THE TRANSCRIPTION FACTOR ZNF658 COORDINATES ZINC-INDUCED REPRESSION OF MULTIPLE GENES THROUGH THE ZINC TRANSCRIPTIONAL REGULATORY ELEMENT (ZTRE)

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Introduction
Cellular zinc homeostasis requires carefully orchestrated transcriptional activation and repression. In contrast to other systems, such as bacteria and Saccharomyces cerevisiae, a transcription factor with the specific function of repressing transcription in response to excess zinc has not been reported in mammals. We previously showed that the presence of the zinc-responsive element (zinc transcriptional regulatory element, ZTRE), a palindromic sequence within the promoter regions of SLC30A5 (ZnT5), SLC30A10 (ZnT10) and CBWD genes confers transcriptional repression in response to increased extracellular zinc concentration.

Aim/Objective
The present study was aimed at determining if ZNF658 binds to the ZTRE and thus mediates transcriptional repression of zinc-regulated genes that include the ZTRE in their promoter regions.

Materials and Methods
ZNF658 was initially identified through MALDI-TOF mass spectrometry of a ZTRE-dependent band in SLC30A5 promoter using EMSA. RT-qPCR and reporter-gene assay (using ZTRE-containing promoter-reporter constructs) were used to assess the effect of knockdown of ZNF658 and response to increased zinc concentration in Caco-2 cells at both transcript and promoter levels. Further analysis using EMSA showed that ZNF658 binds specifically to the ZTRE.

Result/Discussion
The expression of ZNF658 gene was reduced to about 60% on the average using 2 siRNAs. ZNF658 knockdown abrogated or attenuated the reduction in ZnT5, ZnT10 and CBWD mRNA levels and reduced activity of corresponding promoter-reporter constructs observed in response to increased extracellular zinc. Using EMSA, we demonstrated that a myc-tagged recombinant ZNF658 protein binds specifically to the ZTRE.

Conclusion
Together, these findings demonstrate that ZNF658 is involved in mediating transcriptional repression of multiple genes in response to zinc, thus revealing the identity of the first mammalian transcription factor with such a function. This discovery is a key step in building understanding of the integrated network of gene regulatory responses to zinc that will explain cellular zinc homeostasis at the molecular level.