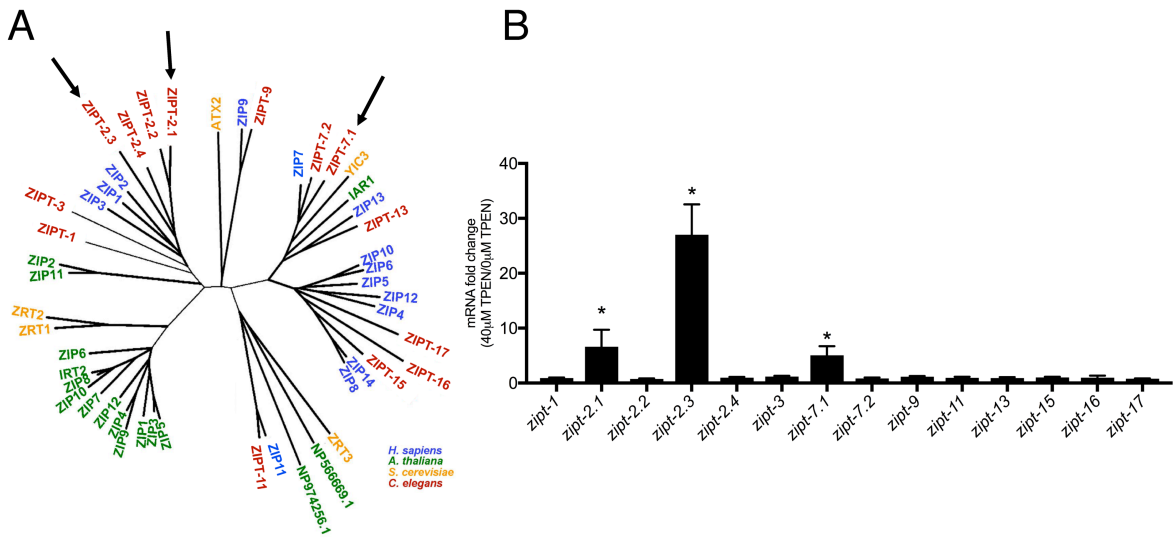
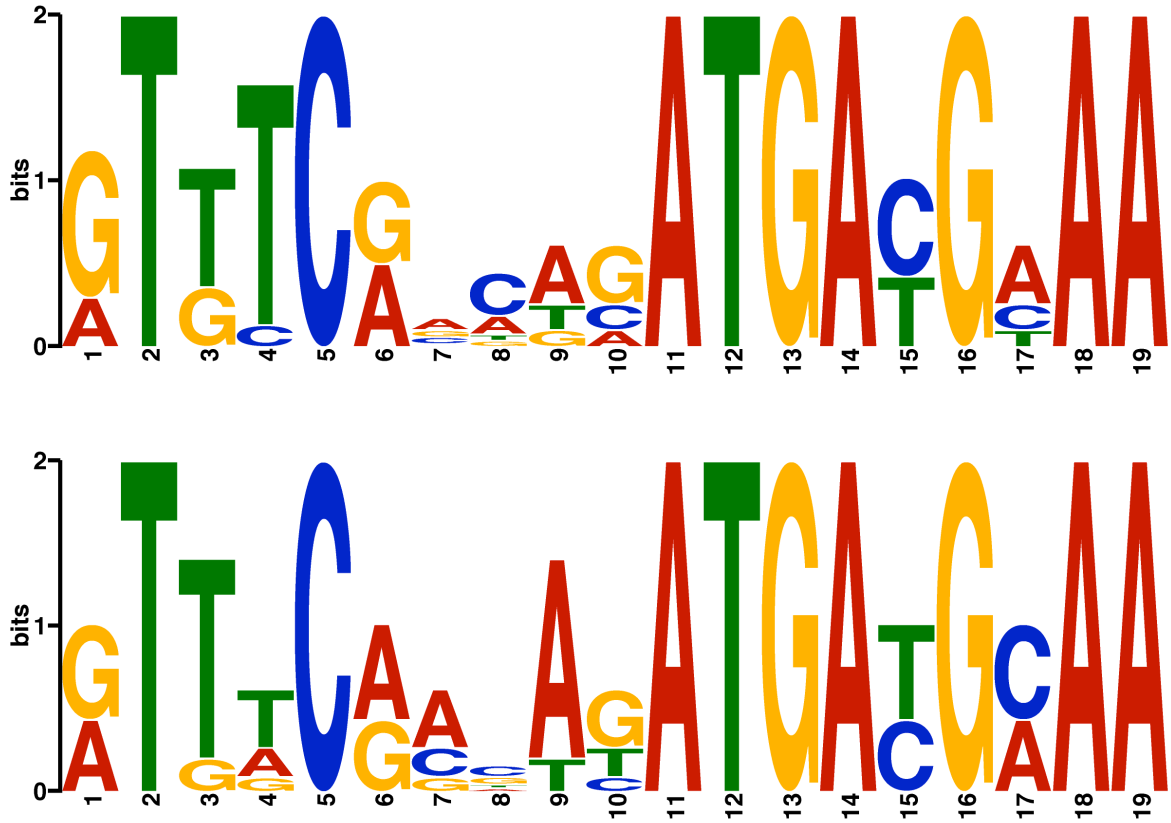
SUPPLEMENTAL TABLES AND FIGURES



