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Opinions and Hypotheses

A unique mechanism regulating gene expression in 1-cell embryos

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Abstract. After fertilization, the genome of zygotes is transcriptionally silent. The timing of the initiation of transcription is species-specific and occurs at the mid-1-cell stage in mice. Recent analyses using high-throughput sequencing (HTS) have identified thousands of genes transcribed at the 1-cell stage, and the pattern of expression among these genes appears to be unique. In this article, we show the result of an additional analysis using HTS data from a previous study, and present the hypothesis that an extremely loose chromatin structure causes promiscuous gene expression in 1-cell embryos.

Key words: 1-Cell embryos, Gene expression, Transcription, Zygote

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For individual organisms, life begins as a zygote after fertilization. The zygotic genome is not transcribed immediately after fertilization; rather, the initiation of transcription occurs later, with species-specific timing. Transcription in the early stages post-fertilization has been called zygotic gene activation (ZGA). In the 1980s, transcription was thought to be initiated at the 2-cell stage in mice [1, 2]. However, Matsumoto *et al*. [3] found that a transgene derived from the genome of spermatozoa was expressed at the late 1-cell stage. Furthermore, a sensitive method for detecting transcription (viz., detecting the incorporation of BrU in macromolecules) revealed that a low level of transcription is initiated at the mid-1-cell stage [4]. Thus, gene activation at the 1-cell stage is known as minor ZGA, whereas an increased level of transcription at the 2-cell stage is called major ZGA. The genes to be expressed must be critically important for regulating subsequent gene expression during development. However, the mechanism that determines which genes are first transcribed has not yet been elucidated.

Transcripts in 1-cell embryos comprise those derived from oocytes and those newly transcribed from the zygotic genome. Oocyte-derived mRNA (i.e., maternal mRNA) is synthesized and accumulated during the days before fertilization, whereas mRNA transcribed from the zygotic genome is synthesized only several hours after fertilization [4, 5]. Thus, the amount of newly synthesized mRNA from the zygotic genome is much less than the accumulated maternal mRNA in 1-cell embryos, which makes it difficult to identify the genes transcribed at the 1-cell stage by comparing the transcriptomes of oocytes and 1-cell embryos. Previous studies using microarrays were unable to identify the genes transcribed in 1-cell embryos [6–8]. Recently, transcriptome analyses by RNA sequencing (RNA-seq) found hundreds of candidates for genes transcribed at the 1-cell stage [9, 10]. Although these studies help to characterize gene expression after fertilization, candidates were determined on the basis of the reads per kilobase per million (RPKM) of the transcripts being only 1.5-fold [10] or 2-fold [9] higher in 1-cell embryos than in MII-stage oocytes, a criterion unlikely to be robust in the face of experimental variation.

Recently, we found that splicing mechanisms do not function adequately in 1-cell embryos [11]. Taking advantage of this property, we identified 4,039 genes transcribed at the 1-cell stage, based on the RPKMs of intron regions being 4-fold higher in 1-cell embryos than of those whose transcription had been inhibited with 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole [11]. When we changed this criterion to 1.5-fold on the grounds that 4-fold might be too conservative, 11,470 genes were obtained as candidates transcribed at the 1-cell stage. Gene expression pattern analysis in 1-cell embryos has revealed that many genes are highly expressed only at the 1-cell stage, and that housekeeping genes, which are highly expressed in various cells, are not highly expressed at this stage [12]. Thus, we demonstrated a unique gene expression pattern in 1-cell embryos, but we did not clarify the mechanism by which this expression pattern is induced. Here, we discuss the mechanism for the regulation of gene expression in 1-cell embryos.

