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Review article

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The maternal-to-zygotic transition: reprogramming of the cytoplasm and nucleus

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Abstract

A fertilized egg is initially transcriptionally silent and relies on maternally provided factors to initiate development. For embryonic development to proceed, the oocyte-inherited cytoplasm and the nuclear chromatin need to be reprogrammed to create a permissive environment for zygotic genome activation (ZGA). During this maternal-to-zygotic transition (MZT), which is conserved in metazoans, transient totipotency is induced and zygotic transcription is initiated to form the blueprint for future development. Recent technological advances have enhanced our understanding of MZT regulation, revealing common themes across species and leading to new fundamental insights about transcription, mRNA decay and translation.

Sections

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Introduction

The maternal-to-zygotic transition (MZT; also called the oocyte-to-embryo transition) represents a complete reprogramming of the newly formed embryo through an overhaul of cytoplasmic transcripts and proteins and the reprogramming of the chromatin to enable zygotic genome activation (ZGA; also called embryonic genome activation) (Fig. 1a). These interconnected processes collectively reshape the oocyte gene expression programme into that of a totipotent embryo, which later differentiates into various cell types to form an organism (Box 1). Consequently, efforts to achieve a comprehensive molecular understanding of how MZT affects early developmental events and gene regulation are providing fundamental insights into the regulation of transcription, mRNA decay and translation, and have important implications for human fertility, development and disease.

Upon fertilization, the newly formed zygote inherits the cytoplasm of the oocyte and the nuclei from highly differentiated sperm and oocyte. Early embryonic development involves reprogramming these parental cells to a new embryonic state of transient totipotency. The capacity of the oocyte cytoplasm to reprogramme the nucleus is evident in somatic cell nuclear transfer (SCNT) reprogramming shown by Gurdon and colleagues[1] and parallels the process of induced pluripotent stem cell generation[2]. The reprogramming of the embryo first occurs in the absence of zygotic transcription and is instead driven by maternally deposited proteins and the translation of maternally deposited mRNA transcripts[3,4]. Remodelling of translation is central to this process[5] as it regulates, through the ribosome, both the protein and mRNA contents of the developing embryo. Cytoplasmic reprogramming also entails the dramatic repression and clearance of maternal transcripts during the MZT, largely mediated by regulating poly(A) tail length. The nuclear reprogramming of the embryo begins immediately after fertilization, characterized by a remodelling of the epigenetic landscape to establish a naïve chromatin state[6–8]. This enables the onset of ZGA, which occurs at varying times across species but is reproducible within a species[9–15] (Table 1 and Fig. 1b). Zygotic transcription begins with the activation of a small number of genes, followed by the transcription of thousands of genes[16]. Ultimately, the zygote assumes developmental control through initiating de novo transcription.

Technological advances have greatly enhanced our ability to interrogate fundamental processes underlying the MZT. Techniques such as mass spectrometry and high-resolution structural analysis have provided new mechanistic insights into translational[17] and transcriptional activation[18–20]. Recent microscopy advances have also been instrumental, including super-resolution single molecule imaging to interrogate transcription factor (TF) behaviour in reprogramming[21,22]; imaging methods with high temporal resolution to visualize transcription[23–26] and translation dynamics[27,28]; and expansion microscopy to investigate how chromatin organization influences transcription[29]. Advances in genomics methods, such as metabolic RNA labelling sequencing approaches[30], are helping to disentangle maternal and zygotic RNA dynamics. Low-input genomics methods are especially critical given the limited number of cells in early embryos undergoing MZT and have been essential in mapping early mouse embryo DNA and RNA modifications[31–35] and genome architecture[36–38]. Furthermore, ribosome profiling methods have furthered our understanding of MZT post-transcriptional regulation[39–44] and identified key regulators of ZGA[45–48]. Taken together, these approaches are revealing how gene expression dynamics and subcellular localization are precisely regulated, offering a deeper understanding of the MZT.

In this Review, we discuss how maternal factors and nuclear chromatin are remodelled to create a state of transient totipotency. We emphasize common themes during MZT, highlighting work from fruit flies (*Drosophila melanogaster*), zebrafish (*Danio rerio*), frogs (*Xenopus tropicalis* and *Xenopus laevis*) and mice (*Mus musculus*). As the role of *trans*-acting factors in regulating maternal transcript clearance has been reviewed recently[3,4], we emphasize the equally important role of translation-dependent clearance mechanisms. Whereas early MZT studies focused on dissecting the timing of ZGA onset and other developmental events (Box 1), here we instead highlight recent work that has leveraged the unique transition from transcriptional silence to robust transcription to yield fundamental mechanistic insights into transcriptional activation that have implications beyond ZGA[16].

Reprogramming the cytoplasm

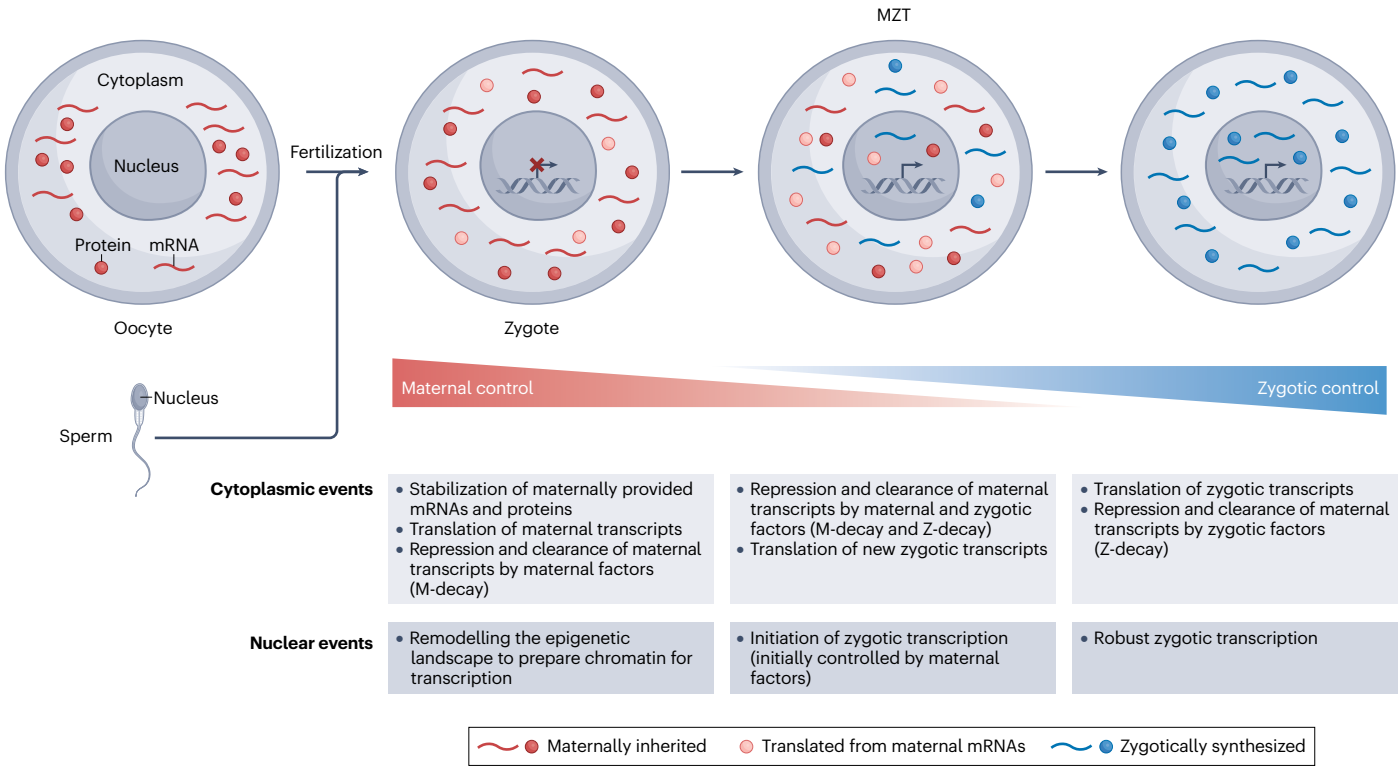
In the absence of transcription, the fertilized egg initially relies solely on maternal proteins and RNAs deposited into the oocyte. The earliest cytoplasmic reprogramming events in the embryo therefore entail post-translational regulation of maternally provided proteins and activation of maternal mRNA translation. Subsequently, as development proceeds, the embryo further reprogrammes its cytoplasm via post-transcriptional and post-translational mechanisms that enable the clearance of the maternally provided factors to prepare the embryo for future development.

Control of maternal factors and mRNA translation

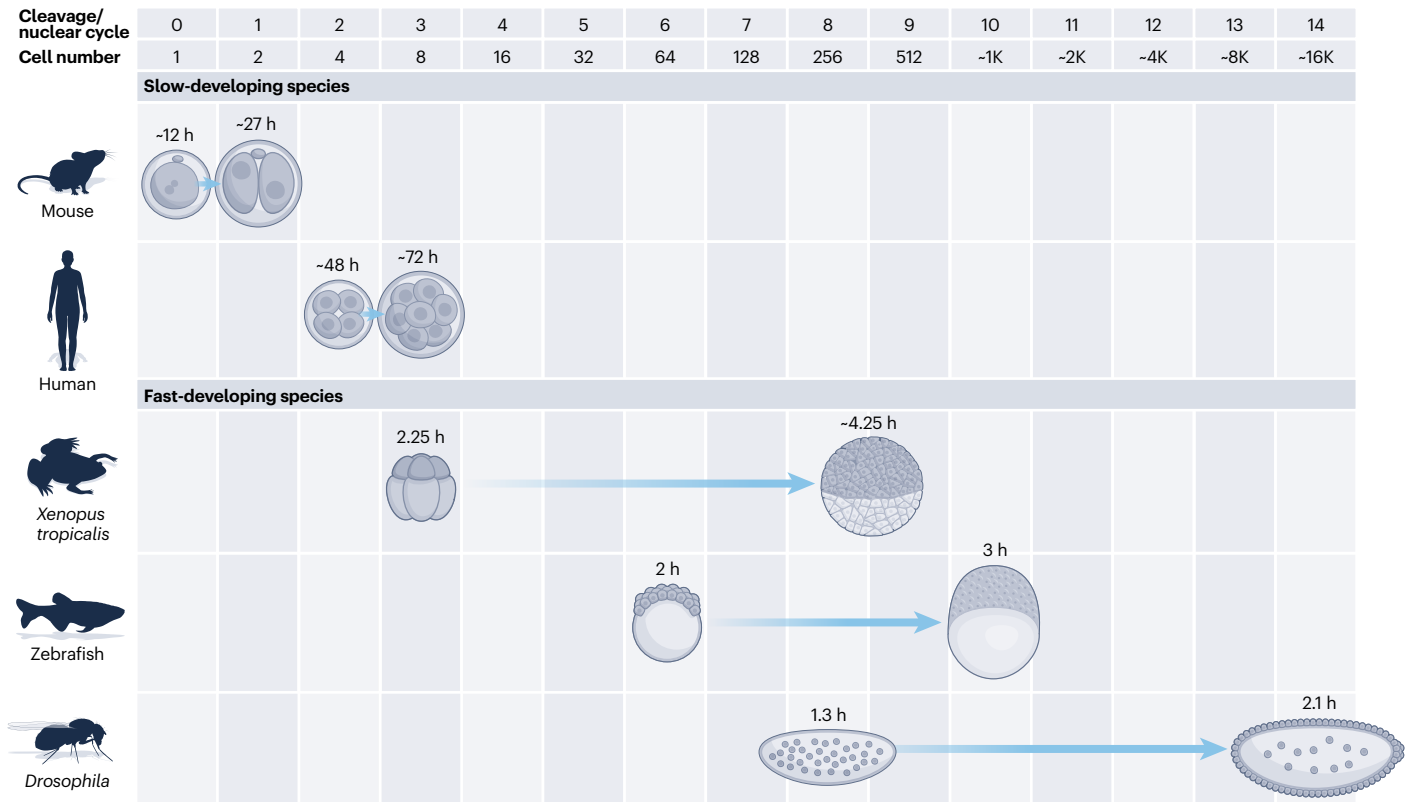
Transcripts deposited in the oocyte are exceptionally stable owing to factors such as specialized RNA binding proteins (RBPs) that protect mRNAs from decay; binding of translational repressor proteins, such as cytoplasmic polyadenylation element binding protein (CPEB) which binds to cytoplasmic polyadenylation element (CPE) motifs in the 3′ untranslated regions (UTRs) (see the section 'Translation-dependent mechanisms of mRNA clearance' for how translation levels affect mRNA stability); and a unique regulatory regime that enables the stability of short, deadenylated poly(A) tails[49–55]. Although poly(A) deadenylation typically drives RNA decay in somatic cells by triggering 5′ decapping and degradation[56], deadenylated mRNAs are uniquely stable in oocytes

