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The Mos Pathway Regulates Cytoplasmic Polyadenylation in *Xenopus* Oocytes

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Cytoplasmic polyadenylation controls the translation of several maternal mRNAs during *Xenopus* oocyte maturation and requires two sequences in the 3′ untranslated region (UTR), the U-rich cytoplasmic polyadenylation element (CPE), and the hexanucleotide AAUAAA. c-mos mRNA is polyadenylated and translated soon after the induction of maturation, and this protein kinase is necessary for a kinase cascade culminating in cdc2 kinase (MPF) activation. Other mRNAs are polyadenylated later, around the time of cdc2 kinase activation. To determine whether there is a hierarchy in the cytoplasmic polyadenylation of maternal mRNAs, we ablated c-mos mRNA with an antisense oligonucleotide. This prevented histone B4 and cyclin A1 and B1 mRNA polyadenylation, indicating that the polyadenylation of these mRNAs is Mos dependent. To investigate a possible role of cdc2 kinase in this process, cyclin B was injected into oocytes lacking c-mos mRNA. cdc2 kinase was activated, but mitogen-activated protein kinase was not. However, polyadenylation of cyclin B1 and histone B4 mRNA was still observed. This demonstrates that cdc2 kinase can induce cytoplasmic polyadenylation in the absence of Mos. Our data further indicate that although phosphorylation of the CPE binding protein may be involved in the induction of Mos-dependent polyadenylation, it is not required for Mos-independent polyadenylation. We characterized the elements conferring Mos dependence (Mos response elements) in the histone B4 and cyclin B1 mRNAs by mutational analysis. For histone B4 mRNA, the Mos response elements were in the coding region or 5′ UTR. For cyclin B1 mRNA, the main Mos response element was a CPE that overlaps with the AAUAAA hexanucleotide. This indicates that the position of the CPE can have a profound influence on the timing of cytoplasmic polyadenylation.

Oocytes of many animals contain translationally dormant mRNAs that are activated in a stage-specific and sequence-specific manner in early development. Such maternal mRNAs encode a variety of products that are important for the initial cell divisions, the establishment of embryonic polarity, and the induction of certain cell lineages (reviewed in references 4, 9, 16, 33, and 35). Although a number of mechanisms are probably responsible for the translational control of maternal mRNA, one that appears to be widespread among metazoans is cytoplasmic poly(A) elongation. In this case, a number of mRNAs that are quiescent in oocytes contain relatively short poly(A) tails, usually fewer than 20 nucleotides. In response to a cue such as reentry into meiosis or fertilization, the poly(A) tails of specific mRNAs are elongated and thereby promote translation. For the most part, the details of this process have emerged from studies of *Xenopus* and mice. During oocyte (meiotic) maturation, two cis-acting sequences in the 3′ untranslated regions of responding mRNAs are required for cytoplasmic polyadenylation, the UUUUUAAU-type cytoplasmic polyadenylation element (CPE), and the hexanucleotide AAUAAA. Other mRNAs that undergo cytoplasmic polyadenylation after fertilization require a poly(U)12–27 CPE, as well as the hexanucleotide (29; reviewed in reference 24).

One factor that is essential for cytoplasmic polyadenylation during maturation is the cytoplasmic polyadenylation element binding protein (CPEB) (8, 31). Because this 62-kDa protein is bound to the CPE both before and after polyadenylation oc-

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analyze the features of mRNAs that determine the timing of cytoplasmic polyadenylation, we have ablated c-mos mRNA by the injection of an antisense oligonucleotide, thus preventing one of the earliest events in oocyte maturation, Mos synthesis. This prevented the polyadenylation of the “late” mRNAs, histone B4 and cyclins B1 and A1. However, because injected cyclin B protein induced polyadenylation even in the absence of Mos, it may be that cdc2 kinase, which is activated by cyclin B, is more directly responsible for late polyadenylation. Mutational analysis indicated that mRNAs that are targets of the Mos-dependent mRNAs contains a cis-acting “Mos response element” that is important for their cytoplasmic polyadenylation. In cyclins B1 and A1 RNA, this element corresponds to a CPE that overlaps the hexanucleotide AUAUAAA. This indicates that CPEs, based on their position relative to the hexanucleotide, can play different regulatory roles in polyadenylation. This observation, in conjunction with the effects of Mos and cdc2 on polyadenylation, is discussed.

