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# Regulation of hepatic suppressor of cytokine signaling 3 by zinc

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**Abstract**

Promoter analysis of the family of suppressors of cytokine signaling (SOCS) revealed that the human SOCS3 gene contains four binding sites for the metal regulatory transcription factor 1 (MTF-1) located within 1600 bp relative to the transcription start site. A series of experiments were carried out with human hepatoma cells (HepG2) and C57BL/6 mice to examine the effect of zinc on the regulation of SOCS3. In addition, we tested the role of MTF-1 in the regulation of SOCS3 expression using EMSA, chromatin immunoprecipitation assay and siRNA. Lastly, the role of the zinc transporter SLC39A14 on the basal expression of SOCS3 was evaluated. Results indicate that SOCS3 expression is regulated by zinc through an MTF-1-dependent mechanism. In addition, results from siRNA experiments suggest that SLC39A14 is required for basal expression of SOCS3. Further studies are needed to determine whether zinc status affects SOCS3 function.
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*Keywords:* Zinc; Inflammation; Liver; MTF-1; SLC39A14; SOCS3; IL-6; mice

## 1. Introduction

Zinc is an essential trace element that is involved in a wide variety of processes including enzyme activity and regulation of gene expression, cell cycle, apoptosis and immune response [1–5]. Severe zinc deficiency is rare; however, mild to moderate zinc deficiency is quite prevalent worldwide [6,7]. Zinc deficiency is common among the elderly and contributes to degenerative changes associated with aging such as immune senescence, cognitive dysfunction and chronic inflammation [8,9]. Zinc is known to block inflammation; however, the mechanisms by which this occurs are not completely understood. It has been suggested that the induction of the zinc finger protein A20 by zinc causes subsequent inhibition of Nuclear factor kappa-B (NFκB), thus suppressing the generation of inflammatory cytokines [10]. In addition, zinc is believed to inhibit inflammatory response through its antioxidant activity [11]. Little is known regarding the role of zinc in the inhibition of inflammatory response mediated by the Janus-Kinase-Signal transducers and activators (JAK/STAT) pathway. The termination of JAK/STAT signal occurs, in part, through the action of suppressors of cytokine signaling (SOCS) [12]. The SOCS family consists of eight members (SOCS 1-7 and CIS [cytokine-induced SH2-domain containing protein]) with conserved SH2 and SOCS-box domains [11]. Most SOCS are known to be negative regulators of the signal initiating their expression; therefore, SOCS proteins are part of a classical negative feedback loop [12]. SOCS proteins inhibit JAK/STAT signal by either promoting proteasome degradation of receptor complexes, inhibiting Janus tyrosine kinase activity or by binding phoshotyrosine residues on activated cytokine receptors [13–15]. In order to determine whether the SOCS proteins are potentially regulated by zinc, we screened the promoter region (2 kb upstream of transcription start site) of the members of the SOCS family for the presence of metal regulatory elements (MREs), a 12-bp motif that allows genes to be regulated by zinc [16]. Four MREs were found within 1.6 kb upstream of the transcription start site of SOCS3 ([17], www.genomatix.de). In addition, two MREs were found at the promoter region of CIS and only one MRE in SOCS1, SOCS2 and SOCS6 genes. Along with SOCS1, SOCS3 is one of the best characterized members of the SOCS. SOCS3 plays an important role in the inhibition of the activation of STAT1 and STAT3 by IL-6. Croker et al. [18] demonstrated that liver or macrophage specific SOCS3 knockout causes prolonged activation of STAT1 and STAT3 after IL-6 stimulation in mice [18]. In this study, we set out to examine the role of zinc in the regulation of SOCS3.

## 2. Methods

### 2.1. Cell culture and treatments

HepG2 cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were grown in a 5% $CO_2$-enriched atmosphere in DMEM medium containing 22 mM glucose supplemented with 10% fetal bovine serum, non-essential amino acids, 2 mM L-glutamine, 100 μg/ml streptomycin and amphotericin

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B (Sigma, St. Louis, MO, USA). Cells were seeded in 12-well plates ($1\times10^5$ cells per well) and allowed to grow to 60–80% of confluence before they were treated. In some experiments, intracellular zinc depletion was produced by treating cells with the zinc chelator *N,N,N′,N′*-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) (Sigma). Intracellular zinc depletion was also caused by incubating the cells in medium with fetal bovine serum treated with Chelex-100 (BioRad, Hercules, CA, USA) [19]. To observe the effects of zinc, cells were exposed to various levels of $ZnCl_2$. Lastly, to assess whether zinc induces the expression of SOCS3 at the transcriptional levels, cells were exposed to actinomycin-D (5 μg/ml) (Sigma) for 60 min before $ZnCl_2$ addition.

### 2.2. Assay of lactate dehydrogenase (LDH) activity

LDH release into culture medium was assayed using the LDH cytotoxicity assay kit from Cayman Chemical (Ann Arbor, MI, USA). Cytotoxicity was expressed as percentage of total LDH activity, which was obtained from the cells treated with 1% Triton X-100.

### 2.3. RNA interference

Cells were transfected with either 16 nM (final concentration) non-targeting siRNA control (All-star negative control, Qiagen, Valencia, CA, USA) or siRNAs targeting the gene of interest (MTF-1 or SLC39A14) (flexitube; Qiagen). The transfection reagent HiPerFect (Qiagen) was used for this procedure according to manufacturer's instruction. To knockdown the expression of SLC39A14 and MTF-1, cells were treated with siRNA for 72 h.