Supplemental Figure 1. Phylogenetic tree of ZIP family members, and transcriptional regulation of *C. elegans zipt* genes by zinc deficient conditions. (A) A dendrogram showing 14 predicted *C. elegans* ZIP family members (red) identified by PSI-BLAST and all predicted ZIP proteins from the mammal *Homo sapiens* (blue), the plant *Arabidopsis thaliana* (green), and the yeast *Saccharomyces cerevisiae* (yellow). ZIPT-2.1, ZIPT-2.3 and ZIPT-7.1 are indicated by arrows. Notably, all fourteen human ZIP proteins cluster with highly related *C. elegans* proteins. For genes that encode multiple protein isoforms, only one isoform is listed. (B) A population of mixed-stage, wild-type animals were cultured with 0 or 40 μM TPEN for 16 hours. RNA levels of all 14 *zipt* genes were analyzed by qRT-PCR. Values are the ratio of mRNA levels at 40 μM TPEN/ 0 μM TPEN, an indication of transcriptional activation, and are the average of 3 biological replicates; error bars display standard deviation. The *zipt-2.1*, *zipt-2.3* and *zipt-7.1* genes displayed significantly increased mRNA levels at 40 μM TPEN (* P<0.05).

Species	Gene	Upstream	Downstream
C. elegans	*zipt-2.1*	GAACTTTCGATGATCTGAAA	CAAAATTTGAGCCGGTTACC
C. brenneri	*zipt-2.1*	GATCTGTAAGAGTGAAAAGA	CAATGGTTTTTTCAGTTTTC
C. briggsae	*zipt-2.1*	TATGATGATCTGAAAAGTAT	CCTTTTGCCGAGCCGCCTCC
C. japonica	*zipt-2.1*	GTGGTTAGCTCCGAAACAAA	ATGAAAAAGAGTGTAGAGAG
C. elegans	*zipt-2.3*	TTAAACACCGAAACACGACA	CAGGCAAAAACCTATAAAAC
C. brenneri	*zipt-2.3*	AAAATAACTCGAAGAATTCA	CAGTCATTTGGTCGGTTGGT
C. briggsae	*zipt-2.3*	TCGAAAAACCCTGCTTATTG	CAGTCGTTTGGTCAACCTAT
C. japonica	*zipt-2.3*	TCCATAAACTCATAAAGCTA	CCGTCGCGGTCAAACCTATA
C. elegans	*zipt-7.1*	GGTGTTATAACACGTCAGCG	TTGGCGTTTTTCCTTGGAGA
C. brenneri	*zipt-7.1*	GAGGCGCTATTACACGTCA	TGGCGAGAAAGAACAAATAG
C. briggsae	*zipt-7.1*	GGGTGTTATAACACGTCAGT	TGGCGCAGAGGAGAGAACAA
C. japonica	*zipt-7.1*	ACACGTCATCCAACTCTTTC	TTGGCGTGAACAAACACCTT

Supplemental Figure 2. Sequences flanking LZA elements did not display evolutionary conservation. Based on the LZA elements identified by MEME (Figure 1D), we aligned the sequences twenty base pairs upstream and twenty base pairs downstream of LZA elements in the promoter regions of *zipt-2.1*, *zipt-2.3*, and *zipt-7.1* in the *Caenorhabditis* species *elegans*, *brenneri*, *briggsae*, and *japonica*. Identical residues are highlighted in black. Only 5 of these 120 positions are conserved in all four species (4%), whereas Figure 1D shows that in the 19 base pair LZA elements 42 of the 57 positions are conserved in all four species (74%).



