Crosstalk Between the Endoplasmic Reticulum and mTOR Signaling

How Stress Makes Our Hearts Grow Bigger

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The majority of adult cardiac myocytes are terminally differentiated cells and thus not able to proliferate. Still, they retain some plasticity, which allows them to grow in response to various physiological and pathological stimuli. This complex growth process, termed cardiac hypertrophy, is a major determinant of heart adaptation to environmental cues.[1] To support their growth, cardiac myocytes must increase the production of proteins, lipids, and nucleotides, and at the same time also suppress catabolic pathways, such as autophagy. mTORC-1 (mammalian target of rapamycin complex 1) plays a central role in regulating all these processes and therefore controls the balance between anabolism and catabolism in response to environmental conditions.[2] The mTOR pathway becomes activated in response to physiological stimuli, such as physical exercise, and pathological stimuli, like pressure overload, and leads to a global increase of protein synthesis and cardiac growth. On the contrary, pharmacological or genetic inhibition of mTORC-1 blocks hypertrophic response and preserves cardiac function,[3] indicating that mTORC-1 inhibition is beneficial for the myocardium. Although the importance of mTOR signaling in promoting (heart) muscle growth is well appreciated by basic scientists and bodybuilders alike, the upstream regulatory mechanisms, that are responsible for the activation of mTORC-1, are not completely understood.

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Clearly, global increase in protein quantity should be counterbalanced by cellular ability to properly fold these proteins. Primary site of protein synthesis, folding, and secretion in eukaryotic cells is the endoplasmic reticulum (ER). Large amounts of newly synthesized proteins pose a higher demand on protein quality control machinery and can cause ER stress, which is known to globally inhibit protein synthesis.[4] Remarkably, a crosstalk between ER stress and mTORC-1 signaling during cardiac growth response is largely unknown. A well-designed study by Blackwood et al[5] in this issue of *Circulation Research* now establishes a new signaling axis between ER stress and mTORC-1 activation, explaining how proteostasis is achieved during hypertrophic growth.

Accumulation of misfolded proteins in the ER is sensed by ATF-6 (activating transcription factor 6), an ER-transmembrane protein, which is then translocated to the Golgi apparatus and cleaved by site-1 and site-2 proteases. The resulting 50 kDa cytosolic fragment of ATF-6 has a DNA-binding domain and a transcriptional activation domain and is subsequently translocated to the nucleus.[6] There, it binds to ER stress response elements of numerous genes involved in resolving ER stress and restoring ER homeostasis.[7]

ATF-6 has been particularly well studied in the context of myocardial ischemia/reperfusion; a condition known to affect protein misfolding in the ER and cause ER stress.[8,9] In such a setting, activated ATF-6 was demonstrated to increase the expression of ER-resident proteins that help restore ER proteostasis. Furthermore, it also induces the transcription of many antioxidative stress genes, thereby reducing the ischemia/reperfusion damage in the myocardium.[10] However, the role of ATF-6 has not been addressed yet in the context of cardiac hypertrophy, which also causes ER perturbations and imbalanced proteostasis.[11]

To address this issue, Blackwood et al[5] performed transverse aortic constriction (TAC) in mice, which surprisingly increased the protein levels of activated 50 kDa ATF-6 in mouse hearts. Before this observation, ATF-6 was not known to be induced by any growth stimulus in the myocardium. The authors also generated cardiac-specific *Atf6* conditional knockout mice, which in contrast to control mice, exhibit blunted heart growth and impaired fractional shortening after TAC. Similar results were also observed in mice subjected to exercise; 4-week voluntary wheel running activated ATF-6 and increased heart weight in control, but not *Atf6* conditional knockout mice. ATF-6 can thus be activated by both pressure overload and exercise and is required for cardiac growth.

To identify ATF-6 target genes required for cardiac growth, transcriptome profiling was performed on mice that express activated ATF-6. One of the candidate genes that emerged from this sequencing approach was *RHEB* (Ras homologue enriched in brain), a small GTPase that activates mTORC-1 in response to growth stimuli. Overexpression of ATF-6 was sufficient to induce RHEB expression in myocardium by binding 2 ER stress response elements in its promoter region. RHEB was also shown to be induced by TAC and exercise in control mice, but not in *Atf6* conditional knockout mice hearts, thus proving that ATF-6 is necessary for its upregulation during cardiac hypertrophy.