Fig. 1 | An overview of the MZT. a, The maternal-to-zygotic transition (MZT) is a reprogramming of the embryonic cytoplasm and the nucleus. Upon fertilization of an oocyte by the sperm, early development is driven by maternally provided proteins, as well as by newly translated proteins from maternal transcripts. Maternal mRNAs are cleared by maternal factors (maternal decay (M-decay)) or zygotic factors (zygotic decay (Z-decay)). The reprogramming of the nucleus enables zygotic genome activation (ZGA), which results in zygotic transcripts that eventually change the composition of the cytoplasm to be zygotic. **b**, Timing of ZGA onset in fast-developing and slow-developing embryos is depicted in hours post fertilization and cleavage/nuclear cycle number. The first embryo of each pair denotes the timing of the first reproducible transcription events, whose detection often requires highly specialized or sensitive methods; the second embryo indicates the timing of the large-scale onset of zygotic transcription. These events have traditionally been called 'minor' and 'major' waves of ZGA, respectively. Recent studies in several species also suggest even earlier transcription events, although many of these may be spurious (Table 1). Note that the timings depicted for *Xenopus* ZGA are for *Xenopus tropicalis*; in *Xenopus laevis*, robust ZGA begins at cleavage cycle 12, at ~5 h post fertilization.

a **The embryonic cytoplasm and nucleus are reprogrammed during the MZT**



b **Timing of initial and robust zygotic transcription in fast-developing and slow-developing species**



Box 1 | The developmental context of the MZT

Genome activation timing varies across species (Table 1 and Fig. 1b) and can be divided into fast-developing and slow-developing embryos[403]. Fast-developing (non-mammalian) embryos develop in externally laid eggs that initiate zygotic genome activation (ZGA) shortly after fertilization to ensure rapid development and reduce predation[404]. Early cleavage cycles are rapid and alternate between DNA S and M phases, omitting G phases[403,405]. In slow-developing embryos (mammals), ZGA (often called embryonic genome activation) occurs during preimplantation development[403,406]. Their slower cell cycles include G phases from the start[403]. In mammals, zygotic transcription begins later in absolute time compared with fast-developing species, with the first zygotic transcripts detected ~12 h post fertilization in mouse embryos[13] (Fig. 1b). Next-generation sequencing enhances mRNA detection sensitivity. Recent studies in some species have detected zygotic transcripts earlier than the canonically appreciated zygotic transcription events[30,399,400] (Table 1); however, these early transcripts may arise from promiscuous transcription events influenced by relaxed chromatin, yielding non-functional mRNAs[166].

In early embryos, initial cell divisions occur without cell growth, with each division resulting in a higher nuclear-to-cytoplasmic volume ratio (N:C ratio)[346,407]. Embryos shift from increasing cell numbers to body axis elongation through gastrulation at the mid-blastula transition (MBT), where cell cycles lengthen owing to a prolonged S phase and the introduction of G phases, and in *Drosophila melanogaster* cellularization occurs[232,344,401]. In fast-developing species, the first zygotic transcription events precede the MBT, but large-scale ZGA coincides with MBT[232,344,401]. Therefore, MBT occurs during maternal-to-zygotic transition (MZT), but these terms describe two distinct transitions.

During MZT, cells gain unique molecular identities and positional information, preparing for the first cell movements and lineage commitments during germ-layer specification[344,401,403]. In species where primordial germ cells segregate early in development, their MZT dynamics differ from the rest of the embryo[103,408–411]. Failure of ZGA or MZT can result in gastrulation failure in many organisms and preimplantation failure in mammals[185,344,406,412].

and very early embryos[57,58], which is important given their transcriptionally quiescent state and, thereby, their inability to replenish mRNAs (Fig. 2a). This stability is driven partially by a lower decapping activity in *X. laevis*[59,60] and potentially by protection of the mRNA cap by the eIF4E1b cap binding protein in zebrafish[49]. Furthermore, the mRNA 5-methylcytosine (m^5c) modification is prevalent in maternal transcripts across species and enhances RNA stability. Disrupting this m^5c landscape impairs embryo development in *D. melanogaster* and zebrafish[61,62], supporting the importance of enhanced mRNA stability in oocytes and early embryos.

Reprogramming of the maternally provided cytoplasm involves activation of translation of oocyte-provided mRNAs and occurs through several mechanisms, some of which begin even before fertilization. For example, CPEB is phosphorylated upon resumption of oocyte meiosis, which switches its function from a translational repressor to a translational activator[54,63] through the recruitment of specialized poly(A) polymerases that lengthen poly(A) tails[43,64–67] (Fig. 2a). In early embryos, poly(A) tail length is strongly correlated with translational efficiency[40,43,58,64]. The cytoplasmic poly(A)-binding protein PABPC enhances translation[44] and is present at limiting levels (Fig. 2a); thus, transcripts with the longest poly(A) tails are more likely to bind PABPC and undergo translation[44]. The exact timing of re-adenylation and translation is strongly influenced by the number and position of 3′ UTR CPEs and/or other motifs[47,48,54,58,66,68–70]. Re-adenylation is universally important for embryogenesis; blocking it, for example with the adenosine analogue cordycepin (3′-dA), halts early embryonic development[67,71,72]. In addition to poly(A) tail length changes, maternal transcripts undergo other remodelling events, including partial 3′ UTR degradation before re-adenylation[72–74], which potentially increases translational efficiency[74], and the addition of G residues in poly(A) tails, which increases transcript stability by preventing deadenylation by the CCR4–NOT complex[72,75].

Regulation of maternally provided proteins also has a key role in cytoplasmic reprogramming and activating translation. As discussed above, post-translational modification of CPEB promotes translation through a switch in protein function. However, post-translational modifications can have a broader impact on translation and the protein landscape; for example, in *D. melanogaster*, PNG kinase-dependent phosphorylation of many maternally provided translational repressors leads to their inactivation and degradation[41,76,77]. Lastly, ribosomal translational activity increases in early embryos through the activation of 'dormant' ribosomes[17,78]. In zebrafish and *X. laevis* oocytes and earliest-stage embryos, ribosomes exist primarily as 'dormant' monosomes because specialized proteins (Dap1b, eIF5a, eEF2 and Hapb4 in zebrafish) block critical ribosome sites (the polypeptide exit channel, the exit and peptidyl sites, the aminoacyl site and the mRNA-entry channel) (Fig. 2b). Release of these dormancy factors has been suggested to allow dormant ribosomes to become translationally active polysomes. Conservation of dormancy factors across species from *D. melanogaster* to mammals suggests that inactive ribosomes are a universal feature of early embryogenesis[17]. This limited ribosome availability creates a unique regulatory framework whereby transcripts compete for active ribosomes and limited PABPC (Fig. 2). The resulting low translation rates in early embryos may protect transcripts from translation-dependent decay, creating a reservoir of mRNAs that can be activated once translation ramps up. In the future, it would be interesting to explore whether specific membraneless compartments in the oocyte safeguard these maternal mRNAs for later use in the embryo.

Clearance and remodelling of the maternal programme

The initiation of translation not only marks the start of embryonic development but also signals the beginning of the end for many maternally deposited mRNAs. Maternal transcript clearance involves translation-dependent and translation-independent mechanisms and can be driven by the maternal programme (maternal decay (M-decay)) or the zygotic programme (zygotic decay (Z-decay), which is dependent on ZGA)[79] (Table 1 and Fig. 3a,b). Transcript susceptibility to M-decay or Z-decay may depend on mRNA function or temporal expression requirements for each gene. For example, transcripts regulated by the earlier M-decay include those needed for oogenesis or during early cell cycles pre ZGA but whose prolonged expression could be detrimental to development and gastrulation[4,80]. Many decay mechanisms converge on poly(A) tail shortening, often through recruitment of the CCR4–NOT deadenylase complex (Fig. 3c). As described above, the direct effect

of deadenylation in early embryos is translational repression[40,43,47,58,64] owing to limiting levels of PABPC[44]. Deadenylation ultimately results in destabilization and clearance of deadenylated transcripts, but only during later embryonic stages. The post-transcriptional regulation of many transcripts allows the embryo to integrate distinct signalling pathways and cellular processes to coordinate embryogenesis. The embryo also reprogrammes the maternal proteome, an aspect of the MZT that has received less attention.

Translation-independent mechanisms of mRNA clearance. A large fraction of maternal mRNAs are destabilized by small RNAs, particularly microRNAs (miRNAs). For example, zebrafish *miR-430* (ref. 81) and its *Xenopus* spp. homologue *miR-427* (refs. 11,82) trigger Z-decay[81,82] and attenuate zygotic transcript levels[81,83]. These miRNAs primarily target 3′ UTRs[84] but can also target 5′ UTRs[85–88] and coding sequences[86,88]. miRNAs bring Argonaute to the mRNA, which recruits TNRC6, a translational repressor and an adaptor for the CCR4–NOT deadenylation complex[84] (Fig. 3c). This complex triggers translational repression[39,40] and mRNA deadenylation[81]. The unrelated *miR-309* serves a similar function in *D. melanogaster*[89], suggesting convergent evolution of miRNA-mediated maternal clearance. Importantly, these miRNAs are all found in multicopy clusters[81,82,89] — the zebrafish *miR-430* locus has >300 promoters and encodes 1,800 mature miRNAs[29,90], and the *X. tropicalis miR-427* locus has >170 miRNA repeats[11] — that enable rapid accumulation of miRNAs and swift clearance of maternal transcripts during the MZT. *miR-430* is also zygotically expressed in other fish species[91] and *miR-430/427* may have independently undergone expansion in multiple vertebrate species[11,82,92]. Although miRNAs help to ensure robust cytoplasmic reprogramming in fast-developing organisms (such as *D. melanogaster*, zebrafish and *Xenopus* spp.) (Box 1), miRNA processing factors are not required in mice for maternal clearance[93], perhaps because their slower development does not necessitate such strong clearance dynamics. However, mammalian homologues of *miR-430/427* (ref. 94) (*miR-290–295* in mouse and *miR-371–373* in human, also *miR-302*) are expressed in early mouse embryos[95] and mammalian stem cells[96,97], can facilitate reprogramming in vitro and help to maintain pluripotency[98,99]. These observations highlight the parallels between cytoplasmic reprogramming to support transient totipotency during MZT and somatic reprogramming. miRNA-mediated post-transcriptional regulation provides an elegant evolutionary strategy to regulate many transcripts through small changes in the 3′ UTR creating new target sequences in mRNAs: the speed and extent of regulation of transcripts containing the same target site could be controlled simultaneously, with different target sites modulating different cellular processes. Other small RNAs involved in maternal transcript clearance include endogenous short interfering RNAs (endo-siRNAs) in mouse[93,100] and *Caenorhabditis elegans*[101,102], and Piwi-interacting RNAs (piRNAs) in *D. melanogaster*[103]. Similar to miRNAs, these small RNAs function via the highly conserved Argonaute/Piwi protein family, making Argonaute proteins the universal player in small RNA pathways and maternal RNA clearance (Fig. 3b).

