Zygote

cambridge.org/zyg

The identification and classification of candidate genes during the zygotic genome activation in the mammals

Kaiyue Hu[1], Wenbo Li[2], Shuxia Ma[1], Dong Fang[1] and Jiawei Xu[2]

[1]Luoyang maternal and Child Health Hospital, 206, Tongqu Road, Luoyang, Henan, 47100 China and [2]The First Affiliated Hospital of Zhengzhou University, 40, Daxue Road, Zhengzhou, Henan, 450052 China

Research Article

Cite this article: Hu K *et al*. (2024) The identification and classification of candidate genes during the zygotic genome activation in the mammals. *Zygote*. **32**: 119–129.
doi: 10.1017/S0967199423000631

Received: 12 June 2023
Revised: 17 November 2023
Accepted: 18 November 2023
First published online: 22 January 2024

Keywords:
Candidate genes; Conserved genes; Ribosomal RNA; Maternal genes; Non-maternal genes; Zygotic genome activation

Corresponding author:
Jiawei Xu; Email: fccxujw@zzu.edu.cn

Summary

Zygotic genome activation (ZGA) is a critical event in early embryonic development, and thousands of genes are involved in this delicate and sophisticated biological process. To date, however, only a handful of these genes have revealed their core functions in this special process, and therefore the roles of other genes still remain unclear. In the present study, we used previously published transcriptome profiling to identify potential key genes (candidate genes) in minor ZGA and major ZGA in both human and mouse specimens, and further identified the conserved genes across species. Our results showed that 887 and 760 genes, respectively, were thought to be specific to human and mouse in major ZGA, and the other 135 genes were considered to be orthologous genes. Moreover, the conserved genes were most enriched in rRNA processing in the nucleus and cytosol, ribonucleoprotein complex biogenesis, ribonucleoprotein complex assembly and ribosome large subunit biogenesis. The findings of this first comprehensive identification and characterization of candidate genes in minor and major ZGA provide relevant insights for future studies on ZGA.

Introduction

Embryonic development in mammals starts with the fertilization of an oocyte by a sperm cell, followed by the formation of the pluripotent zygote and differentiation into a new individual (Shpargel *et al.*, 2014). During this process, the embryo undergoes dramatic morphological changes, coupled with widespread epigenetic reprogramming, of which maternal-to-zygotic transition (MZT) represents the most critical biological event. Notably, MZT involves two main stages, namely the degradation of maternal products and the activation of zygotic genomes. During this process, embryos complete the conversion of maternal to zygotic control (Tadros and Lipshitz, 2009; Sha *et al.*, 2019). The mature oocyte is in a transcriptional silencing state initially (Moore *et al.*, 1974), once fertilized, the genome is rapidly activated. In addition, zygotic RNA accumulates following the gradual degradation of maternal products.

In the last few decades, numerous studies have revealed the importance of maternal products in embryonic development (Nüsslein-Volhard and Wieschaus, 1980; Driever and Nüsslein-Volhard, 1988). The initiation events of early embryonic development in mammals are mainly controlled by maternal effectors, which are encoded by maternal effect factors. These factors accumulate during oogenesis, a phenomenon that makes it possible for zygotic genome activation (ZGA), embryo cleavage and blastocyst development with an inner cell mass and trophectoderm cells. To date, more than 30 mouse genes harbouring maternal mutation effects (effects caused by maternal gene mutation) have been documented including the factor *Nelfa* (Hu *et al.*, 2020), *X-linked Huwe1* (Eisa *et al.*, 2020), and *Argonaute 2* (Zhang *et al.*, 2020). Functionally, these genes regulate the fusion of female and male nuclei, the elimination of maternal material, the activation of the embryonic genome, the cleavage of the zygote and the densification of the embryo (Li *et al.*, 2010; Zheng and Liu, 2012). Moreover, they have been shown to play important roles in oogenesis, meiotic maturation, preimplantation and post-implantation embryonic development (Innocenti *et al.*, 2022).

ZGA is considered the most essential step in regulating early embryo development. It has been demonstrated in previous studies that ZGA disorders result in embryo arrest beyond the 2-cell stage. For example, the growth of *Btg4* knock-out embryos was arrested at the 2-cell stage (Yu *et al.*, 2016), and similar effects were observed in embryos that lacked specific factors such as *Nanog*, *Soxb1* and *Pou5f3* in zebrafish (Lee *et al.*, 2013). Additional evidence has shown that *Dux4*, a classical ZGA gene, also plays a vital function in ZGA in 2-cell-like cells (De Iaco *et al.*, 2017; Hendrickson *et al.*, 2017; Whiddon *et al.*, 2017). Although subsequent research has demonstrated that the loss of *Dux4* would be dramatically compensated for by some alternative substitution factors (Chen and Zhang, 2019), it undoubtedly remains an essential regulatory factor in the process of ZGA in the mouse embryo.

ZGA is the critical step for the successful development of an embryo (Vastenhouw *et al.*, 2019). To date, very large numbers of researchers have described the role of DNA methylation (Messerschmidt *et al.*, 2014; Iurlaro *et al.*, 2017), post-translational modifications of histones (Dahl *et al.*, 2016; Xia *et al.*, 2019), chromatin accessibility (Wu *et al.*, 2016) and the effect of pioneer transcription factors (Duan *et al.*, 2021; Riesle *et al.*, 2023) and some others in the process of ZGA. Despite a series of epigenetic modifications reportedly associated with ZGA such as DNA modifications and histones, the core issue of embryo development is still gene activation, transcription and translation. Moreover, it has been reported that only a proportion (12–15%) of the genome sequence, instead of the whole genome, is activated with transcription factors in the subsequent process (Rizvi *et al.*, 2017; Eckersley-Maslin *et al.*, 2018). Namely, numerous genes are silenced or repressed, and only few genes are activated in the specific process. In other words, it is meaningful but difficult to determine which genes participate in this process and how they interact with each other during this regulation network.

Therefore, the present study sought to identify candidate genes in ZGA and provide a detailed list of those genes, with the aim of setting up a platform for future exploration of the underlying elusive mechanism. Additionally, we also devoted our efforts to identifying the conserved genes between human and mouse with the aim of generating relevant insights to guide future studies on the ZGA process in human embryos. Taken together, these findings are expected to broaden the knowledge of the underlying mechanisms of ZGA and deepen the understanding of the process of early embryo development.

Materials and methods

Collection of transcriptome data

We comprehensively searched PubMed, Embase and Web of Science databases. All RNA-seq data were retrieved on embryo development with the strategy 'RNA-seq and embryo and *Mus musculus* or *Homo sapiens*', published up to 21 January 2023 as this study aimed to screen candidate genes in ZGA, including minor ZGA and major ZGA, and to distinguish whether they were maternal factors. Therefore, each dataset should have included at least four stages: oocyte stage, zygote stage, early 2-cell stage and late 2-cell stage in mouse. Similarly, in human, the datasets needed to include the oocyte stage, zygote stage or 2-cell stage, 4-cell stage and 8-cell stage. Finally, we used a two-step filter for database retrieval. First, we searched all the embryo transcriptome datasets related to human and mouse that included all four stages mentioned to ensure reliability. Second, we selected the datasets reported in high-quality articles. In addition, the time of publication, and the sequencing platform were also taken into consideration. Finally, only the datasets GSE101571 (Wu *et al.*, 2018; human) and GSE71434 (Zhang *et al.*, 2016; mouse) were chosen for further analysis. The search and selection processes are showed in Figure 1. Also the stages involved in ZGA are presented in Figure 2.

According to our search strategy, 1524 records were searched in mouse and 469 records were searched in human; 43 datasets were included in mouse and 14 datasets were included in human after duplicates were removed. Based on the fact that the preimplantation embryo development contains during several different stages, such as oocyte (GV), zygote (PN5), 2-cell, 4-cell, 8-cell, morula, and blastocyst, in this study we focused on ZGA-related processes during mouse and human preimplantation embryo development. Therefore, the dataset must have contained the transcriptome data of each stage related to ZGA, and it was better to compare the gene expression at the same level, which resulted in only one dataset being eligible ultimately both in mouse and in human.

