An expanded role for AMP kinase: self-renewal of the cardiomyocyte

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Abstract

Our work on atrophic remodeling of the heart has caused us to appreciate a simple principle in biology: from the cell cycle to the Krebs cycle, there is no life without cycles. While the potential for cellular regeneration receives much attention, the dynamics of intracellular protein turnover have received only selective consideration. Although the concept of the "dynamic state of body constituents" has existed since the 1940s, the idea that heart muscle cells renew themselves from within is relatively new. The rationale is as follows. For the last 30 years, we (and many others) have elucidated the interaction of metabolic pathways for energy provision and contraction of the heart. Work in the field has uncovered novel metabolic regulators of enzyme action, yet much less attention has been given to the impact of myocardial energy metabolism on myocardial protein turnover. We therefore began to consider metabolic signals as putative regulators of myocardial protein synthesis and degradation. In a broad sense, we sought to establish mechanisms underlying the self-renewal of intact cardiomyocytes, because we have observed that atrophic remodeling of the heart simultaneously activates pathways of intracellular protein synthesis and degradation. We determined how metabolic signals regulate protein degradation, and tested the hypothesis that there is a direct link between intermediary metabolism and protein degradation and that the specific molecular mechanisms involve 5′ AMP-activated protein kinase (AMPK) regulation of ubiquitin ligases. We review our first results on metabolic signals as regulators of myocardial protein turnover that seek to broaden the role energy substrate metabolism from a provider of ATP to a regulator of self-renewal of the cardiomyocyte.

Keywords: AMPK; protein degradation; cardiac metabolism

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Introduction

Our work on atrophic remodeling of the heart has led us to appreciate a simple principle in biology: from the cell cycle to the Krebs cycle, there is no life without cycles. While *cellular* regeneration of the heart receives much attention [1], the dynamics of *intracellular* protein turnover have received only selective consideration [2]. Undoubtedly stem (or precursor) cells contribute to the replacement of cardiomyocytes after injury, but they contribute little to cardiomyocyte renewal during normal aging [3]. Although Schoenheimer's concept of the "dynamic state of body constituents" has existed for some time [4], the idea that heart muscle cells renew themselves from within is relatively new. We will begin to address this concept with a review on the

transcriptional role for 5′ AMP-activated protein kinase (AMPK) on protein degradation pathways.

For the last 50 years many laboratories have elucidated the interaction of metabolic pathways for energy provision and contraction in the heart. Work in the field has uncovered novel metabolic regulators of enzyme action, yet the impact of myocardial energy metabolism on myocardial protein turnover has not been considered. After we discovered that cardiac atropohy is not a simple mirror image of hypertrophy [5], we are now proposing that metabolic signals are putative regulators of myocardial protein synthesis and degradation. In a broad sense we seek to establish mechanisms underlying the self-renewal of the intact cardiomyocyte. The rationale arises from our observation that atrophic remodeling of the heart simultaneously activates pathways of intracellular protein synthesis and degradation [5] and the following considerations.

First, a large number of models already exist that identify molecular targets of myocardial hypertrophy and atrophy [6,7]. Secondly, metabolism is the first responder to any form of stress [8]. We have evidence suggesting that the process of metabolic remodeling precedes, triggers and sustains both structural and functional remodeling of the heart [9]. We propose that modulation of metabolic stresses provides a means to remove damaged or redundant proteins and replace them with new, functional proteins (Fig. 1). Furthermore, the identification of metabolic signals which govern cardiac remodeling will set us also on the path to develop novel strategies aiming at specific metabolic intermediaries as modulators of cardiomyocyte size.

Intracellular protein turnover in perspective

The depressing statistics on heart failure are widely known [10]. Yet in spite of broad and formidable efforts, there is no cure in sight because the cellular and molecular mechanisms are still not completely understood. Accepted features in the development of heart failure are cardiac hypertrophy and impaired ATP production, which develop in response to both endogenous (genetic) and exogenous (environmental) changes. We propose that metabolic remodeling (which is potentially reversible) precedes, triggers and sustains structural and functional remodeling [11]. In order for the heart to adapt to various types of stress, individual heart muscle cells change or "remodel" both