Table 1 | Species-specific regulation of the maternal-to-zygotic transition

	Drosophila melanogaster (fly)	*Danio rerio* (zebrafish)	*Xenopus tropicalis* and *Xenopus laevis* (frog)	*Mus musculus* (mouse)	*Homo sapiens* (human)
Regulators of maternal clearance					
Regulators of M-decay	Smaug[80], BRAT[a,113], Aubergine/piRNAs[103], codon optimality[135], AU-rich elements[b,c,114]	TUT4/7 (ref. 129), m^{6}A[a,104,105], codon optimality[135,136], Upf1/ORF-mediated decay[150], C-rich elements[b,110]	TUT4/7 (ref. 129), EDEN-BP/Celf1 (ref. 111), AU-rich elements[57,117] codon optimality[135]	endo-siRNAs/AGO2 (refs. 99,100), BTG4 (refs. 121,122), ZFP36L2 (ref. 118), PABPN1L[123], codon optimality[135]	BTG4 (refs. 126,132)
Regulators of Z-decay	miR-309 (ref. 89), BRAT[a,113], Pumilio[113,114,395]	miR-430 (ref. 81), m^{6}A[a,104,105], Hnrnpa1[b,109], AU-rich elements[b,110,116], Pumilio[b,116]	miR-427 (ref. 82)	TUT4/7 (ref. 131), endo-siRNAs/AGO2 (refs. 99,100), PABPN1 (ref. 396), m^{6}A (refs. 35,107,108)	TUT4/7 (ref. 132)
Regulators of ZGA					
Maternally deposited transcription factors with demonstrated or suggested pioneering ability that regulate early zygotic gene expression	Zelda[169], GAF[179], CLAMP[180,181]	Nanog[45], Pou5f3 (refs. 45,172), Sox19b (refs. 45,172)	Foxh1 (ref. 177), Pou5f3 (ref. 176), Sox3 (ref. 176), Vegt[178,397], Otx1 (ref. 178)	OBOX[184], NFY[193], NR5A2 (refs. 187,188,190)	TPRX[48]
Timing of ZGA					
Stage when zygotic transcription is first observed[d]	Nuclear cycle 8 (refs. 12,398) [nuclear cycle 6 (ref. 399)]	64-cell stage[10] [2-cell stage[30]]	8 cell (*X. tropicalis*)[11]	1 cell[13]	2–4 cells[15] [1 cell[400]]
Embryo stage where robust ZGA is observed	Nuclear cycle 14 (ref. 349)	1,000-cell stage[401]	Stage 8 (*X. tropicalis*: between cleavage cycles 8 and 9; *X. laevis*: cleavage cycle 12)[11,344]	2 cells[13,14]	4–8 cells[402]

BRAT, Brain tumour; endo-siRNA, endogenous short interfering RNA; m^{6}A, *N*^{6}-methyladenosine; M-decay, maternal decay; ORF, open reading frame; piRNA, Piwi-interacting RNA; Z-decay, zygotic decay; ZGA, zygotic genome activation. [a]Some regulators have been implicated in both M-decay and Z-decay and are therefore listed in both rows. [b]Regulators implicated in maternal transcript clearance and/or stability but without an established mechanism. [c]Unclear whether M-decay or Z-decay. [d]Spurious zygotic transcription, perhaps due to open chromatin, has been observed in some species. The timing of those observed events is denoted in square brackets.

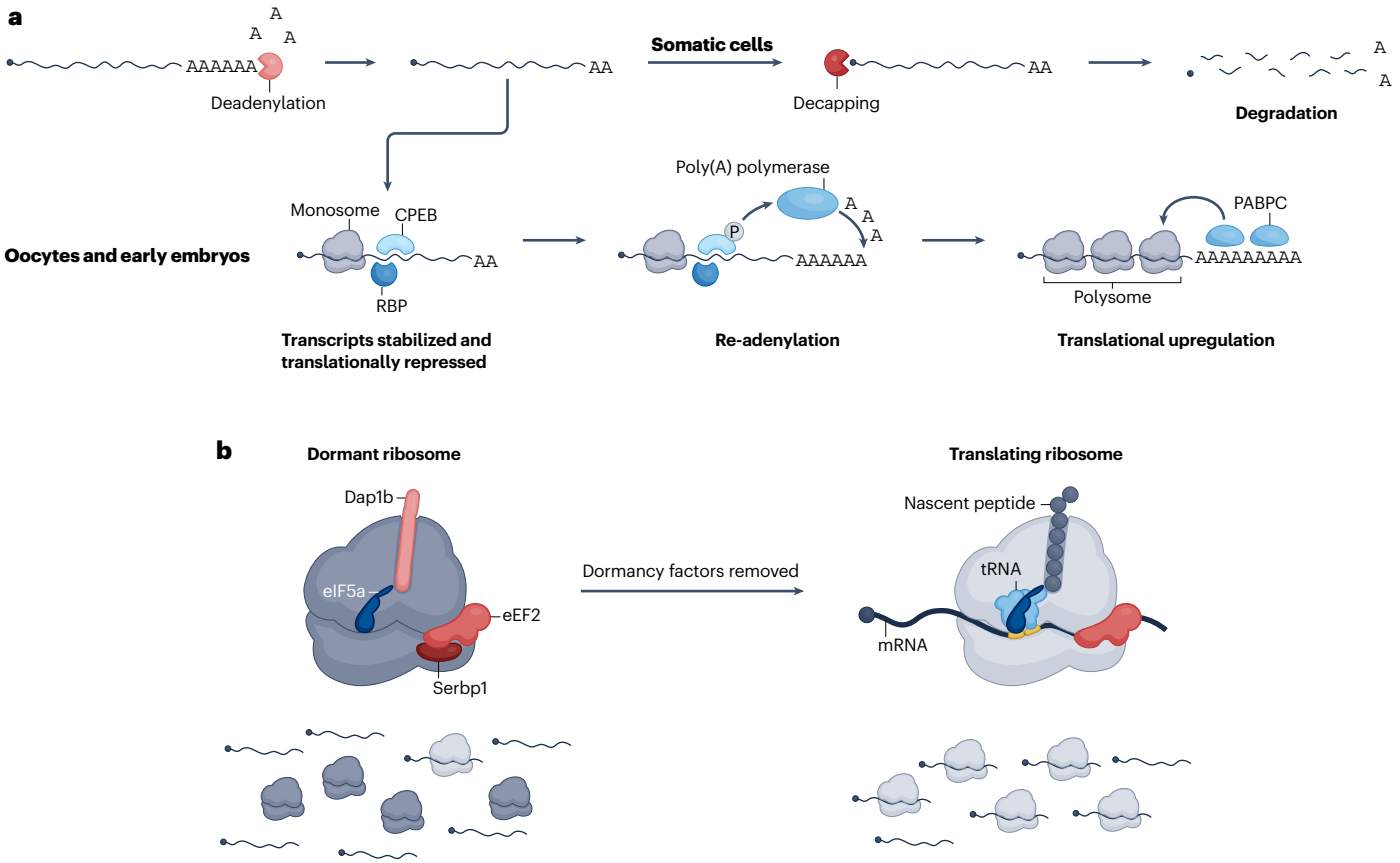


Fig. 2 | Regulating the stability and translation of maternally deposited transcripts. a, In somatic cells, transcript deadenylation is the rate-limiting step for decay. However, deadenylated transcripts remain stable in oocytes and early embryos due to protective RNA binding proteins (RBPs) and overall low translation levels that protect mRNAs from translation-dependent decay. Cytoplasmic polyadenylation element binding protein (CPEB) represses translation initially, until its later activation by phosphorylation leads to recruitment of a poly(A) polymerase and cytoplasmic polyadenylation of transcripts. Longer poly(A) tails, which better compete for the translation-promoting poly(A)-binding protein PABPC, lead to translational upregulation. Poly(A) tail length and translational efficiency are coupled in pre-gastrulation embryos. **b**, Model (based on data from zebrafish and *Xenopus laevis* embryos)[17] depicting how ribosome dormancy affects translation levels in early embryos. Initially, ribosomes are largely repressed by dormancy factors, leading to transcript competition for a limited pool of active ribosomes. Ribosome activation through the removal of dormancy factors leads to translational upregulation and exposes mRNAs to translation-dependent decay. Dormant ribosomes are coloured dark grey; active ribosomes are coloured light grey.

mRNA modifications also facilitate transcript clearance during the MZT (Fig. 3b). In zebrafish, N^6-methyladenosine (m^6A) marks about one-third of maternal transcripts and induces mRNA deadenylation and subsequent decay[104,105]. m^6A co-regulates deadenylation alongside *miR-430*, demonstrating that multiple destabilizing programmes can act additively to clear maternal mRNAs. Recent innovations in low-input genomics methods have enabled profiling of m^6A in mouse oocytes and embryos, revealing that m^6A shapes the oocyte transcriptome[106] by initially promoting stability in late-stage oocytes but later destabilizing transcripts in two-cell embryos[35,107,108].

RBPs mediate target specificity and timing of transcript clearance by recognizing specific RNA motifs or structures[109,110] (Table 1 and Fig. 3b). For example, in *X. laevis*, EDEN-BP/Celf1 binds the embryonic deadenylation element (EDEN) motif to induce deadenylation[111]. In *D. melanogaster*, several RBPs collaborate to regulate maternal transcripts. To drive M-decay, Smaug[42,80,112] and Brain tumour (BRAT)[113] collectively destabilize thousands of mRNAs, with each protein targeting a non-overlapping set of transcripts. BRAT[113] and Pumilio[114] later regulate Z-decay[113,114]. There are several commonalities in RBP-mediated maternal clearance across species. Many RNA motifs and RBPs overlap across species, such as AU-rich elements (AREs) and/or ARE binding proteins[57,110,114–118]. Maternally provided RBPs are often activated post-transcriptionally or post-translationally; for example, dephosphorylation of *X. laevis* EDEN-BP/Celf triggers its activity[119]. Many RBPs, including *D. melanogaster* RBPs[112,120] and BTG4 (refs. 121,122) and PABPN1L (ref. 123) in mice, induce deadenylation by recruiting the CCR4–NOT deadenylase complex, which initially reduces protein output before eventually triggering RNA decay[40,43,47] (Fig. 3c). Some RBPs also recruit translational repressors[124,125]. The developmental importance of these RBPs is underscored by the observation that homozygous mutations of human *BTG4* are linked to infertility, and human zygotes derived from *BTG4*-deficient oocytes do not undergo cleavage divisions[126].

In addition to motifs that serve as RBP binding sites or miRNA target sites, other RNA motifs strongly affect transcript stability.

For example, in early embryos of multiple species, U-rich elements in UTRs are stabilizing and C-rich elements are destabilizing[101,110,116]. The exact mechanisms by which these motifs exert differential stability remain unclear, although they could involve specific RBP binding (such as the poly(C) binding protein)[101,110], their sequence similarity to other important motifs (such as the CPE motif)[70] or their effect on translation[70,87,127].

Transcript uridylation[128] also drives maternal clearance. At MZT onset, many species (although not *D. melanogaster*) add uridyl residues to the 3′ end of transcripts with very short (<25 nucleotides) poly(A) tails to mark them for decay[72,129–132]. In zebrafish and *X. laevis*, maternally deposited terminal uridyltransferases TUT4 and TUT7 drive M-decay[129], whereas mouse TUT4/7 expression shapes the transcriptome during oogenesis[133], with zygotic expression facilitating Z-decay[131] (Fig. 3b). TUT4/7-mediated mRNA clearance is required for gastrulation in zebrafish and *X. laevis*[129] and development past the four-cell stage in mice[131].

