Non-coding stem-bulge RNAs are required for cell proliferation and embryonic development in C. elegans

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Supplementary figure legends

Figure S1. sbRNAs from the Nematoda phylum. (A) Phylogenetic distribution of sbRNAs identified in nematodes, adapted from Boria and co-workers (J Mol Evol 70, 346-358, 2010). Species phylogeny is represented as a cladogram with arbitrary branch lengths. sbRNAs that were synthesised and tested in vitro are indicated. (B) Nucleotide sequences and predicted secondary structures of representative sbRNAs from four nematode species that were synthesised and tested in vitro. (C) sbRNAs can substitute for Y RNAs in a cell-free DNA replication initiation system. Template nuclei from late G1 phase cells were incubated with protein fractions QA and ArFT, supplemented with the indicated RNAs from C. elegans (CeN133 and CeN135) and other nematode species. Human U2 snRNA and hY1 RNA served as negative and positive controls, respectively. Proportions of replicating nuclei were determined by immunofluorescence microscopy. Mean values ± standard error of the mean (SEM) are shown for four experiments (n=4). * P < 0.01 when compared to background level with no RNA added, as determined by Student’s t-tests.

Figure S2. Design of antisense Morpholino oligonucleotides to bind sbRNAs. Nucleotide sequences and predicted secondary structures of six sbRNAs in C. elegans. The positions of antisense morpholino oligonucleotides (MOs) are indicated by black lines along the complementary nucleotide sequences on the RNAs.
A

Clade I
- C. elegans: 19 sbRNAs identified
- C. briggsae: 26 sbRNAs tested
- C. remanei: 19 sbRNAs
- C. brenneri: 8 sbRNAs
- C. japonica: 4 sbRNAs

Clade III
- A. caninum: 16 sbRNAs
- N. brasiliensis: 6 sbRNAs
- H. contortus: 0 sbRNAs

Clade IV
- P. pacificus: 23 sbRNAs
- H. glycines: 0 sbRNAs
- G. pallida: 1 sbRNA
- M. hapla: 5 sbRNAs
- M. incognita: 10 sbRNAs

Clade V
- A. suum: 0 sbRNAs
- B. malayi: 0 sbRNAs
- T. spiralis: 0 sbRNAs

(9) [see Fig. 2]

B

Hc41 (83nt)
- Mh6 (72nt)
- Mi4 (92nt)

Pp11 (79nt)

C

% of nuclei replicating in vitro

none hU2 hY1 CaN133 CaN135 Hc41 Pp11 Mi6 Mi4

Figure S1
Table S1. Durations of S phase and mitosis in AB and P₁ blastomeres in wild-type and MO-loaded *C. elegans* embryos. Values are mean duration (in seconds) ± SEM. The number of embryos (n) analysed for each condition is given in brackets. *These values are significantly different from the coMO-loaded and WT embryos (P < 0.05, Student's t-tests).
### Durations of S phase and mitosis in C. elegans WT and MO-loaded embryos.

| Treatment       | S phase | mitosis  | S phase | mitosis  | asynchrony |
|-----------------|---------|----------|---------|----------|------------|
| None (WT)       | 603.2 ± 14.0 (12) | 174.6 ± 7.3 (13) | 758.8 ± 19.8 (12) | 168.2 ± 5.9 (15) | 149.4 ± 6.8 (15) |
| coMO            | 634.6 ± 17.0 (7)  | 154.4 ± 14.4 (12) | 789.0 ± 22.8 (7)  | 161.0 ± 6.1 (12) | 148.1 ± 9.3 (12) |
| CeN77 MO        | 780.2 ± 31.4 (5)* | 182.7 ± 21.9 (7)  | 1073.2 ± 47.8 (5)* | 186.0 ± 14.2 (6) | 450.7 ± 43.8 (9)* |
| MOs combined    | 754.5 ± 43.4 (7)* | 188.5 ± 9.7 (11)  | 970.0 ± 50.8 (7)* | 184.3 ± 12.8 (10)| 362.5 ± 26.2 (11)* |
**Non-coding stem-bulge RNAs are required for cell proliferation and embryonic development in *C. elegans***

