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Regulation of cobalamin-like synthase gene expression through a zinc-responsive transcriptional element

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Maintenance of Zn homeostasis is essential for health and cellular function; however, the transcriptional control of Zn responsive genes is currently poorly characterised. A novel Zinc Responsive Transcriptional element (ZRE) within the promoter region of the *SLC30A5* Zn transporter gene was identified and an *in silico* search for the ZRE identified 6 human paralogous COBW domain-containing (*CBDW*) genes as potentially responsive to Zn. Furthermore, comparative genomic analysis of prokaryotic *CBWD* homologues has revealed a potential role in metal homeostasis and trafficking[1].

The abundance of the *CBWD* transcript in Caco-2 cells, normalised to *GAPDH*, was measured by RT-qPCR to be ~50% lower ($P<0.001$) in cells treated for 24 h with 100 μM Zn (0.48 ± 0.06, n 6) compared with 3 μM Zn (1.00 ± 0.09, n 6). Zn-regulated *CBDW* transcription and the role of the ZRE was investigated using plasmid constructs comprising the region −1078 to +98 of the *CBDW3* gene, relative to the start of transcription, upstream of the β-galactosidase reporter gene in the vector pBlueTOPO (Invitrogen). Reporter gene expression was lower ($P<0.001$) at 100 μM Zn (0.73 ± 0.04, n 23) compared with 3 μM Zn (1.00 ± 0.04; n 24) in transfected Caco-2 cells treated with Zn for 24 h. The response of the reporter gene to Zn was attenuated ($P<0.01$) by mutating the ZRE to a random sequence (1.00 ± 0.03 at 3 μM Zn; 0.90 ± 0.02 at 100 μM zinc; n 12), consistent with the ZRE having a role in mediating the transcriptional response to Zn.

Human CBWD3 protein, incorporating a C-terminal FLAG tag, was expressed transiently from a plasmid construct comprising the full open reading frame of the human *CBDW3* gene in the vector pCMV6Entry (Origene) in chinese hamster ovary cells treated for 24 h with 3 or 100 μM Zn. Western-blot analysis using an anti-FLAG antibody indicated greater abundance ($P<0.05$) of the recombinant protein resolved by SDS-PAGE at the expected molecular weight (~44 kDa) in cells cultured at the higher Zn concentration (1.00 ± 0.12 at 3 μM Zn; 2.57 ± 0.36 at 100 μM Zn; derived by densitometric quantification of band intensity, n 3–4). Preliminary observations indicated increased abundance at the lower Zn concentration of an anti-FLAG immunoreactive band of a lower molecular weight (~30 kDa), possibly indicative of Zn-dependent cleavage.

These observed effects of Zn availability on the expression of the *CBDW* genes and CBDW protein are consistent with the view that the human CBDW proteins play a role in Zn homeostasis. Further studies, including the identification of CBDW protein-binding partners, description of tissue and sub-cellular distribution and effects of Zn and other divalent metals on these measures, may help to elucidate this role.

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1. Haas CE, Rodinov DA, Kropat J *et al*. (2009) A subset of the diverse COG0526 family of putative metal chaperones is linked to zinc homeostasis in all kingdoms of life. *BMG Genomics* **10**, 470.