**Article**

**Brd4 and P300 Confer Transcriptional Competency during Zygotic Genome Activation**

**Graphical Abstract**

**Highlights**
- Live imaging reveals that the first zygotic transcription is stochastic in zebrafish
- Transcriptional competency depends on developmental time, independent of cell division
- P300 and Brd4 activity are necessary for genome activation and zygotic development
- P300 and BRD4 are sufficient to trigger premature genome activation

**Authors**
Shun Hang Chan, Yin Tang, Liyun Miao, ..., Ariel A. Bazzini, Miguel A. Moreno-Mateos, Antonio J. Giraldez

**Correspondence**
mamormat@upo.es (M.A.M.-M.), antonio.giraldez@yale.edu (A.J.G.)

**In Brief**
Genome activation after fertilization is a cornerstone of development. Chan et al. show that the writers and readers of histone acetylation, p300 and Brd4, are limiting factors required to activate the genome. Genome activation is characterized by a gain of H3K27Ac acetylation and a stochastic activation at the first transcribed locus miR-430.

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Brd4 and P300 Confer Transcriptional Competency during Zygotic Genome Activation

Shun Hang Chan,1 Yin Tang,1 Liyun Miao,1 Hiba Darwich-Codore,1 Charles E. Vejnar,1 Jean-Denis Beaudoin,1 Damir Musaev,1 Juan P. Fernandez,1 Maria D.J. Benitez,1 Ariel A. Bazzini,1,4,6 Miguel A. Moreno-Mateos,1,5,* and Antonio J. Giraldez1,2,3,7,*

1Department of Genetics, Yale University School of Medicine, New Haven, CT 06510, USA
2Stem Cell Center, Yale University School of Medicine, New Haven, CT 06510, USA
3Yale Cancer Center, Yale University School of Medicine, New Haven, CT 06510, USA
4Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA
5Present address: Centro Andaluz Biología del Desarrollo, Universidad Pablo de Olavide/Consejo Superior de Investigaciones Científicas, Sevilla 41013, Spain
6Present address: Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA
7Lead Contact
*Correspondence: mamormat@upo.es (M.A.M.-M.), antonio.giraldez@yale.edu (A.J.G.)

SUMMARY

The awakening of the genome after fertilization is a cornerstone of animal development. However, the mechanisms that activate the silent genome after fertilization are poorly understood. Here, we show that transcriptional competency is regulated by Brd4- and P300-dependent histone acetylation in zebrafish. Live imaging of transcription revealed that genome activation, beginning at the miR-430 locus, is gradual and stochastic. We show that genome activation does not require slowdown of the cell cycle and is regulated through the translation of maternally inherited mRNAs. Among these, the enhancer regulators P300 and Brd4 can prematurely activate transcription and restore transcriptional competency when maternal mRNA translation is blocked, whereas inhibition of histone acetylation blocks genome activation. We conclude that P300 and Brd4 are sufficient to trigger genome-wide transcriptional competency by regulating histone acetylation on the first zygotic genes in zebrafish. This mechanism is critical for initiating zygotic development and developmental reprogramming.

INTRODUCTION

Upon fertilization, the metazoan genome is transcriptionally silent. Understanding the mechanisms that awaken the genome remains a fundamental question in biology. Genome activation occurs during the maternal-to-zygotic transition (MZT), when developmental control shifts from maternally provided proteins and RNAs to the zygotic nucleus. This transition is crucial to reprogram the differentiated nuclei from the sperm and the oocyte into a transient totipotent state where different cell types can be specified, and failure to activate the genome during this transition causes developmental arrest across different species (Artley et al., 1992; Edgar and Datar, 1996; Newport and Kirschner, 1982a; Schultz et al., 1999; Zamir et al., 1997). While mechanisms of zygotic genome activation (ZGA) differ across species, the timing and the number of divisions that precede genome activation are highly reproducible within species, suggesting a robust temporal regulation. Nevertheless, the mechanisms that control when and how the genome becomes activated remain poorly understood.

While the genome is silent, fertilized embryos are competent to transcribe exogenous DNA in zebrafish, Xenopus, and mouse (Harvey et al., 2013; Newport and Kirschner, 1982a; Wiekowski et al., 1993). Thus, the lack of endogenous transcription may reflect a silent chromatin state, possibly because of maternally deposited repressors or the lack of specific activators (Newport and Kirschner, 1982b). It has been proposed that the maternally deposited histones function as potential repressors (Almouzni and Wolffe, 1995; Joseph et al., 2017; Newport and Kirschner, 1982b; Pruleau et al., 1994), which are titrated down by the exponential increase in DNA content during the early cell cycles, providing a switch in transcriptional competency. Yet, it is unclear whether this relative decrease in histone levels is necessary and sufficient to mediate the switch in transcriptional competency during genome activation, as many genes in Drosophila are activated in a time-dependent manner in haploid embryos (Blythe and Wieschaus, 2016; Edgar et al., 1986; Lu et al., 2009). An alternative possibility for the switch to transcriptional competency is an active mechanism mediated by proteins transcribed from maternal RNAs. Indeed, inhibiting translation of maternal mRNAs blocks the expression of zygotic genes as well as cell division in Xenopus and Drosophila (Edgar and Schubiger, 1986; Lund and Dahlberg, 1992). Recent studies have uncovered transcription factors (TFs) required for activating the first zygotically expressed genes, such as Zelda in Drosophila (Harrison et al., 2011; Liang et al., 2008; Nien et al., 2011; ten Bosch et al., 2006); Pou53, Sox19b, and Nanog in zebrafish (Lee et al., 2013; Leischner et al., 2013); and NF-Ya (Lu et al., 2016) and DUX TFs in mammals (De Iaco et al., 2017; Hendrickson et al., 2017; Iturbide and Torres-Padilla, 2017; Whiddon et al., 2016).
Figure 1. Zygotic Genome Activation Begins with the miR-430 Locus in a Gradual and Stochastic Manner

(A) Schematic illustrating strategies used to visualize global transcription using Click-iT chemistry (left) and the miR-430 locus using CRISPR-dCas9-3xGFP (dCas9) (right).

(B) 

(C) 

(D) 

(E) 

(F) 

(G) 

(H) 

(I) 

(J) 

(K) 

Legend continued on next page
et al., 2017). While these TFs provide specificity, their binding alone is not sufficient to trigger transcriptional competency, as many bound genes are not activated during the MZT (Leichtsenring et al., 2013). Other events coincide with genome activation, including chromatin remodeling at promoters and acquisition of histone marks H3K4me3 and H3K27me3 (Akkers et al., 2009; Bogdanovic et al., 2012; Dahl et al., 2016; Li et al., 2014; Lindeman et al., 2011; Liu et al., 2016; Vastenhouw et al., 2010; Zhang et al., 2016), changes in DNA methylation pattern (Bogdanovic et al., 2016; Guo et al., 2014; Jiang et al., 2013; Lee et al., 2015; Messerschmidt et al., 2014; Potok et al., 2013), establishment of topologically associated domains (Du et al., 2017; Hug et al., 2017; Kaaj et al., 2018; Ke et al., 2017; Stadler et al., 2017), and acquisition of H2AZ nucleosomes in the promoter of zygotic genes (Murphy et al., 2018; Zhang et al., 2014b). While these might contribute to transcriptional competency, their direct roles and sufficiency in genome activation are unclear. Thus, although some gene-specific elements have been identified and chromatin architecture emerges during ZGA, the mechanisms that prepare the silent embryonic genome for transcriptional competency remain poorly understood.

