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EMBRYONIC GENOME ACTIVATION

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1. ABSTRACT

Genome activation is one of the first critical events in the life of the new organism. Both the timing of genome activation and the array of genes activated must be controlled correctly. Genome activation occurs in a stepwise manner, with some genes being transcribed well in advance of the major genome activation event, in which most housekeeping genes become activated. Changes in chromatin protein content, particularly histone proteins, and chromatin structure appear to regulate the availability of the genome for transcription and provide for specificity of transcription. Gene enhancers are not initially required for transcription, but become necessary as the chromatin structure is modified. Changes in transcription factor content or activity are also required, and protein synthesis is essential for genome activation during both early and later phases of transcriptional activation. Both the changes in chromatin structure and availability of transcription factors are regulated by cell cycle-dependent mechanisms, thus providing the necessary coordination between these processes and other processes such as DNA replication and cleavage.

2. INTRODUCTION

Oocytes come in many different shapes, sizes, and compositions. Despite this variety, all oocytes have as their primary purpose the uniting of the two parental genomes into a new embryonic genome, followed by the appropriate initiation of the developmental program driven by that newly formed embryonic genome. Indeed, the remarkable successes observed recently in cloning by somatic cell nuclear transfer (1-4) are attributable to the inherent and specific capacity of the oocyte to transform differentiated nuclei (either gamete-derived or somatic cell-derived) into embryonic nuclei and program them appropriately. This truly remarkable power of the oocyte derives wholly from an as yet poorly understood array of maternally supplied nucleic acids, proteins, and other macromolecules deposited in the oocyte during oogenesis. These maternally supplied factors are responsible for supporting embryonic metabolism, may direct crucial early developmental events such as axis formation and cell fate determination, and govern the process of genome activation by entirely post-transcriptional mechanisms. This last process, i.e., genome activation, is of paramount importance, because failure to regulate correctly the timing of activation and the types of genes expressed can lead to developmental arrest.

Recent studies have provided important insights into the molecular mechanisms that likely regulate genome activation in mammals. The available data indicate that genome activation occurs in a stepwise manner and that multiple mechanisms and levels of control are employed by the embryo to govern this crucial process. This review will focus on the role of nuclear proteins and chromatin structure in controlling the availability of the DNA as a transcriptional template, the role of stage-specific changes in transcription factors in controlling the transcriptional capacity of the early embryo, and the role of the cell cycle in coordinating chromatin changes and transcription factor content to provide for overall temporal control of genome activation.

3. THE STEPWISE NATURE OF GENOME ACTIVATION

Our understanding of the timing of genome activation in a number of mammalian species has changed dramatically in recent years. For example, it was formerly believed that genome activation in the mouse occurred during the 2-cell stage, with a minor activation event at the early 2-cell stage and a major activation event at the late 2-cell stage (5-7). This view stemmed largely from available data from protein gel analyses. These studies revealed a small class of proteins exhibiting transiently induced synthesis at the early-mid 2-cell stage, and a much larger number of proteins being induced at the late 2-cell stage, along with the de novo synthesis of many housekeeping mRNAs (8-25).

Early studies employing metabolic labeling to detect pol(A)$^+$ and poly(A)$^-$ RNA synthesis revealed increased transcription at the 2-cell stage, but also indicated possible transcription during the 1-cell stage (26). Subsequently, nuclear transplantation studies revealed that the 1-cell stage cytoplasm undergoes an important transition, so that the early 1-cell stage cytoplasm does not support transcription within exogenous nuclei, whereas the late 1-cell stage cytoplasm is permissive for transcription in such nuclei. Results of these studies suggested that transcription might occur before the 2-cell stage (27). Thereafter, a number of studies employing transgenic mice revealed that specific transgenes indeed are transcribed at the late 1-cell stage, and other studies revealed transcription of endogenous genes at the late 1-cell stage (28-35). Br-UTP incorporation by embryonic nuclei in permeabilized embryos reveals that the amount of gene transcription occurring during the late 1-cell stage is about 30-40% of that observed for 2-cell embryos in G2 (28). More recently, it was observed that the late 1-cell stage supports the transcription of some 2-cell stage-specific genes in transplanted 2-cell stage nuclei (*U2afbp-rs*, *ERV-L*, and the 70 kDa TRC complex), but not others (*Alberich*, *eIf-1A*) (Wang, Chung, & Latham unpublished). These studies thus reinforce the view that a subset of genes are activated at the late 1-cell stage, and then many more genes are activated at the 2-cell stage. Many other genes, including numerous housekeeping genes, appear to be transcribed at low levels during the 2-cell and 4-cell stage, and then are upregulated dramatically during the 8-cell stage (9, 36).

