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The molecular foundations of the maternal to zygotic transition in the preimplantation embryo

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The maternal to zygotic transition is the first major transition that occurs following fertilization, and entails a dramatic reprogramming of gene expression that is essential for continued development. Although the major reprogramming of gene expression occurs during the 2-cell stage, transcription is evident in the 1-cell embryo, with the male pronucleus supporting a significantly higher level of transcription than the female pronucleus. This difference is likely due to differences in chromatin structure as a consequence of the protamine–histone exchange. Although the 1-cell embryo is transcriptionally competent, transcription and translation appear uncoupled. This transcription, however, may mark promoters for efficient utilization in the 2-cell embryo. Genome activation in the 2-cell embryo is accompanied by a requirement for an enhancer for efficient transcription and the more efficient utilization of TATA-less promoters. These changes in promoter utilization could contribute substantially to the reprogramming of gene expression. Superimposed on genome activation is the development of a chromatin-mediated transcriptionally repressive state that is relieved by either inducing histone hyperacetylation or inhibiting the second round of DNA replication. Since genome activation appears to be a relatively opportunistic process, the development of the transcriptionally repressive state may be a major determinant in establishing the appropriate gene expression profile that is essential for continued development.

Key words: chromatin remodelling/genome activation/preimplantation development/promoter utilization/reprogramming gene expression

TABLE OF CONTENTS

Biological functions of the maternal to zygotic transition
Time of genome activation
Role of recruitment of maternal mRNAs in genome activation
Differences in transcriptional activity between male and female pronuclei
Potential uncoupling of transcription and translation in the 1-cell embryo
Function of transcription in the 1-cell embryo
Developmental changes in enhancer and TATA box requirements for gene expression
Chromatin-based nature of the transcriptionally repressive state
Biological function of the transcriptionally repressive state
References

Biological functions of the maternal to zygotic transition

The first major developmental transition that occurs following fertilization is the maternal to zygotic transition (MZT) in which the developmental programme that is initially directed by maternally inherited proteins and transcripts is replaced by a new programme as the consequence of the expression of new genes. This transition is also called zygotic gene activation (ZGA) or embryonic genome activation. Genome activation occurs in the mouse by the 2-cell stage (Schultz, 1993), and in bovine, ovine and human species by the 4- to 8-cell stages (Telford *et al*., 1990). Thus, genome activation occurs relatively early in development with respect to the number of cell divisions when compared with lower species such as *Xenopus* or *Drosophila* where genome activation occurs after ⩾12 rounds of DNA replication (Newport and Kirschner, 1982). On an absolute time scale, however, it occurs much later than in these organisms in which genome activation occurs within a few hours following fertilization.

The MZT has at least three functions that are required for the continued progression of development. The first function is to destroy oocyte-specific transcripts, such as that for the RNA-binding protein MSY2 (Yu *et al*., 2001), that are not subsequently expressed. The destruction of these mRNAs restricts the period of time in which these genes can function. For example, MSY2 is one of the most abundant proteins in the oocyte, comprising ~2% of total oocyte protein, and it has been proposed to regulate mRNA stability in the growing oocyte. The degradation of MSY2 protein and mRNA that is initiated following fertilization and is essentially compete by the late 2-cell stage may be linked to the

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continued degradation of the maternal pool of mRNA. Oocyte maturation initiates the destruction of maternal RNA and continues through the 2-cell stage, by which time most maternal mRNAs are degraded by >90% (e.g. actin). The seemingly non-specific degradation of the maternal mRNA pool may be one mechanism that drives the developmental switch from oocyte to embryo.

The second function of the MZT is to replace maternal transcripts that are common to the oocyte and early embryo, e.g. actin, with zygotic transcripts. If these transcripts are not replaced by zygotic transcripts, it is readily apparent that development will shortly come to a halt due to the inability of the embryo to execute its basic cellular functions. While the expression of these does not result in reprogramming of gene expression in the classical sense, their expression is nonetheless essential.

The third function of this transition is to promote the dramatic reprogramming in the pattern of gene expression that is coupled with the generation of novel transcripts that are not expressed in the oocyte (Latham *et al*., 1991). This reprogramming of gene expression is likely the molecular underpinning for the transformation of the differentiated oocyte into the totipotent blastomeres that are present at the 2-cell stage in the mouse. The identity of these genes and the molecular mechanisms that underlie this reprogramming are initiating a new wave of attention, as such knowledge may provide the 'Rosetta stone' to decipher the mysteries of reprogramming that must occur for successful cloning of mammals.

This short review, which is essentially confined to the mouse, will discuss several aspects of the molecular underpinnings of the MZT that result in the reprogramming of gene expression. Several of the major points highlighted in the review are presented schematically in Figure 1.

