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Genomic Characterization of the Zinc Transcriptional Regulatory Element Reveals Potential Functional Roles of ZNF658

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Abstract

The zinc transcriptional regulatory element (ZTRE) is a newly reported binding motif for human zinc finger protein ZNF658, which alters gene expression in response to cellular zinc. The ZTRE has two nucleotide components—the palindromic flanking pairs and the bridging "N" bases between these flanks that range in number from 0 to 100. There are 12 pairs of ZTRE flanks (designated A-L). Three thousand five hundred twenty-five genes contain one or more ZTREs − 1000 to + 200 bp from their transcriptional start site (TSS). ZTRE-E is observed at a greater frequency, and ZTRE containing 25 bridging bases are less frequent, within − 200 bp from the TSS. The genes with ZTREs in this range are enriched in processes that may compensate zinc deficiency, while other genes with ZTREs outside this range are enriched in transcriptional activation processes. The division of ZTREs into two groups may imply a dual role of ZNF658, similar to the homologous yeast protein Zap1, via binding to low or high affinity sequences dependent upon cellular zinc. The KLF/Sp1-family binding motif is prevalent within the ZTRE "N" bridging bases, suggesting ZNF658 may compete with Sp1-like transactivators to suppress transcription.

Keywords

ZNF658; Zinc transcriptional regulatory element (ZTRE); Zap1; Transcriptional regulation

Introduction

The Cys_2His_2 class of zinc (Zn) finger proteins (ZFPs) accounts for about 3% of the human genome and is the largest class of putative transcription factors (TFs). The majority of these ZFPs have unknown functions, and those that have been characterized have diverse properties—indicating a potentially vast regulatory network that is largely unstudied [1]. ZFPs are also considered ideal for the development of certain molecular medical applications, such as induced transcriptional activation or repression of target genes, or in the fusion of ZF peptides with other functional protein domains to manipulate gene products

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[2]. For certain Cys_2His_2 ZFPs, some of their ZFs act as Zn sensors, and when Zn levels reach a certain threshold relative to their Zn-binding affinity, the TF will activate and bind to DNA. In eukaryotes, these are Zap1 (*Saccharomyces cerevisiae*), Loz1 (*Schizosaccharomyces pombe*), and MTF-1 (*Homo sapiens*, conserved among metazoan species) [3]. To this list, we may also add the recently sequenced factor ZNF658, which has been shown to bind to DNA and regulate gene transcription in response to cellular Zn levels [4].

ZNF658 has a relatively large amount of ZFs (21), which suggests a high degree of Zn sensitivity. It binds to the variable-length motif known as the Zn transcriptional regulatory element (ZTRE), whose sequence is 5′-CMCDCCYN$_{0–100}$RGGHGKG-3′ (M=C or A; D=A, G, or T; Y=C or T; R=A or G; H=A, C, or T; K=T or G) [5]. The nucleotides in the 7-mer 5′- and 3′-flanks are required to be palindromic for ZNF658 binding. There are no reported criteria for the middle “N” bases, which can range in number from zero to potentially 100 [5]. This variability seen in some TF binding to palindromic motifs can be a consequence of TF dimerization [6, 7]. The Zn-dependent activation of ZNF658 binding to the ZTRE was verified in *SLC30A5* (ZnT5), *SLC30A10* (ZnT10), and *CBWD* genes [5]. Introducing ZNF658 siRNA to the transcriptome of Caco-2 cells had significant effects on the up- or downregulation of 124 genes in a microarray panel, including a large number of rRNA genes [4].

ZNF658 and Zap1 in *S. cerevisiae* are homologous proteins whose essential DNA-binding ZF domains are aligned and highly similar [4]. Zap1 binds to an 11-mer palindromic motif, the Zn-responsive element (ZRE). Zap1 is the main regulator of Zn homeostasis in *S. cerevisiae* and has low activity in Zn-adequate cells and high activity during Zn deficiency [8]. Zap1 is known to initiate a “Zn-sparing” response by sensing deficiency and repressing levels of certain Zn-binding proteins, while simultaneously increasing expression of replacement proteins that have lower Zn requirements [9]. Severely Zn-deficient cells increase levels of Zap1 via autoregulation; this increase enables the activation of target genes by allowing Zap1 to bind to lower binding affinity ZREs [10]. These lower affinity ZREs are found closer to target gene transcriptional start sites (TSSs) and are also sites of Zap1-mediated repression. This repression is thought to be a result of Zap1-blocking transcription initiation sites [11].

The two independent activation domains of Zap1, AD1 (dominant) and AD2, can sense Zn levels and initiate DNA binding and may also be relevant to our understanding of ZNF658 [12]. Zn binding to AD1 causes a conformational change in Zap1 that inhibits the ability of AD1 to activate transcription via coactivator recruitment; a similar mechanism governs AD2 [13]. There is a region of similarity at the AD1 domain between the aligned sequences of Zap1 and ZNF658 [4].