**MATERIALS AND METHODS**

Oocyte preparation and injection. To obtain full-grown oocytes, *Xenopus laevis* females were injected with pregnant mare serum gonadotropin (50 IU). At 2 to 3 days later, ovaries were dissected and treated with collagenase and dispase (13), and stage VI oocytes were selected. Maturation was induced by incubating the oocytes in Barth’s medium containing progesterone (10 μM).

High-pressure liquid chromatography-puriﬁed sense and antisense c-mos oligonucleotides were made to match a sequence in the 5′ part of the 24-bp 3′ untranslated region (UTR) of *c-mos* mRNA (nucleotides [nt] 2265 to 2289 of the c-mos cDNA; sense [ATCTAGTACATATCCATTGCA] and antisense [complementary sequence]). This antisense oligonucleotide has been shown to block Mos protein function (30). The oligonucleotides were dissolved in water at 3 μM followed by extractions with phenol-chloroform and chloroform. Approximately 70 nl of this solution was injected into each oocyte, and the oocytes were incubated overnight. Some oocytes were injected with a 30- nl solution of radioactively labeled RNAs that were synthesized in vitro and suspended in water at a concentration of 0.1 to 0.5 μM. Clam cyclin B, synthesized in bacteria (a gift of J. V. Rudner, Harvard Medical School) was used for oocyte injection at 2 μg/ml (50 nioocytes).

After incubation, the oocytes were collected in microcentrifuge tubes (four or five oocytes per sample); all excess buffer was removed, and they were frozen on dry ice. The samples were stored at −80°C.

Plasmid construction and RNA synthesis. Plasmids pSmos (mos), pB4 (B4), and pscyclin B1 (sB1) have been described previously (31, 32). The following DNA oligonucleotides were used in the construction of new templates: 5′-B4.3F, GAAGCTTAGGCTGATATACTTTTAACT; 3′-B4, GGATCTTCTTAAAGACAAAGATTACCTT; 3′-B4 DNA, TCCGACCATGACGGTTCTCTG; 5′-ACGCTGACGGTTCTCTG; 5′-sB1, GGAGATCTGTTTGAGCTTTATTAAAAACC; 3′-sB1, GGAGATCTGTTTGAGCTTTATTAAAAACC; 5′-sB1, GGGATCCTTTAGGTTTTTAATGTTTTACTGG; 3′-sB1, CGGGATCCTGTTGGCACCATGTGC.

The plasmids were linearized with *Dra I* and transcribed with T3 RNA polymerase. For the M series of mutations, the corresponding primers were the 3′ probe, which initiates a series of poorly understood events that result in the cytoplasmic polyadenylation and translational activation of c-mos mRNA. Newly synthesized Mos kinase, in turn, activates MAP kinase kinase by phosphorylation, which activates MAP kinase by phosphorylation, which activates MAP kinase. MAP kinase activation is necessary for activation of cdc2 kinase (a component of MPF). Progesterone-mediated activation of cdc2 kinase probably also involves other substrates of Mos, in addition to Mos-independent signals (reviewed in reference 25).

To characterize the temporal relationships between some of these events and cytoplasmic polyadenylation, we performed several time course experiments, as shown in Fig. 2. cdc2 activation, as manifested by the in vitro phosphorylation of the model substrate histone H1 (and hence is referred to as H1 kinase activity), occurred by 210 min after progesterone addition. This activation was coincident with, and indeed is necessary for, oocyte maturation (scoring by GVBD) as noted previously (reviewed in references 5 and 25). Mos protein, which is barely detectable in untreated oocytes by Western blotting, became visible at 30 min after progesterone addition and was clearly evident by 90 to 120 min. CPEB, the RNA binding protein that is required for cytoplasmic polyadenylation (8, 31), was present both before and after progesterone addition but underwent a phosphorylation-induced mobility change by...
Mos mRNA was injected into oocytes, which were subsequently cultured in progesterone-containing medium. The Northern blot in Fig. 3 shows that full-length c-mos mRNA was cleaved by the injected antisense oligonucleotide (this is due to endogenous RNase H activity) whereas the sense oligonucleotide had no effect. As expected, in the oocytes in which c-mos mRNA was ablated, Mos protein accumulation, H1 kinase activation, and GVBD were all prevented (Fig. 3 and data not shown). The cytoplasmic polyadenylation of cyclin B1 mRNA was also inhibited by c-mos mRNA degradation of CPEB were detected by Western blotting; cytoplasmic polyadenylation of endogenous cyclin B1 mRNA and histone B4 mRNA was detected by Northern analysis of total RNA. The same blot was hybridized with cyclin B1 probe, stripped, and rehybridized with histone B4 probe. Unfortunately, the RNA sample in the 60-min lane was not completely dissolved when loaded on the gel. A radioactive Mos 3′ UTR fragment (sMos) was injected into some oocytes that were then incubated in a medium containing progesterone. Total RNA was isolated at several time points and analyzed by electrophoresis on a denaturing polyacrylamide gel. sMos was visualized by phosphorimaging. The length of the poly(A) tail is indicated (A₀ and Aₘₐ).

