

The authors next examined how PGC-1 α might affect expression of utrophin^{5,7}. A cytoskeletal protein located at the adult NMJ, utrophin has a high degree of sequence similarity to dystrophin. Raising utrophin levels is a potential approach to the treatment of DMD¹¹. In the *mdx* mouse, the mouse model of DMD, a two- to threefold increase in the expression of utrophin ameliorates pathology.

Previous studies have shown that promoter activity and levels of utrophin are increased by transfection of PGC-1 α into muscle fibers *in vitro* and *in vivo*⁷. Handschin *et al.* proposed that PGC-1 α may be a useful target in DMD therapies^{5,7}. To test this hypothesis, the authors crossed a transgenic mouse line overexpressing PGC-1 α with the *mdx* mouse and observed a substantial reduction in the number of nuclei centrally located in muscle fibers, a marker of regeneration. At five weeks of age, the muscle of the transgenic *mdx* mice showed less damage than muscle from the *mdx* mice. Creatine kinase levels in the serum of the transgenic *mdx* mice were reduced by 55% compared to *mdx* mice, also indicating much less muscle damage. The transgenic *mdx* mice were also

more tolerant of exercise, confirming that these muscles were more functional.

These effects were partly due to the increased levels of utrophin, but Handschin *et al.* suggest that there may also be other beneficial effects of PGC-1 α in the *mdx* mice. It has been shown that PGC-1 α overexpressing transgenic mice show less atrophy after damage experimentally induced by nerve cutting and PGC-1 α may help correct the metabolic crisis in dystrophin-deficient cells by increasing the number of mitochondria⁶. It is likely, however, that utrophin expression, rather than generalized NMJ gene activation, is the main therapeutic target of PGC-1 α activity in the transgenic *mdx* mice, as NMJ abnormalities are not often associated with DMD. While further studies involving the crossing of the transgenic line with the utrophin/dystrophin-null mice are needed to determine how much of the effect can be attributed to increased utrophin levels, increasing PGC-1 α expression seems a promising strategy for DMD therapy.

One approach would be to target PPAR transcription factors, which are known to regulate PGC-1 α and are the targets of many approved drugs¹². It remains to be seen which PPAR pro-

tein (α , β , or γ) controls the level of PGC-1 α and can easily be targeted by drugs. The currently available agonists of certain PPAR forms could easily be tested on the *mdx* mice.

Although the approach of Handschin *et al.* does not completely rescue the *mdx* mouse⁵ targeting PGC-1 α , combined with other pharmacological approaches⁴, holds new hope for individuals with DMD.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/naturemedicine/.

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Eat your heart out

Richard N Kitsis, Chang-Fu Peng & Ana Maria Cuervo

The presence of autophagic morphology in failing heart muscle cells has suggested that autophagy causes heart failure. Instead, it seems that the opposite is true: autophagy is critical for normal heart function (pages 619–624).

Heart failure is a common and lethal condition, yet why the heart muscle fails remains a mystery. Alterations in myocardial metabolism, defects in calcium handling, alterations in myosin isoforms, cytoskeletal abnormalities and exces-

sive cardiac myocyte apoptosis have all been implicated as causal factors. Macroautophagy (referred to herein as autophagy, from the Greek meaning ‘self-eating’) is a cellular process in which proteins and organelles are catabolized in lysosomes to provide cells with amino acids and energy (ref. 1 and **Fig. 1**). In this way, autophagy helps cells survive starvation and stress. On the other hand, an association between autophagic morphology and cell death in diseased tissues has begged the question as to whether autophagy can cause cell death². Several studies have noted autophagy in failing hearts^{3,4}. It is unclear, however, whether this process is a cause of heart failure, a compensatory mechanism or merely an epiphenomenon. Nakai *et al.* investigated these possibilities using mice defective for autophagy exclusively in cardiac myocytes⁵. The data indicate that, rather than causing heart failure, autophagy is critical for normal cardiac function.

To study the role of autophagy in normal and failing heart physiology, Nakai *et al.* used mice with conditional knockout for the autophagy-related gene 5 (*Atg5*)⁵. The *Atg5* protein is essential for the formation of the autophagosome, the double-membrane vesicle that carries proteins and organelles destined for autophagy to the lysosome (**Fig. 1**). The authors first removed *Atg5* from cardiac myocytes in adult mice; as expected, these cells exhibited reduced autophagy. Within several days of gene deletion, the animals developed severe heart failure characterized by left ventricular enlargement, contractile dysfunction, abnormal calcium transients and transition to the fetal program of gene expression. In addition, *Atg5*-deficient cardiac myocytes underwent hypertrophy, an enlargement in cell size. It is unclear, however, whether this hypertrophy resulted from reduced autophagy or occurred in response to the deterioration of contractile function.