Therefore, Zap1 is a ZFP-TF that may be an evolutionary precursor to ZNF658. The dual activator/repressor mechanism of Zap1 is related to preferential binding to high and low affinity ZREs based on cellular Zn status. Additionally, ZNF658 has been shown to repress the expression of certain genes under conditions of high Zn, and silencing its expression caused a wide variety of in vitro transcriptomic changes [4]. Based on our genomic analysis

of predicted ZNF658 binding sites in the human genome, we have identified a significant difference in composition between ZTREs that occur closest to gene TSSs and those that do not, as well as the functional annotations of these gene sets. This suggests ZNF658 may provide a similar response to Zap1 in Zn deficiency, as activator or repressor via preference for lower binding affinity ZTREs that are found closer to gene TSSs.

Materials and Methods

Analyses were performed using GENCODE reference annotation for the human genome, Release 29 (GRCh38.p12), standard chromosomes [14]. All bioconductor packages were executed in R Studio (v1.1.463) [15–17]. Gene promoters considered as the region − 1000-bp upstream and + 200-bp downstream from gene transcriptional start sites (TSSs). Geneious was used to determine genomic positions of the ZTRE motif, 5′-CMCDCCYN$_{0–100}$RGGHGKG-3′ (Geneious; v10.2.3) [18]. Background ZTREs were defined as those which fit the sequence format of ZTRE but do not occur in this defined promoter range. Overlaps between ZTRE positions and gene promoters were recorded using GenomicRanges (v1.34.0) and GenomicFeatures (v1.34.1) [19]. Multiple Em for Motif Elicitation (MEME, v5.0.2) and TOMTOM *Homo sapiens* COmprehensive MOdel COllection (HOCOMOCO, v11) were accessed via MEME suite [20]; only motifs that occurred in 100 or more ZTRE “N” base sequences were reported. Ggseqlogo (v0.1) was used to plot sequence logos from MEME data [21]. BLASTp (BLOSUM62 scoring algorithm) was used to identify homologous amino acid sequences [22]. DAVID Bioinformatics Resources (v6.8) functional annotation clustering was used to identify enrichment of gene cohorts [23, 24]. Panther GO Biological Process (v13.1) Fisher’s exact testing with Bonferroni correction for multiple testing was used to identify significantly overrepresented annotated gene ontologies (α = 0.05) [25, 26].

Statistical analyses were performed using R (v3.5.0) [15]. Chi-square test was used to determine whether there is a significant association between ZTRE Flank ID and ZTRE location, based on whether ZTREs are located in the range − 200 bp to TSS versus other promoter ranges (α = 0.05); this test assumes independence between two categorical variables in the null hypothesis. Two-proportion Z-test was used to determine significant difference between ZTRE-E in the range − 200 bp to TSS versus background, and versus all promoter ZTREs (α = 0.05); and also between ZTREs containing 25 bridging bases in the range − 200 bp to TSS versus all ZTREs in the promoter range; this test assumes no significant difference between two population proportions in the null hypothesis. Wilcoxon rank-sum tests were used to compare the number of bridging bases between independent groups of ZTREs, first between ZTREs in background versus ZTREs in promoter, and then between ZTREs in the range − 200 bp to TSS versus other ZTREs in the promoter range (α = 0.05); this test assumes that the two groups come from the same distribution in the null hypothesis.

Results

Genomic Identification of ZTREs

The ZTRE sequence, 5′-CMCDCCYN$_{0–100}$RGGHGKG-3′, was used to search the human genome (M=C or A; D=A, G, or T; Y=C or T; R=A or G; H=A, C, or T; K=Tor G). There is a total of 85,436 ZTREs, and 5200 of these were found in the promoters (− 1000 to + 200 bp from TSS) of 3525 genes (Supplementary Table 1). Of these 3525 genes, 61.4% (2165) are protein coding. The mean distance from the ZTRE start site to TSS for protein coding genes is − 222-bp upstream. Based on previous reports, the maximum number of bridging bases used in this study was set to 100; 41.6% of ZTREs have 0–50 bridging bases.

Analysis of Palindromic Flanking Regions

The ZTRE consists of 12 possible pairs of 7-mer palindromic 5′- and 3′-flanking sequences. We named these ZTRE Flank ID A through L based on descending background frequency (Supplementary Table 2, Fig. 1). There is significantly more ZTRE-E, CCCGCCCN$_{0–100}$GGGCGGG, in gene promoters than background (Z = − 79.46, P < 2.2e-16), and there is also significantly more ZTRE-E in the range − 200 bp to the TSS versus all promoter ranges together (Z = 12.88, P < 2.2e-16). Overall, there is a significant association between ZTRE Flank ID and whether it is located in the range − 200 bp to the TSS versus other promoter ranges (χ^2 = 439.97, df = 11, P < 2.2e-16).

Analysis of Bridging “N” Bases

The most frequently occurring number of promoter (and background) bridging bases is 25 (Fig. 2). However, there are significantly fewer ZTREs containing 25 bridging bases in the range − 200 bp to TSS versus all ZTREs in the promoter range − 1000 to + 200 bp (Z = − 2.53, P = 0.00566). Wilcoxon rank-sum tests indicate that there are significantly more bridging bases in ZTREs found in the promoter range than in background (U = 200,660,000; Z = − 11.725, P < 2.2e-16), but significantly fewer bridging bases in ZTRE are found in the − 200 bp to TSS range than in all promoter ranges (U = 5,162,000; Z = − 1.807, P = 0.03538).