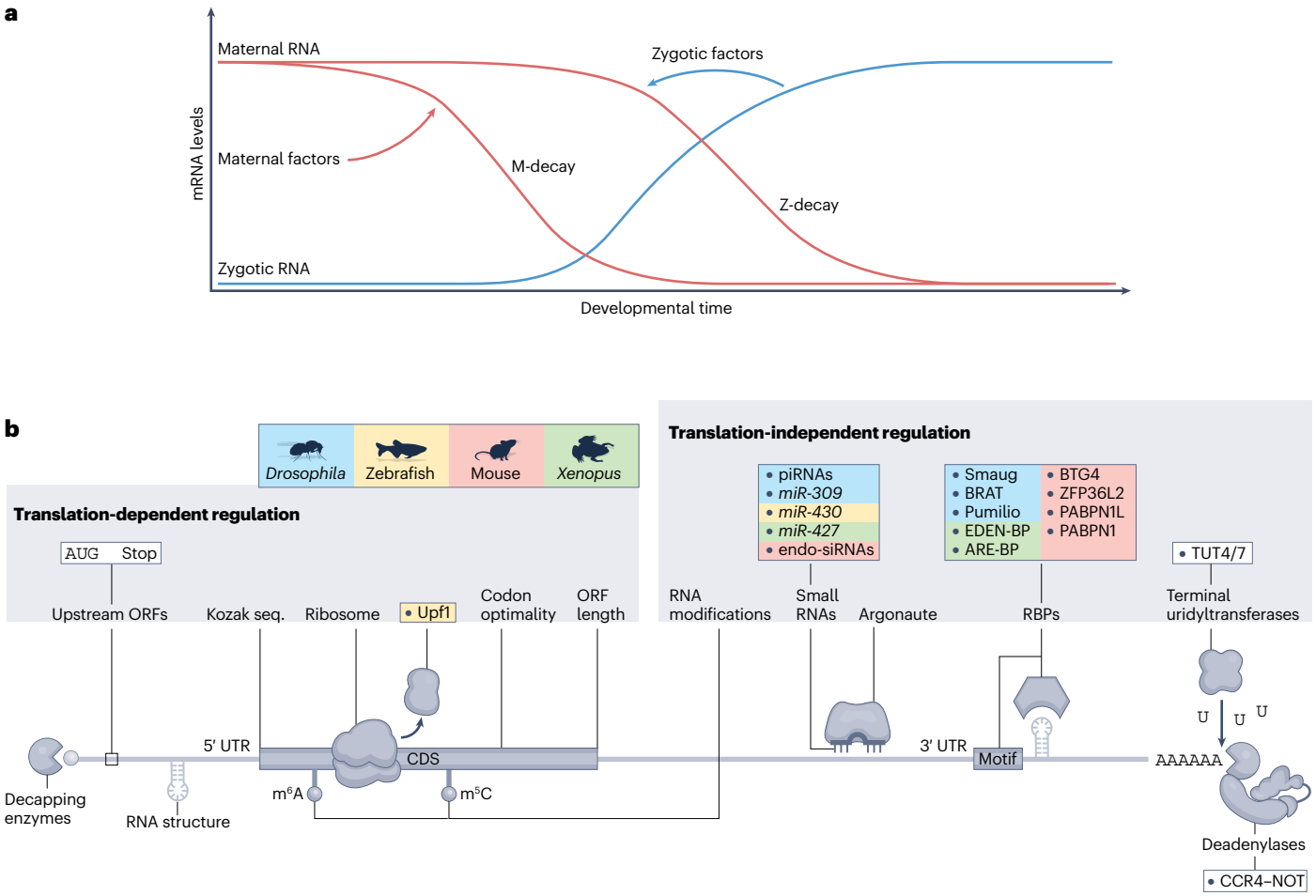
Overall, it is becoming evident that these decay mechanisms comprise a regulatory toolkit that enables embryos to repress translation or induce mRNA decay, depending on the exact developmental time to meet the demands of the growing embryo.

Translation-dependent mechanisms of mRNA clearance. During translation, mRNAs are extensively bound by ribosomes, which are increasingly recognized as universal regulators of transcript stability[5]. Codon usage bias has recently been linked to ribosome dynamics and its effect on transcript stability and translation efficiency[134–136]. Transcripts enriched with optimal codons have long poly(A) tails and are efficiently translated[135,136], whereas ribosomes stall at non-optimal codons and trigger transcript deadenylation through a conformational change that involves recruitment of the NOT5 subunit of the CCR4–NOT complex to the ribosome E-site[137] (Fig. 3c). Codon optimality is a conserved regulator of transcript dynamics during MZT[135,136]. The increase in translational activity in the embryo exposes transcripts to ribosomes that preferentially clear mRNAs with non-optimal codons, which gives rise to an intrinsic mRNA decay rate and regulates M-decay independently of zygotic transcription[134–136,138]. Differential decay rates between optimal and non-optimal transcripts are lost when translation is blocked by cycloheximide, highlighting the central role of translation in decay[135]. The prevailing view is that tRNA availability is a key contributor to codon optimality. We speculate that changes in tRNA availability during the MZT or later in embryonic development[139,140] could trigger differential transcript stability between maternal and zygotic states, or across different cell types. However, tRNA levels explain only part of this effect and it is likely that encoded amino acids and peptide sequences[141,142] also influence mRNA stability. Disentangling their respective effects on transcript stability remains a major challenge.

Other mRNA features that lower translation rates also negatively impact mRNA stability. In zebrafish embryos, mRNAs with long open reading frames (ORFs), upstream ORFs or weak Kozak sequences exhibit lower translation initiation[143–150] and are bound by the surveillance factor Upf1, which promotes ORF-mediated decay by recruiting decapping and deadenylation factors[150] (Fig. 3c). The absence of Upf1 on highly translated mRNAs suggests that the helicase activity of the actively translating ribosome displaces Upf1, thereby suppressing decay[150]. The conserved role of ORF-mediated decay in regulating mRNA stability in human cells[150] indicates that this is likely a universal mechanism for regulating transcript levels and further highlights the key role of the ribosome in cytoplasmic reprogramming. RNA secondary structure can also modulate translation (Fig. 3b), and thereby stability, by acting as internal ribosome entry sites[151] or by inhibiting ribosome recruitment[152]. Powerful new methods described in preprints, such as NaP-TRAP and polysome profiling with massively parallel reporter assays[87,127], that measure how specific sequence elements (such as UTRs or peptide sequences) affect translational efficiency will further identify transcript features that contribute to translation-dependent maternal transcript dynamics.

Importantly, transcript stability is controlled by multiple regulators that often work simultaneously. Destabilization by non-optimal codons or long ORFs can be counteracted by long 3′ UTRs, which promote stability by reducing deadenylation[136,150]. A transcript that requires slow translation for efficient protein folding could use 3′ UTRs or other stabilizing elements to reduce decay. High codon optimality of a transcript reduces its sensitivity to ORF-mediated decay[150] or maternal clearance factors, such as *miR-430* in zebrafish or Pumilio in *D. melanogaster*[120,138]. Remodelling of mRNA structure[153] by the translating ribosome during the MZT can affect how stability-regulating motifs are interpreted[154]. Lastly, mRNA regulatory features with opposite effects can provide temporal stability control; for example, a zebrafish transcript may be stable early in embryogenesis due to U-rich elements that protect against M-decay, but *miR-430* target sites and AU-rich elements may cause its destabilization and drive Z-decay at later stages[110]. Taken together, translation-independent and translation-dependent decay clearance mechanisms, from both maternal and zygotic programmes, provide a finely tuned system of regulatory elements that enable precise control of maternal transcripts in response to the cellular and developmental needs of the embryo.

Reprogramming of the maternal proteome. Similar to maternal transcripts, oocyte-deposited proteins are stable. In mammalian oocytes, cytoplasmic lattice structures have a key role in sequestering and stabilizing oocyte proteins[155]; mutations in genes encoding cytoplasmic lattice proteins are linked to female infertility, highlighting the importance of this protein stability[156]. However, protein dynamics during the MZT remains an understudied area of research. Matched mRNA translation and proteome data from embryos often show discordant patterns, indicating that translation data cannot predict the proteomic landscape[41,46,157]. Recent advances in low-input proteomics methods now enable proteome changes during the MZT to be quantified, promising exciting new research avenues. In *D. melanogaster*, *X. laevis* and mouse, protein turnover during early embryogenesis is much lower than transcript turnover; many oocyte-inherited proteins persist well after ZGA, with many maternally provided proteins found in eight-cell mouse embryos[157–160]. In zebrafish, maternally deposited proteins can persist for many days, allowing zygotic mutants for essential genes to survive through the early stages of development[161]. However, specific maternally provided proteins, such as Smaug[80,112] and the translational repressor complex ME31B–TRAL–Cup in *D. melanogaster*[76,125], are systematically cleared by the ubiquitin–proteasome system[159,162], which is also essential for proper MZT progression in mouse[163]. Other proteins seem to be present at constant expression levels throughout MZT but are highly translated, suggesting that high protein synthesis rates can counteract maternal protein degradation[41]. Protein re-localization from the cytoplasm to the nucleus is also emerging as an important aspect of reprogramming the embryo proteome towards a zygotic programme[160,164]. Despite these new insights, protein dynamics during the MZT deserve more attention.


a
Maternal RNA
Zygotic factors
Maternal factors
M-decay
Z-decay
Zygotic RNA
mRNA levels
Developmental time
b
Drosophila
Zebrafish
Mouse
Xenopus
Translation-dependent regulation
AUG
Stop
Upstream ORFs
Kozak seq.
Ribosome
• Upf1
Codon optimality
ORF length
5′ UTR
CDS
m6A
m5C
Decapping enzymes
RNA structure
Translation-independent regulation
RNA modifications
• piRNAs
• miR-309
• miR-430
• miR-427
• endo-siRNAs
Small RNAs
Argonaute
3′ UTR
Motif
• Smaug
• BRAT
• Pumilio
• EDEN-BP
• ARE-BP
• BTG4
• ZFP36L2
• PABPN1L
• PABPN1
RBPs
• TUT4/7
Terminal uridyltransferases
U
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Deadenylases
• CCR4–NOT
c Decay mechanisms involving the CCR4–NOT complex
Translation-independent
miRNAs
Ago
TNRC6
miRNA
CDS
miRNA seed
AAAAAAA
CCR4–NOT
AAAAAAA
RBPs
CDS
Smaug
RNA hairpin
AAAAAAA
CCR4–NOT
AAAAAAA
Translation-dependent
Codon optimality
Nascent peptide
CDS
Non-optimal codon
AAAAAAA
CCR4–NOT
tRNA
AAAAAAA
ORF-mediated
Upf1
CDS
AAAAAAA
CCR4–NOT
CCR4–NOT
AAAAAAA

Fig. 3 | Molecular mechanisms governing maternal transcript clearance. **a**, Maternal transcript clearance events can be driven by maternally deposited proteins and proteins translated from maternal mRNAs (maternal decay (M-decay)) or zygotically expressed factors such as microRNAs (miRNAs) or proteins (zygotic decay (Z-decay)). **b**, Translation-dependent (left) and translation-independent (right) mechanisms govern maternal transcript stability and clearance dynamics during the maternal-to-zygotic transition (MZT). Different elements or features of an mRNA transcript and *trans*-acting factors that regulate decay during the MZT are shown. Universal mechanisms or regulators used by multiple species are coloured grey. Species-specific regulators are highlighted in other colours (*Drosophila melanogaster*, blue; zebrafish, yellow; *Xenopus* spp., green; mouse, red) (Table 1). **c**, Different mechanisms can recruit the CCR4–NOT complex, leading to deadenylation and decay. Translation-independent decay mechanisms include recruitment via Argonaute (Ago)-bound miRNA and TNRC6 or via RNA binding proteins (RBPs) (*D. melanogaster* Smaug is shown as an example), although miRNAs and RBPs can also directly affect translation independent of decay. Translation-dependent decay mechanisms include recruitment via non-optimal codons and Upf1. Phosphorylated Upf1 recruits CCR4–NOT on long open reading frames (ORFs) but is counteracted by the higher translation rates of shorter ORFs, thereby preventing Upf1-induced deadenylation. BRAT, Brain tumour; CDS, coding sequence; endo-siRNA, endogenous short interfering RNA; m^5c, 5-methylcytosine; m^6A, N^6-methyladenosine; piRNA, Piwi-interacting RNA; UTR, untranslated region.

