—Review—

Activation of the Early Zygotic Genome in the Mouse Embryo

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After fertilization, mammalian eggs enter embryonic cell cycles, which simply divide the egg into smaller cells without any growth between cell divisions and dependent on time elapsed from fertilization. During this early embryo development until implantation, the first event is zygotic gene activation or embryonic gene activation. The activation of the zygotic genome has been shown to indicate the transition from maternal to embryonic control during the embryo development. In other words, stimulation of fertilization induces zygotic gene activation in the egg and sequentially fertilizing eggs develop under the control of the zygotic genome.

To date, the zygotic gene activation in the mouse embryo has been classified into two phases: minor activation at the early 2-cell stage and major activation at the late 2-cell stage, at both of which the synthesis of specific polypeptides is inhibited by α-amanitin, an agent that inhibits the activity of RNA polymerase [1]. Minor activation of the zygotic genome clearly occurs during the short G_1 phase of the 2-cell stage [1, 2], which is independent of the first DNA replication [3, 4]. On the other hand, major activation of the zygotic genome appears during the long G_2 phase of the 2-cell stage [1], the transcriptional activity of which is dependent on the first DNA replication [3, 4]. However, zygotic gene expression has recently been confirmed to begin in late 1-cell stage mouse embryos [5–8]. This suggests that zygotic gene activation can occur earlier than during the previously believed 2-cell stage.

Herein, I provide a brief and recent general introduction into the zygotic gene activation and its presumed role in the mouse development. I review predominantly the aspects of onset of zygotic gene activation in the mouse 1-cell embryo. For more detailed discussions of zygotic gene activation in the mouse and other species, see refs 9 (for mouse), 10 (for rat), and 11 (for hamster, rabbit, pig, sheep, cow, and human).

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Traditional Two Phases of Zygotic Gene Activation during the 2-Cell Stage: Minor and Major Activation of Zygotic Genome

As mentioned above, two phases of zygotic genome activation in the mouse embryo have been traditionally thought to initiate during the second cell cycle at the 2-cell stage: minor activation phase occurs during G_1 (before DNA replication) and major activation occurs during G_2 (before mitosis) [1]. This is supported by the sequential changes in α-amanitin-sensitive protein synthetic pattern.

The minor activation of the zygotic gene is mainly coupled to synthesis of α-amanitin-inhibited peptide complexes of about 68, 70, and 73 $\times$ 10^3 *Mr*, the expression of which is only restricted to 2-cell embryos and decreases to the 4-cell stage. Some of these early 2-cell specific protein complexes comprising about 4–10% of the total protein synthesis in embryos, were thought to be identical to two of the mouse heat-shock proteins, hsp68 and hsp70 by two-dimensional gel (2D) electrophoresis and comparative peptide mapping [12, 2]. However, the detailed analysis using two-dimensional isoelectric focusing/SDS-polyacrylamide gel electrophoresis indicates that these proteins are different from heat shock proteins with respect to their isoelectric points [13]. This protein family is presently referred to as the transcription-requiring complex (TRC). Hence, the TRC has provided a sign to mark the first transcriptional activation of the zygotic genome during the early embryo development, since it is found out immediately after the first cleavage of early mouse embryos.

On the other hand, the major activation of the zygotic genome begins about 6 h after first cleavage and a prominent change in the synthesis of proteins occurs

[1, 2], coinciding largely with elimination of maternal mRNAs [14–16]. The products of genes expressed in this phase are required for maintaining of sequent cell proliferation. In addition, the requirement of enhancer sequence elements in transient expression investigations, and the incidence of stage-specific gene expression suggest that the selective transcription mediated by RNA polymerase II operates in this phase [17–19].