Identification of candidate genes during ZGA

Generally, the candidate genes were identified by comparing the calculated gene expression levels at different stages. Numerous transcription events occurred during ZGA. Here, we hypothesized that if a gene was significantly upregulated during ZGA, then this gene was highly likely to play a role in this process. Therefore its upregulation might be positively correlated with its importance. Notably, only genes with reliable sequence annotation were allowed for further analysis. The levels of gene expression were calculated based on the RPKM or FPKM values (Log2 RPKM or Log2 FPKM) as the previous study described (Sha *et al.*, 2020). For genes with RPKM or FPKM values that were less than 1, we added +1 to the value of each gene to obtain positive results.

Screening criteria of candidate genes during ZGA

In mouse, minor ZGA completes in the early 2-cell (Early 2C) whereas major ZGA occurs in the late 2-cell (Late 2C). Therefore, in the minor ZGA, we selected out the genes if Expression (Early 2C) > Expression (PN5) +1; for major ZGA, genes would be selected if Expression (Late 2C) > Expression (PN5) +1. In human, ZGA occurs at the 4–8-cell stage. Therefore, we defined the 4- and 8-cell stages as minor ZGA and major ZGA, respectively. Genes for ZGA in human were screened in a similar fashion to that in mouse.

Distinction between maternal and non-maternal genes

Next, we differentiated the identified key genes into maternal and non-maternal categories. We adopted a previously described method (Sha *et al.*, 2020) to stratify the mRNA as maternal if the gene had an RPKM or FPKM value of GV stage >2, whereas those with RPKM or FPKM value at GV stages < 0.5 were considered non-maternal mRNAs (Li *et al.*, 2018; Wu *et al.*, 2018). For convenience, we defined genes corresponding to maternal and non-maternal mRNAs as maternal genes and non-maternal genes, respectively. Last, genes with RPKM or FPKM values between 0.5 and 2 were classified into the uncertain group.

Evaluation of gene age and the identification of conserved genes

New gene emergence is so far assumed to be mostly driven by duplication and divergence of existing genes. Generally, the older the gene, the more conservative it is. Therefore, to identify conserved genes between human and mouse, we first identified the orthologous genes and then assessed the gene age. Previous studies have used phylostratigraphic approaches to classify gene ages and divided human and mouse genes into 20 groups (P1–P20; Domazet-Lošo and Tautz, 2008, 2010; Neme and Tautz, 2013). All loci, based on Ensemble Gene ID, from mouse and human assigned to their respective phylostrata and gene age data can be obtained from published research (Neme and Tautz, 2013). Then the age of genes identified in ZGA in mouse and human was obtained by converting gene names. Each gene could correspond to its gene age. Next, we used gene ages to distinguish conserved genes. Specifically, based on a previous protocol (Gao *et al.*, 2018), genes in the P1–P10 and P11–P20 group were considered older and relatively younger genes, respectively. Those in the P1–P10

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

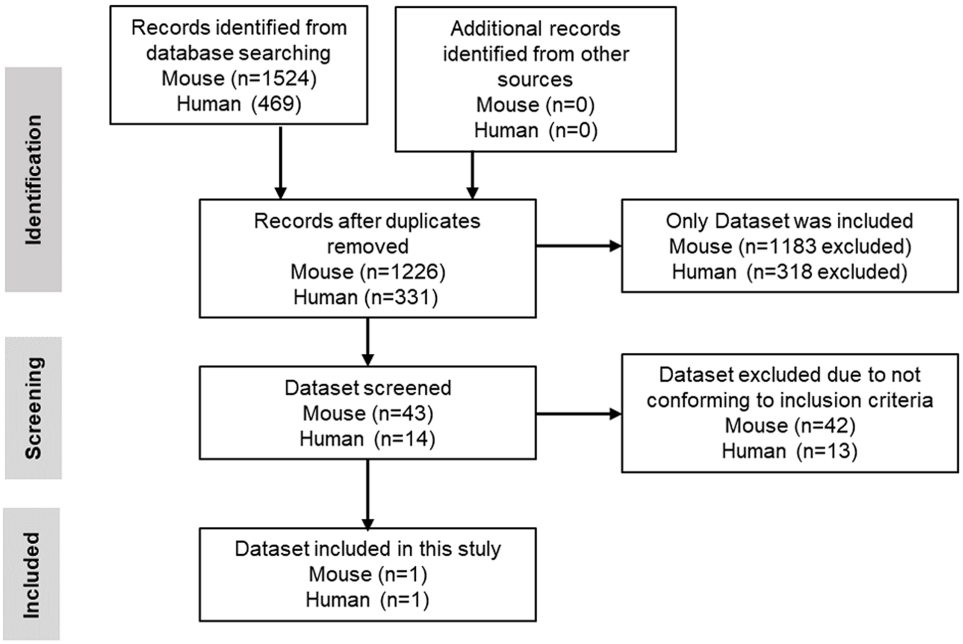

Figure 1. Flow diagram showing the dataset selection process.

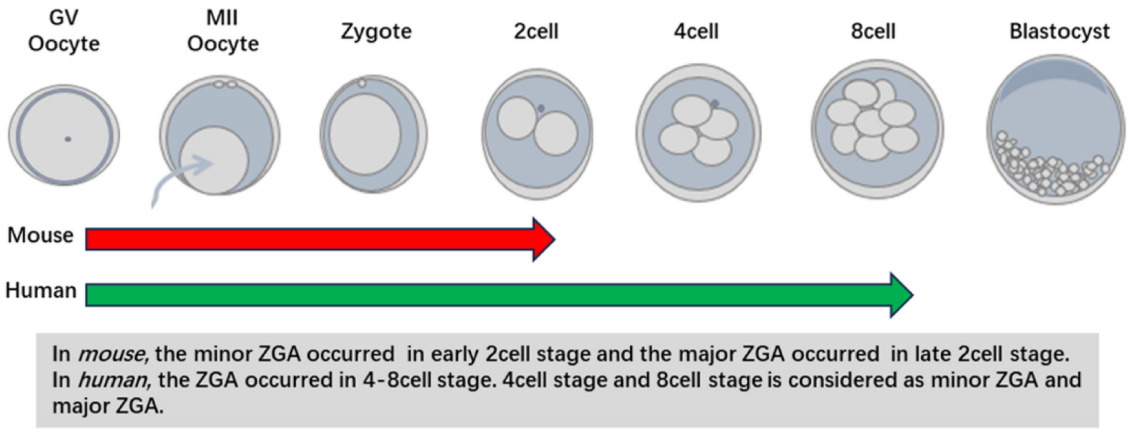

Figure 2. Stages involved in ZGA in mouse and in human.

group were regarded as conserved genes and subjected to further analysis. The gene age data published previously is presented in Table S1.

Pathway analysis

Bioinformatics analysis was mainly focused on signaling pathways. Gene ontology (GO) pathway analysis is functional analysis associating differentially expressed mRNAs with GO categories. Pathway analysis was performed using the 'Pathview' and 'org. Rn.eg.' functions (rat and human genome-wide annotation). The *P*-value of the enriched pathway was derived from the Metascape tool (http://metascape.org/; Zhou *et al.*, 2019). which is a convenient, independent, free site providing comprehensive functional annotation analysis, with the default settings requiring enriched terms to include ≥ 3 candidates, a *P*-value ≤ 0.01, and enrichment factor ≥ 1.5.

Enrichment analysis

Given a gene list, pathway/process enrichment analysis applies the standard accumulative hypergeometric statistical test to identify ontology terms, in which input genes show significant presence. Compared with other GO-based enrichment analysis tools, Metascape provides additional arguably better ontology terms including ones from Broad's Molecular Signatures Database (MSigDB), as well as automatically clusters resultant terms to reduce redundancy.

Enriched terms clustering

As ontology terms, especially within GO, heavily overlap, output terms typically show large degrees of redundancy. Metascape adopts a similar idea as the Database for Annotation, Visualization and Integrated Discovery (DAVID) and automatically clusters all resultant terms into groups based on their similarities. As a result, Metascape can review one term group at a time. Metascape can also

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

uncheck boxes for terms that represent a biological process too broad to be useful so that they are ignored in the export. Terms are hyperlinked to web pages that give their detailed definition.