ZGA: a nuclear reprogramming event

Concurrently with cytoplasmic reprogramming, the early embryo reprogrammes the terminally differentiated sperm and oocyte nuclei into a totipotent state compatible with zygotic transcription. Here, we describe nuclear remodelling at all scales that enable ZGA (Fig. 4A,B), a molecular model for transcriptional activation (Fig. 4C) and the prevailing models for ZGA timing (Fig. 5). Conventionally, ZGA has been viewed as two 'waves': the initial minor wave encompassing the earliest transcriptional events ('first ZGA events'), followed by the major wave with robust transcription ('robust ZGA' or 'large-scale ZGA')[3] (Fig. 1b). This notion of two distinct waves would suggest that transcriptional activation is discontinuous, but recent experiments measuring zygotic gene expression suggest that zygotic transcripts accumulate in a more gradual and continuous process[9–12,15,30]. To emphasize that ZGA unfolds gradually[3], we therefore avoid using these terms in this Review. It is important to note, however, that there are indeed notable mechanistic differences between the earliest and later-transcribed ZGA genes, suggesting molecular changes in transcriptional regulation as ZGA unfolds[90,165–167]. These differences, as well as differences between maternal and zygotic transcripts, are described further below.

By the time of large-scale ZGA, the nuclear reprogramming of the embryo is well underway. By contrast, cytoplasmic reprogramming remains in progress, with maternal transcripts still dominating the transcriptome owing to delayed, ZGA-dependent modes of maternal mRNA clearance (that is, Z-decay). For example, in zebrafish embryos, zygotic transcripts comprise only ~10% of the transcriptome even 2.5 h after the onset of large-scale ZGA[30]. Maternal influence thus persists long after ZGA, with embryo development under both maternal and zygotic control for some time before the embryo gains full control of its own development (Fig. 1a).

Mechanisms of nuclear reprogramming at ZGA

Activation of ZGA by (pioneer) transcription factors. During nuclear reprogramming, pioneer transcription factors (PFs), chromatin remodellers (CRs) and histone modifying enzymes together reprogramme and prime chromatin to promote zygotic transcription (Fig. 4A). PFs can bind condensed, nucleosomal DNA, facilitating chromatin remodelling and accessibility for subsequent binding by other factors[168]. The *D. melanogaster* pioneer factor Zelda was the first identified ZGA regulator[169–171], followed by Nanog, Pou5f3 (OCT4 homologue) and Sox19b (SOX2 homologue) in zebrafish[45,172]. Underscoring their collective role in regulating ZGA, the combined loss of two or three zebrafish factors leads to a stronger developmental defect than loss of any single factor[173–175]. Similarly, in *X. tropicalis*, Pou5f3 and Sox3 together remodel chromatin to establish ZGA[176], alongside Foxh1 and germ layer-specific TFs[177,178].

ZGA-initiating TFs often display a temporal hierarchy. In *D. melanogaster*, Zelda acts earliest, followed by GAF[179] and CLAMP[180,181]. Subsequently, zygotic Opa activity during gastrulation regulates later developmental patterning[182,183]. In mouse, OBOX family TFs act earliest, with the maternal-zygotic knockout (by genetic deletion of the *Obox* gene cluster) causing a two-cell to four-cell arrest[184], resembling the two-cell arrest observed using chemical inhibition of transcription[185]. OBOX factors are highly translated in one-cell zygotes and regulate the accessibility and transcriptional activation of the earliest transcribed zygotic genes[184]. The maternal knockout alone does not display a phenotype, however, indicating that maternal OBOX proteins cannot be the only regulators of ZGA[184]. In humans, knockdown of TPRX family proteins, which share homology with the mouse OBOX proteins[186], results in downregulation of many ZGA genes, although it remains unclear whether TPRX proteins act as pioneer factors to regulate chromatin accessibility[48]. In mice, the pioneer factor NR5A2 and/or other orphan nuclear receptors have also been implicated as major ZGA regulators, because chemical inhibition of NR5A2 results in downregulation of ~70% of ZGA genes and two-cell arrest[18,187]. However, a recent study indicates that embryos derived from oocytes with conditional genetic deletion of *Nr5a2* are viable[188], suggesting that either maternally provided NR5A2 is dispensable for ZGA or it functions redundantly with other factors, such as related nuclear receptor proteins (NR5A1 or NR2C2) that are also inhibited by the NR5A2 chemical inhibitor[187]. NR5A2 is strongly upregulated in two-cell embryos, contributing to the four-cell to eight-cell transcriptional programme, and is required for development past the morula stage, indicating that it regulates gene expression programmes for extended periods during embryogenesis, beyond ZGA[188–192]. Although other mouse TFs with demonstrated pioneering activity, such as NFY[193] and the zygotically expressed DUX[194–197], also contribute to chromatin accessibility and early gene expression, single knockout (*Dux*) or knockdown (*Nfya*) mice survive beyond ZGA[193,198,199]. However, double or triple-knockouts or knockdowns may have more pronounced effects on mouse ZGA and development[200] due to the combinatorial and potentially compensatory action of these factors, as observed in zebrafish[173–175]. Altogether, it is becoming increasingly clear that many factors involved in ZGA also have later functions in development and that a complex process such as ZGA is regulated by the coordinated action of multiple TFs.

Upon binding to nucleosomal DNA, PFs initiate chromatin opening through multiple mechanisms[168]. Some PFs recruit CRs such as SWI/SNF, which can evict nucleosomes, to further open chromatin[201]. CRs therefore also likely have critical roles in ZGA[46,202,203]. Additionally, PFs promote histone acetylation by recruiting the histone acetyltransferases (HATs) p300 or CBP[173,204,205]. Ultimately, pioneering activity locally

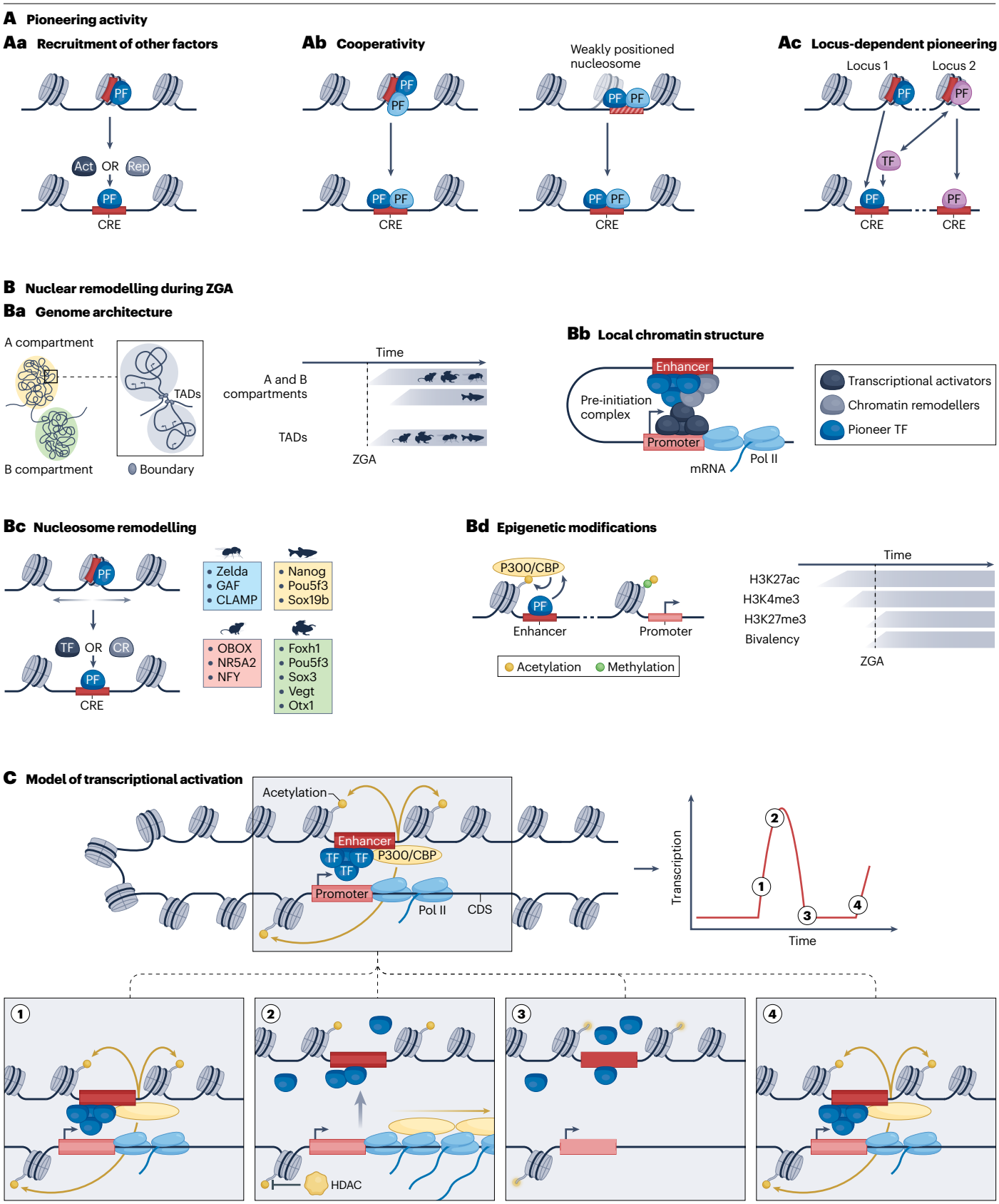
A Pioneering activity
Aa Recruitment of other factors
PF
Act
OR
Rep
CRE
Ab Cooperativity
Weakly positioned nucleosome
Ac Locus-dependent pioneering
Locus 1
Locus 2
TF
B Nuclear remodelling during ZGA
Ba Genome architecture
A compartment
B compartment
TADs
Boundary
Time
A and B compartments
ZGA
Bb Local chromatin structure
Enhancer
Pre-initiation complex
Promoter
mRNA
Pol II
Transcriptional activators
Chromatin remodellers
Pioneer TF
Bc Nucleosome remodelling
TF
CR
Zelda
GAF
CLAMP
Nanog
Pou5f3
Sox19b
OBOX
NR5A2
NFY
Foxh1
Pou5f3
Sox3
Vegt
Otx1
Bd Epigenetic modifications
P300/CBP
H3K27ac
H3K4me3
H3K27me3
Bivalency
Acetylation
Methylation
C Model of transcriptional activation
Pol II
CDS
Transcription
HDAC

Fig. 4 | Transcriptional competency is achieved by nuclear remodelling. **Aa**, Pioneer transcription factors (PFs) can bind *cis*-regulatory elements (CREs) on nucleosomal DNA, making them accessible for other factors, such as activators ('Act') or repressors ('Rep'). **Ab**, Pioneering activity can require cooperative interactions. PF binding sites can be suboptimal (striped CRE) at weakly positioned nucleosomes, requiring increased PF concentration and/or cooperative action. **Ac**, PFs can act as a 'regular' transcription factor (TF) depending on the specific locus. **B**, Nuclear remodelling on multiple scales. **Ba**, Global genome architecture, in the form of A ('active') and B ('inactive') compartments and topologically associating domains (TADs), arises during the maternal-to-zygotic transition (MZT) in most species. **Bb**, Local chromatin forms enhancer–promoter contacts to initiate zygotic transcription. **Bc**, At CREs, PFs change the nucleosome landscape, making them accessible to other TFs and chromatin remodellers (CRs). Maternally deposited zygotic genome activation (ZGA)-regulating TFs with demonstrated or suggested pioneering ability in different species are listed (*Drosophila melanogaster*, blue; zebrafish, yellow; *Xenopus* spp., green; mouse, red). **Bd**, Histone tail modifications are also remodelled and form bivalent modifications in some species. Bars represent the timing of acquisition of different marks relative to the onset of ZGA. Histone H3 acetylated at lysine 27 (H3K27ac), mediated by histone acetyltransferases (HATs) p300/CBP, is essential for ZGA. **C**, Our hypothesized transcription regulation model postulates a central role for acetylated histones: transient promoter–enhancer contacts initiate transcription (step 1); productive transcriptional elongation by RNA polymerase II (Pol II) 'kicks' enhancers away from promoters, and acetylated nucleosomes retain enhancer–promoter contact memory (step 2); after histone deacetylase (HDAC)-mediated deacetylation, the transcriptional burst is terminated (step 3); and transcriptional re-initiation requires renewed enhancer–promoter proximity and histone acetylation (step 4). This proposed model will require testing in the future. H3K4me3, histone H3 trimethylated at lysine 4; H3K27me3, histone H3 trimethylated at lysine 27.