Differences in “N” Base Motifs

The “N” bridging base sequences were analyzed for recurring motifs to predict binding similarities of ZNF658 to other TFs and also to define further differences between the two ZTRE location-based cohorts (Fig. 3, Table 1). A version of the repeating GC-rich sequence (motifs 2, 4, and 8) containing the Sp1/KLF TF family binding motif occurs in all sets of ZTREs and has the highest E values. Motifs 1 and 3, which are significant in all ZTREs taken together, do not retain their significance when the ZTREs are separated into groups. ZTREs closest to TSSs exhibit significant enrichments for binding sites similar to NFY family TFs and androgen receptors (motifs 5 and 6). In the other group of ZTREs, motif 7 shares similarity with the binding sites of three relatively unstudied TFs: ALX1 (associated with autosomal-recessive frontonasal dysplasia), DUX4 (associated with facioscapulohumeral dystrophy), and ZFP28 (associated with circadian rhythm).

Functional Gene Annotation Differs with ZTRE Position

A distinction in the ontologies of the DAVID functional annotation clusters can be seen based upon which of the two location-based groups of ZTREs these genes contain in their promoter (Table 2). For genes with ZTREs in the range − 200 bp to the TSS (1112 mapped DAVID IDs), there is significant enrichment in pleckstrin homology (PH) domain-containing genes, microtubule/kinesin transport, and leucine zipper domain (bZIP) annotation clusters. For genes with ZTREs in other ranges, there is significant enrichment in RNA pol II transcription, transcription and activation processes, and PWWP domain-containing gene annotation clusters. Significantly enriched clusters in each group of genes are unenriched in the other. Nervous system development was the single enriched GO biological process observed for genes with ZTREs in the range − 200 bp to the TSS.

Homology of ZNF658 with Zap1, Sp1, and MTF-1

We compared the amino acid sequence of ZNF658 to Zap1 and MTF-1 and also to Sp1 since the Sp1/KLF binding motif recurred throughout the ZTRE “N” bases (Table 3). MTF-1 then Zap1 exhibits the highest total bit scores, indicating a high degree of similarity. (For reference, the total bit score between MTF-1 and Zap1 is 235. These larger total bit scores are due in part to the 21 repetitive ZF sequences in ZNF658.) MTF-1 ZFs 1–3 are homologous with ZFs 12–16 of ZNF658; these are the essential DNA binding domains of each TF. The AD1 domain of Zap1 is 26% identical and 35% similar to a corresponding region in ZNF658; the percent identity versus sequence length comparison for AD1 falls into the “twilight zone” of indeterminate homology [27]; however, the *E* value (0.008) indicates some statistical significance. The three ZFs of Sp1 are homologous to ZF regions of ZNF658, and when the three are queried together, they are 62% similar to a continuous region of ZNF658. ZF1 of Sp1 is 76% identical to ZF16 of ZNF658 (*E* = 2e-07). The essential DNA-binding Zn finger domains in both Sp1 and MTF-1 also act as a nuclear localization signal (NLS) [28, 29] and may have a similar role in ZNF658.

ZTREs Associated with Ogo et. al. (2015) Genes

Of the 124 genes in Caco-2 cells that exhibited fold changes in the expression of their products following the introduction of ZNF658 siRNA, and under normal Zn conditions [4], 10 have ZTREs in the range − 1000 to + 200 bp from their TSS (*CGB5, DIRC2, F2RL1, IGF2, ITPRIPL2, MBNL3, PAK2, PRPF40A, SESTD1*, and *SOX15*). *F2RL1* and *SESTD1* both contain ZTRE-E in the range − 200 bp to the TSS. There is no derivable relationship between the measured fold changes of these gene products and the characteristics of the ZTREs that occur near these genes.

Discussion

The large number of Zn fingers and thus potential Zn sensitivity of ZNF658, combined with the significant bisection in ZTRE motifs that we have shown here, suggest that this TF may have a dual regulatory function that is dependent on cellular Zn concentration. The mechanism of this function may be analogous to the Zn-activated TF in *S. cerevisiae*, Zap1. The DAVID functional annotation of gene clusters (Table 2) contain several pieces of

information which supports two (or more) distinct categories of ZTREs: namely, one for low Zn (in the range − 200 bp to TSS) and one for adequate Zn (in the other promoter locations).

During Zn deficiency, Zap1 induces *CKI1* and *EKI1* genes to maintain phospholipid synthesis [30]; this adaptation process may be analogous to the enriched set of genes that code for proteins containing Pleckstrin homology domains, which are known to bind phospholipids and are involved in their processing [31]. Zap1 also upregulates the *TSA1* gene, whose product Tsa1 is a protein chaperone that operates under conditions of Zn deficiency. Tsa1 stabilizes the accumulation of Zn-dependent apoproteins in cells, and shields them from misfolding and aggregation until Zn levels increase [30]. In human neurons, these unfolded and misfolded proteins are moved outward by anterograde transport, mediated by microtubule motor activity [32], which is also a significantly enriched functional annotation for genes with ZTREs in the range of − 200 bp to the TSS. In *Arabidopsis thaliana* and *Triticum aestivum*, Zn deficiency also leads to increased expression of bZIP (basic-leucine zipper domain) transcription factors [33]. It is not known whether there is a similar Zn-dependent effect on these TFs in humans; however, the bZIP family are well-characterized as repressors, and this further supports the idea that ZTREs closer to gene TSSs are associated with Zn deficiency and transcriptional “off” regulatory functions. Meanwhile, the top two significantly enriched DAVID annotation clusters for promoter ZTREs not in the − 200 bp to the TSS range are associated with RNA Pol II activation and transcriptional activation. These genes could represent a cascading “on” signal for when Zn levels are adequate enough to resume normal cellular protein synthesis.