These studies collectively reveal that genome activation in the mouse is not a single event, but rather occurs in a stepwise manner, with at least four periods of major gene induction, corresponding to the late 1-cell stage, early 2-cell stage, late 2-cell stage, and 8-cell stage. For other species, including the rabbit, cow, rhesus macaque, and human, for which the major genome activation event occurs around the 4-8 cell stage, recent studies have also revealed a limited degree of gene transcription at earlier stages, confirming a stepwise pattern of genome activation for a variety of mammalian species (37-45). The discovery that genome activation occurs in a stepwise manner has important implications for considering the possible mechanisms that may regulate the process. Specifically, any mechanism to account for the control of genome activation must provide not only temporal control, but also control over the array of genes activated at each stage, and also must encompass multiple transitions that span several cleavage divisions, rather than comprising just a single global transcriptional switch. Thus, the overall process of genome activation is likely to be complex, with multiple mechanisms directing the activation of specific subsets of genes over time.

4. CHANGES IN HISTONE CONTENT ACCOMPANYING GENOME ACTIVATION

4.1. Changes in Histone Protein Expression

One of the key parameters governing what genes are available for transcription and when those genes become available is the temporal control of chromatin structure. A major determinant of chromatin structure is the type of histones associated with the DNA (review, 46). Core histones, which package the DNA into nucleosomes, include histones 2A, 2B, 3, and 4. Other linker histones, including a number of variants of histone H1 and specific developmental forms, such as B4 and HMG1 in *Xenopus*, associate with the DNA in between the nucleosomes and are responsible for condensing the chromatin. The tight association of DNA with core histones and the linker histones can render the DNA effectively inaccessible to transcription factors. The positioning of nucleosomes along the DNA strand can be altered by other DNA binding proteins, and this positioning can in turn affect the ability of other proteins to bind. From a transcriptionally silent, condensed chromatin state, however, the association of DNA-binding proteins with specific sequences likely requires an initial relaxation of the DNA association with histones. During development, different linker histones associate with the chromatin of oocytes, early embryos, and later somatic cells. These differences in linker histones affect chromatin structure because the different linker histones differ in their overall basicity and tightness of association with the DNA. The binding of core histones to DNA can be modulated by phosphorylation and acetylation. Of these, acetylation of the lysine residues in the histone tails reduces the contact of the DNA with the core histones and provides access to the DNA by other DNA binding proteins (review, 47).

A number of recent studies have documented dramatic stage-specific changes in the expression of specific histone isoforms as well as changes in post-translational modification, most notably histone acetylation. One important transition observed in many organisms, including sea urchins and frogs, is a switch from expression of oocyte-specific or cleavage stage-specific forms of histone H1 to expression of somatic forms (48, 49). In *Xenopus*, somatic cell nuclei transplanted to oocytes undergo histone H1 replacement, with the somatic variants H1 and H1(0) being replaced with the oocyte-spcific linker histones B4 and HMG1. The H1(0) form is released preferentially (50). A molecular chaperone, nucleoplasmin, has an important role in mediating the active removal of the somatic type linker histones (50). Along with the removal of somatic type linker histones, other ooplasmic factors, such as the chromatin-remodeling

nucleosomal ATPase ISWI, actively participate in the removal of other DNA associated proteins, e.g., transcription factors (51). Collectively, these observations in the *Xenopus* system indicate that oogenesis is likely associated with an active remodeling of chromatin to establish the chromatin state characteristic of the oocyte, that the factors involved in this remodeling persist for a period of time in the oocyte and early embryo, and that a normal part of post-fertilzation development must include the eventual reversal of this process, so that the oocyte-specific chromatin state is converted to an embryonic chromatin state.

The mouse also expresses an early maternally-encoded, oocyte-specific form of histone H1, which declines in expression during development from the 4-cell to 8-cell stage (52). Another form, histone H1^o is also expressed in the oocyte and early embryo. The somatic form of histone H1 was reported to appear first at the 4-cell stage (53). More recently, the somatic form has been reported to be expressed in 2-cell stage embryos (54) and even in 1-cell stage embryos (55). In the latter study, the somatic form was not observed associated with the metaphase II spindle, but was associated with the two pronuclei. Histone H1 synthesis in the 1-cell mouse embryo is transcription independent and apparently driven by maternally encoded mRNA (56). Histone H1 synthesis declines during the 2-cell stage and then recovers during the 4-cell stage (56), consistent with a transition from expression of maternally encoded histone H1 transcripts to expression of embryonic transcripts, with an enhanced prevalence of the somatic form.

