Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression

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Abstract
The maternal mRNAs that drive meiotic progression in oocytes contain short poly(A) tails and it is only when these tails are elongated to stimulate translation. Cytoplasmic polyadenylation requires two elements in the 3′-UTR (3′-untranslated region), the hexanucleotide AAUAAA and the CPE (cytoplasmic polyadenylation element), which also participates in the transport and localization, in a quiescent state, of its targets. However, not all CPE-containing mRNAs are activated at the same time during the cell cycle, and polyadenylation is temporally and spatially regulated during meiosis. We have recently deciphered a combinatorial code that can be used to qualitatively and quantitatively predict the translational behaviour of CPE-containing mRNAs. This code defines positive and negative feedback loops that generate waves of polyadenylation and deadenylation, creating a circuit of mRNA-specific translational regulation that drives meiotic progression.

Translational control of meiotic progression
Vertebrate immature oocytes are arrested at the PI (prophase of meiosis I). During this growth period, the oocytes synthesize and store large quantities of dormant mRNAs, which will later drive the oocyte’s re-entry into meiosis [1,2]. Not only does meiotic progression require the translational activation of specific mRNAs at specific phases of the cell cycle, but also the extent of translational activation is finely regulated, resulting in differential rates of product accumulation that, combined with the control of protein degradation, establish phase-specific peaks of expression of the factors that drive meiotic progression. The resumption of meiosis in Xenopus is stimulated by progesterone (Figure 1), which carries the oocyte through two consecutive M-phases (MI and MII), without an intervening S-phase [3], to a second arrest at MII. The key activity driving meiotic progression is the MPF (maturation-promoting factor), a heterodimer of CDC2 (cell division cycle 2 kinase) and cyclin B [4,5], which induces metaphase entry. In PI-arrested oocytes (stage VI), this heterodimer is initially formed as an inactive pre-MPF, with cyclins B2 and B5 [6,7], and is activated by the dual-specificity CDC25 phosphatase as the result of new synthesis of Mos [2,9].

Mechanism of translational regulation by cytoplasmic polyadenylation
The most extensively studied mechanism for maintaining repressed maternal mRNAs in arrested oocytes and for activating translation during meiotic resumption is mediated by the CPEB (CPE (cytoplasmic polyadenylation element)-binding protein; for reviews, see [1,10,11]). Cytoplasmic polyadenylation requires two elements in the 3′-UTRs (3′-untranslated regions) of responding mRNAs: the hexanucleotide AAUAAA (Hex) [12], which is bound by the CPSF (cleavage and polyadenylation specificity factor) [13], and the nearby CPE, which recruits CPEB [14–16]. CPEB is phosphorylated and activated by the kinase Aurora A [17] as a result of progesterone-induced GSK-3 (glycogen synthase kinase-3) inactivation [18] (but see [19]). This CPEB phosphorylation increases its affinity for CPSF [20], which,

Key words: C3H4, cytoplasmic polyadenylation element-binding protein (CPEB), deadenylation, meiosis, translation, Xenopus oocyte.

Abbreviations used: APC, anaphase-promoting complex; ARE, AU-rich element; CDC2, cell division cycle 2 kinase; CPE, cytoplasmic polyadenylation element; CPEB, CPE-binding protein; CPSF, cleavage and polyadenylation specificity factor; CSF, cytostatic factor; eIF4G, eukaryotic translation initiation factor 4G; MPF, maturation-promoting factor; MII, metaphase entry; PI, prophase of meiosis I; Pum, Pumilio.

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in turn, recruits the cytoplasmic poly(A) polymerase GLD-2 [21,22] and decreases the affinity for PARN [poly(A)-specific ribonuclease] [23]. In addition, embryonic poly(A)-binding protein is recruited to the CPE-regulated mRNAs by a transient association with the polyadenylation complex, promoting the recruitment of eIF4G (eukaryotic initiation factor 4G) and displacing maskin [24].