The “N” bases of the ZTRE match the binding sites for several TFs related to Zn (Table 1). NF-Y transcription complex associated with motif 5 (Fig. 3), which has global transcription effects and occurs in 30% of gene promoters, is co-expressed with the ZFPs Sp2 and ZNF143 [34]. Motif 6 is similar to the binding site for androgen receptors, which are suppressed with elevated Zn levels in the context of prostate cancer [35]. A deletion in *DUX-4* is associated with facioscapulohumeral dystrophy; muscle strength of those afflicted has been shown to improve following zinc (and other) supplementation [36].

Sp1-like proteins are defined as having a high degree of sequence similarity with the Sp1 DNA-binding domain, comprised of three Kruppel-like Zn fingers. The KLF/Sp1-like proteins have similarity in this domain that ranges from 68 to 96%; ZNF658 exhibits 62% homology in this domain (Table 3). The view that Sp1 binds to all such GC-rich motifs (Table 1) may be overly simplified, and a more likely scenario is that many different TFs compete for these sites based on binding affinity that can be influenced by environmental factors, e.g., Zn levels [37]. Indeed, many KLF/Sp1-like proteins have dual activator/repressor roles under various cellular stresses, similar to the potential role of ZNF658 that we propose here.

When considering the role of Zap1, and the role of ZNF658 that we have proposed here as an initiator of cellular changes based on Zn status, it is not surprising that indirect (nonspecific) effects could account for many of the changes in expression levels that were observed in the ZNF658 knockdown gene expression panel performed by Ogo et al. [4]. *F2RL1* and *SESTD1* may be of particular interest because of the presence of ZTRE-E in the

range − 200 bp to the TSS. It is of interest going forward to design experiments that specifically target the expression of genes with different permutations of the ZTRE in their promoter (e.g., ZTRE-E versus ZTRE-A), under varied Zn concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Najafabadi HS, Mnaimneh S, Schmitges FW, Garton M, Lam KN, Yang A, Albu M, Weirauch MT, Radovani E, Kim PM, Greenblatt J, Frey BJ, Hughes TR (2015) C_2H_2 zinc finger proteins greatly expand the human regulatory lexicon. Nat Biotechnol 33:555–562. 10.1038/nbt.3128 [PubMed: 25690854]
2. Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem 79:213–231. 10.1146/annurev-biochem-010909-095056 [PubMed: 20192761]
3. Choi S, Bird AJ (2014) Zinc'ing sensibly: controlling zinc homeostasis at the transcriptional level. Metallomics 6(7):1198–1215. 10.1039/c4mt00064a [PubMed: 24722954]
4. Ogo OA, Tyson J, Cockell SJ, Howard A, Valentine RA, Ford D (2015) The zinc finger protein ZNF658 regulates the transcription of genes involved in zinc homeostasis and affects ribosome biogenesis through the zinc transcriptional regulatory element. Mol Cellular Biol 35(6):977–987. 10.1128/mcb.01298-14 [PubMed: 25582195]
5. Coneyworth LJ, Jackson KA, Tyson J, Bosomworth HJ, van der Hagen E, Hann GM, Ogo OA, Swann DC, Mathers JC, Valentine RA, Ford D (2012) Identification of the human zinc transcriptional regulatory element (ZTRE): a palindromic protein-binding DNA sequence responsible for zinc-induced transcriptional repression. J Biol Chem 287(43):36567–36581. 10.1074/jbc.M112.397000 [PubMed: 22902622]
6. Huang Y-H, Jankowski A, Cheah KSE, Prabhakar S, Jauch R (2015) SOXE transcription factors form selective dimers on noncompact DNA motifs through multifaceted interactions between dimerization and high-mobility group domains. Sci Rep 5:10398 10.1038/srep10398 [PubMed: 26013289]
7. Peirano RI, Wegner M (2000) The glial transcription factor Sox10 binds to DNA both as monomer and dimer with different functional consequences. Nucleic Acids Res 28(16):3047–3055 [PubMed: 10931919]
8. Lyons TJ, Gasch AP, Gaither LA, Botstein D, Brown PO, Eide DJ (2000) Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. P Natl A Sci 97(14):7957–7962
9. Merchant SS, Helmann JD (2012) Elemental economy: microbial strategies for optimizing growth in the face of nutrient limitation. Adv Microb Physiol 60:91–210. 10.1016/b978-0-12-398264-3.00002-4 [PubMed: 22633059]
10. Wu CY, Bird AJ, Chung LM, Newton MA, Winge DR, Eide DJ (2008) Differential control of Zap1-regulated genes in response to zinc deficiency in Saccharomyces cerevisiae. BMC Genomics 9: 370 10.1186/1471-2164-9-370 [PubMed: 18673560]
11. Bird AJ, Blankman E, Stillman DJ, Eide DJ, Winge DR (2004) The Zap1 transcriptional activator also acts as a repressor by binding downstream of the TATA box in ZRT2. EMBO J 23(5):1123–1132 [PubMed: 14976557]