Zygotic Gene Activation Occurs as Early as the 1-Cell Stage

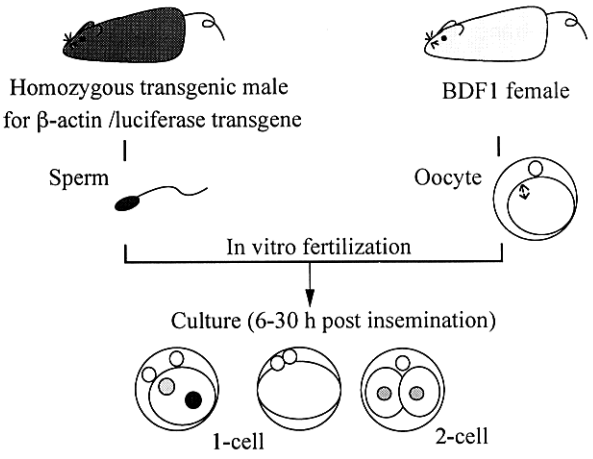
Very few studies directly analyzed the onset of early zygotic gene activation in the 1-cell mouse embryo. This was largely because of the difficulty of such determination and measurements, especially in which the amount of material is limited for biochemical and molecular analyses. For instance, the total RNA content of unfertilized eggs and 2-cell embryos is 0.35 and 0.24 ng per embryo, respectively [20]. At first, Clegg and Pikó [21, 22] observed that stable and low levels of transcription of poly (A)$^+$ RNA occurred at the late 1-cell stage using incorporation into RNA of [^{3}H]adenosine as a precursor. However, the authenticity of their observation was not certain, because RNA polymerase activity was undetectable in the pronuclei of the 1-cell mouse embryo [23]. Therefore, the starting point of early zygotic gene activation gene has been controversial.

Recently, two lines of experiments have been made on the onset of early zygotic gene activation at the 1-cell stage. Nuclear transfer studies have indicated that the cytoplasm of the late 1-cell mouse embryo becomes transcription-permissive [5]. Circular plasmid DNA injected as a reporter gene into the male pronucleus of the mouse embryo is expressed in G_2 of the 1-cell [6]. However, the former study is based on the TRC transcription in manipulated embryos reconstituted with late 1-cell-stage cytoplasm and α-amanitin treated nuclei from the 2-cell embryo. In the latter study, the transient gene expression of exogenous plasmid DNA was estimated at about 7×10^4 copies, a very high copy number. Accordingly, neither studies indicated whether the endogenous embryonic gene was already activated during G_2 of the first cell cycle.

The onset of transcription and translation of the endogenous embryonic gene during the 1-cell stage has been clearly demonstrated in one study [7], based on obtaining a paternal endogenous marker that is distinguishable from the maternal gene and which is constitutively expressed throughout early mouse embryo development under the control of a stage-nonspecific promoter. Transgenic mice carrying firefly luciferase cDNA regulated by the chicken β-actin promoter have already been produced [24]. The β isoform of actin is a major component of the cytoskeleton and it is abundant in many cells [25–27]. The chicken β-actin promoter is a strong constitutive promoter [28]. In addition, there is no marked endogenous background activity of luciferase in mammalian cells [29]. Thus, the initiation of paternal chromosomal gene activation was examined using expression of the firefly luciferase gene as an endogenous marker gene during the 1 and 2-cell stage embryos developed by in vitro fertilization with sperm of the homozygous transgenic mice (Fig. 1).

Since the founder transgenic mouse carried about four copies of the transgene, transgenic mice carrying about eight copies of it as determined by densitometry using a Bio Image, were considered to be homozygous for the transgene. The transgene was found in all progeny obtained from nontransgenic females that had mated with each of these transgenic males, indicating their homologous nature. These homozygous male progenies were used in the following experiments. The fertilization rate of the oocytes at 6 h post insemination with sperm of the homozygous transgenic mice was 90.6% (2810/3103) throughout these experiments. The first cleavage division was examined in 200 embryos (Fig. 2), and M phase was estimated to last from 14 to 18 or 19 h post insemination.

To determine the onset of transcription of the pater-



1) Detection of RNA from paternal gene by RT-PCR
2) Detection of luciferase from paternal gene by photon-count imaging

Fig. 1. Design of experiments.

