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Regulation of the ATF3 gene by a single promoter in response to amino acid availability and endoplasmic reticulum stress in human primary hepatocytes and hepatoma cells

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ABSTRACT

Activating transcription factor 3 (ATF3) is a highly regulated protein that is implicated in a wide range of pathological conditions including inflammation and transformation. Transcription from the ATF3 gene is induced by several stress-induced signaling pathways, including amino acid limitation (amino acid response, AAR) and ER stress (unfolded protein response, UPR). Induction of ATF3 transcription by these pathways is mediated by ATF4 and cJUN recruitment to enhancer elements within the ATF3 gene. Although a canonical promoter (promoter A) has been studied by numerous laboratories, a second promoter activity (promoter A1), 43 kb upstream of the first, has been reported to respond to stress-induced signaling and to be critical for ATF3 expression in certain transformed cells. The results of the present study show that in normal human hepatocytes and HepG2 human hepatoma cells both basal as well as AAR- and UPR-induced transcription occurs almost exclusively from promoter A. This selectivity between the two promoters correlated with increased binding of ATF4, recruitment of RNA polymerase II, and the expected histone modifications in the promoter A region of the gene. Time course studies of ATF3 transcription activity revealed that the temporal kinetics for ATF3 induction differ between the AAR and UPR, with the former being more transient than the latter. Collectively, the results document that ATF3 expression in normal and transformed human liver originates from the canonical promoter A that responds to multiple stress signals.

1. Introduction

Mammalian cells respond to dietary insufficiency of protein or amino acids (AA) through activation of several signaling cascades collectively referred to as the amino acid response (AAR) [reviewed in 1]. The best characterized AAR pathway senses uncharged tRNA levels which activate the general control non-derepressible-2 (GCN2) kinase. The primary substrate for GCN2 is the alpha subunit of the eukaryotic translation initiation factor eIF2. Phospho-eIF2 (p-eIF2) leads to a partial suppression of global protein synthesis, but also causes increased translation of selected mRNA species, including that for activating transcription factor 4 (ATF4) [1,2]. Expression array analysis has shown that AA deprivation, via ATF4 action, leads to induction of hundreds of genes that mediate a spectrum of cellular processes [3–7]. There are three other eIF2 kinases that are activated by a wide range of cellular stresses [8]. For example, endoplasmic reticulum (ER) stress triggers ATF4 synthesis through activation of the eIF2 kinase PKR-like endoplasmic reticulum kinase (PERK) [9,10]. ATF4 mediates an increase in transcription from genes that contain an enhancer sequence composed of a half-site for CAAT enhancer binding protein (C/EBP) family members and a half-site for the ATF family of transcription factors [11,12]. These enhancer sequences are referred to as a C/EBP-ATF response element (CARE). Most, but not all [13], functional CARE sites respond to ATF4 regardless of which eIF2 kinase was activated [reviewed in 1] and collectively, the p-eIF2-ATF4 dependent pathways are often referred to as the Integrated Stress Response (ISR). The products of these CARE-containing genes control a broad range of physiological processes. Characterization of individual genes as well as chromatin immunoprecipitation (ChIP)-sequencing has led to the identification of a consensus CARE sequence (5′-TGATGXAAX-3′) [14].

Abbreviations: AA, amino acid; AAR, AA response; ATF, activating transcription factor; C/EBP, CAAT/enhancer binding protein; CARE, C/EBP-ATF response element; CRE, cyclic AMP response element; IF2, eukaryotic initiation factor 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GCN2, general control non-derepressible 2; HisOH, histidinol; ISR, integrated stress response; PERK, PKR-like endoplasmic reticulum kinase; RT-qPCR, real time quantitative PCR; Tg, thapsigargin; TSS, transcription start site; UPR, unfolded protein response

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Table 1
PCR primers.