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Because RHEB is a known mTORC-1 activator, the authors then investigated the mTOR pathway in their knockout model after TAC and observed a substantial decrease in the activation of this central signaling hub. They followed up on this exciting finding with comprehensive loss-of-function and gain-of-function experiments and convincingly showed that RHEB is both necessary and sufficient for cellular growth in vitro. Finally, ectopic expression of RHEB also restored cardiac growth in *Atf6* conditional knockout mice by enhancing mTORC-1 signaling in vivo.

Mechanistically, ATF-6 has been previously shown to induce various genes; some of them reside in the ER (eg, GRP78 [78 kDa glucose-regulated protein]) and some reside in other organelles (eg, catalase, localized to peroxisomes, or RHEB, localized to lysosomes). Investigating whether these genes are differentially regulated by various ER stress-inducing treatments revealed that their upregulation is fascinatingly very selective—RHEB was highly induced only by the growth stimulus and not by oxidative stress, whereas just the opposite was observed for catalase. This treatment-specific induction was ATF-6–dependent and relied entirely on its binding to ER stress response elements of the abovementioned genes.

Finally, the authors propose a timeline of molecular events that culminate in ATF-6/RHEB/mTORC-1 induction. The initial event is a growth stimulus (eg, TAC), which in a very short time leads to mTORC-1 activation. Subsequent increases in protein synthesis and protein folding demand activate ATF-6, which is responsible for transcriptional upregulation of canonical ER-resident chaperones. In addition, ATF-6 also enhances RHEB expression, which further supports mTORC-1 activation and sustained protein synthesis during cardiac growth (Figure).

This is the first study in a cardiac setting, which connects the transcriptional induction mediated by ATF-6 to growth-promoting mTORC-1 functions. A similar link has been found before in cancer cells,[12] supporting a general mechanism in various cellular systems. The discovery of ATF-6-RHEB-mTORC-1 signaling axis thus expands the complexity of cardiac proteostasis but also poses additional questions. What remains unanswered is the mechanism by which mTORC-1 is initially activated during the hypertrophic response to pressure overload. What molecular events are preceding its activation and how can they occur so rapidly after a growth stimulus? Studies in skeletal muscle provide evidence that increased contraction itself activates mTORC-1, thus explaining how increased muscle use promotes protein anabolism.[13] Although outcomes of physiological and pathological hypertrophy are very different, they both seem to activate ATF-6 and mTORC-1. Future research in this field should therefore improve our understanding of how mTORC-1 integrates distinct signals like insulin, amino acids, mechanical force, and ER stress reported in the current article.

Furthermore, the detailed consequences of ATF-6–dependent mTORC-1 activation on canonical mTOR functions, such as protein synthesis, nucleotide biogenesis, metabolism, or autophagy, remain unclear. Because it is now generally accepted that mTORC-1 controls the translation of specific mRNA networks,[14] it would be exciting to define