Results

Identification of candidate genes in minor ZGA and major ZGA in mouse

We identified the key genes expressed during the ZGA process by calculating expression levels [Log2 (RPKM/FPKM+1)] of all annotated genes from the aforementioned databases. Then we further analyzed which ones were involved in both minor ZGA and major ZGA processes, here termed co-expressed factors. Out of 20,000 genes screened in mice, 432 and 3829 were identified as key in minor and major ZGA, respectively. Notably, out of 432 genes in minor ZGA, 352 genes are the co-expressed factors, indicating that the majority of the 432 genes plays a certain role during both minor ZGA and major ZGA (Figure 3A). In addition, several previous studies have demonstrated that early embryonic development is entirely dependent on and driven by maternal factors (Innocenti *et al.*, 2022). Therefore, we further examined whether the factors identified in minor ZGA and major ZGA were maternal genes. So all the genes selected were classified into two groups based on their expression level in the GV oocyte [(FPKM/RPKM > 2 or FPKM/RPKM < 0.5)]. Our results showed that 196 and 85 genes fell into the maternal and non-maternal categories in minor ZGA, while 1988 and 1222 genes were maternal and non-maternal in the major ZGA process (Figure 3B).

Next, we aimed to identify the most important candidate genes in the ZGA process by calculating the difference in expression levels between the two stages in minor ZGA (Early 2C vs PN5) and major ZGA (Late 2C vs PN5). It should be noted that if the expression level of a gene is significantly increased then this gene is more likely to be critical in the ZGA process. As presented in Table 1, genes including *Snora7a*, *Snora81*, *Snora74a*, *Rn4.5s*, *Amd1*, *Zscan4f*, *Zscan4a*, *Zscan4c*, *Zscan4b* and *Zfp352* were identified to be some of the most important candidate genes in minor ZGA, as well as *Mir8099-2*, *Snora78*, *Snora81*, *Mt2*, *Mt1*, *Guca1a*, *Obox3*, *Obox6*, *Vimp*, and *Cdk2ap1* in major ZGA. Interestingly, we also found identical genes between minor and major ZGA gene lists, including *Gcsh* and *Ctsl* (Table S2), indicating that these genes might play critical roles in both minor ZGA and major ZGA. In addition, the top 100 candidate genes in major ZGA are additionally listed in Table S2.

Identification of candidate genes in minor ZGA and major ZGA in human

To explore the candidate genes in ZGA in human, similarly we first evaluated the expression level of each gene [Log2 (RPKM/FPKM+1)] in minor ZGA and major ZGA, then further analyzed which ones were co-expressed factors in both minor ZGA and major ZGA. As shown in Figure 4A, only 60 genes were identified as co-expressed factors, a number that was markedly lower than that identified in mouse (60 vs. 352). We attributed this discrepancy to the small number of minor ZGA (130 vs. 432). Conversely, when it came to the major ZGA, in total, 3566 genes were chosen that were not significantly different from those in mouse (3566 vs. 3829; Figures 3A and 4A).

Next, we also characterized whether the genes selected were maternal genes. As depicted in Figure 4B, indications implied that there were 85 maternal genes and 27 non-maternal genes in minor ZGA, while 1971 and 1130 genes were classified as maternal genes and non-maternal genes, respectively, in major ZGA. Notably, more maternal than non-maternal genes were recorded in both minor ZGA and major ZGA, indicating that the former category plays a certain role at the stage of zygotic genome activation stage, followed by degradation of maternal factors. Then, in major ZGA, we found that the number of both gene categories in human was comparable with that in mouse (1971–1988 for maternal and 1130–1222 for non-maternal genes), indicating that the number of key transcripts across species is approximately consistent during major ZGA, regardless of whether they were from maternal or the non-maternal groups.

Next, we also identified the most important candidate genes in the ZGA process in human. As shown in Table 2, the top 10 candidate genes identified in minor ZGA and major ZGA were outlined. In summary, *SNAR-C3*, *S100A1*,*TMSB10*, *MBD3L2*, *LOC101927482*, *FRG2C*, *G0S2*, *RRAD*, *MBD3L3* and *LXN* in minor ZGA, and *KHDC1L*, *H2AFZ*, *MBD3L2*, *DUXA*, *LOC100506790*, *MBD3L3*, *BIK*, *TCEAL9*, *ZSCAN4* and *NANOGNB* in major ZGA were standout examples. The top 100 candidate genes in major ZGA are additionally listed in Table S3.

Identification of genes conserved between mouse *and* human

Generally, different genes play unique roles in biological evolution, while conserved genes play roughly the same role. Next, we screened the lists of candidate genes related to the ZGA process in both human and mouse and explored the conserved genes. Genes in major ZGA were markedly upregulated but only slightly upregulated in minor ZGA, and more genes were involved in major than minor ZGA; we only included genes identified in major ZGA in subsequent analysis. In addition, we set the fold change for each gene to three-fold to screen out the most significant candidate genes.

As shown in Figure 5A, we compared the candidate genes identified in the two lists, of which 135 genes were considered to be orthologous genes, while 887 and 760 genes were thought to be specific to human and mouse, respectively. Next, we explored the age of all the 135 genes and used the resulting ages to determine which ones were conserved. Considering that the age data (Neme and Tautz, 2013; see Materials and methods for more details) of mouse and human are not identical, we investigated the gene age of the 135 genes with both the two gene age datasets. In total, 115 and 116 genes in mouse and human, respectively, were successfully mapped, while the remainder failed to match. Most of the genes (109 in mouse and 107 in human) were grouped as older genes, with only a few falling into the younger category (Figure 5B), and the identified common genes had a high degree of conservation. A summary of the conserved genes identified here is provided in Table S4.

Signal pathways analyses of the genes identified in the major ZGA

Biological development comprises a complex network of regulatory mechanisms, in which different genes play specific roles. To obtain a more comprehensive understanding of the roles of these genes in major ZGA, we conducted GO and pathway enrichment analyses on all conserved genes ($n = 135$) using Metascape. Results revealed the enrichment of 20 signal pathways, among which those regulating 'rRNA processing in the nucleus and cytosol ($n = 21$)', 'ribonucleoprotein complex biogenesis ($n = 26$)',

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

Table 1. List of candidate genes identified in minor ZGA and major ZGA in mouse*

	Gene	Oocyte	Zygote	Early 2-cell	Late 2-cell	Fold change	Category
Minor ZGA	*Snora7a*	0	**0**	**57533.1**	0	**15.81212966**	**Non-maternal**
	Snora81	0	**0**	**725.19**	574.706	**9.504203253**	**Non-maternal**
	Snora74a	0	**0**	**231.222**	0	**7.859360845**	**Non-maternal**
	Rn4.5s	0	**0**	**46.8939**	55.7259	**5.581770014**	**Non-maternal**
	Amd1	0.127472	**0.666257**	**59.9236**	129.917	**5.192318353**	**Non-maternal**
	Zscan4f	0.449601	**1.59728**	**93.351**	159.21	**5.182964351**	**Non-maternal**
	Zscan4a	0.0984696	**0.457244**	**39.3866**	69.8893	**4.792562329**	**Non-maternal**
	Zscan4c	0.595843	**1.71532**	**70.3166**	101.664	**4.71504379**	Uncertain
	Zscan4b	0.0945571	**0.288384**	**32.4962**	51.5039	**4.700362883**	**Non-maternal**
	Zfp352	0	**0.622242**	**39.0539**	224.849	**4.625881766**	Uncertain
Major ZGA	*Mir8099-2*	0	**0**	0	**64838**	**15.98457422**	**Non-maternal**
	Snora78	0	**0**	0	**1507.85**	**10.55923367**	**Non-maternal**
	Snora81	0	**0**	725.19	**574.706**	**9.169188438**	**Non-maternal**
	Mt2	1.13292	**0.798252**	11.9356	**813.017**	**8.822319905**	Uncertain
	Mt1	0.960658	**0.844275**	9.65903	**762.392**	**8.693226465**	Uncertain
	Guca1a	0	**0**	8.6284	**327.534**	**8.359898873**	**Non-maternal**
	Obox3	0.042464	**0.364362**	12.6351	**446.529**	**8.357610883**	**Non-maternal**
	Obox6	0.267919	**0.0654915**	4.30065	**309.995**	**8.189228491**	**Non-maternal**
	Vimp	**16.7101**	**0.152345**	2.53189	**259.901**	**7.822785954**	**Maternal**
	Cdk2ap1	**3.45267**	**0.500025**	2.95344	**321.446**	**7.747927217**	**Maternal**

*Table shows the RPKM value for each stage of the genes identified; values for the stages associated with the ZGA are bold.