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These data show that cardiac myocyte autophagy is required for normal cellular function in the basal state. It remains unknown, however, why the heart fails when autophagy is suddenly reduced. Possibilities include the loss of amino acids and energy, and the accumulation of abnormal proteins and organelles. In fact, Nakai *et al.* describe abnormal sarcomeres and mitochondria, accumulation of markers of ER stress, poly-ubiquitinated proteins, and apoptosis.

Having shown that the heart fails when autophagy is suddenly reduced in adulthood, the authors next asked whether autophagy is required throughout heart development. To answer this question, they deleted *Atg5* on embryonic day 8, when the heart is still a straight tube. Interestingly, the embryonic knockout mice were born and lived to adulthood without any detectable heart abnormalities, presumably due to compensatory mechanisms which also perform cellular maintenance.

Noting the reduced capacity for autophagy, Nakai *et al.* considered whether the hearts of the embryonic *Atg5* knockout mice were more susceptible to stress. The investigators tested this possibility by subjecting these mice to experimental models of heart failure. First, they performed surgical constriction of the aorta, which simulates the effects of high blood pressure on the heart. Aortic constriction precipitated fulminant and lethal heart failure in *Atg5* knockout mice, compared to controls. The authors found, however, that *Atg5* knockouts and wild-type mice exhibited similar amounts of cardiac hypertrophy resulting from aortic constriction. This suggests that autophagy is not critical for hypertrophy in this model and is consistent with the observation that autophagy is suppressed during the development of hypertrophy even in wild-type mice. In a second experimental model in which heart failure is induced by β -adrenergic stimulation, *Atg5* knockouts also exhibited marked cardiac functional deterioration compared to controls. Although it remains unclear exactly how the inhibition of autophagy exacerbates heart failure in these models, the fact that mice with reduced autophagy did worse—not better—than wild-type mice provides strong evidence that autophagy is a protective, rather than pathogenic, mechanism in this syndrome.

This study addresses a longstanding question concerning the role of autophagy in the pathogenesis of heart failure. A protective role for autophagy has also been observed in models of ischemia and ischemia-reperfusion in cultured heart cells^{6,7}. The situation may be more complicated in intact animals, however,

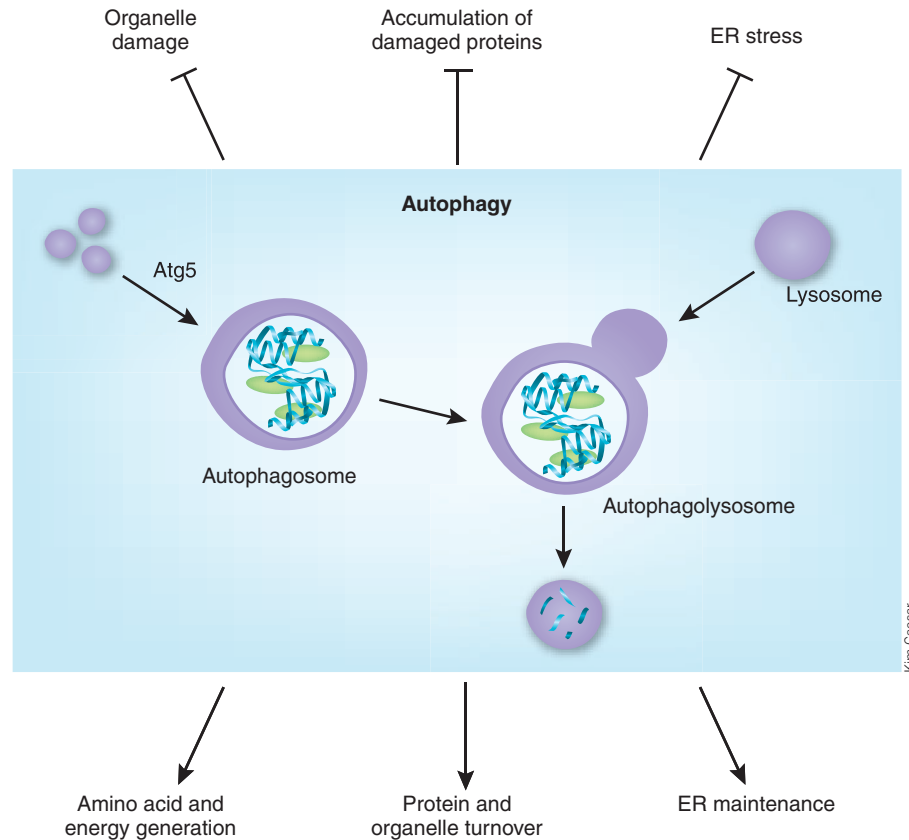


Figure 1 Autophagy is an intracellular process in which proteins and organelles are transported in double-membrane vesicles called autophagosomes through the cytoplasm to lysosomes for degradation. Autophagy disposes of damaged proteins and organelles, which can injure the cell. In addition, it generates amino acids and energy that are used by cells under conditions of starvation and stress. Deletion of *Atg5*, which encodes a protein essential for the formation of autophagosomes, inhibits autophagy and the ability of cells to respond to the energy requirements and to clear cellular components during stress conditions.

as autophagy appears to be protective during myocardial ischemia but deleterious during reperfusion⁷.