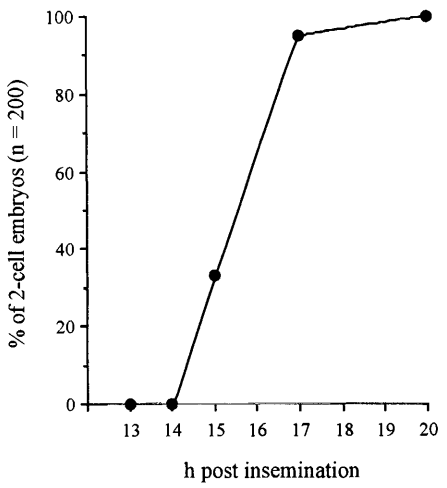


Fig. 2. Timing of first cleavage division expressed as a cumulative percentage from a total of 200 eggs fertilized *in vitro*.

nally derived endogenous luciferase gene in the early mouse embryos, groups of about 200 eggs fertilized *in vitro* were collected for 2- or 3-h periods several times post insemination. Total RNA was isolated from each group with 36 μg baker's yeast tRNA as a carrier using acid guanidium-phenol-chloroform [30], then digested with RNase-free pancreatic DNase I. Subsequently, the total RNA was extracted with phenol/chloroform, precipitated, and resuspended in 5 μl of water. Reverse transcription-polymerase chain reactions (RT-PCR) using 1 μl of water containing total RNA, which corresponded to 40 eggs, were performed with the Perkin-Elmer Cetus GeneAmp Thermostable rTth Reverse Transcriptase RNA PCR kit (N808-0069; Perkin-Elmer Cetus, Norwalk, CT) following the manufacturer's recommendations. The RT-PCR-amplified products were electrophoresed on 2.5% agarose gels and blotted onto nylon membranes (Hybond N; Amersham International plc., Bucks, U. K.). DNA on the membrane was prehybridized for 2 h, then hybridized with the labeled probe using the enhanced chemiluminescence (ECL) gene detection procedures (RPN. 2100; Amersham International plc., Bucks, U. K.). Membranes were washed and exposed to an X-ray film using the ECL gene detection system. As shown in Fig. 3, transcripts of the luciferase gene were detected in 1-cell embryos as early as 13 h post insemination, when embryos morphologically showed the formation of visible nucleoli within both pronuclei migrating to the center of the cytoplasm.

After transferring into modified phosphate-buffered saline [31] supplemented with 500 μM D-luciferin, the luciferase activity expressed in these embryos was identified for 30 consecutive minutes (integration period) using an inverted microscope and an imaging photon counter (ARGUS-50; Hamamatsu Photonics K.K., Hamamatsu, Japan). The transgenic embryos 16, 19, 21, 23, and 25 h post insemination were imaged to determine the luciferase gene expression in early embryo development. The luciferase activity examined by the photon-count imaging was absent at the 1-cell stage but first appeared 23 h (Fig. 4-1, -2) and 25 h post insemination in 47% (9/19) and 75% (18/24) of the embryos, respectively.

These findings demonstrated that transcription of the paternal chromosomal luciferase gene, which was stably integrated into the chromosome, was identified in the late 1-cell mouse embryo. It is clearly suggested that RNA is transcribed from the paternal endogenous gene by functional RNA polymerase II at the late 1-cell stage of the mouse embryo. Furthermore, the zygotic gene may already be activated in the late 1-cell embryo, although only RNA is synthesized and that the zygotic gene activation, including the synthesis of polypeptides, may begin during the early 2-cell stage.

As same as this approach, the onset of the transgene in transgenic embryos carrying integrated heat-shock protein (HSP) 70.1 promoter-driven firefly luciferase cDNA has been discussed in a recent paper [8]: it is demonstrated that the transcription of transgene begins at the 1-cell stage and that it is intensely expressed in the early 2-cell stage. Undoubtedly, the above findings shown by two investigations using individual transgenic mice suggest that an earlier phase of the zygotic gene activation exists at the 1-cell stage before two phases, that is minor and major activation, classified at the 2-cell stage. At present, whether the earlier phase of the zygotic gene activation at the 1-cell stage is included in minor activation and the degree of independence of the three phases of the zygotic gene activation remain unknown. We cannot yet answer this question because the kind of genes expressed during the first and second cell cycle remain to be identified.