However, a short poly(A) tail by itself is not sufficient to repress translation fully, but CPE also mediates active translational repression (masking) of maternal mRNAs in unstimulated oocytes [25,26]. This translation inhibition requires CPEB, which recruits an eIF4E-binding protein named maskin [27], precluding the association of eIF4G with eIF4E and therefore the 40S ribosomal subunit recruitment. A different model where CPEB recruits 4E-T, which in turn binds to an oocyte-specific eIF4E isoform, has also been suggested as a CPE-dependent repression complex [28]. As a result of cytoplasmic polyadenylation, maskin and eIF4E, and/or 4E-T and the oocyte-specific eIF4E, dissociate and the mRNA becomes translationally active [29]. Another trans-acting factor recruited by repressed CPE-containing 3′-UTRs is Xenopus Pumilio (XPum), an RNA-binding protein that interacts with CPEB [30,31]. Thus CPEB binds to two different groups of proteins, one including XPum, maskin, RCK/XP54 and PARN mediating translational repression and the other including CPSF, Symplekin, XGef and GLD-2 driving polyadenylation and translational activation in response to progesterone [19,21–23,32]. In addition to the Aurora A phosphorylation of CPEB described above, all three, CPEB, maskin and XPum, are regulated by other phosphorylation events [18,19,27,31,33,34].

A combinatorial code of cis-acting elements defines the time and extent of translational control

Individual CPE-containing mRNAs display specific translational behaviour during meiosis, suggesting that individual features within their 3′-UTRs determine their response to CPEB-mediated translational control. Thus, not every CPE-containing mRNA is masked [25,35] and the activation of CPE-containing mRNAs does not occur en masse at any one time. Instead, the polyadenylation of specific mRNAs is temporally regulated [33,36,37]. Despite the knowledge accumulated on the composition and regulation of the protein complexes that mediate translational repression and activation of CPE-containing mRNAs, the 3′-UTR features that define whether an mRNA is a target for CPEB-mediated translational repression and how the time and extent of cytoplasmic polyadenylation-dependent translational activation is controlled were still unclear.

In two recent studies [7,38], we have performed a systematic analysis of the combinations of cis-acting elements that define, qualitatively and quantitatively, the differential translational control of CPE-regulated mRNAs. The results of these studies allow us to postulate a set of rules that can be...
used to predict the translational behaviour of CPE-containing mRNAs during meiosis (Figure 2).

(i) Translational repression requires a cluster of at least two CPEs, irrespective of its position along the 3′-UTR, where the distance between adjacent CPEs defines the extent of repression with an optimal distance of 10–12 nt. This implies that the recruitment of maskin must be mediated by a CPEB dimer and that the efficient repression mediated by multiple CPEs corresponds to the recruitment of this heterotrimer rather than multiple CPEB–maskin heterodimers.

(ii) Translational activation requires, at least, a single consensus CPE or a non-consensus CPE together with a PBE (Pum-binding element). The CPE must be closer than 100 nt from the Hex, but not overlapping.

(iii) The distance CPE–Hex determines the extent of polyadenylation and translational activation (either ‘weak’ or ‘strong’), with an optimal distance of 25 nt, which would represent the more relaxed positioning of the CPEB–CPSF complex interacting, respectively, with the CPE and the Hex. Other less optimal distances would probably involve bending of the RNA, introducing tension that would destabilize the binding of the CPSF–CPEB complex. Additional PBEs or CPEs have a positive effect except for an overlapping CPE, which has a negative effect.
(iv) 'Early' or CDC2-independent cytoplasmic polyadenylation requires CPE(s) non-overlapping with the Hex (such as in mos, cyclin B2 and B5, C3H4 and emi1 mRNAs), whereas 'Late' or CDC2-dependent polyadenylation is driven by at least two CPEs, with one of them overlapping the Hex (such as in cyclin B1, B4, emi2 and cyclin E mRNAs).

This effect is directly mediated by the fact that a CPE overlapping with the Hex has a dominant-negative effect in polyadenylation and subsequent translational activation detected only in the presence of low CPEB levels. Thus, during the PI to MI transition, where the levels of CPEB are very high, multiple CPEs are occupied, including the one overlapping the Hex, preventing the recruitment of CPSF to the Hex. However, after CDC2 is activated at MI, most of the CPEB is degraded [33] and stochastically only one CPE would be occupied. Because the non-overlapping CPE has a higher affinity for CPEB than the overlapping CPE–Hex, that would imply that now the single CPEB would be preferentially recruited to CPE and is free to recruit CPSF to the Hex and promote polyadenylation (however, see [39]).