In this study, we combine live imaging and RNA-expression analysis during embryogenesis to interrogate the cellular and molecular mechanisms that mediate ZGA in zebrafish. We find that transcription competency during ZGA is achieved via the function of P300 and Brd4, two factors that are required and sufficient to prepare the genome for transcriptional activation.

RESULTS

Genome Activation Initiates at the miR-430 Locus in Zebrafish

The MZT represents a major switch in the transcriptional competency of the genome. Despite the progress made in understanding this universal transition (Lee et al., 2014; Schulz and Harrison, 2019), it is not fully understood how genome activation first begins. For example, in zebrafish, transcription might begin simultaneously across the genome and synchronously across all cells in the embryo, or the process may be sequential or stochastic. To address these questions, we first analyzed global transcription during MZT using metabolic RNA labeling, Click-IT (Jao and Salic, 2008), an approach similar to previously published work in zebrafish embryos (Heyn et al., 2014). Instead of 4-thio-UTP, 5-ethyluridine (EU) was injected at the one-cell-stage zebrafish embryos and incorporated into nascent RNA as the embryo progressed through development. Newly transcribed RNAs were then detected by Click-IT chemistry (Jao and Salic, 2008) through either imaging or sequencing (Click-IT-seq) (Figures 1A, 1B, S1A–S1J, S2C, and S2D; STAR Methods). This analysis expanded previous studies (Heyn et al., 2014; Lee et al., 2013) and identified 2,669 genes transcribed by 4 h post-fertilization (hpf) in zebrafish that had an increase in exon or intron signal when compared with embryos treated with the RNA polymerase II (Pol II) inhibitor trip-tolide (Figure S1F; Tables S1 and S2; STAR Methods). To characterize the spatiotemporal dynamics of transcription across the embryo during genome activation, we assayed for poised RNA polymerase II (Ser5P RNA Pol II) (Figures 1C, 1E, and S2B) and for RNA synthesis using Click-IT imaging at single-cell resolution. We first observed poised RNA Pol II and nascent transcription in two foci at the 64-cell stage (2 hpf) (Figures 1B, 1C, and 1E). As a control, embryos treated with RNA Pol II inhibitors trip-tolide (Titov et al., 2011) or α-amanitin (Kane et al., 1996; Lindell et al., 1970) lacked EU-labeled nascent transcripts (Figure 1B). Ser5P RNA Pol II signal colocalized with foci of active transcription and was limited to specific stages of the cell cycle during late interphase and early prophase (Figure S2A), indicating that Pol II activity is regulated during the cell cycle.

Previous studies suggested that miR-427/430, a microRNA family that regulates the clearance of maternal mRNAs (Giraldez et al., 2006; Lund et al., 2009), is a potential candidate for one of the earliest transcribed loci in Xenopus and zebrafish (Heyn et al., 2014; Lund et al., 2009) (Figure 1D). To test this, we adapted CRISPR-dCas9-GFP-mediated labeling of endogenous loci (Ma et al., 2015) by coinjecting dCas9-3xGFP with 2 guide RNAs (gRNAs) at the one-cell stage. These 2 gRNAs target dCas9-3xGFP at 20 sites on the endogenous miR-430 locus, a repetitive gene with 54 copies within 17 kb in chromosome 4 (Figures 1A, 1C–1E, and S2A–S2C; Video S1). We observed that miR-430 loci colocalize with the earliest detected transcription and Ser5P RNA Pol II signal in 64-cell-stage embryos (Figures 1C, 1E, and S2B), consistent with previous observations (Heyn et al., 2014). This signal is highly specific to miR-430, as
Developmental Time, Independent of Cell Division

To test whether lengthening of cell cycle could affect transcriptional output across the genome, we uncoupled developmental time and cell division by blocking DNA replication (Figure 2A). Chk1 blocks the formation of the origin of replication and slows cell divisions during the midblastula transition (MBT) (Collart et al., 2013, 2017). Premature Chk1 expression through mRNA injection at the one-cell stage stops cell division in Xenopus (Collart et al., 2017) and zebrafish (Figures 2B and 2C), arresting embryos between 4- and 16-cell stages throughout the first 6 h of development. Chk1-injected embryos (Chk1OE) increased miR-430 transcription at 2 hpf (Figure S3E) because of the longer cell cycle and subsequent increased duration of Pol II activity (Yonaha et al., 1995). However, stopping the cell cycle by Chk1 expression is not sufficient to cause premature and widespread genome activation at this developmental time as assayed by Click-iT labeling of transcription (Figure S3E), consistent with a previous study that analyzed a few genes upon extending the cell cycle by 5–10 min with Chk1 expression (Zhang et al., 2014a). These results suggest that slowdown of the cell cycle during MBT contributes to transcriptional output but is not sufficient to trigger premature genome activation.

Transcriptional Levels Are Modulated by the Nuclear/Cytoplasmic Ratio

Single nucleus analysis of transcription revealed significantly lower Click-iT signal in nuclei from Chk1OE embryos than that in time-matched WT embryos (Figures 2D and 2E). This is consistent with previous studies and indicates that embryos with low N/C ratio have lower transcription levels as compared with triptolide-treated embryos (Amodeo et al., 2015). Despite the low N/C ratio, Chk1OE embryos activated their genomes over time at 4 hpf, unlike control triptolide-treated embryos, as shown by Click-iT imaging and Click-iT-seq (Figures 2D–2G, S3C, and S3D). To further measure transcription of Chk1OE embryos directly, we used Click-iT-seq of nascent RNAs to quantify exonic and intronic sequences. The latter provides better signal-to-noise ratio and thus increases the sensitivity of Click-iT-seq, as most maternally deposited mRNAs are spliced and the introns are degraded early on (Lee et al., 2013). Because Chk1OE embryos have a lower number of cells (and DNA template) than time-matched WT embryos, total Click-iT-seq read levels are also lower. Thus, to identify the genes activated in Chk1OE embryos, we compared Click-iT-captured gene expression between Chk1OE embryos and control triptolide-treated Chk1OE embryos, which have the same amount of DNA template. Compared with triptolide-treated Chk1OE embryos, Chk1OE embryos activated 67.8% of the zygotic genes (1,218 zygotic and 591 maternal zygotic genes upregulated ≥4-fold; Table S3; STAR Methods; Figures 2F and 2G). These results indicate that dilution of maternal repressors through changes in the N/C ratio are not obligatory for genome activation and thus, suggest that activation of the zygotic genome depends on developmental time, independent of cell division.
the 2-fold difference in active DNA template, and (2) compensated the difference in the number of embryos collected by adding α-amanitin-treated diploid embryos to the diploid samples (Figure 3A). This allowed us to keep the number of embryos collected per sample constant and control for the same maternal mRNA background. At 512-cell stage, diploid embryos displayed higher transcription levels than haploid embryos, as the majority of the genes (85%) were expressed at a higher level in diploid than in haploid embryos with 1,088 genes beyond 4-fold (Figures 3B, 3C, and S4A–S4C; Table S4). The lower gene expression in haploid relative to diploid samples is consistent with a transcriptional repressive role for the low N/C ratio. At 1K-cell stage, the difference in transcription competency between the haploid and diploid genome was reduced with only 163 genes displaying a 4-fold difference (Figures S4G–S4I), suggesting that the repressive effect brought about by the low N/C ratio is relieved over developmental time, consistent with the observed transcriptional activation at 4 hpf in Chk1OE embryos despite the low N/C ratio. To further examine the repressive effect of low N/C ratio, we compared the relative
transcriptional activation, lower N/C ratio poses repressive effect on transcriptional activation, and individual genes overcome this repression differentially over developmental time during ZGA.