### 2.4. mRNA quantification by quantitative reverse transcriptase polymerase chain reaction (PCR)

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Isolated RNA was treated with Turbo DNase (Ambion, Austin, TX, USA). Quantitative PCR (qPCR) was performed using the iScript one-step reverse transcriptase PCR kit with SYBR Green (BioRad). Predesigned primers for SOCS3 and 18s detection were purchased from Qiagen. Values were normalized to 18S rRNA.

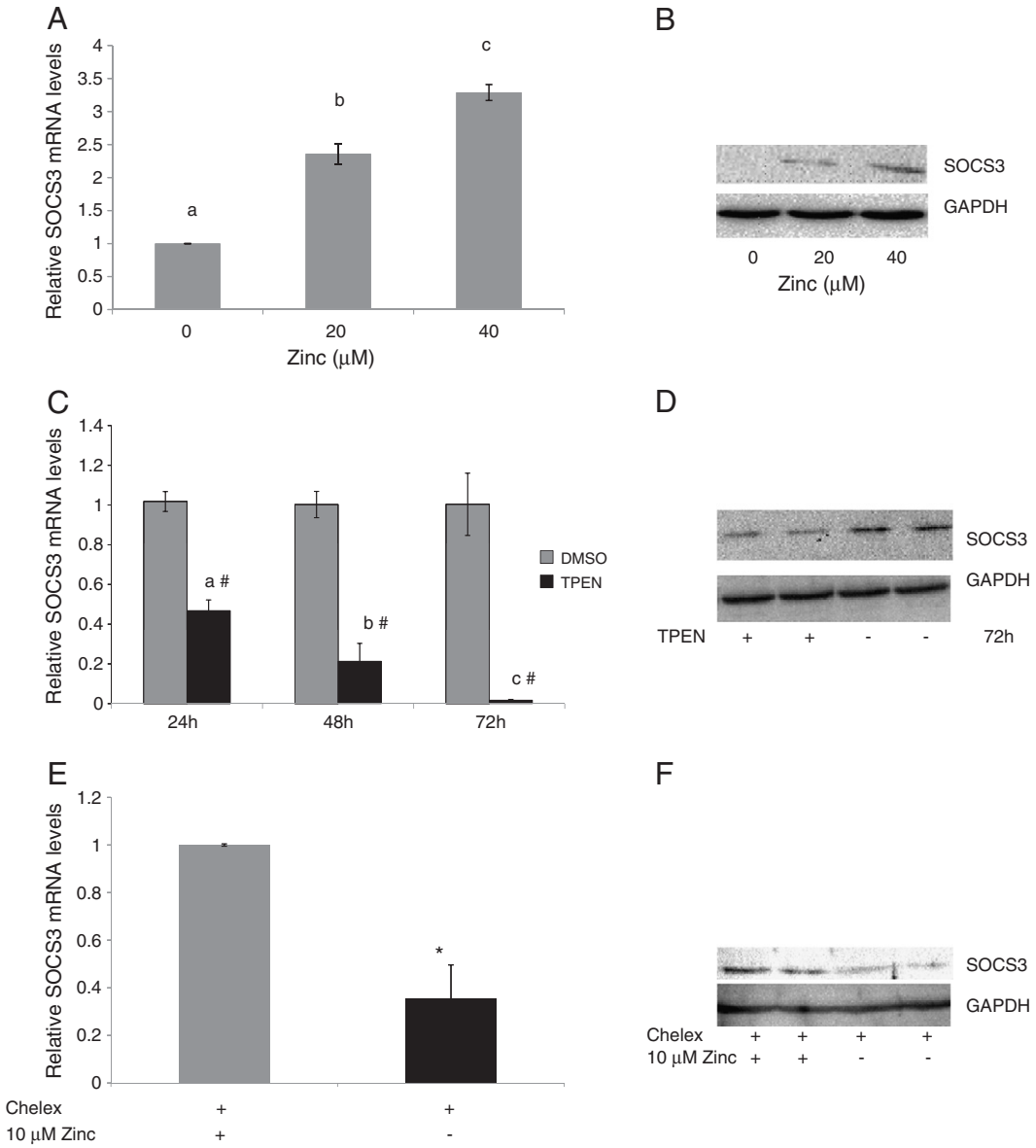


Fig. 1. Effect of zinc depletion and excess on the expression of SOCS3 in HepG2 cells. (A and B) Cells were exposed to increasing amounts of ZnCl2 (0 μM, 20 μM, and 40 μM) for 3 h. (C and D) Cells were incubated in medium with either 4 μM TPEN or dimethyl sulfoxyde (DMSO) for various time intervals (24 h, 48 h, and 72 h). (E and F) Cells were incubated in zinc depleted medium (prepared with fetal bovine serum treated with Chelex-100) supplemented with 0 μM or 10 μM ZnCl2 for 72 h. Abundance of SOCS3 mRNAs was measured by qPCR and normalized to levels of 18S rRNA. Values are means±S.D ($n=3$). Values with different letters are significantly different ($P<.05$) according to one-way ANOVA analysis and Student Newman–Keuls test (Fig 1A). Values with different letters are significantly different ($P < 0.05$) according to two-way ANOVA analysis and Student Newman–Keuls test (Fig 1C). #Significantly different ($P<.05$) from DMSO at same time point according to two-way ANOVA analysis and Student Newman–Keuls test (Fig 1C). Two-way ANOVA analysis showed significant interaction ($P<.05$) between time and treatment.