opens chromatin for subsequent binding and activation by other TFs and transcriptional machinery[173,176,206–209] (Fig. 4A,B). For example, PFs can displace nucleosomes at developmental enhancers, making them accessible for later activation by non-PFs required for developmental patterning, such as Dorsal and Bicoid in *D. melanogaster*[206,207,210–212] or Eomes in zebrafish[173]. Some loci are bound by multiple TFs but require only one of the factors for chromatin opening, with the other TFs acting as 'regular' TFs (Fig. 4A). These roles can switch at different loci[173,174,206], supporting the view that pioneer function is not solely an intrinsic protein property but strongly depends on the specific circumstances including nucleosome position, TF concentration, genomic sequence and the chromatin context[213–215]. Contrary to the canonical view that nucleosomes impede TF binding, they can instead facilitate PF binding. The *D. melanogaster* pioneer factor Zelda and the zebrafish PFs bind sites with high intrinsic nucleosome occupancy[173,207,208], and *X. laevis* Foxh1 preferentially binds nucleosomal sites rather than linear DNA[216]. Strongly positioned nucleosomes facilitate zebrafish PF activity, and therefore lower PF concentrations, or fewer TFs, are required for opening[173], perhaps by stabilizing motifs in a better conformation for binding or participating directly as part of the binding complex (Fig. 4A). Many PFs preferentially bind motifs at specific positions relative to the nucleosome, and thus nucleosomes enhance binding specificity, which is partially provided by the nucleosome–DNA interface, although other mechanisms exist[18,19,168,217–219]. Additionally, nucleosomes can promote TF co-occupancy, further highlighting their positive effect on pioneering activity[220]. Epigenetic marks such as histone modifications also influence pioneering activity[221], with histone H3 trimethylated at lysine 9 (H3K9me3) impeding binding[222] and H3 acetylated at lysine 27 (H3K27ac) promoting TF cooperativity[223], illustrating how the epigenetic landscape influences ZGA (see the section 'Epigenetic reprogramming').

Multiple pioneer factors can act synergistically to open chromatin at some sites, and pioneering ability can depend on other expressed factors[19,173,174,222,224–226] (Fig. 4A). Conversely, TFs can compete with and antagonize each other. For example, Pou5f3 in zebrafish has a repressive function at some sites by blocking precocious expression of Nanog[174]. *D. melanogaster* GAF[227] and *X. tropicalis* Foxh1 (ref. 177) can also exert repressive effects in specific contexts. This repression could be due to direct repressor recruitment[177] or through competition with other TFs for limited transcriptional machinery. Alternatively, this counterintuitive repressive effect of a PF could be due to the displacement of a nucleosome, which could subsequently alter the position of a neighbouring nucleosome and thereby affect the affinity of other TFs to their binding sites. How multiple TFs integrate the nucleosomal landscape to orchestrate transcriptional activation across the genome is a fundamental question for the field to address.

Epigenetic reprogramming. Fertilized embryos inherit differentiated chromatin from the gametes, which is reprogrammed into a naive state essential for zygotic development. Here, we highlight several epigenetic reprogramming events in early embryos, primarily focusing on commonalities across species. We note that we cannot exhaustively review all epigenetic marks and the differences among all species, and refer readers to recent reviews that cover species-specific differences in depth[228–232].

In vertebrates, DNA cytosine methylation produces 5mC, which is strongly linked to transcriptional repression. After fertilization, mammalian embryos erase the global DNA methylation pattern, creating a hypomethylated, totipotent state. 5mC is actively and rapidly removed from the paternal genome at the one-cell stage whereas maternal 5mC is removed more gradually. Mammalian embryos gradually re-establish methylation after preimplantation development[6]. Unlike mammals, zebrafish and *X. laevis* embryos do not rapidly reprogramme 5mC methylation post fertilization[233–235], perhaps due to gradual resetting of the methylation pattern during the many cell cycles before ZGA occurs. In zebrafish, maternal methylation is reprogrammed to the paternal pattern, even in haploid embryos, indicating that the genome or epigenome encodes the eventual methylation pattern[233].

Histone variants are dynamically reprogrammed in early embryos and collectively regulate the global (H1 variants and H3.3) and local (H2A.Z) chromatin landscape to prepare the embryo for ZGA. H1 linker histones modulate chromatin compaction, with embryo-specific variants maintaining a less compact, naive state and facilitating rapid cell divisions. These H1 variants are replaced by somatic variants during ZGA[236–242]. Sperm chromatin (except in zebrafish) is compacted primarily by protamines, with a few nucleosomes and modifications retained at a subset of developmentally important loci[243,244]. The H3 variant H3.3 is critical for protamine-to-histone reprogramming. Maternal H3.3, which is deposited independently of DNA replication, incorporates into the paternal genome post fertilization[245–247]. The H3.3 chaperone HIRA is essential for *X. laevis* gastrulation[248] and is also required for reprogramming the maternal genome in mouse[247]. H3.3 is broadly distributed in mouse zygote and oocyte genomes but incorporation of canonical H3 proteins (H3.1 and H3.2) by the CAF1 histone chaperone

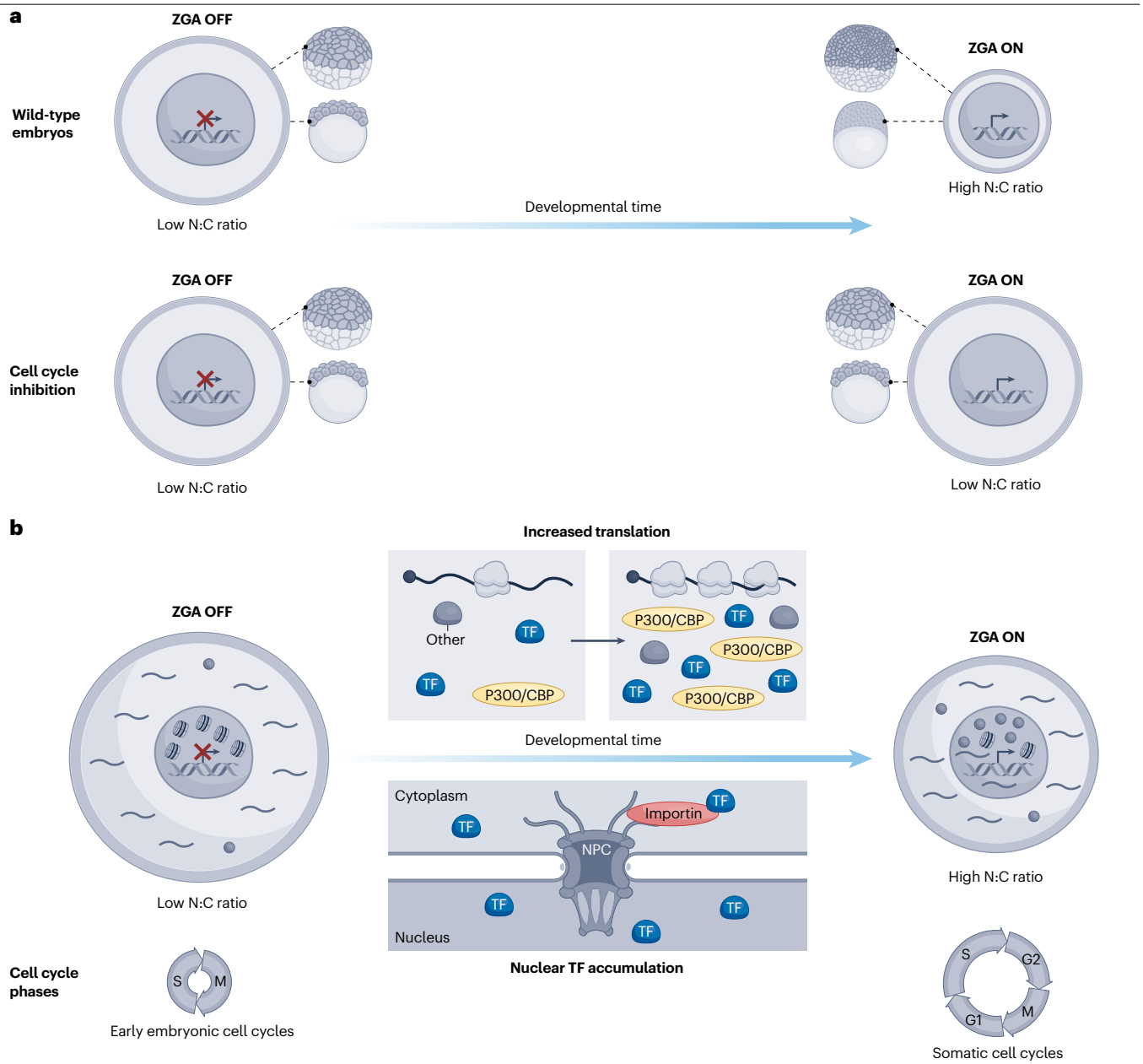

Fig. 5 | Elapsed developmental time is the key regulator of ZGA onset timing. **a**, Wild-type embryos of fast-developing species (zebrafish and *Xenopus* spp.) initiate zygotic genome activation (ZGA) at a high nuclear-to-cytoplasmic volume ratio (N:C ratio). When the cell cycle is stalled in pre-ZGA zebrafish and *Xenopus laevis* embryos (by checkpoint kinase 1 (Chk1) overexpression[272] or cycloheximide treatment[45,272,348], for example), the N:C ratio remains unchanged; however, ZGA can initiate at a similar developmental time to wild-type embryos, even when at a lower N:C ratio. This suggests that an increased developmental time window is what creates a permissive state for transcription rather than an increased N:C ratio owing to cell division. **b**, During development, increased translation of transcription factors (TFs) (blue), acetyltransferases (yellow) and other factors (dark grey) prepares the nucleus for ZGA. Additionally, increased nuclear pore complex (NPC) maturation and Importin affinities for TFs regulate the nuclear import of these proteins. The cell cycle duration is tightly coupled to the N:C ratio and maternally deposited histone proteins are diluted with every cell cleavage. Overabundant histones can control ZGA timing by competing with TFs for DNA binding. Early cell cycles in fast-developing species include only S and M phases; as the cell cycle lengthens, G phases are added.

leads to a more restricted distribution by the two-cell stage[249]. Interestingly, maternal H3.3 stores improve SCNT efficiency and suppression of CAF1 improves in vitro reprogramming efficiency[250,251]. These observations suggest that canonical H3 acts as a reprogramming barrier, whereas the embryonic H3.3 landscape promotes transient totipotency. The H2A variant, H2A.Z, also primes chromatin for gene activation during ZGA in *D. melanogaster* and zebrafish, especially at housekeeping genes[252,253]. H2A.Z is enriched at the transcription

start sites (TSSs) of zygotic genes pre ZGA in *D. melanogaster*[252] and, alongside H3K4me1, forms 'placeholder' nucleosomes in zebrafish[253]. Placeholder nucleosomes maintain hypomethylated states ready for early gene activation and their absence keeps developmental genes repressed[253]. A recent preprint has shown that in zebrafish embryos, H2A.Z also marks a subclass of enhancers that are labelled with H3K4me2 and are hypomethylated (low DNA methylation) due to their activity in gametes; these enhancers can be activated independently of pioneer factors[254]. However, H2A.Z may function redundantly with other mechanisms to promote ZGA; even though H2A.Z accumulates at gene promoters during ZGA in mice and affects levels of developmental genes, knockdown of *H2A.Z* does not impact the onset of ZGA[255].