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**Supplementary Table S2.**

Nucleotide sequences of nematode sbRNAs. Mutated nucleotides are underlined.

| Name  | Length | Sequence |
|-------|--------|----------|
| CeY   | 109 nt | GGGCCUCGGUCCGAGUUACAUUGUCUCCAAUUGUGUGUGUGUGUUUUCUUUAGGAACCUCGGUUCUCCAAUUGUGACCUUGCAGGCUCCUUUU |
| Ce1   | 81 nt  | CCGACCACCCGUGCAGUUCUUGGCCGCUUUAACAAUUGUGAUAUCCCAUCUCCACCAGACCUCC |
| Ce2   | 85 nt  | GUGGCCGUGCUGCGGCUUCAAUGUGAUCUUCUGCUUACCCAGACCUUCC |
| Ce3   | 155 nt | CACUUCGGUCCGAGUAGGUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| Ce4   | 120 nt | CAGUUCGGUCCGAGUAGGUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| Ce5   | 121 nt | CAGUUCGGUCCGAGUAGGUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| Ce6   | 83 nt  | GUAUCGGUCCGAGUAGGUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| Ce7   | 98 nt  | GUUCAAAAUAUAUAUAUCGGUCCGCGCAUAUGGUUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| CeN71 | 78 nt  | CGAAUUCUCUGCCGUAUCGGGUUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| CeN72 | 101 nt | AUCAUCGGUCCGAGUAGGUAUCUCAAAUUGCUUACUCCACUUAACAAUUGGCUUCAAACCUU |
| CeN73-1 | 136 nt | CACAUCCGUAAGCAGGAGACCGCATCUCAGGACGACCUCGUGUCC |
| CeN73-2 | 136 nt | CACAUCCGUAAGCAGGAGACCGCATCUCAGGACGACCUCGUGUCC |

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CeN74-1 (83 nt):
GUCUCGGUCCGGUCAGUUGGGUUAUCGUAUUCUCUCCUUGGGAUUACCUCACCUUACGCAUCA
ACUUGACCGUGCCGUUUU

CeN74-2 (81 nt):
GUACUCGGUCCGGUCAGUUGGGUUAUCGUAUUCUCUCCUUGGGAUUACCUCACCUUACGCAUCA
ACUUGACCGUGCCGUUUU

CeN75 (71 nt):
AAUACCGUCCGGAGUCCGUGGUGGUAUCUGAGAAAGCCCCCAUCGACACCAACUUGACCGGAUAAA
AUUUU

CeN76 (80 nt):
CAGACAGGCGUCCGGAGUCCGUGGUGUACCUUUAGAAACCUCACCCUCACCCUCACCGACCAACUU
UUGACCGGUUCCGUUUU

CeN77 (70 nt):
CAAUUCGGUCCGGAGUCCGUGGUAUCUUUUCACUAACCCACCCACAUUGACAAACUUACUUGACCGGCUU
UUU

CeN133 (100 nt):
CCCCAUCCGUCGAAGUUGAUGGGUUACCCAUUGAAUCUCUGUUUCUUUUAAGGAACUCACCAU
CCCCAUACUAUCACUUUGACGAAAAACUUUUU

CeN133 mU US (100 nt):
CCCCAUCCGUCGAAGUUGAUGGGUUACCCAUUGAAUCUCUGUUUCUUUUAAGGAACUCACCAU
CCCCAUACUAUCACUUUGACGAAAAACUUUUU

CeN133 mt Loop (100 nt):
CCCCAUCCGUCGAAGUUGAUGGGUUACCCAUUGAAUCUCUGUUUCUUUUAAGGAACUCACCAU
CCCCAUACUAUCACUUUGACGAAAAACUUUUU