Time of genome activation

Knowledge of *when* ZGA occurs is critical to understanding the molecular basis of *how* ZGA occurs. Data from several lines of experimentation indicate that ZGA has definitely occurred by the 2-cell stage; these include detection of a paternally derived variant of β_2-microglobulin (Sawicki *et al*., 1981), the synthesis of paternally derived intracisternal particles (Szollosi and Yotsuyanagi, 1985), and the expression of a paternally derived β-actin promoter-driven luciferase reporter transgene (Matsumoto *et al*., 1994). In addition, the expression of a variety of plasmid-borne reporter genes bearing a range of different promoter elements is readily detected in the 2-cell embryo (Dooley *et al*., 1989; Bonnerot *et al*., 1991; Wiekowski *et al*., 1991; Vernet *et al*., 1992; Mélin *et al*., 1993; Ram and Schultz, 1993; Matsumoto *et al*., 1994).

Results of more recent studies demonstrate that the 1-cell embryo is transcriptionally active and that RNA polymerase I, II and III are functional. For example, luciferase activity is detected in G_2 of the 1-cell embryo following injection of the male pronucleus (PN) with an Sp1-dependent-driven luciferase reporter gene during early S phase (Ram and Schultz, 1993); the reporter gene did not contain an enhancer. The level of expression is ~20% of that observed when the reporter gene is injected into the nucleus of a mid 2-cell blastomere. Interestingly, little, if any, expression is detected in G_2 if the female PN is injected during S phase. This difference in transcriptional activity between the male and female PN is discussed below.

Functional RNA polymerase I and III are also present in the 1-cell embyro (Nothias *et al*., 1996). Injection of a chloramphenicol acetyltransferase reporter gene under the control of the RNA polymerase I-dependent ribosomal DNA promoter into the male PN of S phase-arrested 1-cell embryos (the embryos were incubated in the presence of aphidicolin, which inhibits DNA polymerases α and δ) revealed accumulation of the appropriate transcript by G_2 of the 1-cell embryo. The amount of this transcript is ~20% of that maximally accumulated when the cleavage-arrested embryos are cultured to a time that corresponded chronologically to the 2-cell stage and then analysed for expression. A similar result is obtained when the S phase-arrested 1-cell embryos are injected with a plasmid bearing the RNA polymerase III-dependent adenovirus VA1 RNA gene. In this case, the amount of transcript accumulated by G_2 of the S phase-arrested 1-cell embryo is ~30% of that maximally accumulated.

The expression of the total endogenous pool of genes was assessed by monitoring the incorporation of BrUTP, which is a substrate for RNA polymerase II, by antibodies to BrdU that also cross-react with BrU incorporated into RNA (Bouniol *et al*., 1995; Aoki *et al*., 1997). The results of these studies clearly demonstrated that endogenous genes are expressed, and moreover, that the male PN supports ~4- to 5-fold higher levels of transcription than the female PN, a result reminiscent of the higher level of expression of a plasmid-borne reporter gene following injection into the male PN when compared with injection into the female PN. Quantification of the signal reveals the amount incorporated by both pronuclei of the G_2 stage 1-cell embryo is ~40% that of the 2-cell blastomere in G_2 (Aoki *et al*., 1997). Lastly, transcription is first detected shortly after the initiation of DNA replication, and suggests that DNA replication may be linked to initiation of transcription. Consistent with this is the observation that inhibiting the first round of DNA replication results in a 40% decrease in transcription in the 1-cell embryo, as assessed by BrUTP incorporation (Aoki *et al*., 1997).

The linkage between initiation of transcription and DNA replication may be that the disruption of nucleosomes, which inherently inhibit transcription (Wolffe and Hayes, 1999), during DNA replication may provide a window of opportunity for the maternally derived transcription machinery to gain access to promoters that were previously not accessible. Access to these promoters could provide a mechanism in which a maternally derived transcription machinery could reprogramme the pattern of gene expression. As discussed below, such access may also mark promoters for transcription in the 2-cell stage.

Role of recruitment of maternal mRNAs in genome activation

A number of maternal mRNAs, as detected by two-dimensional gel electrophoresis, are recruited following fertilization/oocyte activation but prior to the initiation of transcription (Xu *et al*., 1994). While the identity and function of many of these proteins remain elusive, they constitute another, albeit poorly characterized, component of the maternal legacy to the early embryo.

