DEVELOPMENTAL GENETICS 22:31–42 (1998)

REVIEW ARTICLE

Mouse Embryos Do Not Wait for the MBT: Chromatin and RNA Polymerase Remodeling in Genome Activation at the Onset of Development

ERIC M. THOMPSON,* EDITH LEGOUY, AND JEAN-PAUL RENARD
Unité de Biologie du Développement, Institut National de la Recherche Agronomique, Jouy-en-Josas, France

ABSTRACT In *Xenopus* and *Drosophila* embryos, activation of the zygotic genome occurs after a series of rapid nuclear divisions in which DNA replication occupies most of the cell cycle. In these organisms, it has been proposed that zygotic transcription does not begin until a threshold nucleocytoplasmic ratio has been obtained in which repressive factors are titrated out and interphase becomes long enough to allow synthesis of transcripts. In mammalian embryos, however, a model of threshold nucleocytoplasmic ratios does not seem to apply, as beginning with the 1-cell stage, there are regulated cell cycles with the expression of zygotic transcripts during the cleavage period. By taking advantage of the slower kinetics at the onset of mouse development, we have characterized changes in chromatin structure and the basal transcription machinery throughout the transition from transcriptional incompetence, to minor activation of the zygotic genome during the 1-cell stage, and through major genome activation at the 2-cell stage. Further maturation of chromatin structure continues through subsequent cleavage cycles as a foundation for the first cellular differentiations in the blastocyst. The epigenetic chromatin modifications that occur during the cleavage period may have long range and inheritable effects and are undoubtably important in the ability of the mammalian oocyte to remodel previously defined nuclear structures and cell fates. Dev. Genet. 22:31–42, 1998. © 1998 Wiley-Liss, Inc.

Key words: Histone H4 acetylation; *brahma;* scaffold attachment regions; high-mobility group (HMG) proteins; transgenic mice

INTRODUCTION

Sexual reproduction involves the formation of a new functional genome from two different cells, the paternal and maternal gametes, that have evolved separately and differently up to the point of fertilization. In many animals, gametes are transcriptionally inactive at fertilization, and development is initiated under the control of maternal factors stored during oogenesis. This maternal stock is largely replaced by zygotic gene products during the transition from maternal to zygotic control of development and more than 80% of the first neosynthesized zygotic transcripts are homologous to those that were stored in the oocyte [Davidson, 1986]. The formation of the zygotic genome and the establishment of its control over the developmental process involves both the activation of transcription and the coordinated expression of specific genes in a defined chronological order.

In the life cycle of a typical eukaryotic cell, mitosis is also a period of transcriptional inactivity followed by the activation of transcription and the re-establishment of requisite patterns of gene expression. Entry into mitosis involves extensive nuclear and chromatin rearrangement; interphase chromosomes are progressively condensed into tightly packed structures, and the nuclear membrane disappears. The mature oocyte, using a similar strategy, shuts down transcription by maintaining its chromatin in the metaphase II state. Instead of delivering genetic information as a set of metaphase II chromosomes, however, the sperm arrives in a transcriptionally inert state through the packaging of DNA in specialized basic proteins, including various protamines and histone variants. During early embryonic cleavage stages, the reorganization of the nucleus towards a transcriptionally active state also involves variations on the theme of the core histone octamer and somatic linker histone H1. For example, somatic linker histone is functionally replaced by embryonic linker

*Correspondence to: Eric M. Thompson, Unité de Biologie du Développement, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas, France; E-mail: thompson@biotec.jouy.inra.fr

Received 5 September 1997; accepted 20 October 1997

TABLE 1. Timing of Major Zygotic Gene Activation in Model Organisms

Organism	Time postfertilization	No. Cleavages	No. Nuclei	Reference
Drosophila	2 hr, 10 min (25°C)	14	~6,000	Edgar *et al.* [1986]
C. elegans	2 hr, 30 min (20°C)	3–5	28	Schauer and Wood [1996]
Zebra fish	2 hr, 40 min (28°C)	10	~1,000	Kane and Kimmel [1993] Zamir and Yarden [1997]
Xenopus	7–8 hr (20°C)	11	~4,000	Newport and Kirchner [1982]
Mouse	25 hr (37°C)	1	2	Bolton *et al.* [1984]

histone B4 [Kleinschmidt *et al.*, 1985; Smith *et al.*, 1988; Dimitrov *et al.*, 1994] and the high-mobility group protein HMG1 [Nightingale *et al.*, 1996] in *Xenopus* embryos, and by an HMG1 homologue, HMG-D, in *Drosophila* [Ner and Travers, 1994]. In both organisms, somatic linker H1 becomes functionally active upon major activation of the zygotic genome (ZGA) at the mid-blastula transition (MBT). The latter two organisms have become models of choice in demonstrating the importance of chromosome and chromatin structure in the developmental regulation of gene expression. Both yield large numbers of eggs for biochemical dissection of the mechanisms by which chromatin exerts regulatory effects on early gene expression and in *Drosophila*, the large number of mutant strains available further facilitates functional analysis.

At first glance, the early mammalian embryo, with considerable constraints on the amount of experimental material available, and the scarcity of mutant strains, appears as the poor cousin to *Xenopus* and *Drosophila* in the study of chromatin and gene expression at the onset of development. Further inspection, however, using the mouse embryo as a primary example, shows fundamental differences from *Xenopus* and *Drosophila* in the unfolding of early developmental events in mammalian embryos. Instead of beginning with a rapid series of synchronous divisions, in which DNA replication occupies virtually the entire cell cycle, there are regulated cell cycles with the expression of zygotic transcripts. This expression does not take place on a background of cytoplasmic gradients, such as bicoid in *Drosophila* [Driever and Nusslein-Volhard, 1988]. There is also a much clearer chronological separation between ZGA and the first cellular differentiations; in the mouse, ZGA occurs at the 2-cell stage, with the first cellular differentiations in the blastocyst 3 days later.

The increasing availability of reagents for probing chromatin structure at the cell biological level, combined with advances in in situ molecular analyses, can partially overcome the experimental handicaps facing the mammalian embryologist. This enables exploitation of the slower kinetics of early mammalian development (Table 1) aimed toward understanding the chromatin dynamics that regulate nuclear function as a new genome is formed and becomes operational. This paper reviews data on the interplay between chromatin structure, the basal transcriptional machinery, and architectural transcription factors, in regulating gene expression at the onset of mammalian development, with emphasis on the mouse model.

ACTIVATION OF THE ZYGOTIC GENOME: A MULTISTEP PROCESS

In the mouse, fusion of the transcriptionally silent gametes at fertilization is followed by a lag period of transcriptional incompetence that lasts about 20 hr. This has been determined from time-controlled nuclear transfer experiments of transcriptionally competent nuclei into nucleated 1-cell embryo recipients [Latham *et al.*, 1992]. Transcription begins during S-phase of the first cell cycle as demonstrated by microinjection of reporter genes into pronuclei [Ram and Schultz, 1993], expression of integrated transgenes [Matsumoto *et al.*, 1994; Christians *et al.*, 1995], and incorporation of BrUTP into nascent transcripts [Bouniol *et al.*, 1995; Aoki *et al.*, 1997]. Transcription has also been detected at the 1-cell stage in other mammalian species, including the cow [Gagné *et al.*, 1993] and rabbit [Christians *et al.*, 1994]. This initial zygotic transcription is weak, and results in minor synthesis of a small set of polypeptides that manifest a transient increase restricted to the 2-cell stage [Conover *et al.*, 1991; Latham *et al.*, 1991; Christians *et al.*, 1995; Davis *et al.*, 1996]. Following this minor activation of the genome, a complete change in the pattern of protein synthesis occurs during the cleavage period, with a rapid degradation of maternal transcripts as early as the late 2-cell stage in the mouse [Van Blerkom and Brockway, 1975; Flach *et al.*, 1982; Howlett and Bolton, 1985; Taylor and Piko, 1987; Latham *et al.*, 1991], or after several divisions in other mammalian species [Telford *et al.*, 1990]. Zygotic transcription is then required for further development and the amount of neosynthesized transcripts increases 5- to 10-fold by the time the first cellular differentiations appear at the blastocyst stage [Taylor and Piko, 1987]. Thus, three transitions characterize the activa-

tion of the mammalian zygotic nucleus: (1) the acquisition of a transcriptionally permissive state during the first S phase, (2) the onset of a minor ZGA phase initiated in the 1-cell embryo and lasting over one or several cleavage cycles depending on the species, and (3) a major ZGA, that in all mammalian species studied, is coincident with the transition from maternal to zygotic control of development.