Microinjection of the somatic form of histone H1 into early embryos leads to assembly of the injected protein into chromatin (57). Transplantation of nuclei containing the somatic H1 form into early mouse embryos can result in the transfer of the somatic H1 form to maternal chromatin (55). Similarly, bovine somatic type H1 is undetectable in early embryos until the 8-cell stage, and transplantation of somatic H1 containing morula stage bovine embryo nuclei into ooplasts leads to loss of the somatic H1 from the transplanted nuclei (58). Thus, just as in *Xenopus*, the mammalian ooplasm directs the removal of the somatic form from transplanted nuclei, and the maternal metaphase II chromatin and maternal and paternal pronuclei likely possess less of the somatic form than later stage nuclei.

The reason for this developmental difference in histone H1 isoform expression has not been established. One possibility is that the presence of the somatic form in the early embryo might disrupt the normal pattern of gene expression. Alternatively, the transition to greater expression of the somatic form might be associated with establishing a transcriptionally repressive state during the 2-cell stage. Earlier studies have revealed that a transition occurs during the 2-cell stage to make transcriptional enhancers necessary for efficient transcription (59, 60). This may confer upon the embryo the ability to regulate gene transcription correctly and be responsible for the downregulation of transiently-induced genes that are activated at the early 2-cell stage (reviewed, 61). However, microinjection of the somatic form into early embryos does not disrupt development (57), or expression of the transiently induced 70 kDa Transcription Requiring Complex genes, which is indicative of early genome activation (54). This indicates that the assembly of somatic H1 into early embryonic chromatin probably does not prevent expression of genes required for developmental progression, and is unlikely to be responsible, at least by itself, for the initiation of the transcriptionally repressive state and downregulation of transiently activated genes. An increase in incorporation of somatic H1 into embryonic chromatin during the 4-cell or 8-cell stage, however, may support a transcriptional program at the 8-cell stage that leads to the more somatic epithelial cell phenotype characteristic of compacted embryos. Expression of somatic H1 in the developing oocyte could adversely affect oogenesis, or might permit the deposition of gene products in the oocyte that may be detrimental during the period preceding the major genome activation event. Thus, the transition in histone H1 isoform expression in the early embryo may reflect requirements of the oocyte rather than specific function in the early embryo, and experimentally induced precocious expression of the somatic form may be inconsequential during the period of relative transcriptional silence.

Other changes in histone protein expression occur at earlier stages. Synthesis of histone H2A and H3 is up-regulated between the 1-cell and 2-cell stages and is partially inhibited by alpha-amanitin treatment at both stages (56). Synthesis of histone H4 is transcription independent during these stages (56). Thus, whereas maternal transcripts appear to be wholly responsible for expression of H1 and H4 during the 1-cell and 2-cell stages, some expression of H2A and H3 from the embryonic genome may occur, and this may contribute to chromatin changes related to genome activation during these stages. It should also be noted that the overall regulation of histone protein synthesis in early embryos differs dramatically from regulation in somatic cells. In somatic cells, histone synthesis is temporally regulated and coupled to S phase by transient transcription followed by translation and subsequent mRNA degradation. By contrast, histone synthesis in the 1-cell embryo is largely uncoupled from S phase and is regulated at the level of maternal mRNA recruitment, with the exception of some expression of embryonic histone H2A and H3 genes. This reliance on maternally encoded mRNAs provides the necessary mechanism to support histone transitions during the early period of transcriptional silence.

4.2. Changes in Histone Acetylation

Changes in the post-translational modifications of histones are very striking during early cleavage stages. The core histone H4 exhibits especially notable changes in acetylation. Although stored in a diacetylated form in *Xenopus*, H4 becomes deacetylated upon assembly into chromatin after fertilization (49). The mouse 1-cell embryo also contains a supply of diacetylated histone H4 (acetylated on lysines 5 and 12), but it has not been determined whether this diacetylated form is the replication form and incorporated into chromatin. An early

incorporation of diacetylated histone H4 followed by deacetylation may help establish or maintain a transcriptionally repressed state. An increase in acetylation is seen for histone H4 during the period leading to genome activation (34, 62, 63). In addition, increased acetylation is seen for histones 2A and 3 (62). Interestingly, an enrichment for acetylated histones H2A, H3, and H4 is observed at the nuclear periphery specifically at the 2-cell stage (34, 62, 63). The unusual staining pattern can be enhanced with trichostatin A, or inhibited with aphidicolin, but is not affected by alpha-amanitin or cytochalasin D (34, 62). DNA replication appears to be facilitated by increased histone acetylation, as BrdU labeling reveals enhanced replication at the nuclear periphery that can be accelerated by inhibitors of histone deacetylase (28). These observations indicate that DNA replication facilitates the creation of the specialized domain of enhanced histone acetylation at the nuclear periphery, and that the enhanced degree of histone acetylation in turn enhances DNA replication. This functional link between a domain of increased histone deacetylation and DNA replication at the 2-cell stage provides a potentially important mechanism by which DNA replication and the widespread activation of gene transcription may be coordinated (see below).

