D, Wolff R, Kragh S, Williamson P (1985) RNA synthesis in male pronuclei of the sea urchin. *Biochim Biophys Acta* 824, 349-359
- Prescott DM, Bender MA (1962) Synthesis of RNA and protein during mitosis in mammalian tissue culture cells. *Exp Cell Res* 26, 260-268
- Prioleau MN, Huet J, Sentenac A, Méchali M (1994) Competition between chromatin and transcription complex assembly regulates gene expression during early development. *Cell* 77, 439
- Ram PT, Schultz RM (1993) Reporter gene expression in G2 of the 1. cell mouse embryo. *Dev Biol* 156, 552-556

- Rattner JB, Lin CC (1985) Radical loops and helical coils coexist in metaphase chromosomes. *Cell* 42, 291-296
- Reuter G, Spierer P (1992) Position-effect variegation and chromatin proteins. *BioEssays* 14, 605-612
- Riggs AP, Pfeifer GP (1992) X-chromosome inactivation and cell memory. *Trends Genet* 8, 169-174
- Robl JM, Gilligan B, Critser ES, First NL (1986) Nuclear transplantation in mouse embryos: assessment of recipient cell stage. *Biol Reprod* 34, 733-739
- Roseman RR, Pirrotta V, Geyer PK (1993) The *su(Hw)* protein insulates expression of the *Drosophila melanogaster* White gene from chromosomal position effects. *EMBO J* 12, 435-442
- Roth SY, Schulman IG, Richman R, Cook RG, Allis CD (1988) Characterization of phosphorylation sites in histones H1 in the amitotic macronucleus of *Tetrahymena* during different physiological states. *J Cell Biol* 107, 2473-2482
- Roth SY, Shimizu M, Johnson L, Grunstein M, Simpson RT (1992) Stable nucleosome positioning and complete repression by the yeast $a2$ repressor are disrupted by amino-terminal mutation in histone H4. *Genes Dev* 6, 411-425
- Rupp RAW, Weintraub H (1991) Ubiquitous Myo D transcription at the mid-blastula transition precedes induction-dependent Myo D expression in presumptive mesoderm of *X laevis*. *Cell* 65, 927-937
- Rutledge RG, Neelin JM, Seligly VL (1988) Isolation and expression of cDNA clones coding for two sequence variants of *Xenopus laevis* histone H5. *Gene* 70, 117-126
- Saitoh Y, Laemmli UK (1994) Metaphase chromosome structure: bands arise from a differential folding path of the high AT-rich scaffold. *Cell* 76, 609-622
- Saitoh H, Goldberg I, Wood WR, Earnshaw WC (1994) Sc II, an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J Cell Biol* 127, 303-318
- Salik J, Herlands L, Hoffman HP, Poccia D (1981) Electrophoretic analysis of the stored histone pool in unfertilized sea urchin eggs: quantification and identification by antibody binding. *J Cell Biol* 90, 385-396
- Saunders WC, Chue C, Goebel M, Craig C, Clark RF, Powers JA, Eissenberg JC, Elgin S, Rothfeld NF, Earnshaw WC (1993) Molecular cloning of a human homologue of *Drosophila* heterochromatin protein HP1 using anti-centromere autoantibodies with anti-chromosome specificity. *J Cell Sci* 104, 573-585
- Scarlato V, Arico B, Goyard S, Rici S, Manetti R, Prugnola A, Manetti R, Polverino-De-Laureto P, Ullman A, Rappuoli R (1995) A novel chromatin-forming histone H1 homologue is encoded by a dispensible and growth-regulated gene in *Bordetella pertussis*. *Mol Microb* 15, 871-881
- Schild C, Claret FX, Wahli W, Wolffe AP (1993) A nucleosome-dependent static loop potentiates estrogen-regulated transcription from the *Xenopus* vitellogenin B1 promoter in vitro. *EMBO J* 12, 423-433