Recruitment of maternal mRNAs following fertilization may be linked to the initiation of transcription in the 1-cell embryo. This

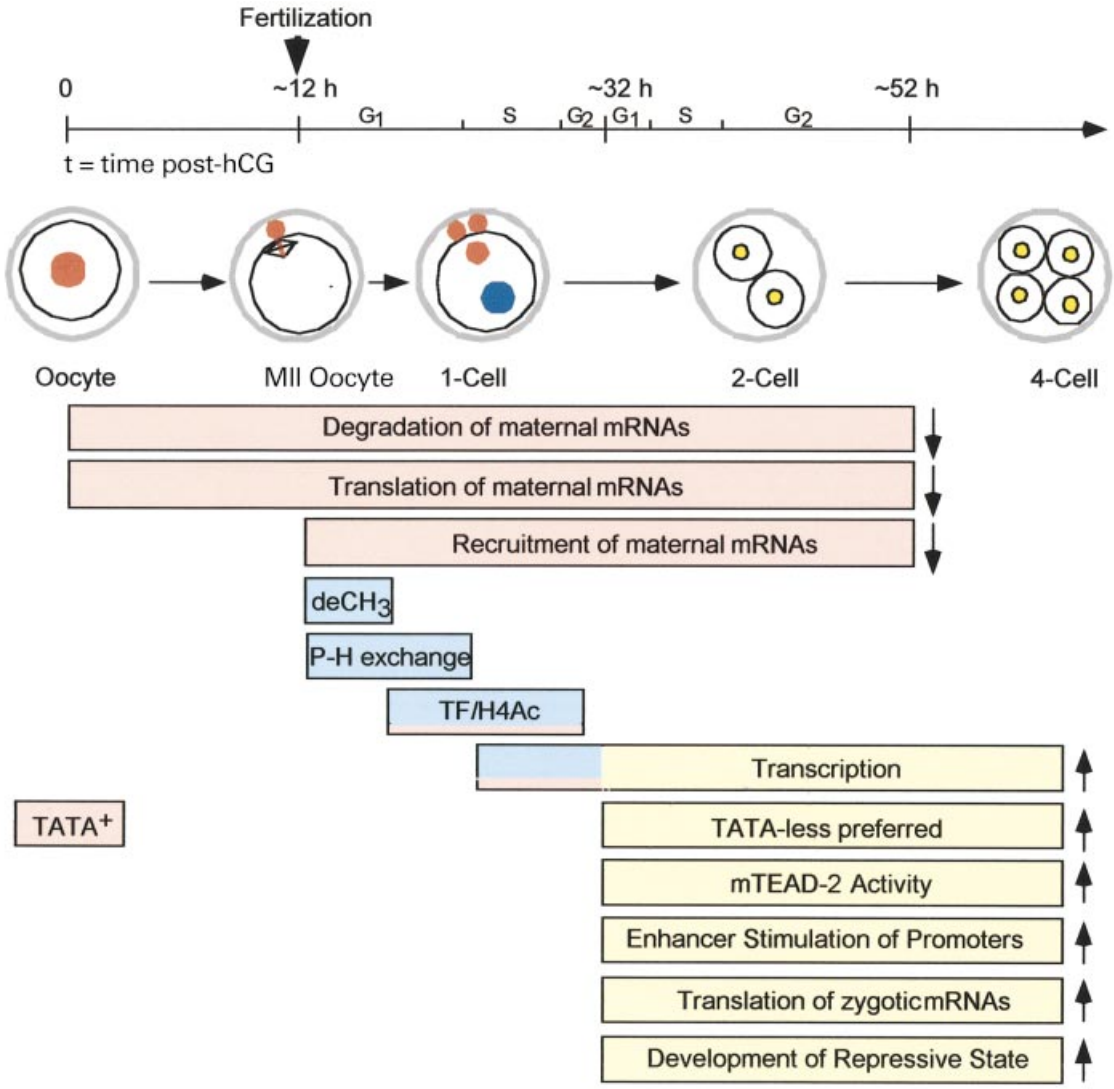

Figure 1. Schematic diagram representing several of the major discussion points. The time line refers to the time post-hCG. Red refers to maternal events, blue refers to paternal events, and yellow to zygotic events. $deCH_3$ refers to demethylation of the paternal genome, and P-H refers to the protamine–histone exchange that occurs in the male PN. $TATA^+$ refers to the preference for TATA-containing promoters. The difference in transcriptional activity supported by the male and female PN, and the difference in concentration of transcription factors (TF) and hyperacetylated histone H4 (H4Ac) is depicted by the difference in width of the blue and pink rectangles. The times shown for when these events initiate and terminate are approximate. The direction of the arrowhead at the end of the event indicates whether the magnitude increases or decreases with further development. mTEAD-2 activity refers to the specific transcription co-activator (see text). MII = metaphase II.

recruitment is coupled with polyadenylation of the poly(A) tail of the maternal mRNAs (Oh *et al*., 2000; Fuchimoto *et al*., 2001). Polyadenylation is inhibited by 3′-deoxyadenosine (3′dA), since it is converted to 3′dATP which, when incorporated into the mRNA's poly(A) tail, prevents elongation of the poly(A) tail due to the lack of a 3′ hydryoxyl group. 3′dA can inhibit polyadenylation in 1-cell mouse embryos, as evidenced by its ability to inhibit the polyadenylation of the maternal cyclin A2 mRNA that occurs following fertilization (Fuchimoto *et al*., 2001). When 1-cell embryos are cultured in medium containing 3′dA, BrUTP incorporation is inhibited by ~80% in both male and female PN (F.Aoki and R.M.Schultz, unpublished observations). This effect is specific, since little inhibition is observed when 3′deoxyguanosine (3′dG) is used.