Primer	Sequences
GAPDH, mRNA	Forward: 5′- TTGGTATCGTGGAAGGACTC-3′ Reverse: 5′-ACAGTCTTCTGGGTGGCAGT-3′
ATF3, mRNA	Forward: 5′-CAGTCACTGTCAGCGACAGACCC-3′ Reverse: 5′-TCTTCTTCAGGGGCTACCTCGG-3′
ATF3, hnRNA, promoter A1	Forward: 5′-GGGGACGATGGCAGAAGCACT-3′ Reverse: 5′-GGAGGCTTCCTGACCAAACACCT-3′
ATF3, hnRNA, promoter A	Forward: 5′-CATCACAAAAGCCGAGGTGGGG-3′ Reverse: 5′-CAGTGGCTGCGAGCGAAACA-3′
ATF3 P1, chip assay	Forward: 5′-CTGTCTTTTCCTCTTCTTCTAAGGGCAC-3′ Reverse: 5′-CAGCCTTTGAGAGATCATTAGGTTTGG-3′
ATF3 P2, chip assay	Forward: 5′-ATCAGTGTCAAGCCCCTCACTCAG-3′ Reverse: 5′-GCTTCCCTTCGAGCCATCATCTA-3′
ATF3 P3, chip assay	Forward: 5′-GATGGGATCAGATGGGAAGATGTGA-3′ Reverse: 5′-TTGGGGCAAGGTGCTGAAAATC-3′
ATF3 P4, chip assay	Forward: 5′-CCGTTCCAAAGCGAAGAAGTAGGT-3′ Reverse: 5′-CTGTATTCGTGCCCAGAATGCTAGA-3′
ATF3 P5, chip assay	Forward: 5′-GTTCCTTGGTTCTGCCGCTCTC-3′ Reverse: 5′-TCCGAGATTCGAGCTGAGACCTC-3′
ATF3 P6, chip assay	Forward: 5′-GACTTTGGACACCTTCCCCACAC-3′ Reverse: 5′-TGGTCATTTTCTGGAGCTTCAGGA-3′
ATF3 P7, chip assay	Forward: 5′-TGAGGGCTATAAAAGGGGTGATGC-3′ Reverse: 5′-GCGAGAGAAGAGAGCTGTGCAGTG-3′
ATF3 P8, chip assay	Forward: 5′-ACTTCTTCTAAGCCACCGCTGCTC-3′ Reverse: 5′-GACCTCCGTCACCAGGAACCTTT-3′
ATF3 P9, chip assay	Forward: 5′-CACAATGCAGTGGTTGGACCAGAT-3′ Reverse: 5′-TGGCTCCTTTTCTCCCCACTACAC-3′
ATF3 P10, chip assay	Forward: 5′- AAGGTGGGGGGATCTGAGAGAATA-3′ Reverse: 5′- TGACATCACCACTACCAACAGGAGAC-3′

As might be expected given the commonality of ATF4 action during AA and ER stress, the transcriptional programs activated have extensive overlap, but significant differences are also observed [15].

The ATF3 gene is subject to complicated transcriptional regulatory mechanisms from a spectrum of stress signals [16,17]. The AAR and UPR both increase ATF3 expression via p-eIF2-ATF4 signaling [18,19]. In a feedback mechanism, the increased ATF3 expression functions as a suppressor of ATF4 action [20]. Whereas most of the published ATF3 gene analysis studies have focused on control from a canonical promoter (herein termed promoter A), evidence has been reported that a second promoter (promoter A1) exists about 43 kb upstream of promoter A [21–23]. Miyazaki et al. showed that in HCT116 cells, ER stress [triggered by thapsigargin (Tg) or tunicamycin (Tm)] induced expression from both promoters, and in some transformed cells promoter A1 was the primary source of ATF3 expression [22]. Both promoters are also functional in adult T-cell leukemia cells, as reported by Hagiya et al. [23]. In previous reports, we have characterized AAR- and UPR-induced transcription from the human ATF3 gene in HepG2 human hepatoma cells, but, like most other laboratories, measured activity from promoter A only [19,24–26].