**Translation of Maternally Provided mRNAs Controls ZGA**

We hypothesized that transcriptional competency might be regulated by a developmental timer that depends on the translation of maternal mRNAs or the function of maternally deposited proteins on the chromatin. To test this model in zebrafish embryos, we took a similar approach as prior studies in Drosophila and Xenopus (Edgar and Schubiger, 1986; Lund and Dahlberg, 1992) to block mRNA translation initiation and elongation with α-amanitin.

expression of individual activated genes in both haploid and diploid conditions (STAR Methods; Figures S4P and S4Q). Stacked bar plot comparing the normalized expression of genes between stage-matched haploid and diploid condition indicates a spectrum of N/C ratio dependence across the activated genes (Figures 3C, S4P, and S4Q). In particular, we observe that genes less affected by N/C ratio are significantly shorter in gene length than those more affected by N/C ratio (Figure 3D). Some examples of genes less affected by N/C ratio include aplnrβ, mxtx2, and ddit4; examples of genes more affected by N/C ratio include vgl4l, asb11, and fbxo5 (Figures 3E and S4R). Together, these results indicate that while high N/C ratio is not obligatory for
pateamine A (PatA) and cycloheximide (CHX) (Bordeleau et al., 2006; Low et al., 2005; Schneider-Poetsch et al., 2010) before transcription is detected. PatA+CHX treatment at the 8- or 32-cell stage significantly reduced translation and arrested the cell cycle at the 16- and 64-cell stage, respectively (Figures 4A, S5A, and S5B) (Beaudoin et al., 2018), maintaining a low N/C ratio over time similar to that observed in Chk1OE embryos (Figure 2C). We observed global transcriptional activation by 4 h when translation was inhibited by the 64-cell stage when compared with α-amanitin-treated controls (2,213 genes upregulated ≥4-fold; Table S3; STAR Methods; Figures 4B, 4C, and S5C–S5F). Examples of these include mxtx2, klf17, her5, and aplnrb (Figure 4D). Similar to Chk1OE embryos, PatA+CHX-treated embryos exhibited lower Click-iT-seq read levels than time-matched WT embryos at 4 hpf because of their lower number of nuclei (i.e., DNA template) per embryo. However, blocking translation 30 min earlier, by the 16-cell stage, reduced transcriptional competency (to only 256 genes upregulated ≥4-fold; Table S3; STAR Methods; Figures 4B, 4C, and S5C–S5F). Based on these results, we conclude that the maternal factors translated by the 64-cell stage (2 hpf) are sufficient to trigger global genome activation for 82.9% of the zygotic transcripts and overcome the repressive effect on low N/C ratio. These results are consistent with observations in Xenopus where blocking translation of maternal mRNAs allows transcription of individual genes (Lund and Dahlberg, 1992). Despite the constant N/C ratio over that time, global activation was only observed at 4 hpf, suggesting that the maternal factors translated by the 64-cell stage still require additional time to induce transcriptional competency, possibly by regulating the chromatin during that time.

**P300 and Brd4 Functions Are Required for ZGA**

To identify temporal regulators of genome activation, we analyzed the chromatin marks labeling the first active genes and interrogated their function using chemical inhibitors for writers and readers of these marks. Two lines of evidence indicate that genome activation coincides with the acquisition of...
H3K27Ac and requires writing and reading of this mark by bromo-domain-containing proteins P300/CBP and Brd2–4, respectively. First, we analyzed the first active gene (miR-430) for histone modifications H3K27Ac, H3K4me1, and H3K4me3 at dome stage (4.3 hpf) using public datasets (Bogdanovic et al., 2012). miR-430 was the top-labeled locus with each of these marks (Figures 5A, S6A–S6C, and S6G). Consistent with these results, imaging analysis revealed that the earliest H3K27Ac and H3K4me3 signals colocalized with the miR-430 locus (Figures 5C and S6H). At later stages, among the different histone marks, H3K27Ac showed the best correlation with transcription as assayed by Click-iT-seq (r = 0.53, Spearman correlation, p = 3.9 \times 10^{-50}, rank

Figure 5. H3K27Ac Correlates with Transcriptional Activation during ZGA

(A) Genome tracks representing normalized Click-iT-seq signal and histone mark level at the miR-430 locus. ChIP-seq data (Bogdanovic et al., 2012). RPM for Click-iT-seq/ChIP-seq (STAR Methods).

(B) Time-resolved single-nucleus confocal imaging analysis of H3K27Ac from 256-cell to sphere stage reveals a positive correlation with the level of Click-iT signal. Both H3K27Ac and Click-iT signal intensity are presented in a heatmap color scale. Scale bar represents 5 μm.

(C) Single-plane confocal image labeled for DAPI, H3K27Ac, dCas9-miR-430, and Click-iT. Note the colocalization of H3K27Ac with Click-iT-labeled transcription activity at the miR-430 locus (n = the fraction of analyzed nuclei that shows the same colocalization of H3K27Ac with Click-iT-labeled transcription at the miR-430 locus as the representative nucleus, >3 independent embryos are imaged). Scale bar represents 5 μm.

(D) Schematic illustrating the selective pharmacologic inhibition of the activity of the BET bromodomain proteins (BRD2–4) and CBP/P300 by JQ1 and SGC, respectively (top). Embryos treated with JQ1 and SGC both arrest before gastrulation similar to those treated with triptolide, consistent with a loss of zygotic transcription (bottom) (Ac, H3K27 acetylation; TFs, transcription factors).

(E) Click-iT imaging analysis in wild-type (WT), triptolide, JQ1, and SGC-treated embryos reveals a significant reduction in transcription by the treatment of JQ1 and SGC. Click-iT signal intensity is presented in a heatmap color scale. Scale bar represents 5 μm.

(F) Biplot comparing intron expression levels of genes measured by Click-iT-seq in triptolide- (left) and JQ1-treated (right) embryos with WT embryos at 4 hpf. Dashed lines represent 4-fold change.

(G) Genome tracks representing normalized Click-iT-seq signal measured at 4 hpf in WT, triptolide, JQ1, and SGC-treated embryos for examples of zygotic genes. RPM (STAR Methods).

See also Figure S6.
Figure 6. P300 and BRD4 Are Limiting Factors Regulating Zygotic Genome Activation

(A and B) Single-nucleus imaging analysis of H3K27Ac and Click-iT signal comparing embryos with and without early expression of P300 and BRD4 at 32-cell (A) and 1K-cell stage (B). Scale bar represents 5 μm. WT, wild-type embryos; P300+BRD4, embryos with early expression of P300 and BRD4 protein; triptolide, embryos treated with triptolide. Both H3K27Ac and Click-iT signal intensity are presented in a heatmap color scale. Inset highlights the single-plane confocal image of region with Click-iT signal colocalizing with high H3K27Ac signal (n = number of incidence among the total number of nuclei imaged).