5. ASPECTS OF PRONUCLEAR STRUCTURE THAT MAY AFFECT TRANSCRIPTION

5.1. Differences Between Maternal and Paternal Pronuclei

Recent studies have revealed striking differences between maternal and paternal pronuclei with respect to genome activation. Labeling mouse embryos with Br-UTP to detect nascent transcripts *in situ* reveals that the paternal pronucleus incorporates about 3-4-fold more Br-UTP than the maternal pronucleus (28). Moreover, the maternal pronuclei in parthenogenetic embryos incorporate an amount of Br-UTP that is intermediate between and equal to the sum of label incorporated into the maternal and paternal pronuclei of normal fertilized embryos (28). Other studies have revealed that microinjection of reporter constructs into the two pronuclei results in a much greater rate of transcription following paternal pronuclear injection than maternal pronuclear injection (64). In addition, the level of histone acetylation also differs between the pronuclei (65). Immediately after fertilization, hyperacetylated H4 was associated with paternal chromatin, but not maternal chromatin. This difference persists in pronuclei throughout G1, but is lost by S/G2. It is suggested that the initial difference may affect both histone-protamine exchange and contribute later to the differential transcriptional activity of the two pronuclei (65). Nuclear transplantation studies reveal that the higher level of gene transcription in paternal pronuclei is not due to an exclusion of repressive factors, but likely is the result of an inherent property of maternal pronuclear chromatin structure (64). The enhanced rate of transcription observed in the paternal pronucleus declines following DNA replication and passage to the 2-cell stage, but this repression can be relieved by treatment with sodium butyrate to increase histone acetylation (64, 66). These observations indicate that there may be limiting quantities of essential transcription factors in the fertilized oocyte, that the paternal pronucleus possesses a competitive advantage over the maternal pronucleus for accumulating these factors and subsequently activating transcription, and that DNA replication and passage to the 2-cell stage repress transcription on paternally derived chromatin as the transcriptionally repressive state becomes established. Interestingly, not all genes possess an inherently more active chromatin structure when paternally inherited. Studies have revealed that for at least one imprinted gene, H19, a heterochromatic chromatin structure is inherited in the embryo via the sperm (67). The initial difference between maternal and paternal pronuclear transcription may have additional relevance to genomic imprinting, because such differences in gene transcription may be accompanied by differences in specific protein–DNA associations, which in turn may affect changes in DNA methylation, or differences in the timing of gene replication during S phase, which may also affect parental allele expression (review, 68, 69).

Maternal and paternal pronuclei also exhibit differences in DNA methylation. During the first 9-10 hours after fertilization, the alpha-actin and *Igf2* genes, and the TKZ751 transgene all exhibit a dramatic reduction in DNA methylation in the paternal pronucleus (70). The TKZ751 transgene does not become demethylated in the maternal pronucleus and may even become more heavily methylated (70, 71). The demethylation of these genes in the paternal pronucleus appears to be the result of an active mechanism, because it occurs before DNA replication and also in embryos inhibited from undergoing DNA replication by aphidicolin treatment (71). Thus, the paternal pronucleus contains an active demethylase that is absent from the maternal pronucleus, the paternal pronucleus possesses a factor that attracts the demethylase, or the paternal chromatin is more accessible to this activity. Previous studies with the RSVIgmyc transgene indicated that the demethylation activity observed in mouse 1-cell embryos is apparently sensitive to DNA sequence and extent of DNA methylation (72). These observations demonstrate the potential for differential epigenetic modification of parental genomes post-fertilization that may affect gene transcription at later stages. Consistent with this, earlier studies revealed that ooplasmic factors modify the paternal genome in a strain-dependent manner and thus affect paternal genome function at later stages (73-75).