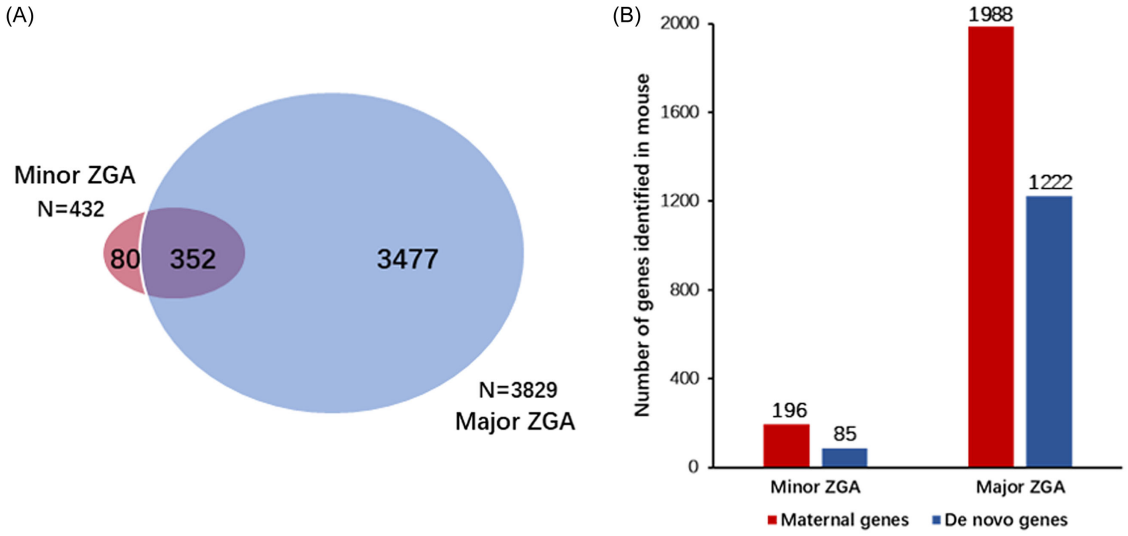

Figure 3. Candidate genes identified in minor ZGA and major ZGA in mouse. (A) Candidate genes identified in minor ZGA and major ZGA in mouse. (B) Maternal genes and non-maternal genes identified in minor ZGA and major ZGA in mouse.

'ribonucleoprotein complex assembly ($n = 12$)' and 'ribosome large subunit biogenesis ($n = 9$)' were significantly enriched (Figure 6A). Interestingly, all these pathways were related to the ribosome, and many more genes were contained in these pathways than the others, indicating that the genes associated with the ribosome and rRNA had made a large contribution to major ZGA; this observation is consistent with results previously reported (Shen *et al.*, 2022).

In addition, GO enrichment analysis of the specific genes ($n = 760$) identified in mouse were significantly enriched in 'ribonucleoprotein complex biogenesis', 'ribonucleoprotein complex subunit organization', and 'mRNA processing' (Figure 6B). In

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

Table 2. List of candidate genes identified in two processes in ZGA in human*

	Gene	Oocyte	2-cell	4-cell	8-cell	Fold change	Category
Minor ZGA	*SNAR-C3*	0	**0**	**15.8134**	0	4.07153959	**Non-maternal**
	S100A11	19.20703333	**1.1426865**	**21.65550085**	540.0815	3.402368698	**Maternal**
	TMSB10	397.0661667	**8.559745**	**96.1312695**	387.017	3.344891777	**Maternal**
	MBD3L2	0	**0.808905**	**14.362915**	2993.645	3.086263435	**Non-maternal**
	LOC101927482	6.4735	**0.2215975**	**9.2587**	4.37996	3.070007002	**Maternal**
	FRG2C	0.006915133	**0.186125107**	**7.677665**	7.36316	2.871050705	**Non-maternal**
	G0S2	2.379845	**0.09715885**	**6.0443**	0.93966	2.682683933	**Maternal**
	RRAD	2.721403333	**0**	**4.868852**	0.995944	2.553078327	**Maternal**
	MBD3L3	0	**0.8689175**	**9.90054**	1600.61	2.544124816	**Non-maternal**
	LXN	8.770456667	**0**	**4.498135**	2.5230725	2.458942331	**Maternal**
Major ZGA	*KHDC1L*	0	**0.7566055**	6.128905	**27582.55**	13.9387303	**Non-maternal**
	H2AFZ	31.80910333	**2.462925**	8.47638	**12132.115**	11.77467123	**Maternal**
	MBD3L2	0	**0.808905**	14.362915	**2993.645**	10.69305263	**Non-maternal**
	DUXA	0.008167267	**0.03839195**	1.43304495	**1297.26**	10.28801252	**Non-maternal**
	LOC100506790	0	**0**	1.513743	**876.532**	9.777307925	**Non-maternal**
	MBD3L3	0	**0.8689175**	9.90054	**1600.61**	9.743104286	**Non-maternal**
	BIK	8.314286667	**1.083791**	0.636949	**1747.475**	9.712670867	**Maternal**
	TCEAL9	4.863233333	**0.160115**	3.92782	**956.1925**	9.688397459	**Maternal**
	ZSCAN4	0.22626	**1.1185015**	3.7883135	**1595.87**	9.557987003	**Non-maternal**
	NANOGNB	0	**0**	0.085399	**719.716**	9.493287064	**Non-maternal**

*Table shows the RPKM value for each stage of the genes identified; values for the stages associated with the ZGA are bold.

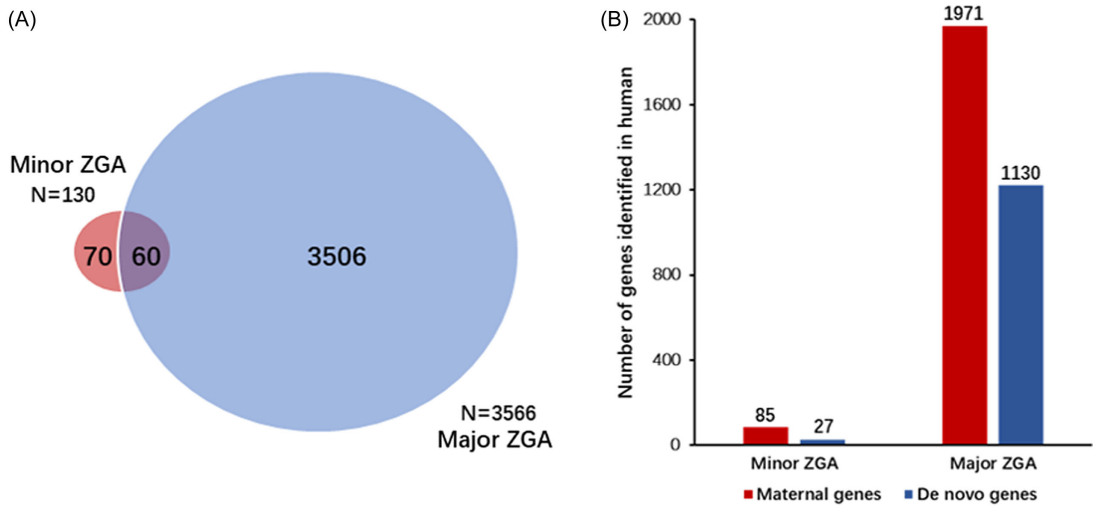

Figure 4. Candidate genes identified in minor ZGA and major ZGA in human. (A) Candidate genes identified in minor ZGA and major ZGA in human. (B) Maternal genes and non-maternal genes identified in minor ZGA and major ZGA in human.

human, the specific genes ($n = 887$) identified are predominately enriched in 'metabolism of RNA', 'ribonucleoprotein complex biogenesis', 'mitochondrial gene expression', and 'mitochondrion organization' (Figure 6C). Overall, it is worth suggesting that the ribosome-related signal pathway is a major signal pathway in major ZGA in both human and mouse.