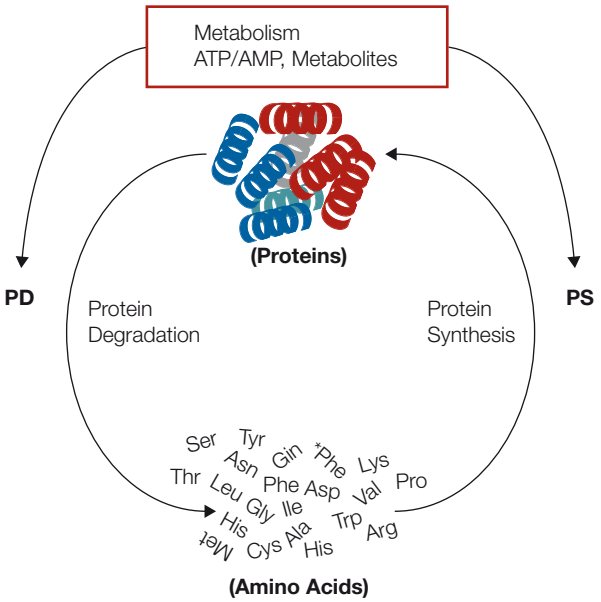

Fig. 1 *The balance of protein synthesis (PS) and degradation (PD) determines size and function of cardiomyocytes. Damaged, misfolded, or useless proteins are degraded to amino acids that are used for the synthesis of new, functional proteins.*

metabolically and structurally. Excessive remodeling results in the enlargement of cardiomyocytes, which translates into an overall increase in heart size. The transition from hypertrophy to heart failure, or the transition from adaptation to maladaptation of the heart, remains elusive. Consequently it seems to us critical to know more about the mechanisms that control the rebuilding of the cardiomyocyte.

Our most recent ideas advance a new understanding of cardiac metabolism as an integral part of the self-renewing myocyte as highlighted below. These ideas result from our investigations on switching of metabolic genes and atrophic remodeling of the cardiomyocytes in response to mechanical unloading.

We are operating under the premise that during steady state conditions rates of myocardial protein synthesis (PS) and protein degradation (PD) are equal [12,13]. The intrinsic mechanism of self-renewal of the cardiomyocyte requires the regulated degradation of damaged, misfolded, or useless proteins and their replacement by new and functional proteins (Fig. 1). Protein turnover therefore constitutes a major line of defense for protein quality control of the cardiomyocytes [14]. The rate of myocardial protein turnover is much faster than it is generally assumed, with the half-life of individual myocardial proteins ranging from several hours to several days [12]. The term "self-

renewal of the cardiomyocyte" gives exciting new meaning to the concept of "cardiac plasticity" [6].

We do not know at present to what extent biochemical signals regulate protein degradation and protein synthesis. We have preliminary evidence which suggests that metabolic signals, i.e., changes in intracellular metabolite levels in response to stress, may activate pathways of protein degradation and protein synthesis [5,15].

Lastly, and perhaps most importantly, for nearly a century the study of cardiac metabolism has concerned itself with energy substrate metabolism and contraction of the heart [16,17]. This focus has culminated in a recent review proclaiming that the failing heart is an "engine out of fuel" [18]. We have questioned this concept because the non-ischemic, failing heart is always well supplied with nutrients, and the heart is actually drowning in fuel [19]. Not surprisingly, attempts to restore normal contractile function in the failing heart by metabolic interventions have not been consistently successful [20,21]. It is much more likely that intermediary metabolism, rather than impaired fuel supply, is the culprit. We consider altered fuel metabolism (leading to either a decrease or an increase of certain metabolic signals) as a root cause for altered rates of intracellular protein turnover and, hence, self-renewal of the cardiomyocyte.

Taken together, we propose that the "metabolic" approach to myocardial protein synthesis and degradation provides a new framework that will expose new regulators driving self-renewal of cardiomyocytes from within. We focus on the ubiquitin proteasome system (UPS).

The ubiquitin proteasome system and AMPK

Intracellular protein degradation is a complex and highly controlled process that is integrated with the environment of the cell. We have recently identified a metabolic signal that regulates protein degradation in the heart and the corresponding mechanisms by which it does so, specifically pertaining to the ubiquitin proteasome system (UPS). Our studies suggest that the adenine nucleotides ATP and AMP are metabolic signals that regulate protein degradation. AMP-activated protein kinase (AMPK) supports energy provision in the cell by sensing changes in the ratio [ATP]:[AMP]. Therefore, our working hypothesis was that metabolic signals (decrease in [ATP]:[AMP]) and the subsequent activation of AMPK, regulate protein degradation. We have tested the hypothesis by modulating AMPK *in vitro* and *in vivo* to define the mechanisms by which AMPK is involved in protein degradation [22].