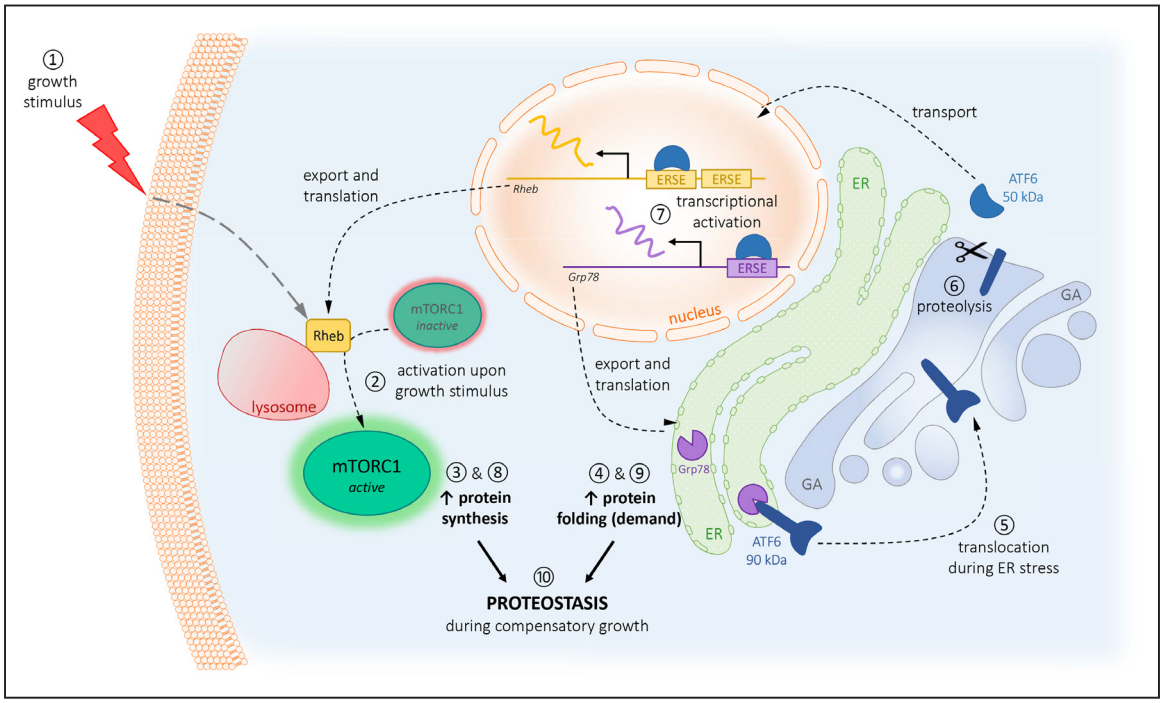

Figure. ATF-6 (activating transcription factor 6) induces the transcription of mTORC-1 (mammalian target of rapamycin complex 1) activator RHEB (Ras homologue enriched in brain) and mediates compensatory cardiac growth. Growth stimuli (1) activate mTORC-1 (2), resulting in the increase of both protein synthesis (3) and protein-folding demand (4). The latter causes endoplasmic reticulum (ER) stress, which leads to the translocation of ATF-6 to Golgi apparatus (GA; 5), where it undergoes regulated proteolysis (6). The released cytosolic fragment of ATF-6 functions as a transcription factor (7) and induces the expression of RHEB, thereby promoting mTORC-1 activation and protein synthesis required for cellular growth (8). ATF-6 also induces its canonical target genes, like GRP78 (78 kDa glucose-regulated protein), which support protein folding (9). Thus, protein synthesis and folding combined contribute to proteostasis during compensatory cardiac growth (10). ERSE indicates ER stress response element.

translationally regulated transcripts downstream of the AFT-6-RHEB-mTORC-1 axis during pathological and physiological remodeling.

Moreover, the proposed mechanism of ATF-6/RHEB/mTORC-1 induction represents a positive feedback loop, suggesting there could be an additional molecular mechanism, which restricts cardiac growth in time. Although the findings of this article were restricted to compensatory cardiac growth, it would be worthwhile to explore the same mechanism and its therapeutic potential in pathological hypertrophy, which often precedes heart failure.

Finally, how does ATF-6 selectively activate the appropriate genes depending on the type of cellular stress? Results reported here clearly suggest that growth stress results in preferential binding of ATF-6 to *RHEB* promoter region, causing increased transcription of *RHEB*, which is necessary for supporting cardiac growth. In contrast, oxidative stress does not result in the increased binding of ER stress response elements in RHEB transcript. Additional mechanisms and still unidentified interaction partners of ATF-6 could be responsible for its stimulus-dependent binding to DNA. Discovering such interacting proteins will shed additional light on the complex interplay of protein synthesis, ER stress, and gene transcription.

Though several mechanistic questions remain to be addressed, the study presented here provides fundamental evidence that ATF-6 is activated in the heart by various growth stimuli. Because ATF-6 can transcriptionally induce RHEB, an mTORC-1 activator, the authors also define a noncanonical growth-promoting function of ATF-6.

Studying the biology of ATF-6-RHEB-mTOR signaling in the myocardium can potentially reveal novel ways to treat dysregulated molecular signaling in cardiovascular diseases. Considering that the field of combined mTOR and ER stress research is still in its early stages, significant progress most likely awaits.

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Disclosures

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