The Biological Role of the Zygotic Gene Activation in the Early Embryo Development

The onset of zygotic gene activation is rather well understood as mentioned earlier, but little is known about the zygotic gene activation for early embryo development at the molecular level. Accordingly, the genes that

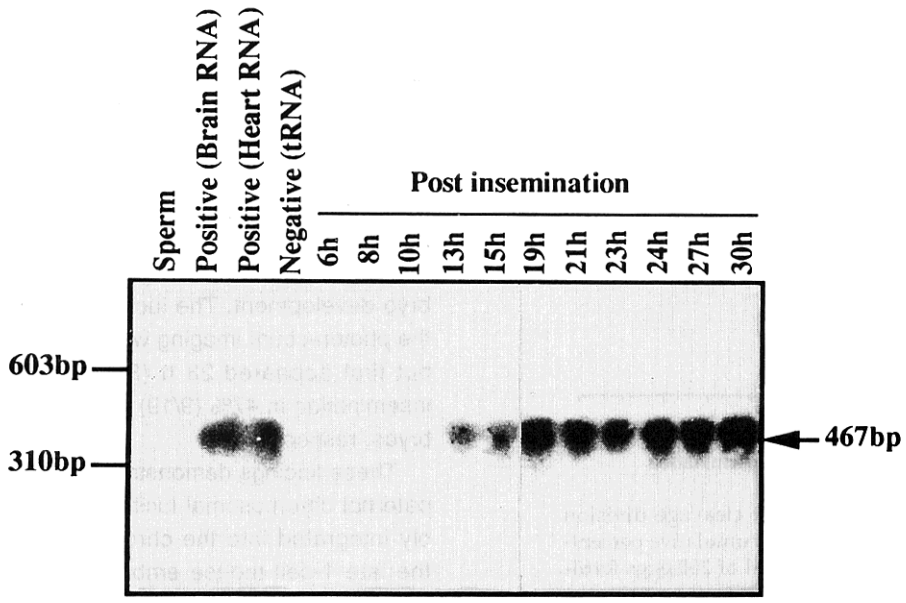


Fig. 3. Transcription of luciferase gene in transgenic embryos during the 1- to 2-cell stage. Sperm represents extraction isolated from 3×10^4 spermatozoa collected from the cauda epididymides of homozygous transgenic male. Brain and heart RNA from a transgenic mouse were used as positive controls, and yeast tRNA as a negative control. Total RNA was isolated from 200 *in vitro* fertilized eggs at various times postimplantation, then digested with DNase. Reverse transcription-polymerase chain reactions (RT-PCR) using one-fifth of the extracted solution was performed. Two 21-mer oligonucleotides matching the sequence of firefly luciferase cDNA were selected as primers (5'-ATGTTCATACTGTTGAGCAAT-3'; Fig. 1) for reverse transcription and PCR amplification, and as forward primers (5'-ATGGAAGACGCCAAAAACATA-3'; Fig. 1) for PCR amplification of reverse transcribed products. RT-PCR-amplified products were electrophoresed and subjected to Southern blot analysis using 1.8 kbp firefly luciferase cDNA as a probe.

are expressed at each of the three phases of the zygotic gene activation from the 1- to 2-cell stage, how first zygotic gene activation occurs after the stimulation of the sperm penetrating into the oocyte, and how the firstly activated genes affect their functions on the sequential zygotic gene activation by specific pathways, remain to be determined.

Considering the aspects of the cell cycle [32] and the regulation of transcription [33], early mouse embryo development may be regulated by a chain of three phases of the zygotic gene activation that begins with an interaction of the sperm with specific sperm receptor(s) located in the plasma membrane of the oocyte (Fig. 5). The binding of the sperm generates signal inductions inside the oocytes by mediating its receptor, followed by activation of a specific signal transduction pathway. The change in phosphorylation of maternal derived proteins, that may be transcription factors or posttranscriptional factors, by activated protein kinases and phosphatases plays a critical role in the transcription of early response genes during G_2 of the first cell cycle, which sequentially results in the transcriptional state that induces transcription of the delayed response genes that encode transcription factors in the early 2-cell stage. Finally, the transcription of many late response genes begins in the late 2-cell stage and then fertilized eggs enter the embryonic cell cycle as mentioned earlier.

