

Plasmid Constructs:

All plasmids were sequenced to verify sequence integrity.

Yeast three hybrid plasmid constructs: The last 48 nucleotides of the Mos UTR were inserted 3' of the MS2 sites in a modified pIII^A MS2-2 plasmid. A *Xho* I and a *Nar* I site were inserted 3' of the tandem MS2 sites in pIII^A MS2-2, a kind gift from Dr. Marv Wickens (Bernstein et al., 2002) using QuikChange (Stratagene) to generate pIII^A MS2-2.1. The last 48 nucleotides of the Mos 3' UTR, with the disrupted CPE were excised from pGEM GST M1 48 (Charlesworth et al., 2002) with *Bam*H I and *Xba* I, blunted using Klenow and ligated into *Xho* I-digested, Klenow blunted pIII^A MS2-2.1. The remnants of the 5' *Bam*H I site reconstituted the 5' *Xho* I site, which was utilized for diagnostic purposes for cloning orientation.

Xenopus Musashi plasmid constructs: PCR primers were designed to amplify the full length Nrp1b sequence (NCBI Accession M34895) with a 5' *Cla* 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 Nrp1b/*Xenopus* Musashi was amplified using Platinum Pfx (Invitrogen) and the PCR product digested with *Cla* I and *Xba* I and ligated into *Cla* I/*Xba* I-digested pXen1 (MacNicol et al., 1997). To create the N-Msi N-terminal RNA-binding domain fragment of Musashi, a PCR primer was designed to contain a stop codon after amino acid 199, followed by an *Xho* I site. The N-terminal of Musashi was amplified using Pfu (Stratagene) using the same forward primer that amplified the full length Musashi. The PCR product was digested *Cla* I/*Xho* I and ligated into *Cla* I/*Xho* I-digested pXen1. The Musashi binding mutant (bm) was constructed based on previous Musashi binding mutants (Imai et al., 2001), using site directed mutagenesis (QuikChange, Stratagene) to create the F63L-F65L-F68L triple mutant,

using the pXen N-Msi plasmid as the starting template. Both pGEM GST N-Msi and pGEM GST N-Msi bm were linearized with *EcoR* I prior to *in vitro* transcription with SP6 as previously described (Howard et al., 1999).

EMSA competition constructs: The wild type competition plasmid was pGEM Mos 82. The last 82 nts of the Mos 3' UTR were cloned into pGEM4Z (Promega) using a 5' *BamH* I site and a 3' *Xba* I site. To make the PRE deletion mutant, pGEM Mos 82 Δ PRE, QuikChange was used to loop out the 5' 21 nt of the PRE, which left the CPE intact, using pGEM Mos 82 as the template. For the point mutant competition plasmids, dinucleotide substitutions were introduced into pGEM GST M1 48 (Charlesworth et al., 2002) using QuikChange. GST was excised by digesting with *Nco* I and *Xho* I, and the plasmid was re-ligated after Klenow treatment. Plasmids were linearized with *Xba* I prior to transcription with SP6.

GST reporter plasmid constructs: The pGEM GST β -globin and pGEM GST β -globin/PRE plasmids have been previously described (Charlesworth et al., 2002). The Mos Musashi consensus site and polyadenylation hexanucleotide were disrupted by dinucleotide substitution, AUAGU to AUccU, and AAUAAA to AAgAAA respectively, in the pGEM GST β -globin/PRE plasmid using QuikChange. The resulting pGEM GST β -globin/PRE Msi mt and pGEM GST β -globin/PRE hex mt plasmids were linearized with *Pst* I prior to transcription with SP6.

Tethered assay plasmid constructs: The pMSPN, pMS2-Xdazl, pLuc-MS2 and pLuc-MS2 plasmids were kind gifts of Dr. Niki Gray (Collier et al., 2005) and the *Renilla* control plasmid was a kind gift of Dr. Nancy Standart (Minshall et al., 2001). To create the MS2-musashi fusion

protein plasmid, pMS2-msi, primers were designed to amplify the full length Musashi sequence from pXen-Msi, with 5' Xho I and 3' BamH I sites. Full length *Xenopus* Musashi was amplified using Pfu (Invitrogen), the PCR product was digested with Xho I and BamH I, and ligated into Xho I and BamH I-digested pMSPN.

Polyadenylation and PCR Assays. RNA-ligation coupled RT-PCR was performed as described previously (Charlesworth et al., 2004). To analyze polyadenylation of reporter constructs, primers to the GST region are used (Charlesworth et al., 2004). The primers used to analyze the novel mRNAs are as follows: Bub3 (NCBI BC073086) GAG AGG AAA CCC TAC AAG ACT GTG; cdk2/Eg1 (NCBI BC106636, this sequence was found to have an open reading frame identical to *Xenopus* cdk2/Eg1 amino acid sequence, and the last 188 nt of the 3'UTR matched the cdk2 mRNA (Stebbins-Boaz and Richter, 1994)) TGT GTG TTT TAG TCT GTA CCA TTC; Eg3 (NCBI Z17205) CCC ATC TGT TTT TTT ATT GTC TGT GAA TG; TATA binding protein 2 (NCBI AY753184) CGA CAT AAG TCC CTC CAT TAA GAC A; Xotch (NCBI M33874) TGT ATC GAG CAG AAA TGT AGT TTA. To analyze mRNAs that were co-associated with GST-Musashi in vivo, the primers were: Mos, GAG AAT CAC AGT TCC ACA GCA ACC (forward) and AGA CAG TTC CCC CAA CAG AAG C (reverse); and Protein Phosphatase Inhibitor 2 (IPP2) (accession number BI444584) CGT GTC ATT AGC AAG CCA GAG AC (forward) and GCA ATC AAC TGT CTG GCG AGT C (reverse).

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