

ATF6 and Thrombospondin 4

The Dynamic Duo of the Adaptive Endoplasmic Reticulum Stress Response

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A Thrombospondin-Dependent Pathway for a Protective ER Stress Response

Lynch et al
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Thrombospondins are secreted, nonstructural, extracellular matrix proteins that are upregulated in the heart and other tissues in response to ischemic injury and pathology. Roles for thrombospondins after they are secreted have been examined in a variety of disease models, including myocardial pathology. However, a recent study published in the journal *Cell* shifts this paradigm by focusing on roles for intracellular thrombospondins; these authors showed that thrombospondin 4 (Thbs4) can function from within cells to protect the heart by enhancing adaptive aspects of the endoplasmic reticulum stress response that are mediated by activating transcription factor 6 (ATF6). Although this study was performed in the cardiac context, the results add to our understanding of protein folding and quality control in all tissues. Moreover, the findings underscore the potential widespread therapeutic benefit of enhancing adaptive responses that are regulated by ATF6.

The study by Lynch et al,¹ which was performed in the laboratory of Dr Jeffery Molkentin, used a series of molecular approaches, including in vivo gain-of-function and loss-of-function models in mice, to demonstrate new roles for Thbs4 in the management of endoplasmic reticulum (ER) protein quality control in the heart. The ER is the site of synthesis and folding of most secreted and membrane proteins, which constitute at least 35% of all proteins.² Secreted and membrane proteins are synthesized by ER-bound ribosomes, cotranslationally translocated across the ER membrane, and folded in the lumen of the ER, after which they are transported to the Golgi, where they are sorted to their final destinations.^{3,4} The ER lumen is populated with chaperones, protein disulfide isomerases, protein oxidoreductases,

and other proteins that are responsible for the proper protein folding. This elaborate machinery requires an optimal ER environment for efficient protein folding. Conditions such as myocardial ischemia, hypertrophy, and heart failure alter this environment in ways that reduce protein folding, leading to the accumulation of misfolded and potentially toxic⁵ proteins that can cause ER stress.⁶

The ER stress response averts the potential proteotoxicity associated with the accumulation of misfolded proteins in the ER during ER stress.^{7–9} Three ER transmembrane proteins, protein kinase RNA-like ER kinase, inositol-requiring protein-1, and ATF6, serve as sensors of misfolded proteins in the ER as well as effectors of the response to ER stress (Figure A). Although the mechanisms by which these sensors detect misfolded proteins are not completely understood, one of the first mechanisms to be described involves the ubiquitous ER luminal chaperone, glucose-regulated protein 78 kDa (GRP78), which plays numerous roles in ER protein folding and quality control.^{10,11} When protein folding in the ER is optimal, GRP78, which has a C-terminal ER retention motif, or KDEL, binds to the ER luminal domains of the 3 proximal sensors and keeps them inactive^{12,13} (Figure A). During ER stress, GRP78 relocates from the proximal sensors to misfolded proteins (Figure B). This relocation of GRP78 has different effects on the 3 sensors. In the case of ATF6, which is a major topic of the study by Lynch et al, the relocation of GRP78 releases ATF6 from the ER, permitting its transport to the Golgi (Figure C), which is facilitated by Golgi localization signals.¹³ In the Golgi, site-1 and site-2 proteases (S1P and S2P) cleave the trans-ER membrane region of ATF6 (Figure D). This liberates the N-terminal, cytosolic domain of ATF6, which translocates to the nucleus, where it binds to and induces nearly 400 genes in the myocardium¹⁴ (Figure E); many of these genes are known to contribute to restoring ER protein folding capacity and enhancing cardioprotection¹⁵ via pleiotropic-adaptive responses¹⁴ (Figure F). Thus, the conditional interaction of ATF6 with the ER resident GRP78 is a determinant of ATF6 location and, thus, ATF6 activity. Lynch et al found that ATF6 location and activity also can be determined by its interaction with Thbs4.

