[Cell Cycle 5:13, 1422-1425, 1 July 2006]; ©2006 Landes Bioscience

Perspective

Egad, More Forms of Gene Regulation: The *gadd45a* Story

Ashish Lal

Myriam Gorospe*

Laboratory of Cellular and Molecular Biology; National Institute on Aging; Intramural Research Program; National Institutes of Health; Baltimore, Maryland USA

*Correspondence to: Myriam Gorospe; Box 12, LCMB, NIA-IRP, NIH; 5600 Nathan Shock Dr.; Baltimore, Maryland 21224, USA; Tel.: 410.558.8443; Fax: 410.558.8386; Email: myriam-gorospe@nih.gov

Original manuscript submitted: 05/12/06
Manuscript accepted: 05/15/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=2902

KEY WORDS

posttranscriptional gene expression, mRNA turnover, translation, DNA damage, ribonucleoprotein complex, RNA-binding protein, AUF1, TIAR

ABBREVIATIONS

AUF1	AU-rich element-binding factor 1
MMS	methyl methanesulfonate
RBP	RNA-binding protein;
RNP	ribonucleoprotein complex
TF	transcription factor
TIAR	T-cell-restricted intracellular antigen-1 (TIA-1)-related protein

ACKNOWLEDGEMENTS

We are grateful to S. Fugmann, A.J. Fornace Jr., and S.C. Cunningham for insightful comments. This research was are supported by the National Institute on Aging-Intramural Research Program, National Institutes of Health.

ABSTRACT

Despite the historical hegemony of transcription, mounting evidence supports the importance of posttranscriptional gene regulation via processes such as mRNA splicing, localization, turnover, and translation. However, each of these steps is still largely viewed as an exclusive proposition, whereby a particular gene under given circumstances is controlled by a single specific regulatory mechanism. Our recent investigation of *gadd45a* expression in response to genotoxic stress illustrates a more complex scenario, wherein transcriptional changes operate in concert with mRNA turnover and translational regulation. *gadd45a* thus joins a handful of reported genes whose levels are potently altered in response to cellular damage or mitogenic cues through the coordinated action of DNA- and RNA-binding proteins. Eliciting cellular responses that are strong, swift, and versatile, gene regulation by multiple factors acting on different levels is emerging as the norm, rather than the exception, for a growing collection of gene products which critically influence cellular homeostasis.

The growth arrest- and DNA damage-inducible (*gadd*) 45 gene family, comprising *gadd45α*/*gadd45a*, *gadd45β*/*gadd45b*/*myd118*, and *gadd45γ*/*gadd45g*/*cr6*, is widely expressed in mammalian cells responding to stress stimuli. *gadd45a*, the founding member,[1] encodes a 21-kDa, predominantly nuclear protein whose levels increase in response to a variety of agents, including hypoxia, ionizing radiation, oxidants, ultraviolet light, and growth-factor withdrawal. In cultured cells, Gadd45a elicits pleiotropic effects, influencing G_2/M cell cycle progression, access to DNA on damaged chromatin, genomic stability, nucleotide excision repair, apoptosis, and signaling through the mitogen-activated protein (MAP) kinases p38 and c-Jun N-terminal kinase (JNK).[2-10] In mice, targeted inactivation of *gadd45a* enhanced tumorigenesis and caused genomic instability, alterations in growth control, and autoimmunity.[11-13] The activities of Gadd45a are thought to be mediated through its interaction with several proteins, such as the proliferating cell nuclear antigen (PCNA), the cyclin-dependent kinase (cdk) inhibitor p21, the p38 and JNK upstream kinase MEKK4/MTK1, the G_2 kinase Cdk1/Cdc2, and histones.[2-4,14,15]

gadd45a expression is rapidly and strongly induced in stressed cells. For over one decade, the mechanisms of *gadd45a* induction have been the focus of intense study leading to the identification of numerous transcription factors (TFs) responsible for its transcriptional upregulation and repression. More recently, several posttranscriptional processes have also been shown to influence *gadd45a* expression. Far from functioning in a mutually exclusive fashion, this multilevel regulation appears to be essential for the robust and timely induction of Gadd45a. Here, we review the transcriptional and posttranscriptional regulators of *gadd45a* expression (Table 1) and discuss the rationale of tandem gene regulation on both sides of the nuclear membrane.