FIG. 2. Time course of events during oocyte maturation. Stage VI Xenopus oocytes were incubated with progesterone, samples were taken at different time points, and a number of parameters were examined. GVBD was scored by the appearance of a white spot on the animal pole of the oocyte; cdc2 activity was detected by assaying the phosphorylation of histone H1 in extracts in vitro (H1 Kinase); Mos protein was assayed by Western blotting; phosphorylation and degradation of CPEB were detected by Western blotting; cytoplasmic polyadenylation of endogenous cyclin B1 mRNA and histone B4 mRNA was detected by Northern analysis of total RNA. The same blot was hybridized with cyclin B1 probe, stripped, and rehybridized with histone B4 probe. Unfortunately, the RNA sample in the 60-min lane was not completely dissolved when loaded on the gel. A radioactive Mos 3′ UTR fragment (sMos) was injected into some oocytes that were then incubated in a medium containing progesterone. Total RNA was isolated at several time points and analyzed by electrophoresis on a denaturing polyacrylamide gel. sMos was visualized by phosphorimaging. The length of the poly(A) tail is indicated (A₀ and Aₘₐ).
protein. This induction was inhibited by antisense Mos oligonucleotide (Fig. 4A).

To further investigate the correlation between translation and polyadenylation we fractionated oocyte extracts into free (supernatant, untranslated) and polysomal (pellet, translated) mRNP. Progesterone treatment of normal oocytes induced a shift of the histone B4 and cyclin B1 mRNAs into the polysomal fraction, in addition to being polyadenylated, as expected. The association of histone B4 mRNA with the polysomes was reduced by antisense Mos oligonucleotide injection, while the polyosomal association of cyclin B1 mRNA was completely abolished (Fig. 4B). These data indicate that in the absence of Mos both polyadenylation and translation of histone B4 and cyclin B1 are inhibited.

Mos-responsive cytoplasmic polyadenylation elements. To determine the features of the histone B4 and cyclin B1 mRNAs that make their polyadenylation dependent on Mos translation, radioactive 3'UTR fragments containing the signals for cytoplasmic polyadenylation (CPE and the hexanucleotide AAUAAA) were injected into oocytes preinjected with a Mos antisense oligonucleotide. As shown in Fig. 5, polyadenylation of the histone B4 (sB4; nt 881 to 910 of the mRNA) and c-mos mRNA fragments (sMos; nt 3117 to 3137) were only slightly inhibited by the antisense oligonucleotide (the antisense oligonucleotide is not directed against sMos, since it covers nt 2265 to 2289). This demonstrates that cytoplasmic polyadenylation per se is not dependent on Mos protein. However, the polyadenylation of an injected cyclin B1 mRNA fragment (sB1) was prevented in oocytes lacking Mos, similar to the endogenous cyclin B1 mRNA. This was a surprising result because both cyclin B1 and histone B4 RNAs were polyadenylated with nearly the same kinetics during oocyte maturation (Fig. 2). To examine whether the dependence of histone B4 mRNA polyadenylation on Mos was conveyed by regions of the mRNA outside the sB4 sequence, we performed an additional series of experiments. RNAs containing the full 3'UTR only (nt 849 to 932) or encompassing the complete mRNA (nt 1 to 932) were injected into oocytes in which c-mos mRNA had been destroyed. Figure 6 shows that only the polyadenylation of the probe containing the full B4 mRNA sequence was inhibited in the absence of Mos. Thus, a histone B4 RNA Mos response element in the 5'UTR or coding region is necessary for the repression of polyadenylation until, presumably, Mos is synthesized to such a level that it can activate polyadenylation.