5.2. Chromatin Structure of Sperm and Possible Relationship to the Paternal Pronucleus

The dramatic differences between paternal and maternal pronuclear gene transcription may be explained on the basis of initial differences in chromatin structure. The two pronuclei differ with regard to their need for chromatin re-packaging. The paternal genome undergoes a much greater degree of nuclear swelling than the maternal genome, and unlike the maternal genome, the paternal genome must be stripped of sperm chromatin packaging proteins, i.e., protamines, and re-packaged with histones. Moreover, the sperm chromatin is packaged in a highly organized manner, consisting of specific DNA loop domains, with the loops attached to a nuclear matrix (76-

79). Within the sperm head, specific chromosomes may occupy specific positions, and this may have implications for chromatin decondensation during such procedures as intracellular sperm injection (80, 81). Specifically, the sex chromosomes were observed to occupy positions within the apical region of the human sperm head, and to exhibit delayed decondensation after ICSI into hamster eggs (81). Telomeres are selectively associated with the sperm nuclear periphery (82). Exogenous DNA can also occupy a specific spatial association with the sperm nuclear matrix (83).

Sperm can be treated with the ionic detergent alkyltrimethylammonium bromide (ATAB), which removes the acrosomal contents and perinuclear theca, but leaves the nuclear envelope intact, and dithithreitol (DTT), which disrupts the nuclear matrix. Microinjection of such treated sperm nuclei into metaphases eggs that are subsequently activated reveals that such disruption of the association of the sperm DNA with the nuclear matrix destroys the developmental potential of the paternal chromatin, as indicated by the ability of the embryos to develop to term (78, 84). Omitting protease inhibitors during the detergent treatment also destroys developmental potential (84). In contrast, detergent treatment with protease inhibitor but without DTT only partially reduces developmental potential (78, 84). These observations indicate that some aspect of sperm chromatin structure is critical for embryogenesis. One intriguing possibility to account for this is that specific genes are made available for early transcription as a consequence of sperm chromatin organization. Thus, particular genes in the sperm may be associated with particular DNA-binding proteins, which in turn could affect which DNA sequences are associated with the nuclear matrix versus the loop domains. These associations may in turn determine which sequences in the paternal genome have immediate access to maternally supplied transcription factors, which could allow them take on a more open chromatin configuration as the paternal chromatin is re-packaged.

Other studies have revealed that sperm chromatin is organized into nuclease hypersensitive domains (85) and that the sensitivity of sperm DNA to nuclease attack differs markedly from that of somatic cell nuclei (86). *In vitro* incubation of epididymal sperm and exposure to stress conditions can activate endogenous nucleases, leading to reduced fertilizing ability, and this functional loss can be inhibited with nuclease inhibitors (87). Exposure to exogenous DNA is also associated with nuclease activation (88). The ability of exogenous DNA to incorporate into sperm DNA has been taken as evidence that some sites of sperm DNA may not be uniformly and tightly packaged with protamines (85). These observations thus raise the interesting possibility that some of the enhanced transcriptional capacity of the paternal pronucleus may be attributable to unique properties and structural features of the sperm nucleus, and perhaps factors associated with regions lacking tight associations with protamines. Such sites in the sperm chromatin may also serve as possible entry sites for other proteins to associate with the DNA, disrupt chromatin structure, and initiate such processes as DNA replication, gene transcription or transcriptional repression, and transcriptional reprogramming. These observations also raise the interesting possibility that disruption of the nuclear matrix may diminish developmental potential by exposing to nucleolytic attack DNA domains that would otherwise be protected by chromatin structure and association with the nuclear matrix. These observations also indicate a potentially critical role for early DNA repair activities, which may be especially required within the paternal pronucleus.

6. REGULATION OF TRANSCRIPTION FACTOR CONTENT

The preceding sections discussed important changes in the chromatin structure of the early embryo that are likely to play important roles in genome activation. Nevertheless, following nuclear transfer the ability of transcriptionally competent nuclei (that presumably have undergone these changes in chromatin structure) to undergo transcription depends on the developmental stage of the recipient cytoplasm (27). This indicates that changes in chromatin structure probably constitute only part of the overall regulatory mechanism governing genome activation. An equally important role in genome activation is likely to be played by changes in the availability, content, or activity of transcription factors.

The availability of the appropriate RNA polymerase activity is of obvious importance to transcriptional activation. RNA polymerasse II exhibits a number of differentially phosphorylated forms, including the IIA form, which is hypophosphorylated in the carboxy terminal domain (CTD) and essential for initiation, and the IIO form, which is hyperphosphorylated in the carboxy terminal domain and essential for transcript elongation for most genes. In mouse, rabbit and *Xenopus*, the CTD becomes hyperphosphorylated during oocyte maturation, becomes hypophosphorylated soon after fertilization, and then exhibits an increase in phosphorylation during the transition to the 2-cell stage (89, 90). The latter change in phosphorylation is associated with an abrupt increase in nuclear localization (89). Thus, changes in the phosphorylation status of RNA polymerase II may be critical for genome activation.

