Minireview

Regulation of Gene Expression at the Beginning of Mammalian Development*

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Life begins for most animals when sperm fertilizes an egg to form a zygote. What do we know about the mechanisms that activate zygotic gene expression in mammals and thereby turn on the developmental program? Historically, answers to this question have relied heavily on studies done with fertilized eggs from frogs and flies (8–11) and on studies of gene expression in animal viruses and differentiated cells. Even with the most convenient and well-characterized mammalian developmental system, the mouse, the major impediment to studies on zygotes is their limited availability (–30 females) and small size (100–1000 times smaller than those from frogs or flies). One solution to this problem has been to inject unique DNA sequences in the form of plasmid DNA into the nuclei of oocytes and cleavage stage embryos. Replication and expression of genes encoded in extrachromosomal DNA respond to the same signals that regulate these functions in cellular DNA (2). They require specific cis-acting regulatory sequences and the trans-acting proteins that activate them and occur only when the host cell executes the same function with its own genome. These results, taken together with analyses of endogenous gene expression and results from nuclear transplantation studies, reveal several novel features of zygotic gene expression at the beginning of mammalian development (Fig. 1). These include the presence of a time-dependent mechanism for regulation of transcription and translational activation of a chromatin-mediated repression of promoter activity, the developmental acquisition of enhancer-dependent and TATA-box-dependent transcription, and identification of transcription factors that are active at the onset of mammalian development.

Activation of Zygotic Gene Expression in Mice

A growing mouse oocyte, arrested at diplotene of its first meiotic prophase, transcribes and translates many of its own genes, thereby producing a store of proteins sufficient to support development to the 8-cell stage (3, 4) (Fig. 1). Transcription of injected genes at this stage requires specific promoter elements, such as binding sites for Sp1, E2F, and TBP1 (5–7), or an oocyte-specific promoter such as ZP3 (8, 9). When an oocyte matures into an egg, it arrests in metaphase of its second meiotic division where transcription stops and translation of mRNA is reduced (10). Fertilization of the egg triggers completion of meiosis and formation of a 1-cell embryo containing a haploid paternal pronucleus derived from the sperm and a haploid maternal pronucleus derived from the oocyte. Each pronucleus then undergoes DNA replication before entering the first mitosis to produce a 2-cell embryo containing two diploid “zygotic” nuclei, each with a set of paternal and a set of maternal chromosomes.

Formation of a 2-cell mouse embryo marks the transition from maternal gene to zygotic gene dependence. Maternal mRNA degradation is triggered by meiotic maturation and ~90% completed in 2-cell embryos, although maternal protein synthesis continues into the 8-cell stage (11–13). ZGA is recognized by the sensitivity of protein synthesis to α-amanitin, a specific inhibitor of RNA polymerases II and III. ZGA involves synthesis of about 40 proteins (14) and is not evident until 2–4 h after completion of the first mitosis, concurrent with S-phase in 2-cell embryos (13–18). Zygotic protein synthesis increases 8–10 h later during G2-phase (15), suggesting that transcription of zygotic genes by RNA polymerase II occurs in two phases (Fig. 1), an early phase that is restricted to 2-cell embryos and a much stronger late phase that is required for further development (14, 16, 18).

Zygotic Clock

One of the most striking features of early ZGA is that its onset is delayed by a time-dependent mechanism referred to as the zygotic clock rather than by a particular cell cycle event. Early ZGA in the mouse occurs ~24 h after fertilization, regardless of whether or not the 1-cell embryo has completed S-phase and formed a 2-cell embryo (13, 16, 17). In contrast, late ZGA does not occur without formation of a 2-cell embryo (19). Thus, when 1-cell embryos that have not yet formed pronuclei are incubated in aphidicolin, a specific inhibitor of replicative DNA polymerases, they arrest development as they enter S-phase, but early ZGA still occurs at the time when they would have become 2-cell embryos (Fig. 1). Expression of plasmid-encoded genes injected into these arrested 1-cell embryos also is delayed until ZGA (17, 20).

Although the bulk of both transcription and translation of mouse zygotic genes occurs after ZGA, this is not true of all zygotic genes. Some early genes (eg, those encoding enzymes required for early embryonic development) may be expressed at the 1-cell stage. However, their expression is transient and is not evident until later during G2-phase (26, 27), although most of it does not occur until the 2-cell stage (28, 29). Moreover, the expression of some zygotic genes is delayed until ZGA (18, 20).2

Translation of nascent mRNA appears to be delayed until the 2-cell stage, suggesting that the zygotic clock regulates translation as well as transcription. Expression of one transgene was not detected until 10 h after its mRNA first appeared (24), and expression of luciferase activity from a plasmid injected into S-phase-arrested 1-cell embryos was not detected until ~12 h after the appearance of luciferase mRNA.2 In contrast, luciferase activity appeared coincident with its mRNA when DNA was injected into arrested 2-cell embryos. Delayed translation may result from failure to export nascent mRNA to the cytoplasm (23) or mRNA instability in 1-cell embryos (28). The net result is that transcription is delayed until ~14 h post-fertilization and translation until ~24 h (Fig. 1).