Discussion

Recent research evidence has confirmed that activation of the zygotic genome is not a single event, but a process through which embryo transcripts are constantly activated. The ZGA process is divided into two stages based on the level of RNA synthesis. The first large-scale synthesis of RNA during embryonic development

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

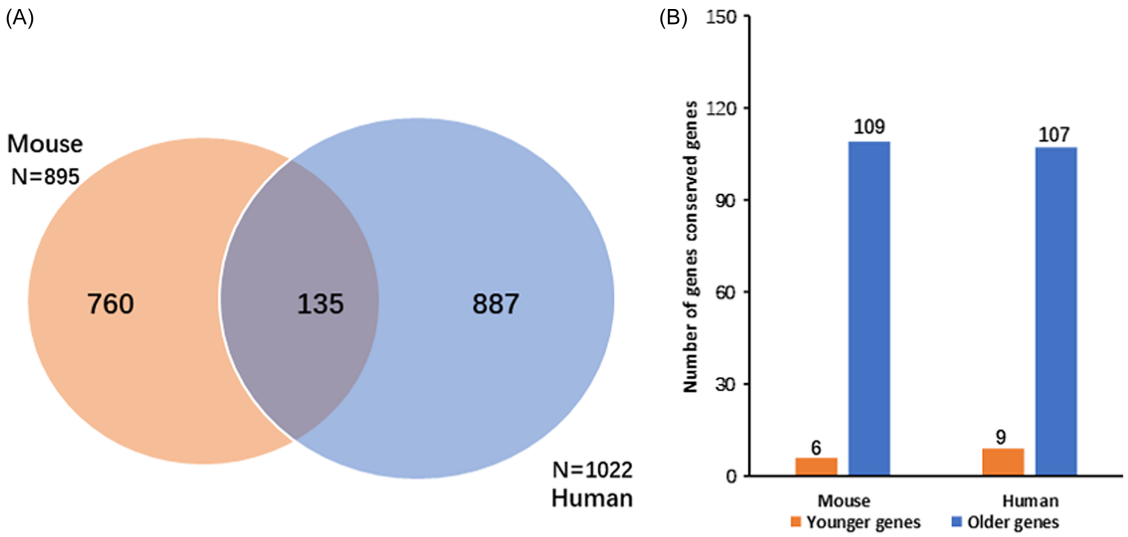

Figure 5. Conserved genes in major ZGA between human and mouse. (A) Candidate genes identified in major ZGA in mouse and human. (B) Distribution of gene age of the orthologous genes.

is called major ZGA, which is followed by a preceding small wave named minor ZGA (Hamatani *et al.*, 2004). The two stages differ in both the number and content of transcripts. Researchers have, for a long time, focused on major ZGA while ignoring the role of minor ZGA. However, it should be noted that major ZGA cannot be successfully activated by inhibition of minor ZGA, namely the inhibition of minor ZGA prevents major ZGA. However, when the inhibition is reversed, transcription activities are observed and characterized as minor rather than major ZGA (Abe *et al.*, 2018). Consequently, both ZGA's main and secondary waves play a crucial role in embryo development.

How does the ZGA program occur? How many genes are important for the ZGA? We are trying to answer these questions. In the present study, we identified the key genes expressed in minor and major ZGA across both human and mouse systems. It should be emphasized that 1222 non-maternal genes were screened from more than 20,000 genes in mouse in our study, which is roughly the same as the number ($n = 1312$; Li *et al.*, 2018) of the genes identified in the previous work, affirming the reliability of our results. Compared with that research, we additionally identified both non-maternal and maternal genes across minor ZGA stages (top 10 on the list) and major ZGA stages (top 100 on the list). Conversely, to further confirm the reliability of our results, we also compared the candidate genes identified during major ZGA with two additional datasets, GSE53386 (Fan *et al.*, 2015) and GSE71257 (Yu *et al.*, 2016). It is worth noting that the majority of the top 100 genes (Table S2) could be successfully matched in Table S5 based on datasets GSE53386 and GSE71257, with the expression levels increased significantly. The reason these datasets were not included in this comprehensive analysis is because they only contained data for three stages in mouse, including oocytes, zygotes and late 2-cell stages and not for the early 2-cell stages. Considering the possible limitations of the results based on a single dataset, with the same method mentioned in this paper, we next calculated and compared the gene expression level of each gene at different stages in the other two datasets (GSE53386 and GSE71257) and screened out the upregulated genes in major ZGA in mouse. The results showed that 3987 and 3541 genes were upregulated in major ZGA, which is similar to the results in our study (3987 in GSE53386, 3541 in GSE71257, and 3829 in our study), and most of the top 100 genes identified in major ZGA can be mapped with the genes identified based on the other two datasets (Table S5).

Generally, most of the current knowledge regarding early mammalian development mainly comes from mouse, due to this system's characteristics of rapid reproduction, easy obtainment, as well as fewer ethical concerns. However, biological development is stage specific and the timing of the ZGA is not exactly the same. For example, ZGA occurs at the 4–8-cell stage in human (Li *et al.*, 2010; Wu *et al.*, 2016), but at the 8–16-cell stage in cattle and sheep (Schultz, 2002; Chen *et al.*, 2012). This indicates that the process in human cannot be directly inferred from that in mouse. However, extensive experiments were limited to be carried out due to the preciousness and scarcity of the embryos. To overcome this problem, we identified the conserved genes in ZGA and presented the gene list with the aim of pinpointing targets for future exploration of the underlying mechanisms in mouse, and prediction of their role in human embryonic development. In a word, our findings provide relevant insights to guide further explorations on human embryonic development.

Although our findings are encouraging, the study had some limitations. First, we analyzed gene expression using the FPKM or RPKM values provided in the database, which might introduce some errors. However, as more than 40,000 genes were obtained in this study, it is difficult to experimentally analyze gene expression using methods such as quantitative real-time polymerase chain reaction (qPCR). Second, as some genes on our list have demonstrated their important roles in recently published work, such as the Mt1/Mt2 (Shi *et al.*, 2018), *Obox* (Ji *et al.*, 2023), *Zfp352* (Mwalilino *et al.*, 2023), *Zscan5b* (Ogawa *et al.*, 2019), *Zscan4* (Cheng *et al.*, 2020; Srinivasan *et al.*, 2020) in mouse and *DUX4* (Vuoristo *et al.*, 2022), *ZSCAN4* (Vuoristo *et al.*, 2022), and *NANOGNB* (Dunwell and Holland, 2017) in human (Table 3), we did not pick any other candidate genes for functional validation. Moreover, it was unreliable to validate one by one. Third, as mentioned above, ZGA proceeds in two phases, minor and major ZGA, and the pattern of gene expression is dramatically changed between these two phases (Abe *et al.*, 2015; Yamamoto and Aoki,