A key point of the current study is that autophagy in the heart plays important roles not merely during stress, but also in the basal state⁵. This notion is consistent with observations that inhibition of autophagy in the liver⁸ and brain^{9,10} elicits marked cellular damage and death, even in the absence of a stressor. These data support a role for autophagy in cellular housekeeping under basal conditions in diverse tissues.

One of the most interesting features of this work is the observation that inhibition of cardiac myocyte autophagy beginning in the embryo is tolerated through adulthood, while inhibition of autophagy beginning in adulthood results in massive cell damage. It is unclear which compensatory mechanisms may operate during embryonic heart development when autophagy is inhibited, but they could include upregulation of other autophagic or nonautophagic proteolytic pathways^{11,12}.

Defects in autophagy can damage heart muscle by several distinct mechanisms, depending on which step is impaired. For example, the inability to form autophagosomes by *Atg5*-deficient cells leaves damaged cellular components in the cytosol where they might further injure organelles. In contrast, in the cardiomyopathy of Danon's disease¹³, autophagosomes form but lysosomal clearance is compromised. Despite the successful sequestration of damaged cellular components within autophagosomes, the accumulation of these vesicles perturbs the cytoskeleton, causing a severe cardiomyopathy. Thus, the failure to either initiate autophagy or to complete the process can result in heart failure.

Our understanding of the relationship between autophagy and heart disease is still in its infancy and many issues remain poorly defined. We know from the study by Nakai *et al.* that autophagy protects from heart failure, although we do not know precisely how. These data raise the possibility that manipulation of

autophagy might be exploited to therapeutic advantage in failing hearts. Augmentation of autophagy might be beneficial when autophagosome clearance is normal. Inhibition of autophagosome formation, on the other hand, might lessen the burden of autophagosome accumulation in patients with defective clearance such as those with the cardiomyopathy of Danon's disease.

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MicroRNAs loom large in the heart

How microRNAs (miRNAs) influence heart development and disease is a topic of growing interest. miRNAs regulate gene expression post-transcriptionally, typically by binding to mRNAs and inhibiting their translation. Three recent papers show that miRNAs are essential for heart development and function, regulating the expression of genes involved in electrical conductance, hypertrophy and contractility.

In mice with deletion of the muscle-specific miRNA *miR-1-2*, Zhao *et al.* observed a range of heart abnormalities (*Cell* **129**, 1–15). Some embryos died from heart structure defects, whereas surviving adults had electrical conduction defects and, unusually, mitotically active cardiomyocytes. The researchers identified several potential *miR-1-2* target mRNAs, including *Irx5*, which they showed is a direct target of *miR-1-2*. Since *Irx5* encodes a homeobox transcription factor known to regulate cardiac repolarization, it may be the culprit for the electrical conduction defect seen in adult mice.

Muscle-specific miRNAs also regulate hypertrophic growth of heart muscle, report Carè *et al.* in this issue of *Nature Medicine* (**13**, 613–618). The researchers showed that expression of *miR-1* and another muscle-specific miRNA, *miR-133*, is decreased in human and mouse hypertrophic heart tissue. In functional studies, the researchers showed that both miRNAs block cardiomyocyte hypertrophy. Most notably, suppression of *miR-133* expression in mice using an oligonucleotide “antagomir” resulted in cardiac hypertrophy. In search of a molecular mechanism for how *miR-133* controls heart size, the researchers showed that the transcripts of *Rhoa*, *Cdc42* and *Whsc2* are direct targets of this miRNA.

In the third study, Van Rooj *et al.* showed that the muscle-specific *miR-208* controls expression of the β -MHC gene, a regulator of cardiac contractility (*Science*, doi: 10.1126/science.1139089). Mice with *miR-208* deleted developed slight defects in heart function as they aged. More strikingly, these mice failed to undergo cardiac hypertrophy under inducing conditions, and β -MHC expression was not upregulated as expected during the hypertrophic response. Repression of β -MHC expression by the thyroid hormone receptor is important for regulating cardiac contractility. The researchers showed that *miR-208* targets THRAP1, a regulator of the thyroid hormone receptor, suggesting a regulatory circuit by which *miR-208* controls β -MHC expression.

From these and other recent papers, it seems clear that miRNAs have a pivotal role in regulating gene expression in the heart. Unraveling the regulatory circuits involved may be challenging, given that a single miRNA can regulate the expression of many mRNA targets. As important regulators of heart function, miRNAs may represent attractive targets for treating heart disease. – **Michael Basson**



Histological section of mouse heart (stained for Masson's trichrome).

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