The zygotic clock is not simply the time required to convert sperm and egg chromatin into a transcribable form but a mechanism that involves trans-acting factors that are either required for transcription or suppress translation. Since RNA polymerase I-, II-, and III-dependent promoters follow the same time course when injected into S-phase-arrested 1-cell embryos,2 the zygotic clock may regulate the activity of a general transcription factor such as the TBP that is required by all three polymerases (29). This regulation may occur through post-translational modification of the target protein(s), because inhibitors of translation do not prevent transcription of either zygotic genes (30) or plasmid genes.2 Protein kinase activity may be involved because ZGA is sensitive to specific inhibitors of protein kinase A (13). In Xenopus embryos, the absence of functional TBP delays transcription of some promoters until the “midblastula transition” (31, 32).

One advantage of the zygotic clock is to delay ZGA until chromatin can be remodeled from a condensed meiotic state to one in

2 J-Y. Nothias, M. Miranda, and M. L. DePamphilis, manuscript in preparation.

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1 The abbreviations used are: TBP, TATA box binding protein; ZGA, zygotic gene activation; TAF, TBP-associated factor.
which selected genes can be transcribed. Since the paternal genome is completely packaged with protamines that must be replaced with histones, some genes might be prematurely expressed if ZGA were not prevented. Cell-specific transcription requires that newly minted zygotic chromosomes repress most, if not all, promoters until development progresses to a stage where specific promoters can be activated by specific enhancers or transactivators.

**Repression at the Beginning of Mammalian Development**

The transition from a 1-cell to a 2-cell mouse embryo is marked by the appearance of repression that reduces the activity of any promoter (6, 17, 20, 33–35) or replication origin (36) injected into either embryo from 20- to >500-fold. This repression is produced sometime between S-phase in a 1-cell embryo and formation of a 2-cell embryo and increases as development proceeds to the 4-cell stage (35). Repression is not observed when DNA is injected into the paternal pronucleus in an S-phase-arrested 1-cell embryo; the activities of both promoters and replication origins injected under these conditions are equivalent to their enhancer-stimulated activities in 2-cell embryos. However, repression is observed when DNA is injected into the maternal pronucleus of a 1-cell embryo or into the cytoplasm of early 1-cell embryos rather than simply excluded from paternal pronuclei. Repression in 2-cell embryos can act on any nucleus, regardless of its parental origin or ploidy. Two-cell embryos constructed to contain only maternal or paternal nuclei with one or two sets of chromosomes were equivalent to 2-cell embryos with zygotic nuclei in terms of their ability to repress an injected gene (35). However, repression occurs in 2-cell and 4-cell embryos regardless of whether or not these embryos continue development or are arrested in S-phase under the same conditions used to arrest 1-cell embryos. Therefore, the absence of repression in paternal pronuclei in S-phase arrested 1-cell embryos is neither unique to S-phase nor to experimental conditions.

Treatment of mouse embryos with butyrate suggests that repression is mediated through chromatin structure. Butyrate inhibits histone deacetylase, thereby inducing hyperacetylation of core histones, which increases the accessibility of DNA to transcription factors and reduces the ability of nucleosomes to interact with histone H1 (37, 38). Plasmid DNA injected into mouse ova as assembled into chromatin (20, 28). Butyrate relieves repression of this DNA in the maternal nucleus of oocytes, activated eggs, and 1-cell embryos, as well as in 2-cell embryos regardless of nuclear origin or ploidy, but butyrate does not stimulate promoter activity in the paternal pronuclei in 1-cell embryos where repression is not observed (33, 34). Furthermore, butyrate does not change the pattern of endogenous protein synthesis. Thus, butyrate appears to stimulate plasmid gene expression by altering its chromatin structure rather than by increasing synthesis of transcription factors which would activate promoters injected into either pronuclei.

Changes in chromatin structure may result from changes in the levels of histone H1 and the acetylated state of core histones. Incorporation of labeled amino acids reveals that histone H1 synthesis begins in late 1-cell embryos, although histone H1 is not detected by antibodies until the late 4-cell stage (40). Since early histone synthesis is insensitive to a-amanitin and the antibodies were made against somatic histones, these data likely reflect two histone pools, maternal and zygotic. Binding of histone H1 to chromatin leads to chromatin condensation with concomitant repression of transcription (41). In transcriptionally active genes, this repression is countered by acetylation of core histones, because histone H1 binds poorly to hyperacetylated chromatin (37, 38).