Other transcription factors are also expressed as maternally derived proteins in the early embryo. These include factors that support the transcription of a wide array of genes, such as Sp1, CBP, TBP (91, 92) and other more specialized factors, including a transcription inhibitory protein, *Maid* (93). Changes in phosphorylation status are observed for some of these proteins. For example, Sp1 DNA binding activity is decreased by phosphorylaton in a number of systems (94-99). Sp1 is phosphorylated, and thus presumably inactivated by phosphorylation upon oocyte maturation, but an increase in hypophosphorylated Sp1 is observed between G1 and G2 of the 1-cell stage (91), and this pool of Sp1 may become activated later. Oocytes and early embryos express an E1A-like activity (100). Other transcription factors for which maternal mRNAs have been detected in oocytes and early embryos

include *B-myb* and *Max* (101, 102). In addition, mRNAs encoding other nuclear proteins that may affect transcription have been detected in the oocyte or early embyro, including those encoding DNA methyltransferase 1 and the HP-1 like chromobox protien *M31* (103, 104).

In addition to the maternally inherited supply of transcription factors, available data indicate that a combination of continuous synthesis of these factors utilizing maternal mRNAs, and stage-specific *de novo* expression of other transcription factors following stage-specific maternal mRNA recruitment is responsible for providing the embryo with the entire array of transcription factors needed at the time of genome activation. In mouse embryos, protein synthesis may play a role in providing an adequate supply of RNA polymerase activity. As discussed above, the hypophosphorylated IIA form of RNA polymerase II associates with preinitiation complexes, while the hyperphosphorylated IIO form is essential for transcriptional elongation (105). The IIO form, but not the IIA, is present during the early 1-cell stage, and both forms exist in the late 1-cell stage embryo (89). Cycloheximide treatment reduces the amount of the IIO form, but not the IIA form, at the late 1-cell stage (89). Cycloheximide treatment does not appear to have a major effect on either the amount of IIA form present or on the normal translocation of RNA polymerase to the nucleus at the 2-cell stage (89). Thus, protein synthesis may be required for the expression of an essential protein kinase to generate the IIO form.

Cycloheximide treatment also produces a 4-fold decrease in nuclear staining for TBP and a 30% reduction in nuclear staining for Sp1 (106). During normal development increases in Sp1 and TBP content and activity are observed during the 1-cell stage (59, 106). Data obtained from microinjection of reporter constructs reveal that a transcriptional co-activator appears during development to the 2-cell stage in the mouse, and this is associated with the ability of transcriptional enhancers to overcome gene repression that is established during the same period (107). In addition, the transcription factor *mTEAD2*, which appears to be wholly responsible for the TEF-1-like activity that appears at the 2-cell stage, is encoded entirely by maternally derived transcripts that are translationally recruited during the 2-cell stage (92, 108).

Other changes in transcription factor content may underlie a striking shift in promoter utilization. The mouse eIF-1A gene, which is strongly induced during the 2-cell stage but then declines somewhat in expression thereafter, uses both a TATA-containing and TATA-less promoter. About 70% of the transcripts in the fully grown oocyte are derived from the proximal TATA-containing promoter (109). Following genome activation there is a switch in promoter utilization, such that the TATA-less promoter is more efficiently used, and by the blastocyst stage only about 5% of the transcripts are derived from the TATA-containing promoter. This change in promoter utilization is also observed for plasmid-borne reporter genes; the TATA box is not required for promoter activity or enhancer-mediated activation of transcription in early mouse embryos or ES cells, but is required for efficient expression in the oocyte (110). This shift in promoter utilization may reflect a difference in transcription factor content that permits the oocyte to express its unique array of transcripts, as well as to promote the expression of the battery of genes that accompanies genome activation, e.g., housekeeping genes that are typically TATA-less.

Protein synthesis appears to be critical for at least two phases of embryonic genome activation. Cycloheximide treatment beginning near the end of the 1-cell stage and continuing into the 2-cell stage prevents the major genome activation event, so that all of the housekeeping mRNAs analyzed remain expressed at a level equivalent to what is observed in alpha-amanitin treated embryos (111). More recently, it was found that cycloheximide treatment from the early to the late 1-cell stage prevents the ability of 2-cell stage donor nuclei to direct the expression of 2-cell stage-specific transcripts following transfer to late 1-cell stage cytoplasm, indicating that protein synthesis may be essential for acquisition of the transcriptionally permissive state during the 1-cell stage (Wang, Chung, Latham, unpublished).