The global nature of this substantial inhibition in transcription suggests involvement of some core component of the transcription machinery. One possible candidate is RNA polymerase II. The carboxy-terminal domain (CTD) of RNA polymerase exists in a hypophosphorylated form (IIA) that is required for transcription initiation, and a hyperphosphorylated form (IIO) that is required for elongation. The IIO form is present in the oocyte, and following fertilization it is converted to the IIA form (Bellier *et al*., 1997). This transition correlates with RNA polymerase II translocation from the cytoplasm to both PN (Bellier *et al*., 1997). This translocation, however, appears to occur in 3′dA-treated embryos. Likewise, the PN accumulation of the general transcription factors TBP and Sp1 also occurs in these treated embryos (F.Aoki, unpublished results). Thus, the linkage

between maternal mRNA recruitment and initiation of transcription in the 1-cell embryo remains unresolved.

Differences in transcriptional activity between male and female pronuclei

The expression of either endogenous genes or plasmid-borne reporter genes is significantly higher in the male PN when compared with the female PN. These differences likely reflect underlying differences in chromatin structure due to the protamine–histone exchange that occurs in the male PN, but not the female PN. This exchange appears to be initiated shortly after insemination and is complete prior to DNA replication (Nonchev and Tsanev, 1990). The glutathione that is generated during oocyte maturation reduces the disulphide bonds in the protamines, which facilitates their dissociation from the sperm DNA. Inhibiting the maturation-associated increase in glutathione inhibits the protamine–histone exchange and male PN formation (Perreault, 1992). In lower species, hyperphosphorylated forms of nucleoplasmin, which is a histone-binding protein, facilitate histone exchange in in-vitro systems (Ohsumi and Katagiri, 1991; Leno *et al*., 1996). Whether nucleoplasmim is present and facilitates the protamine–histone exchange in mammalian oocytes is not known.

The protamine–histone exchange may provide another window of opportunity for maternally derived transcription factors to gain access to their *cis*-cognate DNA-binding sequences before these sequences become sequestered into nucleosomes. The higher nuclear concentration of transcription factors, e.g. Sp1, TBP, oct-4, ets-1, in the male PN than in the female PN is consistent with this notion (Worrad *et al*., 1994; R.M.Schultz, unpublished observations), and may provide the basis for the higher transcriptional activity observed in the male PN. It is unlikely that the female PN is inherently less transcriptionally active than the male PN, since the extent of BrUTP incorporation by the female PN in parthenogenetically activated oocytes is equivalent to that in inseminated oocytes (Aoki *et al*., 1997).

Differences in histone acetylation and DNA methylation between the male and female PN may also contribute to differences in their transcriptional activity. Histone acetylation is highly correlated with the presence of transcriptionally permissive chromatin (Turner, 2000). In contrast, DNA methylation is associated with repression of transcription that is likely mediated by DNA-methyl binding proteins. These proteins recruit histone deacetylases that in turn promote histone hypoacetylation and the formation of transcriptionally non-permissive chromatin (Bird and Wolffe, 1999).

The male PN of the 1-cell embryo contains a higher concentration of hyperacetylated histone H4 than the female PN (Adenot *et al*., 1997). This difference, which is observed before S phase, lost by G_2 of the first cell cycle, and occurs in S phase-arrested 1-cell embryos, may be coupled to the protamine–histone exchange, which would provide the paternal chromatin with the opportunity to out-compete the maternal chromatin for hyperacetylated histones. This initial difference in chromatin structure could, in turn, account for the preferential binding of transcription factors to paternal chromatin and contribute to the attainment of higher concentrations of transcription factors in the male PN.

Non-histone proteins may also play a role in initiating transcription in the 1-cell embryo. The high-mobility group protein 1, HMG-1, interacts via an AT-hook domain with AT-rich regions of DNA that are often found associated with scaffold or matrix-associated regions (Bustin and Reeves, 1996). HMG-1 is present in the PN of the 1-cell embryo (Thompson *et al*., 1995), and injection of HMG-1 into fertilized oocytes advances the onset of transcription, as detected by BrUTP incorporation, by a couple of hours (Beaujean *et al*., 2000). Interestingly, injecting a peptide that contains the AT-hook domain also accelerates the onset of transcription, whereas injecting antibodies that recognize this peptide, and hence will inhibit endogenous HMG-1, delays the onset of transcription in the 1-cell embryo. Moreover, associated with microinjecting HMG-1 is an increase DNase I sensitivity as detected by TUNEL labelling (Beaujean *et al*., 2000). Taken together, these results suggest that HMG-1 can promote a change in chromatin structure that is transcriptionally more permissive.