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

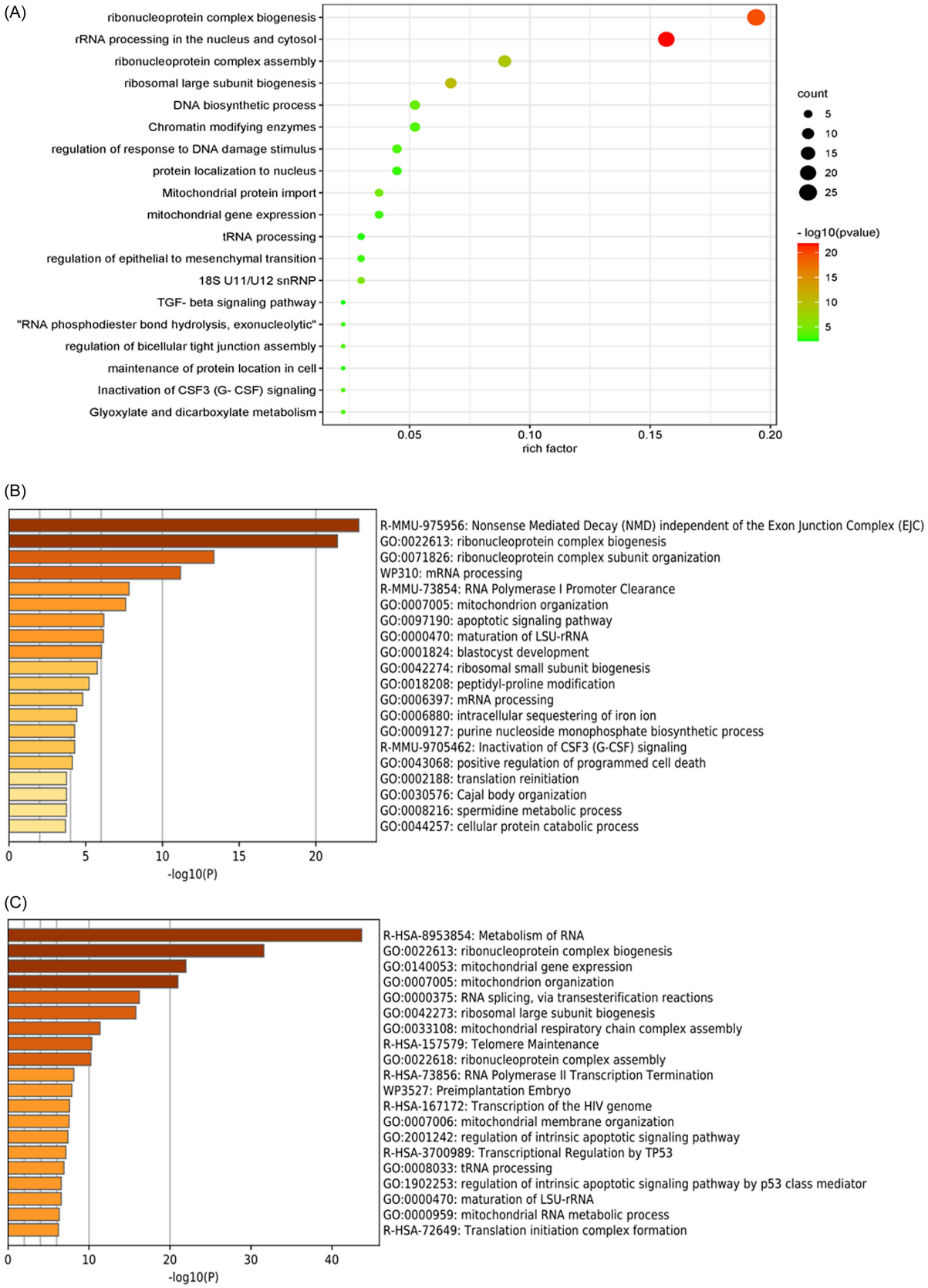

Figure 6. Signaling pathway analyses of genes identified in the major ZGA. (A) Signal pathway analysis of the conserved genes identified in the major ZGA. (B) Signaling pathway analysis of the specific genes identified in the major ZGA in mouse. (C) Signalimg pathway analysis of the specific genes identified in the major ZGA in human.

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

Table 3. Genes on our list that had been already validated in other studies

	Gene	PMID	Year	Journal
	Mt1, Mt2	30074260	2018	*Journal of Cellular Biochemistry*
	Obox3	37459895	2023	*Nature*
Mouse	*Zfp352*	37778747	2023	*Genes to Cells*
	Zscan5b	31155506	2019	*Stem Cell Reports*
	Zscan4f	32668244	2020	*Cell Reports*
	DUXA	35402882	2022	*iScience*
Human	*ZSCAN4*	35402882	2022	*iScience*
	NANOGNB	28446706	2017	*Open Biology*

2017). So, in this research, if the expression level of a gene was significantly increased, this gene was considered to be potentially a key factor in the ZGA process. However, some factors must be admitted, such as the expression level showing just a slight change during ZGA, that may also be of great importance to ZGA. Last, the candidate genes identified may not only play a critical role in ZGA, but also may be of great significance in early embryonic development, such as the formation of totipotent blastocysts. For instance, genes *Mga* (Washkowitz *et al.*, 2015) and *Myc* (Wang *et al.*, 2010; Wan *et al.*, 2013) whose expression levels were increased in ZGA, were also reported to have roles in maintaining pluripotency in the mammalian embryo.

Overall, we identified potential key regulators in minor ZGA and major ZGA both in human and mouse and generated two respective lists of those genes. Moreover, we also made a list of conserved genes in major ZGA, and revealed that their functions were mainly related to ribosomal RNA in the biological process. In summary, our findings provided a platform for future studies on ZGA, and made it more convenient, rapid and easier for other researchers to select one or several genes from the whole genome for subsequent research, and contribute to revealing candidate genes and the regulatory mechanisms in this special process.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0967199423000631

Acknowledgements. We thank all participants for the work, members of Jiawei Xu for the research design, Wenbo Li, Shuxia Ma, and Dong Fang for database supervision and validation. Other authors contributed to the manuscript revision, read it, and all the authors approved the submitted version.

Funding statement. This study was supported by the National Natural Science Foundation of China (31870817), the National Key R&D Programme of China (2019YFA0110900 and 2019YFA0802200), and the Science and Natural Science Foundation of Henan Province (22100026/20).

Competing interests. The authors declare that they have no competing interests.

References

Abe, K., Yamamoto, R., Franke, V., Cao, M., Suzuki, Y., Suzuki, M. G., Vlahovicek, K., Svoboda, P., Schultz, R. M. and Aoki, F. (2015). The first murine zygotic transcription is promiscuous and uncoupled from splicing and 3′ processing. *EMBO Journal*, **34**(11), 1523–1537. doi: 10.15252/embj.201490648

Abe, K. I., Funaya, S., Tsukioka, D., Kawamura, M., Suzuki, Y., Suzuki, M. G., Schultz, R. M. and Aoki, F. (2018). Minor zygotic gene activation is essential for mouse preimplantation development. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(29), E6780–E6788. doi: 10.1073/pnas.1804309115

Chen, Z. and Zhang, Y. (2019). Loss of DUX causes minor defects in zygotic genome activation and is compatible with mouse development. *Nature Genetics*, **51**(6), 947–951. doi: 10.1038/s41588-019-0418-7

Chen, C. H., Chang, W. F., Liu, C. C., Su, H. Y., Shyue, S. K., Cheng, W. T., Chen, Y. E., Wu, S. C., Du, F., Sung, L. Y. and Xu, J. (2012). Spatial and temporal distribution of Oct-4 and acetylated H4K5 in rabbit embryos. *Reproductive Biomedicine Online*, **24**(4), 433–442. doi: 10.1016/j.rbmo.2012.01.001

Cheng, Z. L., Zhang, M. L., Lin, H. P., Gao, C., Song, J. B., Zheng, Z., Li, L., Zhang, Y., Shen, X., Zhang, H., Huang, Z., Zhan, W., Zhang, C., Hu, X., Sun, Y. P., Jiang, L., Sun, L., Xu, Y., Yang, C., *et al.* (2020). The Zscan4-Tet2 transcription nexus regulates metabolic rewiring and enhances proteostasis to promote reprogramming. *Cell Reports*, **32**(2), 107877. doi: 10.1016/j.celrep.2020.107877

Dahl, J. A., Jung, I., Aanes, H., Greggains, G. D., Manaf, A., Lerdrup, M., Li, G., Kuan, S., Li, B., Lee, A. Y., Preissl, S., Jermstad, I., Haugen, M. H., Suganthan, R., Bjørås, M., Hansen, K., Dalen, K. T., Fedorcsak, P., Ren, B. and Klungland, A. (2016). Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature*, **537**(7621), 548–552. doi: 10.1038/nature19360