DEVELOPMENTAL REGULATION OF THE TRANSCRIPTIONAL MACHINERY

Since zygotic transcription initially depends on the time elapsed after oocyte activation, it has been proposed that a "zygotic clock" [Schultz, 1993] controls the processing of maternally inherited factors to convert sperm and oocyte chromatin into transcriptionally competent matrices accessible to transactivating factors [reviewed in Nothias *et al.*, 1995]. The molecular nature of this clock is unknown but it is proposed to regulate protein–protein interactions between three components required for transcriptional activation in vivo: activators bound to enhancer sequences, protein complexes such as the TATA-binding protein (TBP), and associated transcription activating factors (TAFs), and the functional assembly of the RNA polymerase holoenzyme bound to the site of transcription initiation [reviewed in Struhl, 1996].

A functional RNA Pol II is already present in 1-cell mouse embryos [Latham *et al.*, 1992] and can gain access to promoters in the absence of DNA replication [Aoki *et al.*, 1997]. Since transcription factors, such as SP1, and basal components of the transcriptional machinery (TBP) are detectable in pronuclei [Worrad *et al.*, 1994], transcription may be initiated rapidly after fertilization. However, the large protein complex that constitutes the transcriptional machinery can also directly influence all steps in transcription, from initiation to termination. Phosphorylation of the carboxylterminal domain (CTD) of the largest subunit of RNA Pol II is now known to be critical in regulating the initiation of transcription [Emili and Ingles, 1995; Dahmus, 1996]. The CTD contributes to interaction of the polymerase with DNA, transcription activators, TBP, and has recently been implicated in the RNA processing events that accompany transcription [McCracken *et al.*, 1997]. The nonphosphorylated form enters the preinitiation complex, and phosphorylation occurs subsequent to interaction with the promoter and before transcription initiation [Laybourn and Dahmus, 1990]. As the polymerase undergoes the cycle of initiation, elongation, and termination of transcription before reinitiating a new round of transcription, the CTD undergoes a parallel cycle of phosphorylation and dephosphorylation [Corden, 1995].

Using Western blotting and confocal microscopy with monoclonal antibodies targeted against the hyperphosphorylated form of the largest subunit of Pol II, and two isoforms of the CTD, we have shown that maternally stocked Pol II undergoes major post-translational changes in the 1-cell embryo [Bellier *et al.*, 1997]. The CTD, which is predominantly hyperphosphorylated in transcriptionally inactive unfertilized oocytes, is markedly dephosphorylated 2–3 hr after pronuclei become visible, but before the onset of minor ZGA. This was observed in both mouse and rabbit embryos. A second transition then occurred with re-phosphorylation of the large Pol II subunit. This form, however, was not phosphorylated to the same state as that normally observed after activation of the initiation complex by TFIIH-associated kinase [Dubois *et al.*, 1997], which renders the complex competent for elongation [Cismowski *et al.*, 1995; Hoejmakers *et al.*, 1996]. The presence of this embryo-specific phosphorylated form was observed transiently in the 1-cell mouse embryo but persisted to major ZGA at the 8–16 cell stage in rabbit embryos. In the latter species, the delayed timing of major ZGA allowed demonstration of a gradual nuclear translocation of Pol II spanning the entire period of minor ZGA. This nuclear translocation was independent of both the first cycle of DNA replication and of protein synthesis. In both rabbit and mouse, a phosphorylation pattern similar to that observed at later developmental stages and in somatic cells was established at the onset of major ZGA.

These observations suggest that the basal transcriptional machinery, a legacy of the transcriptionally active maturing oocyte, requires a series of modifications to become fully functional at major ZGA. The embryo-specific phosphorylated isoform observed throughout minor ZGA may provide a biochemical basis for a better understanding of how a "zygotic clock" regulates the process of gene activation [Schultz, 1993]. These post-translational modifications occur mainly in the cytoplasmic compartment of the pronuclear (mouse), or first cleavage stages (rabbit), concurrently with changes in nuclear architecture inherent in the formation of a functional zygotic nucleus. It may be that the transitions in phosphorylation and nuclear translocation of Pol II act as temporal regulators to postpone the onset of major ZGA until completion of appropriate chromatin remodeling by the embryo.

CHROMATIN REMODELING UP TO FORMATION OF THE NEW ZYGOTIC GENOME

Modulation of acetylation of the N-terminal tails of core histones has long been thought to play a role in regulating gene expression [Allfrey *et al.*, 1964]. Recently, a direct link between histone acetylation and the transcriptional process was established [Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996]. In mammalian somatic cells, histone H4 is acetylated in the order lysine 16, followed by lysine 8 or 12, and then by lysine 5 [Turner and Fellows, 1989], an order that is also observed in mouse embryonic stem cells [Keohane

et al., 1996]. Higher levels of histone acetylation are generally correlated with more transcriptionally active genetic loci [Hebbes *et al.,* 1988, 1994; Jeppesen and Turner, 1993] and acetylation of histones facilitates transcription factor binding to nucleosomal DNA [Lee *et al.,* 1993; Vettese-Dadey *et al.,* 1996]. In the 1-cell mouse embryo, it is possible to examine how histone acetylation might be involved in early chromatin remodeling during the transition from transcriptional quiescence to activation of the zygotic genome.

Both microinjection of reporter genes [Ram and Shultz, 1993; Wiekowski *et al.,* 1993] and incorporation of BrUTP [Aoki *et al.,* 1997] have shown that the male pronucleus is more transcriptionally active than the female pronucleus in 1-cell mouse embryos. When histone deacetylases are inhibited by butyrate, resulting in core histone hyperacetylation, reporter gene expression from the female pronucleus then becomes equivalent to that observed from the male pronucleus [Wiekowski *et al.,* 1993], suggesting some chromatin regulation of this differential expression. We have investigated whether differential acetylation of the separate parental genomes might underly chromatin control of these transcriptional differences.

During spermatogenesis, hyperacetylation of histone H4 relaxes nucleosomal structure [Christensen *et al.,* 1984; Grimes and Smart, 1985], leading to nucleosome disassembly and competitive protamine DNA binding [Oliva *et al.,* 1987]. In mature mouse sperm, histones have not been detected [O'Brien and Bellvé, 1980] and, using an antibody directed against histone H4 acetylated on lysine 5 (H4–L5), we were unable to detect hyperacetylated H4 in mouse sperm [Adenot *et al.,* 1997]. It has been calculated from rates of histone H4 synthesis in maturing mouse oocytes that the cytoplasmic pool of H4 is sufficient to allow 2 to 3 rounds of DNA replication [Wassarman and Mrozak, 1981], and newly synthesized histones, both in unfertilized oocytes and in zygotes, rapidly undergo post-translational modifications with part of the pool becoming mono-, di-, or triacetylated [Kaye and Wales, 1981; Kaye and Church, 1983]. Maternal metaphase II chromatin itself is packaged in nucleosomes with very low levels of H4–L5 staining [Adenot *et al.,* 1997]. Thus, prior to fertilization, the transcriptionally inert chromatin of both gametes has low to undetectable levels of hyperacetylated chromatin.