(C) Box and whisker plots quantifying the mean fluorescence intensity for H3K27Ac and Click-iT signal in the conditions described in (B). Two-sample t test of H3K27Ac signal: P300+BRD4 > WT, p = 0.0068; P300+BRD4 > triptolide, p = 0.0013; WT/C24 Trip, p = 0.2482. Two-sample t test of Click-iT signal: P300+BRD4 > WT, p = 0.0068; P300+BRD4 > triptolide, p = 0.0013; WT/C24 Trip, p = 0.2482.

(D) Stacked bar plots comparing the total number of read count of zygotic and maternal zygotic genes in wild-type (WT) embryos and embryos with early expression of P300 and BRD4 protein (P300+BRD4) at 1K-cell stage.
correlation independence test) (Figures 5B and S6D–S6F). Second, we analyzed the effect of different small-molecule inhibitors targeting readers or writers for H3K4me3 and H3K27Ac (Figure S6B). Among these drugs, treatment with JQ1 (an inhibitor of bromodomain and extratemporal motif [BET] bromodomains BRD2/4) (Filippakopoulos et al., 2010), or SGC-CBP (an inhibitor of histone acetyltransferase P300 and CBP) (Hammitzsch et al., 2015; Hay et al., 2014) resulted in a significant reduction of zygotic transcription (Figures 5E–5G and S6J–S6P), which in turn blocked gastrulation (Figure 5D). For example, intron analysis revealed that 84% of zygotically transcribed genes were reduced in expression ≥4-fold (Table S3; STAR Methods) by JQ1 when compared with WT embryos (Figure 5F). This effect was also apparent for housekeeping and developmental genes (Figures 5G and S6P). These results suggest that the targets of JQ1 and SGC-CBP (BRD2/4 and P300/CBP) are required to initiate transcriptional competency across the zygotic genome.

**Brd4 and P300 Are Sufficient to Activate Zygotic Transcription Prematurely**

Based on the chemical inhibitors that block transcriptional competency after fertilization, we hypothesized that the writing (P300) and reading (Brd4) of histone acetylation are required to achieve transcriptional competency and that these activities are limiting during the initial stages of the MZT. In this model, only when these activities have reached a certain threshold does the genome become competent for activation. To test this model, we first analyzed the level of translation of these factors using ribosome profiling before (i.e., 0 hpf and 2 hpf) and after ZGA (i.e., 5 hpf). This analysis revealed high levels of translation for the maternally deposited p300 and brd4 mRNA before zygotic transcription begins (Figures S7A–S7D), but their translation diminished after ZGA (Figures S7A–S7D), suggesting a temporal regulation of their translation during MZT. Next, we analyzed whether a premature increase in Brd4 and P300 levels can regulate the timing of genome activation. Injection of P300 and BRD4 proteins at the one-cell stage led to the premature activation of the zygotic genome, and an increase in H3K27Ac coincided with Click-iT signal, revealing premature miR-430 expression at the 32-cell stage (Figure 6A). Click-iT analysis of zygotically transcribed mRNAs revealed an increase in transcriptional output and H3K27Ac by 1K-cell stages (Figures S7L–S7P). To determine whether the level and activity of P300 and BRD4 are responsible for limiting transcriptional competency in translation-inhibited embryos, we introduced P300+BRD4 into embryos treated with PatA+CHX at 8-cell stage. Quantification of zygotic transcription using Click-iT-seq revealed an increase in zygotic transcripts compared with WT embryos, with 623 genes activated ≥4-fold (Figures 6G and 6H; Table S3; STAR Methods) and an increase in H3K27Ac signal (Figures S7L–S7P) by the injection of P300+BRD4 in the translation-inhibited embryos. Together, our results suggest that P300 and Brd4 are highly translated maternal factors that function as a temporal switch to confer transcriptional competency via histone acetylation to the fertilized egg, regulating the timing and transcriptional output during ZGA.

**DISCUSSION**

Our results provide two major insights into the mechanisms that activate the zygotic genome after fertilization. First, transcriptional competency depends on the translation of maternal mRNAs and can be achieved at low N/C ratio over developmental time without diluting maternal repressors by progressive cell divisions (Figure 7). We found that Pol II is regulated during the cell cycle (Figure S2). This is consistent with the increase in transcription output with the lengthening of the cell cycle (Collart et al., 2013; Zhang et al., 2014a) and could contribute to the transition from stochastic to global transcription (Stapel et al., 2017). However, stopping the cell cycle does not cause premature genome activation (Figure S3E), which is evidence that the slowdown of the cell cycle that takes place during MZT is not sufficient to activate the zygotic genome. These results are consistent with previous studies that used low levels of Chk1 to slow down the cell cycle by 5–10 min (Zhang et al., 2014a). Furthermore, stopping the cell cycle by either inhibiting the formation of the origin of replication (Chk1<sup>OE</sup>) or by blocking translation demonstrates that transcriptional competency is acquired over time and does not require the titration of histones by replicating DNA (Figures 2D–2G, 4A, 4C, and 4D). While these results demonstrate that a high N/C ratio is not obligatory for
transcriptional competency, comparing transcription levels in haploid and diploid embryos reveals that haploid embryos express lower levels of zygotic genes (Figure S4), consistent with histones having a repressive effect on transcription in vivo and in vitro (Almouzni and Wolffe, 1995; Amodeo et al., 2015; Dekens et al., 2003; Jevtić and Levy, 2015; Joseph et al., 2017; Newport and Kirschner, 1982b; Prioleau et al., 1994). Consistent with these observations, haploid Drosophila embryos activate a large set of genes dependent and independent of the N/C ratio (Blythe and Wieschaus, 2016; Edgar et al., 1986; Lu et al., 2009). We speculate that the increase in histone acetylation observed during MZT relieves the repressive activity of histones, providing a temporal regulator of transcriptional activation during MZT.