Supplemental Figure 3. The LZA weight matrix computed using different sequences. (Top) MEME was used to compute a weight matrix using the 12 LZA elements shown in Figure 1D (this matrix is identical to Figure 1E). Three of these genes were experimentally demonstrated to be induced by zinc deficiency (*C. elegans zipt-2.1*, *zipt-2.3*, and *zipt-7.1*), whereas nine of these genes were not analyzed experimentally but were only analyzed using bioinformatics. (Bottom) MEME was used to compute a weight matrix using the seven LZA elements shown in Figure 4B. All seven of these genes were experimentally demonstrated to be induced by zinc deficiency. The two weight matrices are highly similar but not identical. The height of each nucleotide represents the frequency scaled in bits.

Rank	Gene	Strand	Start	End	p-value	q-value	Matched Sequence
1	*zipt-7.1*	+	233	252	2.23E-10	0.0153	GTTTCGCCAGATGATGCAAT
2	*6R55.2*	-	555	574	1.42E-09	0.0296	ATGTCGACAGATGACGCAAT
3	*H11L12.1*	+	625	644	1.42E-09	0.0296	ATGTCGACAGATGACGCAAT
4	*T16G12.1*	-	558	577	2.16E-09	0.0296	ATGTCGACAGATGATGCAAT

5	*T16G12.3*	+	1747	1766	2.16E-09	0.0296	ATGTCGACAGATGATGCAAT
6	*D2024.10*	-	2882	2901	3.28E-09	0.0375	ATTTCACAAGATGATGAAAC
7	*F45E4.6*	-	187	206	1.05E-08	0.0905	GTTTCGTCAGATGATGCAAA
8	*plp-1*	+	1058	1077	1.05E-08	0.0905	GTTTCGTCAGATGATGCAAA
9	*zipt-2.3*	+	2167	2186	1.97E-08	0.137	ATGTCAATTCATGACGAAAC
10	*srw-7*	-	182	201	2.20E-08	0.137	GTTACAACATATGACGCAAT
11	*R08F11.4*	+	556	575	2.20E-08	0.137	GTTACAACATATGACGCAAT
12	*Y54G2A.21*	+	1506	1525	2.76E-08	0.143	GTGTCGTCATATGATGCAAT
13	*EGAP1.1*	+	168	187	3.13E-08	0.143	GTTACAGAAAATGATGAAAC
14	*ubq-1*	-	1944	1963	3.13E-08	0.143	GTTACAGAAAATGATGAAAC
15	*C33B4.4*	+	2261	2280	3.58E-08	0.154	ATGTCGGGTAATGACGAAAC
16	*C06G3.3*	-	2378	2397	5.36E-08	0.193	GTTTCGACTGATGATGAATT
17	*srm-2*	-	757	776	5.65E-08	0.193	ATTTCAGAAGATGACACAAT
18	*rme-1*	-	1130	1149	5.88E-08	0.193	ATTCCAGAACATGATGAAAT
19	*mdt-22*	+	1456	1475	6.13E-08	0.193	ATTACGGTAGATGATGTAAC
20	*Y59C2A.1*	-	2631	2650	6.18E-08	0.193	ATTTCAAAAAATGATAAAAC
21	*egl-4*	-	1971	1990	8.68E-08	0.243	ATGTCAGAAAATGACGAAAA
22	*F54C9.11*	-	287	306	8.91E-08	0.243	ATTTCAAGTGATGATATAAT
23	*dkf-2*	-	566	585	9.20E-08	0.243	ATTTCGGAAAATGATGCAAA
24	*gyg-1*	+	788	807	9.20E-08	0.243	ATTTCGGAAAATGATGCAAA
25	*ins-5*	+	922	941	1.04E-07	0.25	GTTTCAACTATTGATAAAAT
26	*F44E7.5*	-	439	458	1.16E-07	0.25	GTTGCGAGAGATGATGCAAT
27	*T22F3.10*	-	1936	1955	1.47E-07	0.25	ATTTCAACAATTGATAAAAC
28	*spe-39*	+	82	101	1.48E-07	0.25	ATTCCAAATGATGATAAAAC
29	*spn-4*	-	488	507	1.48E-07	0.25	ATTCCAAATGATGATAAAAC
30	*klp-4*	-	2323	2342	1.55E-07	0.25	GTTTCAGGAGATGAGGTAAC
31	*F36D1.5*	+	1806	1825	1.57E-07	0.25	ATTTCATTTGTTGATGAAAT
32	*F40F8.5*	-	1972	1991	1.71E-07	0.25	ATTTCGTGGAATGATGTAAT
33	*sru-42*	-	673	692	1.77E-07	0.25	ATTTCAAATAATGACATAAT
34	*H28G03.2*	-	1115	1134	1.80E-07	0.25	ATTTCAAAAAATGACAAAAA
35	*hot-1*	-	742	761	1.82E-07	0.25	ATGCCGAAAAATGACGAAAA
36	*T07A9.12*	-	1315	1334	1.82E-07	0.25	ATGCCGAAAAATGACGAAAA
37	*pqn-98*	-	1886	1905	1.84E-07	0.25	ATTACGACAGTTGACAAAAT