Mos-dependent polyadenylation of cyclin B1 mRNA requires the CPE overlapping with the hexanucleotide. To begin to characterize the sequences that convey Mos dependence to the polyadenylation of the cyclin B1 RNA (sB1), a comparison of its sequence with that of the histone B4 RNA (sB4), whose polyadenylation is Mos independent, is useful. As depicted in Fig. 7, sB1 RNA has two CPEs, the first of which (CPE1) is

FIG. 3. Cytoplasmic polyadenylation of cyclin B1 and histone B4 mRNAs is dependent on Mos protein synthesis. Stage VI oocytes were injected with sense (S) or antisense (AS) Mos oligonucleotide against c-mos mRNA and incubated with progesterone. H1 kinase activation and Mos and CPEB Western blots are as described in the legend to Fig. 2. Lower panels show Northern blots of total RNA probed with the Mos coding region, cyclin B1, and histone B4 probes.

FIG. 4. Mos dependence of translational activation. (A) Cyclin B1 protein levels in oocytes as assayed by Western blotting. The lanes are labeled as in Fig. 3. (B) Northern blots of RNA from fractionated mRNP; T, total RNA; S, supernatant (free mRNP, untranslated); P, pellet (polysomal mRNP, translated). Other labels are as in panel A. The blot was hybridized sequentially with histone B4 and cyclin B1 cDNA probes.

FIG. 5. Mos dependence of polyadenylation of injected 3'UTR fragments. Oocytes were injected with sense (S) or (AS) Mos oligonucleotides as in Fig. 3. Radio-labeled 3'UTR fragments were injected into these oocytes, and they were incubated in progesterone (Progest.), as in Fig. 2. The 3'UTR fragments of c-mos (sMos), histone B4 (sB4), and cyclin B1 (sB1) mRNAs are depicted above each panel to show the relative placement of the CPEs (ovals) and the hexanucleotide (open boxes). P indicates the un injected probe. Polyadenylation was analyzed on denaturing polyacrylamide gels as for sMos in Fig. 2.
Figure 6. Polyadenylation of the injected full-length B4 mRNA is dependent on Mos. Different radioactive fragments of the histone B4 mRNA were injected into oocytes as described in the legend to Fig. 5. As in Fig. 5, the placement of the CPE (ovals) and hexanucleotide (open boxes) is shown. The coding region is depicted as a solid box. The relative sizes of the elements are not drawn to scale. sB4, small 3' UTR fragment of histone B4 mRNA; B4 3' UTR, full 3' UTR of histone B4 mRNA; B4 syn mRNA, synthetic mRNA containing the complete B4 mRNA sequence. Polyadenylation was analyzed on denaturing polyacrylamide gels as in Fig. 5.

Identical to that of sB4 RNA and the second of which (CPE2) overlaps with the AAUAAA hexanucleotide. Deletion of the sequences 3' of the hexanucleotide had no effect on the inhibition of polyadenylation when c-mos mRNA was ablated (xsB1-3). Small regions in the sequence between CPE1 and the hexanucleotide were converted into the corresponding sB4 sequence. Partial escape from the inhibition of polyadenylation in the absence of Mos was observed for the mutations upstream of CPE2 (xsB1-3M1 and xsB1-M3). However, mutation of CPE2 made the polyadenylation of the xsB1-3M2 as independent of Mos as did that of sB4 (compare xsB1-3 and xsB1-3M2 in Fig. 7 with sB4 in Fig. 5 and 6). Deletion of the 5' end of the cyclin B1 RNA sequence resulted in a partial relief of polyadenylation inhibition in the absence of Mos (xsB1-5). As expected, a double deletion of both the 5' and 3' B1 sequences still had only a partial restoration of polyadenylation activity in the absence of Mos (xsxsB1). However, destruction of CPE2 almost completely restored polyadenylation in the absence of Mos in this construct (xsxsB1-M2), similar to that in the xsB1-3M2 RNA. Thus, while sequences upstream of CPE2 contribute to the inhibition of polyadenylation in the absence of Mos, possibly through the formation of secondary structure, it is CPE2 that is indispensable for this repression. In other words, Mos protein, by a mechanism as yet unknown, induces polyadenylation by relieving a repression that acts through CPE2, which overlaps with the hexanucleotide.