TRANSCRIPTIONAL *gadd45a* REGULATION

In order to maintain constitutively low *gadd45a* expression, transcription of this gene is negatively influenced by the action of transcriptional repressors. The proto-oncogene c-Myc was shown to repress both basal and stress-induced *gadd45a* expression;[16,17] accordingly, in rodent cells with c-myc-null status, *gadd45a* expression was transcriptionally derepressed.[18] More recently, a second repressor of *gadd45a* transcription was identified, the zinc finger protein ZBRK1, which bound the *gadd45a* gene on a specific site within intron 3. ZBRK1 was shown to interact with the tumor suppressor BRCA1, and the ZBRK1-BRCA1 corepressor complex inhibited *gadd45a* transcription in unstimulated cells.[19,20] Following stress stimulation, these *gadd45a* transcriptional repressors were

inactivated. Along with the ensuing transcriptional derepression, the transcription of *gadd45a* increased rapidly and potently through the action of transcriptional activators.

Among the transcriptional inducers of *gadd45a* is the tumor suppressor p53, the first TF reported to transcriptionally elevate *gadd45a* expression.[21] p53 was shown to bind a conserved site within intron 3 of the *gadd45a* gene and was strictly required for the transcriptional upregulation of *gadd45a* in response to ionizing radiation.[21] By contrast, the induction of *gadd45a* following irradiation with ultraviolet light (UV), treatment with the alkylating agent methyl methanesulfonate (MMS), or depletion of nutrients, was only partially dependent on p53; under these conditions, *gadd45a* was transcriptionally upregulated by additional TFs. One of these, WT1, was shown to bind to the proximal *gadd45a* promoter region and induced *gadd45a* transcription; WT1 function was positively influenced by p53, although p53 did not directly bind the *gadd45a* proximal promoter region nor did it form direct protein-protein associations with WT1.[22] MMS and UV treatments also transcriptionally upregulated *gadd45a* through the actions of TFs Oct-1 and NF-YA, which associated with regulatory elements (Oct-1 and CAAT binding motifs) within the proximal promoter region.[23-25] In addition, UV irradiation upregulated *gadd45a* levels transcriptionally through the binding of FoxO3a and Egr-1 to response elements within the *gadd45a* proximal promoter.[26,27] In a differentiation model, the association of C/EBPα with the proximal *gadd45a* promoter was similarly reported to stimulate its transcription.[28] The *gadd45a* transcriptional repressors and inducers function in concert: in unstimulated cells, there is no transcriptional activation but there is transcriptional repression, whereas after stimulation, there is transcriptional derepression (inactivation/dissociation of the repressor) coupled with transcriptional activation.

Table 1 **DNA-binding proteins and RNA-binding proteins governing the expression of *gadd45a* in response to damaging agents**

Regulatory Protein	Function	Binding Site	Binding After Damage
Myc	Transcriptional repression	DNA (promoter)	Decreased
ZBRK1	Transcriptional repression	DNA (intron 3)	Decreased
p53	Transcriptional induction	DNA (intron 3)	Increased
WT1	Transcriptional induction	DNA (promoter)	Increased
Oct-1	Transcriptional induction	DNA (promoter)	Increased
NF-Y	Transcriptional induction	DNA (promoter)	Increased
FoxO3a	Transcriptional induction	DNA (promoter)	Increased
Egr-1	Transcriptional induction	DNA (promoter)	Increased
CEBPα	Transcriptional induction	DNA (promoter)	Increased
AUF1	mRNA degradation	mRNA (3′UTR)	Decreased
Nucleolin	mRNA stabilization	mRNA (3′UTR)	Increased
TIAR	Translational repression	mRNA (3′UTR)	Decreased

Their influence upon Gadd45a production and their mode of regulation are indicated.