12. Bird AJ, Zhao H, Luo H, Jensen LT, Srinivasan C, Evans-Galea M, Winge DR, Eide DJ (2000) A dual role for zinc fingers in both DNA binding and zinc sensing by the Zap1 transcriptional activator. EMBO J 19(14):3704–3713. 10.1093/emboj/19.14.3704 [PubMed: 10899124]
13. Frey AG, Eide DJ (2012) Zinc-responsive coactivator recruitment by the yeast Zap1 transcription factor. Microbiologyopen 1(2):105–114. 10.1002/mbo3.8 [PubMed: 22950018]
14. Frankish A, Diekhans M, Ferreira A-M, Johnson R, Jungreis I, Loveland J, Mudge JM, Sisu C, Wright J, Armstrong J, Barnes I, Berry A, Bignell A, Carbonell Sala S, Chrast J, Cunningham F, Di Domenico T, Donaldson S, Fiddes IT, García Girón C, Gonzalez JM, Grego T, Hardy M, Hourlier T, Hunt T, Izuogu OG, Lagarde J, Martin FJ, Martínez L, Mohanan S, Muir P, Navarro FCP, Parker A, Pei B, Pozo F, Ruffier M, Schmitt BM, Stapleton E, Suner M-M, Sycheva I, Uszczynska-Ratajczak B, Xu J, Yates A, Zerbino D, Zhang Y, Aken B, Choudhary JS, Gerstein M, Guigó R, Hubbard TJP, Kellis M, Paten B, Reymond A, Tress ML, Flicek P (2018) GENCODE reference annotation for the human and mouse genomes. Nucleic Acids Res:gky955-gky955. 10.1093/nar/gky955
15. R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna URL https://www.R-project.org/
16. Team R (2016) RStudio: Integrated Development for R. RStudio, Inc Boston, MA URL http://www.rstudio.com/
17. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, Irizarry RA, Lawrence M, Love MI, MacDonald J, Obenchain V, Oleś AK, Pagès H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, Morgan M (2015) Orchestrating high-throughput genomic analysis with bioconductor. Nat Methods 12: 115–121. 10.1038/nmeth.3252 [PubMed: 25633503]
18. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649. 10.1093/bioinformatics/bts199 [PubMed: 22543367]
19. Lawrence MHW, Pagès H, Aboyoun P, Carlson M, Gentleman R, Morgan M, Carey V (2013) Software for computing and annotating genomic ranges. PLoS Comput Biol 9:e1003118 10.1371/journal.pcbi.1003118 [PubMed: 23950696]
20. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS (2009) MEME suite: tools for motif discovery and searching. Nucleic Acids Res 37(suppl_2):W202–W208. 10.1093/nar/gkp335 [PubMed: 19458158]
21. Wagih O (2017) ggseqlogo: a versatile R package for drawing sequence logos. Bioinformatics 33(22):3645–3647. 10.1093/bioinformatics/btx469 [PubMed: 29036507]
22. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402 [PubMed: 9254694]
23. Huang d W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4(1):44–57. 10.1038/nprot.2008.211 [PubMed: 19131956]
24. Huang d W, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37(1):1–13. 10.1093/nar/gkn923 [PubMed: 19033363]
25. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25(1):25–29. 10.1038/75556 [PubMed: 10802651]
26. The Gene Ontology Consortium (2017) Expansion of the gene ontology knowledgebase and resources. Nucleic Acids Res 45(D1):D331–d338. 10.1093/nar/gkw1108 [PubMed: 27899567]
27. Rost B (1999) Twilight zone of protein sequence alignments. Protein Eng 12(2):85–94 [PubMed: 10195279]

28. Ito T, Kitamura H, Uwatoko C, Azumano M, Itoh K, Kuwahara J (2010) Interaction of Sp1 zinc finger with transport factor in the nuclear localization of transcription factor Sp1. Biochem Bioph Res Co 403(2):161–166. 10.1016/j.bbrc.2010.10.036

29. Günther V, Lindert U, Schaffner W (2012) The taste of heavy metals: gene regulation by MTF-1. Biochim Biophys Acta 1823: 1416–1425. 10.1016/j.bbamcr.2012.01.005 [PubMed: 22289350]

30. Wang Y, Weisenhorn E, MacDiarmid CW, Andreini C, Bucci M, Taggart J, Banci L, Russell J, Coon JJ, Eide DJ (2018) The cellular economy of the Saccharomyces cerevisiae zinc proteome. Metallomics 10:1755–1776. 10.1039/C8MT00269J [PubMed: 30358795]

31. Maffucci T, Falasca M (2001) Specificity in pleckstrin homology (PH) domain membrane targeting: a role for a phosphoinositide-protein co-operative mechanism. FEBS Lett 506(3):173–179 [PubMed: 11602240]

32. Chevalier-Larsen E, Holzbaur ELF (2006) Axonal transport and neurodegenerative disease. BBA-Mol Basis Dis 1762(11):1094–1108. 10.1016/j.bbadis.2006.04.002