CeN135 (74 nt):
AUCAGGCAUAGGUGCCGAGUCGGGUGGUAUCUGAGAAACCACCCACCGGCAAAACUUACUUGACU
UCUGUUUUU

CeN135 mt US (74 nt):
AUCAGGCAUAGGUGCCGAGUCGGGUGGUAUCUGAGAAACCACCCACCGGCAAAACUUACUUGACU
UCUGUUUUU

CeN135 mt Loop (74 nt):
AUCAGGCAUAGGUGCCGAGUCGGGUGGUAUCUGAGAAACCACCCACCGGCAAAACUUACUUGACU
UCUGUUUUU

Hc41 (83 nt):
CAUGUUGGUCGGCGGUGGUAAGGGUUAUCAAUUCUGCGUCCCCUGGGAUUACCUCACCUUACGCGA
CUUUGACCUACUAUUUU

Pp11 (79 nt):
AUGCCAAAGGUCGGAAGGUCGUGGGUUAUCGCGGACACUAGGUAUCCAGCCACAGGCACCCACCGGCUA
UCUGCCUUUGUUGGUUU

Mh6 (72 nt):
GCGAAAGGUCGGAAGGUGGUGGUAUCUAGUACUGCAAUUACGAAAUUGGUUUAAACUAACCUUGACCCCA
CUUUGACGAAACCUUUGGUUU

Mi4 (92 nt):
GCAAAAGGUCGGAAGGUGGUGGUAUCUAGUACUGCAAUUACGAAAUUGGUUUAAACUAACCUUGACCCCA
CUUUGACGAAACCUUUGGUUU
**Supplementary Table S3.**
Nucleotide sequences of DNA oligonucleotides used as primers for qRT-PCR.

| Primer | Forward Sequence | Reverse Sequence |
|--------|------------------|------------------|
| CeY fwd | CCGAGTTTCATGGTCTCCAA | GTCAAGATGAGGTTGGAACC |
| Ce1 fwd | GTCCGGAGTTTGGGTGAC | GTCTATGGGGGGATTCAAT |
| Ce2 fwd | GCCGTCGCGTCTGCGTTT | GGGAGATGGATTCAAGAAAT |
| Ce3 fwd | CTTCGGTGCCGAGATATTG | TGGGAAGGGTGGTTGTATTC |
| Ce4 fwd | CAGTTCCGGTCCGAGTTGAT | AAGCATTAATACCACTGTG |
| Ce5 fwd | CAGTTCCGGTCCGAGTTGAT | TGAACCATTGTTAAGCATT |
| Ce6 fwd | GTATCGGTCCGGCAGCAGT | AATGTAACGTCAAGTTGAT |
| CeN71 fwd | TATTATATATCGGTCCGGC | GAAGTGGAATAGGAATG |
| CeN72 fwd | CGAATTCCTGCGGTCCCGA | TCGTATGGGCTGTTGAGAA |
| CeN74-1 fwd | GGCGTCAGTGGGTTACCA | AAGGGGTTGTATTGTAG |
| CeN74-2 fwd | AGGTGGCTGCCAATCCGTC | AAGGGTGTATTGTAG |
| CeN74-3 fwd | GCCGTCAGTGGGTTATCGTA | TCAAGTTGATGCCGATGGGA |
| CeN75 fwd | CAATACGGTCCGAGCTCGGT | GTCAGTGGGCTTCTCAG |
| CeN76 fwd | CAGACAGCGCTGGTCCCGGA | CAAGTTGATGCCGATGGGA |
| CeN77 fwd | CAAATCCGGTCCGAGTCAAT | GTCAGTGGGCTTCTCAG |
| CeN133 fwd | TCCGAAGTTGATGGGTTACC | AAAGAACAGAGAGAATTCAA |
| CeN135 fwd | ATCAAGGCGATGGGTTACC | GATGGGCTTCTCAG |
**Supplementary Table S4.**

Nucleotide sequences of DNA oligonucleotides used as primers for template synthesis by PCR. The first two primers for each template are in the forward orientation (fwd1, fwd 2) and the third is in the reverse orientation (rev). Sequences for the SP6 promoter are underlined.