Histone modifications (Fig. 4B) commonly follow an erase and rewrite strategy, whereby parental signatures are erased and then replaced with zygotic ones. H3K4me3 is canonically found in sharp peaks at TSSs of active genes and this signature is globally lost after fertilization in *D. melanogaster*, zebrafish and *X. tropicalis*[165,204,256–258]. Canonical H3K4me3 peaks may hinder reprogramming, as demonstrated in *X. laevis* SCNT embryos[259]. Mouse zygotes lack sharp H3K4me3 domains; instead, they inherit broad H3K4me3 domains that are paradoxically associated with transcriptional repression[31,32], similar to non-canonical H3K4me3 domains recently reported in *C. elegans* oocytes and embryos[260]. The canonical sharp H3K4me3 pattern is gradually re-established in all species by the time large-scale ZGA occurs[31,32,204,261–263] and H3K4me3 deposition does not depend on zygotic transcription[264]. However, it is unclear whether H3K4me3 has a direct role in ZGA because early transcription events can precede H3K4me3 deposition[165,204] and it is dispensable for transcription in other contexts[265]. H3K27me3, which is associated with gene repression and silencing, is also largely erased post fertilization across species[34,256,258,266–268]. H3K27me3 levels, similar to H3K4me3, increase globally during embryogenesis, although promoter H3K4me3 often precedes H3K27me3 (refs. 33,257,261,262,266). ZGA precedes the appearance of 'bivalent' promoters (those marked by both H3K4me3 and H3K27me3) which poise genes for later activation in development[33,34,261,262]. In zebrafish, bivalent domains are pre-marked by placeholder nucleosomes[253], forming a blueprint for future differentiation.

Parental histone mark retention can regulate gene expression. In mice, despite global erasure of DNA methylation, imprinted sites that retain parental methylation patterns show allele-specific expression[6], and oocyte-inherited H3K27me3 contributes to imprinting[269]. Maternal H3K27me3 in *D. melanogaster* and histone H2A ubiquitination (H2AUb) in mouse embryos prevent precocious expression of developmental genes, such as *Hox* genes[267,270]. Some inherited modifications, such as maternal H4K16ac in *D. melanogaster*, facilitate chromatin accessibility before ZGA[271], whereas inter-nucleosome accessibility in zebrafish is independent of histone acetylation across several core histone tails[173].

Histone acetylation universally regulates transcription-permissive chromatin and always precedes genome activation (Fig. 4B). The most studied mark, H3K27ac is initially erased after fertilization, increases before zygotic transcription and persists during transcriptional initiation in most species[204,272–274]. Mouse one-cell zygotes, however, are globally hyperacetylated through the action of P300/CBP HATs and have broad H3K27ac domains (similar to the broad H3K4me3 domains) which return to canonical sharp H3K27ac patterns by the two-cell stage[275]. PFs that regulate ZGA in *D. melanogaster* and zebrafish can establish histone acetylation across all core histones[173,204] at target enhancers and promoters[173,204] by recruiting P300/CBP[173]. Inhibition of P300/CBP in multiple species significantly compromises ZGA[256,272,275,276], whereas its overexpression in zebrafish induces premature ZGA[272]. Although the catalytic HAT activity of CBP/P300 is dispensable for ZGA in *D. melanogaster*[277], in zebrafish it is sufficient to activate transcription independently of PFs when recruited to specific loci[173]. This sufficiency is important as it suggests that the main function of these TFs is to recruit HATs to enhancers and promoters. It resolves a long-standing question about the relationship between histone acetylation and transcription, indicating that acetylation is essential for transcriptional activation. However, histone acetylation does not occur in isolation; histone deacetylases (HDACs) constantly reduce and refine acetylation levels. This deacetylation ensures proper lineage-specific gene expression in *X. tropicalis*[278] and represses later developmental genes in mouse[275]. Therefore, histone acetylation primes ZGA and orchestrates the precise timing and location of gene expression. These observations have led us to propose a new model that highlights the role of histone acetylation in transcription initiation (see the section 'Mechanism of transcriptional activation').

Chromatin remodelling. During the MZT, three-dimensional chromatin organization undergoes comprehensive remodelling (Fig. 4B) (reviewed in depth elsewhere[7,8,279]). The highly defined oocyte chromatin organization transitions to a naive state pre ZGA, before gradually regaining complexity[7,37,38]. Broadly, chromatin partitions into A ('active') and B ('inactive') compartments[280]. In *D. melanogaster* embryos, the heterochromatin protein 1α (HP1α) is vital for de novo B compartment formation, suggesting independent mechanisms for A and B compartment segregation[281,282]. Conserved HP1α (refs. 281,283) likely plays an important part in chromatin structure across species. A/B compartments emerge around ZGA in *D. melanogaster*[284,285], *X. tropicalis*[286], mice[37,38] and human embryos[287], whereas in zebrafish[288–290] they do not emerge until after MZT (Fig. 4B).

Strong topologically associating domains (TADs) form concomitantly with ZGA in all species but form independently of transcription[37,284–286,288] (except in human embryos, in which blocking ZGA affects TAD formation)[287]. TADs range in size from ~10 kb to a few megabases and act as regulatory scaffolds[291] that are thought to promote frequent promoter–enhancer interactions for robust target gene expression. They feature sharp boundaries formed by architectural proteins such as CTCF and cohesin[287,292] and the PF Zelda contributes to locus-specific TAD boundary formation[284,293]. Disruptions in TAD organization lead to severe developmental defects in humans such as limb malformations in the form of brachydactyly (short digits), syndactyly (finger fusion) and polydactyly[294].

The lack of compartmentalization and TADs before ZGA suggests that relaxed chromatin is a hallmark of developmental reprogramming and totipotency. Indeed, loss of cohesin enhances SCNT[295] and loss of CTCF enhances in vitro reprogramming[296], indicating that a flexible chromatin structure enables reprogramming factors to change regulatory interactions and induce pluripotency[295,296]. Furthermore, during embryogenesis and SCNT, some chromatin-modifying proteins exhibit the erase and rewrite strategy, transiently dissociating to generate relaxed chromatin[297,298], which also facilitates the pluripotent to totipotent-like transition in embryonic stem cells[299].

Zelda can promote promoter–enhancer interactions before TAD formation[300], and several studies show a limited correlation between gene expression and TAD structure[301–304]. Therefore, TADs likely play a bigger part in preventing promiscuous promoter–enhancer

interactions than actively promoting specific ones. The impact of gene expression on chromatin organization at the molecular level remains unclear. New techniques to visualize chromatin with nucleosome-level resolution, such as chromatin expansion microscopy (ChromExM)[29] and ChromEM tomography (ChromEMT)[305], promise to provide additional insight into three-dimensional chromatin reprogramming during MZT.

Mechanism of transcriptional activation

TF and co-activator binding, enhancer activation via histone acetylation and pre-initiation complex formation at *cis*-regulatory elements (CREs) are all key for transcriptional activation (Fig. 4B). Intrinsically disordered regions in TFs aid transcriptional activation by non-specifically binding to DNA and reducing the target search space[306], or by forming nuclear membraneless 'condensates' or 'hubs' to increase the local concentration of TFs, co-activators and RNA polymerase II (Pol II)[307,308]. Dynamic clustering of TFs and co-activators at enhancers facilitates Pol II recruitment and transcriptional activation[309–311]. In *D. melanogaster*, Zelda hubs modulate the nuclear microenvironment and activate transcription[205,212,312,313], while in zebrafish, pioneer factors cluster at the *miR-430* locus (and other loci) before transcriptional activation[29,307]. A new high-resolution microscopy technique enabled the visualization of Nanog–Pol II interactions at the *miR-430* locus[29], supporting the classic model of TF-mediated Pol II recruitment. Future applications of such techniques promise more direct insight into TF clustering and the mechanism of Pol II recruitment by TFs and co-activators.

Histone acetylation is essential for transcriptional activation by promoting recruitment of transcription co-factors and Pol II. Furthermore, in vitro studies showed that acetylation at *cis*-regulatory regions disrupts chromatin-induced phase separation, whereas acetylation-reader bromodomain proteins such as BRD4 induce distinct droplet phases of acetylated chromatin, facilitate pre-initiation complex formation and form condensates with Mediator in mouse embryonic stem cells[308,314–316]. Restoring acetylation to enhancer and/or promoter regions can activate zygotic transcription in zebrafish embryos[173]. Thus, acetylation specifically promotes enhancer-mediated transcriptional activation.

Transcription initiation relies on enhancer–promoter interactions[317], but how close these interactions must be to initiate and sustain transcription remains unclear[318–321]. Imaging studies are inconsistent and seemingly contradictory, potentially due to locus-specific behaviour influenced by the chromatin microenvironment, microscopy resolution limitations or a need to integrate the acetylation status or TF occupancy when studying transcriptional output. Enhancer–promoter contacts are proposed to occur transiently according to the 'kiss-and-run' model[322]. This model was updated by the recent 'kiss-and-kick' model, based on ChromExM and genomics methods during ZGA, which suggests that productive transcriptional elongation kicks the enhancer away from the promoter, thereby disrupting this transient interaction[29]. This updated model is consistent with findings that nascent RNA can displace inactive chromatin from the region of active transcription[323,324]. We propose a further integrated model of transient enhancer–promoter contacts (Fig. 4C), whereby histone acetylation provides a memory of contact information and eliminates the need for continued promoter–enhancer proximity. HDACs can erase the initial contact memory, terminating the current transcriptional burst. Transcription can be re-initiated via renewed transient enhancer–promoter proximity and acetylation. Future experiments combining high-resolution microscopy of specific enhancer–promoter interactions with histone marks and transcription will be required to test this model.

Timing of zygotic transcription

In fast-developing species, initial cleavage cycles (nuclear cycles in *D. melanogaster*) occur without cell growth, leading to decreasing cytoplasmic volumes and resulting in a higher nuclear-to-cytoplasmic volume ratio (N:C ratio). This ratio is crucial for determining the cell cycle length, which slows just before gastrulation. Large-scale ZGA coincides with the mid-blastula transition (MBT) when the cell cycle slows (Box 1), prompting research into the potential co-regulation of cell cycle duration and ZGA onset. We discuss the effect of cell cycle length, N:C ratio, developmental time and translation on zygotic transcription in fast-developing species (Fig. 5). The contribution of these features is less studied in slow-developing mammalian embryos, in which cell cycles are slow from the onset of fertilization.

Early cell cycles are driven by cyclin and cyclin-dependent kinase (Cdk) activity. Remodelling of the cell cycle at the MBT involves destabilizing the Cdk-activator Cdc25 (refs. 325–328) and activating checkpoint kinase 1 (Chk1), which blocks replication origin formation[326,329]. Longer cell cycles facilitate robust zygotic transcription, as shorter cycles limit the transcription time window. Yet extending interphase by precocious Chk1 activity does not trigger early onset of large-scale ZGA[272,330]. Early zygotic transcription events contribute to the lengthening of cell cycles[331,332], indicating that transcription and cell cycles influence each other.

The N:C ratio directly impacts MBT timing by extending the cell cycle but whether it has a direct role in regulating zygotic transcription timing remains ambiguous. In *X. laevis*, cell cycle lengthening results, in part, from the titration of maternally deposited replication factors (Cut5, RecQ4, Treslin and Drf1 (ref. 333)) and the phosphatase PP2A-B55 (ref. 334) relative to the increasing amount of DNA via cell divisions. Another proposed mechanism for regulating transcriptional onset is the dilution of histones, which compete with TFs for DNA binding; histone overexpression or depletion modulates ZGA onset in zebrafish and *X. laevis*[335,336]. H3 tails can also act as a competitive inhibitor of Chk1 in *D. melanogaster*[337,338]. Changing the N:C ratio or ploidy affects gene activation timing[272,339–345]. However, embryos adjust the cell cycle number and duration in response to ploidy changes[346,347], confounding the interpretation of whether the N:C ratio regulates ZGA onset directly, or indirectly through cell cycle lengthening. In *X. laevis*, a cell-size gradient along the animal to vegetal axis correlates with ZGA timing, supporting the notion that an N:C ratio threshold determines ZGA onset[341,348]. However, differential localization of maternal TFs[178] could contribute to differences in ZGA timing. We hypothesize that differential translation of maternal mRNAs across the animal to vegetal axis could drive changes in cell cycle speed and the onset of ZGA through independent mechanisms. Indeed, blocking cell cycle progression can lead to transcriptional activation at a lower N:C ratio[272,348–350], indicating that a high N:C ratio is not absolutely required to activate transcription (Fig. 5a). The direct effect of the N:C ratio on transcriptional activation is gene-dependent, with live imaging[343] and gene expression analyses[272,345,351] in haploid embryos indicating that some genes sense N:C ratio whereas others respond mainly to cell cycle duration. We propose that because each gene has specific requirements for TFs or nucleosome positioning, they respond differently to varying levels of histones and TFs, and thereby exhibit differential sensitivity to changes in the N:C ratio.