Second, we demonstrate that the activities of histone acetylation writer P300 and reader Brd4 are necessary and sufficient to modulate transcriptional competency during MZT. We show that transcriptional competency increases over developmental time, independent of cell division or continuous protein translation, and we propose that this activity depends on histone acetylation through P300 and Brd4. Our study provided two lines of evidence that support the role of P300 and Brd4 in regulating transcriptional activation in zebrafish embryos. First, functional inhibition of both factors by JQ1 and SGC reduces transcriptional activation in zebrafish embryos. First, functional inhibition of both factors by JQ1 and SGC reduces transcriptional activation in zebrafish embryos. First, functional inhibition of both factors by JQ1 and SGC reduces transcriptional activation in zebrafish embryos.
transcriptional competency in embryos with reduced translation (Figures 6 and S7), demonstrating that their activity is sufficient to trigger transcriptional competency. Interestingly, the level of activation correlates with the acquisition of histone acetylation (Figures 5, 6, S6A–S6F, and S7L–S7P), and our ribosome profiling analysis revealed high level of translation of the maternally deposited p300 and brd4 mRNA before transcription first begins (Figures S7A–S7D). Consistent with the role of histone acetylation during ZGA, H4K8Ac, H3K18Ac, and H3K27Ac coincide with genome activation in Drosophila (Harrison and Eisen, 2015; Li et al., 2014). Our findings provide a key functional link between the activity of the enhancer regulators, P300 and Brd4, (Pradeepa, 2017) with transcriptional competency during MZT. However, it remains unclear whether transcriptional competency is regulated at the level of protein abundance, or their activity instead, which can be modulated by additional factors such as casein kinase II (CK2) and phosphatase 2A (PP2A) (Chiang, 2016). Protein quantification with specific antibodies would further provide insights on its temporal dynamics. Interestingly, western blot analysis indicated that Brd4 protein was abundantly provided maternally (data not shown). These results are consistent with prior analysis of Brd4 in zebrafish (Toyama et al., 2008) and suggest alternative levels of regulation beyond protein abundance such as post-translational modification or changes in nuclear localization. It has been shown that the increase in the volume of the nucleus modulating the nuclear import machinery can cause premature onset of zygotic transcription (Jevtic and Levy, 2015). It will be interesting to test whether these manipulations affect the levels of Brd4 and P300 in the nucleus. Previous studies in zebrafish have shown that sequence-specific TFs Nanog, SoxB1, and Pou5f1 are required for initiating a significant fraction of the first wave of ZGA (Lee et al., 2013; Leichsenring et al., 2013). The acquisition of acetylation marks at endogenous enhancers could increase accessibility to these key TFs, in turn mediating the loading of RNA polymerases to their distal target promoters. Alternatively, the threshold activity of histone acetylation writers and readers (P300 and Brd4) may trigger activation at those sites preloaded with pioneer factors and allow recruitment for additional TFs required for the activation of specific genes. Currently, the temporal relationship between histone acetylation and TF binding is not resolved (Harrison and Eisen, 2015; Li et al., 2014). Answering this question will require loss-of-function studies for different TF activators during MZT, combined with high-resolution imaging or chromatin immunoprecipitation methods amenable for low-input DNA. Nanog interacts and recruits P300 and BRD4 to the chromatin in embryonic stem cells (ESCs) (Boo et al., 2015; Fang et al., 2014) and thus may provide specificity to the activity of these general regulators of enhancer activity. We propose that P300 and Brd4 activity serves as a switch to regulate transcriptional competency after fertilization.

One of the first events the embryo must accomplish is the reprogramming of the differentiated sperm and oocyte nuclei into a transient totipotent state receptive to various differentiation programs. It is worth noting remarkable parallels between genome activation, cellular reprogramming, and stem cell maintenance (Giraldez, 2010; Lee et al., 2014). For example, Brd4 and P300, Nanog and Oct4 are shared between these different developmental programs. miR-430 in zebrafish reprograms the transcriptome during MZT to a transient totipotent state (Giraldez et al., 2006; Judson et al., 2009; Lee et al., 2013; Subramanyam et al., 2011), whereas its homolog in mouse, miR-295/miR-302, facilitate cellular reprogramming of differentiated cells into induced pluripotent stem cells (Giraldez et al., 2006; Judson et al., 2009; Lee et al., 2013; Subramanyam et al., 2011). It was reported that miR-295/miR-302 expression is controlled by a super enhancer in ESCs (Hnisz et al., 2013). Interestingly, miR-430 is abundantly labeled with H3K27Ac and H3K4me1, which typically mark super enhancers (Hnisz et al., 2013). These parallels prompt the question of whether other factors controlling super enhancer function and reprogramming regulate genome activation in vertebrates.

In summary, our results address the long-standing question of the mechanism by which ZGA is achieved and identify key molecular factors regulating transcriptional competency, illuminating an essential step required to induce transient totipotency in embryogenesis and initiate zygotic development in zebrafish.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

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AUTHOR CONTRIBUTIONS
S.H.C. and A.J.G. conceived the project. S.H.C. performed embryonic experiments and image analysis with the support of H.D.-C. and M.A.M.-M. Y.T. performed RNA sequencing analysis with the contribution of C.E.V. L.M. and D.M. performed western blots and DNA measurements. S.H.C. and J.-D.B. performed Click-IT-seq. S.H.C. and H.D.-C. performed Click-IT staining. S.H.C. and J.P.F. performed chemical drug screening. C.E.V. performed data processing. M.D.J.B. and L.M. generated haploid embryos. A.A.B. performed ribosome profiling. S.H.C., Y.T., M.A.M.-M., and A.J.G. performed data analysis and, together with the other authors, interpreted the results. A.J.G. supervised the project with the contribution of M.A.M.-M. S.H.C., M.A.M.-M., and A.J.G. wrote the manuscript with input from the other authors.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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REFERENCES

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# STAR METHODS

## KEY RESOURCES TABLE

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Antonio J. Giraldez (antonio.giraldez@yale.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Zebrafish Embryo Production

Zebrafish wild-type embryos were obtained from natural matings of 12-month-old adult zebrafish of mixed wild-type backgrounds (TU-AB, and TL strains). Wild-type adults were selected randomly for mating. Zebrafish were maintained in accordance with AAALAC.
research guidelines, under a protocol approved by Yale University IACUC. All zebrafish and embryo experiments were carried out at 28°C. Embryos were grown and staged according to standard protocols to ensure all embryos were at the same expected developmental stages before sample collection. Embryos between 0 to 6 hours post-fertilization (hpf) developmental stages were randomly collected per sample as specified across different experiments.

**Haploid Zebrafish Embryo Production**

Haploid embryos were generated using a modified version of the protocol by Kroeger et al. (Kroeger et al., 2014). The testes from 7 male males were collected into 700 μl of Hank’s solution and macerated. 350 μl of the sperm mix was irradiated at 1000 J/m² in a UV crosslinker. Eggs from individual females were divided into two groups and used for separate inseminations with either intact zebrafish sperm or UV-irradiated sperm to produce diploid or haploid embryos respectively.

**METHOD DETAILS**

**Constructs, sgRNA Design, and In Vitro Transcription**

Zebrafish chk1 ORF was amplified from cDNA from 64 cell-stage embryos using primers 5’-TTTTCCATGGCTGTGCTT-3’ and 5’-TTTCCGGCTCAATCAATGGCAAAACCTTGG-3’. The resultant PCR product was digested with restriction enzymes NcoI and SacII and ligated into the plasmid pT3TS-zCas9 (Jao et al., 2013). The final construct was confirmed by sequencing and corresponded in sequence to chk1 protein XP_021324451.1.

dCas9-3xGFP was generated as follows: 3xGFP was PCR amplified from plasmid pHAGE-TO-dCas9-3XGFP (Ma et al., 2015) using primers 5’ TTTCGGCGTCTACTCGAGTTTGTACAGTTC-3’ and 5’-TTTCCGGTGAATCTCCTGCAACAGAGGACGTTGCTGCTCT-3’. pHAGE-TO-dCas9-3XGFP was a gift from Thoru Pederson (Addgene plasmid # 64107). PCR products were digested with restriction enzymes Agel and SacII and ligated into the pT3TS-dCas9 plasmid, which is based on pT3TS-zCas9, containing point mutations that catalytically inactivate Cas9, also called dead Cas9 (dCas9) (Gilbert et al., 2013; Jinek et al., 2012).

sgRNAs against the miR-430 locus were designed using an updated version of the CRISPRscan (crisprscan.org) tool (Moreno-Mateos et al., 2015). Two different sgRNAs were used in combination with dCas9-3xGFP to label miR-430 locus:

- **sgRNA 1 specific oligonucleotide:** 5’-taatacgactcactataGAGGGTACCGATAGAGACAAgttttagagctagaa
- **sgRNA 2 specific oligonucleotide:** 5’-taatacgactcactataGAGGGTACCGATAGAGACAAgttttagagctagaa

sgRNA 1 and sgRNA 2 target 11 and 9 sites in the miR-430 locus at chromosome 4, respectively. sgRNAs were designed as previously described (Moreno-Mateos et al., 2015; Vejnar et al., 2016). Briefly, a 52-nt oligo containing the SP6 (5’-atttaggtcataGAGGGTACCGATAGAGACAAgttttagagctagaa) or T7 (5’-taatacgactcactata) promoter, 20-nt of specific sgRNA DNA-binding sequence, and a constant 15-nt tail (small case and underlined) for annealing was used in combination with an 80-nt reverse universal oligo to add the sgRNA irreversible 3’ end (5’-AAAAACACCACCTGGTGCCACTTGTCTTCAGTTGATAAGCAGCGTACTGAGCTTCTAAC). A 117-bp PCR product was generated and purified using QIAquick PCR purification kit (Qiagen). The purified product was used as a template for T7 or SP6 in vitro transcription (AmpliScribe-T7-Flash transcription kit from Epicentre; MAXIscript™ SP6 Transcription Kit from ThermoFisher Scientific, over a 6–7 h of reaction). In vitro transcribed sgRNAs were DNase-treated, precipitated with sodium acetate/ethanol and checked for RNA integrity on a 2% agarose gel stained with ethidium bromide.

To generate dCas9-3xGFP and Chk1 capped mRNAs, the DNA templates were linearized using XbaI (dCas9-3xGFP and Chk1) and mRNA was synthesized using the mMessage mMachine T3 kit (Ambion/ ThermoFisher Scientific). In vitro transcribed mRNAs were DNase I treated and purified using the RNeasy Mini Kit (Qiagen).

**DNA Quantification by qPCR**

5 embryos were manually deyolked in Ringer’s solution (116mM NaCl; 1.8mM CaCl₂, 2.9mM KCl; 5mL HEPES). Genomic DNA was extracted with PureLink Genomic DNA Kits (Thermo Fisher Scientific) following manufacture’s instruction and eluted with 500 μl of MilliQ water. To quantify nuclei, 3 μl of the genomic DNA was used in a 20 μL reaction containing 1 μl of primers amplifying repetitive miR-430 loci (Forward: 5’- CAAATGTGAAAATCCCATC-3’; Reverse: 5’- AAGGGTGACTGTTGCTATG-3’), using power SYBR Green PCR Master Mix Kit (Applied Biosystems) and a Viia 7 instrument (Applied Biosystems).

PCR cycling profile consisted of incubation at 50°C for 2 min, followed by a denaturing step at 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min.

**Embryo Injections and Treatments**

All injections and drug treatments were carried out on wild-type one-cell stage dechorionated embryos, unless otherwise noted. Experimental samples were then collected at the specified developmental stages/time as described in the text and figure legends. Varying amounts of mRNA per embryo were used as follows: 160 pg (Chk1), 25 pg (dCas9-3xGFP), 300 pg and BRD4 proteins were purchased from Protein One (P2004-01) and Reaction Biology Corp. (RD-21-153), respectively, and 200 pg of P300 and 630 pg of BRD4 protein were injected into each embryo.

JQ1 treatment: embryos were bathed in 43.8 μM JQ1 (1:100 dilution from 4.38mM working stock in DMSO) to inhibit BET family of bromodomain proteins including BRD2, BRD3 and BRD4.
SGC-CBP30 treatment: embryos were bathed in 20 μg/ml SGC-CBP30 (Sigma-aldrich, 1:500 dilution from 10mg/ml working stock in DMSO) to inhibit the bromodomain-containing transcription factors CREB binding protein (CBP) and Histone acetyltransferase p300 (EP300). Pol II inhibition: embryos were bathed in 5.8 μM triptolide (1:1,000 dilution from a 5.8 mM working stock in DMSO) or injected with 0.2ng of α-amanitin at one-cell stage to inhibit RNA polymerase II. Triptolide treatment was initially used in some experiments due to its convenience and sufficiency in inhibiting transcription by simply bathing the embryos in the drug. However, it became apparent that there is minor but detectable leaky transcription in triptolide-treated samples, and hence α-amanitin treatment was preferred to provide a more robust inhibition of transcription and hence a better negative control, especially for experiments that require a higher sensitivity to assay the onset of transcription.

Translation inhibition: embryos were collected at the one-cell stage. To limit the amount of translation of maternal mRNAs, embryos were transferred to media containing 10 μM pateamine A (PatA, purchased from D. Romo at Baylor University) and 50 μg/mL cycloheximide (CHX, Sigma Aldrich) at the 8-cell stage or the 32-cell stage, respectively. Because these treatments stop development, collection of these embryos was timed by monitoring the development of untreated sibling embryos when they reached the appropriate stage in this case sphere stage (4hpf). To evaluate the effect of translation inhibition by the treatment of PatA and CHX, 25pg dCas9-3xGFP mRNA was injected in one-cell stage embryos prior to the PatA and CHX treatment at 32-cell stage. Embryos with and without the PatA and CHX treatment were imaged using confocal microscopy to compare the dCas9-3xGFP signal at individual nucleus. Additional evaluation of the effect of translation inhibition treatment was performed by co-injecting 100pg DsRed mRNA and 0.4ng of Alexa Fluor® 488 histone H1 conjugate protein (ThermoFisher Scientific, H-13188) in one-cell stage embryos prior to the CHX treatment at 8- and 32-cell stage respectively. Embryos with and without the CHX treatment were imaged using fluorescent microscopy to compare the DsRed and H1Alexa488 signal.

Chk1 treatment: 160pg of Chk1 mRNA was injected in one-cell stage embryos to inhibit/slow down DNA replication by inducing degradation of the limiting replication initiation factor Drf1 (Collart et al., 2017). Embryos were collected when untreated sibling embryos reached the appropriate developmental stages: 64-cell stage (2hpf) or sphere stage (4hpf).

Treatments of different small molecule inhibitors targeting readers or writers for H3K4me3/H3K4me1 and H3K27Ac to test their effect on gastrulation: embryos with chorion were bathed in the titrated concentrations of each small molecule inhibitors (UNC1999, Sinefungin, MM-102, SGC-CBP30 and JQ1) as indicated in Figure S6I at one-cell stage until 6 hours post-fertilization (hpf) when the embryos were examined under microscopy for any gastrulation arrest phenotypes.

dCas9-Labeling of Endogenous miR-430 Locus

To label the miR-430 locus, the two previously described sgRNAs were injected at 100pg each in combination with 25pg dCas9-3xGFP into dechorionated embryos at the one-cell stage. Embryos were image either live or fixed in 4% paraformaldehyde and processed for antibody staining against GFP.

miR-430 Transcription Labeling by Live Imaging

To visualize nascent transcription of miR-430 by live imaging, a molecular beacon (MBmiR430 : Dabcyl-5’-GCTGAACAGAGGTGACTAAGTCAGC-3’-Lissamine) was specifically designed to target the primary miR-430 transcript. The molecular beacon (MBmiR430) was obtained from Gene Tools and resuspended in nuclease-free water. The molecular beacon was designed with a stem-loop structure, where the single-stranded loop region (5’-ACAGAGGTGACTAAGTCAGC-3’) is antisense to the repetitive single stranded regions of the predicted structure of the primary miR-430 transcript. The single-stranded loop region is flanked by two 5-nt sequences (5’-GCTGA-3’ at the 5’ end and 5’-TCAGC-3’ at the 3’ of the molecular beacon) that are self-complementary and form the double-stranded stem. The molecular beacon is modified at the 5’ end with a fluorophore and the 3’ end with a quencher to improve the signal to noise ratio. 0.5pmole of MBmiR430 was injected into wild type embryos at the one-cell stage. To trace individual nuclei during live imaging analysis, 0.4ng of Alexa Fluor® 488 histone H1 conjugate (ThermoFisher Scientific, H-13188) was co-injected with MBmiR430.