De Iaco, A., Planet, E., Coluccio, A., Verp, S., Duc, J. and Trono, D. (2017). DUX-family transcription factors regulate zygotic genome activation in placental mammals. *Nature Genetics*, **49**(6), 941–945. doi: 10.1038/ng.3858

Domazet-Lošo, T. and Tautz, D. (2008). An ancient evolutionary origin of genes associated with human genetic diseases. *Molecular Biology Evolution*, **25**(12), 2699–2707. doi: 10.1093/molbev/msn214

Domazet-Lošo, T. and Tautz, D. (2010). A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns. *Nature*, **468**(7325), 815–818. doi: 10.1038/nature09632

Driever, W. and Nüsslein-Volhard, C. (1988). The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell*, **54**(1), 95–104. doi: 10.1016/0092-8674(88)90183-3

Duan, J., Rieder, L., Colonnetta, M. M., Huang, A., McKenney, M., Watters, S., Deshpande, G., Jordan, W., Fawzi, N. and Larschan, E. (2021). CLAMP and Zelda function together to promote *Drosophila* zygotic genome activation. *eLife*, **10**. doi: 10.7554/eLife.69937

Dunwell, T. L. and Holland, P. W. H. (2017). A sister of NANOG regulates genes expressed in pre-implantation human development. *Open Biology*, **7**(4). doi: 10.1098/rsob.170027

Eckersley-Maslin, M. A., Alda-Catalinas, C. and Reik, W. (2018). Dynamics of the epigenetic landscape during the maternal-to-zygotic transition. *Nature Reviews. Molecular Cell Biology*, **19**(7), 436–450. doi: 10.1038/s41580-018-0008-z

Eisa, A. A., Bang, S., Crawford, K. J., Murphy, E. M., Feng, W. W., Dey, S., Wells, W., Kon, N., Gu, W., Mehlmann, L. M., Vijayaraghavan, S. and Kurokawa, M. (2020). X-linked *Huwe1* is essential for oocyte maturation and preimplantation embryo development. *iScience*, **23**(9), 101523. doi: 10.1016/j.isci.2020.101523

Fan, X., Zhang, X., Wu, X., Guo, H., Hu, Y., Tang, F. and Huang, Y. (2015). Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos. *Genome Biology*, **16**(1), 148. doi: 10.1186/s13059-015-0706-1

Gao, L., Wu, K., Liu, Z., Yao, X., Yuan, S., Tao, W., Yi, L., Yu, G., Hou, Z., Fan, D., Tian, Y., Liu, J., Chen, Z. J. and Liu, J. (2018). Chromatin accessibility landscape in human early embryos and its association with evolution. *Cell*, **173**(1), 248–259.e15 e215. doi: 10.1016/j.cell.2018.02.028

Hamatani, T., Carter, M. G., Sharov, A. A. and Ko, M. S. (2004). Dynamics of global gene expression changes during mouse preimplantation development. *Developmental Cell*, **6**(1), 117–131. doi: 10.1016/s1534-5807(03)00373-3

Hendrickson, P. G., Doráis, J. A., Grow, E. J., Whiddon, J. L., Lim, J. W., Wike, C. L., Weaver, B. D., Pflueger, C., Emery, B. R., Wilcox, A. L., Nix, D. A., Peterson, C. M., Tapscott, S. J., Carrell, D. T. and Cairns, B. R. (2017). Conserved roles of mouse DUX and human DUX4 in activating

cleavage-stage genes and MERVL/HERVL retrotransposons. *Nature Genetics*, **49**(6), 925–934. doi: 10.1038/ng.3844

Hu, Z., Tan, D. E. K., Chia, G., Tan, H., Leong, H. F., Chen, B. J., Lau, M. S., Tan, K. Y. S., Bi, X., Yang, D., Ho, Y. S., Wu, B., Bao, S., Wong, E. S. M. and Tee, W. W. (2020). Maternal factor NELFA drives a 2C-like state in mouse embryonic stem cells. *Nature Cell Biology*, **22**(2), 175–186. doi: 10.1038/s41556-019-0453-8

Innocenti, F., Fiorentino, G., Cimadomo, D., Soscia, D., Garagna, S., Rienzi, L., Ubaldi, F. M., Zuccotti, M. and SIERR. (2022). Maternal effect factors that contribute to oocytes developmental competence: An update. *Journal of Assisted Reproduction and Genetics*, **39**(4), 861–871. doi: 10.1007/s10815-022-02434-y

Iurlaro, M., von Meyenn, F. and Reik, W. (2017). DNA methylation homeostasis in human and mouse development. *Current Opinion in Genetics and Development*, **43**, 101–109. doi: 10.1016/j.gde.2017.02.003

Ji, S., Chen, F., Stein, P., Wang, J., Zhou, Z., Wang, L., Zhao, Q., Lin, Z., Liu, B., Xu, K., Lai, F., Xiong, Z., Hu, X., Kong, T., Kong, F., Huang, B., Wang, Q., Xu, Q., Fan, Q., *et al.* (2023). OBOX regulates mouse zygotic genome activation and early development. *Nature*, **620**(7976), 1047–1053. doi: 10.1038/s41586-023-06428-3

Lee, M. T., Bonneau, A. R., Takacs, C. M., Bazzini, A. A., DiVito, K. R., Fleming, E. S. and Giraldez, A. J. (2013). Nanog, Pou5f1 and SoxB1 activate zygotic gene expression during the maternal-to-zygotic transition. *Nature*, **503**(7476), 360–364. doi: 10.1038/nature12632

Li, L., Zheng, P. and Dean, J. (2010). Maternal control of early mouse development. *Development*, **137**(6), 859–870. doi: 10.1242/dev.039487

Li, Y., Zhang, Z., Chen, J., Liu, W., Lai, W., Liu, B., Li, X., Liu, L., Xu, S., Dong, Q., Wang, M., Duan, X., Tan, J., Zheng, Y., Zhang, P., Fan, G., Wong, J., Xu, G. L., Wang, Z., *et al.* (2018). Stella safeguards the oocyte methylome by preventing *de novo* methylation mediated by DNMT1. *Nature*, **564**(7734), 136–140. doi: 10.1038/s41586-018-0751-5

Messerschmidt, D. M., Knowles, B. B. and Solter, D. (2014). DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. *Genes and Development*, **28**(8), 812–828. doi: 10.1101/gad.234294.113

Moore, G. P., Lintern-Moore, S., Peters, H. and Faber, M. (1974). RNA synthesis in the mouse oocyte. *Journal of Cell Biology*, **60**(2), 416–422. doi: 10.1083/jcb.60.2.416

Mwalilino, L., Yamane, M., Ishiguro, K. I., Usuki, S., Endoh, M. and Niwa, H. (2023). The role of Zfp352 in the regulation of transient expression of 2-cell specific genes in mouse embryonic stem cells. *Genes to Cells: Devoted to Molecular and Cellular Mechanisms*, **28**(12), 831–844. doi: 10.1111/gtc.13070

Neme, R. and Tautz, D. (2013). Phylogenetic patterns of emergence of new genes support a model of frequent *de novo* evolution. *BMC Genomics*, **14**, 117. doi: 10.1186/1471-2164-14-117

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature*, **287**(5785), 795–801. doi: 10.1038/287795a0

Ogawa, S., Yamada, M., Nakamura, A., Sugawara, T., Nakamura, A., Miyajima, S., Harada, Y., Ooka, R., Okawa, R., Miyauchi, J., Tsumura, H., Yoshimura, Y., Miyado, K., Akutsu, H., Tanaka, M., Umezawa, A. and Hamatani, T. (2019). Zscan5b deficiency impairs DNA damage response and causes chromosomal aberrations during mitosis. *Stem Cell Reports*, **12**(6), 1366–1379. doi: 10.1016/j.stemcr.2019.05.002

Riesle, A. J., Gao, M., Rosenblatt, M., Hermes, J., Hass, H., Gebhard, A., Veil, M., Grüning, B., Timmer, J. and Onichtchouk, D. (2023). Activator-blocker model of transcriptional regulation by pioneer-like factors. *Nature Communications*, **14**(1), 5677. doi: 10.1038/s41467-023-41507-z