Immediately upon entry of the sperm into the oocyte, paternal chromatin appears to outcompete maternal chromatin for the pool of hyperacetylated H4 in the oocyte, as we observe that sperm chromatin becomes intensely labeled with anti H4–L5 antibodies, while maternal metaphase II chromatin remains relatively unstained. This is in sharp contrast to what is observed in activated oocytes, where, in the absence of sperm chromatin, maternal chromatin becomes markedly stained for H4–L5 [Adenot *et al.,* 1997]. The observed difference in acetylation of maternal chromatin in parthenogenetic compared to normal embryos, and the difference in paternal compared to maternal chromatin in fertilized zygotes, raises questions as to what role the early acquisition of different functional states of paternal chromatin might play in imprinting mechanisms [Solter, 1988].

Differential levels of H4 acetylation of paternal and maternal chromatin is maintained throughout most of the long (9-hr) G1 phase of the first cell cycle with the two pronuclei attaining equivalence just prior to or in the early phases of DNA replication. If DNA replication is blocked by aphidicolin, equivalence in acetylation is attained with exactly the same chronological profile as in control embryos, indicating that the observed modifications in acetylation are replication independent. Inhibition of histone deacetylase activity by trichostatin A, resulted in increased hyperacetylation in both male and female pronuclei, showing that histone acetyltransferases and deacetylases are functional during G1 of the first cell cycle and suggesting that different acetylation levels between the two pronuclei are actively maintained during this time. Paradoxically, at the points in S and G2 when global transcriptional differences have been observed between the pronuclei, the levels of acetylation in male and female pronuclei are equivalent. It has been proposed that the replacement of protamines by histones during sperm decondensation provides a window of opportunity for the binding of transcription factors to paternal DNA [Aoki *et al.,* 1997], accounting for the transcriptional differences. However, this fails to explain why female pronuclei in parthenogenetic eggs show higher levels of transcription than female pronuclei in zygotes, as in neither case is there a protamine-histone exchange on maternal chromatin. Alternatively, in the absence of the male pronucleus, the female pronucleus could accumulate higher levels of transcription factors. This would still not explain why acetylation levels of female chromatin that had already been packaged in histone octamer nucleosomes increases in parthenogenetic embryos. Instead, we propose that it is the differences in histone acetylation levels between male and female pronuclei over the duration of G1, which result in preferential recruitment of transcription factors into active chromatin configurations in the male pronucleus, leading to higher transcriptional activity in the paternal pronucleus during S/G2.

Another important question in early development is how genes are marked or selected to become active. Jeppesen [1997] recently proposed that patterns of histone acetylation could be a mechanism for maintaining cell memory through mitosis. Nucleosomal acetylation states are transmitted to newly assembled chromatin after replication [Perry *et al.,* 1993], and hyperacetylated regions of chromosomes at metaphase appear in the same chromatin domains during interphase [Jeppesen and Turner, 1993; Surrallés *et al.,* 1996]. While 10–20% of the cellular content of the

general transcription factor TFIID remains attached to mitotic chromosomes [Segil *et al.*, 1996], many transcription factors are dissociated from chromatin [Martinez-Balbas *et al.*, 1995], though some, such as AP-2 and the serum response factor, p67 srf, do remain attached. Thus, TFIID could serve as a basal marker for transcriptional activation that could be reamplified by subsequent attachment of other transcription factors as the nucleus reforms, or histone acetylation could signal regions of chromatin which were available for transcription in the previous cycle and need to be reactivated in G1. The two mechanisms, which are not mutually exclusive, could act synergistically.

Our results in preimplantation mouse development support the idea that histone acetylation may be involved in cell memory but suggest added requirements. At the beginning of the first cell cycle, neither paternal nor maternal chromatin are marked with patterns of histone H4 hyperacetylation. However, by the first mitosis, chromosomes showed banding patterns of histone H4 hyperacetylation, suggesting that regions of the genome are already marked before major ZGA at the 2-cell stage. The question remains though as to how information is transferred from the transcriptionally inactive and unmarked gametic chromatin to the banding patterns observed on chromosomes at syngamy. Are these messages sequence encoded, or do some of the proteins involved in chromatin remodeling in the early zygote provide the link to the distinct patterns of H4 acetylation observed during the first cell cycle?

MATURATION OF CHROMATIN DURING AND AFTER MAJOR ZYGOTIC GENOME ACTIVATION

When 1-cell mouse embryos are microinjected with episomal DNA templates, and cleavage is arrested by blocking DNA replication with aphidicolin, cDNAs are expressed equally well from templates with and without enhancers [Martinez-Salas *et al.*, 1989]. When the same microinjection experiments are performed at the 2-cell stage, enhancers are required to relieve expression from weak promoters. Injection of various constructs at the 2-cell stage also revealed a certain permissiveness for transcription, suggesting that the early embryo contains trans-activating elements to express genes that it normally does not transcribe [Bonnerot *et al.*, 1991]. On the other hand, when embryos are microinjected at the 1-cell stage and traverse the first mitosis, there is an irreversible repression of gene activity that cannot be alleviated by an enhancer [Martinez-Salas *et al.*, 1989]. Incubation with butyrate increases the activity of weak promoters injected into 2-cell embryos, but is unable to restore activity to promoters alone, or promoters coupled to enhancers, which have been injected into 1-cell embryos that then complete the first mitosis [Wiekowski *et al.*, 1993]. It was concluded from these experiments that the repression of expression from episomal templates probably reflected repressive changes in chromatin structure during the earliest cleavage phases.

To explore this hypothesis in native embryonic chromatin, we developed an integrated transgenic model in which the murine hsp70.1 promoter directed luciferase expression from constructs that were flanked with scaffold attachment regions (SAR+) or remained unflanked (SAR−) [Thompson *et al.*, 1995a,b]. The endogenous hsp70.1 gene is expressed constitutively during G1 of the 2-cell stage, repressed to basal levels during S-phase of the same cell cycle, and can be thermally induced at the blastocyst stage [Christians *et al.*, 1995]. SARs are AT-rich DNA sequences which bind to nuclear matrix preparations and frequently map to or near the domain boundaries of gene loci. Developmentally regulated loci flanked by SAR sequences include *Adh*, *ftz*, and *Sgs-4* in *Drosophila* [Gasser and Laemmli, 1986] and the β-globin locus in humans [Jarman and Higgs, 1988]. SARs may be involved in mediating chromatin accessibility through synergistic action with enhancers [Forrester *et al.*, 1994] or via interaction with histone H1 (closed conformation) or proteins such as the high mobility group protein HMG-I/Y, capable of displacing histone H1 from AT-tracts (open conformation) [Zhao *et al.*, 1993].

SAR− transgenic lines reproduced the preimplantation expression profile of the hsp70.1 endogene but SAR+ lines showed significantly higher expression levels at the 2-cell stage than SAR− lines and continued to express luciferase above basal levels through to the 4-cell stage before finally being repressed at the 8-cell stage. Upon induction of the transgenes by heat shock at the blastocyst stage, SAR+ lines showed a per copy expression 11-fold higher than SAR− lines. At both the 2-cell and blastocyst stages, copy number dependent expression was observed in SAR+ lines but not in SAR− lines.

When core histones were hyperacetylated by incubating embryos in the reversible histone deacetylase inhibitor, trichostatin A, neither type of transgenic line showed any increase in expression at the 2-cell stage. At the 4-cell stage, both SAR+ and SAR− lines averaged a 25-fold increase in expression in response to core histone hyperacetylation. At the 8-cell stage the response to hyperacetylation differed; while SAR− lines dropped back to a 10-fold response, SAR+ lines exhibited a 160-fold increase in transgene expression. This differential response to hyperacetylation persisted to the blastocyst stage with a 30-fold response in SAR+ lines compared to a 3-fold response in SAR− lines.