CeY:
- ATTTAGGTGACACTATAAGGCGTCCGAGTT (34 nt);
- GGGCTCGGTCAGGTTCTATAGTGCTCAATGGTGAATGCTCTCTAGAATTCAATTTTTAAGGACCGCTCTGAGGT (87 nt);
- AGGAGCGGTCAAAGTAGTTTCCAGGGATGAGATGAGGT (37 nt)

CeN71:
- ATTTAGGTGACACTATAGCGAATTCCTGCGGTCC (34 nt);
- CGAATTCCTGCGGTCCGGATCGTATGGGTTATCAATTCTCAACCACCCCATACGAATGTA (59 nt);
- AAAATCCGGTAGTCAATGGATTCTGGATATGAGGGTTGTT (37 nt)

CeN72:
- ATTTAGGTGACACTATAAGATCATCGGTCCGGTGTT (34 nt);
- ATCATCGGTCCGGTGTTGATGGGTTATTATCGTGTGCTGATCGACATCCACCAGTCC (82 nt);
- AAAACATCGGTCAAAGTTGAGATGAGATGAGGT (37 nt)

CeN73-1:
- ATTTAGGTGACACTATATAGCAATTCGTCGCTCC (34 nt);
- CACATCGGTCCGGAGTATGTTGATGTTCAATAGGAGGGGTTGTT (36 nt)

CeN74-2
- ATTTAGGTGACACTATAAGATCATCGGTCCGGCGTC (34 nt);
- GTATCGGTCCGGTGTTGATGGGTTATTCATTCTGCCTCCAGGAGGCGTTGCTCC (62 nt);
- AAAAGCAGTGCAAGTTGAGATGAGATGAGGTTG (37 nt)

CeN76
- ATTTAGGTGACACTATAGCAATTCGTCGCTCC (34 nt);
- CAGACAGCGTGGTTGCTCCAGGTGGTATCCTCTTTGGAAGCCCCTCCGTCGCAAC (61 nt);
- AAAACAGGAAACGTCGCAAGTTGAGATGAGATGAG (37 nt)

CeN77
- ATTTAGGTGACACTATAAGCAATTCCGTCGCTCC (35 nt);
- CATTCCGGTGCCATATGTTTATCTTTCTTTAAGGAAAAACCGCTATAG (50 nt);
- AAAACGCGCCTGCAATATGAGGGGG (36 nt)

CeN133:
- ATTTAGGTGACACTATAGCAATTCGTCGCTCCAAG (34 nt);
- CCCCATCGGTCCGAATGTAGGGTACATCCTCTCTCTCTTTAAGGAATCCACCATCCC (81 nt);
- AAAATGGTTTTCGATCGAGTTGGAGTGGGAG (37 nt)

CeN133 mt US
- ATTTAGGTGACACTATAAGGCGTCCGAGAG (34 nt);
- CCCCATCGGTCCGAATGTAGGGTACATCCTCTCTCTCTCTTTAAGGAATCCACCATCCC (81 nt);
- AAAATGGTTTTCGATCGAGTTGGAGTGGGAG (37 nt)
CeN133 mt Loop:
ATTTAGGTGACACTATAGCCCATCGGTCCGAAG (34 nt);
CCCCATCGGTCCGAAGTTGATGGGCGCAAAATTGAATTCTCTCTTTTAAAGAACTACCCATCCC
ATCAACATCAAC (81 nt);
AAAAATGTTTTTCGATCAAGTTGATGGGATGGGAT (37 nt)

CeN135:
ATTTAGGTGACACTATAGATCAGGCATAGGTCCG (34 nt);
ATCAGGCATAGGTCCGGAGTCGGTGGGTATCTGAAGAAACCACCAACGAAGCAACT (55 nt);
AAAAACAGGAAGTCAAGTTGTTGCTCTGGGATTCGTTG (32 nt)

CeN135 mt US:
ATTTAGGTGACACTATAGATCAGGCATAGGTCCG (34 nt);
ATCAGGCATAGGTCCGGAGTTTCGTTGTTATCTGAAGAAACCACCAACGAAGCAACT (60 nt);
AAAAACAGGAAGTCAAGTTGTTGCTCTGGGATTCGTTG (37 nt)