Rizvi, A. H., Camara, P. G., Kandror, E. K., Roberts, T. J., Schieren, I., Maniatis, T. and Rabadan, R. (2017). Single-cell topological RNA-seq analysis reveals insights into cellular differentiation and development. *Nature Biotechnology*, **35**(6), 551–560. doi: 10.1038/nbt.3854

Schultz, R. M. (2002). The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Human Reproduction Update*, **8**(4), 323–331. doi: 10.1093/humupd/8.4.323

Sha, Q. Q., Zhang, J. and Fan, H. Y. (2019). A story of birth and death: mRNA translation and clearance at the onset of maternal-to-zygotic transition in mammals. *Biology of Reproduction*, **101**(3), 579–590. doi: 10.1093/biolre/ioz012

Sha, Q. Q., Zhu, Y. Z., Li, S., Jiang, Y., Chen, L., Sun, X. H., Shen, L., Ou, X. H. and Fan, H. Y. (2020) Characterization of zygotic genome activation-dependent maternal mRNA clearance in mouse. *Nucleic Acids Research*, **48**(2), 879–894. doi: 10.1093/nar/gkz1111

Shen, W., Gong, B., Xing, C., Zhang, L., Sun, J., Chen, Y., Yang, C., Yan, L., Chen, L., Yao, L., Li, G., Deng, H., Wu, X. and Meng, A. (2022). Comprehensive maturity of nuclear pore complexes regulates zygotic genome activation. *Cell*, **185**(26), 4954–4970.e20. doi: 10.1016/j.cell.2022.11.011

Shi, F., Li, H., Wang, E., Chen, Z. J. and Zhang, C. (2018). Melatonin reduces two-cell block via nonreceptor pathway in mice. *Journal of Cellular Biochemistry*, **119**(11), 9380–9393. doi: 10.1002/jcb.27255

Shpargel, K. B., Starmer, J., Yee, D., Pohlers, M. and Magnuson, T. (2014). KDM6 demethylase independent loss of histone H3 lysine 27 trimethylation during early embryonic development. *PLOS Genetics*, **10**(8), e1004507. doi: 10.1371/journal.pgen.1004507

Srinivasan, R., Nady, N., Arora, N., Hsieh, L. J., Swigut, T., Narlikar, G. J., Wossidlo, M. and Wysocka, J. (2020). Zscan4 binds nucleosomal microsatellite DNA and protects mouse two-cell embryos from DNA damage. *Science Advances*, **6**(12), eaaz9115. doi: 10.1126/sciadv.aaz9115

Tadros, W. and Lipshitz, H. D. (2009). The maternal-to-zygotic transition: A play in two acts. *Development*, **136**(18), 3033–3042. doi: 10.1242/dev.033183

Vastenhouw, N. L., Cao, W. X. and Lipshitz, H. D. (2019). The maternal-to-zygotic transition revisited. *Development*, **146**(11). doi: 10.1242/dev.161471

Vuoristo, S., Bhagat, S., Hydén-Granskog, C., Yoshihara, M., Gawriyski, L., Jouhilahti, E. M., Ranga, V., Tamirat, M., Huhtala, M., Kirjanov, I., Nykänen, S., Krjutškov, K., Damdimopoulos, A., Weltner, J., Hashimoto, K., Recher, G., Ezer, S., Paluoja, P., Paloviita, P., *et al.* (2022). DUX4 is a multifunctional factor priming human embryonic genome activation. *iScience*, **25**(4), 104137. doi: 10.1016/j.isci.2022.104137

Wan, M., Liang, J., Xiong, Y., Shi, F., Zhang, Y., Lu, W., He, Q., Yang, D., Chen, R., Liu, D., Barton, M. and Songyang, Z. (2013). The trithorax group protein Ash2l is essential for pluripotency and maintaining open chromatin in embryonic stem cells. *Journal of Biological Chemistry*, **288**(7), 5039–5048. doi: 10.1074/jbc.M112.424515

Wang, J., Zhang, M., Zhang, Y., Kou, Z., Han, Z., Chen, D. Y., Sun, Q. Y. and Gao, S. (2010). The histone demethylase JMJD2C is stage-specifically expressed in preimplantation mouse embryos and is required for embryonic development. *Biology of Reproduction*, **82**(1), 105–111. doi: 10.1095/biolreprod.109.078055

Washkowitz, A. J., Schall, C., Zhang, K., Wurst, W., Floss, T., Mager, J. and Papaioannou, V. E. (2015). Mga is essential for the survival of pluripotent cells during peri-implantation development. *Development*, **142**(1), 31–40. doi: 10.1242/dev.111104

Whiddon, J. L., Langford, A. T., Wong, C. J., Zhong, J. W. and Tapscott, S. J. (2017). Conservation and innovation in the DUX4-family gene network. *Nature Genetics*, **49**(6), 935–940. doi: 10.1038/ng.3846

Wu, J., Huang, B., Chen, H., Yin, Q., Liu, Y., Xiang, Y., Zhang, B., Liu, B., Wang, Q., Xia, W., Li, W., Li, Y., Ma, J., Peng, X., Zheng, H., Ming, J., Zhang, W., Zhang, J., Tian, G., *et al.* (2016). The landscape of accessible chromatin in mammalian preimplantation embryos. *Nature*, **534**(7609), 652–657. doi: 10.1038/nature18606

Wu, J., Xu, J., Liu, B., Yao, G., Wang, P., Lin, Z., Huang, B., Wang, X., Li, T., Shi, S., Zhang, N., Duan, F., Ming, J., Zhang, X., Niu, W., Song, W., Jin, H., Guo, Y., Dai, S., *et al.* (2018). Chromatin analysis in human early development reveals epigenetic transition during ZGA. *Nature*, **557**(7704), 256–260. doi: 10.1038/s41586-018-0080-8

Xia, W., Xu, J., Yu, G., Yao, G., Xu, K., Ma, X., Zhang, N., Liu, B., Li, T., Lin, Z., Chen, X., Li, L., Wang, Q., Shi, D., Shi, S., Zhang, Y., Song, W., Jin, H., Hu, L., *et al.* (2019). Resetting histone modifications during human parental-to-zygotic transition. *Science*, **365**(6451), 353–360. doi: 10.1126/science.aaw5118

https://doi.org/10.1017/S0967199423000631 Published online by Cambridge University Press

Yamamoto, R. and Aoki, F. (2017). A unique mechanism regulating gene expression in 1-cell embryos. *Journal of Reproduction and Development*, **63**(1), 9–11. doi: 10.1262/jrd.2016-133

Yu, C., Ji, S. Y., Sha, Q. Q., Dang, Y., Zhou, J. J., Zhang, Y. L., Liu, Y., Wang, Z. W., Hu, B., Sun, Q. Y., Sun, S. C., Tang, F. and Fan, H. Y. (2016). BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. *Nature Structural and Molecular Biology*, **23**(5), 387–394. doi: 10.1038/nsmb.3204

Zhang, B., Zheng, H., Huang, B., Li, W., Xiang, Y., Peng, X., Ming, J., Wu, X., Zhang, Y., Xu, Q., Liu, W., Kou, X., Zhao, Y., He, W., Li, C., Chen, B., Li, Y., Wang, Q., Ma, J., *et al.* (2016). Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature*, **537**(7621), 553–557. doi: 10.1038/nature19361

Zhang, J. M., Hou, W. B., Du, J. W., Zong, M., Zheng, K. L., Wang, W. J., Wang, J. Q., Zhang, H., Mu, Y. S., Yin, Z., Ding, C. M., Sun, Q. Y., Liu, Z. H. and Kong, Q. R. (2020). Argonaute 2 is a key regulator of maternal mRNA degradation in mouse early embryos. *Cell Death Discovery*, **6**(1), 133. doi: 10.1038/s41420-020-00368-x

Zheng, W. and Liu, K. (2012). Maternal control of mouse preimplantation development. *Results and Problems in Cell Differentiation*, **55**, 115–139. doi: 10.1007/978-3-642-30406-4_7

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C. and Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, **10**(1), 1523. doi: 10.1038/s41467-019-09234-6