38	*Y73F8A.24*	-	741	760	1.91E-07	0.25	GTGTCACTTTTTGATGAAAT
39	*sre-50*	+	1053	1072	1.99E-07	0.25	ATTTCAATTATTGACGTAAT
40	*C50E10.1*	-	72	91	1.99E-07	0.25	ATTTCAATTATTGACGTAAT
41	*nhr-249*	+	1265	1284	2.03E-07	0.25	GTTTCGTCAATTGATGTAAA
42	*kin-33*	-	107	126	2.04E-07	0.25	ATGACGAAAAATGACGAAAA
43	*sdz-21*	+	1798	1817	2.14E-07	0.254	GTGTCAAGTGATGATGAATC
44	*M05D6.6*	+	45	64	2.18E-07	0.254	ATTTCATTATATGACGAAAA
45	*gbh-2*	-	2610	2629	2.18E-07	0.254	ATTTCATTATATGACGAAAA
46	*trp-4*	-	355	374	2.22E-07	0.254	GTTTCGTTTGTTGACAAAAT
47	*B0365.1*	+	605	624	2.51E-07	0.281	GTGTCAAAGGATGACCAAAT
48	*lin-14*	-	2886	2905	2.55E-07	0.281	GTGTCAAAAGATGAAGAAAA
49	*fbxa-21*	+	2620	2639	2.58E-07	0.281	ATGTCAAAAATTGACGAAAA
50	*srv-11*	+	1144	1163	2.68E-07	0.283	GTTTGAAAATATGACGAAAC

Supplemental Table 1. Genes identified by searching for LZA elements in *C. elegans* promoters. The Rank column is the ranking of each gene based on similarity to the LZA weight matrix. The Gene column is the Wormbase ID or gene name. *zipt-7.1* and *zipt-2.3* are shown in red. The strand column indicates orientation of the LZA element relative to the transcribed strand. The Start and End columns define the location of the LZA element in base pairs from the ATG start codon. The p-value is defined as the probability of a random sequence of the same length as the motif matching that position of the sequence with as good or better a score. The q-value is defined as the false discovery rate if the occurrence is accepted as significant. The Matched Sequence is the LZA element nucleotide sequence. The list is sorted by p-value [32].