The male PN also undergoes a very rapid DNA demethylation that is completed within 4 h following fertilization (Mayer *et al*., 2000; Santos *et al*., 2002). The demethylation is global in that both imprinted and non-imprinted genes become demethylated as determined by bisulphite mutagenesis (Oswald *et al*., 2000). Demethylation is also an active process, and not a consequence of DNA replication, since it occurs in 1-cell embryos treated with aphidicolin to inhibit DNA replication (Oswald *et al*., 2000). Little demethylation occurs in the female PN during this time. Again, the protamine–histone exchange could provide a window of opportunity for the enzymes involved in DNA demethylation to gain preferential access to the male PN. This loss of DNA methylation could in turn relieve the repression attributed to DNA methylation and ultimately lead to the observed higher level of transcription that is supported by the male PN when compared with the female PN.

Potential uncoupling of transcription and translation in the 1-cell embryo

Although the 1-cell embryo is clearly transcriptionally active, it is not apparent if these transcripts are efficiently translated. For example, following addition of α-amanitin to 1-cell embryos in G_2, the synthesis of α-amanitin-sensitive polypeptides called the transcription-requiring complex (TRC) that are the hallmark of ZGA is not observed in the developing 2-cell embryos (F.Zeng and R.M.Schultz, unpublished results). Likewise, although the transcript of a paternally derived luciferase transgene is detected in the 1-cell embryo, luciferase activity is not, but is detectable in the 2-cell embryo (Matsumoto *et al*., 1994). As discussed above, luciferase activity is detected in G_2 of the 1-cell embryo following injection of a plasmid-borne luciferase reporter gene (Ram and Schultz, 1993). However, the level of expression is very low on an absolute basis and hence may reflect poor translation of the expressed transcripts.

Further support for a possible uncoupling of transcription from translation comes from the analysis of expression of plasmid-borne reporter genes (Nothias *et al*., 1996). Whereas injection of the reporter gene into the nucleus of a 2-cell blastomere results in detectable luciferase activity shortly after injection, injection of the plasmid into the PN of a 1-cell nucleus reveals that while luciferase mRNA is readily detected during G_2 of the 1-cell stage,

luciferase activity is not detected. However, luciferase activity is detected at a time that corresponds to the early 2-cell stage. The coupling of transcription with translation is apparently rapidly established following the cleavage of 1-cell embryos, since although low levels of expression of a luciferase transgene driven by the hsp70 promoter are observed in a small fraction of 1-cell embryos in G_2, luciferase activity is detected immediately after cleavage in virtually all of the embryos (Christians *et al*., 1995).

The molecular basis for the apparent uncoupling of transcription and translation is unknown. The observation that synthesis of the TRC is observed in G_2 of the 1-cell embryo following transplantation of a 2-cell nucleus to an enucleated 1-cell embryo (Latham *et al*., 1992) suggests that the uncoupling is not due to either the rapid degradation of nascent transcript or their complexing with proteins to render them 'masked'. A splicing deficiency is also unlikely to be responsible, since detection of the spliced transcript is detected in 1-cell embryos shortly after microinjection of a plasmid-borne reporter gene containing the SV40 small intron in the 3′UTR (F.Zeng and R.M.Schultz, unpublished results). However, the inability (or low ability) of nascent transcripts to be exported to the cytoplasm has not been excluded.

The biological significance of an uncoupling of transcription from translation may be to protect the early embryo from the promiscuous expression of genes that could ensue as a consequence of the dramatic chromatin remodelling events that occur while the maternal and paternal genomes are sculpted into a chromatin structure that is present in the 2-cell embryo. Formation of this 'mature' chromatin structure may be essential to support the appropriate pattern of gene expression required for continued development.

Function of transcription in the 1-cell embryo

If transcription in the 1-cell embryo does not result in the production of functional transcripts, what purpose could it serve? One possibility described above is that it serves no obvious purpose but is simply the consequence of the chromatin remodelling that occurs during PN formation. Another possibility is that it serves to mark promoters that will be rapidly utilized following cleavage to the 2-cell stage with the formation of a zygotic nucleus. As mentioned above, it appears that genes are rapidly expressed following cleavage to the 2-cell stage.