These observations collectively indicate that protein synthesis (a) is required to maintain the normal level of expression of some transcription factors like RNA polymerase II, Sp1 and TBP that are also expressed maternally, (b) is required for the stage-specific appearance of other specific factors such as *mTEAD2* or kinases that modify transcription factors, and (c) is essential for genome activation to occur. A requirement for protein synthesis in the process of genome activation provides a useful mechanism for contributing to the control of the timing of genome activation. Stage-specific recruitment of maternally inherited mRNAs can provide for time-dependent appearance of key transcription factors, or for the stage specific appearance of kinases or phosphatases that may regulate transcription factor activities. The stage-specific recruitment of maternal mRNAs could in turn be governed by the phosphorylation of mRNA binding proteins and mRNA polyadenylation factors, which can be under the control of cell cycle proteins such as *cdc2*-dependent kinases (112). Evidence in favor of such a mechanism was obtained from studies in which 1-cell stage mouse embryos were treated with cordycepin to inhibit the poly(A) tail elongation that is associated with maternal mRNA recruitment. Cordycepin treatment during the first 10 hours of the 1-cell stage greatly inhibited the transcription-dependent incorporation of Br-UTP during the subsequent 4 hour period (Aoki and Schultz, unpublished observations). As a control to exclude a possible direct effect on gene transcription rather than mRNA polyadenylation, embryos treated with 3-deoxyguanosine exhibited no reduction in Br-UTP incorporation. These data indicate that stage-specific maternal mRNA translational recruitment *per se*, as opposed to ongoing mRNA translation, plays an important role in transcriptional activation during the 1-cell stage.

7. ROLE OF THE CELL CYCLE IN CONTROLLING NUCLEAR AND CYTOPLASMIC EVENTS CONTRIBUTING TO GENOME ACTIVATION

A role for both changes in chromatin structure and stage-specific synthesis of transcription factors utilizing mRNAs in the overall control of genome

activation may require that the nuclear events, involving chromatin reorganization and histone acetylation, and the cytoplasmic events, related to protein synthesis and post-translational transcription factor activation, be coordinated with each other. One could argue that such coordination is not necessarily needed, so that the timing of genome activation is governed solely by cytoplasmic or nuclear events, but not both. In this case, one set of events would establish the potential for activation, and the other set of events would permit overt activation. Under such a scheme, however, the embryo could be at risk for incomplete or incorrect genome activation. For example, if genome activation were governed solely by nuclear events, a lack of control of cytoplasmic production of essential transcription factors might lead to precocious production followed by premature degradation via normal protein turnover, and hence an insufficiency of such factors. Conversely, strictly cytoplasmic controls might create a potential for activating an inappropriate array of genes if activating transcription factors are produced before nuclear reprogramming and establishment of the transcriptionally repressive state compatible with accurate gene regulation. It seems reasonable, therefore, to propose that the coordination of nuclear and cytoplasmic events would provide the most precise manner of control over the specificity and timing of genome activation.

Progression through the cell cycle could provide an ideal means of coordinating nuclear and cytoplasmic events. Progression through S phase, for example, could provide a means of regulating changes in chromatin structure, while cell cycle dependent kinases could regulate the activities of key transcription factors and mRNA masking, polyadenylation, or translation factors, thereby affecting the translational recruitment of maternal mRNAs. The available data indeed indicate that progression through the cell cycle can indeed affect genome activation.

Treatment of 1-cell stage mouse embryos with aphidicolin, an inhibitor of DNA replicative polymerases, reduces gene transcription, as revealed through Br-UTP incorporation (28). Aphidicolin treatment also inhibits expression of the eIF-1A gene (31). These results indicate that the first round of DNA replication may facilitate genome activation. Other studies have revealed that the expression of reporter constructs injected into pronuclei can be reduced by progression through the S and/or G2 phase of the first cell cycle (33, 60, 64, 113), and that treatment with aphidicolin during the 1-cell stage prevents this repression. These apparently disparate results can be reconciled with a model in which the first round of DNA replication permits chromatin remodeling that is both necessary for transcription of some genes, and that also establishes a transcriptionally repressive state in which gene enhancers and the factors that bind to them become necessary for transcription. Consistent with a role of chromatin structure in the repression of some injected reporter genes, treatment with sodium butyrate to enhance histone acetylation alleviates the repression (64, 66). In some transgene constructs, enhancer elements are able to provide long-range stimulation of transcription in 1-cell embryos only after completion of the first S phase, and achieve this effect most likely by acting as a locus control region to affect local chromatin structure (114).