Primer	Sequence
ama-1 Forward	ATCGGAGCAGCCAGGAACTT
ama-1 Reverse	GACTGTATGATGGTGAAGCTGG
mCherry Forward	CCGCAAAACTAAAGGTAACTAAAG
mCherry Reverse	TCTGGTATATCTGCCGGATG
zipt-1 Forward	TCTCTGTTCATGCTCTGTTCG
zipt-1 Reverse	GAACTAGACGAACTCCAAGGC
zipt-2.1 Forward	GATCAAAATGACAGCGGATGG
zipt-2.1 Reverse	ATGGGTAACCGGAATTCTACG
zipt-2.2 Forward	GTTGGCTTGCAGATTTCTATGG
zipt-2.2 Reverse	CCATGAGACTGTAGATGAGGATG
zipt-2.3 Forward	ATATGAATCAGACGCCGAAGG
zipt-2.3 Reverse	TGAAGGATGTTGGAGTGGTGG
zipt-2.4 Forward	TCATGAAACTACACCTCCATTGG
zipt-2.4 Reverse	TGTTTTCGTCAGTGCAGTCG
zipt-3 Forward	ATTCAGATGACAGCGGAGAAG
zipt-3 Reverse	ATTACAAATATTCCGATTCCAGCG
zipt-7.1 Forward	AGCTGGAAATACTCTTGGATGG
zipt-7.1 Reverse	CAGTAACGGCTTGCAAACG
zipt-7.2 Forward	CATGAGGATCACGGACACAG
zipt-7.2 Reverse	GCAGAGATGAGAAGTGTAGCC
zipt-9 Forward	TTAGTCAGTATATTCGGCGCTG

zipt-9 Reverse	GTCGTGATGGTTGTCTTGATG
zipt-11 Forward	TCTTGATGTTGCAGCTCTGG
zipt-11 Reverse	CAAAAGAATCCGCCTCCATG
zipt-13 Forward	GCGTGTGCCTATTTGAACTTG
zipt-13 Reverse	TCGTGAGGAATTTCGTGGAG
zipt-20 Forward	GTCGGAATTGGAGAACTCGG
zipt-20 Reverse	AAGTGAGACGTTTAGGGCTTC
zipt-21 Forward	ACTACACAGAAGCCGTCAAC
zipt-21 Reverse	ACTACATCCCGAGACAATTGC
zipt-22 Forward	TTGTATGTTGCCTGGGTGG
zipt-22 Reverse	TGCTCAAACCATCCTAATCCG
GFP Forward	GGAACTACAAGACACGTGCTG
GFP Reverse	GTTGTATTCCAATTTGTGTCC
F44E7.5 Forward	GTCCAAAGCAAGAAGTCAAGTG
F44E7.5 Reverse	TGAGCCGTTTGTAGAGTTTAGAG
R08F11.4 Forward	AGTCATCCTGTTTGGGTCTTG
R08F11.4 Reverse	GCTAGATCCATCAACGTCGG
srw-7 Forward	GCTGCATTTCATTAGTGGTCAG
srw-7 Reverse	AGCCATCACTACAACCAATTTTG
D2024.10 Forward	GAGGGACCTTTGGAAACAAGA
D2024.10 Reverse	AATTGTTCCGTTGTGCAGC
HSPCB Forward	TCTGGGTATCGGAAAGCAAGCC
HSPCB Reverse	GTGCACTTCCTCAGGCATCTTG
hZIP2 Forward	TCATGGTGCAGAACAGATCAG
hZIP2 Reverse	AATGCCAGCGACTCCAAA
hZIP13 Forward	TCATGGTGCAGAACAGATCAG
hZIP13 Reverse	AATGCCAGCGACTCCAAA

Supplemental Table 2. Primers used for qRT-PCR reactions described in the Materials and Methods. Each primer is listed in the 5' to 3' direction.

SUPPLEMENTAL METHODS

Phylogenetic analysis

To analyze evolutionary conservation, we used *C. elegans* ZIPT proteins to search ORFs of *S. cerevisiae*, *A. thaliana* and humans using PSI-BLAST. The orthologs identified by this search were analyzed by reciprocal PSI-BLAST; each was used to search *C. elegans* ORFs to determine the most similar proteins. Multiple sequence alignment of ZIPT proteins was carried out using ClustalW, and the resulting alignment was used to generate a phylogenetic tree using MEGA (1).

1. Tamura,K., Dudley,J., Nei,M. and Kumar,S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.*, **24**, 1596–1599.