POSTTRANSCRIPTIONAL *gadd45a* REGULATION

Once the *gadd45a* gene is transcribed, the *gadd45a* pre-mRNA must first be spliced, and the resulting mRNA capped at the 5' end, polyadenylated, transported through the nucleoplasm, and exported out of the nucleus. After it reaches the cytoplasm, the *gadd45a* mRNA must elude ribonucleolytic degradation, bear possible episodes of storage in subcytoplasmic domains, and eventually engage with the translational machinery to serve as a template for the synthesis of Gadd45a protein. In order for the stress-triggered transcriptional induction of *gadd45*a to lead to elevated Gadd45a protein levels, all of the intervening posttranscriptional processes must also function appropriately. Like transcription, posttranscriptional processes can be regulated negatively through repressors and positively via inducers.[29]

Recent studies from our laboratory have identified several posttranscriptional repressors of *gadd45a*. Among the RNA-binding proteins (RBPs) examined, two were found to associate with the *gadd45a* mRNA via specific recognition of its AU-rich 3'-untranslated region (UTR). The first, AUF1 (AU-rich element RNA-binding factor 1), is a family of four proteins (p37, p40, p42, p45) arising through alternative splicing.[30] The second, TIAR [T-cell-restricted intracellular antigen-1 (TIA-1)-related protein], is a set of two proteins of 42/50 kDa that arises also via alternative splicing.[31,32] In unstimulated cells, AUF1 and TIAR proteins (each representing a family of related RBPs) were found prominently in complex with the *gadd45a* mRNA. AUF1 was found to render the *gadd45a* mRNA unstable, thus further maintaining low transcript levels, while TIAR was found to inhibit the association of the *gadd45a* mRNA with the cell's polysomes, thereby suppressing its translation.[33] The combined action of these RBPs effectively suppressed Gadd45a biosynthesis, as they potently reduced *gadd45a* mRNA abundance in the cell and excluded any remaining *gadd45a* transcripts from the translational machinery. In cells treated with MMS or UV, there was a rapid and dramatic dissociation of AUF1 and TIAR from the *gadd45a* mRNA. These events were linked to striking increases in the stability of the *gadd45a* mRNA, leading to its accumulation in the cell, and to marked enhancements in the association of the *gadd45a* mRNA with the actively translating polysomes. The dissociation of AUF1 and TIAR from the *gadd45a* mRNA represents a novel example of *posttranscriptional derepression* in mammalian cells, resulting in mRNA stabilization and increased translation. These two posttranscriptional steps were found to be essential for the swift and robust elevation in Gadd45a levels in cells responding to DNA damage.[33]

The existence of posttranscriptional inducers of *gadd45a* expression has also been proposed,[34-36] but such regulatory protein(s) are less well characterized. A likely candidate is nucleolin, an RBP that promotes mRNA stability; the association of nucleolin with the *gadd45a* mRNA increased in cells responding to arsenic and was linked to elevations in *gadd45a* mRNA half-life.[37,38] Our best efforts to identify additional RBPs which either directly enhance the stability of *gadd45a* mRNA or actively recruit this transcript to heavy polysomes in order to promote its translation have been fruitless thus far. Particular attention was given to HuR, a stress-regulated RBP which has been implicated in both the stabilization and the translational enhancement of other transcripts,[39] but HuR was not found to interact with the *gadd45a* mRNA in either untreated or stress-treated cells.[33] However, unlike *gadd45a* transcription, where genotoxicity-induced dissociation of repressor proteins (c-Myc, ZBRK1) was to be followed by increased function of activating TFs (p53, Oct-1, WY1, FoxO3a, etc), the removal of posttranscriptional