33. Evens NP, Buchner P, Williams LE, Hawkesford MJ (2017) The role of ZIP transporters and group F bZIP transcription factors in the Zn-deficiency response of wheat (Triticum aestivum). Plant J 92(2): 291–304. 10.1111/tpj.13655 [PubMed: 28771859]

34. Dolfini D, Zambelli F, Pedrazzoli M, Mantovani R, Pavesi G (2016) A high definition look at the NF-Y regulome reveals genome-wide associations with selected transcription factors. Nucleic Acids Res 44(10):4684–4702. 10.1093/nar/gkw096 [PubMed: 26896797]

35. To KP, Do M-H, Cho Y-S, Kwon S-Y, Kim SM, Jung C (2018) Zinc inhibits expression of androgen receptor to suppress growth of prostate cancer cells. Int J Mol Sci 19(10). 10.3390/ijms19103062

36. Passerieux E, Hayot M, Jaussent A, Carnac G, Gouzi F, Pillard F, Picot M-C, Böcker K, Hugon G, Pincemail J, Defraigne JO, Verrips T, Mercier J, Laoudj-Chenivesse D (2015) Effects of vitamin C, vitamin E, zinc gluconate, and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: a double-blind randomized controlled clinical trial. Free Radical Bio Med 81:158–169. 10.1016/j.freeradbiomed.2014.09.014 [PubMed: 25246239]

37. Lomberk G, Urrutia R (2005) The family feud: turning off Sp1 by Sp1-like KLF proteins. Biochem J 392 (Pt 1):1–11. 10.1042/BJ20051234 [PubMed: 16266294]

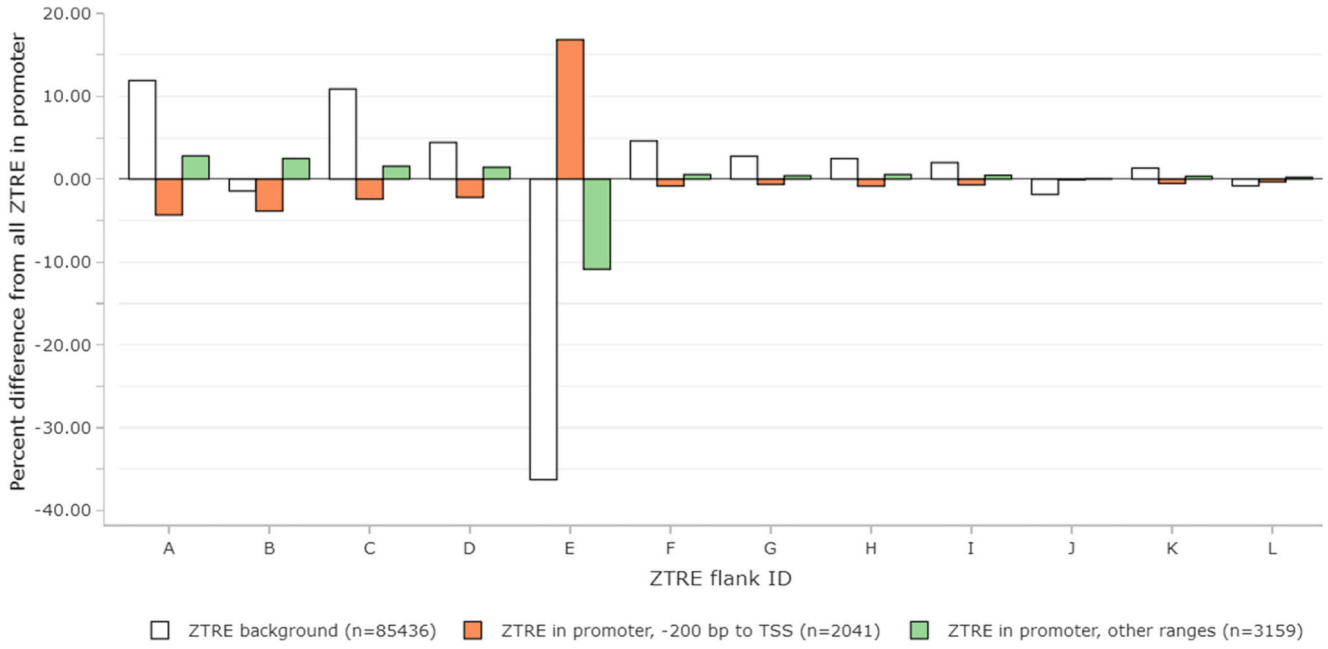

Fig. 1.