Retention of transcription factors on the chromosomes of the fertilized oocyte during cleavage could provide one component of this molecular memory. The DNase I hypersensitivity of some promoters is retained in mitotic chromatin, even though transcription factors required for the expression of the gene are not. For example, HSF1, which is required for the expression of the hsp70 gene, is absent from mitotic chromatin that nevertheless retains the DNase I hypersensitivity profile characteristic for the hsp70 promoter (Martinez-Balbás *et al*., 1995). Analysis of transcription start sites during mitosis *in vivo* by ligation-mediated PCR is also consistent with a conformationally distorted chromatin conformation for genes that would normally be transcribed following entry into interphase. In contrast, genes not destined for expression are present in a normal chromatin conformation (Michelotti *et al*., 1997).

Maternal to zygotic transition in preimplantation embryo

More recent studies using chromatin immunoprecipitation reveal that transcription factors critical for the recruitment of productive transcription complexes at promoters are retained on mitotic chromatin (Christova and Oelgeschlager, 2002). Chromatin immunoprecipitation of asynchronous HeLa cells indicates that TFIID, TFIIB and RNA polymerase II are associated with active, but not inactive RNA polymerase II promoters. When the experiments are conducted with mitotic chromatin, both TFIID and TFIIB, but not RNA polymerase II, remain associated with mitotic chromatin. The association of these transcription factors, in particular TFIID, which is the only sequence-specific general DNA-binding transcription factor for RNA polymerase II, with mitotic chromatin suggests that following cleavage of 1-cell embryos to the 2-cell stage, TFIID that is recruited to promoters during the 1-cell stage and remains associated with the chromosomes could rapidly nucleate the formation of productive transcription complexes in the 2-cell embryo. Although it remains unknown if such retention occurs during passage through the first cleavage division, if so, it could provide a mechanism to reprogramme the pattern of gene expression that occurs during the 2-cell stage.

Developmental changes in enhancer and TATA box requirements for gene expression

Enhancers, which are located more distal to the transcription start site than promoters, are proposed to have two functions, namely to recruit RNA polymerase to the promoter and/or to relieve chromatin-mediated repression of a weak promoter (Majumder and DePamphilis, 1995). Results of experiments using a luciferase plasmid-borne reporter gene driven by the thymidine kinase (tk) promoter that either had or did not have the F101 enhancer, provided the first evidence that an enhancer is required for efficient transcription following genome activation during the 2-cell stage and that the function of this enhancer is to relieve chromatin-mediated repression that develops concomitant with genome activation (Wiekowski *et al*., 1991, 1993; Henery *et al*., 1995) (Figure 2).

Injection of this plasmid into the male PN of 1-cell embryos in which DNA replication was inhibited by aphidicolin results in high levels of luciferase expression when assayed at a time that corresponds to the 2-cell stage, whether or not the F101 enhancer was present. In stark contrast, efficient luciferase expression was only detected following injection of the plasmid into the nucleus of a 2-cell blastomere if the enhancer was present. Moreover, the ability of the enhancer to stimulate the promoter increased between the 2- and 4-cell stages, as evidenced by a greater fold-stimulation of luciferase expression. These results were the first to suggest that a transcriptionally repressive state develops during the course of genome activation, and that the role of the enhancer is to relieve this repression. The development of a transcriptionally repressive state may be a general property of preimplantation embryos since an increasing degree of repression with development, as evidenced by a decrease in expression driven by a basal promoter and the increased stimulation in expression in response to the presence of an enhancer, is also observed in rabbit preimplantation embryos (Delouis *et al*., 1992; Christians *et al*., 1994). As discussed below, the repression of transcription appears to be mediated at the level of chromatin structure.

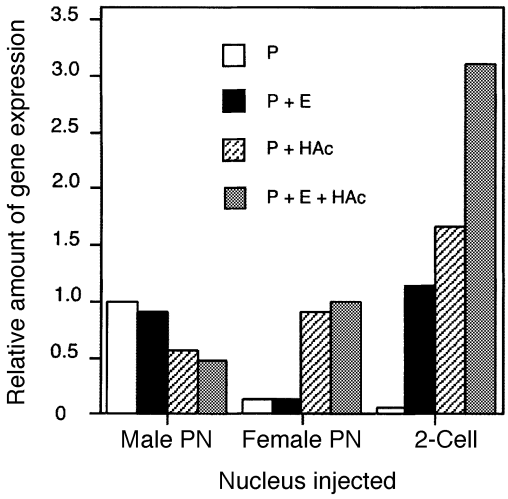

Figure 2. Summary of differences in transcriptional activity between male and female PN, and the role of an enhancer to relieve chromatin-mediated repression of transcription based on the expression of a plasmid-borne reporter gene driven by the tk promoter (P) ± F101 enhancer (E). Histone hyperacetylation (HAc) was induced by culturing the embryos in the presence of butyrate, which inhibits histone deacetylases. Either the male pronucleus (male PN), female pronucleus (female PN) or zygotic nucleus (2-cell) was injected. Note that the male PN supports a significantly higher level of transcription than the female PN ± the enhancer. Although histone hyperacetylation does not stimulate transcription from the male PN, it does so from the female PN. When only the promoter is present, the 2-cell nucleus supports a very low level of transcription relative to the male PN, i.e. it is repressed, but the presence of an enhancer relieves this repression. Inducing histone hyperacetylation has only a small (3-fold) enhancement of expression when the enhancer is present compared with the much larger stimulation when only the promoter is present. Thus, changes in chromatin structure can substitute for the enhancer requirement. (Data from Wiekowski *et al*., 1993.)