Click-iT Labeling of Zygotic Transcription for Imaging

Click-iT™ RNA Alexa Fluor™ 594 Imaging Kit (C10330) was adapted for application in zebrafish embryos. Embryos were injected with 50 pmols of Click-iT® RNA (5-ethylidyne-uridine) (EU, E10345) or in kit form with Click-iT Nascent RNA capture kit, C-10365, and collected at the times/developmental stages indicated in the text and figure legends. After collection, embryos were fixed using a 4% paraformaldehyde (PFA) solution in 1x phosphate-buffered saline (PBS) at 4°C overnight. Fixed embryos were washed three times with 1x PBS and permeabilized using 0.5% Triton X-100 (PBS-T) at room temperature for a total of 30 minutes, followed by dehydration with serial dilutions of Methanol (25%, 50%, 75%, 100% Methanol diluted with 1x PBS-T). Dehydrated embryos were incubated at -20°C for at least 2 hours before rehydration with serial dilutions of Methanol (75%, 50%, 25%, 0% Methanol diluted with 1x PBS-T). Rehydrated embryos then underwent antibody staining, as described below, before proceeding with the manufacturer’s EdU-labeling visualization protocol. Briefly, embryos were incubated with a 1x working solution of Click-iT reaction cocktail, containing the Alexa Fluor 594 azide and CuSO4 for 1 hour in dark at room temperature. After removal of the reaction cocktail, embryos were washed once with Click-iT reaction rinse buffer. Embryos were then washed three times with 1x PBS-T and stained with DAPI, followed by dissection and mounting on glass slides in ProLong™ Diamond Antifade Mountant (ThermoFisher Scientific, P36966).
Click-iT Captured Nascent Zygotic Transcripts for RNA-Sequencing
The Click-iT® Nascent RNA Capture Kit (ThermoFisher Scientific, C10365) was adapted for application in zebrafish embryos. To capture nascent RNAs, 50 pmols of Click-iT® EU (5-ethyl Uridine) was injected in one-cell stage embryos and allowed to incorporate into the nascent zygotic transcript until the time point/developmental stage indicated in the text and figure legends. At these time points, total RNA from 35 embryos was extracted using TRIzol reagent (Invitrogen). The EU-incorporated RNAs were biotinylated and captured following the manufacturer’s instructions. Briefly, the EU-labeled RNA was biotinylated with 0.25mM biotin azide in Click-iT reaction buffer. The biotinylated RNAs were precipitated with ethanol and resuspended in nuclease-free water. The biotinylated RNAs were washed with DynaMags, rehydrated embryos were washed three times with 1x PBS and permeabilized using 0.5% Triton X-100 (PBS-T) at room temperature for a total of 30 minutes, followed by dehydration with serial dilutions of Methanol (25%, 50%, 75%, 100% Methanol diluted with 1x PBS-T). Dehydrated embryos were incubated at -20°C for at least 2 hours before rehydration with serial dilutions of Methanol (75%, 50%, 25%, 0% Methanol diluted with 1x PBS-T). Rehydrated embryos were washed with 1x PBS-T and incubated for 2-3 hours in blocking solution (1x PBS-T, 10% Bovine Serum Albumin), followed by overnight incubation at 4°C with the primary antibodies specified in the text and figure legends. The primary antibodies included rabbit anti-Histone H3 (acetyl K27) antibody 1:1,000 (Abcam, ab177178), rabbit anti-RNA polymerase II CTD repeat YSPTSPS (phospho SS) antibody 1:1,000 (Abcam, ab5131), mouse anti-GFP Tag antibody.
Western Blot

Ten embryos (H3, H2B n=5; H2A, H4) were manually deyolked, snap frozen in liquid nitrogen and boiled at 95 °C for 5 min in 15 μL of water, 7.5 μL of 4x NuPAGE LDS Sample Buffer (ThermoFisher Scientific), 3 μL DTT (Sigma-Aldrich). Samples were run on 4–12% polyacrylamide NuPAGE Bis-Tris gels (ThermoFisher Scientific) for 45 min at 180 V and wet electrotransferred onto a nitrocellulose membrane (GE LifeSciences) for 70 min at 30 V. Membranes were incubated in blocking solution (5% milk in PBS-T) for 2 h. Then primary antibodies were diluted in blocking solution and incubated with the membrane overnight at 4 °C Anti-H3 1:10,000 (ab1791; Abcam), H4 1:1,000 (ab10158; Abcam), H2A 1:1,000 (ab18255; Abcam), H2B 1:3,000 (ab1790; Abcam), Actin 1:5,000 (ab8227, Abcam). Secondary antibody Goat Anti-Rabbit IgG Antibody, (H+L) HRP conjugate (AP307P; Millipore) was incubated with the membrane at 1:10,000 for 1 h at RT. Membranes were analyzed by chemiluminescent detection and X-ray film (E3012, Denville Scientific).

RNA-seq and Click-it-seq Analysis and Normalization

Total RNA from 20 embryos per condition at indicated time point/developmental stage were snap frozen in Liquid Nitrogen and the RNA was extracted using Trizol reagent (Invitrogen). Samples were treated with Epicentre Ribo-Zero Gold, to deplete ribosomal RNA, or subject to pull-down by oligo dT beads, to enrich for poly(A)+ RNA. TruSeq Illumina RNA sequencing libraries were constructed with the membrane at 1:10,000 for 1 h at RT. Membranes were analyzed by chemiluminescent detection and X-ray film (E3012, Denville Scientific).

Determination of Zygotic and Maternal-Zygotic Genes

To calculate per gene RPKMs, the number of reads mapped to each gene from the Click-it RNA-seq experiment were summed and normalized by gene length and the total number of reads mapped to the mitochondrial protein-coding genes, unless otherwise specified. Intron regions were defined as genic regions that are not covered by any extended exon (exon extending 15nt on both ends) on
the same or any other gene. Only uniquely mapped reads were used for intron analysis. All zygotic and maternal zygotic genes were defined using criteria in Table S1. Genes in each category must satisfy all the criteria in the column.