Cyclin A1 mRNA polyadenylation is Mos dependent. Cyclin A1 mRNA is known to be polyadenylated in maturing oocytes (27). Interestingly, the single CPE of this mRNA overlaps with the AAUAAA hexanucleotide, similar to CPE2 in cyclin B1 mRNA, which raises the possibility that the cytoplasmic polyadenylation of cyclin A1 mRNA is also Mos dependent. Indeed, ablation of c-mos mRNA by antisense oligonucleotide injection prevented the polyadenylation of endogenous cyclin A1 mRNA (Fig. 8, top). Similarly, an injected cyclin A1 3' UTR fragment also was not polyadenylated in the absence of Mos (Fig. 8, bottom). Taken together, our data show that the position of the CPE relative to the hexanucleotide AAUAAA has a profound effect on cytoplasmic polyadenylation and demonstrate that Mos regulates the function of CPEs overlapping with their hexanucleotide.

cdc2 kinase induces cytoplasmic polyadenylation of cyclin B1 mRNA and histone B4 mRNA. Mos protein activates a kinase cascade that includes MAP kinase and cdc2 kinase (Fig. 1). However, active cdc2 can also induce Mos protein synthesis and MAP kinase activation via a feedback loop (7). To dissect the pathway by which Mos induces cytoplasmic polyadenylation, we activated cdc2 kinase by injecting cyclin B into oocytes in which the feedback to Mos synthesis was blocked by the injection of an antisense oligonucleotide. As can be seen in Fig. 9, lanes 1 to 4, the ablation of c-mos RNA prevented progesterone-induced Mos synthesis, cdc2 kinase activation (H1 kinase), CPEB phosphorylation, and cyclin B1 and histone B4 mRNA polyadenylation, as shown previously (Fig. 3). In addition, we show here that in the absence of Mos, MAP kinase did not undergo tyrosine phosphorylation, which is required for its activation (12). We note that the identity of this protein was confirmed by probing this Western blot with MAP kinase antibody (data not shown).

To obtain the data shown in Fig. 9, lanes 5 to 7, maturation was induced by the injection of clam cyclin B protein in the absence of progesterone. In control oocytes, this protein activated cdc2 kinase (H1 kinase), as expected. Mos protein synthesis and MAP kinase tyrosine phosphorylation (i.e., activation) were induced, presumably by the feedback loop, as was the phosphorylation of CPEB. The cyclin B1 and histone B4 mRNAs were polyadenylated as well (lanes 5 and 6). However, in oocytes whose c-mos mRNA had been ablated by a preinjection of antisense oligonucleotide (lane 7), the injection of cyclin B1 protein resulted in cdc2 activation and CPEB phosphorylation but not in Mos protein synthesis or MAP kinase activation. Importantly, both cyclin B1 and histone B4 mRNAs were polyadenylated, which demonstrates that cdc2 kinase can induce cytoplasmic polyadenylation of these mRNAs, independently of Mos or MAP kinase. This indicates that cdc2 kinase is an intermediate in the regulatory pathway from Mos to cytoplasmic polyadenylation.

Discussion

In this report, we have shown that during the normal course of oocyte maturation, embryonic histone B4 and cyclin B1 and A1 mRNAs require Mos synthesis before they can undergo cytoplasmic polyadenylation. When c-mos mRNA is ablated by antisense oligonucleotide injection, these mRNAs are not polyadenylated and their translation is inhibited. Mutational analysis revealed that specific sequences within these mRNAs are necessary for Mos-controlled polyadenylation. For histone B4, this region resides in the 5' UTR or coding region; for cyclins B1 and A1, it is an overlapping CPE and hexanucleotide (UUUUUAUAAAA). These sequences or, rather, the factors that might bind to them are probably not controlled directly by Mos but are most probably controlled by cdc2 kinase.

We report that cyclin B1 and histone B4 mRNAs are polyadenylated at GVBD. An injected Mos 3' UTR fragment is polyadenylated approximately 2 h earlier. Sheets et al. (27) reported that Mos mRNA is polyadenylated at the same time as cyclin B1 mRNA, but this might be because their time points are 2 h apart. Moreover, the accumulation of cyclin B1 protein during oocyte maturation is also consistent with a translational activation at GVBD, long after Mos accumulation has started (11) (Fig. 2).