The Thbs family is composed of 5 members whose expression and secretion from numerous cell types, including cardiac myocytes, are increased during pathology and tissue damage.¹⁶ Thbs1 and Thbs2 monomers can assemble as homotrimers and heterotrimers, whereas the remaining Thbs family members form homopentamers or heteropentamers. Most studies have focused on the functions of secreted Thbs, which interacts with various structural extracellular matrix proteins and contributes to matrix remodeling, during the course of pathology and responses to tissue injury. Under most conditions, Thbs is expressed at low levels in cardiac

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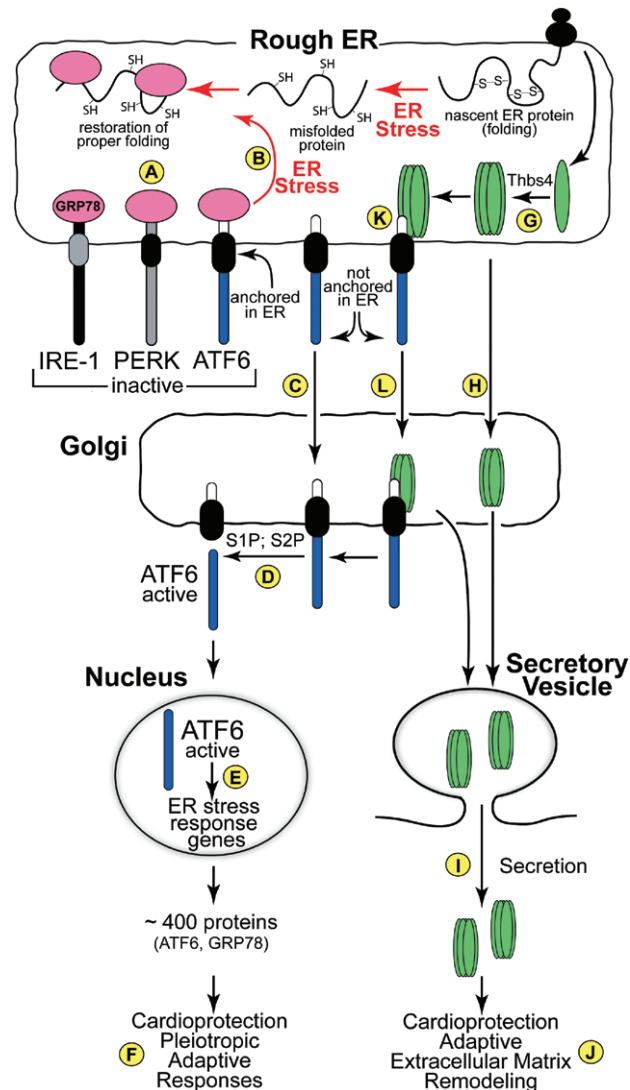


Figure. Roles of glucose-regulated protein 78 (GRP78) and thrombospondin 4 (Thbs4) in activating transcription factor 6 (ATF6) activation. The endoplasmic reticulum (ER)-transmembrane proteins, inositol-requiring protein-1 (IRE-1), protein kinase RNA-like ER kinase (PERK), and ATF6, are sensors of protein folding in the ER. When ER protein folding is optimal, the ER luminal domains of each sensor are associated with the ER chaperone, GRP78 (A). GRP78 is an ER resident protein by virtue of its C-terminal KDEL sequence, which facilitates its retrieval from the Golgi to the ER via its binding to the KDEL receptor. Because it binds to GRP78, ATF6 is anchored in the ER, which keeps ATF6 from being activated. However, when ER protein folding is impaired, for example, during ER stress (red arrows), GRP78 relocates to misfolded proteins as they accumulate (B). Under these conditions, GRP78 is no longer bound to ATF6 in the ER, which allows ATF6 to relocate to the Golgi (C). In the Golgi, ATF6 is cleaved by site-1 and site-2 proteases (S1P and S2P) (D) to form an active transcription factor that induces ER stress response genes (E), including ATF6 and GRP78, which primarily mediate pleiotropic adaptive or protective responses (F). Thrombospondin-4 (Thbs4) is synthesized as monomers in the ER, which assemble into pentamers (G) that transit from the ER lumen to the Golgi (H), then to secretory vesicles, from which Thbs4 is eventually secreted (I). Extracellular Thbs4 binds to structural extracellular matrix (ECM) proteins and modulates the ECM in response to tissue damage, contributing to cardioprotective and adaptive ECM remodeling (J). The study by Lynch et al¹ showed that Thbs4 can bind to ATF6 (K) and contribute to its activation, most likely by facilitating its translocation to the Golgi (L).

myocytes and at moderate levels in other cell types in the heart.¹⁷ Thbs1 and Thbs2 knockout studies in mice have shown that these forms of Thbs protect against myocardial infarction (MI) injury and pressure overload-induced hypertrophy.¹⁸ Thbs4 has captured recent attention because it is expressed primarily in cardiac and skeletal muscle and is further upregulated in MI, hypertrophy, and heart failure.^{19,20}