The differential expression profiles of SAR+ and SAR− lines, and their differential response to histone hyperacetylation suggest that, during the preimplantation period, the hsp70.1 gene is regulated by changes in chromatin structure rather than through alterations in the concentration of hsp70.1-related transcription factors. This was tested by microinjection of hsp70.1

luciferase plasmids at the 1-, 2-, and 4-cell stages. The resulting expression from the transient templates showed that hsp70.1-related transcription factors were never limiting.

The stimulatory effect of SARs on preimplantation gene expression was very well correlated with the presence of HMG-I/Y in embryonic nuclei. HMG-I/Y is an architectural transcription factor with an affinity for AT-rich DNA, cruciform DNA [Zeleznik-Le *et al.*, 1994] and non-B-form DNA [Nissen and Reeves, 1995]. HMG-I/Y can bind preferentially to certain AT sequences on the surface of nucleosomes [Reeves and Nissen, 1993] and can induce localized changes in rotational setting and helical periodicity of DNA on the surface of nucleosomes [Reeves and Wolffe, 1996]. HMG-I/Y alters DNA bending at the human interferon-β (IFN-β) enhancer [Falvo *et al.*, 1995] and interacts synergistically with NF-κβ and activating transcription factor 2 to stimulate induction of interferon-β transcription [Thanos and Maniatis, 1992; Du *et al.*, 1993]. In the preimplantation mouse embryo, HMG-I/Y transcripts are stocked in oocytes and the protein shows a diminishing concentration of well-defined foci in the nucleus from the 1-cell stage through to the 8-cell stage. Zygotic HMG-I/Y transcripts are expressed at the blastocyst stage, and the protein is located in a large number of foci throughout the nuclei. Whenever SAR+ lines showed increased expression over SAR− lines, HMG-I/Y was present in embryonic nuclei, and when hyperacetylation relieved repression of SAR+ expression at the 8-cell stage, the nuclear concentration of HMG-I/Y went from virtually undetectable to significant amounts.

Assembling the microinjection, gene expression, and immunofluorescence data, we propose the following maturation of chromatin structure in the regulation of hsp70.1-luciferase transgene expression. At the 2-cell stage, nucleosomes are positioned or acetylated such that key promoter elements, probably the Sp1 sites [Metzger *et al.*, 1994], are accessible to transcription factors. The presence of HMG-I/Y in 2-cell nuclei, and its affinity for AT-rich sequences of SAR, stimulates luciferase expression in SAR+ lines. Cleavage to the 4-cell stage is accompanied by local changes in nucleosome positioning and/or degree of acetylation which reduce transcription factor access to the promoter. Somatic histone H1 becomes detectable in the nuclei of 4-cell embryos [Clarke *et al.*, 1992] at the same time as HMG-I/Y is decreasing in concentration, with the result that SAR+ expression decreases, but does not attain, the basal levels of SAR− lines. At the 4-cell stage, hyperacetylation of core histones, allows recovery of an open chromatin conformation and permits per copy expression levels, that in the case of the SAR+ lines, exceed those in the 2-cell embryo. At the 8-cell stage, HMG-I/Y is present at very low levels in the nucleus and histone H1 completes the organization of a chromatin structure which reduces all transgenic lines to basal levels of expression. Hyperacetylation of core histones increases nuclear HMG-I/Y content, as well as transgene expression, most notably in SAR+ lines, but this is no longer sufficient to obtain the constitutive per copy levels observed at the 2-cell stage. Constitutive expression remains low in blastocysts but can again be somewhat increased by histone hyperacetylation. Though HMG-I/Y is present in normal blastocyst nuclei, the chromatin conformation of the transgene after passage through the 8-cell stage is unfavourable for interactions with SAR sequences. Upon heat shock of blastocysts, the heat shock factor (HSF) complex is phosphorylated and binds HSE elements in the promoter. In SAR+ lines, initial opening of the locus by the HSF complex allows cooperative interactions of SAR with HMG-I/Y to create a more extended open chromatin conformation resulting in increased expression levels.

More global modification of chromatin and nuclear structure are suggested by immunofluorescence studies of acetylated isoforms of histone H4 during the 2- to 8-cell transition [Thompson *et al.*, 1995b]. At the 2- and 4-cell stages, treatment with trichostatin A resulted in a strong increase in histone acetylation at the nuclear periphery but not in the nuclear interior. This may suggest that the majority of nuclear histone acetyltransferase activities are confined to the nuclear periphery during these stages. At the 8-cell stage, trichostatin A treatment still increased acetylated H4 staining most strongly at the nuclear periphery, but staining also increased significantly in the nuclear interior, while in blastocysts, the same treatment no longer strongly increased staining at the periphery and contrast with staining in the nuclear interior was further reduced. In *Xenopus* embryos, inhibition of histone deacetylases is unable to cause accumulation of hyperacetylated histone H4 in early cleavage stages until after major ZGA at the MBT [Dimitrov *et al.*, 1993; Almouzni *et al.*, 1994]. In the mouse, we observed a capability to hyperacetylate histone H4 at the 1-cell stage prior to minor ZGA [Adenot *et al.*, 1997] and demonstrated a locus-specific transcriptional effect as early as the 4-cell stage [Thompson *et al.*, 1995b]. These results suggest that the ability to modulate core histone acetylation levels may be an important prerequisite to a regulated program of zygotic gene expression.

MOLECULAR MACHINES FOR REMODELING EMBRYONIC CHROMATIN

Packaging DNA into heterochromatin-like structures has been described as epigenetic regulation of gene expression during development. In *Drosophila*, the silencing of segmentation and homeotic genes required to maintain segment identity is ensured by the polycomb-group proteins (Pc-G). The polycomb protein (Pc), along with the heterochromatin protein HP1, share a chromodomain that is critical for silencing. Repression of transcription has been proposed to occur by packag-

ing of large chromosomal domains into structures inaccessible to the transcriptional machinery [reviewed in Shaffer *et al.*, 1993; Orlando and Paro, 1995; Pirrotta, 1997]. Human and murine homologues to HP1 [Pearce *et al.*, 1992; Saunders *et al.*, 1993] and Pc-G proteins [reviewed in Schumacher and Magnuson, 1997] suggest conservation of chromatin remodeling mechanisms among different species. In *Drosophila*, chromodomain containing proteins are present early in development, before cellularization of the blastocyst [Kellum *et al.*, 1995]. In mammals, only *eed*, a Pc-G gene, has been found to be expressed during preimplantation development [Schumacher *et al.*, 1996], but other murine homologues of the *Drosophila* Pc-G family are expressed before the regulation of Hox genes is established, suggesting recruitment for chromatin packaging earlier in development [Pearce *et al.*, 1992; van der Lugt *et al.*, 1996].

Analysis in *Drosophila* of mutations that sustain homeotic gene expression led to the identification of the trithorax-group genes (trx-G) which are antagonistic to Pc-G [reviewed in Kennison, 1993]. The *brahma* gene, a member of trx-G, was of particular interest because of its strong homology with the yeast global activator *SWI2/SNF2* [Tamkum *et al.*, 1992]. The yeast protein is part of a large multiprotein complex that destabilizes DNA–histone interactions in vitro and increases the binding of transcription factors. These activities require the DNA-dependent ATPase domain of the SWI2/SNF2 protein. Combining these results with genetic studies showing that the requirement for the SWI/SNF protein is abolished in cells with mutations in genes coding for histones, led to the proposal that the SWI/SNF complex induces chromatin remodeling that promotes transcriptional activation [reviewed in Carlson and Laurent, 1994; Peterson and Tamkum, 1995; Kingston *et al.*, 1996]. These properties also characterize the recently purified human SWI/SNF complex [Kwon *et al.*, 1994; Imbalzano *et al.*, 1994], which contains the *brahma* and SWI2 homologues, *hbrm* [Muchardt and Yaniv, 1993], and *BRG1* [Khavari *et al.*, 1993]. Both human proteins are functional in heteropolymeric complexes [Wang *et al.*, 1996a] in which the protein composition varies with cell type [Wang *et al.*, 1996b]. The modular nature of these protein-protein interactions in a developmental context might allow the embryo to create a wide repertoire of regulators for a large number of promoters using a limited number of proteins.