The present study addresses the following questions. 1) Do both promoters A1 and A contribute to regulation of the ATF3 gene in response to the AAR and UPR pathways in human hepatocytes and HepG2 human hepatoma cells? 2) Do the enhancer-specific factors ATF4 and cJUN contribute to promoter A1 activity? 3) Are there differences in the epigenetic changes at the two promoters associated with stimulation of the ATF3 gene by these two stress pathways? The results indicate that the 24 h time course of transcription from the ATF3 gene is different after activation of the AAR or UPR pathway. The AAR induction is transient, whereas the activation following UPR signaling remains elevated for the entire 24 h period studied. In primary human hepatocytes and HepG2 hepatoma cells, only promoter A appears to be functional, neither the AAR nor the UPR increased transcription from promoter A1. This lack of promoter A1 activity was supported by ChIP analysis that revealed ATF4, RNA polymerase II, and transcription-associated histone changes at promoter A, but not at promoter A1. Collectively, the data indicate that for human liver-derived cells transcriptional regulation of the ATF3 gene by pathways belonging to the ISR occurs largely by promoter A and that unknown regulatory factors contribute to differential temporal control of the gene depending on which of the ISR pathways is activated.

2. Materials and methods

2.1. Cell culture

Primary human hepatocytes were purchased from Corning Life Sciences (Tewksbury, MA). Both the primary hepatocytes and the HepG2 human hepatoma cells (purchased from American Type Tissue

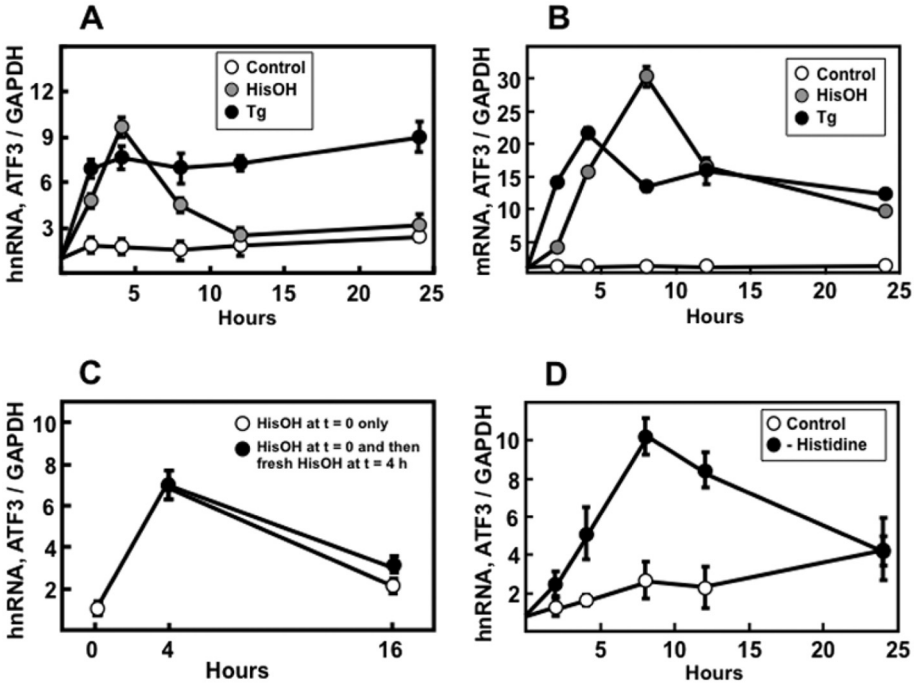

Fig. 1. Temporal analysis of ATF3 transcription activity during the AAR and UPR. HepG2 cells were cultured in DMEM (Control) ± 2.5 mM HisOH or ± 50 nM Tg for 0–24 h. Total RNA was isolated at the indicated times and RT-qPCR was performed to analyze the ATF3 transcriptional activity as measured by hnRNA (A) and steady-state mRNA levels of ATF3 (B). To verify that the decreased ATF3 transcriptional activity in DMEM + HisOH was not the result of HisOH degradation, transcriptional activity was measured in HepG2 cells in which the media in one half of the cells was supplemented with fresh 2.5 mM HisOH at the 4 h time period (C) or in cells incubated in DMEM ± histidine for 0-24 h (D). In all panels, GAPDH mRNA, which is not affected by the AAR or UPR, was used as an internal control. The results shown are the means ± S.D. of at least three replicates. Each experiment was repeated to ensure reproducibility between batches of cells.