The synthesis of Thbs4 has not been studied in detail. However, by analogy to studies of other Thbs family members,^{17,21,22} it can be inferred that Thbs4 monomers (≈ 900 AA, ≈ 100 kDa each), which have no transmembrane motifs, are synthesized on ER-bound ribosomes, followed by folding and assembly into heteropentameric or homopentameric oligomers in the ER lumen (Figure G; shown as homopentamers). Because it does not have a C-terminal KDEL motif to facilitate ER retention, Thbs4 presumably moves rapidly from the ER to the Golgi (Figure H) on its way to secretion via the constitutive secretory pathway (Figure I). Constitutive secretion is a process by which secreted proteins are released from cells at rates dictated primarily by their expression levels.^{3,23} Although Thbs secretion from cardiac myocytes has not been studied in detail, Thbs has been shown to be released from endothelial cells within 60 minutes of its synthesis,²¹ consistent with rapid, constitutive secretion. After its secretion, Thbs contributes to cardioprotection through processes such as adaptive extracellular matrix remodeling (Figure, J). To fold properly, Thbs must be glycosylated. In endothelial cells, the impairment of protein glycosylation in the ER by tunicamycin leads to Thbs misfolding, which results in its retention in the ER.²¹

Targeted disruption of Thbs4 in the mouse heart has been shown to increase the maladaptive effects of pressure overload; these effects have been attributed to the absence of extracellular Thbs4.^{18,24,25} However, the study by Lynch et al represents a paradigm shift, because it identifies a new function for intracellular Thbs4, linking it to ATF6-mediated cardioprotection. To determine the role of Thbs4 in the heart, Lynch et al overexpressed Thbs4 in mouse hearts and showed that it enhanced survival and preserved cardiac function after MI. The effects of pressure overload were unclear, because there was no apparent loss of cardiac function in either control or Thbs4 transgenic mice subjected to transaortic constriction. The authors performed a gene expression analysis to examine the mechanism of Thbs4-mediated cardioprotection. Although the data were not shown, the authors concluded that many genes previously shown to be increased in the hearts of transgenic mice expressing activated ATF6 in cardiac myocytes¹⁴ also were upregulated in the hearts of Thbs4 transgenic mice. The authors showed that Thbs4 increased the levels of cleaved ATF6, as well as numerous ATF6-inducible proteins. This finding led to the authors' hypothesis that Thbs4 might exert at least some of its protective effects by facilitating ATF6

(Continued) Because Thbs4 is retained in the ER during some ER stress conditions that are known to stimulate ATF6 translocation to the Golgi, it may be that there are some conditions under which ATF6 relocation from the ER to the Golgi its activation can take place in a Thbs4-independent manner (C and D).

activation. In support of this hypothesis, the authors showed that Thbs4 interacted with ATF6. To examine whether Thbs4-mediated activation of ATF6 required Thbs4 translocation to the Golgi, they generated a form of Thbs4 with a C-terminal ER retention KDEL motif (Thbs4-KDEL), which should convert Thbs4 into an ER resident protein. Unlike native Thbs4, Thbs4-KDEL did not increase the levels of activated ATF6, presumably because it resided primarily in the ER and thus was unable to facilitate ATF6 transport to the Golgi. The study also showed that the ATF6 activation observed in the hearts of wild-type mice subjected to pressure overload-induced hypertrophy and MI was lost when Thbs4 gene expression was disrupted. Moreover, compared with wild-type mice, the hearts of the Thbs4 knockout mice exhibited reduced cardiac function and increased injury in the hypertrophy and MI models, respectively. These results are consistent with the hypothesis that Thbs4 mediates protection from cardiac pathology, at least partly, by activating ATF6.

The results of this study have broad impact because they suggest that in addition to the roles of secreted Thbs4 in extracellular matrix remodeling after secretion, intracellular Thbs4 can be cardioprotective. Moreover, because Lynch et al showed that Thbs1, which is in a different subfamily and structurally somewhat different than Thbs4, also facilitated ATF6 activation, it is apparent that other members of the Thbs family activate ATF6. In terms of determining the relative importance of intracellular and extracellular Thbs, Lynch et al showed that in contrast to overexpressed Thbs4, the addition of recombinant Thbs4 to culture media did not increase activated ATF6, consistent with the idea that only intracellular Thbs4 can bind to and activate ATF6. However, the relative contributions of intracellular and extracellular Thbs4 in the cardioprotection that were observed *in vivo* remain unknown.