After partial cloning of the murine homologues of the *hbrm* and *BRG1* genes, we examined expression of the murine genes at the onset of development, using reverse transcription-polymerase chain reaction (RT-PCR). Specific antibodies, raised against the divergent N-terminal regions of these otherwise highly homologous proteins, were used to explore the cellular localization of the proteins during the preimplantation period (E. Legouy *et al.*, in press). Transcripts of the two genes are stored as maternal mRNAs in mature oocytes and are maintained in 1-cell and 2-cell embryos. Considerable differences were found in the relative levels of the two transcripts following major ZGA and this was confirmed at the protein level. The mBRG1 protein remained consistently abundant throughout preimplantation development whereas levels of the mbrm protein dropped drastically at the 4-cell stage. Zygotic expression of mbrm then reappeared at the blastocyst stage. Thus far, both genes had been found to be expressed in the same differentiated tissues or cell lines, but our results on their differential expression in early embryos, and the fact that despite having common protein partners, they have not been found in the same complexes [Wang *et al.*, 1996b], suggest distinct roles for the two proteins.

The presence of the two proteins in mature oocytes, and in pronuclei of 1-cell embryos argues for the requirement of both proteins for transcription as early as minor ZGA. Storage of maternal brahma protein has also been described in *Drosophila* and absence of the maternal protein has severe effects on early gastrulation, probably because of down-regulation of segmentation genes. On the other hand, the zygotically expressed *Drosophila* brahma protein only seems to be required later in development, suggesting different targets for the maternal and zygotic products [Brizuella *et al.*, 1994]. This indicates that *brahma* is probably not an essential general activator of transcription in *Drosophila*, as recently suggested by Wilson *et al.*, [1996], where it was found that the yeast SWI/SNF complex was associated with the RNA Pol II holoenzyme. The ubiquitous expression of mBRG1 at the onset of mouse development is consistent with such a proposal, but recent results in the human argue against colocalization of the human SWI/SNF complex with RNA pol II [Reyes *et al.*, 1997].

brahma is the only closely related homologue of *SWI2*, which has been described in *Drosophila*, but *ISWI* [Elfring *et al.*, 1994], another member of the *SWI2* family, has been identified as a component of three chromatin remodeling complexes, NURF [Tsukiyama and Wu, 1995], CHRAC [Varga-Weisz *et al.*, 1997], and ACF [Ito *et al.*, 1997]. As observed for *brahma*, the ISWI protein is abundant during early development in *Drosophila* when chromatin modifications are occurring in the absence of transcription [Elfring *et al.*, 1994]. In vitro, the CHRAC and ACF complexes have been shown to participate in chromatin assembly, suggesting that in addition to nucleosome remodeling during the activation of transcription, the ATPase activity of ISWI is required for other nuclear activities over the course of the cell cycle. In mammals, an *ISWI* homologue has been identified [Okabe *et al.*, 1992], but its participation in chromatin remodeling complexes has yet to be characterized. It will be of considerable interest to study the role of such complexes in the chromatin transitions leading up to minor and major zygotic genome activation in mammalian embryos.

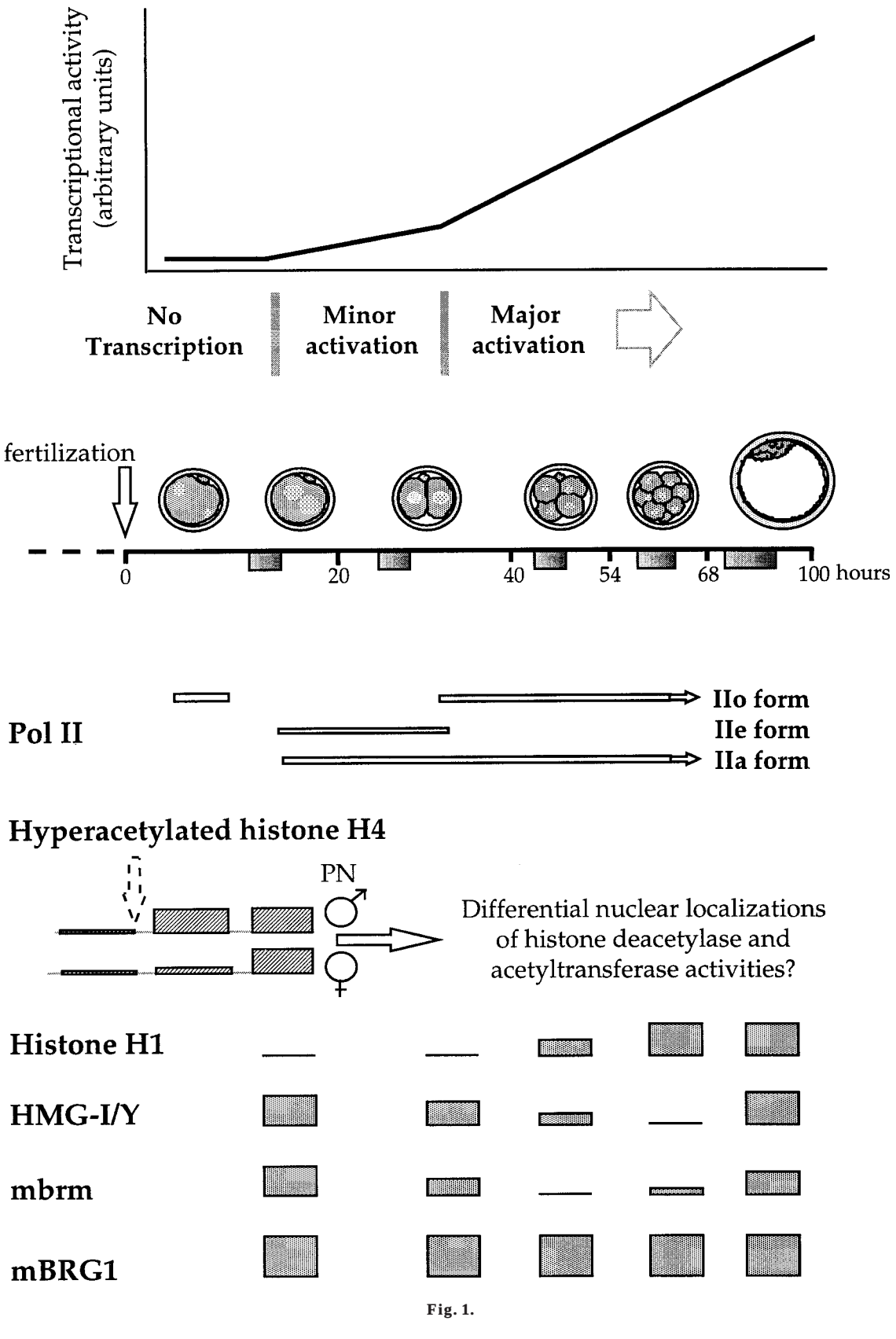
Transcriptional activity
(arbitrary units)
No
Transcription
Minor
activation
Major
activation
fertilization
0
20
40
54
68
100 hours
Pol II
IIo form
IIe form
IIa form
Hyperacetylated histone H4
PN
Differential nuclear localizations
of histone deacetylase and
acetyltransferase activities?
Histone H1
HMG-I/Y
mbrm
mBRG1

Fig. 1.

