Plasmid constructions and RNA synthesis. Standard PCR mutagenesis was used to generate the series of Wee1 3' UTR deletion mutants in the pGEM GST vector from a full length 297 nucleotide Wee1 3' UTR that encoded mutational disruptions in all three CPE sequences (pGEM GST WeeUTR(ϕ), (1)). For the deletion plasmid 256-283, the Wee1 UTR nucleotides 3' of the polyadenylation hexanucleotide were replaced with the sequence found 3' of the polyadenylation hexanucleotide in the β-globin 3' UTR.

PCR mutagenesis was utilized to introduce the 17 nucleotide region Wee1 3' UTR region (Wee17), located between the CPE sequences 5' of the polyadenylation hexanucleotide, into the β-globin 3' UTR cloned downstream of the GST open reading frame in the pGEM GST vector (1). Deletion mutations were introduced within the Wee17 region using QuickChange (Promega) PCR mutagenesis and the β-globin Wee17 plasmid as the starting template in each case. For the 5' TCS boundary mutations, Quickchange was employed using the 5' Δ10 plasmid as the starting template. QuickChange mutagenesis was also employed to introduce diguanosine nucleotide substitutions into the 5' Δ10 sequence (ATTGTCT to AggGTCT) to generate 5' Δ10 TCS/gg and the Wee17 region (TTATTATCTTATTGTCT to TTAggATCTTAggGTCT) to generate Wee17/2gg. The polyadenylation hexanucleotide was disrupted in the β-globin/TCS 3' UTR by nucleotide substitution (changing AATAAA to AAgAAA) using QuickChange mutagenesis. PCR mutagenesis was also employed to introduce flanking CPE sequences into the β-globin/Wee17 3' UTR. The β-globin UTR constructs were subcloned downstream of the GST coding region in the pGEM GST vector.

PCR primers were designed to amplify the full length Pcm-1 3' UTR sequence (NCBI Accession AB025414) with a 5' BamH I site and a 3' Xba I site. cDNA was made from RNA from immature Xenopus oocytes using the reverse PCR primer and Superscript III (Invitrogen). Full length Pcm-1 3'UTR was amplified using Platinum Pfx (Invitrogen) and the PCR product digested with BamH I and Xba I and ligated into BamH I/Xba I digested pGEM GST. To make mutations in the Pcm-1 TCS elements, the TCS elements were disrupted by dinucleotide substitution, changing both TTTGTCT sequences to TggGTCT using QuickChange. QuickChange mutagenesis was also employed to delete the 5' and 3' TCS elements (TTTGTCT) within the Pcm-1 3' UTR to generate Pcm-1 TCS Δ.

Real Time PCR quantitation of injected reporter mRNAs

Real time PCR was performed with LightCycler FastStart DNA Master SYBR Green I (Roche) in a LightCycler 2.0 (Roche) according to manufacturers directions. Specifically, 2 µl of 1:10 diluted cDNA was added in a final volume of 10 µl with a final concentration of 2 mM (GST) or 1.5 mM (Cyclin B1) $MgCl_2$ and 0.5 µM primers. Primers were designed by real time PCR primer design (Genescript Corp, https://www.genscript.com/ssl-bin/app/primer) GST-L1: 5' TAC GGT GTT TCG AGA ATT GC; GST-R1: 5'TCA ATT TGT GGG ATA GCT TCA; cyclin B1-L1: 5' ATG GAA CTT GTG ATG GTG GA; and cyclin B1-R1: 5'CTG TGG AAT CGT GCT GAT CT. PCR amplification parameters were: 95°C 10s, 55°C 5s, 72°C 11s for 45 cycles (GST); or 95°C 10s, 53°C 5s, 72°C 11s 45 cycles (Cyclin B1). PCR integrity was confirmed by melting curve analysis. Standards were created by PCR cloning the fragment of GST and cyclin B1 that was amplified by the real time PCR primers using Pfu (Stratagene) and modified forward primers that had an extra CACC at the 5' end for directional cloning into pENTR (Invitrogen). Fragments were excised by *Not*I/*Asc*I, gel purified and quantitated. Standards were diluted to 50 fg, 10 fg, 2 fg and 0.4 fg (GST) or 10 fg, 1 fg, 0.1 fg and 0.01 fg (cyclin B1) and were included in each run in duplicate. Samples were run in duplicate from each of three experiments. Lightcycler3 (v3.5.28) Data Analysis software calculated the concentrations of the samples relative to the standards. The amount of GST was normalized to cyclin B1 as an internal control to account for any variation in RNA extraction and cDNA preparation between samples.

Reference for Supplemental Information:

1. Charlesworth, A., Welk, J., and MacNicol, A. (2000) *Dev. Biol.* **227**(2), 706-719