Twelve pairs of 5′- and 3′- palindromic flanking sequences of the ZTRE, named ZTRE-A through L-based on their background abundances. Shown are the relative abundances of these sequences as compared to all ZTREs in gene promoters (n = 5200). ZTRE flank IDs sorted according to their distance from the TSS

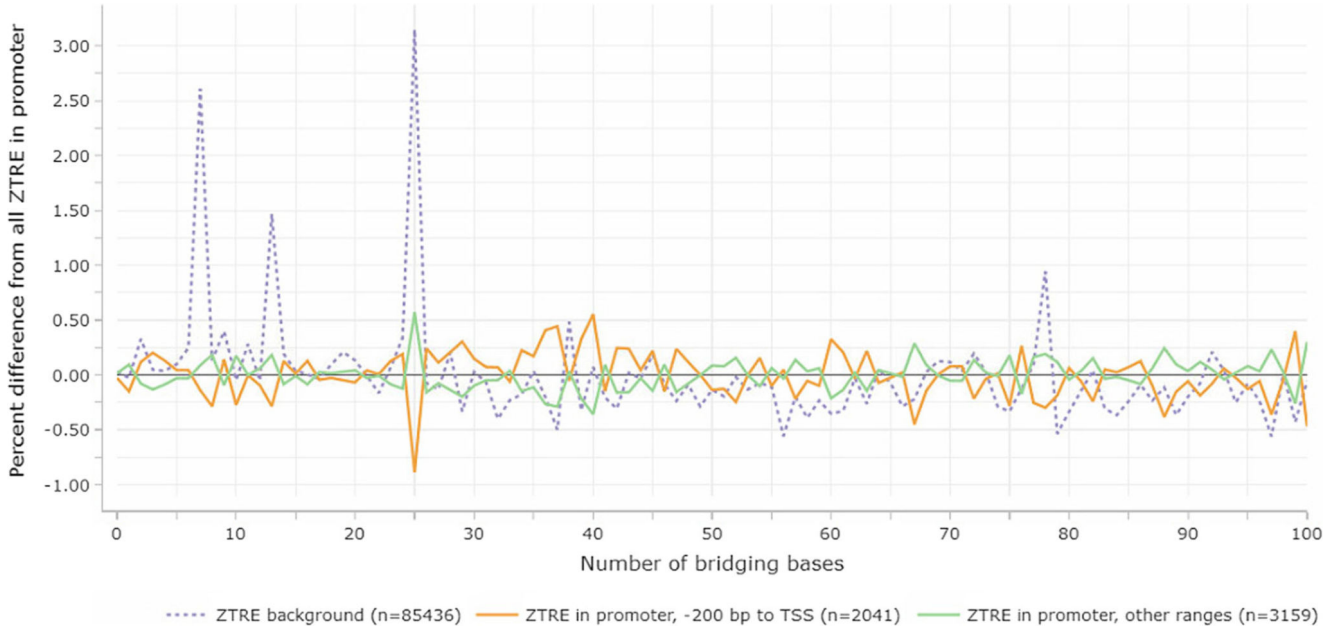

Fig. 2.

Numbers of bridging “N” bases in ZTRE sequences. There is a significant difference in the total number of bridging bases $N_{0–100}$, as well as at N_{25}, for ZTREs located in the range − 200 bp to the TSS, compared to other promoter ZTREs or background ZTREs