CeN135 mt Loop:
ATTTAGGTGACACTATAGATCAGGCATAGGTCCG (34 nt);
ATCAGGCATAGGTCCGGAGTCGGTGGGCGCAATGAAGAAACCACCAACGAAGCAACT (60 nt);
AAAAACAGGAAGTCAAGTTGTTGCTCTGGGATTCGTTG (37 nt)

Hc41:
ATTTAGGTGACACTATAGCATGTGGTCCGGCGTT (34 nt);
CATGTGGTCCGGCGTTGAAGGGTTATCAATTACTGCGTCCCTCTGGGAATTCCCTCAACAC (64
nt);
AAAATAGAGCTGGTCAAGGAGGTGGTTGAGGGAATTC (37 nt)

Pp11:
ATTTAGGTGACACTATAGATGCCAAGGTCCGAAG (34 nt);
ATGCCAAGGTCCGAAGGCTGTGGGTATCGCCACTATGAAGTCCCACAGTCACCACCCT (60 nt);
TACAAACAAAAGGCAGAATGCAAGGTGGTTGCTACTGCGG (37 nt)

Mh6:
ATTTAGGTGACACTATAGGCCAAAGGTCCGGAGT (34 nt);
GGCGAAAGGTCCGGAGTGATTGGGTATCCAAGATTTCCAATTTCAATATACAAATGCT (53 nt);
AAAAGCGAAGGTCAATTAATTGTAATTTGGACTT (37 nt)

Mi4:
ATTTAGGTGACACTATAGGCAGAAAGGTCCGGAGT (34 nt);
GCAGAAAGGTCCGGATGTGGGTATCATGTCAATAAGCAATTTGTTTTAATCTAAAGGCCCAT
GCA (73 nt);
AAAAATCAGTGGTCAGATTCTGGGCTCTTTTTA (37 nt)
**Supplementary Table S5.**

Nucleotide sequences of DNA oligonucleotides used directly as templates for RNA synthesis by *in vitro* transcription. The first oligonucleotide for each template is in the forward orientation (fwd1) and the second is in the reverse orientation (rev). Each oligonucleotide pair was annealed to form a double-stranded DNA template for *in vitro* transcription. Sequences for the SP6 promoter are underlined.

| Template | Forward Oligonucleotide | Reverse Oligonucleotide |
|----------|-------------------------|-------------------------|
| CeN133 US | GATTTAGGTGACACTATAGTTGATGGGTTCTTCCCATCAACTT; | AAGTTGATGGAAGAACCATCAACTATAGTGTCACCTAAAATC |
| CeN133 USLM | GATTTAGGTGACACTATAGTTGATGGGTTACCCCCATCAACTT; | AAGTTGATGGAAGAACCATCAACTATAGTGTCACCTAAAATC |
| CeN135 US | GATTTAGGTGACACTATAGTCGGTGGTTCTTCCCATCGGCTT; | AAGCCGATGGAAGAACCACCAGACTATAGTGTCACCTAAAATC |
| CeN135 USLM | GATTTAGGTGACACTATAGTCGGTGGTTATCCCCATCGGCTT; | AAGCCGATGGAAGAACCACCAGACTATAGTGTCACCTAAAATC |
**Supplementary Table S6.**  
Nucleotide sequences of antisense morpholino oligonucleotides (MOs).

|        | Sequence                             |
|--------|--------------------------------------|
| coMO (standard control MO) | CCTCTTACCTCAGTTACAATTTATA |
| CeN71 MO     | GGTGGTTGAGAATTGATAACCCATA          |
| CeN72 MO     | ACGGTGAATGTGATCAGCGACGACA          |
| CeN74-2 MO   | AGAAATTCCCGAAGGGAGGCAAACCT         |
| CeN77 MO     | AACGCCTCGTCAAGTTGTTGTCATG          |
| CeN133 MO    | ATGTGTAGGGATGGGTAGTTCTTT           |
| CeN135 MO    | GTGGTTTCTTCAGATAACCCACCGA          |