CONCLUSIONS

It was first proposed in *Xenopus* embryos that activation of the zygotic genome was dependent on the titration of a maternally stocked factor(s) that combined stoichiometrically with DNA and became limiting when about 4,000 nuclei had formed [Newport and Kirschner, 1982]. Subsequently, Edgar *et al.* [1986] suggested that in *Drosophila* embryos, the onset of zygotic transcription might depend on a lengthening of the cell cycle in order to allow sufficient time for transcripts to be synthesized. More recently, Pritchard and Schubiger [1996] put forward the idea that "initial activation of zygotic transcription in embryos is a generic noninformational event, with genes initiating transcription at a low and variable level." In their model, transcription would begin in a few nuclei where the nucleocytoplasmic ratio attained a threshold level such that titration of maternal repressors such as tramtrack (*Ttk*) would allow low level expression of certain genes.

The summary of events (Fig. 1) that occurs during activation of the zygotic genome at the onset of mouse development indicates that this model does not apply very well to the mammalian embryo. Achieving an appropriate nucleocytoplasmic dilution of maternal inhibitors would not appear to be the mechanism by which the zygotic genome begins to activate transcription as early as S-phase in 1-cell pronuclei. Instead, we find time-dependent changes in specific phosphorylated isoforms of RNA polymerase II and in the acetylation status of histone H4 in the octamer core of nucleosomes leading up to minor and major activation of the genome. While it is clear that activation of the genome will involve a far greater complexity of events than those outlined here, the data suggest that regulation of the competence of the basal transcriptional machinery and the establishment of transcriptionally functional states of chromatin will be essential controlling elements in this process. The idea, however, that very restricted temporal and spatial control of early gene expression are not essential for correct development of the animal [Pritchard and Schubiger, 1996] may also be true of the preimplantation mouse embryo. In microinjection experiments, mouse embryos are permissive for expression from a wide variety of templates that they normally do not express [Bonnerot *et al.*, 1991], and altered levels of expression of some genes, in response to different culture conditions does not preclude successful completion of development to term [Vernet *et al.*, 1993; Christians *et al.*, 1995]. In fact, as shown in Figure 1, a progressive maturation of chromatin continues through and after major ZGA. Chromatin remodeling proteins such as HMG-I/Y and mbrm, stocked as maternal products, are down-regulated through to the 8-cell stage before reappearing as zygotic products toward the blastocyst stage. Simultaneously, somatic linker histone H1 increases in concentration through to the 8-cell stage. In the mouse embryo, there is also a major genome-wide demethylation that occurs between the 8-cell stage and the blastocyst [Monk *et al.*, 1987]. Since methylation is usually associated with reduced levels of gene expression, higher levels of methylation during the earliest cleavage phases may reduce 'noninformational generic expression' during genome activation until a minimally mature chromatin structure is attained at the 8-cell stage. It may be significant that both demethylated DNA and the establishment of this basic chromatin structure are in place before the first cellular differentiations occur at the blastocyst stage.

The importance of understanding how chromatin is remodeled at the onset of mammalian development is not restricted to a better comprehension of how the embryo progresses through the preimplantation period. Recent evidence suggests that the chromatin remodeling that occurs shortly after fertilization may exert long lasting and inheritable epigenetic effects which only appear in adults [Roemer *et al.*, 1997]. The capacity of the mammalian oocyte to remodel somatic nuclei, as shown in the cloning of a sheep from an adult somatic cell [Wilmut *et al.*, 1997], also opens up new and interesting perspectives in defining how the reorganization of chromatin states may be involved in defining totipotent or restricted cell fates.

Fig. 1. Timing of events during the activation of transcription in the preimplantation mouse embryo. All events are relative to the time scale post fertilization indicated underneath the sketched representations of embryos from the 1-cell pronuclear stage up to the blastocyst. The time scale is not linear. Shaded bars directly beneath the time scale indicate periods of DNA replication. Changes in rates of transcription are shown schematically in the graph at the top. Modulation of the phosphorylation of RNA polymerase II into the IIo, IIa, and embryonic IIe isoforms, is shown by open bars and arrowheads. Differential hyperacetylation of histone H4 in male and female pronuclei (PN) is represented by hatched bars during the first cell cycle. After major activation of the zygotic genome, different patterns of H4 acetylation, particularly in response to inhibition of histone deacetylases, might reflect different localizations of histone deacetylase and acetyltransferase activities in the nucleus. The relative nuclear abundance of histone H1, HMG-I/Y, mbrm, and mBRG1 is represented by shaded boxes. The relative differences shown are schematic and are not meant to be scaled to true quantitative differences. The representation for histone H1 is based on nuclear immunofluorescence data from Clarke *et al.* [1992].

ACKNOWLEDGMENTS

We thank Pierre Adenot, Sylvie Chastant, and Evelyne Campion, from our laboratory, for their contributions to the experimental data presented in this article.

REFERENCES

Adenot PG, Mercier Y, Renard JP, Thompson EM (1997): Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of one-cell mouse embryos. Development 124:4615–4625.

Allfrey VG, Faulkner R, Mirsky AE (1964): Acetylation and methyl-

ation of histones and their possible role in the regulation of RNA synthesis. Proc Natl Acad Sci USA 51:786–794.

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.

Aoki F, Worrad DM, Schultz RM (1997): Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. Dev Biol 181:296–307.

Bellier S, Chastant S, Adenot P, Vincent M, Renard JP, Bensaude O (1997): Nuclear translocation and carboxy-terminal domain phosphorylation of RNA polymerase II delineate the two phases of zygotic gene activation in mammalian embryos. EMBO J 16:6250–6262.

Bolton VN, Oades PJ, Johnson MH (1984): The relationship between cleavage, DNA replication and gene expression in the mouse 2-cell embryo. J Embryol Exp Morphol 79:139–163.

Bonnerot C, Vernet M, Grimber G, Briand P, Nicolas JF (1991): Transcriptional selectivity in early mouse embryos: A qualitative study. Nucleic Acids Res 19:7251–7257.

Bouniol C, Nguyen E, Debey P (1995): Endogenous transcription occurs at the 1-cell stage in the mouse embryo. Exp Cell Res 218:57–62.

Brizuela BJ, Elfring L, Ballard J, Tamkum JW, Kennison JA (1994): Genetic analysis of the *brahma* gene of *Drosophila* melanogaster and polytene chromosome subdivision *72AB*. Genetics 137:803–813.

Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD (1996): *Tetrahymena* histone acetyltransferase A: A homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84:843–851.

Carlson M, Laurent BC (1994): The SNF/SWI family of global transcriptional activators. Curr Opin Gen Dev 6:396–402.

Christensen ME, Rattner JB, Dixon GH (1984): Hyperacetylation of histone H4 promotes chromatin decondensation prior to histone replacement by protamines during spermatogenesis in rainbow trout. Nucleic Acids Res 12:4575–4592.

Christians E, Rao VH, Renard JP (1994): Sequential acquisition of transcriptional control during early embryonic development in the rabbit. Dev Biol 164:160–172.

Christians E, Campion E, Thompson EM, Renard JP (1995): Expression of the *HSP70*.1 gene, a landmark of early zygotic activity in the mouse embryo, is restricted to the first burst of transcription. Development 121:113–122.

Cismowski MJ, Laff GM, Solomon MJ, Reed SI (1995): KIN28 encodes a C-terminal domain kinase that controls mRNA transcription in *Saccharomyces cerevisiae* but lacks cyclin-dependent kinase-activating kinase (CAK) activity. Mol Cell Biol 15:2983–2992.

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.

Conover J, Temeles GL, Zimmermann JW, Burke B, Schultz RM (1991): Stage-specific expression of a family of proteins that are major products of zygotic gene activation in the mouse embryo. Dev Biol 114:392–404.

Corden JL (1995): RNA polymerase II transcription cycles. Curr Opin Genet Dev 3:213–218.

Dahmus ME (1996): Reversible phosphorylation of the C-terminal domain of RNA polymerase II. J Biol Chem 271:19009–19012.

Davidson EH (1986): The nature and function of maternal transcripts. In Davidson EH (ed): "Gene Activity in Early Development, 3rd Edition." London: Academic Press.