**Acquisition of Enhancer Function**

Enhancers provide one mechanism that can overcome chromatin-mediated repression. Promoters consist of transcription factor binding sites located upstream and proximal to the transcription start site, while enhancers consist of transcription factor binding sites distal to the start site that are located in either orientation upstream or downstream of the promoter. Enhancers impose tissue specificity on promoter activity. The ability of enhancers to stimulate promoters during mouse development is not observed until formation of a 2-cell embryo; plasmids injected into growing oocytes or S-phase-arrested 1-cell embryos require a promoter to express a gene, but the promoter is not stimulated by enhancers that function efficiently in 2- and 4-cell embryos (5, 17, 20, 33, 34, 42) (Fig. 1). A similar result is observed with the polyoma virus replication origin (36). Arresting 2- or 4-cell embryos at the beginning of their S-phase under the same conditions used to arrest 1-cell embryos does not affect their ability to utilize enhancers.

A survey of polyoma virus mutants that replicate in undifferentiated mouse embryonal carcinoma or embryonic stem cells identified the F101 polyoma virus enhancer as the most effective in stimulating the activity of promoters injected into 2-cell mouse embryos (20, 42). Stimulation ranges from 20- to >300-fold (17, 20, 33, 34, 42). Its activity depends on DNA binding sites for transcription factor TEF1 (42) and on cellular transcription factors that can be depleted in competition experiments (20, 36). TEF1 is a highly conserved transcription factor in mammals and the prototype of the gene family consisting of three or four proteins that share the same TEA DNA binding domain (43, 44).

Recent studies using in situ hybridization and injection of a TEF1-dependent synthetic promoter suggest that the TEF1 gene family is not expressed until ZGA. Since TEF1 itself is not required for preimplantation development (46), another member of this family may activate enhancers in preimplantation embryos. The ability to use enhancers is not dependent on formation of a zygotic nucleus, because stimulation by enhancers also occurs in 2-cell embryos constructed with nuclei derived exclusively from either the maternal or paternal pronuclei (33). Moreover, the

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3 M. Wielowski, M. Miranda, B. M. Turner, and M. DePamphilis, unpublished data.

4 K. Kaneko, E. Cullinan, M. Miranda, and M. DePamphilis, manuscript in preparation.
F101 enhancer is active if injected into a 1-cell embryo, and the injected pronucleus is then transplanted to a 2-cell embryo (35). Conversely, the F101 enhancer is inactive if injected into a 2-cell embryo, and the injected zygotic nucleus is then transplanted to a 1-cell embryo (35). Therefore, the ability to utilize these enhancers must depend on one or more factors that are not available until formation of a 2-cell embryo.

This hypothesis was tested using plasmids containing a tandem series of yeast GAL4 DNA binding sites located either proximal to the transcription initiation site (GAL4-dependent promoter) or distal to the HSV thymidine kinase promoter (GAL4-dependent enhancer). Each plasmid was co-injected together with an expression vector for GAL4:VP16 protein (34). In the presence of sufficient GAL4:VP16 protein to drive the GAL4-dependent promoter at its maximum rate, the GAL4-dependent enhancer strongly stimulated promoter activity when injected into 2-cell embryos but not when injected into oocytes or into either pronucleus of S-phase-arrested 1-cell embryos. Therefore, enhancer function requires a co-activator that is not available until formation of a 2-cell embryo, presumably because it is expressed during ZGA (Fig. 1). This enhancer-specific co-activator may be a TBP-associated factor (TAF) (48), but it must differ from the TAF that mediates interaction between the basal level transcription complex and GAL4:VP16 bound proximal to the transcription start site. Transcription factors can have multiple activation domains whose function depends on their proximal or distal location to the transcription start site (49). Each domain may interact with a different TAF. Most, perhaps all, promoters that are stimulated by enhancers contain a TATA box. The TATA box binds the basal level transcription complex through its TBP and determines the direction and start site for transcription (50). There are at least 12 examples of eukaryotic promoters that exhibit TATA-dependent stimulation by enhancers or transactivators, suggesting that a major role of the TATA box is to mediate promoter stimulation by an enhancer (Ref. 7 and references therein). Therefore, it is not surprising that disruption of the HSV thymidine kinase promoter’s TATA box element does not affect its efficiency in differentiated mouse cells unless the promoter is stimulated by an enhancer or its natural transactivator, HSV ICP4 (7). Presumably, this stimulation is mediated through TBP. However, it is surprising that this TATA box is not required for promoter activity or stimulation of the promoter by an enhancer or transactivator in cleavage stage mouse embryos and embryonic stem cells (7). Instead, enhancer stimulation of the thymidine kinase promoter in these undifferentiated cells is mediated through transcription factor Sp1. Thus, there appears to be a developmental switch that changes the pathway through which promoters are stimulated by enhancers. This switch could provide a simple mechanism for early embryos to utilize enhancers or transactivators to stimulate the activity of promoters that lack a TATA box but that contain one or more binding sites for Sp1, and then, following cell differentiation, reduce the activity of the same promoter to its basal level. “Housekeeping genes” (genes expressed ubiquitously and at low levels in differentiated cells) are frequently driven by TATA-less promoters containing Sp1 sites and therefore are candidates for this type of developmental control.