- Schulman IG, Wang T, Stargell LA, Gorovsky M, Allis CD (1991) Cell-cell interactions trigger the rapid induction of a specific high mobility group-like protein during early stages of conjugation in *Tetrahymena*. *Dev Biol* 143, 248-257
- Schultz RM (1993) Regulation of zygotic gene activation in the mouse. *BioEssays* 15, 531-538
- Shen X, Yu L, Weir JW, Gorovsky MA (1995) Linker histones are not essential and affect chromatin condensation in vivo. *Cell* 82, 47-56
- Shiokawa K, Fu Y, Nakakura N, Tashiro K, Sameshima M, Hosokawa K (1989) Effects of the injection of exogenous DNAs on gene expression in early embryos and coenocytic egg cells of *Xenopus laevis*. *Arch Dev Biol* 198, 78-84
- Shrader TE, Crothers DM (1989) Artificial nucleosome positioning sequences. *Proc Natl Acad Sci USA* 86, 7418-7422
- Shykind BM, Kim J, Sharp PA (1995) Activation of the TFIIID-TFIIA complex with HMG-2. *Genes Dev* 9, 1354-1365
- Singh PB (1994) Molecular mechanisms of cellular determination: their relation to chromatin structure and paternal imprinting. *J Cell Sci* 107, 2653-2668
- Stargell LA, Bowen J, Dadd CA, Dedon PA, Davis M, Cook RG, Allis CD, Gorovsky MA (1993) Temporal and spatial association of histone H2A variant hv1 with transcriptionally competent chromatin during nuclear development in *Tetrahymena thermophila*. *Genes Dev* 7, 2641-2651
- Strickland WN, Strickland M, Brandt WF, Von Holt C, Lehmann A, Wittmann-Liebold B (1980) The primary structure of histone H1 from sperm of the sea urchin *Parechinus angulosus*. *Eur J Biochem* 104, 567-578
- Strunnikov AV, Lavionoc VL, Koshland D (1993) SMC1: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. *J Cell Biol* 123, 1635-1648
- Sullivan KF, Hechenberger M, Masri K (1994) Human CENP-A contains a histone H3 related histone fold domain that is required for targeting to the centromere. *J Cell Biol* 127, 581-592
- Sun JM, Wiaderekiewicz R, Ruiz-Carrillo A (1989) Histone H5 in the control of DNA synthesis and cell proliferation. *Science* 245, 68-71
- Tafari SR, Wolffe AP (1993) Selective recruitment of masked maternal mRNA from messenger ribonucleoprotein particles containing FRGY2 (mRNP4). *J Biol Chem* 257, 24255-24261
- Takeichi T, Satoh S, Tashiro K, Shiokawa K (1985) Temporal control of rRNA synthesis in cleavage-arrested embryos of *Xenopus laevis*. *Dev Biol* 112, 443-450

- Tamkun JW, Deuring R, Scott MP, Kissinger M, Palacucci AM, Kaufman TC, Kennison JA (1992) Brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* 68, 561-572
- Telford NA, Watson AJ, Schultz GA (1990) Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Mol Reprod Dev* 26, 90-100
- Thompson EM, Legony E, Christians E, Renard JP (1995) Progressive maturation of chromatin structure regulates HSP70.1 gene expression in the preimplantation mouse embryo. *Development* 121, 3425-3427
- Tjian R, Maniatis T (1994) Transcriptional activation: a complex puzzle with few easy pieces. *Cell* 77, 5-8
- Turner BM (1993) Decoding the nucleosome. *Cell* 75, 5-8
- Turner BM, Birley AJ, Lavender J (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell* 69, 375-384
- Ura K, Hayes JJ, Wolffe AP (1995) A positive role for nucleosome mobility in the transcriptional activity of chromatin templates: restriction by linker histones. *EMBO J* 14, 3753-3765
- van Daal A, Elgin SCR (1992) A histone variant, H2AvD, is essential in *Drosophila melanogaster*. *Mol Biol Cell* 3, 593-602