Although we describe three mRNAs whose polyadenylation is Mos dependent, it is not clear which endogenous mRNAs are polyadenylated in the absence of Mos. Perhaps the most likely candidate is the c-mos mRNA itself. The data of Sheets et al. (27), obtained with Xenopus oocytes, and those of Gebauer et al. (6), obtained with mouse oocytes, have shown that...
c-mos mRNA polyadenylation is necessary for Mos synthesis. Thus, c-mos mRNA polyadenylation would have to occur prior to Mos synthesis because Mos protein is almost undetectable in immature oocytes. However, the data presented in Fig. 2 show that some Mos had accumulated even when c-mos mRNA polyadenylation was just beginning. These apparently contradictory results may be explained by recent studies that have examined how polyadenylation induces translation. During maturation, 3' poly(A) addition induces 5' cap ribose methylation, which in turn stimulates translation (13, 14). However, it appears that the process of poly(A) addition, rather than a poly(A) tail per se, is important for cap ribose methylation.
methylation (13). Thus, in the case of c-mos mRNA, it is possible that the initial stages of polyadenylation are sufficient to induce cap ribose methylation and the resulting translational activation.

The observation that some CPE-containing 3’ UTR fragments (sMos, sB4, and xsB1-3M2) are polyadenylated in oocytes that have an ablated c-mos mRNA (Fig. 5 to 7) indicates that the cytoplasmic polyadenylation machinery is activated independently of Mos accumulation. Thus, the polyadenylation of Mos-dependent mRNAs appears to be under dual control, i.e., derepression by Mos and activation by CPEB. Why a dual control? The answer may lie in the importance of translational repression of these mRNAs. The cyclin mRNAs, like many other maternal mRNAs, are stored in a translationally dormant form. Should the translational repression of cyclin A1 or B1 mRNA be “leaky” during this storage period, cyclin protein could build up to such a level as to induce oocyte maturation during the latter growth stages of oogenesis, independently of exposure to hormone and thus not coupled to egg-laying and mating behavior. Since mature oocytes have a limited life span, such spontaneous maturation would cause a sharp reduction in fertility.

Because cdc2 activation can induce polyadenylation of Mos-dependent mRNAs, even in the absence of both Mos and active MAP kinase (Fig. 9), it is probable that cdc2 is a more direct activator of this process than the two other enzymes are. This is also consistent with the timing of cyclin B1 and histone B4 mRNA polyadenylation during progesterone-induced oocyte maturation (Fig. 2). In addition, in a preliminary experiment, we injected an mRNA for a dominant negative cdc2 mutant into oocytes. This prevented both cdc2 activation and Mos accumulation, as reported previously (19). When a bacterially expressed Mos fusion protein was injected into these oocytes, MAP kinase was activated by tyrosine phosphorylation but cdc2 kinase remained inactive, as expected. In this case, cyclin B1 and histone B4 mRNAs were not polyadenylated (data not shown), which suggests that cdc2 kinase is not only sufficient but also necessary for Mos-dependent cytoplasmic polyadenylation.

Although CPEB is essential for cytoplasmic polyadenylation (8, 31) it is unclear what the role of its phosphorylation is. Phosphorylation of CPEB occurs simultaneously with cdc2 kinase activation (Fig. 2, 3, and 9). This implies that CPEB phosphorylation could be involved in the induction of Mos-dependent cytoplasmic polyadenylation. However, because polyadenylation of injected 3’ UTR fragments takes place in the absence of Mos synthesis of CPEB phosphorylation (Fig. 3, 5, and 7), activation of polyadenylation by progesterone does not absolutely require Mos protein or phosphorylation of CPEB (although, as stated above, CPEB itself is dispensable). The phosphorylation of CPEB is correlated with the destruction of most of the protein, as noted previously (8) (Fig. 2 and 9).

A CPE overlapping with a hexanucleotide seems to be the main hallmark of the Mos-response element in cyclin A1 and B1 mRNA (Fig. 7 and 8). The dovetail arrangement of these two elements suggests that the binding of a protein to the CPE might prevent the binding of the hexanucleotide binding factor, possibly CPSF (1). This hypothetical hexanucleotide masking factor could be CPEB or another RNA binding protein recognizing a similar sequence. The release from repression could be mediated by conformational changes caused by phosphorylation of the masking protein or phosphorylation of the hexanucleotide binding factor, resulting in the binding of both
CPEB and the hexanucleotide binding factor and the initiation of polyadenylation.

Irrespective of the mechanisms involved, our data indicate that a CPE-hexanucleotide fusion can confer Mos dependence on polyadenylation and thus regulate the timing of polyadenylation.

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