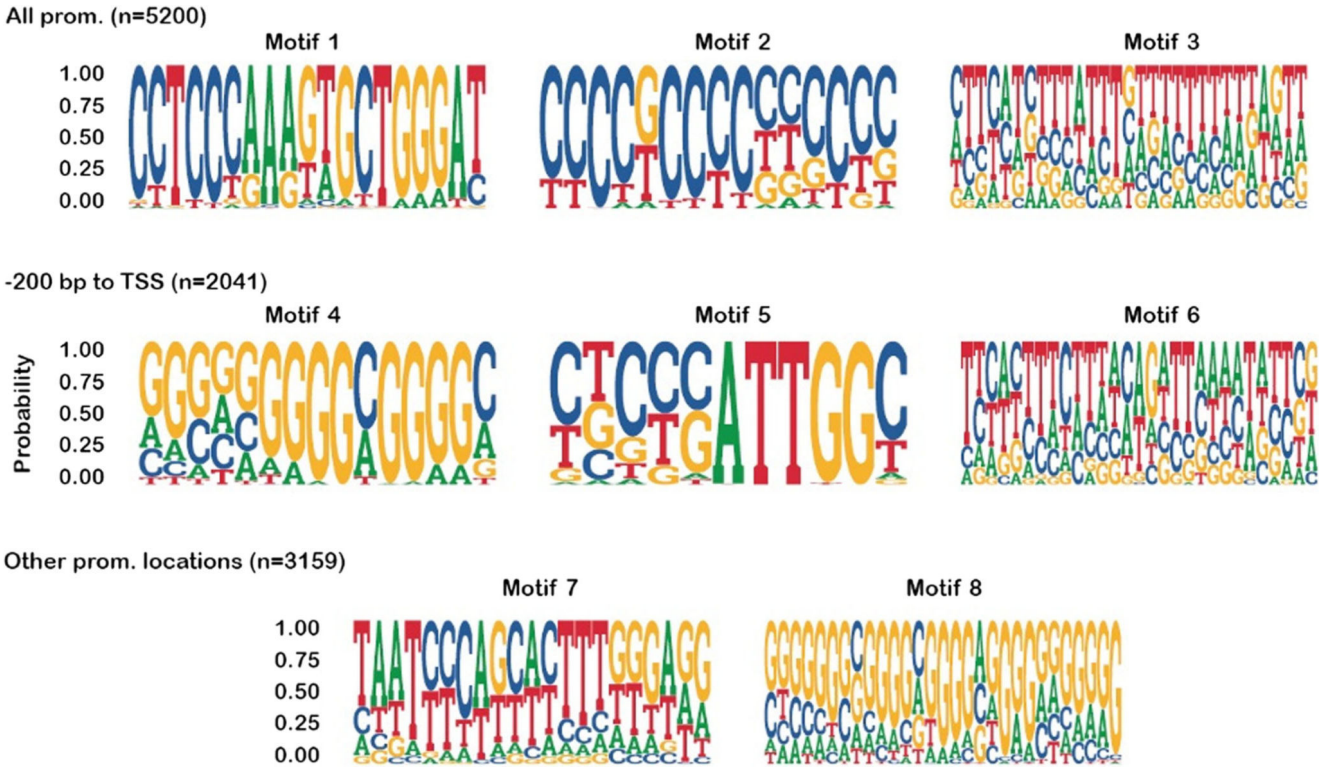

Fig. 3.

Significantly recurring motifs found in ZTRE “N” bases. Top row are all ZTREs found in the promoters of genes (n = 5200). Middle row motifs found in ZTREs in the range − 200-bp upstream to the TSS (n = 2041). Bottom row motifs are other promoter ZTREs (n = 3159). A version of the GC-rich Sp1-like binding motif (4,8, and reverse complement of 2) occurs in all three sets

Table 1

Results from ZTRE “N” base analysis as depicted in Fig. 3. Sp1-like GC-rich binding motifs are common to all groups and have the lowest *E* values, though high statistical significance is noted for all motifs listed. Only motifs with 100 or more sites are reported. TFs with similar binding sites to the motifs identified are also listed

ZTRE set	Motif	Width	ZTRE “N” base motif *E* value	Sites	TOMTOM HOCOMOCO database matches
All in promoter (n = 5200)	1	19	2.00E-177	127	IKZF1 **
	2	15	1.80E-189	1000	PATZ1 ***, Sp1-TF family ***, VEZF1 ***, KLF3 ***, ZN467 ***
	3	29	5.20E-125	385	PRDM6 **, SOX2 **, LEF1 *, FOXJ3 *, IRF1 *
− 200 bp to TSS (n = 2041)	4	15	1.80E-284	673	Sp1-TF family ***, KLF3 ***, KLF12 ***, PATZ1 ***, WT1 ***
	5	11	3.60E-138	229	NFYC ***, NFYA ***, FOXI1 ***
	6	29	5.00E-118	106	ANDR *
Other promoter locations (n = 3159)	7	21	3.1E-310	415	ALX1 *, DUX4 *, ZFP28 *
	8	29	8.20E-181	455	MAZ ***, WT1 ***, Sp1-TF family ***, KLF15 ***, PATZ1 ***

TOMTOM matches *E* value

* $E < 0.1$

** $E < 0.01$

*** $E < 0.001$

Table 2

Enrichment scores for genes with a ZTRE in the range − 200 bp to the TSS, compared to those with ZTREs in other promoter locations. Top DAVID annotation clusters for each cohort are reported. Annotation clusters significantly enriched for genes found in the range − 200 bp to the TSS may be associated with Zn deficiency cellular responses, while enriched genes for other ZTREs are associated with transcriptional activation

Summary: annotation/enrichment scores	Genes with ZTRE -200 bp to TSS	All other ZTRE genes
Number of mapped DAVID IDs	1112	1481
DAVID Pleckstrin homology domain annotation cluster	4.73**	0.54
DAVID microtubule transport annotation cluster	1.81**	0.25
DAVID leucine zipper domain annotation cluster	1.42*	0
DAVID RNA pol II transcriptional activation annotation cluster	0.26	3.56**
DAVID transcription/activation annotation cluster	0.62	2.86**
DAVID PWWP domain annotation cluster	0	2.42**
GO nervous system development	1.41*	–

* $p < 0.01$

** $p < 0.001$

Table 3

Comparison of amino acid sequences of Zap1, Sp1, and MTF- 1 versus ZNF658. Homologous ZF domains are found in all of these proteins. The AD1 domain of Zap1 has regulatory element-binding characteristics which may be analogous to those of ZNF658, though direct homology of the domains is indeterminate

Subject organism	Subject protein	Subject domain (position)	Human ZNF658 homologous region	*E* value	Identity (%)	Positives (%)	Total bit score (BLOSUM62)
S. cerevisiae	Zap1	(all)	–	–	–	–	1649
S. cerevisiae	Zap1	AD1 (258–317)	199–253	8.00E-03	26	35	–
H. sapiens	Sp1	(all)	–	–	–	–	1072
H. sapiens	Sp1	Zn finger 1 (626–650)	886–902 (ZF16)	2.00E-07	76	82	–
H. sapiens	Sp1	Zn finger 2 (656–680)	523–540(ZF3)	4.00E-04	44	72	–
H. sapiens	Sp1	Zn finger 3 (686–708)	440–462(ZF2)	9.00E-06	43	69	–
H. sapiens	Sp1	NLS/Zn fingers 1–3 (626–708)	971–1040(ZFs 19–21)	3.00E-20	49	62	–
H. sapiens	MTF-1	(all)	–	–	–	–	1705
H. sapiens	MTF-1	NLS/Zn fingers 1–3 (112–202)	768–903 (ZFs 12–16)	2.00E-31	44	58	–