The developmental acquisition of an enhancer requirement for efficient plasmid-borne reporter gene expression requires the appearance of a co-activator (Majumder *et al*., 1997). mTEAD-2, which appears to be this co-activator (Kaneko *et al*., 1997; Kaneko and DePamphilis, 1998), is one of the four-member gene family of murine TEA domain genes (mTEAD-1 to -4) (Kaneko and DePamphilis, 1998), which bind to the same DNA sequence as the transcription enhancer factor (TEF)-1. Interestingly, only mTEAD-2 is significantly expressed in the preimplantation embryo, and its expression is derived from maternal mRNAs that are recruited during the 2-cell stage (Kaneko *et al*., 1997; Wang and Latham, 2000). This represents the first example in which a recruited maternal mRNA may participate in the reprogramming of gene expression that occurs during the 2-cell stage.

Analysis of gene expression with the plasmid-borne F101 enhancer, tk promoter-driven plasmid-borne luciferase reporter gene also reveals a change in the requirement for a TATA box for efficient expression. Regardless of the presence of a functional TATA box, high levels of expression are observed following injection of undifferentiated 2- to 8-cell blastomeres and embryonic stem cells with the enhancer-driven plasmid (Majumder and DePamphilis, 1994). In contrast, differentiated cells, such as the oocyte and 3T3 cells, require a TATA box for enhancer-driven expression.

A change in TATA box utilization is also observed for an endogenous gene. eIF-1A, which displays a transient increase in expression during the 2-cell stage (Davis *et al*., 1996), contains both a proximal TATA-containing promoter and a distal TATA-less promoter (Davis and Schultz, 1998). Using a RT–PCR assay that resolves the transcripts generated by these two different promoters, it was noted that while ~70% of the transcripts present in the oocyte are derived from the TATA-containing promoter, by the 2- and 8-cell stages only 25% and <10% respectively result from transcription initiated from the TATA-containing promoter (Davis and Schultz, 2000). This suggests that the change in TATA box utilization may be a general property of genome activation such that TATA-less promoters are more efficiently used.

There are two obvious biological consequences of such a switch in promoter utilization, the first being that many housekeeping genes are regulated by a TATA-less promoter (Bird, 1986). Preimplantation development is accompanied by a dramatic increase in metabolism as manifested by increased rates of oxygen consumption (Kaye, 1986). The more efficient use of TATA-less promoters that accompanies development would provide a convenient mechanism to foster an increased expression of housekeeping genes that in turn could support this increased energy demand. Such a change would also contribute significantly to the reprogramming of gene expression that occurs during the 2-cell stage.

Another consequence of this switch in promoter utilization would be to enhance the expression of genes critical to preimplantation development. For example, oct-4, which is required for the maintenance of totipotent blastomeres of the inner cell mass, is driven by a TATA-less promoter; ablation of oct-4 results in a blastocyst that contains only trophectoderm cells and no functional inner cell mass cells (Nichols *et al*., 1998). The more efficient use of TATA-less promoters that accompanies genome activation could ensure that sufficient levels of oct-4 expression are maintained during preimplantation development.

Chromatin-based nature of the transcriptionally repressive state

Several converging lines of evidence based on the expression of both reporter genes and endogenous genes suggests that the major locus of regulation for the observed repression is mediated by changes in chromatin structure, and not changes in the activity of the transcription machinery *per se*. (See above, however, for evidence that maternally derived transcription factors may also play a significant role.) The first insight was that while the expression of a reporter gene injected into the female PN of an S phase-arrested 1-cell embryo is ~4- to 5-fold less than that observed if the male PN is injected, inducing histone hyperacetylation results in the female PN supporting levels of expression equal to the male PN (Wiekowski *et al*., 1993) (Figure 2).

Inducing histone acetylation also relieves the repression that is present in the 2-cell embryo, as manifested by a 20-fold increase in expression of an enhancerless promoter following injection of a 2-cell blastomere nucleus, but only a 3-fold increase for the enhancer-driven reporter gene. Moreover, the stimulation attributed to the enhancer is ~10- to 12-fold in the absence of histone hyperacetylation, but only ~2-fold in the presence of histone

hyperacetylation (Figure 2). Thus, histone hyperacetylation relieves the repression observed in the 2-cell embryo as revealed by an increased expression of the enhancerless promoter and a reduced stimulation when the enhancer is present (Wiekowski *et al*., 1993).