- van Daal A, White EM, Gorovsky MA, Elgin SCR (1988) *Drosophila* has a single copy of the gene encoding a highly conserved histone H2A variant of the H2A.F/Z type. *Nucleic Acids Res* 16, 7487-7497
- Wang T, Allis CD (1992) Replication-dependent and independent regulation of HMG expression during the cell cycle and conjugation in *Tetrahymena*. *Nucl Acids Res* 20, 6525-6533
- Wenkert D, Allis CD (1984) Timing of the appearance of the macronuclear-specific histone variant hv1 and gene expression in developing new macronuclei of *Tetrahymena thermophila*. *J Cell Biol* 98, 2107-2117
- Wiekowski M, Miranda M, De Pamphilis ML (1991) Regulation of gene expression in preimplantation mouse embryos: effects of zygotic gene expression and the first mitosis on promoter and enhancer activities. *Dev Biol* 147, 403-414
- Wiekowski M, Miranda M, De Pamphilis ML (1993) Requirements for promoter activity in mouse oocytes and embryos distinguish paternal pronuclei from maternal and zygotic nuclei. *Dev Biol* 159, 366-378
- Wilt F (1963) The synthesis of ribonucleic acid in sea urchin embryos. *Biochem Biophys Res Commun* 11, 447-457
- Wilt F (1964) Ribonucleic acid synthesis during sea urchin embryogenesis. *Dev Biol* 9, 299-315
- Winston F, Carlson M (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet* 8, 387-391
- Wolffe AP (1989) Dominant and specific repression of *Xenopus* oocyte 5S RNA gene and satellite I DNA by histone H1. *EMBO J* 8, 527-537
- Wolffe AP (1994a) Insulating chromatin. *Curr Biol* 4, 85-87
- Wolffe AP (1994b) The role of transcription factors, chromatin structure and DNA replication in 5S RNA gene regulation. *J Cell Sci* 107, 2055-2063
- Wolffe AP (1994c) Switched-on chromatin. *Curr Biol* 4, 525-527
- Wolffe AP (1995) Chromatin: structure and function, 2nd ed, Academic Press, London, UK
- Wolffe AP, Brown DD (1986) DNA replication in vitro erases a *Xenopus* 5S RNA gene transcription complex. *Cell* 47, 217-227
- Wolffe AP, Brown DD (1988) Developmental regulation of two 5S ribosomal RNA genes. *Science* 241, 1626-1632
- Wolffe AP, Drew HR (1989) Initiation of transcription on nucleosomal templates. *Proc Natl Acad Sci USA* 86, 9817-9821
- Wormington WM, Brown DD (1983) On set of 5S RNA gene regulation during *Xenopus* embryogenesis. *Dev Biol* 99, 248-257
- Worrad DM, Turner BM, Schultz RM (1995) Temporally restricted spatial localization of acetylated isoforms of histone H4 and RNA polymerase II in the 2-cell mouse embryo. *Development* 121, 2949-2956
- Wu M, Allis CD, Richman R, Cook RG, Gorovsky MA (1986). An intervening sequence in an unusual histone H1 gene of *Tetrahymena thermophila*. *Proc Natl Acad Sci USA* 83, 8674-8678
- Wu M, Allis CD, Gorovsky MA (1988) Cell cycle regulation as a mechanism for targeting proteins to specific DNA sequences in *Tetrahymena thermophila*. *Proc Natl Acad Sci USA* 85, 2205-2209
- Wu M, Allis CD, Sweet MT, Cook RG, Gorovsky MA (1994) Four distinct and unusual linker histones in a mitotically dividing nucleus are derived from a 71Kd polyprotein lack p34 cdc2 sites and contain protein A kinase sites. *Mol Cell Biol* 14, 10-20
- Yoshida M, Horinouoshi S, Beppu T (1995) Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *BioEssays* 17, 423-430
- Zhao K, Hart CM, Laemmli UK (1995) Visualization of chromosomal domains with boundary element-associated factor BEAF-32. *Cell* 81, 879-889