The primary role of enhancers is not simply to provide additional transcription factors to facilitate formation of an active initiation complex but to relieve repression of weak promoters from chromatin structure. Enhancers and butyrate appear to overcome the same problem. For example, the capacity of oocytes, S-phase-arrested 1-cell embryos, and 2-cell embryos to utilize a plasmid-encoded promoter is essentially the same in the presence of butyrate (33). In 2-cell embryos, these high levels of activity also can be achieved by linking the promoter to an embryo-responsive enhancer (34). Furthermore, the need for enhancers in 2-cell embryos does not result from functional changes in the promoter elements recognized by the transcription complex, because the thymidine kinase promoter depends on the same transcription factor binding sites in S-phase-arrested 2-cell embryos as in S-phase-arrested 1-cell embryos (34). Moreover, enhancers do not compensate for low concentrations of transcription factors needed to activate promot-

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5 S. Majumder and M. DePamphilis, unpublished data.
Summary

The maternal to zygotic transition can be viewed as a cascade of events that begins when fertilization triggers the zygotic clock that delays early ZGA until formation of a 2-cell embryo. Early ZGA, in turn, appears to be required for expression of late ZGA, and late ZGA is required to form a 4-cell embryo. ZGA in mammals is a time-dependent mechanism rather than a cell cycle-dependent mechanism that delays both transcription and translation of nascent transcripts. Thus, zygotic gene transcripts appear to be handled differently than maternal mRNA, a phenomenon also observed in Xenopus (55). The length of this delay is species-dependent, occurring at the 2-cell stage in mice, the 4–8-cell stage in cows and humans, and the 8–16-cell stage in sheep and rabbits (4). However, concurrent with formation of a 2-cell embryo in the mouse and rabbit (47, 56), perhaps in all mammals, a general chromatin-mediated repression of promoter activity appears.

Repressor factors are inherited by the maternal pronucleus from the oocyte but are absent in the paternal pronucleus and not available until sometime during the transition from a late 1-cell to a 2-cell embryo. This means that paternaly inherited genes are exposed to a different environment in fertilized eggs than are maternally inherited genes, a situation that could contribute to genomic imprinting. Chromatin-mediated repression of promoter activity prior to ZGA is similar to what is observed during Xenopus embryogenesis (31, 32) and ensures that genes are not expressed until the appropriate time in development when positive acting factors, such as enhancers, can relieve this repression. The ability to use enhancers appears to depend on the acquisition of specific co-activators at the 2-cell stage in mice and perhaps later in other mammals (47, 56), concurrent with ZGA. Even then, the mechanism by which enhancers communicate with promoters changes during development (Fig. 2), providing an opportunity for enhancer-mediated stimulation of TATA-less promoters (e.g. housekeeping genes) early during development while eliminating this mechanism later during development.

The net result of this sequence of events is to impose a directionality at the very beginning of animal development. This directionality is evident from the inability of fertilized mouse eggs to reproduce gene expression in nuclei taken from cells at developmentally advanced stages. For example, nuclei transplanted from mouse embryos that have progressed beyond ZGA (>late 2-cell stage) into enucleated 1-cell embryos do not recapitulate the normal program of gene expression (45) and therefore do not support successful development (21, 39). At least two factors contribute to this phenomenon: the inability of 1-cell embryos to relieve repression once it has been established and their inability to utilize enhancers. Although S-phase-arrested 1-cell embryos can efficiently utilize promoters encoded in plasmid DNA, they cannot relieve repression of the same promoter if it is first injected into a 2-cell embryo and then the injected nucleus transplanted back into an arrested 1-cell embryo (35). Linking the promoter to the 9101 enhancer does not (33) (Fig. 1).

The results described above have opened the door to understanding how the developmental program in mammals is initiated. It should now be possible to identify the roles of specific transcription factors and chromosomal changes in activating specific genes at the beginning of mammalian development.

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