In support of this putative view, the signal transduction pathways in first and second cell cycles of early embryo development have been reported. Proteins phosphorylated by the cAMP-dependent protein kinase (PK-A) appear to be necessary for the zygotic gene activation in the 2-cell embryo [34]. Likewise, G-proteins are strongly implicated to regulate fertilization, egg activation, and transcriptional activation of the zygotic genome [35, 36]. The findings indicating that the Ras/Raf pathway is specifically required for development of mouse

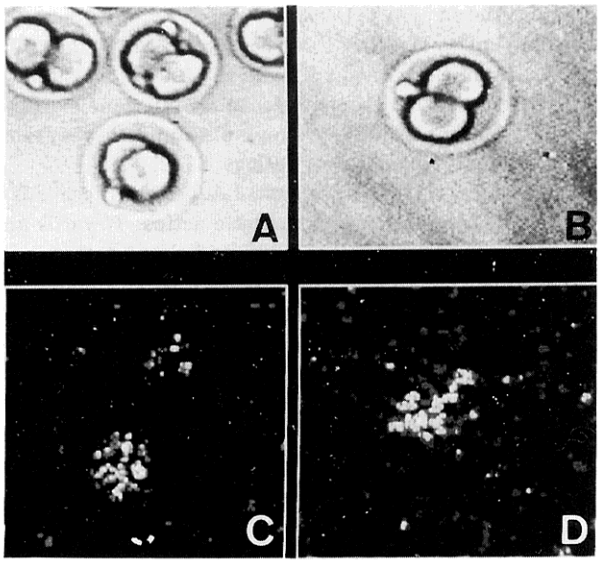


Fig. 4. Luciferase gene expression in preimplantation transgenic embryos detected by photon-count imaging. A and C: Light and bioluminescence images of transgenic 2-cell embryos 23 h postinsemination, respectively. B and D: Light and bioluminescence images of transgenic 2-cell embryo 30 h postinsemination, respectively.

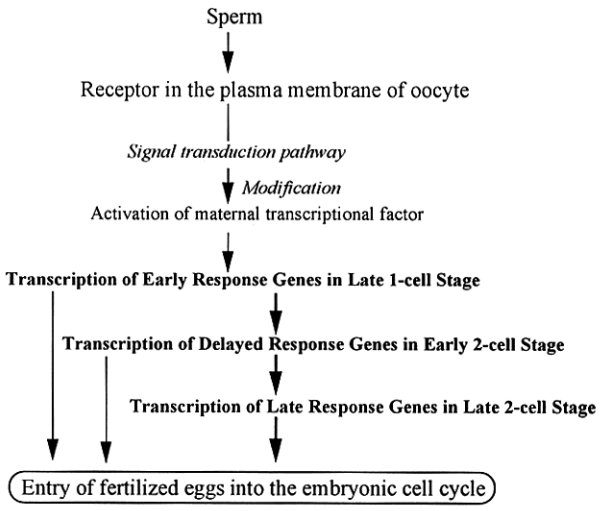


Fig. 5. A hypothetical role of three phases of zygotic gene activation in the early embryo development. For explanation, see text.

embryos through the 2-cell stage, suggest the potential role of Ras/Raf pathway in the regulation of the zygotic gene expression [37]. Furthermore, the requirements for replication and expression of genes are likely to be established on formation of the 2-cell embryo [18, 38]. Although it awaits discovery in the mouse, fertilization-dependent protein tyrosine kinases play an important role in the events of eggs activation in sea urchin eggs [39].

The identification and functional characterization of these overall interactions between genes activated following fertilization remains an interesting question. Ultimately, prospective studies would aim at integrating the regulation of the zygotic gene activation with signal transduction pathways controlling early embryo development. The main key to the role of zygotic gene activation is to identify the genes that demonstrate the specific transcriptional manner during the 1- and 2-cell stage, the attempts of which have merely begun [19, 40–42].

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