Davis W, De Sousa PA, Schultz RM (1996): Transient expression of translational initiation factor e1F-4C during the 2-cell stage of the preimplantation mouse embryo: Identification by mRNA differential display and the role of DNA replication in zygotic gene activation. Dev Biol 174:190–201.

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.

Driever W, Nusslein-Volhard C (1988): The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell 54:95–104.

Du W, Thanos D, Maniatis T (1993): Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. Cell 74:887–898.

Dubois MF, Vincent M, Adamcewski J, Egly JM, Bensaude O (1997): Heat shock inactivation of the TFIIH-associated kinase and change in the phosphorylation sites on RNA polymerase II largest subunit. Nucleic Acids Res 25:694–700.

Edgar BA, Kiehle CP, Schubiger G (1986): Cell cycle control by the nucleo-cytoplasmic ratio in early *Drosophila* development. Cell 44:365–372.

Elfring LK, Deuring R, McCallum CM, Peterson CL, Tamkum JW (1994): Identification and characterisation of *Drosophila* relatives of the yeast transcriptional activator *SNF2/SWI2*. Mol Cell Biol 14:2225–2234.

Emili A, Ingles CJ (1995): The RNA polymerase II carboxy-terminal domain links to a bigger and better "holoenzyme"? Curr Opin Genet Dev 5:204–209.

Falvo JV, Thanos D, Maniatis T (1995): Reversal of intrinsic DNA bends in the IFNβ gene enhancer by transcription factors and the architectural protein HMG I(Y). Cell 83:1101–1111.

Flach G, Johnson MH, Braude PR, Taylor RAS, Bolton VN (1982): The transition from maternal to embryonic control in the 2-cell mouse embryo. EMBO J 1:681–686.

Forrester WC, van Genderen C, Jenuwein T, Grosschedl R (1994): Dependence of enhancer-mediated transcription of the immunoglobulin μ gene on nuclear matrix attachment regions. Science 265:1221–1225.

Gagné M, Pothier F, Sirard MA (1993): Foreign gene expression in activated oocytes and bovine embryos following pAGS-lacZ plasmid microinjection. Theriogenology 39:223.

Gasser M, Laemmli UK (1986): Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster*. Cell 46:521–530.

Grimes SR, Smart PG (1985): Changes in the structural organization of chromatin during spermatogenesis in the rat. Biochim Biophys Acta 824:128–139.

Hebbes TR, Thorne AW, Crane-Robinson C (1988): A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7:1395–1402.

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.

Hoeijmakers JHJ, Egly JM, Vermeulen W (1996): TFIIH: a key component in multiple DNA transactions. Curr Opin Genet Dev 6:26–33.

Howlett SK, Bolton VN (1985): Sequence and regulation of morphological and molecular events during the first cell cycle of mouse embryogenesis. J Embryol Exp Morphol 87:175–206.

Imbalzano AN, Kwon H, Green MR, Kingston RE (1994): Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature 370:481–485.

Ito T, Bulger M, Pazin MJ, Kobayashi R, Kadonaga JT (1997): ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell 90:145–155.

Jarman AP, Higgs DR (1988): Nuclear scaffold attachment sites in the human globin gene complexes. EMBO J 7:3337–3344.

Jeppesen P (1997): Histone acetylation: A possible mechanism for the inheritance of cell memory at mitosis. BioEssays 19:67–74.

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 74:281–289.

Kane DA, Kimmel B (1993): The zebrafish midblastula transition. Development 119:447–456.

Kaye PL, Wales RG (1981): Histones synthesis in preimplantation mouse embryos. J Exp Zool 216:453–459.

Kaye PL, Church RB (1983): Uncoordinated synthesis of histones and DNA by mouse eggs and preimplantation embryos. J Exp Zool 226:231–237.

Kellum R, Raff JW, Alberts BM (1995): Heterochromatin protein 1 distribution during development and during the cell cycle in *Drosophila* embryos. J Cell Sci 108:1407–1418.

Kennison JA (1993): Transcriptional activation of *Drosophila* homeotic genes from distant regulatory elements. Trends Genet 9:75–79.

Keohane AM, O'Neill LP, Belyaev ND, Lavender JS, Turner BM (1996): X-inactivation and histone H4 acetylation in embryonic stem cells. Dev Biol 180:618–630.

Khavari PA, Peterson CL, Tamkum JW, Mendel DB, Crabtree GR (1993): BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. Nature 366:171–174.

Kingston RE, Bunker CA, Imbalzano AN (1996): Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev 10:905–920.

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.

Kwon H, Imbalzano AN, Khavari PA, Kingston RE, Green MR (1994): Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. Nature 370:477–479.

Latham KE, Garrels JI, Chang C, Solter D (1991): Quantitative analysis of protein synthesis in mouse embryos. 1. Extensive reprogramming at the one and two cell stages. Development 112:921–932.

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.

Laybourn PJ, Dahmus ME (1990): Phosphorylation of RNA polymerase IIA occurs subsequent to interaction with the promoter and before the initiation of transcription. J Biol Chem 265:13165–13173.

Lee DY, Hayes JJ, Pruss D, Wolffe AP (1993): A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell 72:73–84.

Legouy E, Thompson EM, Muchardt C, Renard JP (1998): Differential preimplantation regulation of two mouse homologues of the yeast SWI2 protein. Dev Dyn (in press).

Martinez-Balbas A, Dey A, Rabindran SK, Ozato K, Wu C (1995): Displacement of sequence-specific transcription factors from mitotic chromatin. Cell 83:29–38.

Martinez-Salas E, Linney E, Hassall J, DePamphilis 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.

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.

McCracken S, Fong N, Yankulov K, Ballantyne S, Pan G, Greenblatt J, Patterson SD, Wickens M, Bentley DL (1997): The C-terminal domain of RNA polymerase II couples mRNA processing to transcription. Nature 385:357–361.

Metzger V, Renard JP, Christians E, Morange M (1994): Detection of heat shock element-binding activities by gel shift assay during mouse preimplantation development. Dev Biol 165:627–638.

Mizzen CA, Yang XJ, Kokubo T, Brownell JE, Bannister AJ, Owen-Hughes T, Workman J, Wang L, Berger SL, Kouzarides T, Nakatani Y, Allis CD (1996): The $TAF_{II}250$ subunit of TFIID has histone acetyltransferase activity. Cell 87:1261–1270.

Monk M, Boubelik M, Lehnert S (1987): Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development 99:371–382.

Muchardt C, Yaniv M (1993): A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12: 4279–4290.

Ner SS, Travers AA (1994): HMG-D, the *Drosophila melanogaster* homologue of HMG 1 protein, is associated with early embryonic chromatin in the absence of histone H1. EMBO J 13:1817–1822.

Newport J, Kirschner M (1982): A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. Cell 30:687–696.

Nightingale K, Dimitrov S, Reeves R, Wolffe AP (1996): Evidence for a shared structural role for HMG1 and linker histones B4 and H1 in organizing chromatin. EMBO J 15:548–561.

Nissen MS, Reeves R (1995): Changes in superhelicity are introduced into closed circular DNA by binding of high mobility group protein I/Y. J Biol Chem 270:4355–4360.

Nothias JY, Majumder S, Kaneko KJ, DePamphilis ML (1995): Regulation of gene expression at the beginning of mammalian development. J Biol Chem 270:22077–22080.

O'Brien DA, Bellvé AR (1980): Protein constituents of the mouse spermatozoon. I. an electrophoretic characterization. Dev Biol 75: 386–404.

Ogryzko VV, Schlitz RL, Russanova V, Howard BH, Nakatani Y (1996): The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953–959.

Okabe I, Bailey LC, Attree O, Srinivasan S, Perkel JM, Laurent BC, Carlson M, Nelson DL, Nussbaum RL (1992): Cloning of human and bovine homologs of SNF2/SWI2: A global activator of transcription in yeast *S. cerevisiae*. Nucleic Acids Res 17:4649–4655.