ZGA requires transcriptional activators such as PFs, p300/CBP and components of the general transcriptional machinery (such as TBP)[352]

to reach a threshold level through translation. Indeed, transcriptional activation can occur at lower N:C ratios (stalled cell cycle) without repressor dilution in *D. melanogaster* and zebrafish, as long as enough developmental time has passed[272,350]. Furthermore, blocking translation of maternal mRNAs before they have reached a critical concentration blocks zygotic transcription in zebrafish, *D. melanogaster* and *X. laevis*[45,272,348,349]. These observations are consistent with a role for developmental time in genome activation by allowing translation of the necessary maternal factors, including TFs, HATs and other transcriptional regulators (Fig. 5b). Stochastic activation of ZGA within an embryo[272] could be explained by some individual cells accumulating threshold levels of transcriptional regulators earlier than others. Although premature Zelda expression alone does not result in premature ZGA in *D. melanogaster*[353], overexpression of P300 or BRD4 in zebrafish drives earlier ZGA onset[272]. Recent studies implicate increased nuclear abundance of maternal TFs as an important regulator of the ZGA clock; increasing nuclear pore maturity[164] and differential affinity to Importin[160], which regulates nuclear import and is essential for ZGA[354], influences TF nuclear import timing. Collectively, these data indicate that developmental time, by enabling nuclear accumulation of PFs and other transcriptional regulators through translation and regulated import, is the rate-limiting step for competence of genome activation.

Zygotic transcripts

Numerous approaches can be used to differentiate zygotic mRNAs from the more abundant maternal mRNAs in early embryos, such as using intron sequencing reads as a proxy for nascent transcription[45,355,356], uridine analogue-labelled RNA pull-down[10,12,272,276,348,357], metabolic RNA sequencing[30,358] or distinct promoter and/or TSS usage relative to maternal mRNAs[167,264]. Initial zygotic gene expression is often stochastic and heterogeneous but is later averaged spatially or temporally[272,359–361]. Localized gene activation, combined with maternal mRNA degradation, also contributes to spatiotemporal patterning[112,114]. In *Xenopus* spp., ectodermal genes are activated before endodermal genes, with signalling gradients contributing to regional ZGA[176,348]. The earliest zygotic transcripts in fast-developing species are shorter and have fewer introns[10,12,45,114,165,362] than later transcripts, and include miRNAs that clear maternal transcripts[11,81,82,89]. In mice, one of the earliest transcribed genes encodes ZSCAN4, which helps to protect against genomic instability[363,364]. In *D. melanogaster* and mice, transposable elements[12,365,366] are also transcribed early, including short interspersed nuclear elements (SINEs), long interspersed nuclear elements (LINEs) and murine endogenous retrovirus-L (MERVLs) that comprise a large fraction of the mouse genome and are essential for early embryonic development[366–371]. We postulate that evolutionary pressures driving transposon activation soon after fertilization, enabling their spread in the germ line, have been harnessed over time to regulate other zygotic genes. SINE B1 elements are enriched at ZGA genes and contain binding sites for NR5A2 and OBOX[187,190]. LINE-1 elements regulate global chromatin accessibility, activate ZGA genes and are required for *Dux* silencing and rRNA synthesis[367–369]. MERVL is transiently upregulated during ZGA[366] by OBOX and DUX[184,372], and its long terminal repeat has been co-opted as CREs by ZGA-specific genes[370,372,373]. MERVL is subsequently downregulated by LINE-1 RNA activity and by DUX-activated DUXBL, thereby limiting MERVL expression to a defined time window[368,374]. These transposable elements may also be important for reducing precocious transcription across other genomic sites; for example, in mouse embryonic stem cells, endogenous retroviruses can 'hijack' transcriptional condensates away from other loci[375], and in zebrafish, *miR-430* and repetitive element transcription can serve as a 'sink' for transcription regulators[90,376,377]. This framework suggests that the CREs of early zygotic genes can act as negative regulators of later-transcribed genes, controlling overall transcriptional activation timing.

Mechanistic differences in transcriptional regulation between the traditionally called minor and major waves impact transcription as ZGA unfolds, including differences in promoter architecture and TSS usage. The earliest-expressed *D. melanogaster* and zebrafish genes are enriched for TATA boxes, whereas later-expressed genes use different motifs and exhibit increased promoter-proximal pausing[26,90,165]. In mouse embryos, the earliest transcription occurs independently of core promoter sequences, often originating promiscuously from intergenic regions[166]. This may be due to global epigenetic changes leading to spurious transcription. Some genes are expressed only in the one-cell stage[378] and isoform switching takes place throughout early ZGA[379]. These differences suggest that the mechanisms for activating the earliest transcripts differ from those for later-expressed mRNAs, and that there are functional differences through selective splicing or promoter usage among the transcribed genes.

Zygotic transcripts also differ from those provided maternally. In zebrafish and mouse embryos, the TSSs of maternal transcripts feature distinct motifs that differ from those of zygotic transcripts and are precisely positioned relative to the +1 nucleosome[167,264,380]. Maternal and zygotic transcript isoforms can have different splice sites and UTRs[11,72,73,262,379,381]. Changes to the coding sequence can impact protein identity; differences in upstream ORFs, Kozak sequence or ORF length can affect isoform stability and translation[87,127,150]. These transcript differences offer the potential for diverse post-transcriptional regulation and add to proteome diversity between the maternal and zygotic states.

Conclusions and future perspectives

Our field has made extensive progress in understanding the molecular mechanisms that eventually transition developmental control to the embryo. Yet the full orchestration of the cytoplasmic and nuclear reprogramming events, and how they together enable genome activation from an initially silent genome, remains unclear. Integrating genomic techniques with novel imaging methods at the single molecule level will enhance our ability to investigate chromatin organization[29,305] and the dynamics of transcription and translation[23–28]. An ultimate frontier in the field is the high-resolution spatial visualization of translation and transcriptional machinery as well as genome architecture, including enhancer–promoter interactions, to deepen our understanding of how different TFs work together to regulate genome activation and downstream gene expression networks[21,382]. Additionally, elucidating how TFs interact with chromatin in vivo at the nucleosome level will shed light on how a cell integrates the input of multiple TFs and is crucial for understanding the regulation of transcriptional programmes across cell types. Future work should also investigate the combinatorial effect of gene-specific and/or locus-specific responses, local epigenetic modifications and chromatin architecture on gene expression. The identity of ZGA regulators remains an ongoing question. Many more ZGA-regulating factors (PFs and others) will likely be discovered, with future research focusing on how their concerted action influences precise gene expression and embryonic development. Nuclear reprogramming defines which genes are initially transcribed but could also prime subsequent transcription events, even hours or days later, by regulating other TFs through nucleosome positioning and epigenetic remodelling. In the future, further integration of machine learning models with massively

Glossary

CCR4–NOT complex
During maternal-to-zygotic transition (MZT), this multiprotein complex plays a critical role in regulating gene expression by controlling mRNA deadenylation.

Cleavage cycles
The series of rapid mitotic cell divisions that occur in the early embryo following fertilization, essential for increasing cell numbers in the embryo while maintaining a constant overall size, except for *Drosophila* spp. where cleavage cycles occur in a syncytium resulting in a growing number of nuclei in a shared cytoplasm.

Deadenylation
The process by which the poly(A) tail of an mRNA molecule is shortened or removed by deadenylating enzymes, which regulates the stability and lifespan of the mRNA molecule.

Erase and rewrite
A developmental strategy that involves the removal (erase) of maternal signatures to a naive state, followed by the establishment (rewrite) of zygotic signatures.

Histone acetylation
Acetyl groups on histone tails that modify the functional properties of DNA, added by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs).

Maternal decay
(M-decay). Refers to the degradation of maternally deposited mRNAs before zygotic genome activation (ZGA) or independent of zygotically produced factors.

Maternal-to-zygotic transition
(MZT). The transition period during embryogenesis when control of embryonic development transitions from maternal factors to zygotic factors.

Mid-blastula transition
(MBT). A transition phase in embryonic development, characterized by lengthening cell cycles, acquisition of cell motility and, in *Drosophila* spp., cellularization.

Nuclear-to-cytoplasmic volume ratio
(N:C ratio). The relative nuclear-to-cytoplasmic ratio within a cell. During embryogenesis (zygote to gastrulation) the size of the embryo does not change; cell sizes are halved with every cleavage cycle.

ORF-mediated decay
A decay pathway driven by the protein Upf1, whereby the translation status of the main open reading frame (ORF), affected by upstream ORFs and ORF length, influences decay dynamics.

Pioneer transcription factors
(PFs). Specialized transcription factors (TFs) with the unique ability to bind to condensed or inaccessible regions of chromatin, promoting chromatin opening and making these regions accessible for other regulatory proteins.

Protamines
Small proteins that replace histones in sperm (except in zebrafish) and help to compact the sperm genome.

Re-adenylation
(Cytoplasmic polyadenylation). Lengthening of poly(A) tails by specialized poly(A) polymerases in the cytoplasm, which leads to translational upregulation.

Totipotent
The ability of a cell to give rise to all cell types in an organism, including both embryonic and extra-embryonic tissues.

Zygote
Describes a fertilized egg and the earliest developmental stage of a multicellular organism.

Zygotic decay
(Z-decay). Refers to the clearance of maternally deposited mRNAs dependent on zygotically produced factors.

Zygotic genome activation
(ZGA). The process during embryogenesis where the zygotic genome becomes transcriptionally active.

parallel reporter assays and other genomic approaches will also advance post-transcriptional studies to decipher regulatory grammar that shapes mRNA stability and translation during development[110,116].

Studying other organisms will allow us to understand the conserved regulatory principles in MZT and new regulation paradigms[383–386]. For example, bovine ZGA aligns closely with that of human embryos, potentially offering a more accurate model of human development[387,388]. Additionally, unique genome features in certain species, such as the allotetraploid *X. laevis*[389], will provide distinctive insights into how one maternal genome can differentially regulate two evolutionarily distant zygotic genomes in the same embryo. This will help us to better understand how the specific regulatory grammar in the genome leads to quantitative changes in gene expression.

The MZT erase and write strategy extends beyond epigenetic marks to transcripts and proteins. SLAM-seq has revealed that many maternal transcripts are erased and then resynthesized zygotically[30]. This 'rewriting' could represent a need for zygotic isoforms with distinct functions or for untainted molecules without accumulated damage and/or modifications. More sensitive mass spectrometry could also reveal erased and resynthesized proteins[41] and elucidate roles of post-translational modifications, hormones, signalling factors and metabolites during the MZT[390]. Metabolism is a particularly exciting avenue to explore because metabolites serve as substrates for histone modifications that are important for nuclear reprogramming[391]. Indeed, transient nuclear translocation of TCA cycle enzymes[392,393] and lactate[394] modulates key epigenetic modifications during somatic reprogramming[393] and ZGA in mouse and human embryos[392,394]. Investigating how maternal, embryo and yolk metabolites influence chromatin, gene expression, protein function and, ultimately, cell fate promises intriguing insights[391].

Ultimately, we need to remember that the MZT is what initiates the gene regulatory cascade crucial for embryogenesis. It plays a key part not only in developmental biology but also in human fertility and overall human biology. Future research into the epigenetic and mutational factors that contribute to MZT failure will be vital for understanding how specific mutations affect genome activation and human infertility. Furthermore, studying the reprogramming of fertilized eggs has provided fundamental mechanisms for remodelling the cytoplasm and activating genomes to achieve transient totipotency in vivo. The embryo is a dynamic non-steady-state system that has served as a molecular testing ground for various paradigms across the central dogma. Research on this reprogramming will continue to have broad implications beyond MZT and provide fundamental insights into epigenetics, transcription and post-transcriptional regulation.

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