Intracellular protein degradation in cardiomyocytes is controlled by independent but interrelated processes: UPS-mediated proteolysis and autophagy. While autophagy can degrade whole organelles, individual proteins are degraded through the UPS [13]. Ubiquitin ligases confer specificity to the system by the selective ubiquitination of target proteins which are then degraded by the proteasome [2]. Two muscle-specific ubiquitin ligases, muscle atrophy F-box (MAFbx/atrogin-1) and muscle ring finger-1 (MuRF1), are critical regulators of cardiac protein degradation and myocardial mass. Studies *in vivo* have demonstrated that overexpressing atrogin-1 in the heart attenuates the development of hypertrophy [23], while the deletion of MuRF1 results in increased hypertrophy [24]. These experiments highlight the importance of atrogin-1 and MuRF1 in regulating heart size. However, the mechanisms by which the ligases themselves are regulated are not completely understood.

Early studies in the heart *in vivo* demonstrated that nutrient deprivation decreases protein synthesis and increases fractional rates of protein degradation [25]. Starvation decreases the intracellular concentration of ATP and, consequently, AMPK is activated in order to provide energy to maintain normal cellular function. It is well established that AMPK regulates energy substrate metabolism, inhibits protein synthesis [26], and regulates transcription of metabolic genes [27]. Although it has recently been reported that starvation induces autophagy in cardiomyocytes through AMPK [28], a role of AMPK in the cardiac UPS had never been considered before.

In order to investigate the role of AMPK in the UPS, we first verified that substrate deprivation in cardiomyocytes (CM) enhances protein degradation (PD), as has been shown already *in vivo* [25]. Protein degradation was enhanced in CM during starvation, but decreased with bortezomib, a proteasome inhibitor, or with 3-methyladenine (3-MA), an inhibitor of autophagy. These results suggest that, like autophagy [28], proteasome-mediated degradation is important during nutrient starvation in CM. Given the importance of atrogin-1 and MuRF1 in regulating protein degradation

and cardiac size [23,24], we quantified their expression in parallel experiments. Atrogin-1 and MuRF1 levels were significantly increased with starvation, which also correlated with enhanced AMPK activity (Fig. 2). We also found that direct AMPK activation, independent of nutrient starvation, increased both atrogin-1 and MuRF1 expression, which was significantly impaired with AMPK inhibition. Consequently, protein degradation in the heart is increased with AMPK activation, but proteasome-mediated protein degradation downstream of AMPK requires MuRF1 [22]. The conclusions are shown in the schematic (Fig. 3).

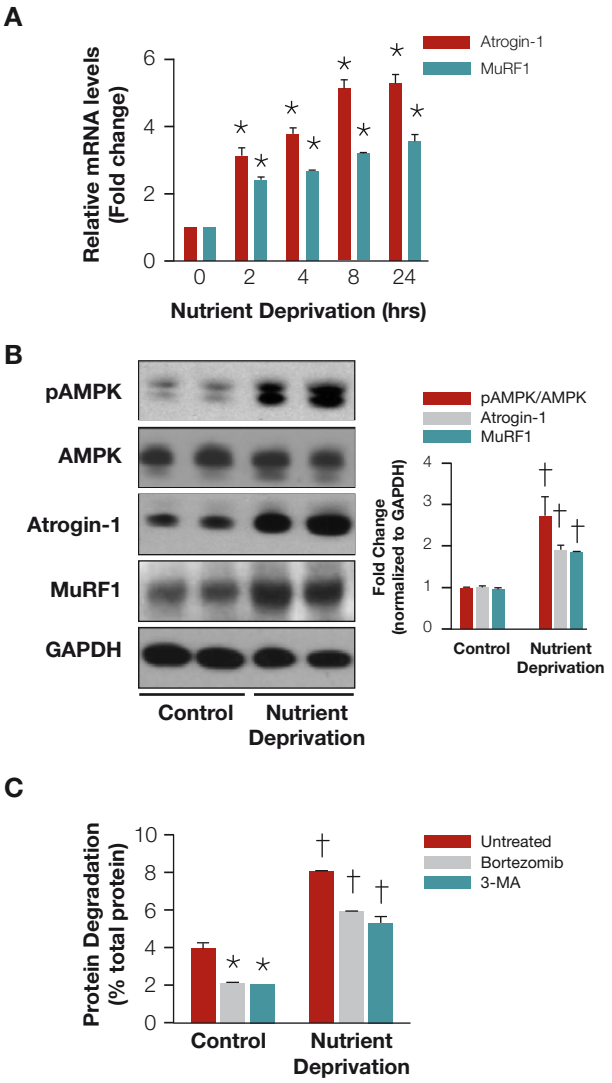

Fig. 2 *Nutrient deprivation increases expression of ubiquitin ligases and enhances protein degradation. (A) Atrogin-1 and MuRF1 mRNA expression in nutrient deprived NRVM. (B) Relative protein levels and quantification in NRVM after 24 hours of nutrient deprivation. (C) Protein degradation in NRVM after 24 hours of nutrient deprivation with 1μmol/L Bortezomib or 10μmol/L 3-methyladenine treatment. Data are mean ± SEM of 3 independent experiments performed in triplicate. *P<0.01 vs control or untreated, †P<0.01 vs control or complete nutrients (reprinted with permission from [22] ©2011 Wolters Kluwer Health).*