The second round of DNA replication also affects gene transcription. A number of genes exhibit transient bursts of transcription during the 2-cell stage in the mouse embryo. Aphidicolin treatment during the second S phase prevents the normal down-regulation of these genes after their induction (31) and results in an elevated rate of gene transcription overall during the 2-cell stage (28). These observations indicate that the second round of DNA replication is also associated with transcriptional repression. Thus, the first round of DNA replication is necessary for gene transcription and also represents the first step in creating a transcriptionally repressive state, and the second round of DNA replication completes the transition to a transcriptionally repressive state.

The repressive effects of the first two rounds of DNA replication may involve a combination of incorporation of deacetylated histones, altered association of DNA with the nuclear matrix, histone-protamine exchange and other specific changes in the paternal chromatin, and incorporation of somatic type histone H1. Although these changes are transcriptionally repressive in nature, they should be viewed as essential components of genome activation, because they are likely to provide the ability to regulate correctly the array of genes that are transcribed. The transcriptionally repressive state may thus sculpt the newly generated gene expression profile to one compatible with further development. Genome activation appears a relatively opportunistic process likely due to extensive chromatin remodeling; genes that are expressed may simply be those for which the necessary transcription factors are present and for which the promoter is accessible. While genes with strong promoters and/or enhancers would be preferentially expressed, many other genes may opportunistically be expressed (especially at basal levels of transcription) during this transition. The development of a transcriptionally repressive state could preferentially reduce the expression of these genes, but permit the continued expression of genes regulated by strong promoters/enhancers and critical for continued development.

Given the apparent need to establish the ability to regulate gene transcription, it is reasonable to expect that cytoplasmic events that would promote gene transcription, such as the recruitment of maternal mRNAs encoding transcription factors, would be coordinated with progression through S phase. Indeed, it has not been determined whether the negative effect of aphidicolin treatment during the 1-cell stage on transcription reflects a failure to alter the chromatin structure to one that is transcriptionally permissive, or instead reflects a lack of cell cycle dependent production of essential transcription factors, which would thus mimic the effects of cycloheximide treatment on the acquisition of transcriptional permissiveness and subsequent genome activation (Wang, Chung, & Latham, unpublished observations).

8. PERSPECTIVE

Given the available data related to the mechanisms for achieving and regulating embryonic genome activation, it is clear that the overall process is likely to be rather complex and to involve molecular events at multiple levels of gene regulation. A combination of nuclear and cytoplasmic events is involved, and these events must be coordinated with one another through bi-directional communication between the two compartments. DNA replication may constitute a critical focal point for regulation. As a consequence of DNA replication, chromatin remodeling occurs, and this at once creates the potential for gene transcription and creates the ability to regulate transcription. The production or post-translational modification of proteins in the cytoplasm, including nuclear lamins, histones, histone acetylases/deacetylases, and protein kinases, also contributes to chromatin remodeling. DNA replication then provides for cell cycle-dependent signals that regulate mRNA recruitment and stage-dependent expression or activation of essential transcription factors, which then can enter the nucleus to promote gene activation. Genome activation does not occur as a one-step process, but rather occurs in a stepwise manner, so that the above regulatory mechanisms must combine to activate specific sets of genes at specific times. Thus, the regulation of embryonic genome activation is not explicable on the basis of a single 'master switch', but rather constitutes a complex cascade of interacting nuclear-cytoplasmic signals initiated at the moment of fertilization and leading to the creation of an embryonic genome with a chromatin structure that is compatible with gene regulation.

Although a great deal has been learned recently abut the mechanisms governing embryonic genome activation, a great deal remains to be learned. Protein synthesis and maternal mRNA recruitment clearly play an important role in genome activation, but we have only a minimal knowledge of the array of maternal transcripts that are recruited and the functions provided by their encoded proteins. Knowledge of how passage through the cell cycle regulates the translation of those mRNAs is likewise minimal. Perhaps the most compelling mystery remaining is the identities of those genes that must be expressed in order to initiate the overall developmental program that leads to embryogenesis. Are these genes expressed in the oocyte as maternal mRNAs, or are they first expressed during the earliest phases of gene transcription, at the time of the first major genome activation event, or at a later stage of development, e.g., the 8-cell or morula stage, or perhaps in the inner cell mass? If the latter is the case, do all of the foregoing events serve merely to support embryonic metabolism until a critical mass of cells is generated to permit the creation of a trophectoderm-enclosed inner cell mass? Determining when the path to creation of a new individual begins and what genes are responsible remains one of the most fundamental questions of mammalian embryology. With the advent of novel methods for identifying temporally regulated genes in the early embryo and for manipulating their expression, it should soon be possible to address these questions.

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