A loosened chromatin structure is involved in transcriptional regulation at the 1-cell stage

Our previous analysis for *cis*-regulatory elements was unable to elucidate the mechanism regulating the gene expression pattern in 1-cell embryos, except for the GC-rich nature of regions upstream of active genes [11, 12]. Since there is no *cis*-regulatory ele-

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ment specific to 1-cell embryos, it is possible that the chromatin structure is involved in regulating gene expression in 1-cell embryos. Generally, genes require enhancers for their active expression. The chromatin structure is essentially repressive for transcription, necessitating the presence of enhancers to help transcription factors access the gene promoters [13]. However, it has been shown by reporter gene assays that enhancers do not increase transcriptional activity in 1-cell embryos, suggesting that transcription is regulated independently of enhancers at this stage [2, 14, 15]. Furthermore, we demonstrated that transcription occurs only via the core promoter in 1-cell embryos, but not in 2-cell embryos [16]. Majumder *et al*. [15] suggested that enhancer-independent transcription is caused by the loosened chromatin structure in 1-cell embryos, since this type of transcription was still observed at the 2-cell stage when the embryos were treated with butyrate (an inhibitor of histone deacetylase), which increases histone acetylation to loosen the chromatin structure. Using fluorescence recovery after photobleaching analysis, we have recently shown that preimplantation embryos at the 1-cell stage form the loosest chromatin structure [17]. A recent analysis of the genome-wide landscape of chromatin accessibility to DNase I demonstrated that the number of DNase I-hypersensitive sites (DHSs) is the fewest at the 1-cell stage and increases during preimplantation development [18], which appears to contradict the hypothesis made by the above-mentioned reports [2, 14–17]. However, in the analysis using DNase I, the DHSs do not necessarily correspond to the sites of the genome with a loosened chromatin structure. In sites with a loosened chromatin structure, transcription factors can easily access the DNA and bind to it, thereby obstructing the DNase I access to the DNA. In this case, the sites of chromatin with a loosened structure are insensitive, rather than sensitive, to DNase I. Furthermore, since the chromatin structure is extremely loose in 1-cell embryos, DNase I might have cleaved an enormously large number of sites to make small fragments of DNA that were removed by size selection (> 50 bp) for high-throughput sequencing, which would underestimate the number of DHSs. Taken together, we propose that 1-cell embryos have a loose chromatin structure that does not require enhancers for gene expression.

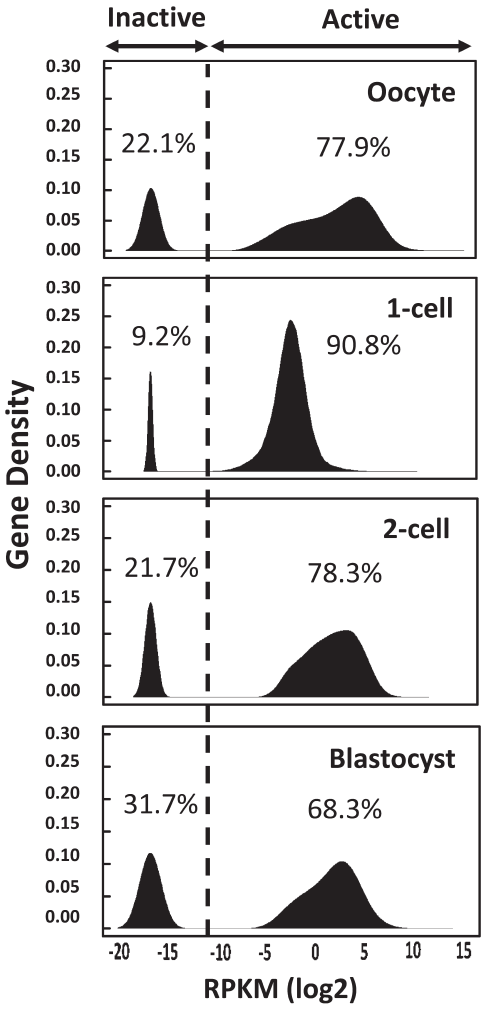

Fig. 1. Histogram of gene expression levels in the preimplantation embryos. The kernel density estimation was calculated to classify all known genes as active or inactive in oocytes, 1-cell and 2-cell embryos, and blastocysts. Horizontal and vertical axes are shown as the log2 of reads per kilobase per million (RPKM) and gene density, respectively. Reads in exons were used to calculate the RPKM values in oocytes, 2-cell embryos, and blastocysts, whereas reads in introns were used for 1-cell embryos. The broken line represents the border between active and inactive genes.