As a liberator of ATF6 from the ER, Thbs4 is a counterpart of GRP78, which anchors ATF6 in the ER. The mechanism responsible for these opposing actions is likely to be complex and will require further study to delineate. However, coupled with previous studies, the study by Lynch et al supports several different possibilities. For example, because overexpressing Thbs4 activates ATF6, and since ATF6 activation requires its liberation from the ER, the overexpressed Thbs4 must liberate ATF6 from the ER by overcoming the anchoring effects of GRP78. This could happen if Thbs4 is able to physically displace GRP78 from ATF6. The relatively close proximity of the GRP78 binding sites on ATF6, which were described previously,¹³ to the Thbs4 binding sites on ATF6, described by Lynch et al, supports this hypothesis, suggesting that the winner of the competition serves as a determinant of ATF6 location. Moreover, the site on ATF6 to which Lynch et al showed Thbs4 binds previously was shown to serve as a Golgi localization sequence that is masked when GRP78 binds to ATF6.¹³ This is consistent with the hypothesis that GRP78 dislocation from ATF6 unmasks a Golgi localization sequence to which Thbs4 can bind (Figure K), which then facilitates ATF6 translocation to the Golgi (Figure L). Thus, the relative amounts of GRP78 and Thbs4 could determine whether ATF6 remains in the ER or whether it relocates to the Golgi. Furthermore, it is possible that Thbs4 overexpression contributes to ATF6

translocation to the Golgi by attracting GRP78 away from ATF6 to assist in the folding of nascent Thbs4. In support of this possibility are previous studies showing that GRP78 binds to Thbs, while it is being synthesized in the ER.^{26,27}

A provocative result from this study was the finding that overexpressed Thbs4 increased the secretion of both atrial natriuretic factor (ANF) and mesencephalic astrocyte-derived neurotrophic factor (MANF; MANF was called ARMET [arginine-rich, mutated in early stage tumors] by Lynch et al) from neonatal cardiac myocytes. The authors suggested that this may be because of the ability of Thbs4 to enhance the secretory capacity of the ER. Although this may be a contributing factor, the ability of Thbs4 to coordinately increase the secretion of both these proteins is unexpected, because ANF and MANF are secreted under different conditions and via different mechanisms. In the case of ANF, because its secretion from ventricular myocytes is constitutive and therefore dictated primarily by its expression levels, increased levels of secreted ANF are usually a reflection of increased cellular levels of ANF.²⁸ Thus, perhaps Thbs4 overexpression increased cellular ANF, which resulted in increased secretion. In contrast to ANF, MANF secretion from ventricular myocytes is not constitutive because like ATF6, MANF binding to GRP78 conditionally retains MANF in the ER. Disruption of this binding has been shown to release MANF from the ER and, because it has no transmembrane domains, MANF is secreted.²⁹ Similar to ATF6, it is the conditional association of MANF with GRP78 that dictates its location. Accordingly, it may be that Thbs4 increases MANF secretion by liberating it from the ER-anchoring effects of GRP78, much like it does with ATF6.

The study by Lynch et al examined ATF6 activation by Thbs4 under basal conditions but did not address whether Thbs4 is required for the activation of ATF6 during activation of the canonical ER stress response. However, previous studies have shown that ER stress decreases Thbs exit from the ER and secretion^{21,30} accordingly, the binding of Thbs4 to ATF6 may not always be required for ATF6 translocation and activation. Thbs4-mediated activation of ATF6 in the absence of ER stress is consistent with the hypothesis that ATF6 may have functions beyond its known roles as a first responder to ER protein misfolding.^{31,32} In support of this hypothesis is a study by Wu et al,³³ which showed that ATF6 was activated during physiological skeletal muscle exercise, and that this activation was required for metabolic adaptation to exercise training. This supports roles for ATF6-mediated adaptive responses beyond those that take place during acute ER protein misfolding. Perhaps, like it does in skeletal muscle, ATF6 contributes to adaptive physiological responses in cardiac muscle that are not necessarily associated with overt activation of ER stress. Moreover, the findings reported by this study raise awareness of the potentially beneficial effects of Thbs4 and ATF6 in treating a vast array of pathologies associated with protein misfolding in the ER.

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Disclosures

None.

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Correction

In the *Circulation Research* article by Doroudgar and Glembotski (Doroudgar S, Glembotski CC. Activating of transcription factor 6 and thrombospondin 4: the dynamic duo of the adaptive endoplasmic reticulum stress response. *Circ Res.* 2013;112:9–12. DOI: 10.1161/CIRCRESAHA.112.280560), the title of the article should have been: ATF6 and Thrombospondin 4: The Dynamic Duo of the Adaptive Endoplasmic Reticulum Stress Response.

This error has been corrected in the online version of the article, which is available at <http://circres.ahajournals.org/content/112/1/9.full>.

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