Perspective

We have shown that AMPK regulates ubiquitin ligases in the rodent heart. The present work extends the long established concept of the "dynamic state of body constituents" [4] to a specific situation when the heart adapts to changes in its metabolic environment. Protein turnover constitutes a major line of defense for protein quality control of the cardiomyocytes [14] and is a major mechanism of adaptation in the heart. Therefore it is of interest to understand how protein degradation is regulated under various circumstances in the heart. Markers of the UPS are upregulated in the heart in several settings of cardiac remodeling [13], but it is not clear exactly how the markers themselves are regulated. AMPK regulates cellular homeostasis in part by inhibiting the mTOR pathway [26] and thus by decreasing protein synthesis, while at the same time AMPK activates autophagy [28].

It is well known that AMPK is a central regulator of fuel homeostasis, but studies have until now predominantly focused on the effects of AMPK activation on energy substrate metabolism [29]. The active subunit of AMPK is highly expressed in the heart, and is preferentially localized to the nucleus [30]. It is therefore not surprising that AMPK also transcriptionally regulates metabolic gene expression. Earlier reports in liver show that AMPK activation represses transcription, but little is known about AMPK-regulated transcription in the heart. AMPK activates transcription [27], and the activation of PGC1α by AMPK leads to increased mitochondrial gene expression [31]. Still, the importance of AMPK in transcription is only now coming into focus. AMPK regulates entire transcriptional programs, and not only transcription of individual genes, by regulating histone 2B [32]. We have now expanded the role of AMPK in both cellular homeostasis and transcriptional regulation in the heart [22].

The AMPK activator and anti-diabetic drug metformin has proven to have beneficial outcomes in heart failure patients with diabetes [21]. The role of protein turnover in hearts of these patients could not be investigated. However, based on our experimental findings, AMPK-regulated protein degradation may be

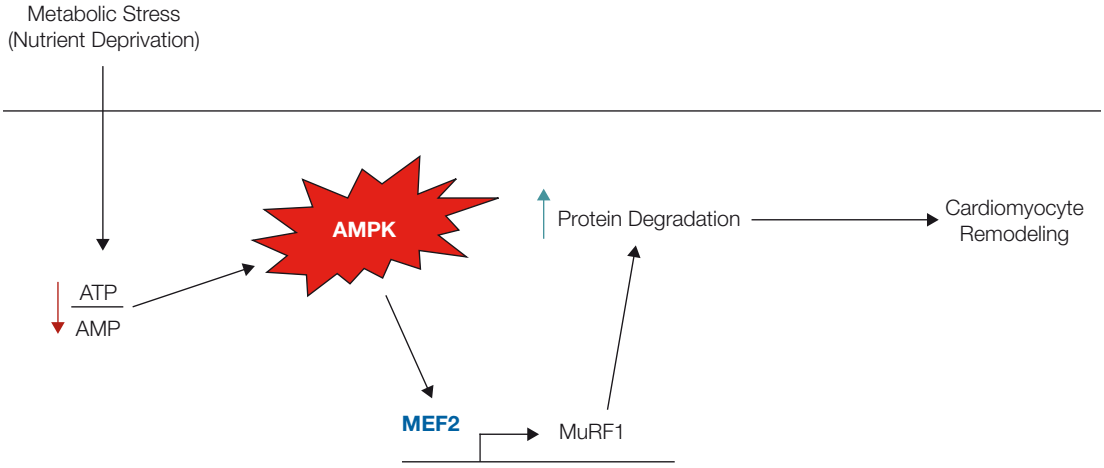

Fig. 3 *AMPK regulates MuRF1 transcription in a MEF2-dependent manner. This leads to increased protein degradation in the cardiomyocyte and increased remodeling (reprinted with permission from [22] ©2011 Wolters Kluwer Health).*

protective because of enhanced protein quality control [14]. Activation of AMPK results in increased rates of protein degradation, and consequently leads to remodeling of the heart. The immediate cardiometabolic environment may determine whether the remodeling is beneficial or detrimental. We speculate that the activation of AMPK results in enhanced availability of intracellular amino acids for either ATP production or the synthesis of new proteins as the heart adapts to a new physiologic state. This self-renewal of the cardiomyocytes would mean an expanded role for 5′ AMP-activated protein kinase in the heart. •

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