If this is the case, we should expect a large number of genes to be promiscuously expressed, and the variation in their expression level should be relatively low. Since many transcription factors would be able to easily access the promoters, enhancers should not have a strong effect on transcription. Indeed, the analysis of our RNA-seq data bears out this hypothesis. When genes are classified as active or inactive based on kernel density estimation, more than 90% of genes are active in 1-cell embryos, whereas only around 70% of genes are active in embryos at other stages and in oocytes (Fig. 1). This analysis also reveals that the variation in expression level is much lower in 1-cell embryos than in cells of other stages; the slope of the histogram is steeper for 1-cell embryos. Therefore, we suggest that a low level of enhancer-independent transcription occurs promiscuously in a large proportion of genes, which is probably caused by a loosened chromatin structure in 1-cell embryos (Fig. 2).

We hope that this hypothesis sheds light on the mechanism that regulates the initiation of the gene expression program in embryos.

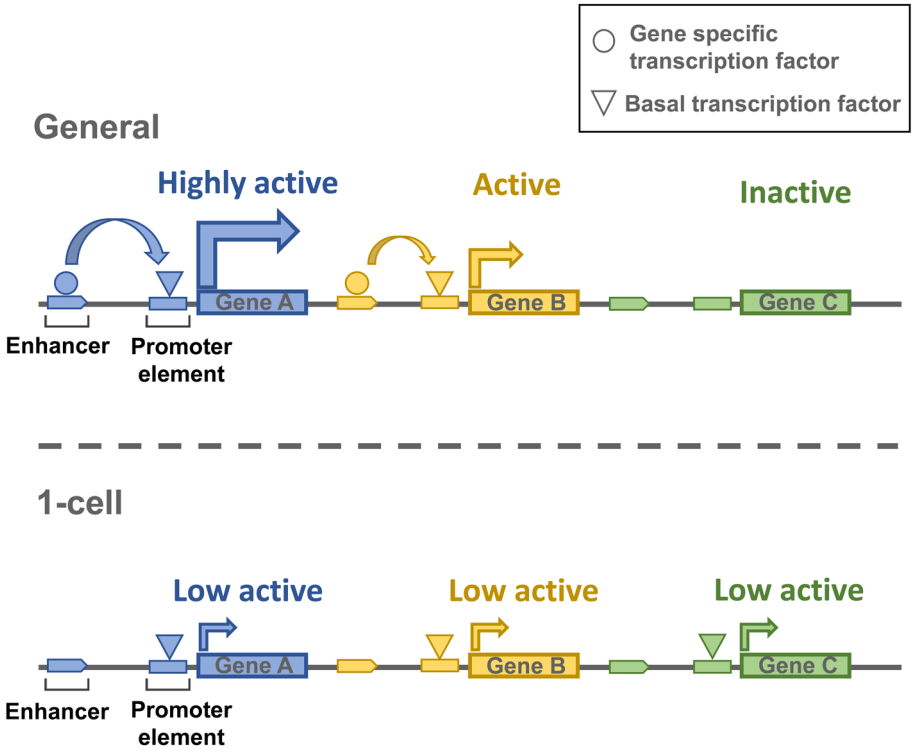

Fig. 2. Diagram of transcriptional regulation in 1-cell embryos. The upper section illustrates the general mechanism of transcription regulation. Gene expression is facilitated by the enhancers, which also regulate the gene expression levels. The lower section depicts enhancer-independent transcription in 1-cell embryos. The chromatin structure is loosened genome-wide, which allows a low level of transcription in most genes independently of enhancers.

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