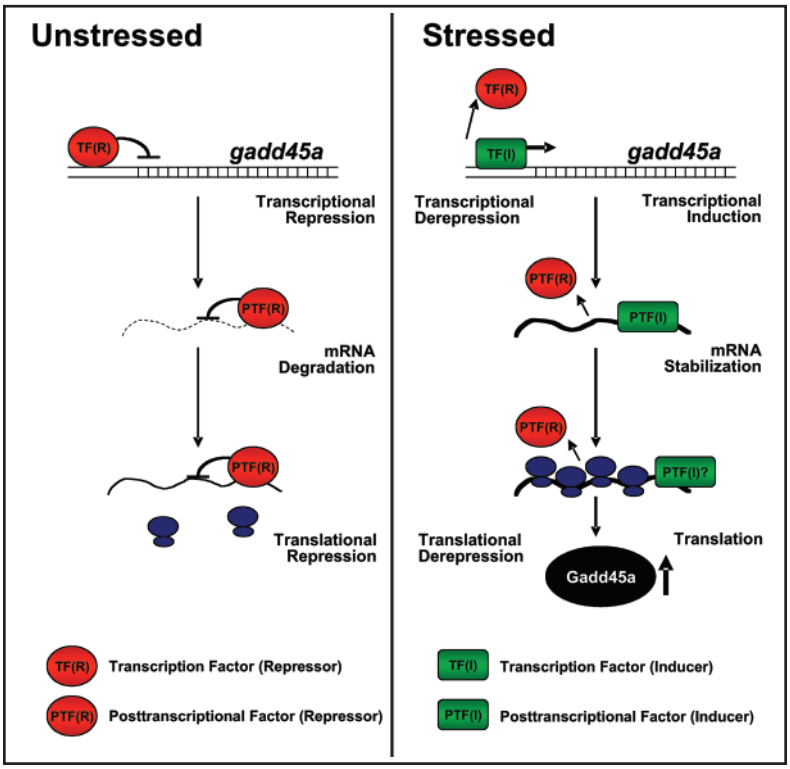

Figure 1. Schematic of the multiple regulatory mechanisms controlling *gadd45a* expression. Left, in unstressed cells, transcriptional repressors block gene transcription, while posttranscriptional repressors promote mRNA degradation or inhibit translation; the net consequences are a potent suppression of Gadd45a production. Right, in response to stress, gene repression at the transcriptional and posttranscriptional levels is relieved, whereupon transcriptional and posttranscriptional inducers activate gene transcription and stabilize the mRNA ('?', as-yet unidentified translational inducer); their joint activities cause a potent increase in Gadd45a levels.

repressors of the *gadd45a* mRNA possibly need not be accompanied by the association of specific RBPs to promote mRNA stabilization and translation. Instead, the AUF1- and TIAR-less *gadd45a* mRNA is likely to be intrinsically stable and recruited to the translational machinery by generic translation factors.

TANDEM GENE REGULATION: INTEGRATING TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL PROCESSES

Collectively, the studies on *gadd45a* gene expression have indentified an extensive group of factors influencing Gadd45a protein levels. In the emerging scenario, a coordinated team of DNA- and RNA-binding proteins ensures the strong and timely expression of Gadd45a: its levels are effectively suppressed in the absence of stress, its induction is rapid and robust upon encountering stress. Taken together, the suppression of *gadd45a* expression (Fig. 1, 'Unstressed') relies upon transcriptional repressors which block transcription (c-Myc, ZBRK1) as well as posttranscriptional repressors that either degrade mRNAs resulting from low-level transcription (AUF1) or block their translation (TIAR). In this capacity, the posttranscriptional repressors function as effective safeguard mechanisms to further avoid the unscheduled production of Gadd45a, preventing the transcripts which escape the transcriptional repression from accumulating in the cytoplasm and from being translated. Following exposure to damaging stimuli (Fig. 1, 'Stressed'), the induction of *gadd45a* expression occurs through combined transcriptional and posttranscriptional derepression (removal or inactivation of the repressors) followed by the tandem action of transcriptional inducers (p53, WT1, Oct-1, NF-Y, FoxO3a, Egr-1, CEBPα) and posttranscriptional inducers (nucleolin). Together, these synchronized processes provide the strength, unidirectionality, specificity, and timeliness needed for effective Gadd45a upregulation.

Gene regulatory circuits which depend upon multiple factors, transcriptional and posttranscriptional, may be particularly well suited to control the expression of proteins like Gadd45a, involved in DNA repair and cell cycle progression. For example, numerous proteins functioning as cell cycle regulators (cdk4, E2F-1, cdk inhibitors p21 and p27, cyclins A, B1, and D1), tumor suppressors (p53, BRCA1), regulators of apoptosis (Bcl-2, Bcl-x, Bax), DNA repair factors (ERCC1), and mitogenic effectors (c-Fos, c-Jun, c-Myc), have been shown to be regulated by both transcriptional and posttranscriptional mechanisms. However, the specific regulatory steps and the DNA- and RNA-binding proteins responsible for controlling their expression are largely unknown. The challenges ahead will be to identify these factors systematically and to understand their coordinated influence upon the expression of target stress genes. The knowledge obtained through these efforts will be particularly valuable as we seek an improved understanding of the gene products, pathways, and networks governing the response of damaged cells.

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