Oliva R, Bazett-Jones D, Mezquit C, Dixon GH (1987): Factors affecting nucleosome disassembly by protamines in vitro. Histone hyperacetylation and chromatin structure, time dependence, and the size of the sperm nuclear proteins. J Biol Chem 262:17016–17025.

Orlando V, Paro R (1995): Chromatin multiprotein complexes involved in the maintenance of transcription patterns. Curr Opin Genet Dev 5:174–179.

Pearce JH, Singh PB, Gaunt SJ (1992): The mouse has a Polycomb-like chromobox. Development 114:921–929.

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.

Peterson CL, Tamkum JW (1995): The SWI–SNF complex: A chromatin remodeling machine? Trends Biochem Sci. 20:143–146.

Pirrota V (1997): PcG complexes and chromatin silencing. Curr Opin Genet Dev 7:249–258.

Pritchard DP, Schubiger G (1996): Activation of transcription in *Drosophila* embryos is a gradual process mediated by the nucleocytoplasmic ratio. Genes Dev 10:1131–1142.

Ram PT, Schultz RM (1993): Reporter gene expression in G2 of the 1-cell mouse embryo. Dev Biol 156:552–556.

Reeves R, Nissen MS (1993): Interaction of high mobility group-I(Y) nonhistone proteins with nucleosome core particles. J Biol Chem 268:21137–21146.

Reeves R, Wolffe AP (1996): Substrate structure influences binding of the non-histone protein HMG-I(Y) to free and nucleosomal DNA. Biochemistry 35:5063–5074.

Reyes JC, Muchardt C, Yaniv M (1997): Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. J Cell Biol 137:263–274.

Roemer I, Reik W, Dean W, Klose J (1997): Epigenetic inheritance in the mouse. Curr Biol 7:277–280.

Saunders WS, Chue C, Goebl M, Craig C, Clark RF, Powers JA, Eissenberg JC, Elgin SCR, Rothfield NF, Earnshaw WC (1993): Molecular cloning of a human homologue of *Drosophila* heterochromatin protein HP1 using anti-centromere autoantibodies with anti-chromo specificity. J Cell Sci 104:573–582.

Schauer IE, Wood WB (1990): Early C. elegans embryos are transcriptionally active. Development 110:1303–1317.

Schultz RM (1993): Regulation of zygotic gene activation in the mouse. BioEssays 15:531–538.

Schumacher A, Magnuson T (1997): Murine *polycomb*- and *trithorax*-group genes regulate homeotic pathways and beyond. Trends Genet 13:167–170.

Schumacher A, Faust C, Magnuson T (1996): Positional cloning of a

global regulator of anterior-posterior patterning in mice. Nature 383:250–253.

Segil N, Guermah M, Hoffmann A, Roeder RG, Heintz N (1996): Mitotic regulation of TFIID: Inhibition of activator-dependent transcription and changes in subcellular localization. Genes Dev 10:2389–2400.

Shaffer CD, Wallrath LL, Elgin SCR (1993): Regulation by packaging domains: Bits of heterochromatin in euchromatin? Trends Genet 9:35–38.

Smith RC, Dworkin-Rastl E, Dworkin MD (1988): Expression of a histone H1-like protein is restricted to early *Xenopus* development. Genes Dev 2:1284–1295.

Solter D (1988): Differential imprinting and expression of maternal and paternal genomes. Annu Rev Genet 22:127–146.

Struhl K (1996): Chromatin structure and RNA polymerase II connection: Implications for transcription. Cell 84:179–182.

Surrallés J, Jeppesen P, Morrison H, Natarajan AT (1996): Analysis of loss of inactive X chromosomes in interphase cells. Am J Hum Genet 59:1091–1096.

Tamkum JW, Deuring R, Scott MP, Kissinger M, Pattatuci AM, Kaufman TC, Kennison JA (1992): *brahma:* A regulator of *Drosophila* homeotic genes structuraly related to the yeast transcriptional activator SNF2/SWI2. Cell 68:561–572.

Taylor KD, Piko L (1987): Patterns of mRNA prevalence and expression of B1 and B2 transcripts in early mouse embryo. Development 101:877–892.

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.

Thanos D, Maniatis T (1992): The high mobility group protein HMG I(Y) is required for NF-κβ-dependent virus induction of the human IFN-β gene. Cell 71:777–789.

Thompson EM, Adenot P, Tsuji FI, Renard JP (1995a): Real time imaging of transcriptional activity in live mouse preimplantation embryos using a secreted luciferase. Proc Natl Acad Sci USA 92:1317–1321.

Thompson EM, Legouy E, Christians E, Renard JP (1995b): Progressive maturation of chromatin structure regulates HSP70.1 gene expression in the preimplantation mouse embryo. Development 121:3425–3437.

Tsukiyama T, Wu C (1995): Purification and properties of an ATP-dependant nucleosome remodeling factor. Cell 83:1011–1020.

Turner BM, Fellows G (1989): Specific antibodies reveal ordered and cell-cycle-related use of histone-H4 acetylation sites in mammalian cells. Eur J Biochem 179:131–139.

Van Blerkom J, Brockway GO (1975): Qualitative pattern of protein synthesis in the preimplantation mouse embryo. Dev Biol 44:148–157.

van der Lugt NMT, Alkema M, Berns A, Deschamps J (1996): The polycomb group homolog *Bmi-1* is a regulator of murine *Hox* gene expression. Mech Dev 58:153–164.

Varga-Weisz PD, Wilm M, Bonte E, Dumas K, Mann M, Becker PB (1997): Chromatin remodeling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature 388:598–602.

Vernet M, Cavard C, Zider A, Fergelot P, Grimber G, Briand P (1993): In vitro manipulation of early mouse embryos induces HIV1-LTRlacz transgene expression. Development 119:1293–1300.

Vettese-Dadey M, Grant PA, Hebbes TR, Crane-Robinson C, Allis CD, Workman JL (1996): Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J 15:2508–2518.

Wang W, *et al.* (1996a): Purification and biochemical heterogeneity of the mammalian SWI/SNF complex. EMBO J 15:5370–5382.

Wang W, Xue Y, Zhou S, Kuo A, Cairns BR, Crabtree GR (1996b): Diversity and specialisation of mammalian SWI/SNF complexes. Genes Dev 10:2117–2130.

Wassarman PM, Mrozak SC (1981): Program of early development in the mammal: synthesis and intracellular migration of histone H4 during oogenesis in the mouse. Dev Biol 84:364–371.

Wiekowski M, Miranda M, DePamphilis ML (1993): Requirements for promoter activity in mouse oocytes and embryos distinguish paternal pronuclei from maternal and zygotic nuclei. Dev Biol 159:366–378.

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997): Viable offspring derived from fetal and adult mammalian cells. Nature 385:810–813.

Wilson CJ, Chao DM, Imbalzano AN, Schnitzler GR, Kingston RE, Young RA (1996): RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. Cell 84:235–244.

Worrad DM, Ram PT, Schultz RM (1994): Regulation of gene expression in the mouse oocyte and early preimplantation embryo: developmental changes in Sp1 and TATA box-binding protein, TBP. Development 120:2347–2357.

Zamir E, Kam Z, Yarden A (1997): Transcription-dependant induction of G1 phase during the zebra fish midblastula transition. Mol Cell Biol 17:529–536.

Zeleznik-Le NJ, Harden AM, Rowley JD (1994): 11q23 translocations split the "AT-hook" cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene. Proc Natl Acad Sci USA 91:10610–10614.

Zhao K, Käs E, Gonzalez E, Laemmli UK (1993): SAR-dependent mobilization of histone H1 by HMG-I/Y in vitro: HMG-I/Y is enriched in H1-depleted chromatin. EMBO J 12:3237–3247.