(v) The presence of AREs (AU-rich elements), a feature of mRNAs regulated by deadenylation [39], further defines the effect on polyadenylation dictated by the different arrangements of CPEs. During meiosis, these AREs recruit a zinc-finger protein named C3H4 that is encoded by a CPEB-regulated mRNA activated during the 'early' wave of cytoplasmic polyadenylation. In turn, C3H4 recruits the CCR4 (CC chemokine receptor 4)–Not deadenylase complex to the ARE-containing mRNAs opposing CPEB activity on mRNAs containing both CPEs and AREs. The effect of the C3H4-mediated deadenylation on the target mRNAs is defined by the arrangements of CPEs. Thus, for an mRNA, such as emi1 mRNA, that was polyadenylated by the 'early' activation of a 'weak' CPE, the deadenylase overrides the polyadenylation inactivating the mRNA after MI. For 'early-strong' CPEs, polyadenylation is displaced to MI, whereas for mRNAs containing a 'late-strong' CPE arrangement, which would be polyadenylated in MI, C3H4 is not able to completely neutralize the polyadenylation but causes a delay in the poly(A) tail elongation until later meiotic stages, generating a third wave of polyadenylation in interkinesis, such as for emi2 and cyclin E mRNAs.

Sequential waves of polyadenylation and deadenylation drive meiotic progression

Meiotic progression is a switch-like irreversible process where the successive meiotic phases are discrete states sustained by multiple positive and negative feedback loops that require protein synthesis [38,40–42] and keep the oocyte from slipping rapidly back and forth between cell cycle phases [41,43]. The hierarchical translation of specific subpopulations of mRNAs at each meiotic phase is regulated through sequential waves of polyadenylation and deadenylation (Figure 3).

In PI-arrested oocytes, the CPE-regulated mRNAs are either inactive with a short poly(A) tail or even actively repressed by a dimer of CPEB. As a result of progesterone stimulation, CPEB is phosphorylated by Aurora A [17] inducing a first wave of 'early' or CDC2-independent cytoplasmic polyadenylation of mRNAs such as the ones encoding the MF components cyclins B2 and B5, the MPF activator Mos and the APC inhibitor Emi1 [7], which are required for the PI–MI transition. The switch-like activation of MPF is sustained by multiple positive feedback loops in the p42 MAPK (mitogen-activated protein kinase)/CDC2 network [40,41], which require protein synthesis [42] and which also target the re-activation of the 'early' wave of polyadenylation through the synthesis and activation of Aurora A [40,44–46]. At the same time, a negative feedback loop, which opposes CPEB activity on mRNAs containing both 'early-weak' CPEs and AREs, is switched on through the 'early' polyadenylation-activated translation of C3H4 mRNA. C3H4 generates a deadenylation wave that inactivates Emi1 translation in MI, allowing for the activation of the APC and the transition to interkinesis. As a result of MPF activation in MI, CPEB is phosphorylated by CDC2 and PLK1 (Polo-like kinase 1), triggering its partial destruction and generating the second wave of 'late' or CDC2-dependent polyadenylation of mRNAs such as the ones encoding cyclins B1 and B4. These cyclins are required to sustain an intermediate MPF
activity during interkinesis, and for the reactivation of MPF in MII [7,20,33,47]. In addition, the partial destruction of CPEB together with the synthesis of C3H4 generates the third wave of ‘late–late’ cytoplasmic polyadenylation. This wave targets mRNAs containing ‘late-strong’ CPEs and AREs, such as the ones encoding the CSF components Emi2 and cyclin E, which are synthesized during interkinesis. CSF, in turn, inhibits the APC, allowing the full re-activation of the MPF, now with cyclins B1 and B4, and maintaining the oocyte arrested in MII until fertilization takes place [38].

Beyond oocyte maturation

This combinatorial code defines 274 possible arrangements of cis-acting elements that result in 20 different translational behaviours during meiosis and early embryonic development. Experimental and computational analysis of these motif patterns identified hundreds of vertebrate mRNAs (up to 20% of Xenopus laevis, mouse or human genomes) potentially regulated by CPEs and AREs [7,38]. The accuracy of these predictions has been validated both by a functional screening to identify CPE-regulated mRNAs activated in PI or MI [38] and by testing randomly selected candidates [7]. Moreover, a phylogenetic hidden Markov model, phastCons, performed across 17 vertebrate genomes indicates the selective pressure to maintain the regulatory configurations, and that this code appears to be universal across vertebrates.

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