The criteria are detailed as follows:

a. Zygotically expressed genes from previous studies. The union of all identified zygotically expressed zebrafish genes reported by the Neugebauer lab ([Heyn et al., 2014] and the Giraldez lab ([Lee et al., 2013]).

b. WT 4h Click-iT compared to WT 2h mRNA-seq R0. This comparison was designed to distinguish between zygotic and maternal zygotic genes. Zygotic genes are expected to be enriched by Click-iT RNA-seq at 4 hpf compared to RibZero purified maternal mRNAs at 2 hpf measured by mRNA-seq. In this comparison, read counts from the Click-iT RNA-seq experiment were normalized to RPKMs using the total exonic reads in the sample (Figures S1B and S1E).

c. Exon RPKM in WT 4h Click-iT (normalized by total). To define additional high confidence zygotic and maternal zygotic genes, a high Click-iT RPKM cut-off was applied. This is aimed to avoid potential background from maternal mRNAs purified by Click-iT. Cut-off of 10 RPKM was determined by minimum expression of zygotic genes identified in previous studies (Figure S1A).

d. WT 4h Click-iT compared to triptolide 4h Click-iT. Zygotic and maternal zygotic genes are expected to have higher exon and intron RPKM in WT embryos compared to triptolide treated embryos (Figures S1B, S1C, S1E, and S1F). Intron signal is more sensitive than exon signal as most maternally deposited mRNAs are spliced and the introns are degraded early on ([Lee et al., 2013]). In this comparison, exonic and intronic read counts were normalized to RPKMs using the total reads mapping to mitochondrial protein-coding genes. As the overall intron RPKMs are ~4-10 fold lower than exon RPKMs (Figure S1F), a 30 RPKM intron cut-off was used corresponding to ~7-fold decrease to the minimum RPKM cutoff used for exons.

e. WT 4h compared to triptolide 4h in mRNA-seq R0 on exon. The additional zygotic and maternal zygotic candidates were further restricted by mRNA-seq as zygotically expressed genes are expected to have greater exon expression in WT embryos compared to embryos treated with triptolide.

f. WT 4h compared to triptolide 4h in mRNA-seq R0 on intron. Zygotically expressed genes were expected to have greater intron expression as newly transcribed zygotically expressed genes are spliced and detectable in WT embryos, unless the gene has no intron or the intron is small, hence this condition is only applied if the intron length is >= 500bp.

Identification of Zygotically Activated Genes

For Click-iT RNA-sequencing assays in which ZGA factors were overexpressed (P300, BRD4), a gene was considered to be activated in the overexpression condition if the gene had at least 4-fold increase in exon or intron RPKM in the overexpression condition relative to triptolide or α-amanitin treated embryos at the same stage. For Click-iT RNA-sequencing assays involving chemical or genetic treatment (chk1, Pateamine A+ Cycloheximide), genes were considered to be activated in the wildtype or treated embryos if those genes had at least 4-fold increase in exon RPKM or intron in the condition relative to the same condition treated with triptolide or α-amanitin. The fold increase was calculated after adding 0.1 RPKM to genes in both conditions in the comparison for a more stringent filter for genes with low RPKM, which effectively sets a 0.3 RPKM cut off. For biplots created in this study, a small 0.1 RPKM was added to the expression of each gene in both conditions to allow the display of genes with zero counts in any condition. For biplots comparing gene expression in chk1 or PatA+CHX treated embryos, 0.01 RPKM was added to each gene given that these embryos have lower gene expression due to the fewer number cells and DNA template per embryo.

ChIP-seq Analysis of Histone Marks

Previously published ChIP-seq data [GSE32483] of H3K27ac, H3K4me3 and H3K4me1 marks at dome stage ([Bogdanovic et al., 2012]) were realigned to the zebrafish GRCz10 genome using Bowtie v1.1.2 ([Langmead et al., 2009]) using parameters -v 3 -best -strata -all -chunksize 1000 -m 5000. Histone mark signal for each gene was calculated by the total number of reads mapped to the gene body region and 1,000 nt upstream of the transcription start site, and normalized by the total number of reads aligned the genome and the length of the gene body plus 1,000 nt upstream. Histone mark signal per Kilobase (RPKM) for each gene was reported and used for the correlation with gene transcription level. The gene length for miR-430 is 17164nt = 16164 + 1000nt. For RPM calculation on gene tracks, ChIP-seq reads were normalized to the total number of reads mapped to the zebrafish genome per million, i.e. RPM = Reads per million mapped reads to the zebrafish genome (Figure 5A and S6G).

Heatmap

Heatmaps were created using R 3.3.3 and package gplots. Fold change of gene expression between different conditions was first taken base 2 logarithm and then capped at -5 to 5. Genes with no expression in one of the conditions in the comparison were assigned infinite fold change and capped by the value mentioned above. Genes with no expression in both of the conditions were taken as no change between conditions.

Filtering and Alignment of Ribosome Profiling Reads

The Illumina TruSeq index adaptor sequence was trimmed from raw reads by aligning its sequence, requiring 100% match of the first five base pairs and a minimum global alignment score of 60 (Matches: 5, Mismatches: -4, Gap opening: -7, Gap extension: -7, Cost-free ends gaps). Trimmed reads were then depleted of rRNA, tRNA, snRNA, snoRNA and misc_RNA from Ensembl and
RepeatMasker annotations using strand-specific alignment performed with STAR 2.6.1c (Dobin et al., 2013) with the following non-default parameters: --alignEndsType Local --seedSearchStartLmaxOverLread 0.8 --outReadsUnmapped Fastx --outSAMtype None. Filtered reads were aligned strand-specifically to the zebrafish GRCz11 genome assembly using STAR with the following non-default parameters: --alignEndsType EndToEnd --seedSearchStartLmaxOverLread 0.8 and the exon-junction coordinates from Ensembl r92 (Cunningham et al., 2019).

**Calculating mRNA Translation Rates**
Translation rate was calculated by counting the number of ribo-seq reads per kilobase per million reads (RPKM) for each effective coding sequence. Effective CDSs exclude the first and last three codons and are shifted 12 nt upstream to position each read at the ribosome P-site location, as described in Bazzini et al. (2014). Using the effective CDS of each transcript allows for computation of translation rate from actively translating ribosomes. Reads overlapping effective CDSs by a minimum of 10 nucleotides, and matching up to five times in the genome (each mapping site counting 1/n, n = number of mapping sites) were included. Replicates for each time-point were combined. To restrict reads to bona fide ribosome protected fragments, only fragments of length 27, 28 and 29 were kept for time-points 2 hpf (AGS000372) and 5 hpf (AGS000372). All lengths were kept for 0 hpf sample (AGS000069) in absence of translation frame observed on metagene analysis.

**QUANTIFICATION AND STATISTICAL ANALYSIS**
No statistical methods were used to predetermine sample size. No data were excluded from the analyses. The experimental findings were verified by independent experimental replicates as indicated in figure legends and text. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment. Box and whisker plots are presented with the box extending from 25th to 75th percentiles and the line in the middle of the box represents the median; and whiskers representing the minimum to maximum value. The mean and error plot are represented with the mean values and s.d. error bars. For unpaired two-tailed t-test was performed and P values were calculated with Prism (GraphPad Software, La Jolla, CA, USA). Rank correlation independence test was performed and P values were calculated for the comparison between transcription level and histone mark signal.

**DATA AND SOFTWARE AVAILABILITY**
Input raw reads are publicly accessible in the Sequence Read Archive under SRP184786. To facilitate data download, internal to lab (AGx) and SRA (SRx) IDs are listed in Table S6 and at https://data.giraldezlab.org. All other relevant data are available from corresponding authors upon reasonable request.