Histone hyperacetylation also relieves the repression that occurs for both eIF-1A and the TRC. Both eIF-1A and the TRC display a transient increase in expression during the 2-cell stage, i.e. they are repressed, and inducing histone hyperacetylation prevents this repression (Davis *et al*., 1996). Although there is a progressive increase in BrUTP incorporation during the 2-cell stage, inducing histone hyperacetylation results in a further increase in BrUTP incorporation (Aoki *et al*., 1997). Thus, inducing histone hyperacetylation unmasks the development of a chromatin-mediated transcriptionally repressive state that is superimposed on genome activation.

The second round of DNA replication may be involved in the repression of expression of specific endogenous genes whose expression transiently increases during the 2-cell stage, as well as total endogenous transcription. Inhibiting the second round of DNA replication prevents the decrease in both the synthesis of the TRC and hsp70, and the decline in abundance of the eIF-1A mRNA (Christians *et al*., 1995; Davis *et al*., 1996). Furthermore, the total amount of BrUTP incorporated (as expressed on a per chromosome basis) by a 2-cell blastomere nucleus in G_2 is ~3-fold less than that incorporated by a blastomere obtained from a 2-cell embryo that is placed in aphidicolin just prior to the second S phase (Aoki *et al*., 1997). The molecular basis for how this round of DNA replication could lead to repression might be that replication would displace productive transcription complexes assembled on their promoters. The factors that constitute the basis for the transcriptionally repressive state would then prevent the formation of stable transcription complexes from reforming and hence reduce the expression of these genes.

What these factors are remains an enigma. Although the expression of a somatic form of histone H1 was an attractive candidate—the formation of a truly transcriptionally repressive chromatin structure is brought about by the addition of histone H1 and only stimulate their promoters if the chromatin contains H1 (Paranjape *et al*., 1994)—two lines of evidence suggest that it is unlikely that H1 solely accounts for the development of the transcriptionally repressive state. Injection of somatic histone H1 into 1-cell embryos that results in expanding the endogenous histone H1 pool to levels comparable with those present in 4-cell embryos, by which time the transcriptionally repressive state is already established, has no effect on TRC expression (Stein and Schultz, 2000). Similarly, injection of plasmid-borne reporter genes that had previously been reconstituted with either core histones or core histones and H1 reveals only a small increase of enhancer-mediated promoter stimulation (Rastelli *et al*., 2001). This system appears to replicate faithfully the regulation of endogenous genes, since reconstitution of the plasmid with acetylated histones effectively relieves the requirement for an enhancer (Rastelli *et al*., 2001). Interestingly, the ability of an enhancer to stimulate transcription is most robust when the plasmid is reconstituted with histones H3 and H4; little enhancer-stimulation is observed when the plasmid is reconstituted with histones H2A and H2B. Thus, it is unlikely that histone H1 initiates the development of the transcriptionally repressive state, although the continued expression of histone H1 could be critical for the maintenance of this state with further development.

Biological function of the transcriptionally repressive state

As described above, the development of the transcriptionally repressive state is manifested by the expression of transiently expressed genes, or the increased expression of a gene in response to histone hyperacetylation or by inhibiting the second round of DNA replication. Using these criteria, analysis of global patterns of gene expression by mRNA differential display reveals that of the 217 genes analysed, 45% are subject to repression during the 2- to 4-cell transition (Ma *et al*., 2001). This result suggests that the repression that develops during the 2-cell stage is quite extensive and is consistent with the results quantifying BrUTP incorporation by the 2-cell embryo, a marker for total endogenous transcription, in response to either inducing histone hyperacetylation or inhibiting the second round of DNA replication.

What could be the function served by the formation of this transcriptionally repressive state? The growing consensus is that genome activation is relatively promiscuous. For example, examination of the genes expressed during the 2-cell stage by either differential display (Ma *et al*., 2001) or analysis of expressed sequence tags derived from a 2-cell library (Ko *et al*., 2000) reveals that ~15% correspond to repetitive sequences, i.e. retrotransposons. It has been previously proposed (Ma *et al*., 2001) that a function of the repressive state is to sculpt the newly generated gene expression profile to make it compatible with further development. Activation of the genome appears to be a relatively opportunistic process due to the extensive remodelling of chromatin structure, and hence the genes that are expressed are simply those for which the necessary transcription factors are present and for which the promoter is accessible. Such a global activation will result in the expression of genes with strong promoters and/or enhancers, as well as many other genes that may be inappropriately expressed, i.e. opportunistically expressed (especially at basal levels of transcription) during this transition. A function of the transcriptionally repressive state that develops would be to reduce preferentially the expression of these inappropriately expressed genes, but permit the continued expression of genes that are regulated by strong promoters/enhancers. The expression of these genes would, therefore, be critical for continued development.

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